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REVIEW ARTICLE

Trillium govanianum (Nagchattri): A promising rare and commercially important medicinal herb from higher altitudes of the Himalayas

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Abstract

Secondary metabolites and bioactive compounds derived from naturally occurring sources have been the prime ingredients in modern health care as well as ancient medicine systems like Ayurveda, Yunani, and traditional folk medicines. A large number of plant species and their extracts are utilized to cure several human ailments. *Trillium govanianum,* belonging to the genus Trillium (family: Melanthiaceae alt. Trilliaceae), is a traditional medicinal herb of the Himalayan region used to cure joint pains, wounds, boils, dysentery, inflammation, menstrual and sexual disorders. *T. govanianum* root extracts have analgesic, anti-inflammatory, cancer-preventing, anti-fungal, and wound-healing activities. The main bioactive compounds present in this species are steroidal saponins. Indiscriminate and commercial harvesting in an unsustainable manner, along with various other biotic pressures, have created a synergistically severe menace toward the subsistence of *T. govanianum* in its natural habitat. Population assessment, conservative and *in vitro* proliferation methods pertaining to mass multiplication and advancement in *ex–situ* and *in–situ* environments are required for sustainable use of this species. In lieu of the significance of this herb, the present study aims at the exploration of phytochemical, ethnomedicinal, ecological, pharmacological, and conservational practices of *T. govanianum* for a better understanding of medicinal activities and sustainable use of this plant. This review summarised the potential resources of *T. govaninanum* in terms of biologically active compounds and their dependence on the local population. It focuses on the medicinal utility of *T. govaninanum* in different diseases and ailments.

Keywords

Trillium govaninanum; Nagchhatri; phytochemistry; ethnomedicinal herb; pharmacology; secondary metabolites

Introduction

The success of the primary health care system depends upon appropriate use along with continuous access to suitable drugs and medicines. To date, traditional medicine is the prime and most inexpensive treatment system available, owing to its easy access to several ailments pertaining to the primary health care system. Secondary metabolites derived from naturally occurring sources are vital in curing various diseases. Earlier civilizations completely depended upon such natural products for health maintenance and disease treatment. Natural sources contribute to about 60% of the total

scientifically approved drugs and medicines (1). WHO reported that the traditional medicine system is the source of medicinal requirements for about 80% of the world's total population (2). These time-honored medicine systems like Ayurveda, Yunani, traditional Chinese medicine, and folk medicine systems prevalent around the globe utilize a wide variety of plant varieties and their extracts in the treatment of different human body disorders and ailments (3,4). 21,000 plant species have been identified by WHO as potential medicinal plants available in developing countries (5).

India has a rich biodiversity with about 11.4% of the total world flora (6) and thus holds tenth rank in biodiversity of flora in the whole world. The Himalayan region holds 50% of the entire country's flora reserve with nearly 4,000 prevalent species (7). The Himalayan and Trans – Himalayan regions are enriched with a vibrant biodiversity consisting of a large number of medicinal and pharmacologically important plant species (3,8), which are native to these regions only. The major source of most medicinal plant species is their wild varieties, which are obtained in the form of raw plant material. Destructive harvesting is a majorly employed collection practice that accounts for habitat destruction and over-exploitation (9). Another major contributor in this regard is anthropogenic activities, which exert pressure on the population and accessibility of plant species having medicinal utility and demand (10).

There has been a major hike in the traditional medicine system throughout the world (11). An exponential growth rate in the global herbal trade, i.e., 15% per annum, has been observed (5). It is estimated to touch the scale of 5 trillion US$ by the year 2050 (12). The trade sector pertaining to medicinal plants includes 1178 species, of which about 242 species of medicinal plants are traded for more than 100 metric tons per year (13). Therapeutic plant varieties are cultivated in the rural expanse of the Himalayan belt. These have proved to be the best possible alternative source of income generation for the people living in these regions (14). One such medicinal plant is *Trillium govanianum* (15).

Trillium govanianum genus *Trillium* (family: Melanthiaceae alt. Trilliaceae) is commonly known as Teen Patra, Nag Chhatri, or Himalayan Trillium. It is a vulnerable perennial rhizomatous medicinal herb plant native to the Himalayan region due to its high therapeutic value and high commercial demand. This species majorly prefers cold, shady, and moist habitats for growth and development (16). The distribution of this species is extended from 2500 to 4000 meters across the Himalayan region. It is distributed among countries like India, Pakistan, Bhutan, Nepal, and China (17-18). This plant species was not included in the list of 960 medicinal plants for trade before 2008, but owing to its distinguished health benefits and high market demand, it has been exploited for illegal trade (19). *T. govanianum* rhizomes are used in folk medicine to cure joint pains, wounds, boils, dysentery, and inflammation, menstrual and sexual disorders. Root extracts of *T. govanianum* bears analgesic, anti–inflammatory, anti–cancer, anti–fungal and wound healing properties. Due to over-exploitation and illegal trade, it has now been listed in the category of threatened medicinal plant by IUCN (International Union of Conservation of Nature) (20).

Review methodology

Search strategy

A systematic search for relevant literature was undertaken in accordance with the Preferred Reporting Items for Systematic Review. Three key databases were searched from the start: PubMed, Scopus, and Ovid (Books, Journals, Cochrane, AMED, Embase, and MEDLINE). The relevant literature, data, and information pertaining to *T. govanianum* have been collected using reliable sources like Science Direct, Springer, PubMed, ChemSpider, Google Scholar, etc. Review and research articles derived from peer–reviewed journals, books, and unpublished theses were also included.

Study selection

The systematic review applied specific inclusion and exclusion criteria. To be included, studies had to (1) be published in English; (2) report the isolation of pure compounds; derivatives or metabolites of *T. govanianum*; (3) evaluate or report the pharmacological activity of these compounds; (4) assess or report the biological activity of the compounds; (5) report the toxicological activity of the compounds; (6) discuss the traditional or folk use of. Exclusion criteria involved systematic reviews, meta-analyses, conference proceedings, abstracts, case reports, letters to editors, and books. Authors independently screened references. Initial screening involved titles, with potentially relevant titles further evaluated through abstracts. When the title and abstract proved inconclusive, the full text was evaluated. Lists were shared and cross-checked. Any differences were settled by discussion among the authors. Studies that did not match the inclusion criteria were excluded.

Data extraction

The authors extracted data using a standardized form. Extracted information from selected articles includes author names, reported plant species, biological plant parts, isolated substances, biological and pharmacological activity, plant extract details (fraction used), experimental methodologies, concentrations, and toxicity research results. The acquired data was first recorded in an Excel sheet and then altered to fit the format necessary for the current publication.

Results and Discussion

Origin

Based on morphological and chromosomal investigations *T. govanianum*, a species found in the Himalayas, is thought to be an intergeneric hybrid of Daiswa and Trillium. *T. govanianum*, an allotetraploid (4x=20), is most likely derived from the 10 GG genome of a plant in the Trillium family and the 10 DD genome of a plant in the genus Daiswa (21). A paleoclimatic analysis of the Himalayan

mountain range and an evolutionary perspective have been used to examine factors related to the development of polyploid species.

Botanical description and ecology

T. govanianum is a perennial herb that grows up to the height of 30 centimeters, having three leaves in a whorl at the apex with a centrally placed flower that is purplish brown in color. It consists of broadly ovate leaves that are acute and have conspicuous petioles. It bears three sepals, three petals, three double stamens, and three – carpel late ovaries (21). The life cycle of *T. govanianum* includes three stages. In the first stage, the plant remains single-leaved for several years. During this time period, it continues to accumulate its rhizome biomass, and upon reaching the threshold, it enters the second stage of its life cycle. Here, it attains non–flowering three-leaf structures, which, after several years, enter into a three-leaf flowering stage (22-24), represented in Fig. 1.

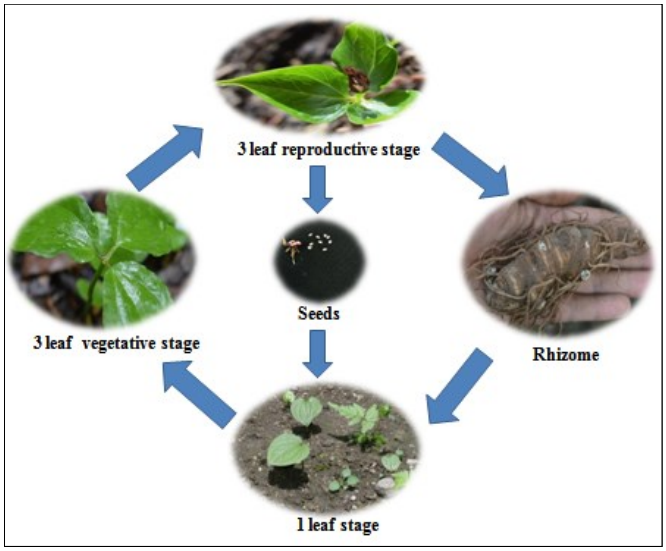


Fig. 1. Life cycle of *T. govanianum.* Courtesy Science direct.

The flowering season is from May to June and is pursued by seed setting and occurs from September to October. 1–2 centimeter diameter, globular, red-colored berry is characteristic of *T. govanianum.* It produces numerous oblong seeds, which are 2.6 mm long and contain a laterally placed pulpy appendage. A tuberous rhizome, present in the underground part, is stout and has adventitious roots (Fig. 2). The rhizomes are creeping type, having prolonged and elongated shapes, and are greyish to brown in color. The external surface appears to be rough, having a 3 – 5 centimeter length and 0.8 to 1.5 centimeter thickness (25). Reproduction in *T. govanianum* is majorly sexual via seeds produced in large quantities; however, asexual reproduction has also been reported to a lesser extent and only in old plants bearing large rhizomes (26). However, detailed reproductive biology and ex-situ conservation need to be systematically investigated and understood. In the rainy season, an endophytic association of various fungal species has been reported (27). These fungal species associated with *T. govanianum* are *Trichoderma* spp. *Alternaria* spp., *Stachybotrys* spp. *Aspergillus* spp., *Rhizopus* spp., *Fusarium* spp., *Mucor* spp., *Phoma* spp. and *Pythium* spp. either with leaves, stems, or rhizomes.

Trillium genus includes 38 North American and 11 Asian species (28). Distribution all around the world includes regions from the western Himalayas to Japan, China, Russia, and North America (5). In the Asian context, *Trillium* species have maximum diversity in Japan, while only two species are present in the Indian Himalayan region (24). Specifically, in the Himalayan region, *T. govanianum* is widespread, along with *T. tschonoski* which is an associated species. Both species have similar phylogenic features and characteristics, which sometimes cause dilemma and, ultimately, adulteration by mixing of species (21). The genus has a long regeneration cycle within the range of 5 – 15 years before attainment of maturity (29).

Geographical distribution of T. govanianum

T. govanianum Wall.ex. D. Don belongs to the family Trilliaceae. This spp. is native to the vicinities of the Himalayas with respect to its occurrence in India, Nepal, China, and Bhutan (15). It has also been reported to be found in the regions of upper Beas Valley, Parbati Valley, Lag Valley, Banjar Valley, and Mohal Khad Watershed regions. A few areas of the Bajar Valley forest area, Great Himalayan National Park area, and Saharan forest areas were also reported to have *T. govanianum* population. These areas

Fig. 2. Field grown 1year old *T. govanianum* with rhizome.

mainly comprise temperate to sub-alpine forests, which are majorly dominated by broadleaved and coniferous species, along with alpine meadows dominated by alpine scrubs and herbaceous species.

Shady areas in temperate regions are mostly preferred by *T. govanianum.* In Asia, the temperate region distribution of this plant extends from Tibet, China, Afghanistan, and Western Asia, while in tropical regions; its distribution is mainly in the Indian subcontinent, East and West Himalayas, Pakistan, and Nepal (30-33). Dad (34) also reported the occurrence of *T. govanianum* in the high altitude grasslands of Bandipora district, Kashmir. In Padhar Valley of Jammu and Kashmir, the altitudinal range of occurrence of this species has been reported to be 2800 – 4000 meters (35).

Dhauladhar mountain range has also been reported to possess the *T. govanianum* species in their forests (18). In the Indian context, its occurrence has been reported in Shimla (36,37), Chamba (38), Parbati Valley in Kullu district (39,40), Sanga Valley and Chotkanda in Kinnaur district (41,42), Kullu districts of Himachal Pradesh, India (43). The distribution of the plant species in the Himalayan region is depicted in Table 1.

Table 1. Geographical distribution of *T. govanianum*

Areas	Region
Upper Beas valley	Dundhi, Hamta, Jamu Dhug, Rani nalla, Fakru, Seri nalla Kothi Jot Kelgu butru, Bakru Thatch and Deusu
Parbati Valley	Thunja, Malana, Pandu Pul, Kheer Ganga
Banjar Valley	Raghupur Jot, Sakiran, Hirb, Rajjandi, Lamba lambhri, Seolsar, Chhera
Lag Valley	Bhabsi, Sruni, Sori and Machak Jot
Mohal Khad Watershed	Nanga Dhardha, Munjhak, Tarapur Garh, Hathipur, Bhubu
Bajar valley forest area	Jahloripass, Sojha, Sarolshar

Vernacular names

T. govanianum belongs to the genus *Trillium* (Family: Liliaceae), commonly known as 'teen patra' or 'nag chhatri'.

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Liliopsida
Subclass	Liliidae
Order	Liliales
Family	Liliaceae
Genus	Trillium L.
Species	*T. Govanianum*

Taxonomic Classification

The vernacular names of the *T. govanianum* in different languages are summarized in Table 2.

Table 2. Vernacular names of the *T. govanianum.*

Language	Name	Reference
Hindi	Nag chhatri, Satva, chotasatwa, *Tri pater*	
Nepali	Nakali satuwa, Nag chhatri	
Pashto	Matar Jarri; Matar zeal	
Urdu	Dood bachha	(97)
Chinese	Xizangyan ling cao	
Pakistani	Matarzela, Teen patra	
English	Himalayan Trillium, birthroot	

Traditional and Ethnomedicinal uses

It is a native species of the Himalayas. *T. govanianum* has been a lesser-known medicinal plant in trade during past decades and has gained importance in commercial utilization these days. The underground part of the plant called the rhizome is a key trade material containing trillarin, which, on hydrolysis, yields diosgenin, which is used in the preparation of steroidal and sex hormones. *T. govanianum* is used in various traditional medicines, has both steroids and sex hormones. In folk medicine, T. govanianum rhizome is used to cure dysentery, back pain, wound healing, inflammation, skin boils, menstrual abnormalities, and sexual dysfunction (37).

The rural communities of Khyber Pakhtunkhwa, particularly those residing in small villages nestled in remote areas and valleys; possess a wealth of traditional knowledge. During the field survey, it was observed that a large number of local people were involved in the digging and collection of this plant species for commercial sale and earning purposes. The local people of these areas, particularly in Dir and Swat Kohistan, sold the collected rhizome at a rate of Rupees 1000 per Kilogram (≈ $11/ Kg) (25). In light of this, conducting ethnomedicinal studies emerges as the most effective means to document and understand the utilization and management of natural resources by the indigenous population. In accordance with these considerations, the current study focuses on ethnomedicinal and pharmacognostic aspects. The objective is to investigate the presence of medicinal plants, uncover their potential traditional uses, and establish pharmacognostic parameters to aid in the accurate identification of crude drugs. This approach aims to contribute to the preservation of indigenous knowledge, promote sustainable resource management, and facilitate the integration of traditional practices with scientific understanding (25).

T. govanianum is one of the significant traditional ethnomedicinal herb plant used in multiple ways by indigenous populations of the Himalayan region for different medicinal purposes, as depicted in Table 3. The root part shows the highest bioactivity (44), and in the case of *T. Govanianum,* rhizomes attached to the adventitious roots play the most important role by serving as the reservoir of the majority of bioactive compounds and secondary metabolites. Crude extracts of rhizomes of *T. govanianum* are the major constituent of tonics prepared for a wide spectrum of health issues as varied as neurasthenia, cancer, hypertension, reproductive disorders, sepsis, giddiness,

Table 3. Ethno – medicinal uses of *T. govanianum*

Medicinal uses	References
Neurasthenia, Cancer, hypertention, arthritis, sepsis, inflammation, giddiness, reproductive disorders	Chauhan et al., (16)
Dysentery	Pant and Samant, (45); Sharma and Samant, (40)
treating menstrual and sexual disorders, wounds	Rahman et al., (25); Rani et al., (38)
curing skin boils	Sharma, (46)
Stomach and joint pains, treating infections	Sharma et al., (39)
headache	Shah et al., (47)
As an analgesic and in curing inflammation	Rahman et al., (48)
As an antibacterial agent	Sagar et al., (27)

arthritis, inflammation, dysentery, and many more. It is also useful for treating menstruation and sexual issues, wounds, as an antibacterial, and curing skin boils (25). It has also been reported to be useful in infections and gastric and joint pains. Consumption of herbal tea prepared from the 10 g of rhizomes of *T. govanianum* is a common practice among the tribes of Jammu and Kashmir, specifically in Rajouri and Poonch districts, as a remedy for headaches (47).

Preliminary phytochemical screening

The methanol extract and its fractions contained secondary metabolites such as glycosides, steroidal saponins, tannins, sterols, and flavonoids, according to preliminary (qualitative) phytochemical testing on *T. govanianum* rhizome has been depicted in Table 4 (58). Notably, the samples exhibited a high concentration of steroids, steroidal glycosides, and saponins.

Table 4. Preliminary phytochemical profile of *T. govanianum*

Phytochemical	Qualitative test	Methanol extract	n-Hexane fraction	Chloroform fraction	Ethyl acetate fraction	n-butanol fraction
Alkaloids	Mayer's test	-	-	-	-	-
	Wagner's test	-	-	-	-	-
Glycosides	Keller Killiani test	+	-	+	+	+
Tannins	Ferric chloride test	+	-	+	-	+
	Lead acetate test	+	-	-	-	+
Flavonoids	Ferric chloride test	+	-	+	-	+
	Sodium hydroxide test	+	-	+	+	+
Carbohydrates	Molisch's test	+	-	+	+	+
Sterols	Liebermann-Burchard test	+	+	+	+	+
	Salkowski's test	+	+	+	+	+
Saponins	Frothing test	+	-	+	+	+

Phytochemistry

The major part of *T. govanianum* bearing phytochemical compounds is the rhizomes, which form the key ingredient for trade. These rhizomes are reported to contain 'Trillarin', which gets hydrolyzed to produce diosgenin along with a large number of high-value bioactive compounds like steroidal glycosides, steroids, steroidal saponins, phenolics, terpenoids, and fatty acid esters (49-54, 5). Due to the presence of these phytochemical compounds, they hold a high therapeutic value in national and international markets. These bioactive compounds or secondary metabolites of *T. Govanianum,* specifically pertaining to the Asian continent, have been reported to have extensive use in cancer treatment (52). These compounds are recognized as chemical markers and are utilized in the qualitative evaluation of the genus (55).

Rehman et al. (56) reported the isolation of four different spirostanol saponins, namely diosgenin, borassoside E, govanoside A, and pennogenin (Fig. 3). Trillarin is considered to be the prime phytochemical compound (57). Another study (58) reported 12 compounds to be isolated from n–hexane fractions using GC/MS. 30% saturated and 70% unsaturated fatty acids were also obtained along with these bioactive compounds. Rahman et al. (59) were able to isolate two known phytoecdysteroids, namely 20 – hydroxyecdysone, along with a completely new type of trihydroxylated fatty acid, named govanic acid, from the chloroform–soluble plant extracts. SPE fractions were used to isolate considerable amounts of quercetin, myricetin, and kaemferol, along with flavonoids, revealing the potential of *T. govanianum* and its bioactive compounds (60). To date, only 9 steroidal saponins have been isolated, and the isolation mechanisms have been detailed from *T. govanianum* rhizomes namely pennogenin – triglycoside, pregna – chacotrioside, pennogenin – tetraglycoside, borassoside E, pennogenin – diglycoside, clintonioside B, protodioscin, govanoside B and borassoside D (55). The phytochemical constituents of *T. Govanianum,* along with their chemical formula and structure are depicted in Table 5.

Water and methanol extracts of *T. govanianum* have been reported to be capable of higher extraction of sugars, glycosides, saponins, flavonoids, and steroids (25) as compared to nonpolar solvent systems (Table 6). Diosgenin is a naturally occurring steroid saponin in various medicinal herb plants and is considered to be the antecedent of a spectrum of synthetic steroidal drugs, thus having a huge utility in the pharmaceutical industry. Studies on this bioactive compound have been conducted against diseases like obesity, hypercholesterolemia, swelling, diabetes, and cancer using in vitro models, which revealed the activity of diosgenin against cancer-causing cell growth by inhibiting

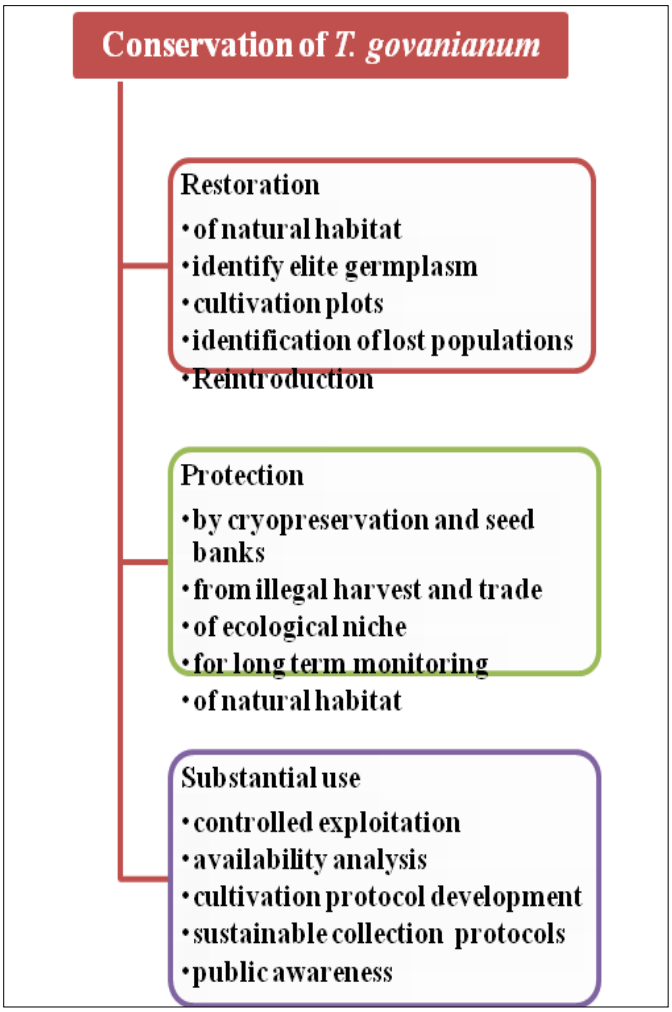


Fig. 3. Graphical representation of conservation methods for *T. govanianum*.

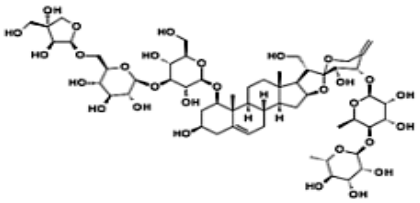
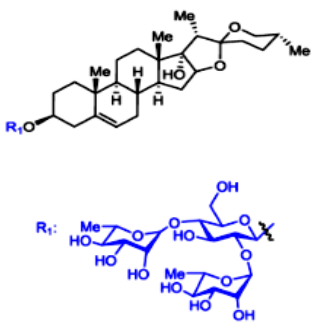
inflammation, multiplicity, oncogenesis, differentiation, and apoptosis (61). Earlier *Dioscorea* spp. and *Trigonella* spp. were considered to be the main source of diosgenin. However, in the contemporary scenario, it has been noted that aq. and MeOH extracts of *T. govanianum* yield greater amounts of diosgenin as compared to Dioscorea spp. and Trigonella spp. (62). The diosgenin content in *T. govanianum* is approximately three times higher than other species having diosgenin content in their secondary metabolites like *Chlorophytum* spp., *Asparagus* spp, *Dioscorea* spp., and *Trigonella* spp. Thus, the scope of its commercial use is higher as its production and extraction from other sources is both time-consuming and expensive (5).

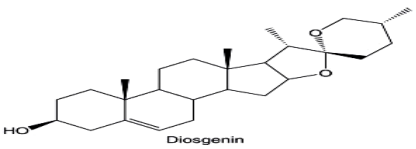
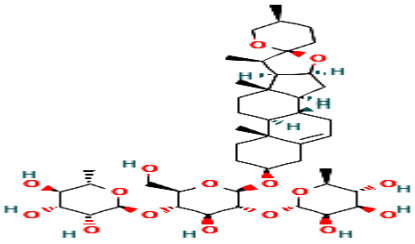
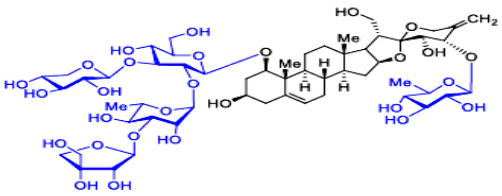
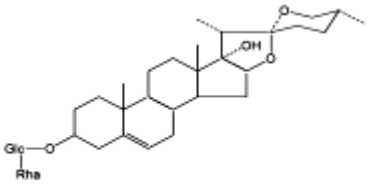
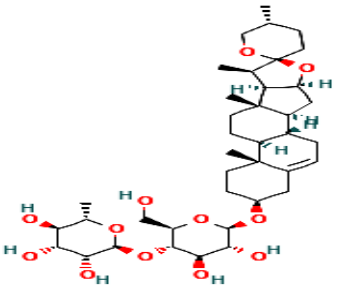
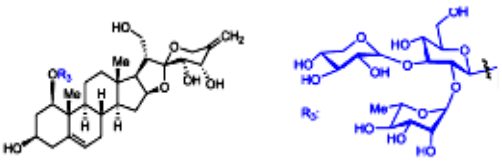
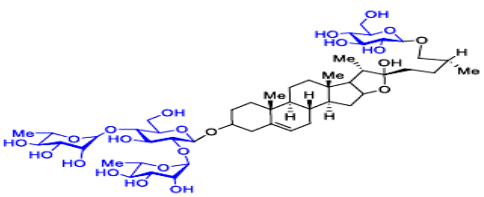
Pharmacological activity

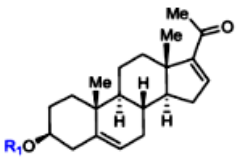
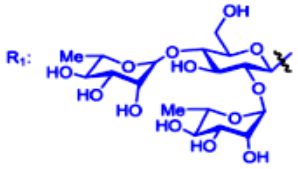
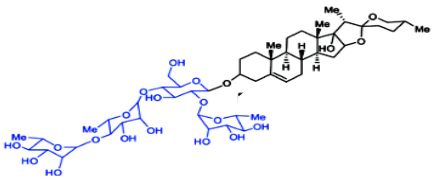
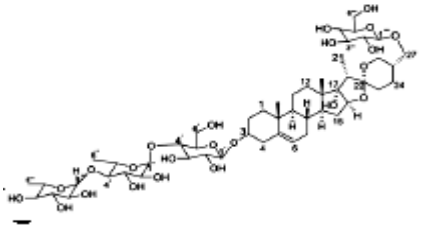
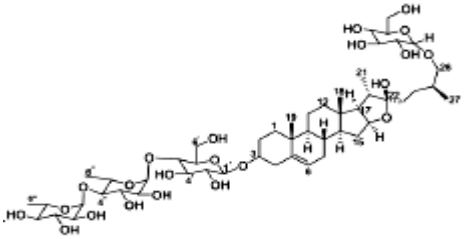
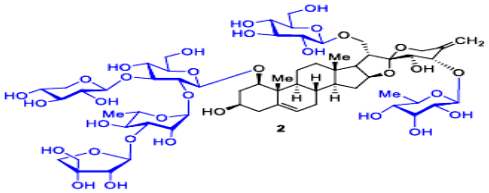
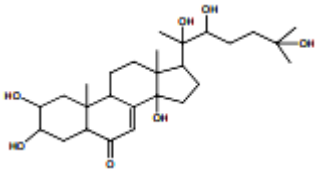
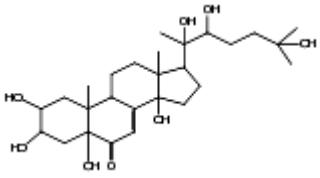
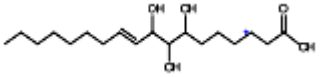
T. govanianum, being an indigenous medicinal herb of Himalayas, is rich in bioactive compounds which form the basic ingredient in various life-saving drugs as these secondary metabolites bear various therapeutic properties as reported from time to time. Steroidal saponins having variable structures are present in the form of secondary metabolites in *T. govanianum* and have utility in the preparation of a large number of drugs, contraceptives in addition to sex hormones and steroids owing to their variable pharmacological activity depicted by its secondary metabolites (5). Majorly, the hydro–methanolic extracts of rhizomes are rich in the secondary metabolites of *T. govanianum.* However, butanol extracts, n – butanol dimethylsulphoxide extracts along with some fatty acids and hexane or chloroform fractions are also other useful solvents for secondary metabolite extraction of rhizomatous extracts of *T. govanianum.* Artemiasalina, human carcinoma cells, cancer cells, human cervical and prostate cancer cells have been tested using methanol, butanol, and hydroalcoholic extracts of *T. govanianum* to study its anticancer activity (60-64). Rani et al. (38) studied the effects of crude rhizome extracts of *T. govanianum* in treating sexual disorders and reported considerably positive results. Similar results were reported for skin infections (65).

Methanol and n–butanol extracts of *T. govanianum* showed good anti–inflammatory activities on carrageenan

Table 5. Phytochemical constituents of *T. govanianum* with their chemical formula and structure

S. No.	Chemical compounds	Chemical formula	Structure	Origin and Ref.
1	Govanside A	$C_{56}H_{88}O_{29}$		Rahman et al. (25)
2	Pennogenin triglycoside	$C_{51}H_{82}O_{21}$		Nohara et al.; (50)

3	Diosegnin	$C_{27}H_{42}O_3$		Ismail et al., (44)
4	Borassoside E	$C_{45}H_{72}O_{16}$		Ismail et al., (44)
5	Govanoside B	$C_{55}H_{86}O_{28}$		Yokosuka and Mimaki, (50)
6	Pennogenin-diglycoside	$C_{28}H_{33}ClO_{16}$		Yokosuka and Mimaki, (50)
7	Borassoside D	$C_{39}H_{62}O_{12}$		Patil et al., (96)
8	Clintonioside B	$C_{44}H_{68}O_{20}$		Kumar et al., (99)
9	Protodioscin	$C_{51}H_{84}O_{22}$		Kumar et al., (99)

				
10	Pregna-chacotrioside	$C_{21}H_{30}O_2$		Yokosuka and Mimaki, (50)
11	Pennogenin-tetraglycoside	$C_{51}H_{82}O_{21}$		‹umar et al., (99)
12	Trilliumoside K	$C_{51}H_{85}O_{23}$		Kumar et al., (99)
13	Trilliumoside L	$C_{51}H_{85}O_{22}$		Kumar et al., (99)
14	Govanoside D	$C_{61}H_{96}O_{33}$		‹ahman et al., (59)
15	20- Hydroxyecdysone	$C_{27}H_{44}O_7$		Rahman et al., (59); Ono et al. (76)
16	5,20- Hydroxyecdysone	$C_{18}H_{45}O_8$		Rahman et al., (59)
17	Govanic acid	$C_{18}H_{35}O_5$		Rahman et al., (59)

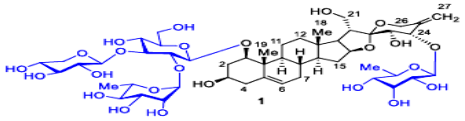
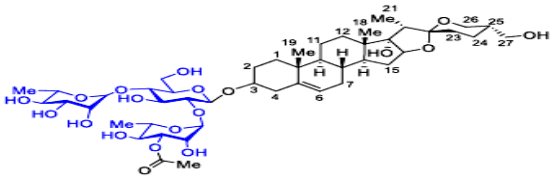
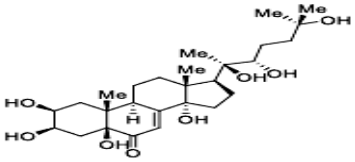
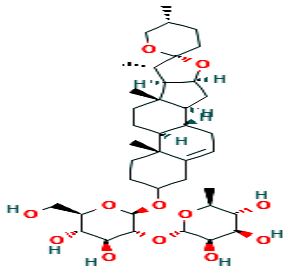
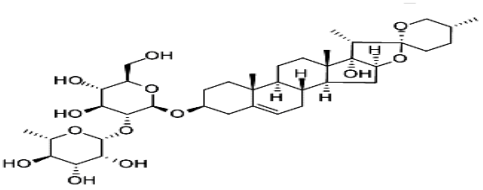
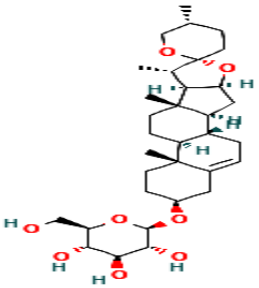
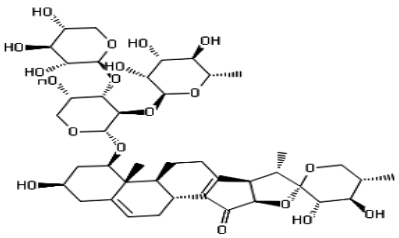
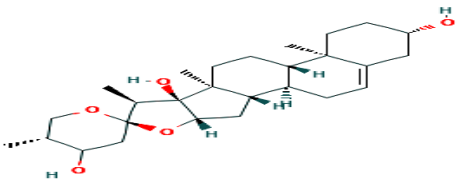
18	Govanoside C	$C_{50}H_{78}O_{24}$		Rahman et al., (59)
19	Govanoside E	$C_{47}H_{74}O_{19}$		Rahman et al., (59)
20	Polypodine B	$C_{27}H_{44}O_8$		Kumar et al., (99)
21	Polyphyllin V	$C_{39}H_{62}O_{21}$		Kumar et al., (99)
22	Polyphyllin VI	$C_{39}H_{62}O_{13}$		Kumar et al., (99)
23	Trillin	$C_{33}H_{52}O_8$		Kumar et al., (99)
24	Deoxytrillenoside B	$C_{42}H_{62}O_{19}$		Kumar et al., (99)
25	24-hydroxypennogenin	$C_{27}H_{44}O_7$		Kumar et al., (99)

Table 6. Different solvent systems for extraction of bioactive compounds of *T. govanianum*

Solvent system	Compound	References
Soxhlet extraction	Diosgenin	Sharma *et al*., (62)
Methanol extract and its fractions	saponins and Steroids	Rahman *et al.*, (25)
Extracts	Steroids and saponins	Sharma *et al*., (62)
Methanol extract	Phenolic and flavonoids	Khan *et al*., (60)
Ethyl acetate and hexane fractions, Methanol	Govanoside A, Borassoside E, Pennogenin, Diosgenin	Rahman *et al*., (59)

induced paw edema (47-48). Mahmood et al. and Qiong et al. (66-67) reported anti – fungal property of dimethylsulphoxide extract and fatty acids of *T. govanianum* in Aspergillus niger, Candida albicans, A. flavus, and C. glabrata. Anti–oxidant activity of hexane and chloroform fractions has been reported by Rahman et al. and Khan et al. (58, 60). Methanol extracts showed good anti–bacterial properties against Escherichia coli, Staphylococcus aureus and Yersinia pestis (27). 48 reported analgesic properties of the methanol extract against Carrageenan-induced paw edema. Methanol extract also showed positive anti–leishmanial activity against Leishmaniatropica (60), and butanol fraction is reported to be β-glucuronidase inhibitory in its action (58).

Anti –oxidant activity

T. govanianum shows the highest scavenging activity in the form of chloroform and hexane fractions (Table 7). However, anti–oxidant property of the extract has been reported to be lower than that of BHT (butylated hydroxytoluene) and ascorbic acid, and the reason behind comparatively lower anti–oxidant property than BHT or ascorbic acid may be attributed to the presence of large sized fat constituents in *T. govanianum* (58).

Anti–cancerous activity

Higher anti–proliferative and cytotoxicity activity have been reported in *T. govanianum* extract on cancer cells of cervical and prostate cancer as compared to the standard anticancerous drug i.e., doxorubicin (58). Lower toxicity of *T. govanianum* extract pertaining to normal cells and significant anti-proliferative characteristics against cancer cells have been observed by Sharma et al. (62), followed by reduced viability. Diosgenin showed cytotoxicity on limited cell lines, like V79 fibroblast and K562 cells (64,68). Steroidal saponin compounds like Diosgenin present in *T. govanianum* are responsible for the anti–cancerous property as reported by Rahman et al. (58). Cytotoxic ability of rhizome and methanol extracts of *T. govanianum* along with serum protein electrophoresis (SPE) fractions have been examined on brine shrimp by Khan et al. (60). Cytotoxicity of methanol extracts against human carcinoma cells were also reported in another study by Khan et al. (63) pertaining to breast, liver, lung and urinary bladder carcinomas. Anti–cancerous activity of *T. govanianum* is depicted in Table 8.

Antifungal activity

Hydro-methanolic extract of *T. govanianum* was studied for its antifungal activity by Rahman et al. (53) against *Trichophyton rubrum* and *Microsporum canis* fungal strains. 80% fungal inhibition was reported in *Trichophyton rubrum* while it was observed to be 75% in *Microsporum canis*, respectively. However, 90% inhibition was observed when chloroform-soluble fractions of *T. govanianum* were used. Saturated and unsaturated fatty acids have also been reported to bear antifungal activities (67). As observed by Rahman et al. (56), Borassoside E and Govanoside A compounds have been seen to demonstrate a range from fine to moderate antifungal activity against *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans,* and *Candida glabrata* spp. Anti–cancerous activity of *T. govanianum* is depicted in Table 9.

In vitro antileishmanial activity

Flavonoids – parasite cell wall complexes showed higher antileishmanial activity (69), and specific flavonoids like quercetin are considered effective anti – trypanosomal and antileishmanial compounds (70-71). Khan et al. (60) studied the antileishmanial ability of *T. govanianum*. MeOH extract and its SPE fractions were examined against *Leishmania tropica*. 70% mortality was observed using MeOH extract, while SPE fractions also revealed comparable results.

Analgesic activity

Methanolic extracts of *T. govanianum* and its solvent-soluble fractions, when subjected to tonic visceral chemical–induced nociceptive pain, showed statistically significant analgesic properties at 50 and 100 mg/kg doses, which was comparable to the standard diclofenac sodium drug activity (48). In the case of acute phasic thermal nociceptive pain, these extracts showed significant attenua-

Table 7. *T. govanianum* extract, fractions, and standards exhibit DPPH free radical scavenging activity

Conc.(µg/ml)	Percent inhibitor (%) ± SEM						
	n-Hexane fraction	Chloroform fraction	Ethyl acetate fraction	Butanol fraction	Crude methanol extract	Ascorbic acid	Butylated hydroxytoluene
1	7.61±2.68	2.63±0.56	0.82±0.64	1.35±0.53	1.35±0.02	10.2±4.06	5.19±0.04
10	6.61±2.64	2.99±0.12	1.46±0.64	2.35±0.24	1.84±0.36	41.5±2.24	16.9±3.87
30	11.0±0.08	6.68±2.13	4.25±0.08	3.39±0.51	2.06±0.15	95.8±0.22	39.0±9.12
50	23.0±0.46	11.0±0.08	22.1±4.61	22.3±2.16	21.5±3.91	96.3±0.15	68.7±8.75
100	23.2±0.42	23.0±0.42	7.67±0.53	6.28±0.80	4.32±0.39	96.4±0.01	90.9±0.44
200	20.4±2.13	20.4±2.13	11.0±0.33	11.1±1.88	6.96±1.46	96.5±0.04	93.1±0.03

Table 8. Anti – cancerous activity of *T. govanianum*.

Part of Plant	Extract	Cell line	Type of cancer	References
Rhizome	Methanol	Hep G2	Liver	Khan et al., (60)
	Ethanol	A549	Lungs	
		MCF7	Breast	
		EJ138	Urinary bladder	
Rhizome	Methanol	HeLa	Cervix	Rahman et al., (25)
		PC – 3	Prostrate	
Rhizome	Methanol	MCF7, MDCK, MDA – MB 123	Breast	Sharma et al., (62)

Table 9. Antifungal activity of *T. govanianum*.

Fungus	Bioactive compounds	References
Aspergillus niger	Govanoside A and borassoside E	Rahman et al., (25)
Aspergillus flavus		
Candida albicans		
Candida glabrata		
Trychophyton rubrum	hydro-methanolic extract	Rahman et al., (59)
Microsporum canis		

tion after 30 minutes compared to normal saline. Studies have reported saponins to be efficient suppressors of iNOS and COX–2 expressions (72). These saponins are, thus, capable of managing prostaglandin E_2 levels efficiently. *T. govanianum* has been reported to have high steroidal and saponin contents in them (73) and thus, are capable of iNOS and COX – 2 expression management (74).

Anti-inflammatory activity

The crude extract of MeOH, as well as its solvent fractions, shows considerable amelioration at 50, 100, and 200 mg/kg when tested against the carrageenan-induced paw edema model. N – butanol fraction of *T. govanianum* showed pain relieving and anti–inflammatory activity at 100 mg/kg level in the early phase of inflammation (48). It also shows considerable oxidative inhibition when in vitro ROS (Reactive oxygen species) whole blood is added to the methanolic extract of *T. govanianum* rhizomes having IC_{50}. These results pinpoint the fact that isolated constituents like diosgenin, pennogenin, and borass side E have significant inhibitory activity, with pennogenin having the highest anti–oxidant activity among the three compounds.

ROS (Reactive oxygen species) and inflammation work in mutual promotion and, thus, collectively contribute towards tissue damage. Additionally, oxidation is an important contributor to several pathogenesis pertaining to chronic inflammatory disease. Several studies have reported the onsite production of ROS, RNS, and hypochlorous acid (HOCl) in case of inflammation. Thus the property of methanol extracts, fractions, and isolated compounds of *T. govanianum* in inhibition of ROS from whole blood may be attributed to its scavenging of ROS or enzyme inhibition in NADPH oxidase, catalase, and peroxidase and superoxide dismutase (SOD) production (48). Furthermore, diosgenin has been reported to show anti–inflammatory properties in an array of plant groups. Maximum anti – inflammatory activity of diosgenin has been observed at the dose of 400 mg/kg, i.e., 82.25% (75).

Antibacterial activity

North American species of Trillium have been well known for their antibacterial properties (52,76,50). In the Indian context, with respect to *T. govanianum*, the antibacterial activity of various extracts namely acetone, ethanol, methanol, and distilled water extracts, have been studied by Sagar et al. (27) in vitro, pertaining to three different human pathogenic bacteria (*Staphylococcus aureus*, *Escherichia coli* and *Yersinia pestis*), followed by agar well diffusion method at different concentrations of *T. govanianum* extracts. The results were considerable in comparison to the standard and commonly used anti–bacterial drugs. The pharmacological properties of *T. govanianum* are depicted in Table 10.

Cytotoxicity

Reports on cytotoxicity of methanol extracts of *T. govanianum* roots/rhizomes in solid–phase extraction fractions are available against human carcinoma cell lines, namely breast cancer (MCF7), liver cancer (HEPG2), lung cancer (A549) and urinary bladder (EJ138) (63). The cytotoxicity evaluation was conducted using 3-(4,5-dimethylthiazol-2-yl) – 2,5 – diphenyltetrazolium bromide cytotoxicity assay and liquid chromatography along with electrospray ionization quadrupole time–to–flight mass spectroscopy analysis of SPE fractions. The MeOH extracts and their SPE fractions revealed considerable cytotoxicity against the human carcinoma cell lines with varying degrees. TGMF1 showed the highest degree of cytotoxicity for the urinary bladder cell lines with IC_{50} = 5µg/mL followed by breast, liver, and lung cell lines, i.e., IC_{50} = 5, 7, and 9µg/mL, respectively. Anticancer activity of Crude methanolic extract and fractions of *T. govanianum* rhizomes against HeLa cells (cervical cancer cells) and PC-3 cells (prostate cancer cells) was evaluated by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay has been depicted in the Table 11. The highest polar components in SPE fraction were present in TGMF1 and thus showed the highest toxicity against the EJ138 cell line. TGMF2 was highly toxic against lung cancer cell line IC_{50} = 6µg/mL. TGMF4, having the least polar components, showed considerable cytotoxicity against the EJ138 cell line. These results are in congruence with other studies (99-100). The cytotoxicity showed by roots of *T. govanianum* may be attributed to the occurrence of saponins and aglycones adding to its putative anticancer potential.

T. govanianum formulations available in the market

There are a large number of pharmaceutical advantages of bioactive compounds and secondary metabolites present

Table 10. Pharmacological properties of *T. govanianum*

Medicinal Use	Target	Solvent	References
Anti – cancer	Artemiasalina, Human carcinoma cells, Cancer cells, Human cervical and prostate cancer cells	Methanol extract, Hydroalcoholic extract, Butanol extract	Rahman et al. (25); Chauhan et al. (16); Khan et al. (60); Khan et al. (63); Sharma et al., (62); Liu, (64).
Sexual disorders	-	-	Rani et al., (38); Chauhan et al., (16); Rahman et al.,(25).
Skin disease	-	-	Lone et al., (65)
Anti – inflammatory	*Carrageenan* induced paw edema	Methanol and n-butanol extract	Chauhan et al. (16); Shah et al. (47); Rahman et al.,(48).
Anti – fungal	*Aspergillus niger* , *Candida albicans, A. flavus* and *C. glabrata*	Dimethylsulphoxide extract, Fatty acids	Mahmood et al., (66); Sharma and Samant, (40); Pant and Samant, (45); Rahman et al., (25); Qiong et al.,(67).
Anti – oxidant	-	Hexane and Chloroform fraction	Rahman et al., (56); Khan et al., (60).
Anti – bacterial	*Escherichia coli, Yersinia pestis* and *Staphylococcus aureus*	Methanol extract	Sagar et al., (27)
Analgesic	*Carrageenan* induced paw edema	Methanol extract	Rahman et al., (48)
Antiseptic, treatment of boils	-	-	Chauhan et al.,(16); Sharma et al., (46)
Anthelmintic	-	-	Lone et al., (65)
Anti-Leishmanial activity	*Leishmania tropica*	Methanol extract	Khan et al., (60)
β-glucuronidase inhibitory	-	Butanol fraction	Rhaman et al., (56)

Table 11. IC_{50} value of different extracts and fractions of rhizomes of *T. govanianum* on cancer cells

Samples	IC_{50} (µg/ml)	
	HeLa cells	PC-3 cells
Crude methanolic extract	3.13±0.71	6.51±0.53
Chloroform	0.83±0.17	2.71±0.34
Ethyl acetate	1.42±0.07	5.14±0.33
Butanol fraction	1.61±0.33	4.03±0.34
Doxorubicin	0.35±0.02	1.37±0.15

in *T. govanianum* and, most prominently diosgenin (C_{27} H_{42} O_3). It is the most active component of the majority of anti –microbial, anti–viral, ant–inflammatory, anti–cancer, CNS modulator, immune–modulator, lipo–modulator, and sexual disorder-related formulations available in the market; some of them are shown in Table 12.

Table 12. Formulations of *T. govanianum* available in the market with their price

Formulations	Price/packet
Diosgen	(Rs 2250 / packet)
Testoprime	(Rs 2250 / packet)
Oribolin – 50	(Rs 750 / packet)
LR3IGF – 1	(Rs 13,000 / box)

Micropropagation

The most cost-effective mass propagation of germplasm can be obtained using the vegetative propagation method for conservation purposes. Initial attempts for ex-situ propagation have been reported to be unsuccessful, with limited or negative micro propagation outcomes (55). However, the study conducted by Chauhan et al., (79) showed considerable success in ex situ regeneration of *Trillium govaninanum*. The apical part of rhizomes showed considerably positive propagation potential with survival efficiency between 71.4 to 90.4% and multiple roots formation at a single bud base. The vegetatively propagated buds showed 60% survival efficiency when replanted. This may be attributed to exposed plantlet tissues, which are prone to microbial attack and have lower endurance in the acclimatization of the newly produced roots.

In vitro propagation revealed the potency of shoot buds of *Trillium govaninanum* as explants. The developmental stage has an important role in the degree of response provided by different explant types in tissue culture. Thus, buds at the highly advanced developmental stage are observed to be most responsive to in vitro propagation. ½ strength MS media was observed to be more suitable for growth than full-strength MS medium, as reported by other authors as well for various other species. The use of activated charcoal showed enhanced performance in leaf area by improving cell growth. It also absorbs various inhibitory compounds, such as phenols and other exudates, in a culture medium (99). A culture medium with BA (2.5 ppm) resulted in a maximum leaf area, which was congruent with various other studies (50). Successful results were obtained in another study using MS media along with growth regulators like 1mg/L TDZ, 0.5mg/L IBA, and 0.5mg/L KN for shoot proliferation (45). Although it has been reported that the *Trillium* genus bears poor ex-situ propagation potential, even then, *Trillium govaninanum* can be comparatively easily propagated by the use of simple ex-situ and in situ methods of mass cultivation, thus providing an effective alternate method to prevent this ethno botanically important species.

Agrotechnology

Cultivation of *T. govanianum* is done through seeds and rhizomatous roots (51). During winters (November-December), plants are raised using rhizomes that have apical buds in soils with pH = 5.6 – 7.5. Moist humus-rich soil with 4-5 tons of vermin compost per hectare is ideal

for its cultivation. Plant to plant (10 cm) and row to row (15 -20 cm) spacing is required for proper growth and development. In the months of February and March, sprouting occurs and requires 2-3 irrigations in the summers. Weeding is required once a week during the initial growth stages and once a month in the later stages. Flowering occurs during the months of May and June, and mature seeds develop in early September. The harvesting of rhizomes is done in mid-September. The part of the rhizome that has the apical bud is used for further propagation, while the rest of the part is used for various medicinal purposes (98). The demand for *T. govanianum* in the traditional and contemporary pharmaceutical industry can only be catered to by the use of proper agro-technological practices. Conservational practices including agro-technological aspects for cultivation of *T. govanianum* have been initiated by CSIR-Institute of Himalayan Bioresource Technology, Palampur, H.P. at Bharmour and Pangi regions of Chamba district and Keylong region in Lahaul and Spiti district of H.P. under CSIR Phytopharmaceutical mission project (HCP0010). This project includes the conservation of this plant species from extinction by identification of elite germplasm along with cultivation and preservation in gene banks.

Threats

The national and international demand for medicinal plants for the preparation of herbal remedies has been rising at an alarming rate, which in turn exerts enormous pressure on the wild species, particularly in the Himalayan region, as it is the major hub of medicinal plants. This condition is further exaggerated due to increasing demand as well as interest due to awareness with respect to traditional ethnopharmaceutics in human health care systems (77). 39 categorized the threatened herbal plant species that are in dire need of conservation to prevent them from being endangered or extinct, particularly in the region of Himachal Pradesh, India. Trillium root extraction before seed development may ultimately result in the extinction of this species due to habitat destruction. Thus, awareness regarding this is a must among natives involved in the collection of these herbal species, which will lead to sustainable availability of this species and will also provide ample time for root development up to a desired threshold so as to bring out high concentrations of its bioactive compounds (15).

Non–scientific extraction and unauthorized trade have led to the depletion of high-value herbal vegetation in the high-altitudinal region of Himachal Pradesh, leading to its scarce availability in the Himalayan region (18). Indigenous harvesting practices in an unmanaged way have led to depletion in the population of *T. govanianum* in the regions of Muniyari (Uttrakhand), where the highest density of population has been reported in earlier studies, followed by Tirthan Valley (Himachal Pradesh) and Tungnath (Uttrakhand) (16). *T. govanianum* is threatened in Pakistan, the region of Chail Valley, and the region of Kashmir, India, owing to improper collection methods as well as over-exploitation (78). The sudden hike in recent years in demand for *T. govanianum* has been attributed to its utility in the treatment of sexual disorders in particular. Unsustainable and destructive gathering leads to habitat destruction and exerts pressure on *T. govanianum* population. This has led to a price hike of these herbs in the traditional medicinal market, nearly up to USD 50 – 315/ kg, as reported by several authors (57; 23). The price of *T. govanianum* has also jumped in the local market from Rs 800/kg to about Rs 2500 to Rs 3000/kg (39).

T. govanianum is a threatened medicinal herb that is vulnerable to trade, over-exploitation, predation, habitat destruction, and climatic changes (24). Among all these factors, over-exploitation poses a major threat to its sustainable existence and requires instant and effective conservation strategies for the species. The major commercial collection of *T. govanianum* has been marked out from the year 2010 in the context of the Indian Himalayas (23). Unauthorized and unsystematic collection methods employed to fulfil the demand of national and international markets for trade is another major factor that contributes towards the depletion of *T. govanianum* populations in its native habitat (24; 15). The trade census of *T. govanianum* in the year 2014 – 2015 has revealed an estimate of 200 – 500 tons of *T. govanianum* rhizome trade (80). However, the actual figures may be far more than actually reported.

T. govanianum in the Indian Himalayas has a specific habitat requirement as it grows under mixed temperate canopies of *Juniperus indica*, *Quercus* spp., *Picea smithiana*, *Abies pindrow*, *Cedrus deodara*, *Juglans regia*, *Betula utilis*, *Rhododendron* spp., *Salix* spp. and sub–alpine forest of *Rhododendron* spp., where thick humus with slow decomposing litter is typical a attribute. This ultimately results in a patchy distribution of *T. govanianum* in favorable pockets of the Himalayas only. *T. govanianum* has a long life cycle (29). The oldest rhizome of *T. govanianum* has been reported to be approximately 30+ years old. Peculiar lifecycle and restricted habitat add up to the vulnerability of this species. Another major factor is the herbivore activity in *T. govanianum* abundant areas where domestic animals and wild deer pose a threat to its existence (23; 81). Reproductive constraints like various inbreeding despairs lead to reduced seed setting or fruit propagation (82) like insufficient pollination, which limits seed production in Trillium species (83), lack or complete absence of vegetative propagation, underdeveloped or lack of commercial propagation methods pertaining to Trillium species which adds to its vulnerability (84).

Mass propagation of *T. govanianum* is being conducted owing to its utility; however, to date, no promising results have been reported in preliminary studies. High-altitude species are under high pressure exerted due to climate change. Specifically, the consequences of climate disturbances on *T. govanianum* have not been reported yet, although studies on other species of the Trillium genus reveal the limiting effect of climate change upon these populations in being capable of recovering on their own from the stress exerted due to environmental conditions. Occurrence of Trillium in spring is directly related to the temperature conditions in the region. However, alteration in this temperature may lead to changes in the phenological events (85).

Conservation

Owing to the cultural, livelihood, and economic significance of medicinal plants, their conservation has been recognized as a priority agenda pertaining to various national and international forums. Himalayas are a cache of medicinal plants biodiversity wharfing several life-saving medicines. Collection practices of *T. govanianum* in order to meet the demand of the market are rampant and lead to worsening its threatened status. A combination of both conventional and in vitro methods is required to be developed and tested before using it for mass propagation and commercial production of *T. govanianum* , in order to enhance the possibilities of its recovery from the present status of threatened species and to curb the risk of its extinction (9). Wild varieties of a large number of medicinal plants are on a decline. Pertaining to *T. govanianum*, due to its limited ecological range, it seems to be unable to respond positively in ex-situ conditions, and thus, in-situ, methods of conservation are more likely to be employed for its sustainable supply and to meet the demands of the national and international markets. Domestication and cultivation in its altitudinal ranges by the natives may help to reduce the pressure on the wild species and will also add to the financial ecology of the people of that region.

Tissue culture methods should be developed and employed for the purpose of mass propagation and to maintain the quality of the plantation. Ex situ conservation methods like education and awareness programs for the natives of the Himalayan region for the conservation and sustainable use of these precious species of medicinal plants are specifically needed. A three-tier conservation approach is the need of the hour, including protection, restoration, and sustainable use of the species, as represented in Fig. 3.

Recent advances

The biotechnological interventions at the genetic level help to understand the biosynthetic pathways of different bioactive compounds and the role of key genes in the production of secondary metabolites. Molecular fragmentation or dissection of different enzymes and genes is important as it helps in the metabolic engineering of the pathways in order to meet the ever-increasing demand for these secondary moieties in the selected plant species. The genetic mechanisms which control the production and flux of metabolites in medicinal plants like *Aconitium heterophyllum* (86) and *Picrorhiza kurroa* (87) pave valuable methodological cues pertaining to the role of biotechnology. All higher eukaryotes and microbes are recognized to have an important pathway for isoprenoid biosynthesis, i.e., MVA (mevalonic acid) pathway. Rhizomes of *T. govaninanum* are reported to have high contents of diosgenin, i.e., nearly 5.99%, and it can be extracted from the hydroalcoholic extract along with other important bioactive compounds by the process of 12 hours of hydrolysis carried out at 85^0C from a solid–liquid ratio of 1.234g/ml. As reported by Mehrafarin et al., (88), diosgenin synthesis from cholesterol occurs in a number of plant varieties, and the cholesterol synthesis pathway is catalyzed by HMGR (hydroxymethylglutaryl Co-A reductase), which is majorly the rate-controlling step.

Quantitative real time–PCR analysis has been employed and recommended by various authors in determining the correlation of genes and degree of expression of secondary steroids in different species of medicinal plants (88-91). HPLC analysis of hydrolyzed rhizome extracts of *T. govaninanum* along with quantitative real time–PCR analysis of steroid pathway of diosgenin content has been conducted by (62). Diosgenin has been recognized to be suitable as a raw precursor in the manufacture of steroidal drugs like testosterone, glucocorticoids, and progesterone and is also employed in the treatment of rheumatism and menstrual flow regulation (92,93). Diosgenin is produced through the steroid biosynthesis pathway (94; 89). Table 13 below represents the percentage of diosgenin extracted from various plant species.

Table 13. Diosgenin content in different plant species

Plant species	Part of plant	Diosgenin content	References
Trigonella spp.	Aerial	0.01%	Dangi et al., (96)
	Seeds	0.5%	
Dioscorea polygonoides		0.2%	
Dioscorea althaeoids		0.2 – 2.3%	
Dioscorea prazeri		1.92%	Nino et al., (97)
Dioscorea villosa		1.3%	
Dioscorea zingiberenzis		0.18 – 0.55%	
Trillium govaninanum		0.7 – 2.4%	Sharma et al., (62)

Sharma et al. (62) conducted the identification of the elite chemotype of *T. govaninanum* with 2.4% diosgenin content. The study aimed at measuring of the degree of expression pertaining to key genes that play a part in steroid biosynthesis. The results reported higher expression levels of SQS (Squalene synthase), HMGR (Hydroxymethylglutaryl Co-A reductase), FPPS (Farnesyl pyrophosphate synthase), CAS (Cycloartenol synthase) and BETA (26-O-Beta-glucosidase) in rhizomes of *T. govaninanum* to be positively correlated to diosgenin content. The vital genes having eminent expression levels pertaining to diosgenin content may serve as appropriate targets for molecular marker development with the aim of genetic improvement of *T. govaninanum* and attaining higher production levels of diosgenin and other steroidal saponins present in *T. govaninanum*.

Toxicity and safety

Plants and plant-based products have long been used as the foundation for a variety of treatments in humans. Since ancient times, *T. govaninanum* has been used for the treatment of diseases, and *in vivo* and *in vitro* studies have verified its role in the inhibition of various pathogenesis. The findings from both in vivo and in vitro studies have demonstrated that TGaqu (possibly referring to an extract from *T. govanianum)* and TGAgNPs (Silver nanoparticles synthesized using *T. govanianum* extracts) exhibit no

toxicity at doses of 200 mg/kg, 400 mg/kg, and 1000 mg/kg. Additionally, these substances showcase robust anti-diabetic and anti-cancer properties, attributed to the presence of phyto-constituents (Gulzar et al., 2023) (95). Patial et al. (96) demonstrated that in macrophages, cytocompatibility cell viability was not affected at any tested concentration of extract, fractions, and pure compounds [diosgenin borassoside D, and govanoside B. The LPS-stimulated macrophages secreted nitric oxide (NO) and pro-inflammatory cytokines (TNFα, IL-1β, and IL-6), which were inhibited by the extract, fractions, and compounds 1, 2, and 7 in a concentration-independent manner (63). No toxic effects of *T. govanianum* were observed, as demonstrated by hematological examination, behavioral observation, and biochemical parameters, suggesting the safe utilization of *T. govanianum.*

Conclusion and Future perspectives

The pharmacological abilities of *T. govanianum* have proved its importance in a variety of life-threatening diseases like cancer. However, over-exploitation, restricted habitat, habitat destruction, narrow distribution range, peculiar life cycle, climatic change, and anthropogenic activities have led to depleting the wild sources and are posing a threat to the very existence of this precious species. Only patchy regions are available due to the selective environmental ecology required for the proper propagation of *T. govanianum*. Conventional methods like vegetative propagation of rhizomes and in-situ, propagation like tissue culture along with domestication and cultivation in the nearby areas having the desired altitudinal range are preferable for a sustained use of *T. govanianum* as well as meeting the market demands without disturbing the wild ecosystem of the species.

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Authors' contributions

RG participated in conceptualization, writing–original draft, methodology, data curation, designed review, writing-review and editing. HS contributes to supervision, writing–original draft, visualization, and writing-review and editing. NS contributes to data curation, resources, writing-review, editing, and visualization.

Compliance with ethical standards

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