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## Ethnopharmacology, phytochemistry and cytotoxicity of emerging biotechnological tool: Mayapple (*Podophyllum hexandrum* L.: Berberidaceae)

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### ABSTRACT

*Podophyllum hexandrum* is a perennial herb and a valuable medicinal plant. Its common name is mayapple and is native to the lower elevations in and surrounding the Himalayan area of Pakistan. It has been used through the ages and in modern time as an intestinal purgative, inhibitor of tumor growth and salve for necrotic and infected wounds. Many important secondary metabolites have been reported to be extracted from the plant among which the most important one is podophyllotoxin as it possesses antitumor property which is used for the treatment of testicular cancer as well as lung cancer. The plant is very badly overexploited which causes decline in the frequency of this species in the past few years. It needs immediate attention for conservation by improving propagation techniques. This article reviews briefly the medicinal, phytochemical, pharmacological and conservation-related aspects of the plant.

## 1. Introduction

### 1.1. Classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Ranunculales

Family: Berberidaceae

Genus: *Podophyllum*

Species: *Podophyllum hexandrum* (*P. hexandrum*)

### 1.2. General distribution

*P. hexandrum* ranges in height of 2000–4500 m and resides in Tibet, Afghanistan, Himalayan areas of Pakistan and India, Bhutan and China[1]. The plant is commercially available from the Central United States and from Virginia and North Carolina[2]. Indian podophyllum is the another name given to this plant and is seen growing on the lower slopes of the Himalayas and forest from Afghanistan eastwards to Central China[3]. In India *P. hexandrum* is mostly found in Alpine Himalayas (3000–4000 mean sea level) of Jammu and Kashmir, Himachal Pradesh, Sikkim, Uttaranchal and Arunachal Pradesh[4]. In Pakistan, it is also seen growing in valleys of Astor, Chitral, Hazara, Dir, Murree Hills, Swat and

in Azad Kashmir[1,5]. Mayapple was introduced to be a part of modern medicine in 1787 and was used as a medicinal source in Pharmacological Department of USA from 1820 to 1910. *Podophyllum* was first used in Origin and History of All the Pharmacopeial Vegetable Drugs as a trade name[6]. It has been described as cathartic, emetic and alterative in first edition of American Dispensatory (1852)[6].

### 1.3. Morphology

Morphologically *P. hexandrum* is an erect perennial herb and it can grow up to 15–40 cm in height. Its rhizome is low to ground bearing many adventitious roots (almost 50 cm long) (Figure 1). The stem length is 30–90 cm. Umbrella-like, lobed leaves are borne on its branches which are mostly 2 to 3 in number; they completely open up after the plant has blossomed and their colour is dark green splotched with brown. The name *Podophyllum* comes from the Greek word “podos” meaning a foot, and “phyllon” meaning leaf. This name is selected for this plant because its leaves look like duck’s foot. White or pale pink, 6-petaled flowers come out on the top of its stem during the beautiful weather of spring (Figure 2), and after some time they are beautifully turned into red berries which are oval in shape and somewhat fleshy (Figure 3). The plant is said to give beautiful flowers during little period from May to August and the flower is a hexa structure consisting of six petals and six stamens as well, and that is the reason to name this plant hexandrum meaning six stamens. The leaves are round in outline, 10–25 cm long, which is cut into 3 deep oval shaped lobes looking like tooth; the number of lobes may be more than 3. It has a pulpy

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fruit with embedded seeds and colour is somewhat reddish to scarlet. The weight of its seed is approximately 20 g. The seeds as well as rhizome are used for the propagation of this plant[7,8].



Figure 1. *P. hexandrum* roots.



Figure 2. *P. hexandrum* flowers.



Figure 3. *P. hexandrum*: fruiting stage.

#### 1.4. Common names

*P. hexandrum* got numerous names in different languages. The most commonly use name is Indian podophyllum. “Kakhri” is name given to this plant in Muzaffarabad, Azad Kashmir Pakistan. “Bankakri” is another name of this plant given by natives of Niti Valley in Central Himalaya. In Nepal, it is known as “Lagu Petra”. “Bantrapushi” or “Giriparpat” is the name given to this plant by Ayurvedal[9-11].

## 2. Chemical constituents

American *Podophyllum* contains 4%–5% podophyllum resin, whereas Indian species contains 7%–16%. The variation in percentage of resin is attributed to seasonal differences, different

sites of growth and age of the plant. The pharmacological activities seen by Indian podophyllum is two times as seen by American drug. Indian podophyllum resin gives a yield of 42%–46% of crystalline podophyllotoxin[12]. Tannin was noticed in the root of this plant by Wallis[13]. The percentage of rough fiber is 10.5%, podophyllin is 11.5% to 15.56%, podophyllotoxin is 3.19% to 4.10%. In addition to these compounds, podophyllolic acid, podophylloquercin, starch, tannin, peltatin, peltatin and picropodophyllin are also present in small amount[2,14,15]. The roots of this species are well known in phamacology as a source of podophyllin resin. The resin yields podophyllotoxin as the major lignan and is produced by dimerisation of phenylpropanoid intermediates[16-18]. Podophyllotoxin content is greater (> 5%) in *P. hexandrum* more than that in *Podophyllum peltatum* (*P. peltatum*) and hence it gains more importance. The roots of *P. peltatum* have only 0.25% podophyllotoxin content[19,20]. The lignans have carbon to carbon structure as they are linked by central carbons of their side chain and they are produced by dimerisation as mentioned above[18,21]. The scientist discovered astragalinal (kaempferol-3-glucoside) (Figure 4) out of this plant while performing their feeding experiments, and noticed that *P. hexandrum* aryltetralin lignans have made huge biosynthetic relationship which was existing amongst *Podophyllum* lignans[18]. So, desoxypodophyllotoxin is changed into podophyllotoxin (Figure 4), and podophyllotoxone is oxidized product of podophyllotoxin; 4'-demethyl derivatives have a same sequence as mentioned above. Although 4'-demethyldeoxy-podophyllotoxin (Figure 4) can be easily changed into 4'-demethylpodophyllotoxin, this compound is never changed into lignans of the 4'-methyl series, for example, podophyllotoxin. The *Podophyllum* lignans biogenetically are grouped into 3,4,5-trimethoxy substituted in the pendent aryl ring, and those with 4-hydroxy-3,5-dimethoxy substitution in the pendent ring. They may arise from same source[22]. Indian mayapple is endangered[23,24]. The different populations of *Podophyllum* contain different amounts of podophyllotoxin and American mayapple (*P. peltatum*) is collected from various locations[25,26]. Wani *et al.* noticed that they also contain glycosides, flavonoids, saponins and terpenes and proteins[27]. Flowering period is from May to August[28].

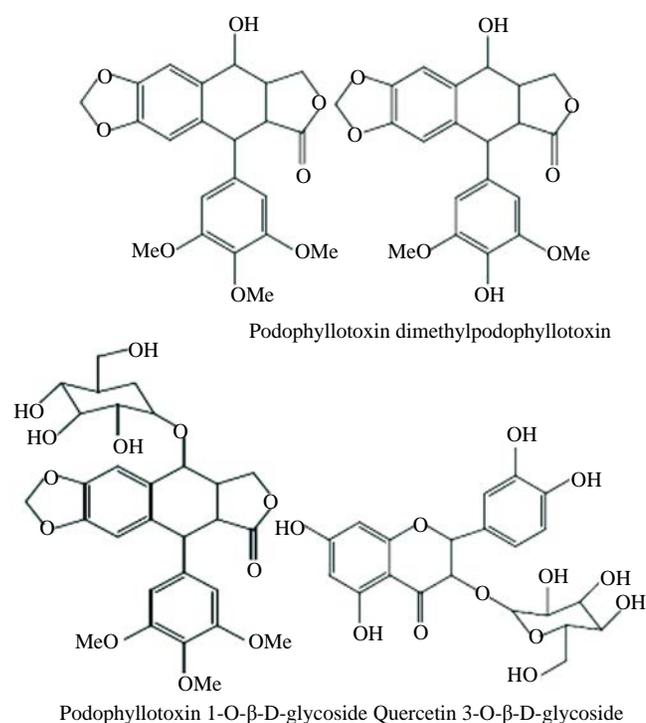


Figure 4. Compounds isolated from *P. hexandrum*[7].

### 3. Pharmacological properties

In old days *P. hexandrum* was famous as Aindri (a divine drug). Podophyllotoxin is a natural plant secondary metabolite which mainly exists in the root of *P. hexandrum* as well as its congeners and it is known to have importance in pharmacology and therapeutics as it contains anticancer, antineoplastic as well as anti HIV activities[3,29,30]. *P. hexandrum* obtained from India is better than its American counterpart, *P. peltatum*. The is because Indian plant has more amount of *P. hexandrum* (4% in the dried roots in comparison to only 0.25% in *P. peltatum*). Podophyllotoxin is present in this plant as mentioned above, and it is also known as a natural lignin and most importantly this lignin contains cytotoxic activity. The cytotoxic activity is said to act in metaphase of mitosis by attaching the microtubules and hence block their proliferation[31]. Podophyllotoxin is included in many pharmacopoeias and used as an antiviral agent due to its antiviral activity. It is used for treating a viral disease called condyloma acuminatum which is caused by human papilloma virus or some other viruses. The podophyllotoxin can be used to kill almost all types of warts and the most beneficial thing is that it has very little side effects and takes little time. Some of the famous categories of viruses like cytomegalovirus and Sindbis virus are also victims of podophyllotoxin and its related compounds. It can also be used for fighting against viruses affecting the anogenital track which attack mostly children. There is self limiting benign disease known as molluscum contagiosum that affects mostly children, young adults, and HIV patients. Podophyllotoxin can also be used for the treatment of this disease. In the field of dermatology, podophyllotoxin is used to cure psoriasis vulgaris. Podophyllotoxin is a superb pharmacological tool because of its antitumor activity as it blocks proliferation in the metaphase of mitosis. The forms of tumors which can be treated by podophyllotoxin are Wilms' tumors and various types of genital tumors (e.g. carcinoma verrucous). It is also used for the treatment of non-Hodgkin lymphoma and other lymphomas. Podophyllotoxin can also penetrate into human skin and lead to acantholysis and cytolysis which was studied by using human bioengineered skin. Podophyllotoxin may be used in cosmetology as its intrinsic activity shows many features related which can be proved beneficial in this case[32].

Etoposide, teniposide and etopophos are the semisynthetic products of podophyllotoxin and they are commonly used in the treatment of cancer and also known to be effective in the treatment of many types of neoplasms, including small cell lung cancers, lymphoma, leukemia, Kaposi's sarcoma, etc. Refractory testicular lymph and myeloid leukemia and leukemia in stomach, ovarian, brain, breast, pancreatic, and large-cell lung cancers can all be cured by using etoposide, combined with other drugs. Teniposide is less beneficial than etoposide but still used for the treatment of lymphoblasts[7,33]. The other uses of this plant in pharmacology and therapeutics are mentioned below and they are based on constituents different from those mentioned above.

#### 3.1. Radioprotective efficacy

*P. hexandrum* also has a radioprotective potential exerted by its rhizome. The radioprotective property of this plant at molecular level along with expression of the patterns of the various proteins associated with apoptosis in the spleen of male Swiss albino mice by immunoblotting were reported by some researchers[34-41]. The radioprotective activities of this plant are used widely. They include free radical scavenging, apoptosis and cell cycle related activities in *in vitro* (Hep G2 cells) as well as *in vivo* models[42-44]. Chawla *et al.* worked on radioprotective properties of this plant and told the effect of variation in aryltetralin[45,46]. Some properties of this plant were also studied and compared by Arora *et al.* who worked on

antioxidant and radioprotective properties of *P. hexandrum* growing at high altitude as well as low altitudes and then compared both of them[41]. The *P. hexandrum* growing on low altitudes is easy to get and cultivated. Its radioprotective properties are multifold. It can also be used to get some other bioactive substances. It is also somewhat cytotoxic and hence it is valuable in case of use in clinical fields for human beings. Dutta *et al.* proposed that *P. hexandrum* is stable as well as able to bind serum proteins; its biodistribution is wide and it possesses antioxidant activity and tissue toxicity in case of radioprotection (G-002M)[47].

#### 3.2. Antifungal activity

*Aspergillus niger* and *Candida albicans* are various forms of fungi, and diseases caused by them can be cured by using this plant. The method used to test its antifungal activity is disc diffusion method. Different minimum inhibitory concentrations were found out for *Candida albicans* (25 mg/mL) and *Aspergillus niger* it is (16.66 mg/mL).

#### 3.3. Insecticidal activity

*Drosophila melanogaster* is an insect which can be killed by using the insect killing power of this plant[48]. There is a compound called podophyllotoxin which was also extracted by the same scientist. The LC<sub>50</sub> value of podophyllotoxin is 0.24 µmol/mL which can be used to kill larvae of *Drosophila melanogaster* and a LD<sub>50</sub> value of 22 µmol/mL against adults. Acetylpodophyllotoxin is another extracted compound from this plant, and it has showed little insect killing activity in both assays, indicating that the 4-hydroxyl group is the reason of the compound to be more potent[48].

#### 3.4. Anticancer activity

The anticancer activity of this plant is associated with its extract podophyllotoxin which is taken from its root. It is very rare to prepare podophyllotoxin biosynthetically, so its importance is beyond doubts. Biotechnological production of the extract is also available[49]. The plant also has antimitotic activity which is showed by its extract called podophyllin. It is used in the treatment of cancer, and mostly used for treating cancers in female called cancer of ovary[50-52]. Podophyllotoxin, podophyllin and berberine are various forms of lignins which are extracted from the rhizome of *P. hexandrum* and all of them have antitumor activities because they can inhibit mitosis at the level of metaphase and block the proliferation of microtubules properly. The different types of cancers which can be properly cured by this plant include lung cancer, testicular cancer, neuroblastoma, hepatoma and other tumors[29,53].

#### 3.5. Cytotoxic activity

Podophyllotoxin and D-glycoside are lignins which are extracted from the rhizome of this plant and they have antitumor activity[54]. Podophyllotoxin is declared to be a pharmacologically active compound and is very important lignin. It exhibited wide range of cytotoxic activities. Some scientists prepared the derivatives of podophyllotoxin and then noticed that these substances were pharmacologically active in case of cytotoxic activity at micromolecular level[55-57]. Uden *et al.* studied the phenylpropanoid and said that it is a derived lignan of podophyllotoxin[33]. Then it is used as first line raw compound for the chemical synthesis of the antitumor agents like etoposide and teniposide. Qi *et al.* reported *in vitro* cytotoxic activities of 4-demethyl-picropodophyllotoxin 7'-O-D-glucopyranoside (4DPG)[58]. 4DPG are very good for the inhibition of cancer cell division and stop the cell cycle in the

mitotic phase. The cytotoxicity of 4DPG is attributed to its ability to inhibit microtubule assembly of cancer cells at a low concentration, thus giving favourable circumstance for apoptosis. These properties made 4DPG to be an effective antitumor drug.

### 3.6. Anti-inflammatory activity

Prakash *et al.* has told about the anti-inflammatory power of extract of *P. hexandrum* in aqueous form[59].

## 4. Ethnomedicinal uses

*P. hexandrum* rhizomes have been used since a long time ago and its historical importance is beyond any doubt while studying a long medicinal history of this plant. The native North American tribes used rhizome powder as a purgative and laxative or to expel worms (anthelmintic). Some forms of the tumours of skin can be cured by this plant as this plant can be used to treat various forms of warts which are responsible for causing some types of skin tumours. This plant is also used by Kashmiri people in traditional system of medicine from time immemorial. In Kashmir it is locally known as "Banwangan" because of its red coloured fruit (berry) which has the size of a small brinjal. Indian podophyllum is also a very fertile plant in historical views because of long history of usage by natives of the Himalayas. The aqueous extract of its roots was used for the treatment of catharsis. It has also been used therapeutically for the treatment of ophthalmia[7].

The plant is used traditionally in treating colds, constipation, septic wounds, burning sensation, erysipelas, mental disorders, plague, allergic and inflammatory conditions of the skin, cancer of brain, bladder and lung, venereal warts, monocytoid leukemia, Hodgkin's disease and non-Hodgkin's lymphoma[60]. While in Muzaffarabad, Azad Kashmir, the root paste of this plant is used for topical application on ulcers, cuts wound and also used to treat vaginal warts[10]. Rhizome is said to have a hepatic stimulating effect that used as a purgative, emetic, and used to cure fever and body pain in district of Battagram, Pakistan[61].

## 5. Cell culture

This plant is cultured under *in vitro* environment and the cell culture of this plant was produced by Chattopadhyay *et al.*[62]. The maximum production of this plant was achieved when its culture was produced in 3 L stirred tank bioreactor and the amount produced was 4.26 mg/L. The substance produced from the cell culture was used to kill the human breast cancer cell line at 1 nmol/L. About 50% cure rate was achieved by using podophyllotoxin for the treatment of breast cancer. The plant is said to have inhibitory effect on the growth of cancer cells in beginning of cell cycle. Woerdenbag *et al.* noticed that the cell suspension cultures obtained from rhizome of *P. hexandrum* (Berberidaceae) can lead to the accumulation of podophyllotoxin in them[63]. The scientists tried to produce podophyllotoxin by plant cell cultures using bioreactors, but the amount of that product is not enough to cope with the demand[53].

## 6. Molecular status

Sultan *et al.* said that for useful conservation and home cultivation of a species, consideration of its genetic diversity is important and one should make the use of different types of markers under consideration[64]. So for the purpose of studying the genetic diversity of this plant a proper plan was made and a well arranged report was presented including morphological characteristics, phytochemical variation and random amplified polymorphic DNA profiles of this plant. Random amplified polymorphic DNA analysis was also

conducted and shown wide degree of genetic diversity and it was used in the study. There was also considerable multiplicity in the amount of marker compounds in the respected samples as noticed by high performance liquid chromatography analysis.

## 7. Side effects

This plant is very famous for its various beneficial use, but there are also some side effects associated to the use of this plant.

The side effects include bloody diarrhea, severe stomach pain, hallucinations, muscle paralysis, kidney failure, breathing failure, neuropathy and encephalopathy. Some weak side effects are also found including confusion, headache, local irritation, low blood pressure, nausea and vomiting[65,66]. This plant can some times be proved to be very harmful and therefore it should be used by considering all aspects under the guidance of a trained practitioner poisonous. The use of this plant is contraindicated in pregnancy[67].

## 8. Future prospects

Podophyllotoxin is a very useful extract from the roots of this plant and gaining fame day by day. Podophyllotoxin is also collected from other sources, but *P. hexandrum* is the main source of podophyllotoxin. There are also some semisynthetic compounds of this podophyllotoxin (the etoposide, teniposide, and etoposide phosphate), and they are very useful in treatment of specific types of cancers. In America Bristol Co. and in Switzerland Sandoz manufactured lots of semisynthetic compounds. Three of these semisynthetic compounds are more commonly used for the treatment of tumors because of their fewer side effects. The isolation of compounds for production of pharmaceuticals from biomass is always very problematic and difficult because there is wide population of species from where the required specimens are to be collected. The supply of this plant is effected and widely destroyed by natural disasters and sometimes exploited by other means also. Therefore the content of bioactive secondary metabolite in plant is also in short supply. Therefore, immediate efforts should be given for generating the trustworthy traditional methods of mass cultivation of *P. hexandrum*. The wild populations of this plant are also presented in many genetically different types and they can grow under different environmental conditions. This growth problem may affect drug profile, and therefore leading to the production of a product which may be less pure and fine. So suitable groups must be selected for continuous supply of reliable form of this plant and associated substances.

The two main species responsible for supplying podophyllotoxin are Himalayan and Indian *P. hexandrum*. Podophyllin is a mixture of *Podophyllum* resin and podophyllotoxin. In 1942 in USA it was said that it can be used for cancer growth inhibition when applied topically, and after some time podophyllotoxin came into knowledge. It was obtained from the rhizomes of this plant and than used against cancerous growth. At that time, M/S Sandoz introduced the product into the market, and that product did a good business. It was already well known that *P. hexandrum* or American *Podophyllum* are available in the Himalayas in large amount. *P. hexandrum* was widely used by the medical doctors who belonged to Britain, and they used this plant in Indian subcontinent for medical purposes. The plant was obtained from the Himalayan region and also exported to England for use in field of medicine. The wide use of this plant in other words led to over-exploitation soon and after almost 50 years the plant was depleted. This plant is included among the endangered species in India. The *P. hexandrum* of Western Himalaya is lessening in amount day by day, and in some places the plant is almost extinct because of anthropogenic activities and overexploitation.

The plant has a very long juvenile phase and hence the limited availability. The other reason for poor availability is that the fruit-setting ability is also not so much good, and it has a very bad effect on the active cultivation of *P. hexandrum*. These all result in low availability of *Podophyllum* resin. There is also another problem *i.e.*, the non-optimal yield after extraction. Podophyllotoxin is not economical if it is used as a chemical starter of other products.

It is generally considered as a special plant because it is rare, and it is also included among the endangered species. The scientists are trying to grow this plant *in vitro* and make use of the technique of breaking the dormancy of seeds by artificial means.

The Plant cells often start spontaneous genetic mutations in case of secondary metabolite accumulation in suspension culture and produce a heterogeneous population of cells. The reason behind the genetic basis of somaclonal variation is unknown because it is not studied in detail. For the wide range production of podophyllotoxin we can make use of genetically stable lines which are themselves high yield producing. Secondary metabolite production can be increased by a well planned method of plant cell differentiation, intracellular organization, cell physiological characteristics and regulatory mechanisms. We must be aware of all these in regards to metabolic routes so that lead to an improvement in product accumulation during cell culture.

The subject of plant biotechnology gives reader a platform to study plant cell cultures, and use them practically and the techniques in a proper way to produce secondary metabolites; it may be considered as a new method, as compared with other more traditional approaches. However, the truth remains that, currently, success in production on a commercial scale has been achieved for only a little substances. It is very unfortunate that the production of podophyllotoxin has not yet declared to be a commercially better way when compared to the plant cell culture approach. Development in technology is needed so that the methods can become commercially acceptable. The advancement of knowledge in phytochemistry in very near future, the regulation of secondary pathways and an ability to express desired traits by transgenics are giving a hope that it will help in economic production of this plant and its medically important derivatives.

After investigating this plant we came to know that plant can be a very useful biotechnological studying tool. Genes that are purposed for plant enzymes can be expressed in fast growing microorganisms. The non-plant genes and enzymes can be used to develop a successful production system, possibly in near future. Expressed sequence tag based investigations are also done by scientists in present time to know the genes involved in this pathway, with a desire to construct a better supply of podophyllotoxin and other anticancer drugs. In this way, metabolic engineering may be used as an important mean for making betterment in the complex regulatory mechanism in the lignin biosynthetic pathway for the production of podophyllotoxin.

### Conflict of interest statement

We declare that we have no conflict of interest.

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