Review Article

Podophyllum hexandrum: An endangered medicinal plant from Pakistan

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ABSTRACT

Podophyllum hexandrum Royle syn *P. emodi* Wall. ex Hook. F & Thoms is an important medicinal plant. It is categorized as globally rare plant in IUCN red list. In Pakistan it is found at higher elevations of Himalaya, Gilgit, Chitral, Azad Kashmir. Plant body contains about 8% of podophylloresin from which several lignans have been isolated. Among them the most important is podophyllotoxin which has cytotoxic and anticancerous properties. Total synthesis of podophyllotoxin is an expensive process and availability of the compound from natural resources is an important task for pharmaceutical companies that manufacture these drugs. In Pakistan, *Podophyllum hexandrum* is on the verge of being endangered due to overexploitation for pharmaceutical purposes. This article briefly reviews botanical, medicinal, phytochemical, pharmacological and molecular attributes of this plant species along with its conservation strategies.

Geographical Distribution

The name *Podophyllum* is taken from “podos” a foot and “phyllon” a leaf, and refers to the resemblance of the leaves to a duck's foot. *Podophyllum hexandrum* Royle belongs to family *Podophyllaceae* and its vernacular name is Bankakri, commonly known as Indian May Apple [1]. It is believed to be originated from the Himalayan region. *Podophyllum hexandrum* is distributed from Indian Himalayas to Bhutan, Pakistan, Afganistan, Nepal, Taiwan and China [2]. In Pakistan, it is found in Himalayan zone, Gilgit, Chitral and Azad Kashmir regions of Pakistan at 2000 to 4000m altitudes. It is categorized as globally rare plant in IUCN red list [3, 4]

Taxonomic and Phenological Description

Podophyllum hexandrum is a Perennial herb which is about 15-40 cm tall. The plant has pretty leaves that are divided into 3 lobes. They completely unfurl after the plant has bloomed and are dark green spotted with brown. In the spring, white or pale pink, 6-petaled flowers are borne at the ends of stout stems; these are followed by fleshy, oval, red berries. They flower from May-August. The flowers have six petals and six stamens, which inspired its species name *hexandrum*, meaning six stamens. Leaves are rounded in outline, 10-25 cm long, deeply cut into 3 ovate, toothed lobes, sometimes further lobed. Fruit is a large scarlet or reddish berry, 2.5-5 cm, with many seeds embedded in pulp. [5]

Bioactivities

Podophyllum hexandrum has been used as a biological source of several metabolites with various bioactivities. Ethanolic rhizome extracts of *Podophyllum hexandrum* have shown significant anti-oxidant activity in wistar rats [6], anti-inflammatory activities of aqueous extract of *Podophyllum hexandrum* have also been seen [7]. The aqueous-ethanolic extract (AEE) of high altitude *Podophyllum hexandrum* has earlier been reported to render a radio protective effect against lethal gamma radiation in *in-vitro* model. AEE has also been reported to possess metal chelating and DNA protecting properties. It has also been seen that *Podophyllum hexandrum* offers radioprotection by modulating free radical flux [8].

Active Constituents

Extensive chemical investigation of *Podophyllum* species revealed presence of a number of compounds like podophyllin, podophyllotoxin, quercetin, 4-demethylpodophyllotoxin, podophyllotoxin glucoside, 4-dimethyl podophyllotoxin glucoside, kaempferol, icropodophyllotoxin, deoxypodophyllotoxin, picropodophyllotoxin, isopicropodophyllone, 4-Methyl deoxypodophyllotoxin, peltatin and S-peltatin [9].

The roots and rhizomes of *P. hexandrum* are known to synthesize a plethora of secondary metabolites besides podophyllotoxin and multifaceted pharmacological applications.

Figure 1: a: Plant body b: Flower c: Fruits

Figure 2: Structure of three health related lignans in May apple

Etoposide and teniposide, the two semi synthetic glycoside derivatives form an integral part of the therapeutic regimen used for chemotherapy and have triggered further studies in the design and the synthesis of other potent anticancer compounds [10, 11, 12]. Of these lignans, podophyllotoxin is the most important for its use in the semi synthesis of anticancer drugs, etoposide and teniposide [11]. Podophyllotoxin acts as an inhibitor of microtubule assembly. These drugs are widely used in treatment of lung cancer, testicular cancer, neuroblastoma, hepatoma and other tumours. Podophyllotoxin also shows antiviral activity and it interferes with critical viral processes [13]. The major active constituent, podophyllotoxin, was first isolated in 1880, but its correct structure was only reported in the 1950s. Many closely related podophyllotoxin like lignans were also isolated, and several of them were introduced into clinical trials, only to be dropped due to lack of efficacy and unacceptable toxicity. Podophyllotoxin content of Himalayan May apple is quite high (4.3%) compared to that of *P. peltatum* (0.25%), the most common species in the America [14]. Extensive research led to the development of etoposide and teniposide as clinically effective agents which are used in the treatment of lymphomas and bronchial and testicular cancer. 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 [15].

Podophyllotoxin is anticancerous in action. It 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 microtubule growth is however, different as they stop degradation and not assembly [16,17,18].

Medicinal Attributes

Podophyllum hexandrum has been described as divine drug in the Indian traditional system of medicine, the Ayurveda and has also been used in traditional Chinese system of medicine [19] for the treatment of number of ailments. In the modern allopathic system of medicine, the plant has been successfully used for treatment of various disorders, monocytoid leukemia, hodgkins lymphoma, bacterial and viral infections [20,21], venereal warts [22], rheumatoid arthralgia associated with numbness of the limbs and pyogenic infection of skin tissues, AIDS associated Kaposi sarcoma and different cancers of brain, lung and bladder [23]. Traditional utility of *P. hexandrum* has also been reported against constipation, cold, biliary fever, septic wound, burning sensation, erysipelas insect bite, mental disorders, rheumatism, plague [24] and also to provide symptomatic relief in some of the allergic and inflammatory conditions of skin [25].

Ethnobotanical Attributes

The ripen fruits of *Podophyllum* species are said to be edible and also used against fever in traditional system of medicine in India. Rhizome is used against various forms of gynaecological disorders in Tibetan system of medicine [26].

Podophyllum 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. Fruits are juicy but insipid. The leaves are edible according to one report but this must be treated with some caution, see notes on toxicity above. High altitude *Podophyllum hexandrum* (HAPH) provides a radio protective 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. These are used against various forms of gynaecological disorders [27, 28, 29].

The crushed leaves and roots are applied on skin diseases. Fruit is eaten in gastric problems [30]. *Podophyllum hexandrum* is also used to cure small tumors and its powder is an antiseptic for the healing wounds [31].

Molecular and Genetic Attributes

There is a need to conserve genetic diversity of this prized medicinal plant *Podophyllum hexandrum*, which may become extinct if reckless exploitation continues. Estimation of the level and distribution of genetic variation in endangered species is a primary objective in implementation of conservation programs [32]. Therefore it is necessary to evaluate the genetic variation from different regions for identification of elite germplasm with high genetic variability that can be used in conservation strategies. In Western Himalaya, two population of *P. hexandrum* have been characterized for their population genetic variation using RAPD analysis [33]. Lata *et al.* [34] studied to find out the suitable genetic marker for the assessment of genetic diversity of *Podophyllum peltatum*. Genetic diversity among the 28 genotypes of *P. hexandrum* distributed in 11 geographical region from Himachal Pradesh, India was analyzed using RAPD markers [35].

Threats

This species has greatly declined in nature due to its high demand and unskilled overexploitation. Seed germination and seedling establishment is very poor under natural conditions. In any population, the plant shows a kind of clumped distribution pattern. Earlier, *P. hexandrum* was used in folk medicine by local healers in small quantities, but commercialization of the plant for its medicinal attributes in recent years has increased demand and consequent exploitation. The size of the wild populations has been declining owing to overexploitation, habitat fragmentation, long dormancy, and low rate of natural regeneration. Some populations in certain pockets have virtually disappeared owing to anthropogenic activities and overexploitation [36]. Furthermore, excessive harvest has resulted in a significant decline of wild populations. Such decline has been

reported in Pakistan with *P. emodi* now considered an endangered species [37].

Conservation Strategies

Podophyllum is becoming rare and is at the risk of danger for being extinction. This exerted huge pressure on the population may result in the extinction of species. So efforts should be made to conserve germ plasm of such valued species. For this certain protective measures should be taken:

Exploitation in wild should be prohibited at Government level in order to conserve this plant in its natural habitat. It could be cultivated commercially by sowing stored seeds in early spring. Seed will germinate in 1-4 months at 15 °C. Transfer it to green house/shady moist place for growth.

Propagation of *Podophyllum* plant is difficult because of low seed number, prolonged dormancy, and laborious harvesting procedure. In an effort to develop a sustainable source of podophyllotoxin, *P. hexandrum* plants can be propagated by vegetative root cuttings. Plant tissue culture technique is an alternative practice for micropropagation and metabolic engineering. In *P. hexandrum*, there are a few reports on in vitro cell suspension culture and plant regeneration via somatic embryogenesis [38, 39]. The biotechnological production of Podophyllotoxin using plant cell culture derived from *Podophyllum hexandrum* may be an attractive alternative. Selection of the best performing cell line, its maintenance and stabilization are necessary prerequisites for its production in bioreactors and subsequent scale-up of the cultivation process to the industrial level. *P. hexandrum* cells have successfully been cultivated in a 3l stirred-tank bioreactor under low shear conditions in batch and fed-batch modes of operation. By tissue culturing and biotechnological techniques *P. hexandrum* can be cultivated artificially on commercial basis. This will reduce the pressure on natural resources.

Conclusion

Podophyllum 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 cultivable land is not yet reported. Standardization 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 required. 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 realize the existing population of *Podophyllum hexandrum* and hence useful in its conservation and sustainable utilization.

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