



Review: Genetic diversity, biodiversity, status and potential of *Trillium govanianum* wall. ex D Don

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Abstract

The Himalayan medicinal herb *Trillium govanianum* is endangered. Nagchhatri is the common name for it. *T. govanianum* rhizome is used to treat diarrhoea, menstruation irregularities, and sexually disorders. It's also used as a wound dressing and as an antibacterial. Plants included glycosides, steroids, saponins, tannins, sterols, and flavonoids, according to phytochemical analyses which are used to medicinal and therapeutic uses. The plant has a lot of anti-inflammatory, anti-cancer, antibacterial, and anti-fungal properties. Biodiversity has always provided humans with a variety of services. Population genetics can help to conserve biodiversity. *Trillium's* genetic diversity is measured using polymorphic markers.

Keywords: pharmacological, antifungal, antibacterial, anticancer, genetic diversity

Introduction

Medicinal plants have been important part of human life from ancient periods. Wild plants are mostly employed as medicine in the traditional method. The traditional medical system is used by more than 80% of the population in all nations. Therapeutic plants have widely recognised as a rich of medicinal ingredients for sickness prevention. The Himalaya is rich in biodiversity due to its diverse ecosystems and climatic conditions. The Indian Himalayas is biodiversity hotspot to a diverse range with flora and fauna. It is made up of five bio geographic regions that occupy an area of 591 thousand square kilometres ^[1]. Medicinal plants were recognised mostly as major source for health-giving substances for prevention of illnesses as well as disorder for millennia across the world. In the Indian Himalayan area, there are around 1748 distinct types indigenous therapeutic plant species ^[2], 675 varieties are natural consumable ^[3], with 279 types of fodder ^[4]. There are also 118 types of aromatic and medicinal plants that produce essential oils ^[5]. People that live in hilly areas rely on natural plant resources. It is hoped to discover good treatments for illness that are cost-effective and have no side effects in a variety of pathological situations. Medicinal plants have been utilised for the prevention, protection and treatment of illnesses from time immemorial. In medicinal plants the *Trillium govanianum* is of the most demanding in the western Himalayan region. *T. govanianum* belongs to family Trilliaceae/Melanthiaceae is a Himalayan plant that grows between 2400 and 4000 metres above sea level ^[6]. *T. govanianum* commonly called as Nagchhatri, or teenpatra (Pakistan), is one of the most significant medicinal plants with a wide range of therapeutic properties. *T. govanianum* contains both sex and steroids hormones, which are utilised in a variety of traditional treatments ^[7]. It's temperate forest undergrowth plant that thrives in cold, gloomy, damp environments. The major portion of the plant that is utilised for medicinal reasons is the rhizome. It's in high demand on the international market because it contains 2.5 percent

diosgenin (a saponin hydrolyzed product), which is utilised in the large scale production for steroid and sex hormones ^[8]. Traditional medicine is gaining popularity globally, and the usage of this medicinal herb is quickly increasing in rich countries. Overexploitation and unreasonable uprooting of this species' population in natural environments, particularly in Himachal Pradesh, has pushed it to the brink of extinction during the last several years ^[6]. The plant was not among the 960 medicinal plant species trafficked in India before 2008, but it is currently illegally marketed due to its remarkable health advantages and growing demand. *Trillium* is recognised as an endangered medicinal plant by the International Union for Conservation of Nature (IUCN) ^[2, 9]. Because many endangered species' higher extinction risk is generally related to low and fragmented groups, population genetics are crucial for threatened species conservation ^[10]. The genetic diversity might help to conserve biodiversity in a variety of ways. The increasing genetic drift and inbreeding within tiny or restricted groups may have also decreased genetic diversity ^[10, 11, 12]. Reduced population size and also the lack of gene transfer may result in genetic variation, reproduction fitness, and even a limited ability to respond to climatic change, all of which increase the chances for extinct.

Geographical Distribution

T. govanianum is a common herb of the Himalayas also known as Himalayan *Trillium*. The distribution of plant species is influenced by geographical factors such as height, slope steepness, and degree of sunshine. *T. govanianum* has been distributed across India, Bhutan, Nepal, Pakistan, Afghanistan and China. In India, mainly distributed in Himachal Pradesh, Sikkim, Uttarakhand, Jammu and Kashmir. It has been documented in the Dhauladhar mountain range forest ^[13], Shimla ^[14], Chamba ^[7], Kullu ^[6, 8, 15] and Kinnaur ^[8, 16]. In Jammu and Kashmir (Bandipora district, Fatehpur, Gulmarg, Kanzalwan, Pahalgam, Poonch, Gurez, Sonamarg, Machil, Sinthan top, Lidderwat, Bangus,

Ramnagar forest range, Doda and Kishtwar.), Sikkim and Uttarakhand (Gangotri, Govind Pasu Vihar, Harsil, Kedarnath, Munsiyari, Pindari, Panchachuli, Raithal, Sukhi, Sayara, Sunderdunga and Tehri-Garhwal in Bhagirathi Valley).

Biodiversity

Biodiversity has always provided humans with a variety of services. Medicinal plants are well-known as a source of income among the various components of biodiversity. The finest example is *T. govonianum*, which is economically important for people's survival in rural areas. The genus *Trillium* (Melanthiaceae family) has 49 perennial species, most of which are found in North America and Asia. Japan boasts the greatest variety for *Trillium* species in Asia. *Trillium govonianum* is a Himalayan herb out of total 11 Asiatic species [17]. The Indian Himalayan area, only two species (*T. govonianum* and *T. tschonoskii*) exist. This species is most abundant in many parts of western Himalayas (Himachal Pradesh, Uttarakhand, Jammu and Kashmir) however it may also be found in the eastern - parts but less frequent [18]. The *Trillium* thrives in a temperate forest cohabit with some plants like *Abies pindrow*, *Betula utilis*, *Cedrus deodara*, *Juglans regia*, *Juniperus* spp., *Picea smithiana*, *Quercus* spp., *Rhododendron* spp., *Salix* spp., or in sub-alpine forest (*Rhododendron* dominant).

Trillium govonianum is a perennial herb preferring shady areas and possesses three ovate green leaves in or whorl at stem, and purple bloom at the apex. The plant reaches a height of 30cm and the leaves are widely oval, sharp, and have a prominent petiole. The flower has three sepals, three petals, and six (3 double) stamens, as well as a three-carpellate ovary. Flowering takes place from May to July, while seed germination takes place between September and October. The fruit is crimson with a globule berry, and the seeds are oval, 0.25 cm long, and have a lateral appendage. The rhizomes is crawling, prolonged, and elongate greyish to brown colour with rough exterior surface reaching 3 - 5cm long and 0.8 - 1.5 cm in depth and numerous adventitious roots [19]. Kullu district is one of Himachal Pradesh twelve districts and area is recognised for its naturally, distinctive, and economically valuable biodiversity. With about 500 kinds of therapeutic plants, the area has a rich medicinal plant biodiversity. Numerous fungus genera (*Alternaria* spp., *Aspergillus* spp., *Fusarium* spp., *Mucor* spp., *Phoma* spp., *Pythium* spp., *Rhizopus* spp., *Stachybotrys* spp., and *Trichoderma* spp.) show endophytic relationship among leaves, stem, and rhizome throughout the summer and rainy season. The number of endophytes is higher in the rainy season than in the summer [20].



Fig: *Trillium govonianum*

Organization of chromosomes

Mehra and Sachdeva (1976) [21] discovered chromosome polymorphism in *T. govonianum* in a plant taken in Gulmarg, Kashmir. In terms of the length, quantity, and location of secondary constrictions, certain chromosomal pairs were discovered to be heteromorphic. *T. govonianum* has 20 chromosomes (2n), according to Pellicer *et al.*, (2014) [22].

Genetic diversity

Molecular markers are highly valuable tools for analysing genetic variation, and they may be the only tools available. Population structure and genetic diversity including an estimate with 4.33 alleles each loci were amplified 290 variants from 14 geographically diverse populations covering eight separate regions (2086-3500m amsl) of the IHR. Low genetic diversity (He: 0.25), high genetic differentiation (GST: 0.23), significant in between population genetic variation (72%), restricted gene flow (0.792), with modest significant separation by distances (Rxy = 0.260; p = 0.030) were found inside the population of *T. govonianum* [23]. Twenty one molecular markers were generated and characterised into 20 accessions of *T. govonianum*. The observed heterozygosity (Ho), expected heterozygosity (He) and Shannon information index (I) reported was 0.46, 0.48 and 0.73 respectively. The 31 alleles amplified by the polymorphic markers ranging from 2 to 4, via an average of 2.6allele each marker [24].

Ethnobotanical uses

In ethnomedicine, *T. govonianum* is often used to cure a number of diseases, including cancer, hypertension, arthritis, inflammation, and reproductive issues [25], as well as diarrhoea [15]. Its use to treat ulcers, sexual and menstrual issues [7, 19], a disinfectant, infection and joints aches [8]. Locals from Rajouri and Poonch regions in Jammu and Kashmir observed to drinking the daily cup of tea made with ten grammes of rhizome every three to four days relieves headaches [26]. Some *Trillium* species' dried rhizomes have long been utilised for immunoregulation, antiinflammatory, antiaging as well as anticancer effects [27]. Rhizomes from this plant species are used by the locals to treat wounds, diarrhoea, skin boils, infections, menstruation, sexual issues, and stomach problems [8].

Therapeutic uses

The presence of the steroids saponin, among the most structurally diverse but widely distributed plant secondary metabolites, is responsible for the species' pharmacological and therapeutic benefits. *T. govonianum* contains anticancerous, antiinflammatory, antifungal, antioxidant and antibacterial properties.

Anticancerous activity

The rhizome of *T. govonianum* is used to treat prostate and cervical cancers, as well as hepatic and colon tumours linked to enhance in β -glucuronidase activities [28]. Few cell lines, such as V79 fibroblasts and K562 cells, have been shown to be cytotoxic to diosgenin [29, 30]. *T. govonianum*'s anticancerous action is due to the presence of diosgenin and other steroidal saponin chemicals [28]. On brine shrimp (*Artemia salina*), the cytotoxicity potential of *T. govonianum* rhizome using methanolic and SPE fractions were evaluated and brine shrimp fatality were observed [29].

The methanolic extract and solid-phase extraction (SPE) fractions of *T. govanianum* rhizome are cytotoxic to four tumor cell lines: MCF7 (breast cancer), HEPG2 (Hepatocellular carcinoma), A549 (lungs tumour), and EJ138 (urinary bladder cancer). IC50 values range from 5 to 16 g/mL [31].

Antifungal activity

Its antifungal susceptibility results showed the steroid saponins 1 (govanoside A) or 2 (Borassoside E) was effective against *Candida albicans* but also *Candida glabrata* with *C. albicans* showing stronger effects. *A. niger* and *A. flavus* reacted to filamentous fungus, whereas *A. fumigates* was unaffected [28]. *T. govanianum* rhizomes were used to isolate three known compounds: pennogenin, borassoside E, and diosgenin. Borassoside E and Govanoside A compounds inhibited *A. flavus* ATCC 9643, *A. niger* ATCC 16888, *C. glabrata* ATCC 90030, and *C. albicans* ATCC 18804 [32]. *T. grandiflourm* rhizome and aboveground section extracts demonstrated considerable anti-*Candida albicans* action *in vitro* [33]. Hydro-methanolic extraction of *T. govanianum* inhibited *Trichophyton rubrum* and *Microsporium canis* by 80%, 75%, etc, whereas chloroform soluble extract inhibited them by 90% [34].

Antiinflammatory and analgesic activities

Rhizomes of *Trillium* species might be a new source of chemicals that can help with inflammation and pain relief. The crude extract of *T. govanianum* has anti-inflammatory and analgesic properties [14, 8]. The crude MeOH extraction, as well as its solvent extracts, showed anxiety and anti-inflammatory effects, as well as a significant reduction in paw edoema and relief of abdominal chemical and acute heat cognition [32]. The majority of anti-inflammatory and analgesic effects are attributed to steroid saponins, which have been discovered to be one of the primary chemical components in therapeutic preparations. According to recent research, saponins also reduce the production with iNOS and COX-2, culminating with a significant decrease in prostaglandin E2 concentrations [35].

Antibacterial activity

Antibacterial activity in acetone, ethanol, methanol, and distilled water rhizome extraction with *T. govanianum* against three pathogenic bacterial strains (*E. coli*, *Yersinia pestis*, and *Staphylococcus aureus*) was assessed *in vitro* by using the agar well diffusion method with various proportions (25%, 50%, 75%, and 100%) in plant extraction. The methanol rhizome extract of *T. govanianum* was shown to be the most efficient in preventing *S. aureus* growth [20]. The resazurin microtiter is used to find minimum inhibitory concentration (MIC) in methanolic extraction with *T. govanianum* rhizome and solid phase extraction (SPE) components, as well as to perform phytochemical analysis [36].

Chemical constituents

Trillarin is found in *T. govanianum* rhizomes, which when hydrolyzed gives diosgenin. Several glycosides, steroids, and steroidal saponins were reported in *Trillium* species. The existence of glycosides, steroidal saponins, tannins, sterols, as well as flavonoids imparts this plant medicinal and therapeutic significance [33, 34]. Borassoside E, diosgenin, govanoside A and pennogenin, are four

spirostanol saponins discovered from *T. govanianum*. Diosgenin is the most bioactive component because it accumulates in the rhizome as "Trillarin." [37]. From SPE fractions, a large quantity of kaemferol, myrecetin and quercetin, was extracted, and chromatograms were obtained using a PDA detector to identify distinct group and types of chemicals. Flavonoids, quercetin, myrecetin, and kaemferol were found in SPE extracts, demonstrating plant capability along various recognised bioactivities [31]. Diosgenin is a steroid saponin that occurs naturally. It is frequently utilised as source for a number of manufactured steroidal medicines in the pharmaceutical industry. Independent studies have been undertaken to determine the significance of diosgenin in the prevention and treatment of metabolic illnesses (dyslipidemia, hypercholesterolemia, obesity, and diabetes), as well as edoema and cancer. Proliferation, inflammation, differentiation, oncogenesis, and apoptosis are all inhibited by diosgenin, which suppresses cancer cell development [38].

Threat and conservational aspect

In Himachal Pradesh, this herb is an endangered and its conservation is a top priority [8]. The increased therapeutic importances as well as rising prices, a large number of plants (about 630 plants) were removed in their native habitat for just a deal worth 1kg dried herb [13]. *Trillium* rhizomes collection occurs in month of June and ended in August, with seeds maturity occurring from September and the end of October. Harvesting rhizome prior to seed germination might result in species extinction in their native habitat. Because of its excessive extraction and illicit export in recent years, higher altitude areas of Himachal Pradesh are now bereft of this plant. It has resulted in the rapid extinction of species in their native habitats, as well as endangered plants in Himalayan areas [21]. In Indian Himalayas, uncontrolled traditional collection custom resulted in population declines, with Munsiyari (Uttarakhand) on the top, which are followed Tirthan Valley of Himachal Pradesh and Tunghnath of Uttarakhand [38]. Because of its vast scale and inappropriate collecting techniques, the plant is endangered in Pakistan's Chail Valley and India's Kashmir [39]. Traditional rhizome vegetative propagation and *in-vitro* approaches are deemed important for fast massive multiplication in *T. govanianum*, which might allow it to recover from its endangered status and reducing the chance of extinction.

Conclusion

The goal of review is to analyse the biodiversity, genetic diversity, ethnobotanically significant as well as endangered plant *T. govanianum*. The increased demand for this plant on a worldwide basis has resulted in large-scale uprooting from its native environments. This species' population has declined as a result of unethical wild harvesting, and it is now designated an endangered plant. *Trillium* is an extremely important plant. It is both medical and herbal in nature. Its leaves and roots, for example, can be used to treat dysentery and diarrhoea since each component contains important pharmacological active chemicals with antibacterial, anticancer, antifungal, and analgesic effects. As a result, it is not incorrect to describe *Trillium govanianum* as a god-given medicinal plant with exceptional therapeutic qualities. Biodiversity has always been one of the finest sources of income since it delivers a variety of services to humanity. Medicinal plants are well-

known among the different components of biodiversity as a source of income. The greatest example is *T. govanianum*, which is commercially cultivated by villages for trade and subsistence. In the last two to three years, increased demand for this species has led to overexploitation in environment. Trade is mainly uncontrolled with in research region. Because the region lacks certification requirements, it loses a significant amount of money. The locals rely heavily on forest resources for their livelihood, particularly medicinal plant gathering and trading. The genetic diversity of each taxon has a significant impact on their long-term existence as well as adaptation. As a result, a genetic diversity evaluation becomes critical for developing conservation measures for risky and endemic plants. Many locals are unaware of plant's purpose, the cause for its unexpected need, or the raw materials eventual destination. For them, the monetary advantage is the most important factor. The most essential thing is to raise awareness and provide multifaceted training to local populations about sustainable exploitation of medicinal plant richness in hillside management for plant resources. It's also important to promote large-scale agriculture in high-altitude locations. The government should build nurseries and give villages with plant material. It will not only aid in its preservation but also provide a source of revenue for them.

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