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REVIEW ARTICLE

A concise review on *Trillium govanianum* (Wall. ex D. Don) Kunth. - An endemic endangered medicinal plant from the Western Himalayas

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Abstract

Trillium govanianum (Wall.ex D.Don) Kunth., also called Nag Chhatri or Teen Patra, is a member of the Melanthiaceae family (Trilliaceae). It is a threatened, endemic, perennial, angiospermic herb primarily indigenous to the Himalayas. It is extensively found in Bhutan, Nepal, the Himalayan area, Pakistan, India and China, with elevations from 2500 to 4000 meters. In traditional medicine, *T. govanianum* rootstocks treat abscesses, dysentery and inflammation while addressing menstrual irregularities and sexual disorders. Functioning as an antiseptic, it contributes to the healing of wounds. The plant showcases pain relief, anti-inflammatory, anticancer potential and antifungal qualities. Analysis of its phytochemical composition has identified a range of compounds, including steroids, saponins, flavonoids, phenolics, terpenoids and fatty acid esters. Notably, the predominant active components are steroidal saponins. This review offers concise perspectives on a range of aspects such as taxonomy, origin and evolution, morphology, reproductive behavior, life cycle, geographical distribution, ecology, genetics, ethnopharmacology, phytochemistry, pharmacognosy, production and trading, conservation, propagation, adulteration and the utilization of *T. govanianum*.

Keywords

adulteration; ethnopharmacology; pharmacognosy; phytochemistry; *Trillium govanianum*

Introduction

Plants that grow in upper Himalayan ranges, especially in the temperate and alpine areas, are rich sources of secondary metabolites (1), out of which *T. govanianum* (Wall. ex D. Don) Kunth. (Fig. 1) is one seasonal rhizomatous herb commonly known as "Nagchhatri" (2) and also known as Himalayan *Trillium* (3).

The plant is distributed in Himalayan ranges, specifically in temperate and alpine areas such as China, Bhutan, Pakistan and India (3). The plant pertained to the family Trilliaceae (4). It is a tuberous-rooted herb that grows up to 30 cm in height with purple-colored flowers and berry-like fruit. The plant has three life phases. The active components present in *Trillium* are reported to have anti-cancer, anti-inflammatory, anti-bacterial and analgesic properties (5, 6). This perennial herbaceous plant has flavonoid and phenolic contents as active substances reported in leaves stems and rhizomes. This plant is shown to help treat sex disorders, infectious problems and kidney disorders.

Fig. 1. *T. govanianum* (A) mature plant with fruit (B) young plant in one leaf stage.

The microscopic examination demonstrated the existence of various calcium oxalate crystals, starch grains, carinal canals and cortical cells. *Trillium* is propagated through seeds or by division of its rhizomatous roots. Rhizomes of *T. govanianum* contain four steroidal saponins, namely govanoside A, borassoside E, pennogenin and diosgenin, out of which diosgenin has various pharmaceutical uses for the deterrence of cancer and aging factors (2). Its rhizomes have been utilized as a traditional medicine for treating dysentery, boils, inflammation and menstrual and sexual issues (7, 8). Essential nutrients, such as carbohydrates, amino acids, lipids and proteins, are responsible for biological activity. Terpenoids and steroidal compounds are also significant phytochemicals reported in this plant (9). It is often grown in moist and shady places under the shade of *Abies* and *Rhododendron* trees (1). Though this plant has several medicinal properties, most commonly, it is used to cure cancer. Hence, it is also renowned as an anticancer plant.

This review provides succinct viewpoints on a variety of topics with regard to taxonomy, origin and evolution, morphology, reproductive behavior and life cycle, geographic distribution, ecology, genetics, ethnopharmacology, phytochemistry, pharmacology, production and trading, biodiversity conservation, propagation strategies, adulteration and traditional use of *T. govanianum*.

Vernacular names and etymology of T. govanianum

T. govanianum is recognized by diverse names all over the world. Its English common name is the Himalayan *Trillium* (three-leaved). It is known by different local names in various parts of the Himalayas. Nagchhatri, Chotasatwa, Teenpatra (three-leaved), Matarjarai, Triflower and Birthroot. Yan Ling Cao is its Chinese common name (4, 10, 11). In Nepal, it is known as Satuwa in the local language (12). The author citation of the plant is Wall. ex D. Don: Abbreviation “Wall” in author citation is the name of the botanist Nathaniel Wolff Wallich (1786-1854), who was the first to describe it, but his taxonomic guidelines were insufficient to support a publication. Wallich's descriptions were eventually systematized by David Don (1799-1841), who met the prerequisites for a legitimate publication. Wallich proposed *"govanianum"* in remembrance of George Govan, the erstwhile head of the Saharanpur Botanical Garden (13).

Cytogenetics of T. govanianum

Based on their cytological studies, various researchers reported that the chromosome number of *T. govanianum* is 20 and it is a natural tetraploid (2n = 4x) (14-18). A study reported the comparative cytological studies on Himalayan *Trillium* (*T. govanianum*) with Japanese and American species (14). The same study concluded that *T. govanianum* is a tetraploid (2n=20) with two pairs of chromosomes (A-E). Except for chromosome C, all chromosomes are similar to those of Japanese and American species in length and kinetochore location. Chromosome C1 has a satellite on the short arm, whereas C2 has a slit-like constriction in the long arm's proximal area. No satellite chromosomes exist in any Japanese species and they are unlikely to exist in the American species. This finding is particularly significant because it confirms that speciation by hybridization and chromosomal doubling was common among species in Eastern Asia but not in North America.

A thorough depiction of the male meiotic behavior of *T. govanianum,* was reported by researchers in 2021 based on their studies on samples from the Bagus valleys of the Kashmir Himalayas (19). According to the study, the lower fertility was caused by meiotic defects such as chromatin stickiness, late bivalent disjunction, chromatin bridges, laggard formation and polysporads with micronuclei. The lower seed set rate could be attributable to the discovery of numerous meiotic abnormalities.

Systematics and phylogeny of T. govanianum

Nagchhatri belongs to the Melanthiaceae family order Liliales (APG system). Members of this family were placed in different taxons like Paridaceae (20), Parideae (21), Smilacineae (22) and Liliaceae (23-26), as both their sepals and petals resembled each other and are frequently large and showy like those of lilies. However, as molecular and genetic studies evolved, scientists began using DNA analysis to understand plant evolutionary relationships better. *Trillium* has been classified as part of the Trilliaceae family by numerous more contemporary taxonomists (27-30) and it is now formally acknowledged as belonging to the Melanthiaceae family (31). Even after using various molecular and bioinformatics approaches, there are still many controversies regarding the positioning of different taxa, one of which is *Trillium*. The placement of *Trillium* in families such as Liliaceae, Trilliaceae and now Melanthiaceae is a matter of controversy. However, as molecular and genetic studies evolved, scientists began using DNA analysis to understand plant evolutionary relationships better. The research results of a group of researchers support categorizing the general Paris and *Trillium* in the Melanthiaceae family (APG VI) (32).

T. govanianum is believed to be an intergeneric hybrid between Daiswa and *Trillium* based on morphological and chromosomal investigations (33). *T. govanianum* shares morphological characteristics with *Daiswa polyphylla* and *Trillium tschonoskii*. *T. govanianum* grows on its own in the Himalayan mountain range and the allied species *daiswa polyphylla* and *Trillium tschonoskii* are also found in the Himalayan habitat. *D. polyphylla* occurs in lowlands- in the Himalayas, Myanmar, Thailand and Yunnan

(33), whereas *T. tschonoskii* is growing in mountain regions-Sikkim, Nepal, Japan and Bhutan (33). *T. govanianum* shares physical characteristics similar to *T. tschonoskii* and *D. polyphylla*. By chromosomal investigation, it could be analyzed that *T. govanianum* is composed of two genomes; one genome is derived from *the Daiswa* group, which is closely associated with *D. polyphylla* and the second genome is derived from *the Trillium* group, which is closely associated with *T. tschonoskii*. Both *D. polyphylla* and *T. tschonoskii* are diploid (33). *T. govanianum* is an allotetraploid (4X=20) that presumably developed from 10 genomes of a *Daiswa* plant and 10 genomes of a *Trillium* plant.

Based on morphological and chromosomal analysis, It was also reported that *T. govanianum* is an intergeneric natural hybrid of *Trillium* and Daiswa. According to Fakuda, this natural allopolyploid originated from the 10GG genome of *Trillium* and the 10DD genome of the genus Daiswa (33). The paleoclimatics in the Himalayan mountain regions are the factors causing natural polyploidy. Insect-mediated pollination is reported to be the enabling outbreeding between Daiswa and *Trillium*. An article reported that the tetraploids with a double set of genomes may help the species for broader habitat (33). Further, it was reported that the diploid species (GG genome) are vanishing from the Himalayas and the tetraploid species are becoming more prevalent. Adverse climatic conditions in the Pleistocene age in Asia, like severe cold and continuous drought, are popping up as reasons to justify these dramatic changes. In intense climatic conditions, diploid GG *Trillium* species perished, as did the diploid *T. tschonoskii,* but the allotetraploid of the former and the tetraploid of the latter survived.

Next-generation sequencing data generated through plastid sequencing indicated that the ancestral genome of current *Paris* and *Trillium* expanded between 59.16 Mya and 38.21 Mya (34). The genome size growth in Paris and *Trillium*'s most recent common ancestor was most likely a slow process that lasted about 20 million years. *P. japonica* and other taxa with thick rhizomes may have diverged due to the isolation of the Japan Islands from the continent of Asia. This long-term isolation, which began at the Oligocene/Miocene border, would have played a significant role in establishing and evolving *P. japonica's* genomic gigantism. Recent research reports support the taxonomic ranking of Parsis as a genus rather than a genera (34).

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Geographical distribution and ecological status of T. govanianum

T. govanianum Wall ex. D. Don (Fig. 1), is a medicinally significant herb found in subalpine, damp and shady environments. The plant's subalpine environment necessitates survival in stressful conditions and a short life cycle (12, 35-38). *Trillium* is a genus with remarkable homogeneity in general look and structure but tremendous variety in individual organs. The physical and geographical differentiations in the Trillium genus were very strongly tied to the forms of divergence in its important life history and demographic variables (39). The 31 species comprising the *Trillium* genus are extensively dispersed over North America, Japan, China, Russia and the western Himalayas (40). Several *Trillium* species have significant medical use worldwide, including *T. tschonoskii, T. erectum, T. govanianum* and *T. grandiflorum* (41). *Trillium* species *(T. govanianum, T. tschonoskii* and *D. polyphylla*) are found in the Indian Himalayas (27, 41). *T. tschonoskii* grows in lowland mountain ranges across Nepal, Bhutan, Sikkim, Yunnan, Japan and Sakhalin (41).

In India, the distribution of *T. govanianum* is mainly restricted to the temperate and sub-alpine zones at 2,400-3,500 meters above sea level (m asl) in the Western Himalayas (Fig. 2) with infrequent existence up to 4,000 m asl (2, 38). Its distribution is restricted to the high-altitude range of the Himalayas, exclusively in India, China, Nepal, Bhutan, Afganistan (3), which indicates that the species show a very narrow distribution in a highly specific habitat. The center of its diversity is the North-West Himalayas (11). In Himachal Pradesh, it grows in forest shade rich in humus (3). The locations that reported the availability of nagchatri from India in literature are compiled in Table 1. The plant is usually located in the well-moist, shady canopy (Fig. 3) under rock crevices below the dense canopy of trees like *Rhododendron* spp., Juglans *Quercus*spp., *Abies* spp., *Betula* spp., *Cedrus*spp., *Picea*spp., *Juglans* spp., Juniperus spp., Salix spp., *Ribes* spp. and *Arundinaria* spp. (12). Common herbaceous species with which *T. govanianum* is observed to be sharing habitat are *Polygonum* spp., *Oxalis* spp., *Anemone* spp., *Viola* spp., *Podophyllum hexandrum* and *Valeriana hardwickii* (Fig. 4). In many of the study reports, it is stated that the roots of this plant show association with the roots of these herbs.

Ecological status

The IUCN recently classified this endemic species as endangered due to its unsustainable harvest from the wild (47). Preserving and restoring genetic variation is essential to successful conservation because it poses a serious threat to the survival of endangered species.

The unselective, unsustainable, or unmanaged collection of species from their natural habitats is causing the species' potential threat and local extinction. A very slow life cycle, overexploitation because of the increased commercial demand in the local market and a very low population density are the commonly observed reasons for the threat to the species after thorough population diversity analysis in various locations of the Indo-Himalayan areas of plant habitats. Scientists have reported that the highest rate of population decline is in the Munsiyari region, with species extinction in some locations (2). Reduction in population density was reported in Tunghnath and Thirthan Valley too. Earlier, the species was in the threatened category (3). Local extinction of the species impacts the overall biological diversity and socioeconomic status of the local inhabitants, as this species is one of the significant seasonal income sources of the villagers.

Table 1. Distribution of *T. govanianum* in the Himalayan region

State/Country	Location	Reference
Pakistan	Khyber Pakhtunkhwa, Dir Upper, Kohistan Valley	(6)
Himachal Pradesh	Lahaul, Kinnaur, Kullu, Rohru, Kangra, Pangi, Bharmour Upper beas valley, Kothi, Banjar valley, Parbati valley	(42, 43)
Uttarakhand	Gangotri, Govind Pasu Vihar, Harsil, Kedarnath, Raithal, Sukhi, Sayara, Tehri-Garhwal in Bhagirathi Valley	(44)
China	Qinba mountain of Shaanxi Province, Tibetan region of S. Xizang	(45)
Jammu & Kashmir	Doda and Kishtwar, Bandipora district, Fatehpur, Gulmarg, Kanzalwan, Phalgham, Poonch, Gurez (Kashmir)	(46)

Fig. 3. *T. govanianum* in its natural habitat.

Life cycle and morphological characters of T. govanianum

Trillium is a genus of perennial herbs whose rhizomes are characterized by being parallel or erect, semi-erect, branching, or somewhat compact, shorter and elongated to bulky and fleshy, with a big terminal shoot or bud at the apex (3). This herb is modest and prefers shaded spots. It has a stocky stem that is 15 to 25 cm purple-red and has plain, broadly oval green leaves that are strongly deflexed. At the top is a green blossom with a rich oxblood red color (3). Its abundant growth typically favors cold, dark and damp environments (3). The plant grows in well-drained, well-moistured soil, usually beneath huge rocks and a dense canopy of trees and shrubs (3).

The *T. govanianum* herb has a center purplish-brown flower and three leaves arranged in a whorl at the top of the stem, reaching a maximum height of 30 cm. There are three broadly oval, sharp leaves with a conspicuous petiole. The plant comprises three sepals, three petals, six (3 double) stamens and a three-carpellate ovary. *T. govanianum's* petals are reddish purple, linear-lanceolate, with an obtuse base and acuminate tip. *T. govanianum* bears a dark pinkish sepal with a linear-wide lanceolate shape, an obtuse base and an acuminate tip (3, 10). Pollen grains are monocolpate and ellipsoidal (10). *T. govanianum* develops yearly in April after snowmelt and flowers in May and June. Lower altitudes experience fruit maturity and leaf senescence in July/August, while higher altitudes experience it in September/October (10).

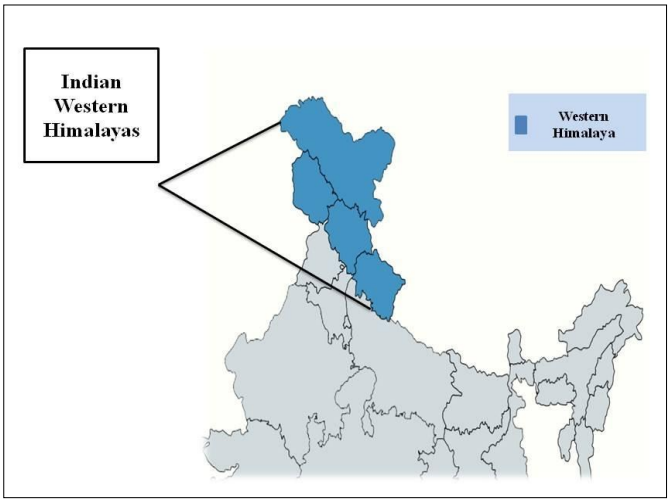

Fig. 2. Picture depicting the geographical distribution of *T. govanianum.*

Fig. 4. *T. govanianum* in natural habitat with co-species.

Every year, in April, following the melting of the snow, *T. govanianum* was among the first plants to emerge; it would flower in May or June. The lifespan is comparatively thirty years (2). The species is usually propagated through rhizomes. Three life phases (Fig. 5 A - C) could be distinguished-the one-leaf vegetative stage, the three-leaf vegetative stage and the three-leaf reproductive stage, with each stage of rhizome life increasing its size. May to June is flowering time and July to October is fruiting time with leaf senescence. Rhizomes become dormant in winter before snowfall (12). A long life cycle with longevity of up to 72 years is a characteristic feature of *T. govanianum*. Asexual reproduction of *Trillium* is less documented (37, 39). The vegetative mode of reproduction from the rhizome is the more common. *T. govanianum* has a long life cycle, which is characteristic of other *Trillium* spp., which have been reported to spend several years in each of the vegetative stages before proceeding to reproduction and reproductive plants may or may not regress to vegetative in the next year (48, 49). The observations of asexual reproduction in *T. govanianum* are interesting because asexual reproduction in *Trillium* is seldom recorded and some species nearly never create new individuals asexually (37, 44). *T. govanianum* can reproduce vegetatively from a single rhizome, but collection procedures eliminate all rhizome-associated genets. Other *Trillium* species have shown self-fertilization (37), but significant inbreeding depression .

Inbreeding depression is reported to be evident in the population because of the prevalence of self-fertilization (37), which leads to a low level of genetic

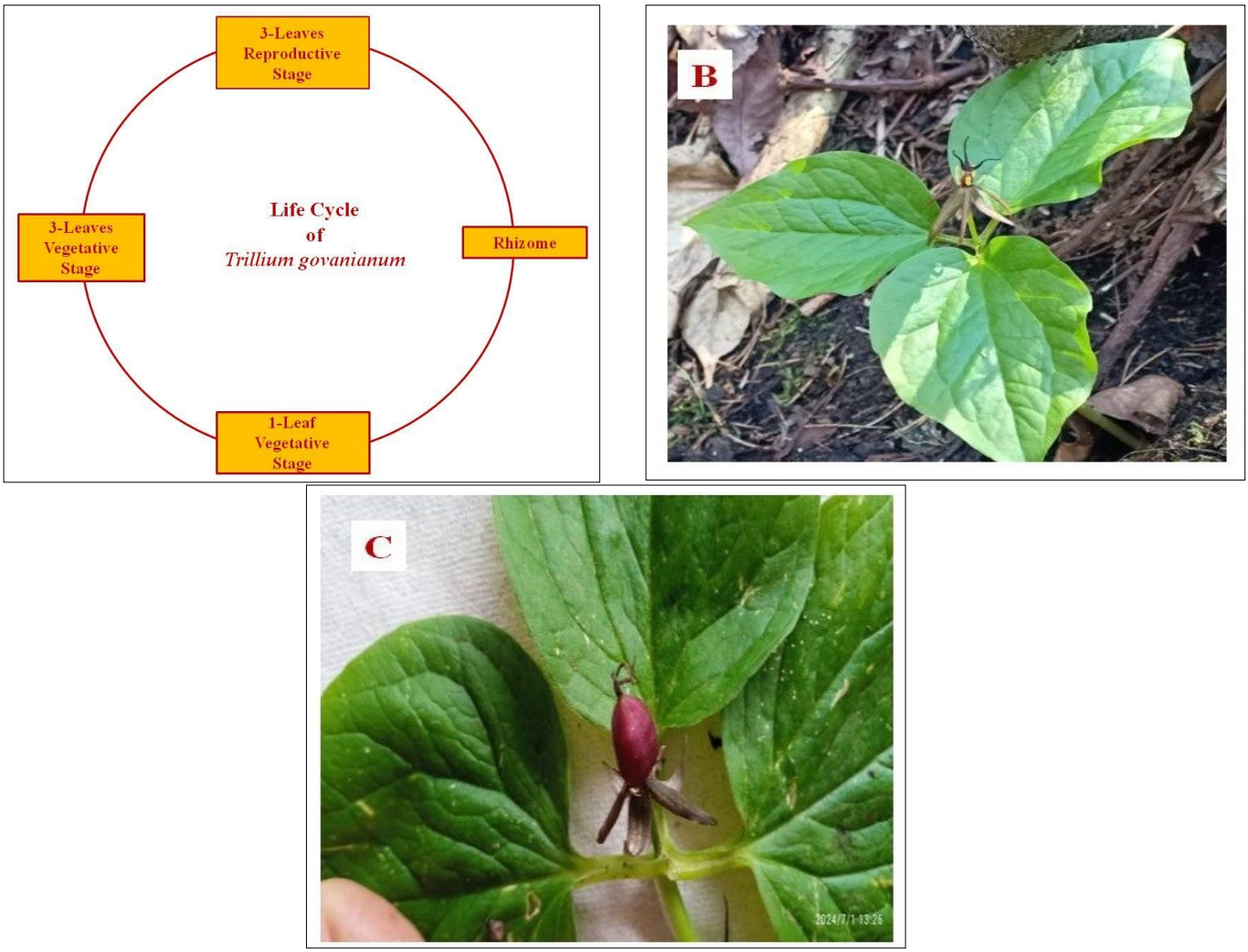

Fig. 5. A. *T. govanianum* life cycle; B and C. *Trillium* plant in reproductive stage (B. flowering sage; C. fruiting stage).

diversity. The plant's growth rate is very low as it takes 7-8 years to reach maturity. 2-3 cm long flowers. The seeds are ovoid, have palpated lateral appendages and the fruit is a globular red berry (38). It is noticeable that the plant remains in one leaf vegetative stage for several years, but it still produces more rhizome biomass. The cycle of several years without blooming ends when the rhizome reaches the threshold of biomass accumulation, at which point flowering stages begin. The plant may return to the previous stage at the start of each new season, most likely if the rhizome's resources have depleted to a certain extent (37). Every year, *T. govanianum* emerges in April following snowmelt and it flowers in May and June.

There have been reports of unsuccessful attempts at ex-situ propagation of *T. govanianum* via rhizome, seed and tissue culture. Seed propagation has had some success (50), but it is complex and time-consuming, rendering it unsuitable for large-scale dissemination (51).

Anatomical studies

***Anatomical details of stem*:** The stem's TS is circular in outline, with a single-layered epidermis of square-shaped cells and an inner, single-layered hypodermis (52). The core cortex is reported as having oval to distorted parenchyma cells with intercellular gaps. The same research has reported the cortical region was made up of six oval-shaped vascular bundles on the outside and three smaller bundles on the inside and the vascular bundles are made up of a crescent-shaped xylem that encloses the phloem (52).

***Anatomical details of leaf*:** Researchers report that the TS of the leaf revealed a classic dicot anatomical organization with midrib and lamina areas (52). The lamina consists of the upper epidermis, which is single-layered and the interior layers, which are less distinct. The midrib consists of a single-layered upper and lower epidermis with trichomes, followed by an inner cortical zone. The cortex is 2-3 layers deep on the adaxial side, with small and compact cells. The abaxial side featured 3-4 layers of cortex cells with higher cell sizes. The cortical region surrounding inner oval-shaped circulatory bundles is made up of the xylem (adaxial side) and phloem (abaxial side) (52).

***Anatomical details of rhizome*:** The rhizome's structure is reported to be circular, with a single-layered epidermis of rectangular to square-shaped cells, followed by 1-2 layers of massive hypodermal cells and a core multilayered cortical zone with starch-containing parenchymatous cells. Further, the literature reports smaller epidermal cells than hypodermal cells and cortex cells made up of parenchymatous cells that range in shape from hexagonal to oval, with intercellular gaps documented in the literature. The study further reports the cortex's middle area with a circular ring of fragmented vascular bundles and less defined vascular tissues.

Economic importance of T. govanianum

Trillium was widely used and traded in national and international markets for millions of years. This plant is currently in high demand due to its increasing use in the trade sector. The market value of the rhizome of *T. govanianum* is connected to the age of the plant (53). The presence of 2.5% diosgenin, a hydrolyzed saponin, makes it highly sought after in the international market for commercial production of steroids and sex hormones (4).

Rhizome commerce has considerably improved the living standards of locals who suffer from poor wages and high unemployment and residents have become reliant on rising income. For the last decades, the prices have grown from Rs. 1000-1500 per kg to Rs. 3000-5000. These days, the market price ranges from Rs. 15000 to 25000, which shows a decrease in supply or a high demand in the market. A hike in income because of high prices is both a reason and a result of over-exploitation. There is a report regarding a well-established clandestine network of *T. govanianum* supply to Tibet (China), which can be related to the demand for the species in Chinese traditional medicine. It requires legal permission to transport rhizomes through Himachal (43). However, economic incentives have pushed residents to risk legal prosecution. Furthermore, accessions from Himachal Pradesh, including *T. govanianum*'s most anthropogenically damaged habitats, were more diverse than accessions from intact Kashmir Himalaya environments. The rhizomes of this plant species could serve as a potential novel source of chemicals beneficial in relieving pain and inflammation. The rhizomes of this plant species may serve as a potential novel source of chemicals helpful in relieving pain and inflammation (10). In traditional medicine, *T. govanianum* rhizome treats diarrhea, backache, wound healing, inflammation, skin boils, menstruation and sexual issues (54-57). *T. govanianum* roots are used to treat external wounds and allergic skin responses. *T. govanianum* roots can heal external wounds and allergic skin reactions (58). The root decoction is useful for sexual difficulties, as an emetic and astringent (59), for impotence and tumors (60) and as a tonic and emetic (61). The root is an alternative medicine with antibacterial, antispasmodic, diuretic, emmenagogue (to stimulate menstruation) and ophthalmic properties. Fresh or dried roots can be boiled in milk, treating diarrhea and dysentery. The raw root is grated and used as a poultice to reduce eye swelling or treat hurting rheumatic joints. The leaves were boiled in lard and used as a poultice to treat wounds and prevent gangrene. An infusion of the root is used to relieve cramps and the plant's popular name, "birthroot," stems from its use to encourage menstruation. A root bark decoction can alleviate earaches in drops (7). The immature edible unfolding leaves are a fantastic addition to salads, tasting similar to sunflower seeds. The leaves can also be used as a pot herb. Phytochemical analysis identified the presence of steroidal glycosides, saponins and sterols. Flavonoids and carbohydrates. Gas chromatography/Mass spectrometric analysis of the n-hexane fraction revealed 12 molecules, including 70% unsaturated and 30% saturated fatty acids. *T. govanianum* has higher diosgenin content than previously researched plants, making it suitable for commercial diosgenin production. More than 40 steroidal saponins with spirostane and furostane-type aglycons have been isolated from the genus (59, 62, 63). From the Kullu region of Himachal Pradesh, only around 637 tons were traded, while there was a report of 0.7 billion plants from the region in 2011 (41). The gathering of the species is so intense in Tirthan Valley and Musiyari. The mediators of locals and global traders are buying the rhizome much cheaper than the global market and the villagers have no idea where the items are being resold. There is a well-established chain for smuggling of the item. There are reports that local plant nursery owners are gathering the species from locals and exporting the items to foreign countries like the UK as part of an existing illegal supply chain.

Phytochemical reports

Reports on chemical investigation found that numerous elements such as steroids, terpenoids, saponins, phenolics, flavonoids and fatty acid esters were extracted from this species, of which steroidal saponins are the most active constituents (62-67).

Diosgenin, a hydrolyzed product of saponins, makes its demand remarkably high, enabling the plant to become the natural resource material for commercializing steroids and sex hormones (4). Researchers reported the presence of various phytochemical constituents like saponins, steroids, terpenoids, phenolics, flavonoids and fatty acids from this plant (68). The major steroidal compounds isolated from the plant are Diosgenin, Pennogenin, Govanoside A, Borassoside E, Sitosteroid and 5-hydroxy-steroids (66, 69) (Fig. 6, Table 2, Table 3) Steroidal saponins could serve as chemical indicators for evaluating the quality of the species.

The steroidal saponins isolated from *Trillium* include govanoside B, pregna-chacotrioside, pennogenin-triglycoside, borassoside E, pennogenin-tetraglycoside, protodioscin, clinioside B, pennogenindiglycoside and borassoside D (69).

T. govanianum rhizome, by phytochemical analyses, exposed the existence of various secondary metabolites in its fractions and methanolic extract, including flavonoids, steroidal saponins, tannins, sterols and glycosides (3). Under hot and cold extraction circumstances, the chemical, nutritional and antioxidant profiles of *T. govanianum* are shown to vary. The hot ethanolic extract contains a high level of total flavonoids & phenolics. Still, the cold-water extract contains a high level of nutrients like amino acids and nucleosides, i.e., both cold and hot ethanolic extracts had greater nutritional energy values (9).

Principal active components of this species are steroidal saponins. Researchers reported 24 saponins with steroidal characteristics in *T. govanianum* utilizing UHPLC-QTOF-MS/MS and for the successful extraction of metabolites from the *T. govanianum* rhizomes by the different procedures (70). govanoside A, govanoside B, pennogenin-[O-β-D-glucopyranosyl-S1 or its isomer, (1β,23S,24S)-1-[O-β-D-glucopyranosyl(1→3) - O - (β-D-xylopyranosyl-(1→2)-Oα-L-rhamnopyranosyl]-23 hydroxyspirosta-5,25-dienyl-24-[O-β-D-6-deoxygulopyranoside] or its isomer, spirosta-5,25-dienyl-[O-β-D-glucopyranosyl-S3 or its isomer, protodioscin, protodioscin

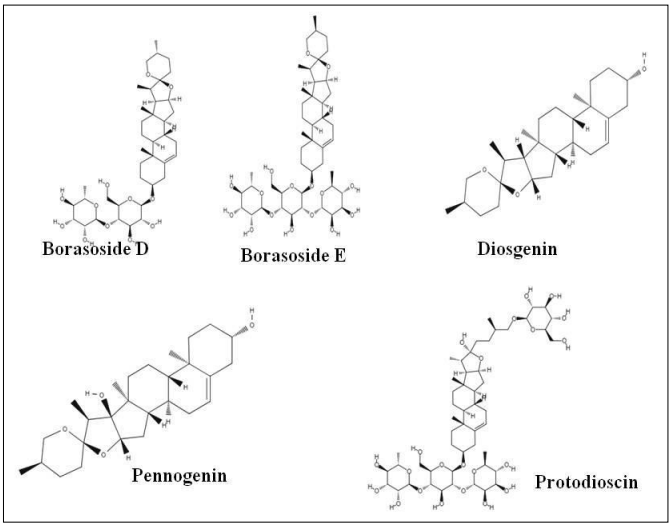

Fig. 6. Chemical structure of major steroidal saponins from the *T. govanianum*. (alkaloids, flavonoids, saponins, etc.) drawn in MolView (https://molview.org/).

isomer, pennogenin-[O-β-D-glucopyranosyl-S4 or its isomer, diosgenin-[O-β-D-glucopyranosyl-S5 or its isomer, pennogenin -[O-β-D-glucopyranosyl-S4 or its isomer, pennogenin - [O-β-D-glucopyranosyl-S4, pennogenin-[O-β-D-glucopyranosyl- S6, gentrogenin 3-O-βchacotrioside or its isomer, pennogenin -[O-β-D-glucopyranosyl-S7 isomer, pennogenin-[O -β-D-glucopyranosyl or its isomer, pennogenin-[O-β-D-glucopyranosyl-S7, pennogenin-[O-β-D-glucopyranosyl-S7 isomer, Borassoside E isomer, Spirosta-5,25-dienyl-[O-β-D-glucopyranosyl-S4 or its isomer, borassoside E isomer, borassoside E , borassoside E isomer , borassoside D isomer and borassoside D are the 24 saponins that are identified by the UHPLC-QTOF-MS/MS method.

Ethnopharmacological uses

Plants have been utilized since antiquity to treat diseases and promote well-being. This plant's rhizomes have traditionally been used to treat various diseases, including inflammatory and sexual issues (56-59). There are reports that the rhizome in very small quantities is used as a remedy for dysentery and stomachic disease, as well as the preparation of steroids and saponins (43). Native uses this plant to cure cancer, hypertension, neurasthenia, giddiness, arthritis, dysentery, inflammation, sepsis and reproductive disorders. *T. govanianum* is used in traditional medicine to treat dysentery, wounds, skin blisters, inflammation, sepsis and menstrual and sexual issues (7, 11, 62). According to reports, the powdered plant is utilized as a body and sexual tonic (60, 61).

Overexploitation of natural habitats to suit the demand of the pharmaceutical business has resulted in significant population reduction. The root infusion is used for sexual dysfunctions, as an emetic and astringent (43), impotence and tumors. The root is used in alternative medicine as an antibacterial, antispasmodic, diuretic, emmenagogue (to stimulate menstruation) and ophthalmic. Fresh or dried roots can be boiled in milk, treating diarrhea and dysentery. The raw root is grated and placed as a poultice to relieve swelling in the eye or on hurting rheumatic joints. The leaves were boiled in lard and used as a poultice to treat wounds and prevent gangrene. An infusion of the root is used to relieve cramps and the plant's popular name, "birthroot," derives from its use to encourage menstruation. A root bark decoction can be applied as drops to relieve earache. The species is used to ease childbirth and treat other female issues. In traditional medicine, *T. govanianum* rhizome treats diarrhea, backache, wound healing, inflammation, skin boils, menstruation and sexual issues (7, 10, 55, 62). *T. govanianum* roots also treat external wounds and allergic skin responses (56). The root decoction is used to treat sexual abnormalities, as an emetic and astringent (63), impotence and tumors (60) and as a tonic and emetic (58). The immature edible unfolded leaves are a fantastic addition to salads, which taste similar to sunflower seeds. The leaves can also be used as a pot herb. In traditional medicine in northern Pakistan, *T govanianum* rhizome is used as an analgesic and anti-inflammatory. *T. govanianum* rhizomes, known as 'beth root,' have been utilized in folk medicine to treat uterine, urinary tract and pulmonary hemorrhages.

Table 3. List of major steroidal saponins reported from *T. govanianum* and their and their pharmacological properties

Name of the phytochemical	Uses	Reference
Govanoside A & Borassoside E	Anti-fungal and Anti-bacterial	(54)
Pennogenin & Borassoside E	Analgesic and Anti-inflammatory	(54)
Glycosides & Saponins	Anticancer	(54)
Borassoside E, Pennogenin, Protodioscin, Tetraglycoside, Govanoside B saponins	Insecticidal	(54)
Borassoside E, Protodioscin & Diosgenin	Anti-diabetic	(71)

Table 2. List of steroidal saponins reported from *T. govanianum*

Name of	Principal phytochemicals reported	References
T. govanianum	Govanoside A, Pennogenin, Diosgenin, Govanoside B, Borassoside E, Borassoside D, Protodioscin, Pennogenin glycosides, Pennogenin tetra glycosides, Pregnachacotrioside, (1β, 23S,24S)-1- [O-β-d-glucopyranosyl (1→ 3)-O-(β-d-xylopyranosyl-(1 → 2)-O-α-l-rhamnopyranosyl]-23 hydroxyspirosta-5,25-dienyl-24-[O-β-D-6-deoxygulopyranoside] or its isomer, Pennogenin- [O-β-d-glucopyranosyl-S4 or its isomer, Pennogenin- [O-β-d-glucopyranosyl-S7 isomer, Pennogenin- [O-β-d-glucopyranosyl or its isomer, Pennogenin- [O-β-d-glucopyranosyl-S1 or its isomer, Borassoside D isomer, Borassoside E isomer, Pennogenin- [O-β-d-glucopyranosyl-S4 or its isomer, prostate-5,25-dienyl- [O-β-d-glucopyranosyl-S3 or its isomer, Pennogenin- [O-β-d-glucopyranosyl-S6, Diosgenin- [O-β-d-glucopyranosyl-S5 or its isomer, Gen-trogenin 3-O-β-chacotrioside or its isomer, Protodioscin.	(19, 70)

Therapeutic uses of T. govanianum

Various reports are available regarding the therapeutic applications of *T. govanianum.* According to a study, the plant has anti-inflammatory, analgesic, anticancer, anti-fungal, anti -oxidant, anti-bacterial, anti-diabetic, anti-aging and anti-tumor properties (Fig. 7) (4).

Reports are there regarding the activity of rhizome extract against microbial infections like fungi and bacteria. The steroidal chemical groups of saponins constitute the predominant secondary metabolites extracted from the genus *Trillium*. Trihydroxy fatty acids and flavonoids have been reported from the rhizome extract and have also been shown to contribute to antimicrobial activity. Numerous saponins, including spirostanol and furostanol saponins, have demonstrated strong potential against fungus strains (6). Comparing their furostanol saponins to spirostanol saponins, the spirostanol saponins reported greater antifungal potential. *Trillium* has yielded over forty steroidal saponins containing spirostan ($C_{27}H_{44}O_2$) and furostane aglycones. It is shown that the strains of microbes like *Aspergillus flavus* ATCC 9643, *Aspergillus niger* ATCC 16888, *Candida glabrata* ATCC 90030 and *Candida albicans* ATCC 18804, all susceptible to the actions of the compounds borassoside E and govanoside A. It denotes the possibility of using this plant for candidiasis and aspergillosis (6).

The plant species' rhizomes cure various illnesses, including cancer, diarrhea, giddiness, inflammation, hypertension, neuropathy and arthritis. Additionally, it is used as an antiseptic to treat wounds, infections, stomach and joint pain and menstrual and sexual issues (4, 7, 68). Compared to the anticancer medication doxorubicin, *T. govanianum* extract showed significant antiproliferative and cytotoxic action on cervical and prostate cancer cells (4). Rhizomes are home to a variety of substances, including four steroidal saponins (govanoside A, borassoside E, pennogenin and diosgenin), of which this species is the only one known to have govaniside A (41).

Various crude extracts of rhizomes have exhibited anti-cancerous action against the HepG2 cell line (liver), A549 cell line (lungs), MCF7 cell lines (breast), EJ138 cell line (urinary/ bladder), HeLa (cervix) and PC-3 (prostate) (6, 69). Crude extracts also exhibited antifungal, antioxidant, anti-inflammatory and analgesic properties (66). The methanol extract is hazardous to brine shrimps and leishmanial (66). The leaves of this species are used to cure dyspepsia and animal parasites (2). Roots contain trillarin, which, when hydrolyzed, yields 2.5% diosgenin (a corticosteroid hormone) (1). The steroidal saponins extracted from *T. govanianum* exhibit strong alpha-glucosidase and alpha-amylase inhibitory activities in addition to mild dipeptidyl peptidase IV inhibitory activity. The best substances to lessen the effects of dipeptidyl peptidase IV, alpha-glucosidase and alpha-amylase are boraxoside E, protodioscin and diosgenin. Consequently, the steroidal saponins and fractions of *T. govaníanum* possess strong hypoglycemic qualities and can be utilized as nutraceutical nutrients to regulate systemic glucose levels (4).

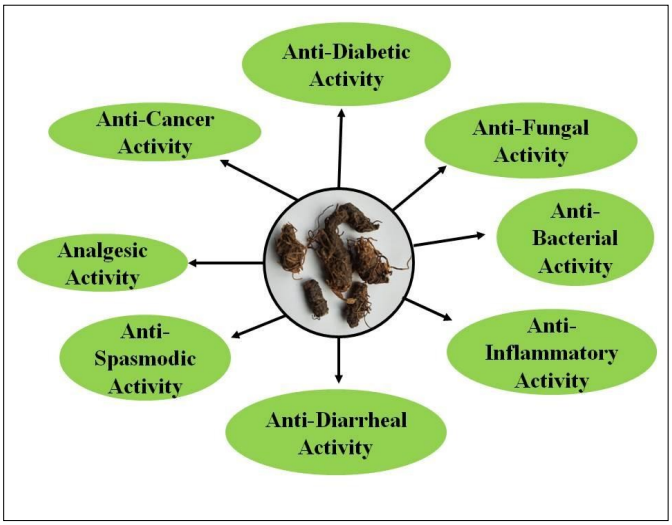

Fig. 7. Pharmacological activities of *T. govanianum.*

T. govanianum rhizome extracts (methanol, ethanol, acetone and distilled water) were tested *in vitro* against three human pathogenic bacteria (*Escherichia coli, Staphylococcus aureus* and *Yersinia pestis*) using the agar well diffusion method at varying concentrations (69). *Trichophyton rubrum* and *Microsporum canis* were the two fungal strains against which the hydro-methanolic extract of *T. govanianum* demonstrated significant inhibitions of 80 and 75 percent, respectively; the chloroform-soluble fraction demonstrated an inhibition of 90 percent (72-74). An ethanolic extract of the plant's rhizome was employed for the antibacterial study, exhibiting a maximal inhibitory zone against a standard isolate of *K pneumoniae* (56). The species' leaves cure livestock parasites and dyspepsia (53). In traditional medicine, *T. govanianum* has been used to treat menstruation and sexual problems, infections, wounds, boils on the skin, inflammation and dysentery (7, 62). Govanoside A and borassoside E compounds showed good to moderate activity against *Aspergillus niger* ATCC 16888, *Aspergillus flavus* ATCC 9643, *Candida albicans* ATCC 18804 and *Candida glabrata* ATCC 90030 (70). It is also used for stomach disorders. It is also used for stomach disorders

In-vitro propagation studies on T. govaniaum

Ex-situ propagation via rhizome, seed and tissue culture has been attempted but has yielded dismal results. Seed propagation has had some success reports (50, 57, 71-72), but the process is complicated and time-consuming, rendering it unsuitable for large-scale propagation (71-72). Chemical inhibition of apical dominance is used in vegetative propagation via the rhizome to induce budding, but this strategy seldom yields buds (53, 73). In a study, (74) lanolin paste containing cytokinins was applied on the cut surface of the rhizome to form a callus before applying gibberellins to encourage shoot growth (71). Moreover, attempt for *in vitro* propagation with negative outcome for *T. govanianum* have also been reported (72).

Genetic diversity studies

although a plant species of tremendous value, its germplasm remains uncharacterized and there are very few reports on genetic diversity in this species. Reports are there (4) regarding the usage of microsatellite markers for genetic diversity assessment of various accessions of *T. govanianum*

from the Western Himalayas (4). Polymorphic microsatellites discovered in this work may be relevant in future genetic analyses of *T. govanianum* and other similar species. Reports are there that the genetic makeup of *T. govanianum* consists of 10 protein sequences and 12 nucleotides (70).

In population diversity studies of species in Himachal Pradesh, the maximum population density has been reported at Kathnag, Chamba and Jamathu, Chamba shows the lowest population density of the species (75, 76). Another study reported a total of 5337 flanking in *T. govanianum*. Simple Sequence Repeat (TGSSR) markers from 7514 SSRs with transcripts (77). They selected 288 TGSSR markers for amplification validation in random genotypes based on their functional relevance and class. The result of the study showed that 180 markers were effectively amplified in the various random genotypes, including 105 polymorphic and 75 monomorphic markers. Furthermore, the study reports that 431 alleles were identified with polymorphism information content (PIC) ranging from 0.15 to 0.50, with an average of 0.43. Interestingly, 105 polymorphic TGSSR markers encompassed the main genes of the steroid saponin and terpenoid production pathways and critical stress-related transcription factors.

A study reported the assessment of intra- and inter-population genetic variation in populations from seven different sites of the Kashmir Himalayas using start codon-targeted (SCoT) and SSR markers (78). Their study results show a very low level of genetic diversity in *T. govanianaum* with very low observed and expected heterozygosity. This study also reveals high levels of inbreeding, habitat fragmentation, population isolation, the bottleneck effect, low gene flow and predominantly asexual reproduction in the species.

Wild trafficking and molecular authentication studies on T. govanianum

The rhizomes of this species, obtained from wild populations, have been traded throughout the Himalayan region and have recently emerged as an important source of revenue for locals in the Indian Himalayas. A unique method for precisely identifying species using DNA barcodes addresses the serious issue of trafficking of this valuable medicinal herb. Reports are there (78) about constructing a reference DNA barcode for *T. govanianum* and *P. polyphylla* (Fig. 8 A & B), a powerful adulterant, to ensure accurate identification (75). The DNA barcode library for *T. govanianum* and *P. polyphylla* was created using ITS, matK, trnH-psbA and rbcL regions. ITS, matK and trnH-psbA are effective reference barcodes for *T. govanianum*, while ITS and trnH-psbA are appropriate for *P. polyphylla*. Phylogenetic trees were generated using the neighbor-joining (NJ) method, ITS, rbcL and trnH-PsbA regions to differentiate *Trillium* species from *Paris* species (78). The Internal Transcribed Spacer (ITS) region successfully distinguished between closely related *Trillium* species (57, 78).

In another study, researchers proved the suitability of ITS and matK regions as barcodes to authenticate the adulteration of *T. govanianum* with *Dioscorea villosa* (79). A group of researchers in another study generated a DNA barcode to generated a DNA barcode to authenticate *T. govanianum* species (52). Their study used plant accessions from various locations in J&K and Tissa, Chamba of Himachal Pradesh. The DNA barcoding produced a 100% identification match between barcode sequences generated in this study and the NCBI database. The psbA-trnH barcode sequences were not in the NCBI database and were submitted for the first time in this study.

During our field surveys, we observed that the rhizome of *Cryptocoryne spiralis* (Retz) Fischer ex Wydler var. *spiralis* (Fig. 8 C1-C3), widely distributed in India, mainly in the western coast, including Maharashtra, North Karnataka, Kerala, the Coromandel Coast and Pondicherry, is utilized as an adulterant to *T. govanianaum* rhizome in the herbal export markets.

We observed during our field study throughout various locations of natural habitats (Fig. 9) as well as local markets in Punjab and Delhi that the rhizome of *Cryptochorine spiralis* is also used as an adulterant for *T. govanianum* because of the morphological similarities between the rhizome and *T. govanianum*.

Existing biodiversity threats for T. govanianum

The rhizomes of this species, obtained from wild populations, have been marketed throughout the Himalayan region and have recently emerged as a substantial source of revenue among indigenous people in the Indian Himalayan region. Mature *T. govanianum* plants are more valuable to gatherers; however, plants of all ages are harvested in present practices. Survival of mature plants is a standard limiter of population recovery, especially for this species, because sexual reproduction occurs only in older plants (79, 80). The short gathering season may limit gatherer demand, yet the gathering rate seems unstable (61). A considerable quantity (637 tons) of collection and trade was reported from the Kullu area of Himachal Pradesh alone in 2011 (42). The species has a restricted geographic distribution and requires a specialized natural terrain, which may also diminish its distribution.

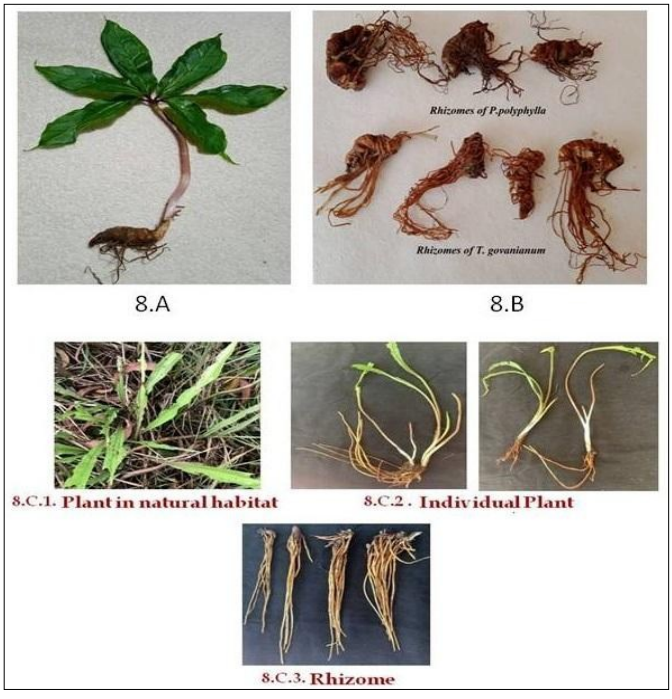

Fig. 8. Plant species used as an adulterant of *T. govanianum*: 8 A. *Paris Polyphylla*, the plant which is used as a common adulterant of *T. govanianum*; 8 B. Rhizomes of genuine samples *(T. govanianum)* and adulterant (*P. polyphylla)*; 8 C.1-3: *Cryptocoryne spiralis* another adulterant species of *T. govanianum.*

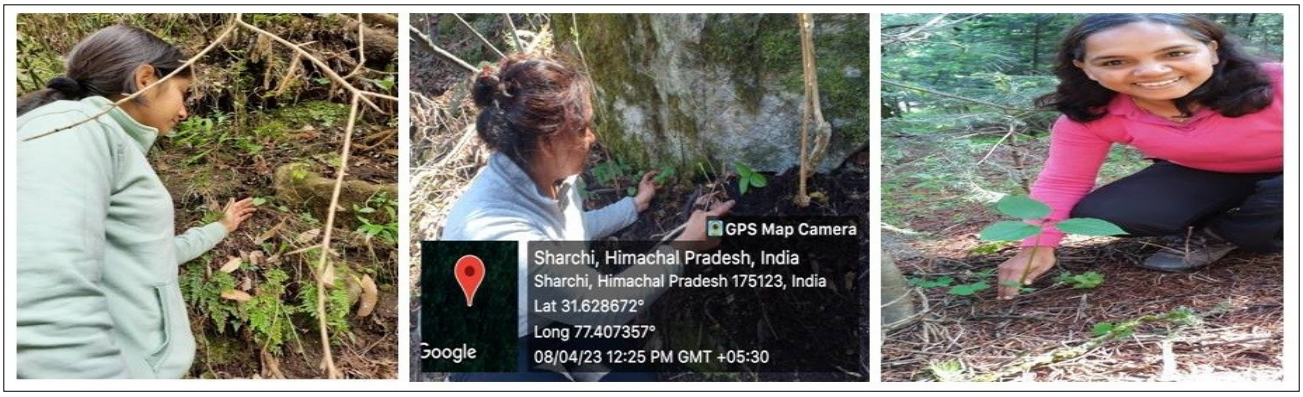

Fig. 9. Authors during their visit to the natural habitat *of T. govanianum*.

Grazing and herbivory are common threats to species diversity (42). Vegetative mode reproduction is one of the reasons for the low biodiversity of plants. Long life cycles to reach maturity, self-fertilization and associated inbreeding depression also led to the loss of biodiversity in addition to over-exploitation of the species (42). The natural habitats of the species from Himachal Pradesh were found to be more diverse than accessions from intact Kashmir Himalaya environments. Unregulated from natural habitat may be diminishing its distribution. *T. govanianum* root systems were typically seen in conjunction.

This connection likely boosts *T. govanianum* survival rates, as root associations typically result in enhanced biomass, growth and reproduction in sub-alpine and alpine species (81, 82). Grazing is one factor affecting the growth of *Trillium* species in their natural habitat (83). Gatherers cannot distinguish leafless plants; hence, grazing can benefit a population by allowing some leafless rhizomes to be neglected during extensive collection (83-86).

The plant propagates using underground rhizomes. The rhizome is the primary plant portion used for therapeutic purposes. In recent years, overexploitation and improper uprooting of this herb from the northwestern Himalayan region, notably from Himachal Pradesh, has left it on the edge of extinction (41). As a result, the various populations of *T. govanianum* in its natural habitats are under significant anthropogenic pressure, causing their decline.

Future perspectives

Recently, a group of researchers reported their study results on the reproductive biology of *T. govanianaum*. They have conducted different breeding experiments in their study, including qualitative and quantitative floral traits. Their findings conclude that some inherent biological traits and environmental conditions limit *T. govanianum* reproduction, though the anemophily is active in the species. The study also revealed the occurrence of both self- and cross-pollination in the species (19).

Recently, a group of researchers reported their study results on the reproductive biology of *T.govanianaum*. They have conducted different breeding experiments in their study, including qualitative and quantitative floral traits. Their findings conclude that some inherent biological traits and environmental conditions limit *T. govanianum* reproduction, though the anemophily is active in the species. The study also revealed the occurrence of both self- and cross -pollination in the species (45).

In another study on conservation genetics approaches (78) to investigate how drastically declining populations in natural habitats impact population genetic diversity and structure of this endangered species across the Kashmir Himalaya, the researchers reported that there is very low genetic diversity, high levels of inbreeding and high genetic differentiation among the populations; likely resulting from habitat fragmentation, population isolation, the bottleneck effect, low gene flow and predominantly asexual reproduction currently operative in the species (75).

Researchers recently reported an efficient and competent way to propagate the valuable endangered medicinal herb (87). This study reports that on MS medium enriched with BAP, Kn and GA3, rhizome bud explants harvested from individuals in identified elite locations demonstrated the highest survival rate and shoot regeneration. The phytochemical analysis they conducted using HPLC techniques revealed that the high altitude samples have higher diosgenin content. Also, a low level of genetic diversity had been reported for this species based on genetic diversity analysis using SSR and ISSR markers.

Targeted and non-targeted metabolomic studies using UPLC and PDA.ELSD and UHPLC-Q-TOF-MS for organ-specific (roots, rhizomes, rhizomatous buds, stems, leaves and fruits) phytochemical profiling of this Himalayan medicinal herb indicated that diosgenin is located only on the rhizomes of the plant. At the same time, dioscin is reported in all parts of the plant in this study (88).

The in-silico studies on the root samples of this herb from the Kashmir province of Pakistan displayed the interaction of key phytochemicals and enzymes. The evaluated biological activity and bioactive phytochemicals in the studied plant extract may lead to the development of new pharmaceuticals (89, 90).

Information on this species' genetic diversity and structure is needed to recognize different accessions for conservation priority. Because of its widespread use, therapeutic effects and complexity, *T. govanianum* requires rigorous quality control techniques to assure safety and efficacy. A rigorous assessment of extinction risk necessitates knowledge of a species' ecology, distribution and stress responses, all lacking in the case of *T. govanianum*. To research the safety profile and establish the efficacy of this medicinal plant, precise identification and standardization are required, which can be achieved through pharmacognostic investigations or molecular authentication rules. Investigation

of the safety profile and establishment of the efficacy of this medicinal plant requires exact identification and standardization procedures, which can be accomplished through pharmacognostic examinations or the development of molecular authentication tools. *T. govanianum* must be preserved because it is a valuable herb in treating several ailments. Establishing conservation strategies for this endemic endangered species should be the highest priority to safeguard it from the potential risk of extinction. Hence, a thorough analysis of the inter- and intra-specific genetic diversity assessment and development of molecular authentication markers for all the available populations is necessary.

Conclusion

Though being a critically important Himalayan species, *T. govanianum* is still not prioritized for conservation. It's a very important Himalayan species still not under the prioritized category for conservation. The plants are susceptible to grazing and harsh weather conditions and their population tends to decrease rapidly. *T. govanianum* is being harvested from its natural habitats on a considerable scale, which poses a significant threat to the species and should be investigated. The herb desperately needs conservation and prioritization because nearly 99% of the natural population dies before seed set due to harsh conditions, grazing and illegal extraction; in nature, the plant multiplies primarily through vegetative reproduction. Survival and germination of the falling seeds are also less and take up to three years, as documented in other species of *Trillium*. As a result, the various *T. govanianum* populations are under significant anthropogenic pressure, causing them to decline. The species is in serious peril and it is time to prioritize the species and implement a conservation strategy. Cultivation will not only serve to sustain the population but also provide a source of income.

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Authors' contributions

VDN was responsible for the conception and design of the study. D and VDN performed material preparation, data collection and analysis and support fieldwork. D compiled the first draft of the manuscript and all authors commented on previous versions. TD and SHK has suggested the critical revisions for the article for significant intellectual content. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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