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Podophyllum L.: An endergered and anticancerous medicinal plant–An overview

OP Chaurasia*[1], B Ballabh[2], A Tayade[1], Raj Kumar[1], G Phani Kumar[1] & SB Singh[1]

[1]Defence Institute of High Altitude Research, Defence Research and Development Organisation, Leh-Ladakh - 194101, Jammu and Kashmir, India

[2]Defence Institute of Bio-Energy Research, Defence Research & Development Organisation, Goraparao, PO- Arjunpur, Haldwani - 263139, Uttarakhand, India

*E-mail: opchaurasia@indiatimes.com

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Podophyllum hexandrum Royle syn *P. emodi* Wall. ex Hook.f. & Thoms. is an endangered and valuable medicinal plant, distributed in the Himalayan zone at an altitudes ranging from 2000 to 4000 m above MSL. It is an herbaceous and rhizomatous perennial plant also found in few pockets of Indian cold desert (Ladakh and Lahaul-Spiti). Its rhizomes and roots contain about 8% of podophylloresin from which several lignans were isolated. Amongst the most important being podophyllotoxin (4% on a dry weight basis) which has cytotoxic and antitumour properties, and also used in the treatment of certain forms of cancer. In past few years, the frequency of this species in nature has declined considerably because of unscientific exploitation to meet the everincreasing demand of pharmaceutical sector. *Podophyllum hexandrum* has an increasing demand in national and international market because of more than double amount of podophyllotoxin content found in it, than *P. peltatum* (American *Podophyllum*).

This article briefly reviews the botanical, medicinal, phytochemical, pharmacological, conservation, market and trade related aspects of the plant. An attempt has also been made to compile and document information on other aspect of *Podophyllum hexandrum*, other species of such as *P. peltatum, P. sikkimenmsis* and also highlight the need for its research and development.

Keywords: Mayapple, Lignan, Etoposide, Teniposide, Podophyllotoxin, *Podophyllum hexandrum*

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The term *Podophyllum* is derived from ancient Greek word means 'foot leaf' and also known as Mayapple because of its fruits ripen in spring. The genus *Podophyllum* is generally represented by two species, the *P. hexandrum* is commonly distributed in the Himalayan regions of Asian continent popularly called as Himalayan Mayapple and the *P. peltatum* commonly distributed in Atlantic North America popularly called as American Mayapple. The *Podophyllum* is represented by three species *P. hexandrum, P. peltatum* and *P. sikkimensis*[1]. *P. hexandrum* shows high range of variation in plant height, shape, number of leaves, fruit sizes and seed colours (Figs. 1-6).

Podophyllum hexandrum Royle is an important medicinal plant known for valuable drug podophylotoxin which is effective against various diseases: purgative, laxative, cholagogue, polyps, alterative, emetic and also useful against warts and tumors growth of skin, anti-cancer[2-3]. Podophyllotoxin, along with α-peltatin, and β-peltatin, are aryltetralin lignans known to have biological activity such as anti-cancer, anti-fungal, anti-viral, anti-mitotic, and immunostimulatory properties[4]. Podophylloresin contains several lignans including podophyllotoxin from which two modern chemotherapeutic drugs etoposide and teniposide have been synthesized which are now being used in the treatment of mild lung cancer and other tumours[5].

Podophyllum peltatum contains lower amounts of podophyllotoxin in comparison to *P. hexandrum*[6]. *Podophyllum* mentioned in pharmacopoeia, dating from 1820, as a cathartic and cholagogue. Because of its severe toxicity the drug was removed from the 12th edition of this Pharmacopoeia, appeared in 1942[7]. The *Podophyllum* has got immense potential in various traditional systems of medicines including Ayurveda, Unani, and Siddha for the remedies of several disorders[8]. The demand of Podophyllotoxin has increased immensely in global market against its limitaed supply due to both intensive collection and lack of cultivation of *Podophyllum*[9].

*Corresponding author

Fig. 1-6; 1, *P. hexandrum* single flowering stage; 2, *P. hexandrum* in flowering stage; 3, *P. hexandrum* in fruiting stage; 4, *P. hexandrum* with ripen fruits; 5, *P. hexandrum* : 3 yrs old rhizomes and 6, *P. hexandrum* : vegetativelypropagating parts

The Indian Mayapple

P. hexandrum is believed to be originated from the Himalayan region. It is distributed from Indian Himalayas to Bhutan, Pakistan, Afganistan, Nepal, Taiwan and China. In India, it is grown in the Himalayan regions between 2000-4500 m above MSL in very restricted locations in Zanskar, Suru Valleys of Ladakh, Kashmir region in Jammu and Kashmir, Lahaul, Spiti, Kangra, Chamba and Kinnaur in Himachal Pradesh, between 2000-4000 m above MSL in Kumaon and Garhwal in Uttarakhand, Sikkim and Arunachal Pradesh[10].

The American Mayapple

Podophyllum peltatum, the American mayapple also known as Mandrake, grows in large colonies " in eastern North America from Quebec and Minnesota to Florida and Texas[11]. Vegetative propagation of mayapple is by rhizome cuttings or by micropropagation using the terminal bud as the source of explant-inducing adventitious buds with 70-90% success rate in soil acclimatization[12]. Mayapple thrive very well under shade and moist conditions where it sprouts first in the spring. More podophyllotoxin yield is, however, reported under the sun than plantings under the shade house[13].

Podophyllotoxin is the preliminary compound for the production of three important clinically useful anticancer drugs etoposide, etopophos and teniposide[14]. So far, podophyllotoxin is isolated from the rhizomes of *P. peltatum* and *P. hexandrum* plants because organic synthesis is not economically feasible. The supply of *P. hexandrum* rhizomes which contain up to 5% of podophyllotoxin on a dry weight basis becomes increasingly limited due to both intensive collection and lack of cultivation[6, 15].

The lignans deoxypodophyllotoxin, yatein and anhydropodorhizol obtained from *Anthriscus sylvestris* (Apiaceae; wild chervil) is a common weed in Northwest Europe and its rhizomes contain considerable amounts of yatein which is precursor of deoxypodophyllotoxin in *Podophyllum hexandrum*[16]. Undifferentiated cultures of plant cells or fungi are also able to convert deoxypodophyllotoxin into podophyllotoxin after feeding[17].

Medicinal uses

The resin or Podophyllin is used as purgative. The content of resin and rhizome varies from 7-15% according to season and locality. Freshly collected rhizomes are reported to contain higher quantity of active principles which may be lost on prolonged storing. *Podophyllum* leaves also contain 7.8-9.7% of resin and it may be used as a main source of drug at the time of slow growth of roots and rhizomes, and rapid decline of natural growth and active regeneration.

Podophyllum rhizomes, roots, fruits are also used as anticancer agents and in the treatment of ulcers, hepatic disorders, wounds/cuts, tuberculosis and constipation[18]. In past few years podophyllin resin and its active principle podpphyllotoxin, have received significant attention for their tumor necrotizing properties. Podophyllin is listed in various pharmacopoeias and formularies. Indian *Podophyllum* has more than double amount of podophyllotoxin content in comparison to American *Podophyllum* (2-8%) however, the active principle α- and β-peltatins isolated from *P. peltatum* which are absent in *P. hexandrum*. Podophyllin has been used in treatment of warty lesions of the skin and neoplasms occurring in the regions of body accessible to topical therapy. It is also used in dermatological infections[19], the simplicity of treatment and absence of severe pain to the patient. Podophyllin is an effective vermifuge, first stimulating, then paralyzing and finally killing *Ascaris*. It also gives symptomatic relief to certain allergic and inflammatory conditions of skin.

Podophyllin is also being used for controlling some forms of cancer; the limiting factor is the severe gastro-intestinal discomfort while used in higher doses. The drug podophyllotoxins has certain cytotoxic effects similar to that of colchicine (mitotic arrest, nuclear fragmentation, other evidence of cellular damage) as mitosis inhibitors and other medicinal applications including uses as anti-malarial and anti-fungal agents with immune modulator activities[4,20]. It has been reported that after the major discovery of the anticancer properties of podophyllotoxin derivatives[11], the US annual demand for *P. peltatum* rhizomes was more than 130 tons in 1970.

High altitude *Podophyllum hexandrum* (HAPH) provides a radioprotective effect in *in-vitro* and *in-vivo* models. The ripen fruits of *Podophyllum* species are said to be edible and also used against fever in traditional system of medicine in India. Rhizomes are called *Ol-mo-se* in Ladakh which means the remedies for women problems. These are used against various forms of gynaecological disorders in Tibetan system of medicine and in Ladakh[21].

Two aryltetralin lignans (4`-*O*-demethyl-dehydropodophyllotoxin and picropodophyllone), isolated from leaves showed strong antifungal activity against *Epidermophyton floccosum*, *Curvularia lunata, Nigrospora oryzae, Microsporum canis, Allescheria boydii* and *Pleurotus ostreatus*. Picropodophyllon also showed activity against *Drechslera rostrata*[22].

The whole plant, but especially the root, is cholagogue, cytostatic and purgative. The plant contains podophyllin, which has an antimiotic effect (it interferes with cell division and can thus prevent the growth of cells). It is, therefore, a possible treatment for cancer, and has been used especially in the treatment of ovarian cancer[4]. The roots contain several important anti-cancer lignans, including podophyllin and berberine[23]. The roots are also antirheumatic[23]. The root is harvested in the autumn and either dried for later use or the resin is extracted[24]. This plant is highly poisonous and should only be used under the supervision of a qualified practitioner[24]. It should not be prescribed for pregnant women[24].

It is currently being used as a lead compound for the semi-synthesis of anticancer drugs etoposide, teniposide, etopophos, which are used for the treatment of lung and testicular cancers and certain leukemias[14, 25]. LPS has been reported to activate pro-inflammatory molecules are known to manifest oxidative stress[14], vascular anomalies like intravascular coagulation, vascular perfusion and hypotension[26] and ultimately the mortality. RP-1 has been reported to have antioxidant property[27], which may be attributed to presence of several molecules in the extract like quercetin and flavanoides, the known inhibitors of oxidative stress[28] and inflammatory response[29].

The aqueous-ethanolic extract (AEE) of high altitude *Podophyllum hexandrum* has earlier been reported to render a radioprotective effect against lethal gamma radiation in *in-vitro* model. AEE has also been reported to possess metal chelating and DNA protecting properties.

The Indian introduced podophyllin, a resin obtained by ethanolic extraction of the *Podophyllum* roots and rhizomes, to colonists for the use as a catharic, an anthelmentic and misuse as a lethal poison. The main constituents in podophyllin are the lignans podophyllotoxin, 4'-demethylpodophyllotoxin and α- and β-peltatin. The colonists also used this resin as an emetic and cholagogue. The clinically applied cytostatics etoposide, teniposide and etopophos. NK 611 is currently used in the clinical trials as a new anticancer agent. In 1947, podophyllin reported as cancer growth inhibitor agent in animals[30]. Due to high cytotoxic constituents podophyllin and

glycosides induce severe gastrointestinal side effects, thus their clinical uses get limited[26]. Sandoz was the first to develop etoposide after preparing several series of glycoside and aglycon derivatives by chemical synthesis in 1966. This semi-synthetic drug was clinically tried in 1971 and it took another 12 yrs before etoposide became Food and Drug Association (FDA) approved. Bristol-Myers Squibb Co. took over license for both etoposide and teniposide during this period. In 1996 the phosphate analogue etopophos was also approved. A detailed history of these compounds has been recently described by Imbert in 1998. Metastatic testicular germ cell tumours are still treated by etoposide for instance bleomycin often in combination with cisplatin[31]. Etoposide is also used in treatment of small cell lung cancer[32-33].

The major active constituent in podophyllin from *P. hexandrum* vary from 32-54% however, a number of other related compounds and their glucosides were isolated from resin (Table 1). The resin also contains quercetin (8%), kaemferol, astragalin (kaempferol-3-glucoside), an essential oil (3.7%) responsible for the odour of podophyllin, wax (8.6%) and mineral salts.

The antioxidant defence with *Podophyllum* sp. treatment in mice can explain to some extent its protective action manifested in terms of survival against whole body lethal irradiation[28]. *P. hexandrum* contains several lignans which possess antitumor activity. Podphyllotoxin is the most active cytotoxic natural product. It is used as starting compound for the synthesis of anticancer drug etoposide and teniposide. Podophyllotoxin acts as an inhibitor of microtubule assembly. These drugs are used for lung cancer, testicular cancer, neuroblastoma, hepatoma and other tumors. Besides this, it also shows antiviral activities by interfering with some critical viral processes[15].

Podophyllum hexandrum referred in Ayurvedic literature for various therapeutic purposes and its aqueous extract is known for anti-inflammatory potential[38]. The peroxyl ion scavenging potentials of methanolic S1 and chloroform fractions S2 of *P. hexandrum* were found to be comparable [i.e. 45.88% (S1) and 41% (S2)] after a 48 hrs interval in a time dependent study, whereas in a 2 hrs study, S2 exhibited significant ($P < 0.05$) antioxidant activity in different metal ion flux states. In the aqueous phase, S2 exhibited non-site-specific reactive oxygen species scavenging activity, i.e. 73.12% inhibition at 500 µg ml^{-1}. S1 exhibited 58.40 ± 0.8% inhibition (at 0.025 µg ml^{-1}) of the formation of reactive nitrite radicals, comparable to S2 (52.45 ± 0.825%), and also showed 45.01% site-specific activity (1000 µg ml^{-1}), along with significant ($P < 0.05$) electron donation potential (50–2000 µg ml^{-1}) compared to S2. Such activities of S1 could be attributed to the significantly ($P < 0.05$) higher levels of podophyllotoxin ß-D-glucopyranoside (16.5 times) and demethyl podophyllotoxin glucoside (2.9 times) compared with S2. These findings clearly prove that aryl-tetralin lignan content influences the radiation protective potential of the *Podophyllum* fractions to a great extent[39]. Various biological acticity/uses of *P. hexandrum* are given in Table 2.

The mechanism of the action of podophyllin and its active constituents on tumors is incompletely understood. It has been found that the necrosis is a direct consequence of a cytotoxic effect on tumour tissue, a rapid and marked reduction of the cytochrome oxidase was observed in tumour homogenates from animals treated with the podophyllin derivatives. Another hypothesis is that the primary locus of action of the drug is the vascular system but no correlation was observed between tumour necrotizing activity and the effect on arterial blood pressure of the drug[53].

The mode of action of Podophyllotoxin

Podophyllotoxin inhibits the formation of the microtubules. *In-vitro*, it binds to tubulin dimers giving podophyllotoxin-tubulin complexes. This stops the further formation of the microtubules at one end but does not stop the disassembly at the other end leading to the degradation of the microtubules. Cells treated with podophyllotoxin are arrested in the metaphase of the mitosis. Its mode of action is comparable to the alkaloid colchicin and for their mode of action these compounds are called spindle poisons. Other spindle poisons in clinical use are paclitaxel and vincristine like alkaloids. These cause the cells to enter the mitosis, but the duplicated chromosomes will not be separated. In this way, the cells cannot duplicate and hence the growth stops. The specific interaction of these compounds with the microtubuli growth is however, different as they stop degradation and not assembly[25, 54-55].

The clinically applied podophyllotoxin derivatives etoposide, teniposide and etopophos have a completely different mode of action. These compounds are topoisomerase II inhibitors. Topoisomerase II is an

enzyme that cleaves double stranded DNA and seals it again after unwinding. It is crucial in the processes of DNA replication and repair. For its function topoisomerase II binds covalently to the broken DNA. Etoposide and other derivatives stabilise the DNA-topoisomerase II complex in such a way that resealing of the DNA strands becomes impossible. Cells that are duplicating their DNA for the mitosis are very sensitive for this mechanism. The overall effect of these anticancer drugs is the arrest of the cells in late S or early G2 phase of the cell cycle[56-57]. A major advantage of the newly introduced etopophos (etoposide phosphate) is the improved solubility in water. Etopophos is a pro-drug of etoposide. After administration the phosphate group is hydrolysed in the human body to yield etoposide, which is bioactive. Because of its hydrophilic property etopophos can be administered much easier[58]. Several new derivatives of etoposide are currently in the clinical phase of studies. For instance NK 611 has improved water solubility and has also a topoisomerase II blocking activity[51-52].

The only known interactions between human CYP 3A4 and lignans are inhibitory effects[59-60] and 3-*O*-demethylation of etoposide and teniposide[61-62]. The mechanism of enzyme inhibition is not clear yet and competitive, non-competitive as well as mutual inhibition of CYPs by lignans has been found. *O*-demethylation is a reaction that eliminates a methyl group from the 3-methoxy group of etoposide and teniposide. It results in a formation of 3-hydroxy group. Deoxypodophyllotoxin, podophyllotoxin and α-peltatin all three possess methoxy groups at the 3 and 5 position.

It can be concluded that now a day lignans are compounds of interest for the pharmaceutical industry. Podophyllotoxin is an important starting compound to prepare semi-synthetic cytostatics. An investigation of related lignans may lead to new cytostatic compounds, which can be at the basis of new anti-tumour drugs. More detailed information on the pharmacological activity of lignans and their derivatives can be found in a number of reviews[14,56, 63-64].

Insecticidal and other activities

Deoxypodophyllotoxin is the main responsible compound for lethal activity on a number of different insect larvae, e.g. *Culex pipiens*, *Epilachna spara* and adult insects such as *Blatella germanica*[65]. A further study showed that deoxypodophyllotoxin has an effect on larvae of the Silkworm, *Bombyx mori* [66].

During a bioguided isolation based on the insecticidal activity against *Drosophila melanogaster*, it was shown that podophyllotoxin was the main constituent of *Podophyllum hexandrum* that was responsible for its insecticidal activity[67].

Further, *in-vitro* and *in-vivo* studies showed that podophyllotoxin and related lignans are active against viruses[67] and different kinds of tumour cells[64]. For other lignans germination inhibitory, antimicrobial and antifungal activity has been reported, but there is no evidence for podophyllotoxin or related lignans concerning these activities[55]. From the results reported so far, it is not possible to define the exact ecological role of these compounds[30].

Other uses

Podophyllum peltatum rhizomes have a long medicinal history among native North American tribes who used a rhizome powder as a laxative or an agent that expels worms (anthelmintic). A poultice of the powder was also used to treat warts and tumorous growths on the skin. Fruits must only be eaten when it is fully ripe[69-72]. Fruits are juicy but insipid[73]. The leaves are edible according to one report but this must be treated with some caution, see notes on toxicity above[74].

Conservation and trade aspects

Podophyllum prefers a moist peaty soil and filtered light or shade[75-76] and grows well in moist open woodland[77]. Due to continuous exploitation and habitat destruction, certain species are becoming rare. About 113 taxa, identified as threatened in Indian Himalaya, only a few species, for example, *Podophyllum hexandrum* have been studied for population dynamics in Western Himalaya[78]. *Podophyllum hexandrum* declared as endangered species under new IUCN criteria[79].

Podophyllum is a hardy plant which thrive upto about -20°C[76], it takes some years to become established but is very long lived in a suitable habitat[73]. Young leaves may be damaged by late frosts but otherwise the plants are quite hardy[73]. Young plants produce only one leaf each year; older plants have 2 or 3 leaves each year[80]. According to some reports about 37.3 tonnes of rhizomes of *P. hexandrum* were uprooted during 1995-2000 in HP.

Under schedule 2-appendix 2 of Export and Import Policy 1997-2002 the export of *Podophyllum* parts and its derivatives and extracts as such obtained from the wild except the formulations made there from, is prohibited.

Convention on International Trade in Endangered Species of wild flora and fauna (CITES)

Exploitation of *Podophyllum* from the wild is prohibited for export from India under CITES. Only cultivated/ artificially propagated plant species is allowed for export under cover of CITES export permit and Legal Procurement Certificate (LPC) or certificate of cultivation from the designated authorities.

Export of endangered and vulnerable plant species requires 'certificate of cultivation' or Legal Procurement Certificate' from the designated authorities of the Forest Department as per MOEF circular dt. 4.10.2000. *Podophyllum* can be cultivated through seeds while sown as soon as it is ripe in a cold frame. Sow stored seed in a cold frame in early spring. The seed germinates in 1-4 months at 15°C. Seedlings need to be transferred to shady part of the greenhouse for at least 2 growing seasons and then plant at permanent positions in the winter when the plants are dormant.

Considering the locality and variability of *P. hexandrum* certain morphological and few genetical differences were reported from Himalayan regions of India, however, still there is a possibility to study the morphological, molecular and biochemical variability among different populations for better and correct identification. Being as an endangered species *P. hexandrum* needs study of its variability and population under different locations with scientific basis and its *ex-situ* and *in-situ* conservation. National Medicinal Plant Board, India has initiated efforts towards conservation of high value rare, endangered and threatened medicinal plants throught the country after its formation in the recent past[81-82].

P. hexandrum receiving considerable attention of pharmaceutical sector for its trade in domestic and international market but due to unorganized medicinal plant sector in India it is difficult to get a regular update of statistics *vis-a-vis* demand and supply, collection and economics. The market demand of *Podophyllum* is usually deviate between rupees 60-100 in India and more than Rupees 500 in outside India. Since, the supply is made from inaccessible areas of Jammu and Kashmir, Himachal Pradesh, Uttarakhand and some parts of North-east hilly states, the cost of collection is very high and it is difficult to compete with American *Podophyllum* (*P. peltatum*) which is offered at lower rates. The annual supply during 1970 was estimated around 50-80 tonnes against over 100 tonnes of demand[3,44-45]. The existing rate per hectare return is estimated Rs. 1, 41, 120.00 at the rate of Rs. 60 per kg[83]. At present, the supply is still very less against heavy demand in Indian and international market. Adultertion to *Podophyllum* is not reported so far because of the only species grown thorought the Himalayan belt[83].

Conclusion

Popophyllum is an endangered but high value medicinal plant from temperate and cold climatic zones of the globe has wide scope of organized scientific study on its different aspects. Though the cultivation practices were standardized at several locations but still quality and quantity of its bioactive compounds under different zones from wild and cultivativable land is not systematically reported. Standardisation of quality and quantity podophyllotoxin contents from cultured plantlets is another area of organized study due to its endangered status. Similarly sustainable harvesting methods are urgently requied. Of course, there is no established variety either developed or under development so that this is another area which need vital attention too. Screening of its chemotypes, diversity for morphological, biochemical and genetic levels will enable the researchers to reliaze the existing population of *Podophyllum hexandrum* and hence useful in its conservation and sustainable utilization.

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