Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Suppl 5, 2011

Review Article

"PODOPHYLLUM HEXANDRUM" – A VERSATILE MEDICINAL PLANT

PARVAIZ H QAZI*, ABILA RASHID, SAMI A SHAWL

Indian Institute of Integrative Medicine (CSIR), (Br) Sanatnagar Srinagar, Kashmir (India). Email: pervaizqazi@yahoo.com

Received: 29 Dec 2010, Revised and Accepted: 11 Sep 2011

ABSTRACT

Podophyllum hexandrum a perennial herb, bearing the common name Himalayan Mayapple known as *Aindri* (a divine drug) in ancient times is native to the lower elevations in and surrounding the Himalaya. It has been reported to be used through the ages and in modern times as an intestinal purgative and emetic, salve for infected and necrotic wounds, and inhibitor of tumor growth. The rhizome of the plant contains a resin, known generally and commercially as Indian Podophyllum Resin, which can be processed to extract podophyllotoxin or podophyllin, a neurotoxin. Podophyllotoxin is the major lignan present in the resin and is a dimerized product of the intermediates of the phenylpropanoid pathway. The starting material of etoposide (Vepeside), an FDA approved anticancer drug is podophyllotoxin and has been used to treat testicular cancer as well as lung cancer by inhibiting replication of cancer cells. Podophyllotoxin finds use as a precursor for the semi-synthetic topoisomerase inhibitors in the treatment of leukemias, lung and testicular cancers, dermatological disorders like warts, rheumatoid arthritis and psoriasis. It also has numerous applications in modern medicine by virtue of its free radical scavenging capacity. An extract of *P. hexandrum* has been shown to provide approximately 80% whole-body radioprotection in mice. Total synthesis of podophyllotoxin is an expensive process and availability of the compound from natural resources is an important issue for pharmaceutical companies that manufacture these drugs. The Indian *P. hexandrum* is superior to its American counterpart, *P. peltatum* in terms of its higher podophyllotoxin content (>5%) in dried roots in comparison to only 0.25% of *P. peltatum*. Thus, taking into consideration present status of *P. hexandrum* in general, it needs immediate attention for conservation, in depth studies for improving propagation techniques and podophyllotoxin production, encouraging its cultivation and a detailed study of its phytochemical diversity, particularly of marker compounds like podophyllotoxin and its glycosides.

Keywords: *Podophyllum hexandrum,* Podophyllotoxin, Etoposide, Testicular cancer

INTRODUCTION

Classification

Kingdom	Plantae
Division	Mangnoliophyta
Class	Magnoliopsida
Order	Ranunculales
Family	Berberidaceae
Genus	*Podophyllum*
Species	*Podophyllum hexandrum*

Fig. 1: *P. hexundrum*

Distribution, phenology and uses of *Podophyllum hexundrum*

Podophyllum hexandrum Royle (Himalayan Mayapple) was known as *Aindri* (a divine drug) in ancient times. Its name in Hindi and Ayurveda is *bantrapushi* or *Giriparpat*. The perennial herb *Podophyllum hexandrum* bearing the common names Himalayan May apple or Indian May apple, is native to the lower elevations of Himalayan countries like Afghanistan, Pakistan, India, Nepal, Bhutan, and in S. W. China [1,2 3,4] . In India *Podophyllum hexandrum* is mostly found in Alpine Himalayas (3000-4000 msl) of Jammu and Kashmir, Himachal Pradesh, Sikkim, Uttaranchal and Arunachal Pradesh [5, 6, 7]. In Kashmir it has been used in traditional system of medicine from time immemorial and is locally known as Banwangun, since its red colour fruit (berry) is of the size of a small brinjal. Indian *Podophyllum* has a long history of usage amongst natives of the Himalayas, an aqueous extract of the roots being a common cathartic. It has also been used as a remedy in ophthalmia. Resin from the Indian plant was analyzed by Thomson in 1890, who reported 56% podophyllotoxin content. Podophyllotoxin was first shown to be the active principle of podophyllin by Podwyssotzki and was obtained in a pure state in 1880. The rhizomes of *Podophyllum hexandrum* are known to contain several lignans which are dimerisation products of phenylpropanoid pathway intermediates linked by central carbons of their side chain [8,9,10]. It is low to the ground with glossy green, lobed leaves on its few stiff branches, and it bears a light pink flower and bright red-orange bulbous fruit. It can be propagated by seed or by dividing the rhizome. It is tolerant to cold temperatures, as would be expected of a Himalayan plant, but is not tolerant to dry conditions.

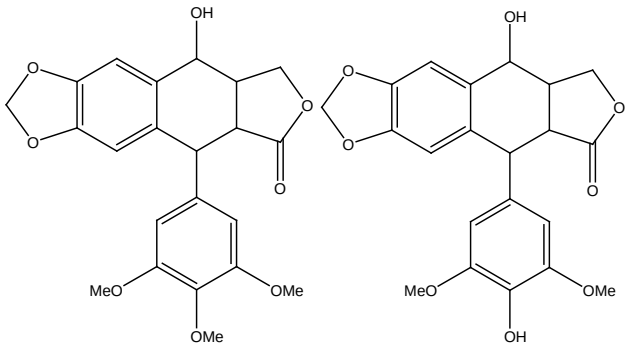
Fig. 2: Shows *Podophyllum hexandrum* growing wild in Kashmir – India

Podophyllum hexandrum grows from 12 to 18 inches high with deeply lobed leaves, fleshy stems, which rise straight up from the soil. 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. The plant has pretty leaves that are divided into 3 lobes. They completely unfurl after the plant has bloomed and are dark green splotched 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. The flower in May-August has 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. It can be propagated by seed or by dividing the rhizome. It is found found at a level of 2800 - 3000 m in the Indian Himalayas, in the wet alpine meadows, in humus rich and shaded localities or near stream banks as an under growth along with other herbs.

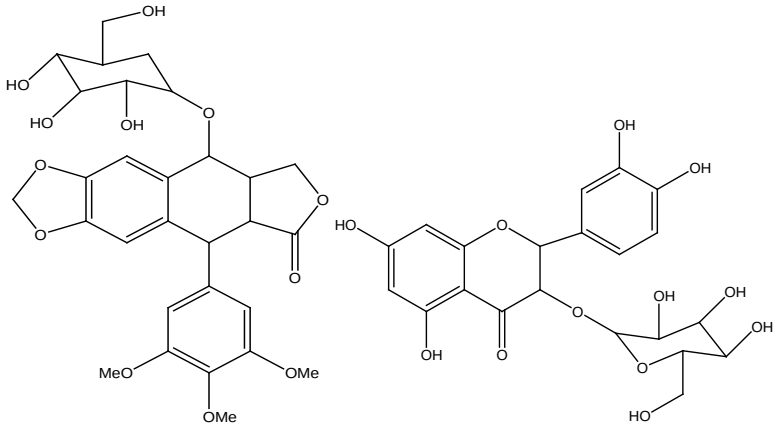
Plants containing lignans have been used since approximately 1000 years ago as folk remedies in traditional medicine of many diverse cultures. Plants with high lignin content have been commonly used in Chinese, Japanese, and the Eastern world folk medicine, for example, *Kadsura coccinea* (Schizandraceae), *Fraxinus sp.* and *Olea europaea* (Oleaceae). The very extensive use in traditional medicine makes the lignans an important family of starting products for the development of new therapeutic agents based on structural modifications of such compounds. *Podophyllum hexandrum,* also being a high lignin content plant has been reported to be used through the ages and in modern times. It has been extensively exploited in traditional Ayurvedic system of medicine for treatment of a number of ailments like Condyloma acuminata, Taenia capitis, monocytoid leukemia, Hodgkin's disease, non-Hodgkin's Lymphoma, cancer of brain, lung, bladder and venereal warts [11-13].

Podophyllum hexundrum is reported to contain a number of compounds with significant pharmacological properties, e.g. epipodophyllotoxin, podophyllotoxone, aryltetrahydronaphthalene lignans, flavonoids such as quercetin, quercetin-3-glycoside, podophyllotoxinglycoside, kaempferol and kaempferol-3-glucoside. The rhizomes and roots of the plant contain anti tumor lignans such as podophyllotoxin, 4'-demethyl podophyllotoxin and podophyllotoxin 4-O-glucoside [14-15]. Among these lignans, podophyllotoxin is most important for its use in the synthesis of anti-cancer drugs etoposide, teniposide and etophos [16]. These compounds have been used for the treatment of lung and testicular cancers as well as certain leukemias [17-18]. In addition, podophyllotoxin is also the precursor to a new derivative CPH 82 that has been tested for rheumatoid arthritis and other derivatives for the treatment of psoriasis and malaria [19-20]. American Podophyllum contains 4–5% podophyllum resin, whereas Indian sps. contains 7-16%. The variation in percentage of resin is attributed to seasonal differences, different sites of growth and age of the plant [21]. In certain areas as much as 20% resin has also been recorded. The highest percentage of resin is obtained in May-June during the flowering stage. Thus Indian podophyllum when collected at the proper season contains 2.5 times more resin compared to its American counterpart. Moreover, this resin has double the amount of podophyllotoxin [22-24]. Podophyllotoxin is commonly extracted from *P. hexandrum* that contains 6–12% resin of which the concentration of podophyllotoxin is around 40%.

Podophyllotoxin is a naturally occurring lignan which is endowed with potent cytotoxicity. It acts as a mitotic spindle poison, binding the microtubules and causing mitotic arrest in metaphase. Total synthesis of podophyllotoxin is an expensive process and availability of the compound from natural resources is an important issue for pharmaceutical companies that manufacture these drugs[25]. Commercially exploitable sources of podophyllotoxin are few and currently it is obtained for drug use from dried rhizome and roots of Podophyllum sps. like *Podophyllum hexandrum* (Indian Podophyllum) and *Podophyllum peltatum* (American Podophyllum). The resin of Podophyllum rhizome is the source of podophyllotoxin.



Podophyllotoxin Dimethylpodophyllotoxin



Podophyllotoxin 1-O-β-D-glycoside Quercetin 3-O-β-D-glycoside

Fig. 3: Shows compounds isolated from *Podophyllum hexandum*

Podophyllin was included in the U.S. Pharmacopoeia in 1820, and the use of this resin for the treatment of venereal warts was described, attributing this action to podophyllotoxin. The destructive effect of this resin on experimental cancer cells in animals was published too. Also, podophyllotoxin is included in many Pharmacopoeias and used as an antiviral agent in the treatment of *Condyloma acuminatum* caused by human papilloma virus - HPV and other venereal and perianal warts. The application of podophyllotoxin cured almost all the warts completely in less time than other strategies and with fewer side effects. Podophyllotoxin and analog compounds are also active against cytomegalovirus and Sindbis virus. Podophyllotoxin is also effective in the treatment of anogenital warts in children and against *Molluscum contagiosum*, which is generally a self-limiting benign skin disease that affects mostly children, young adults, and HIV patients.

Podophyllotoxin has other uses in dermatology: it is a useful agent in psoriasis vulgaris. Antitumor activity is another outstanding property of podophyllotoxin. It is effective in the treatment of Wilms tumors, different types of genital tumors (e.g., carcinoma verrucosus) and in non-Hodgkin's and other lymphomas. Combination therapies are currently being implemented with other chemotherapeutic agents or with other techniques useful in the fight against viral infections and cancer. In this sense, condyloma acuminata respond best to the cryotherapy-podophyllotoxin combination; multiple myeloma responds best to homeotherapy with podophyllotoxin and intermittent local administration of methotrexate and systemic polychemotherapy. In combination with interferon, podophyllotoxin is active in genital human infections caused by vulvar pruritic papillomatosis and together with *cis*-platin is effective in treating neuroblastomas.

Studies on penetration of podophyllotoxin into human bioengineered skin have demonstrated that the lignan induces acantholysis and cytolysis in the skin-equivalent model used for a wide variety of pharmacotoxicological trials. This might apply to claims of efficacy for cosmetic compounds. In combination with vinblastine, it was used as mitotic agent for preparing embryonic chromosomes for trials [26].

The mechanism of action of podophyllotoxin is based on inhibiting the polymerisation of tubulin and arresting of the cell cycle in the metaphase [27-28]. It has also been proposed that that cyclolignanolides of the podophyllotoxin group might work as alkylating agents through their C-9 methylene, rather than as acylating agents[29]. Several papers have been published related to the mechanism of action of this cyclolignan. Schonbrunn et al. 1999 got the crystallization of podophyllotoxin linked to a tubulin fragment. Effects of microtubule damaging agents like podophyllotoxin or colchicine on DNA and cell cycle have been described[31-32]. Chaudhuri et al. 2000 and Pal et al. 2001 have studied the interactions of B-ring of colchicine with α-tubulin and Lopez Perez et al. 2000, have discussed the role of dipole moment in the activity of cyclolignans.

Podophyllotoxin derivatives

Three semisynthetic derivatives of podophyllotoxin etoposide, teniposide and etopophos are widely as used anticancer drugs and show good clinical effects against several types of neoplasms, including testicular and small cell lung cancers, lymphoma, leukemia, Kaposi's sarcoma, etc. Podophyllotoxin shares the property with the anticancer drugs paclitaxel and camptothecin of being virtually insoluble in water[36]. Being more hydrophilic, the glucosides are less toxic than aglucons, but their cytostatic activity also reduces to the same degree. Therefore, research efforts were made to perform numerous modifications on the basic podophyllotoxin skeleton, in order to avoid several side-effects; and this resulted in the clinical introduction of etoposide and teniposide, which are cytostatic (antimitotic) glucosides. Both are 4-glucopyranosyl derivatives of epipodophyllo- toxin, a distereoisomer of podophyllotoxin[37]. These semi-synthetic derivatives of podophyllotoxin play an important role in the treatment of lung cancer, a variety of leukemia and other solid tumors[38-39].

However, they have several limitations, such as poor water solubility, metabolic inactivation and the development of drug resistance. Etoposide is used in combination therapy in refractory testicular lymphoid and myceloid leukemia and in stomach, ovarian, brain, breast, pancreatic, and both small- and large-cell lung cancers. Teniposide is used less often than etoposide and it is mainly used to treat lymphomas. Numerous derivatives varying the topside basic structure have been proposed, synthesized and clinically tested. The successful derivatization of podophyllotoxin into etoposide and teniposide has generated interest in structure optimization to produce new derivatives with a superior pharmacological profile and broader therapeutic uses[40].

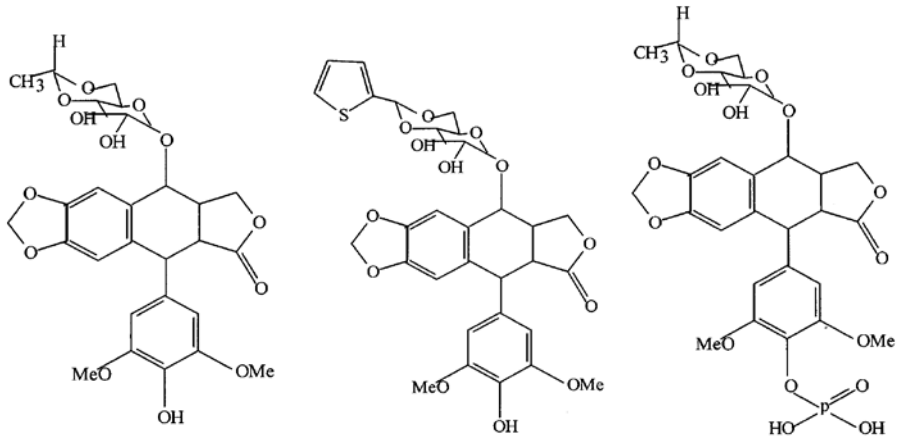
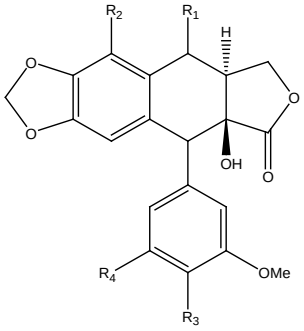


Fig. 5: Etoposide Teniposide Etoposide phosphate

Etopophose is a new etoposide phosphate designed to overcome the limitations associated with the poor solubility of etoposide. Etopophose can be administered intravenously at higher doses and is rapidly converted by phosphatase in the plasma to etoposide, thus constituting an improvement in the treatment[41]. Successful development of anticancer drug etoposide and teniposide from natural podophyllotoxin has focussed attention on Podophyllum as an economic source of lignans. Podophyllotoxin, etoposide and teniposide are mostly manufactured and produced in Switzerland, USA and Japan. However, the Chinese pharmaceutical companies are also main suppliers of these preparations in bulk quantitites. An Indian company CIPLA Ltd, Bombay also worked for the production of etoposide on a commercial scale, from the technology developed by the Indian Institute of Chemical Technology (IICT), Hyderabad,

for converting the semi-synthetic compound from podophyllotoxin.

However, currently the commercial source of podophyllotoxin are the rhizomes and roots of *P.hexandrum* Royle, an endangered species from the Himalayas. Availability of podophyllotoxin isolated from plant has its limitations, due to scarce occurrence of the plant because of intense collection from nature and lack of organized cultivation[42]. One of the major problems for the cultivation of this plant is its long juvenile phase and poor fruit setting ability.



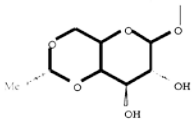
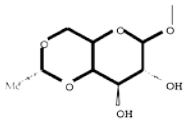
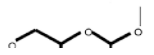
R_1	R_2	R_3	R_4	*Other molecule*	*Name*
- OH	H	- OCH_3	- OCH_3	--	Podophyllotoxin
- OH	- OCH_3	- OCH_3	- OCH_3	--	5-methoxypodophyllotoxin (5-MPT)
- OH	- OCH_3	- OCH_3	H	--	5'-demethoxy-5-MPT
	H	- OH	- OCH_3	-	Etoposide
	H	-$OPO(OH)_2$	-OCH_3	-	Etopophose
	H	- OH	- OCH_3	-	Teniposide

Fig. 6: Shows a list of derivatives from Podophyllotoxin

Moreover, its seeds take long period to germinate[43]. As the yields of podophyllotoxin are low it is an expensive starting compound for the chemical synthesis of its derivatives. In addition the chemical synthesis of podophyllotoxin, the starting material for the anticancer drugs Etoposide and Teniposide is very complicated and rather difficult. Therefore the effective availability of these drugs will ultimately depend upon the supply of raw materials. Biotechnological means for production of podophyllotoxin using *in vitro* techniques of propagation, plant cell and organ cultures is considered as an attractive alternative.

Propagation by conventional and *in vitro* techniques

Seeds of *P. hexandrum* remain dormant for about 10 months under natural conditions[44]. Strategies towards conventional and *in vitro* propagation of *Podophyllum* sp. have been tried. Badhwar et al. 1963, reported 44% germination upon sowing *P. hexandrum* seeds with fruit pulp immediately after collection. Cultivation trials using rhizome as the planting material at higher altitudes (3600m asl) and seeds at a relatively lower altitude (1150m asl) have been reported[45-46]. Researchers at the G.B. Pant Institute of Himalayan

Resources (Garhwal, India) have studied the propagation of *P. hexandrum* by the use of natural stocks, using rhizome cuttings, viable seeds and plants regenerated from embryogenic calli[47]. Nadeem et al. 2007, reported 5 fold improvement in seed germination of *P. hexandrum* following pre treatment with sodium hypochlorite and also improved vegetative multiplication using rhizome segments treated with IBA. The authors also reported *in vitro* multiplication of the Indian *Podophyllum* via multiple shoot formation from zygotic embryos and subsequent rooting.

Plant regeneration via somatic embryogenesis has also been reported[49]. Callus derived from zygotic embryos differentiated globular somatic embryos which developed into plantlets. Anrini M et al. 2009, observed occasional shoot organogenesis in mature root derived callus cultures maintained on MS basal medium supplemented with BA and NAA for 12 weeks. Nodular structures developed on such calli after 12 weeks which developed into bud primordia like structures within another 3 weeks of culture. Further development of bud primordia into adventitious shoot buds was found to occur after 2 weeks of culture on the same medium. A protocol was developed by Indian Institute of Integrative Medicine (CSIR) Srinagar – India for in vitro propagation of *P. hexundrum* starting from callus cultures raised in Gamborg's medium[51]. The institute has also developed agro techniques for mass cultivation of *P. hexundrum*. Attempts of developing in vitro propagation protocols that would provide high-yielding *Podophyllum* clones; and somatic embryogenesis in tissue cultures has also been described by several authors[52-53].

Podophyllotoxin production through Cell / Organ cultures

The formation of secondary metabolites is correlated with the degree of organization of cell structures and is often low and unstable in undifferentiated callus and suspension cultures[54]; for example, several alkaloids which are scarcely synthesized in undifferentiated cultures are produced at higher levels in cultured roots thereby correlating alkaloid production with root organogenesis[55]. But the major constraint associated with *in vitro* root cultures is their slow growth rate. On the other hand, hairy roots transformed with *Agrobacterium rhizogenes* have a fast growth rate, are genetically stable and produce secondary metabolites at levels comparable to or greater than that of intact plants[56-58]. There is a single report on transformation of *P. hexandrum* by different strains of *A. rhizogenes* (A4, 15834 and K599) in which transformed calli obtained from embryo were reported to contain 3 fold more podophyllotoxin compared to controls[59]. However, till date establishment of hairy roots following transformation using *A. rhizogenes* has not been reported.

Cell cultures derived from *P.hexandrum* have been reported to produce podophyllotoxin, 4′-dimethyl-podo- phyllotoxin and podophyllotoxin-4-O-glucoside when the callus was incubated in B5 medium containing 2,4- dichlorophenoxyacetic acid (2,4-D), gibberellic acid and 6-benzylaminopurine[60]. The levels of podophyllotoxin and its derivatives were similar to those in the parent plant. Podophyllotoxin and related derivatives have also been reported in cell cultures of *L. flavum*[61]. However, the total content of a desired compound in cell cultures strongly depends on the culture conditions. Thus optimization of important nutritional and environmental parameters for cell cultivation was done for enhanced production of podophyllotoxin. Cell suspension of P. hexandrum was performed on MS medium containing indoleacetic acid (IAA, 2 mg l^{-1}) on a gyratory shaker 20°C in dark[62]. *P. hexandrum* cells were found to be slow growing and required 30 days to reach a maximum biomass of 8.3 g l^{-1}.

Transformed calli of *P. hexandrum* were obtained by embryo transformation, using different strains of A. rhizogenes, viz. A4, 15834 and K599. HPLC analysis of transformed cultures depicted a three-fold increase in podophyllotoxin content in comparison with controls[63]. Immobilization of *P.hexandrum* cells using calcium alginate in combination with precursor feeding with L-phenylalanine and L-tyrosine was attempted[64]. This, however, did not lead to any improvement in the accumulation of podophyllotoxin, possibly because of the stress environment of the cells, in which they were unable to synthesize podophyllotoxin endogenously. Various phenylpropanoid precursors (phenylalanin, tyrosin, cinnamic acid, caffeic acid, coumaric acid, ferrulic acid, coniferyl alcohol, coniferin, etc.) were applied for the improvement of podophyllotoxin levels in P. hexandrum cell cultures, but only coniferin was found to exhibit a positive and reproducible effect on podophyllotoxin accumulation[64]. Coniferin at a final concentration of 2.1 mM in the culture medium was able to significantly increase the podophyllotoxin accumulation by a factor of 12.8, while concentrations exceeding 4.0 mM led to the inhibition of cellular growth. The addition of coniferin to the culture medium also resulted in rapid accumulation of podophyllotoxin on day 10 of cultivation, although most of the coniferin was transformed into unknown products[65].

Batch cultivation of P. hexandrum was conducted in a 3-l stirred-tank bioreactor using statistically optimized process parameters. A mathematical model was developed using the batch kinetic data and then extrapolated to computersimulate and select the optimum nutrient feeding strategy, which was employed in a fed-batch cultivation for enhanced biomass and product accumulation[66]. A 36% increase in volumetric productivity of podophyllotoxin was achieved in fed-batch cultivation of *P. hexandrum* cells over batch cultivation. Continuous cultivation of P. hexandrum with cell retention was carried out in a 3l bioreactor equipped with a spin filter mounted on the agitator shaft of the bioreactor, which resulted in the accumulation of 53g l^{-1} of biomass and 48.8 mg l^{-1} of podophyllotoxin with a volumetric productivity of 0.8 mg l^{-1} day^{-1} [67]. Podophyllotoxin productivity was increased 1.5-fold in a cell culture of P. hexandrum by optimizing the culture and nutritional parameters in continuous cultivation mode in a 3l bioreactor[68].

Future prospects

The rhizomes and roots of *Podophyllum* species have gained much importance throughout the world as being the main source or the starting material for the alkaloid podophyllotoxin and its semisynthetic compounds, the etoposide, teniposide, and etoposide phosphate since their use in treatment of specific types of cancers. In USA Bristol Co. and in Switzerland Sandoz prepared hundreds of semi synthetic compounds. Out of these only above three are widely used as anti-tumour agents with minimal toxic or side effects.

There are several problems associated with the isolation of compounds for production of pharmaceuticals from biomass collected from wild populations of plants. Destruction of plant populations due to over exploitation or natural calamities affects drug supply and the content of bioactive secondary metabolite in the plant. Therefore immediate thrust has to be given for generating the reliable conventional protocols of mass cultivation of *P. hexundrum*. Moreover, wild populations may be represented by various genotypes growing under different environmental conditions which may affect drug profile leading to problems in the purity of the final product. Thus cultivation of suitable clones would ensure a reliable supply of the material with consistent quality. Thus, there is a need to understand and conserve the genetic diversity of this important medicinal plant.

At present two species of *Podophyllum*, viz. the American Podophyllum or Mayapple (*P. peltatum*) and the Himalayan Podophyllum (*P. hexandrum*) are the main sources of podophyllotoxin. In 1942, podophyllin, a mixture of podophyllum resin and podophyllotoxin was introduced as a topical remedy for cancerous growth in the USA, and later podophyllotoxin was discovered and extracted from the rhizomes for use against cancerous growth. At that time, M/S Sandoz brought the product on the market, and there was a good demand for the product. It was already well known that *P. hexandrum*, a good substitute of the May Apple or American Podophyllum, was abundantly available in the Himalayas in the wild. Thereafter, *Podophyllum hexandrum* was intensely used by the British physicians in India for medical treatment. It was collected from the Himalayan region and also exported to England for medical treatment, which probably led to over-exploitation soon and after half a century or so to extreme depletion and is now endangered in India. The population of *P.*

hexandrum in western Himalaya is declining, and in some areas the plant has almost disappeared as a result of anthropogenic activities and overexploitation[69].

The limited availability of *Podophyllum hexandrum* plant due to its long juvenile phase and poor fruit setting ability has a serious negative impact for the active cultivation of *Podophyllum hexandrum* thus resulting in shortage of Podophyllum resin. Moreover, because of the non-optimal yield after extraction, Podophyllotoxin is an expensive starting compound for the chemical synthesis of its derivatives.

Podophyllum hexandrum is now being considered as a rare and threatened species mainly due to the large scale removal of its underground parts that still continues at rates well over natural regeneration. Attempts have been made to conserve this plant through *in vitro* propagation and artificial breaking of seed dormancy[70,47]. Efforts to collect and maintain germplasm have been mainly centred on clearly defined characters recognizable in a phenotype. However, there is now a paradigm shift in looking for characters or genes using molecular markers.

Therefore, the biotechnological production of Podophyllotoxin using plant cell culture derived from *Podophyllum hexandrum* may be an attractive alternative. Podophyllotoxin content are prone to changes due to environment factors of different ecoregions and stage of harvest. These changes could be controlled by *in-vitro* culture of *Podophyllum hexandrum* for the synthesis of lignan Podophyllotoxin.

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. Scale-up of growth and product yield depends on a multitude of factors, such as growth medium, physicochemical conditions, seed inoculum, type of reactor and processing conditions. The composition of the growth medium, elicitors and precursors, etc. can markedly influence the production. Optimum levels of parameters that facilitate high growth and product response in cell suspensions of *Podophyllum hexandrum* have already been determined by statistical design. *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. The batch kinetic data were used to identify the mathematical model which was then used to develop nutrient feeding strategies for fed batch cultivation to prolong the productive log phase of cultivation. An improvement in the production of podophyllotoxin to 48.8 mg l-1 in a cell culture of *P. hexandrum* has been achieved.

Therefore, Plant biotechnology offers a great opportunity to exploit plant cell culture techniques to produce a whole range of secondary metabolites; and it may be considered as a new approach, as compared with other more conventional methods. However, the fact remains that, at present, success in production on a commercial scale has been achieved for only a few compounds. Unfortunately, the production of podophyllotoxin has not yet proven to be a commercially viable alternative to the plant cell culture approach. Technological developments are required such that the process becomes commercially viable. The recent advancement of knowledge in phytochemistry, the regulation of secondary pathways and an ability to express desired traits by transgenics is expected to help the economic production of such an important pharmaceutical and healthcare product.

Plant cells often undergo spontaneous genetic variation in terms of secondary metabolite accumulation in suspension culture, which results in a heterogeneous population of cells. The genetic basis of somaclonal variation has not yet been extensively studied. However, high yielding genetically stable cell lines would provide a suitable means for the large-scale production of podophyllotoxin. Secondary metabolite production can be improved by a proper understanding of plant cell differentiation, intracellular organization, cell physiological characteristics and regulatory mechanisms. Increasing the awareness in metabolic routes may lead to an improvement in product accumulation during cell culture.

Thus it seems attractive to establish a biotechnological production system outside the plant. Genes encoding plant enzymes might be expressed in fast growing microorganisms. Possibly also non plant genes and enzymes can be used to construct a successful production system. Expressed sequence tag based investigations are currently in progress to identify the genes involved in this pathway, with a view to engineer a better production of podophyllotoxin and related cancer drugs. In this way, metabolic engineering may prove to be an important tool for improving the complex regulatory mechanisms in the lignan biosynthetic pathway for the production of podophyllotoxin.

REFERENCES

1. Airi S, Rawal RS, Dhar U, Purohit AN. Population studies on *Podophyllum hexandrum* Royle- A dwindling, medicinal plant of the Himalaya. Plant Genet. Resour News 1997; 110 : 29-34
2. Gupta R, Sethi KL. Conservation of medicinal plant resources. In: Jain SK and Mehra KL (eds) Conservation of tropical plant resources 1983; 101–107.
3. Giri A, Narasu ML. Transgenic hairy roots: recent trends and applications. Biotechnology Advances 2000; 18: 1-22.
4. Choudhary DK, Kaul BL, Khan S. Cultivation and conservation of Podophyllum hexandrum- An overview. J. Med. Aro. Plant Sci 1998; 20:1071–1073
5. Nayar SL, Chopra IC. Distribution of British pharmacopoeial drug plants and their substitutes growing in India. Pharmaceutical and Drugs Research Committee, Council of Scientific & Industrial Research, New Delhi 1951; 56 pp.
6. Uniyal MR. Uttarakhand Vanaushdh Darshika. Pub. Indian system of Medicine and Homeopathy, New Delhi 1977; (In Hindi)
7. Kumar S, Singh J, Shah NC, Ranjan V. Indian Medicinal Plants Facing Genetic Erosion. Central Institute of Medicinal & Aromatic Plants, Lucknow, CSIR 1997; 205 pp.
8. Kamil MW, Dewick PM. Biosynthesis of lignans alfa and beta-peltetin. Phytochem 1986a; 25(9) : 2089-2092.
9. Kamil MW, Dewick PM. Biosynthetic relationship of aryltetralin lactone lignans to dibenzylbutyrolactone lignans. Phytochem 1986b; 25: 2093-2102.
10. Jackson DE, Dewick PM. Aryltetralin lignans from Podophyllum hexandrum and Podophyllum peltatum. Phytochem 1984; 23(5):1147-1152.
11. Gowdey G, Lee RK, Carpenter WM. Traetise of HIV- related hairy Leuoplakia with *Podophyllum* resin 25% solution. Oral. Pathol. Oral Radiol. Endocrinol 1995; 79: 64.
12. Cobb MW. Human Papiloma Virus infection. J. Am. Acad. Dermatol 1990; 22: 547.
13. Beutner KR, Krog V. Current status of podophyllotoxin for the treatment of warts. Semin. Dermatol 1990; 9: 148.
14. Tyler VE, Brady LR, Robbers JE. Pharmacology. 9th edn. Lea & Febiger 1988; Philadelphia.
15. Broomhead AJ, Dewick PM. Tumor inhibitory aryltralin lignans in *Podophyllum versipelle, Diphyllcia cymosa* and *Diphyllicia grayi*. Phytochemistry 1990; 29: 3831-3837.
16. Issell BF, Muggia FM, Carter SK. Etoposide (VP-16)- Current Status and New Developments. Academic Press 1984; Orlando, USA.
17. Stahelin HF, Wartburg AV. The chemical and biological route form podophyllotoxin glucoside to etoposide: Ninth Cain Memorail Award Lecture. Cancer Res 1991; 51: 5-15.
18. Imbert TF. Discovery of podophyllotoxins. Biochimie 1998; 80: 207-22.
19. Leander K, Rosen B. Medicinal uses for podophyllotoxin. U.S Patent 1988; 4: 788, 216.
20. Lerndal T, Svensson B. A clinical study of CPH 82 vs. methotrexate in early rheumatoid arthritis. Rheumatology (Oxford) 2000; 39: 316.
21. Purohit MC, Bahuguna R, Maithani UC, Purohit AN, Rawat MSM. Variation in podophylloresin and podophyllotoxin contents in different population of *Podophyllum hexandrum*. Curr. Sci 1999; 77: 1078-1080.
22. Fay DA, Ziegler HW. Botanical sources differentiation of *Podophyllum* resin by high performance liquid chromatography. J Liq Chromatogr 1985; 8: 1501–1506.

23. Drew SE, Conway SJ, Jenning P, Helliwell K. Determination of the aryltetralin lignan content of podophyllum resins and roots/rhizomes. J Pharm Pharmacol 1987; 39: 738–739.
24. Thakur RS. Plant drugs: Emerging areas of modern drug research. In: Govil JN, Singh VK and Hashmi S (eds) Glimpses in Plant Research 1993; XI, 371–375.
25. Canel C, Moraes RM, Dayan F, Ferreria D. Podophyllotoxin, Phytochemistry 2000; 54: 115-120.
26. Datt M., Sharma A. Vinblastine – podophylotoxin, a good mitotic agent for preparing embryonic chromosomes. Ind. J. Anim. Sci 2000; 70, 912–913.
27. Buss AD, Waigh RD. In: Wolff, M.E., (Ed.), Natural Products as Leads for New Pharmaceuticals, Wiley, New York 1995; chapter 24.
28. Gordaliza M., Castro M.A, Miguel del Corral JM, San Feliciano A. Antitumor properties of podophyllotoxin and related compounds. Curr Pharm Des 2000a; 6, 1811–1839.
29. Gordaliza M, Miguel del Corral JM, Castro MA, Lo´pez Va´zquez ML, San Feliciano A, Garcı´a-Gra´valos MD, Carpy A. Synthesis and evaluation of pyrazolignans, A new class of cytotoxic agents. Bioorganic and Medicinal Chemistry 1995; 3(9):1203–1210.
30. Schonbrunn E, Phlippen W, Trinczek B, Sack S, Eschenburg S, Mandelkow EM, Mandelkow E. Crystallization of a macromolecular ring assembly of tubulin liganded with the antimitotic drug podophyllotoxin. J. Struct. Biol 1999; 128:211–215.
31. Cowan CR, Cande WZ. Meiotic telomere clustering is inhibited by colchicine but does not require cytoplasmic microtubules. J. Cell Sci 2002; 115:3747–3756.
32. Tseng CJ, Wang YJ, Liang YC, Jeng JH, Lee WS, Lin JK, Chen CH, Liu IC, Ho YS. Microtubule damaging agents induce apoptosis in HL 60 cells and G2/M cell cycle arrest in HT 29 cells. Toxicology 2002;175:123–142.
33. Chaudhuri AR, Seetharamalu P, Schwarz PM, Hausheer FH, Luduena RF. The interaction of the B-ring of colchicines with a-tubulin: a novel footprinting approach. J. Mol. Biol 2000; 303:679–692.
34. Pal D, Mahapatra P, Manna T, Chakrabarti P, Bhattacharyya B, Banerjee A, Basu G, Roy S. Conformational properties of a tubulin tail peptide: implications for a tail–body interaction. Biochem 2001; 40:15512–15519.
35. Lopez-Perez JL, Del Olmo E, De Pascual TB, Merino M, Barajas M, San Feliciano A. A role for dipole moment in the activity of cyclolignans. J. Mol. Struct 2000; 504, 51–57.
36. Greenwald RB, Conover CD, Pendri A, Choe YH, Martinez A, Wu DC, Guan SY, Yao ZL, Shum KL. Drug delivery of anticancer agents: water soluble 4-poly (ethylene glycol) derivatives of the lignan podophyllotoxin. J. Controlled Release 1999; 61:281–294.
37. Stahelin HF, Wartburg AV: The chemical and biological route form podophyllotoxin glucoside to etoposide. Ninth Cain Memorail Award Lecture. Cancer Res 1991; 51:5-15.
38. Uden WV, Pras N, Visser JF, Malingre TM. Detection and identification of podophyllotoxin produced by cell cultures derived from Podophyllum hexandrum Royle. Plant Cell Rep 1989; 8:165–168
39. Holthuis JJM. Etoposide and teniposide: Bioanalysis, metabolism and clinical pharmokinetics. Pharm Weekly 1988; 10:101–116.
40. Pelter A, Ward RS, Ma WY. An asymmetric synthesis of isopodophyllotoxin. J Nat Prod 1994; 57:1598–1602.
41. Schacter L. Etoposide phosphate: what, why, where and how? Semin Oncol 1996; 23:1–7.
42. Gupta R, Sethi KL. Conservation of medicinal plant resources. In: Jain SK and Mehra KL (eds) Conservation of tropical plant resources 1983; 101–107.
43. Handa SS, Chawla AS, Maninder A. Hypoglycemic plants: a review. Fitotherapia 1989; 60:195.
44. Badhwar RL, Sharma BK. A note on germination of *Podophyllum* seeds. Indian Forester 1963; 89:445–447.
45. Singh A. Ecophysiology of *Podophyllum hexandrum* Royle, Ph.D thesis, H.N.B. Garhwal University 1992.
46. Pandey H, Nandi SK, Kumar A, Palni UT, Palni LMS. Podophyllotoxin content in Podophyllum hexandrum Royle plants of known age of seed origin and grown at a lower altitude, Acta Physiologiae Plantarum 2007; 29:121–126.
47. Nadeem M, Palni LMS, Purohit AN, Pandey H, Nandi SK. Propagation and conservation of Podophyllum *hexandrum* Royle: an important medicinal herb. Biol Conserv 2000; 92:121–129.
48. Nadeem M, Palni LMS, Kumar A, Nandi SK. Podophyllotoxin content, above- and belowground biomass in relation to altitude in *Podophyllum hexandrum* populations from Kumaun region of the Indian Central Himalaya. Planta Medica 2007; 73: 388–391.
49. Arumugam N, Bhojwani SS. Somatic embryogenesis in tissue cultures of *Podophyllum hexandrum*", Canadian Journal of Botany 1990; 68:487–491.
50. Anrini M, Sumita J. Biotechnological approaches for the production of potential anticancer leads podophyllotoxin and paclitaxel: an overview J Biol Sci 2009; 1(1): 46-69
51. Phalisteen S, Shawl AS, Ramteke PW, Arif J, Nahida C, Neelofar J, Shabir S. In vitro propagation for mass multiplication of *Podophyllum hexundrum*: A value medicinal plant. Asian J of Plant Science 2006; 5(2):179–184
52. Arumugam N, Bhojwani SS. Somatic embryogenesis in tissue cultures of *Podophyllum hexandrum*. Can J Bot 1990; 68: 487–491.
53. Sharma TR, Singh BM, Sharma NR, Chauhan RS. Identification of high podophyllotoxin producing biotypes of *Podophyllum hexandrum* Royle from north-western Himalaya. J Plant Biochem Biotechnol 2000; 9 : 49–51
54. Sevón N, Oksman-Caldentey KM. Agrobacterium rhizogenes mediated transformation: root cultures as a source of alkaloids. Planta Medica 2002; 68, 859-868.
55. Sevón N, Hiltunen R, Oksman-Caldentey KM. Somaclonal variation in Agrobacterium transformed roots and protoplast-derived hairy root clones of Hyoscyamus muticus. Planta Medica 1998; 64, 37-41.
56. Oksman-Caldentey KM, Hiltunen R. Transgenic crops for improved pharmaceutical products. Field Crops Research 1996; 45, 57-69
57. Sevón N. Tropane alkaloids in hairy roots and regenerated plants of Hyoscyamus muticus. 1997, PhD Thesis, University of Helsinki, Finland,
58. Giri A, Narasu ML. Production of Podophyllotoxin from Podophyllum hexandrum: A potential natural product for clinically useful anticancer drugs. Cytotechnol 2000; 34:17–26.
59. Giri A, Giri CC, Dhingra V, Narasu ML. Enhanced podophyllotoxin production from Agrobacterium rhizogenes transformed cultures of Podophyllum hexandrum. Natural Product Letters 2001a; 15: 229–235.
60. Heyenga AG, Lucas JA, Dewick PM. Production of tumor inhibitory lignans in callus cultures of *Podophyllum hexandrum*. Plant Cell Rep 1990; 9:382–385
61. Wichers HJ, Versluis-de-Hann GG, Marsman JW, Harkes MP. Podophyllotoxin related lignans in plants and cell cultures of Linum flavum. Phytochemistry 1991; 30:3601–3604
62. S, Srivastava AK, Bhojwani SS, Bisaria VS. Development of suspension culture of Podophyllum hexandrum for the production of podophyllotoxin. Biotechnol Lett 2001; 23:2063–2066
63. Giri A, Giri CC, Dhingra V, Narasu ML. Enhanced podophyllotoxin production from *Agrobacterium rhizogenes* transformed cultures of *P. hexandrum*. Nat.Prod. Lett 2001; 15: 229-235.
64. Uden W Van, Pras N, Malingre TM. On the improvement of the podophyllotoxin production by phenylpropanoid precursor feeding to cell cultures of Podophyllum hexandrum Royle. Plant Cell Tissue Organ Cult 1990a; 23:217–224
65. Woerdenbag HJ, Uden W van, Frijlink HW, Lerk CF, Pras N, Malingre TM. Increased podophyllotoxin production in Podophyllum hexandrum cell suspension cultures after feeding coniferyl alcohol as a beta cyclodextrin complex. Plant Cell Rep 1990; 9: 97–100.

66. Chattopadhyay S, Bisaria VS, Bhojwani SS, Srivastava AK. Enhanced production of podophyllotoxin by fed-batch cultivation of *Podophyllum hexandrum*, Can. J.Chem.Eng 2003b; 81(5),1011-1018.
67. Chattopadhyay S, Bisaria VS, Srivastava AK. Enhanced production of podophyllotoxin by Podophyllum hexandrum using in situ cell retention bioreactor. Biotechnol Prog 2003a; 19:1026–1028
68. Chattopadhyay S, Mehra RS, Srivastava AK, Bhojwani SS, Bisaria VS. Effect of major nutrients on podophyllotoxin production in Podophyllum hexandrum suspension cultures. Appl Microbiol Biotechnol 2003c; 60:541–546
69. Bhadula SK, Singh A, Lata H, Kuniyal CP, Purohit AN. Genetic resources of *Podophyllum hexandrum* Royle, an endangered medicinal species from Garhwal Himalaya, India. Plant Genetic Resources Newsletter 1996; 106:26–29.
70. Nautiyal MC, Rawat AS, Bhadula SK, Purohit AN. Seed germination in *Podophyllum hexandrum*. Seed Res 1987; 16:206–209