International Journal of Ecology and Environmental Sciences
www.ecologyjournal.in
Online ISSN: 2664-7133; Print ISSN: 2664-7125; Impact Factor: RJIF 5.28
Received: 26-07-2020; Accepted: 11-08-2020; Published: 21-08-2020
Volume 2; Issue 3; 2020; Page No. 191-196

Podophyllum Hexandrum: The treasure of trans Himalayas

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Abstract
Podophyllum hexandrum Royle (Himalayan Mayapple) was commonly known as Bantrapushi or Giriparpat, Bankakri, Kanda-ri-mokri, Rikhpat, Papra, Aindri (a divine drug) in ancient times. It is also known as 'May apple' as the fruit ripening occurs in the spring season. This plant has already proven its potential, as it shows the properties of antioxidants, antibacterial, antifungal, antiviral, anticancer and insecticidal. *Podophyllum* has also got attention due its remarkable importance in various medicinal systems, either allopathic, homeopathic or amchi, because of its highly extensive therapeutic potential. However, the decline in species frequency for the last few years has drawn attention of many scientific bodies. This review paper gives a brief highlight on the phytochemical and pharmacological properties of *P.hexandrum* and its importance to mankind on pharmaceutical aspects related to this endangered plant.

Keywords: Endangered, pharmacological, medicinal, *P. hexandrum*

1. Introduction
Podophyllum hexandrum is a perennial herb, native to cold and sub alpine regions of trans-Himalayas [1]. The word *Podophyllum* represents the genus consisting of 'podos' a foot and 'phyllos' a leaf which resembles duck's foot [2]. The six petals and stamens of this species had led to its name *hexandrum*. The genus *Podophyllum* comprises of about seven species belonging to family Berberidaceae (to name a few *P. hexandrum*, *P. peltatum* and *P. sikkimensis*) out of which, six species are native to eastern Asia and one species is from North America and Central Asia [3]. Among different species of *Podophyllum*, *P. hexandrum*, is medicinally more valuable, due to higher concentration of podophyllotoxin [2, 4]. *Podophyllum peltatum* contains 4–5% podophyllum resin, whereas *P. hexandrum* contains 7–16% [5, 6, 7]. The variation in resin content is one of the major threats in demolishing the native species from Asia [8]. The chromosomal number of *Podophyllum hexandrum* is 2n = 12. The plant is reasonably abundant in the Great Himalayan National Park Kullu, Himachal Pradesh. In the fringes of the Valley of National Park, the study conducted by (C.P. Kala) shows some scattered population of this important species, locally called as 'Ban Kakdi'. Here, its density is about 0.98 individuals per meter square [9,1]. The genus *Podophyllum* is widely distributed in different regions of China, Sikkim, Nepal, Bhutan, North India, Kashmir, Pakistan, East Afghanistan. It also covers Himalayan regions of Pakistan, India, Afghanistan and China. In Afghanistan (Kashmir to NEFA), South-Tibet, West-China *P. hexandrum* has been extensively exploited by the Ayurvedic system of medicine for treatment of constipation, cold, biliary fever, septic wounds, inflammation, burning sensation, mental disorders, genital warts, monocytoid leukemia, Hodgkin's and non-Hodgkin's lymphoma [5, 10].

2. Geographical Range and Propagation
In Indian Himalayas, the distribution of plant is strictly restricted to temperate regions of cold deserts and sub alpine regions including the areas of Kullu, Lahaul and Spiti. The plant prefers the western Himalaya's harsh climatic conditions; such as low annual precipitation, dry summers, high wind velocity, extreme temperature conditions from very low to high, intense solar radiation, intense snow fall, high UV radiation and extremely xeric conditions which are however suitable for its proper growth [11, 12]. The prolonged and severe winter, 6-8 month of snowfall and 0-4 °C temperature is favorable for growth of this plant [1]. The mature seeds show about 80% viability in early stages that decreases very rapidly after seed desiccation. *Podophyllum* can be propagated through underground rhizomes as well as seeds, but shows no proper germination and remains typically dormant because of its specific habitat. *P. hexandrum* plant has long period of juvenility and has a slow growing habit. The slow growth is attributed to the adaptive strategies employed by the plant to overcome adverse, unpredictable conditions of sub-alpine and alpine regions of the Himalayas. The biodiversity catastrophe labels for global assessments are well informed by local indigenous communities. Delaminating majority of native species by the very poor ecological knowledge hindered the conservation opportunities [13].

3. Classification
Kingdom - Plantae
Division - Magnoliophyta
Class - Magnoliopsida
Order- Ranunculales
Family - Berberidaceae
Genus - Podophyllum
Species - *Podophyllum hexandrum* Royle

4. Botanical Description
The perennial herb attains 30-75cm height. The stem is unbranched, has a long stalk with a single leaf at the terminal end. The Stalk is round, hollow, light green, hairless and slightly

slippery. Leaves are large, dissected or triangular in shape; similar to umbrella, with 5-9 lobes per leaf. The number of leaves in the vegetative as well as reproductive shoots varies in number. Vegetative shoot is generally represented with a single leaf, while reproductive shoots generally bear two and sometimes three to four palmate, orbicular and deeply lobed (three to five) leaves. Flowers are cream-colored with six to nine petals, flowering once in season on single lobe covered by the leaves. The fruits are oblong, berry or egg-shaped with altered pigmentation. It is typically green in color when unripe that turns yellow to dark red when ripe. The pulpy berries contain numerous hard and deep red colored seeds with rough outer seed coat. The pigmentation or coloration of seed changes during maturation from dark brown to deep red. The seeds are poisonous as they contain cytostatic toxic compound known as podophyllotoxin, if consumed directly. Each plant has five to eight vegetative shoots that bear one to four berries in the months of April-May.

5. Why Need for Conservation?

The plant is now considered important owing to its unskilled and overexploitation rate (Fig.I). The decline in number of plants due to habitat fragmentation, low rate of natural regeneration or seed germination and prolonged dormancy has been the major factor causing decrease in the size of its wild populations [14, 15]. The increasing demand for podophyllotoxin contained in its rhizome and poor regeneration practices have also threatened its diversity (Fig.I). The availability of the chemical compound within this species also varies along regional distribution. A noteworthy amount of *Podophyllum* lignans has been observed in the *P. hexandrum* which is present in the Lahaul valley of Himalayas. *P. hexandrum*has been categorized as an endangered species in India. The plant yields two bioactive compounds teniposide and etoposide which are specifically used to cure brain tumors, infancy leukemia and testicular as well as small-cell lung cancer [16]. The plantar warts is cured by the drug Posalfilin that contain bioactive compound podophyllin. As the demand of these compounds are increasing rapidly, the risk of plant extinction is also at peak [17, 18, 19, 15].

Fig 1: *Podophyllum hexandrum* at keylong region (Lahaul valley) [2017-18].

6. Chemical compositions

Podophyllotoxin was extensively used since a long time and proved its potential as an antidote against poisons, or as cathartic, purgative, antihelminthic, vesicant and suicidal agents. Podophyllotoxin (PTOX) or a 2,7'cyclolignan was recognized as the 'active principle' and molecule of immense commercial interest which was obtained primarily from genus *Podophyllum* [20, 21] (Fig.II). The podophyllum resin is used as a curative in different medical aspects like anticancerous, antioxidant, peripheral neuropathy, vomiting, confusional states [22, 23, 24, 10].

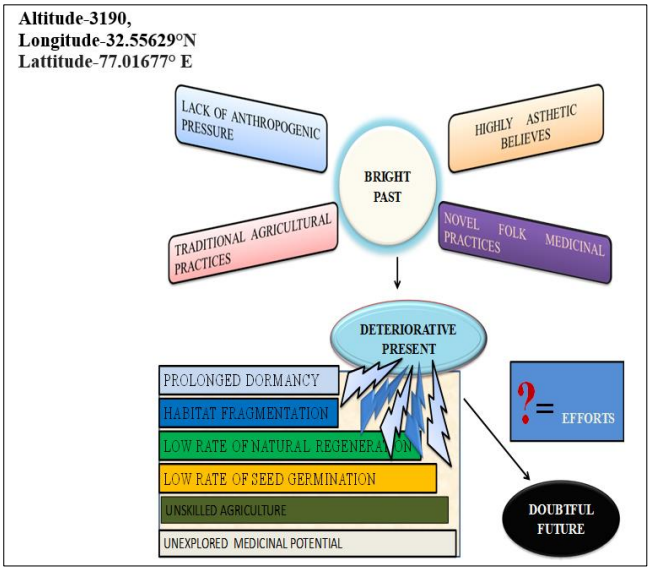


Fig 2: Major concern over species conservation and its deteriorative aspects.

Podophyllotoxin pharmacology, its product and mode of action

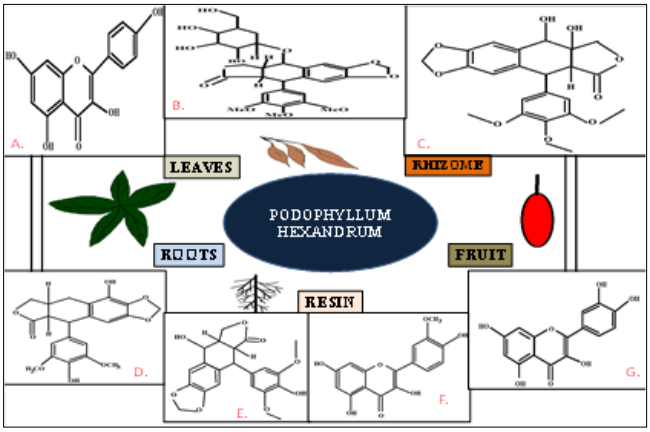
Podophyllin resin derivatives have been used as medicaments for over 250 years. Podophyllotoxin extracted from *Podophyllum pelleta* (Native species of America) has been used as an effective antidote against snakebites. Podophyllotoxin inhibits the tubulin polymerization [25]. During initiation of metaphase, it stops cell division and acts as a meiotic spindle poison. The two podophyllotoxin compounds (teniposide and etoposide) block the phase between the last division and the start of DNA replication (the G1 phase) and they block the replication of DNA (the S phase) [26, 27]. It is applicable to generate single strand break in the DNA as well as retardation of the activity of topoisomerase. Whereas, it promotes some derived products from the oxidation and reduction reactions that ultimately binds to DNA directly [8]. Penile warts, external condyloma along with rheumatoid arthritis and oral hairy leukoplakia (HIV related) can be treated with the application of this compound. Hence it has antiviral, antitumor and radio-protective activity [28, 29]. However, usage of decoction via direct ingestion results in human poisoning [30]. Even their over dosage leads to death because of toxicity in gastrointestinal, haematological and neurological system. However, immature fruit or other plant part consumption is generally nontoxic, as it may cause diarrhoea [31]. Also, the mature ripe fruit are non-toxic. *Podophyllum* resin is an anti-mitotic agent and hence can be used to inhibit mitosis as much as by colchicine treatment. It binds to tubulin, the protein subunit of the spindle micro- tubules, at the same site or greatly overlapping the same site as colchicine. The anti-mitotic action of podophyllotoxin probably results from interference with the movement of the chromosomes. The molecular mechanism of mitosis blockage is the disruption of the micro-tubules of the mitotic spindle via binding of podophyllotoxin to tubulin [8]. Podophyllum is a keratolytic agent with cathartic and caustic actions. *Podophyllum* is caustic but its action differs from most caustics in that, its effect is neither direct

nor immediate rather the disruption of cells and erosion of tissue occur slowly, subsequently arrests cell division and impairs other cellular processes. Podophyllotoxin has an inhibitory effect similar to that of vinblastine (obtained from *Catharanthusrosea* or periwinkle plant) on the release of iodine from the thyroid gland and catecholamine from the adrenal medulla.

7. Pharmacogonological properties

7.1 Anti-bacterial activity

The podophyllotoxin affects a wide range of bacterial species. A few bacterial species were subjected as anti-bacterial pathogenic strains under *in vitro* condition and have been tested worldwide [32]. On screening of its different derivatives, the results indicated that ethyl-2-(3′-methyl-4′-methoxybenzyol-3-1 3″, 4″-dimethoxyphenyl)-cyclopropane-carboxylic acid and ethyl-2-(3′-methyl-4′-methoxybenzoyl)-3-(4″methoxyphenol)-cyclopropane-1 carboxylic acid have significant antibacterial activity against different bacterial strains of *Klebsiella pneumonia, Salmonella typhi, Shigellasonnei, Escherchia coli, Pseudomonas aeruginosa and Streptococcus faecalis* (Fig.III). Antibacterial activity was determined by cup diffusion method on nutrient agar medium [33]. The antibiotics such as Ciprofloxacin, Gentamicin, Penicillin G and Streptomycin were also used along with podophyllotoxin derivatives for comparison during the experiment. The antibacterial activity of Podophyllotoxin derivatives against the test bacteria, as compared to the antibiotics provided positive results.



a. Kempferol
b. podophyllotoxin 4-O-glucoside
c. Picropodophyllin
d. α-peltatin
e. 4'-Demethylpodophyllotoxin
f. isorhamnetin
g. Quercitin

Fig 3: Source of different podophyllotoxin derivatives from plant parts.

7.2 Insecticidal activity

Matairesinol acts as a precursor of Podophyllum lignans, a naturally occurring aryltetralinlignan, isolated from the underground part (Rhizome, roots) of *Podophyllum hexandrum* [34]. In recent investigation of several podophyllotoxin derivatives, the insecticidal activity of these derivatives was higher than toosendanin (a commercial botanical insecticide isolated from Meliaazedarach) and displayed the anti-molting hormone effect (Fig.III). The series of 2β- chloropodophyllotoxin and 2α/β-bromopodophyllotoxin derivatives and 4-(acyloxy) podophyllotoxin derivatives find novel natural product-based insecticidal agents to control the lepidopteran pests [22]. When the series of novel 4α- (acyloxy)-2′(2′,6′)-(di)halogen podophyllotoxin derivatives by introduction of the halogen atom at the C2′ or C2′ and C6′ positions on the E ring as insecticidal agents against the pre-third-instar larvae of oriental armyworm, *M. separata* Walker, in vivo (Fig III). The insecticidal activity of novel 4α- (acyloxy)-2′(2′,6′)-(di) halogen podophyllotoxin derivatives′) against the pre-third-instar larvae *of M. separata* was tested by the leaf dipping method [35]. The corresponding mortality rates caused by these compounds against *M. separata* for 30 days experiment were generally higher than those after 10 and 20 days [36]. This indicated that these compounds are time-dependent but different from other quick acting conventional neurotoxic insecticides such as organophosphates, carbamates, and pyrethroids, showed delayed insecticidal activity [37]. By feeding on treated leaves during the first 48 h, some larvae with slim and wrinkled bodies died slowly. In some cases larvae died during the pupation stage by molting. The malformed pupae and malformed moths with imperfect wings appeared in the treated groups [38]. The result indicates that these derivative compounds exhibited higher insecticidal activity than that of toosendanin, the most potent insecticidal activity showed mortality rates of approximately 70%. The efficiency was increased by modification of the compound by introduction of halogen at different positions of podophyllotoxin that resulted in more potent compounds as compared to their precursor podophyllotoxin and its potent derivatives. This further demonstrated that a chlorine or bromine atom introduced at different position of podophyllotoxin ring also affect the range of invasiveness. As compared with 2β-chloropodophyllotoxin derivatives, the 2′(2′,6′)-(di) chloropodophyllotoxin exhibited higher potent insecticidal and final mortality rates [39]. Other derivatives of podophyllotoxin such as 4α-(acetyloxy)-2β-chloropodophyllotoxin, 4α-(propanoyloxy)-2β chloropodophyllotoxin, 2′- bromopodophyllotoxin, 2α ′-bromopodophyllotoxin, 2β-bromopodophyllotoxin, 4α-(acetyloxy)-2α-bromopodophyllotoxin, 4α-[(m-nitrobenzoyl)oxy]-2α-bromopodophyllotoxin, and 4α-[(1-naphthylacetyl)oxy]-2α-bromopodophyllotoxin,4α-(acetyloxy)-2′-bromo podophyllotoxin showed positive results (Tab. I) [40].

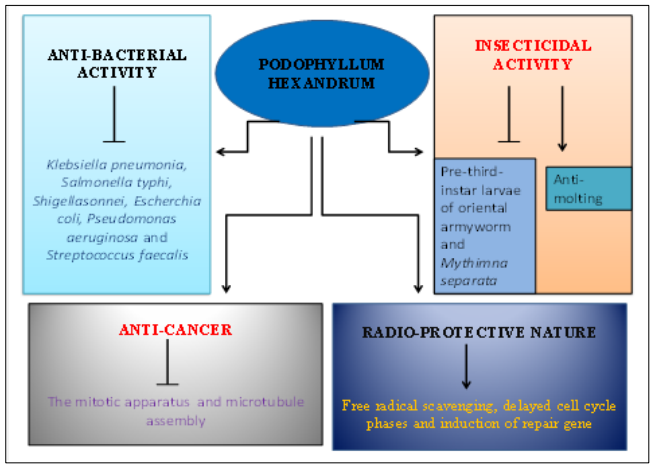


Fig 4: Potential role of *Podophyllum hexandrum.*

7.3 Anti-cancer

The toxin podophyllotoxin alters the cell biosynthesis by blocking the cell differentiation [41]. It inhibited the mitotic apparatus and microtubule assembly thereby causing uncontrolled cell size leading to abnormal mass of cell formation [42, 43] (Table II). The use of conjugates podophyllotoxin analogues and its derivatives (4'-demethyl-epipodophyllotoxin and camptothecin or paclitaxel) has emerged as a frequent strategy in efforts to optimize therapeutic beneficial properties of sequence specificity and tumour cell recognition of DNA damage (Fig.III). The thymidine derivatives of these compounds displayed activities similar to podophyllotoxin and lack etoposide-like activities. These thymidine derivatives exhibited much lower activity (Tab.II). These compounds interfere with the receptor site on the tubulin molecule [44].

Table 1: Chemical composition of various parts of *Podophyllum hexandrum* [13, 47, 48, 49]

Sr. No.	Part	Compound
1.	Roots	Aryltetralinlignans: podophyllotoxin, 4′-demethylpodophyllotoxin, α-peltatin, β-peltatin, desoxypodophyllotoxin, podophyllotoxone, isopicropodophyllone, 4′-demethyldesoxypodophyllotoxin, 4′-demethylpodophyllotoxone and 4′-demethylisopicropodophyllone
2.	Resin	Lactone, podophyllotoxin, picropodophyllin, podophyllic acid, quercetin, isorhamnetin, querceting alactoside
3.	Rhizome	Aryltetrahydronephthalene lignans such as podophyllotoxin4-O-glucopyranoside, 4'-demethylpodophyllotoxin, Podophyllotoxin, dehydropodophyllotoxin, podophyllotoxin 4-O-glucoside
4.	Fruit	Picropodophyllin, 4'-Demethylpodophyllotoxin
5.	Leaves	Epipodophyllotoxin, podophyllotoxone, 4-methylpodophyllotoxin, quercetin, kaempferol aryltetrahydronaphthalene lignans, 4-demethylpodophyllotoxin glycoside, quercetin-3- glycoside, podophyllotoxin glucoside and kaempferol-3-glucoside

7.4 Radio-protective nature

The exposure of radiation or radioactive activities increases risk of total body irradiation (TBI) continuously. It has been widely used as radio-protective compound since many years and its extract has been shown to render 80% total-body radioprotection [45] (Fig. III). The herb contains a wide range of bioactive compounds that exhibit potent antioxidant ability and protect against radiation-induced reactive oxygen species (ROS) mediated damage. It is proved to be a significant free radical scavenging remedy. The radiation induces activity of lipid peroxidation that starts a signaling cascade and activates transcription cascade. The series of reactions involves heat shock transcription factor-1 (HSF-1) and MAPKAP (mitogen-activated protein kinaseactivated protein) kinase-2 leading to activation of Heat shock protein (HSP) -70 in gastrointestinal murine model. The expression of HSF-1 and Hsp70 is inhibited by Pifithrin-α ultimately leading to inhibition of Bcl2 expression required for cell proliferation. Pifithrin-α reduces the duration of DNA repair and upregulates the accessory proteins associated with cell-cycle maintainace and repair. In an experiment conducted on mice, positive results were obtained. The plant extract activated the major cell proliferation genes (Bcl-2, RasGAP and PCNA). The lower expression of cyclins (Cycklin E and CDK2) along with increased expression of p21 gene in the *P. hexandrum* treated and mice showed delay in the G1 phase of cell cycle, which provided extra time for DNA repair. The free radical scavenging, delayed cell cycle phases and induction of repair gene enhanced survival at cellular as well as molecular level [46].

Table 2: Types of Podophyllotoxin, its chemical name and disease treatment [8, 14, 1, 2, 43, 46, 37]

Podophyllotoxin	Chemical name	Treatment
Etoposide (Vepeside)	Demethyl epipodophyllotoxinethylidene glucopyranoside, epipodophyllotoxin or VP-16	It is a highly active and widely used antineoplastic agent. It is active against many tumor types and used primarily as part of combination treatment for testicular tumors and leucopenia. This is most active single agent for small cell lung cancer, brain tumors, wings sarcoma, histiocytosis, kaposis sarcoma and neuroblastoma, acute myelogenous leukemia, germ cell tumors, hodgkins disease, ovarian cancer, rhabdomoysarcoma and newly diagnose glioblastoma multiforma.
Etoposide phosphate	Etophos	Increased convenience of high dose treatment and feasibility of chronic infusion
Teniposide	VM-26	Treatment of lymphomas of acute refractory leukemia and that of brain and bladder tumors. It can be used in single drug therapy for induction of remission.
NK 611	–	The antitumour activity of NK 611 showed to be equal or superior to etoposide.
CPH 82	Reumacon	It has potential for the treatment of rheumatoid arthritis, rheumatoid, psoriatic Arthritis, Juvenile rheumatoid Arthritis, oligoarticular large joint arthritis.

8. Conclusion and future perspective

The podophyllotoxin extracted from *P. hexandrum* (>5%) is much higher than *P. peltatum* in dried roots (0.25%). The synthesis of bioactive compound (podophyllotoxin) is difficult, expensive and time consuming. The availability of the compound from natural resources is a very simple and cost-effective way for the pharmaceutical industries that manufacture this drug from its chemical constituents. So there is a need for exploration and conservation of the species. Findings revealed that *P. peltatum* leaves have significant amount of lignans as glucopyranosides as they can provide continuous source due to their renewable nature. Some other genera can be used as substitute such as *Dysosma, Hyptis*, *Juniperus*, *Thymus Thuja* etc as well as needles of *Juniperus virginiana* provide 4.7% bioactive compound. Owing to the ever-increasing demand of *Podophyllum hexandrum* for podophyllotoxin, it has been subjected to heavy collection from

the wild. *P. hexandrum* population and there is an intense need to take protective measures to conserve this highly valuable herb. This review aimed to summarize the characterization and importance of *P. hexandrum*. Historically, this wild Asian herb was widely accepted and frequently used in amchi medicinal system, because it helped to cure snakebites, condyloma accuminata, lymphadenopathyge poisons and weakness. It was also used by Penobscot Indians for tumor treatment. Recently, it is also used as curative for hepatic diseases, syphilis and as a laxative. It is worthwhile to mention that this tribal species is a frontline medicinal herb with immense potential to be a functional resource for the second-generation medicines. The domestication of plant in plains or any land different from its native habitat is difficult yet the ideal approach. There is a need for strengthening approach for propagation of this herb through vernalization, regulation of photoperiods or applications of plant growth regulators.

9. Acknowledgments

The author would like to thanks Anuj Choudhary for diagram and table preparation and Harmanjot Kaur for manuscript prepration.

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