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REVIEW ARTICLE **OPEN ACCESS**

Sinopodophyllum hexandrum (Royle) T.S Ying: An Endangered Medicinal Plant Species in Indian Himalayan Region – A Review

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ABSTRACT
Indian Himalayan region is one of the biodiversity hotspot areas in the world. There are several endemic species in the region including the much famed Sinopodophyllum hexandrum (Royle) T.S Ying which is of great medicinal importance. It is chiefly distributed in high mountains of Himalayan states and union territories at an altitude of 2200 to 4500 m. The rhizome and roots are used to extract an alkaloid podophyllotoxin. Podophyllotoxin is a baseline chemical compound used for the preparation of semisynthetic derivatives like etoposide, teniposide and etophos that are clinically applied as cytostatic drugs in the treatment of several kinds of cancer. The anti-cancer activity of podophyllotoxin derivatives is attributed to inhibition of tubulin polymerization and thereby arresting the mitotic spindle formation. The massive harvesting and overexploitation has lead to the drastic decline in its populations. It has been declared as a rare and endangered species by the IUCN. The biotechnological advancements over the decades have also focused on production of podophyllotoxin through in-vitro cell culture. However, genes encoding essential plant enzymes can be expressed in fast growing microorganisms. It seems attractive to establish biotechnological production system outside the plant not only to enhance pharmaceutical product generation but also to conserve the endangered species.
Keywords: *podophyllotoxin, cytostatic drugs, in-vitro culture.*

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INTRODUCTION

The Indian Himalayan region is a treasure grove of several medicinal plants. The native people have been using herbal plants containing lignans for the cure and prevention of several health disorders since ancient. Plant lignans are dimerziation products of two phenylpropane units which are linked by β-carbon atom of side chain. Many such plant lignans find applications for the development of new therapeutic agents through their structural modifications. Due to globalization and extensive growth of pharmaceutical sector people are finding many new kinds of medicines for the cure of health problems and many such drugs are often accompanied by side effects. There has been a desirable interest in natural plant products due to their compatibility to the human body without any side effects. India has a very ancient civilization where the use of medicinal plants since the vedic age is an honored tradition that has been accepted even today by various indigenous healthcare systems of medicine like ayurveda, unani, siddha and homeopathy [1]. It has been recognized as one of the top twelve mega biodiversity regions of the world with huge number of about 45,000 species of floral diversity and 6,500 species of faunal diversity. Although most of biodiversity is present in tropical regions and smaller proportion of less than 30% is confined in temperate and alpine regions but the later include species of high medicinal importance to the pharmaceutical industry [2]. The plant wealth of Indian Himalayan region has been acknowledged over the world for its uniqueness and high medicinal value. The inhabitants of the Himalayan region are known to keep a high medicinal reverence on its plant wealth since the vedic period [3]. There are several endemic medicinal plants in the region including *Sinopodophyllum hexandrum* (Royle) T.S Ying which is a small rhizomatous herbaceous species of great medicinal value. It is commonly known as Himalayan May Apple since its fruiting chiefly occurs in the summer month of May. It is believed to be the native to lower elevations of the northern Himalayan region and its adjoining parts. The rhizome and roots of the plant are the source of a resinous substance which is used for the extraction of an alkaloidal product called podophyllotoxin. It is an active ingredient used as a starting compound for the chemical synthesis of drugs that are effective in treatment of various cancers including a variety of leukemia, and even some tumors [4]. It is worthwhile to mention that etoposide is such an important

anticancer drug that it is included in the WHO list of essential medicines. Due to a very high demand throughout world it faces shortages in its supply due to the limited availability of its raw material which is podophyllotoxin lignan obtained from Himalayan May Apple [5]. Although podophyllotoxin is also present in other plant species it is present in sufficient amounts only in *Sinopodophyllum hexandrum* and *Podophyllum peltatum*. The later is commonly known as American Podophyllum. The former is more promising as regards to the active lignan content. It has been found that *Sinopodophyllum hexandrum* of Indian origin bears almost three times more podophyllotoxin than its American counterpart [6]. However there has been massive extraction of its underground parts ever since it came into prominence during with a concomitant decline of its natural populations in the region. The species is currently listed in the red data book as per IUCN criteria [7]. It is also included in negative list of Indian exports by the Ministry of Commerce, Government of India [8]. This review is intended to provide information about *Sinopodophyllum hexandrum* such as morphology, distribution, pharmacology of podophyllotoxin and its derivatives, medicinal uses and also discusses conservation efforts and the potential of podophyllotoxin production through biotechnological processes.

TAXONOMIC DESCRIPTION

Classification

Kingdom: Plantae
Group: Angiosperms
Clade: Eudicots
Order: Ranunculales
Family: Berberidaceae
Genus: Sinopodophyllum
Species: *Sinopodophyllum hexandrum* (Royle) T.S Ying
(Synonyms: *Podophyllum hexandrum* Royle.; *Podophyllum emodi* L.)
Common Name: Himalayan May Apple, Indian May Apple, Indian Podophyllum.
Vernacular Names: *Bakrachimaka, Bankakri*, *Bantrapushi, Bhananbakra, Banwangun* (Kashmiri), *Giriparpat, Hol-mo-se* (Ladakhi), *Papra or Papri.*

MORPHOLOGY

Sinopodophyllum hexandrum is an erect, glabrous, succulent herb, 10 to 40 cm tall with perennial creeping rhizome bearing many adventitious roots. It is usually low to the ground with glossy green, drooping and lobed leaves on its few stiff branches. Stem is one or few arising from underground rhizome, herbaceous, green and fleshy. Leaves are 1-3, usually 2, terminal on shoots, alternate, petiolate and often purple spotted. Leaf lamina is glossy green, rounded, 15-25 cm across, deeply divided into 3 ovate, toothed lobes which are sometimes further lobed. Flower is solitary, white or light pink, large, usually 3.5-5 cm across, hermaphrodite and actinomorphic. Sepals are 3-6, gamosepalous and caducous. Petals are 6, rarely more or less, polypetalous, white or light pink. Stamens are 6, polyandrous and anthers dehisce longitudinally. The fruit is an ovoid berry 2.5-5 cm, scarlet or reddish when ripe with many seeds embedded in the pulp [9,10]. Flowers in May-August [11].

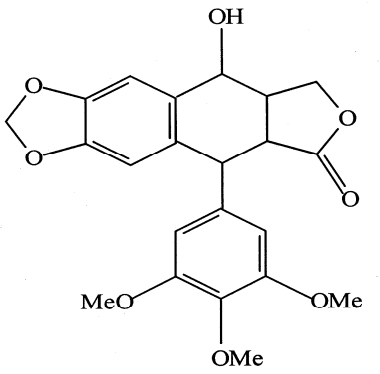
DISTRIBUTION

Indian May Apple grows in the Himalayan region from north-eastern India to Afghanistan and north to south-west China. It is native to the lower elevations of Himalayan region including Afghanistan, Pakistan, India, Nepal, Bhutan and south-west China. In India, *Sinopodophyllum hexandrum* has been distributed in all the Himalayan states and union territories including Ladakh, Jammu and Kashmir, Himachal Pradesh, Uttarakhand, Uttar Pardesh, Sikkim, Assam, Meghalaya, Arunachal Pradesh and Manipur. It is seen growing in the scrub forests and alpine meadows of mighty Himalayas as an under growth together with other herbaceous flora at an altitude range of 2200-4500 m [12]. In Ladakh, it grows wild in Kargil, Zanskar, Zozila, Suru Valley and Gilgit. In Jammu and Kashmir it has been reported from several locations such as Tanmarg (2,200-2,600 m), Mechigaon, Trumba, Dagoum, Pahalgam, Chandanwari, Sheshnag, Pissughati, Daitwas forest, Gulmarg (2,700-3,000 m), Khilanmarg (2,700-3,000 m), Jagran river bank between Kundi and Shikar (3,000- 3,600 m), Kishenganga valley, Kansar, Jhelum basin (2,400-2,700 m), Muzafarabad forest (2,400 m), Lidwas, and Sindh Valley. In Himachal Pradesh, it grows in many locations including Chamba, Kullu, Kangra, Pulga (2,400 m), Chulkot forest (3,000 m), Pangi, Killar pass, Saach valley, Pandrabis (2,400 m) (Bashar), Hiranghati pass (3,600 m), Kala-Tope forest (2,438 m), Keylong, Lahul, Spiti, Matian, Shalli hills, Dencho, Narkunda, Sissoo, Koksar, Dalhousie and Shimla. In Uttarakhand, it is reported from Deoban (2700 m), Konain (Dt. Dehra Dun), Kanjatra (2,600 m), Bhillangana, Panwali

(Dt. Tehri); Jamnotri, Jamunachatti, Barkot (Yamuna valley), Dodital, Gomukh, Kedar-Kanta (3,000-3,300 m) (Uttarkashi), Rudhgaria Gar (4,000-4,300 m), Dasoli, Bhyander, Hemkund (Dt. Chamoli), Mundali (2,300 m), Madhya-Maheshwar, Tungnath, Pindari glacier (Almora), Kutti, Yankti river valley (3,700-4,000 m) and Bogudiar (2,400 m). In Sikkim, it grows wild at Chamnaga (3,600 m), Thangu, Tsomgo, Chanaga and Thangu (3000-4200 m) [13,14]. It has also been reported from various other locations of north-eastern Himalayan states of Assam, Meghalaya, Manipur and Arunachal Pardesh.

PODOPHYLLOTOXIN

The resin of rhizome and roots of Indian May Apple yields an alkaloid podophyllotoxin which accumulates as a secondary metabolite. It is also known by some other unpopular names like podophyllin or podophyllum. The chemical name of podophyllotoxin is 5,8,8a,9-Tetrahydro-9-hydroxy-5(3,4,5-trimethoxyphenyl), furo [3',4':6,7], naphtho [2,3,d]-1,3-dioxol-6 (5aH)-one. The chemical formula of podophyllotoxin is $C_{22}H_{22}O_8$ with a molecular weight of 414.4 g/mol. Podophyllotoxin is available in market with product name and code J. T. Baker: 2898 and Mallinckrodt: 7700.



PODOPHYLLOTOXIN

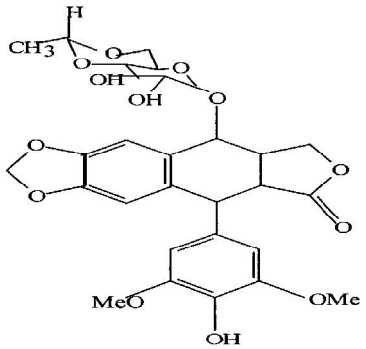
PHARMACOLOGY

The resin from the rhizomes and roots of *Sinopodophyllum hexandrum* possesses many lignans and out of them only podophyllotoxin has been used on commercial scale for the preparation of many drugs and most important among them are the anticancer drugs. Podophyllotoxin and its derivatives containing two semisynthetic podophyllotoxin glycosides inhibit mitogen induced lymphocyte proliferation [15]. The controlled clinical trials have shown that podophyllotoxin exerts a colchicine like effect by arresting mitosis at metaphase resulting death of dividing cells. The anti-mitotic activity of podophyllotoxin is due to the inhibition of microtubule polymerization and assembly of mitotic spindle apparatus. It is found efficacious against many viral infections. Besides its antiviral activity it is also known for insecticidal and phytotoxic activities [16,17]. Another property of podophyllotoxin is to bind with cell proteins and act by inhibition of purine synthesis and its incorporation into RNA [18]. The two important mitochondrial enzymes cytochrome oxidase and succinate oxidase exhibit reduced activity in presence of this plant liganan [19]. Its antiviral activity is effective through factors like tubulin binding, reverse transcriptase inhibition, topoisomerase inhibition and integrase inhibition. It has been established that podophyllotoxin is most notable among the lignans binding tubulin. It can also disrupt the cellular cytoskeleton and interfere with vital viral processes by tubulin binding. However, the semisynthetic derivatives of podophyllotoxin exhibit significant therapeutic activity against several human cancers. These semisynthetic derivatives are demethylated at 4′ and belong to epi series. The hydroxyl in position 4 is part of a glycosidic linkage with glucose and two of the hydroxyl groups at 4′ and 6′ are blocked by acetylization as thianylidene (teniposide) or ethylidene (etoposide). Both these derivatives are known to arrest the cell cycle at G1 and S phase. They also act by topoisomerase II inhibition besides activation of oxidation-reduction reactions to produce derivatives which bind directly to DNA thereby finally result in the arrest of cell cycle. Topoisomerae II relaxes both negative and positive supercoils in DNA by ATP driven reactions. These reactions are mediated by a double-stranded break in one strand of DNA duplex and passing another duplex region through it. The reaction probably represents a non specific recognition of DNA duplex in which enzyme binds any two double stranded segments that cross each other and forms a cleavable complex. The cleavage complex allows one double strand of DNA to pass through a temporary break in another double strand. Both the semisynthetic derivatives of podophyllotoxin not only bind but

also stabilize this DNA complex which prevents the repair of double stranded breaks. It is established that etoposide is particularly cell cycle phase specific inhibitor with predominant activity occurring in late S and G2 phases [20]. It has been found that tris substituted aniline- 4′-O-demethyl-podophyllotoxin derivative also shows high inhibition of DNA topoisomerase II and tumour cell proliferation [21]. It is found to be highly inhibitory in action against a series of tumour cell lines including those which are resistant to the treatment of etoposide. Its action elucidates that it is ten times more potent than etoposide in both topoisomerase II inhibition and killing of tumour cells. Podophyllotoxin is therefore, a very efficacious and useful base line plant lignan for the synthesis of a number of semisynthetic derivatives which are proven to possess anticancer activity against many tumour cell lines [22]. The highly potent semisynthetic derivatives of podophyllotoxin are etoposide, teniposide and etoposide phosphate.

ETOPOSIDE

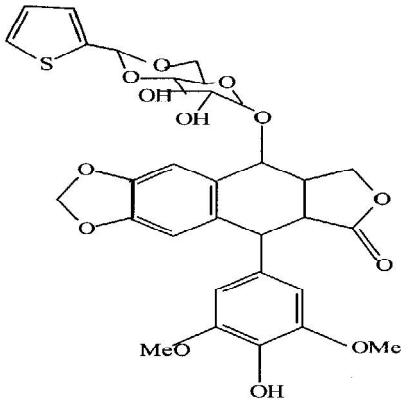
Etoposide is Demethylepipodophyllotoxin-ethylideneglucopyranoside, EPEG, or epipodophyllotoxin with various synonyms such as VP-16, VP–16–213. This semisynthetic derivative of podphyllotoxin is sold by Bristol-Myers Squibb Company of USA as Vepesid, aka VP-16. It is a highly potent and widely used anticancer drug which is usually prescribed in the form of multiple chemotherapy protocols [23,24]. It has been found highly efficacious to treat certain cancers like germ cell tumours, acute lymphocytic leukemia, acute myelogenous leukemia, lung cancer, ovarian cancer, rhabdomyosarcoma, hodgkins disease and glioblastoma multiforma.



ETOPOSIDE

TENIPOSIDE

It is another derivative of podophyllotoxin with a synonym VM-26. Teniposide is generally used in the treatment of acute refractory leukemia besides the bladder and brain tumors. It can be used in single drug therapy for induction of remission but it is less frequently used rather than etoposide.

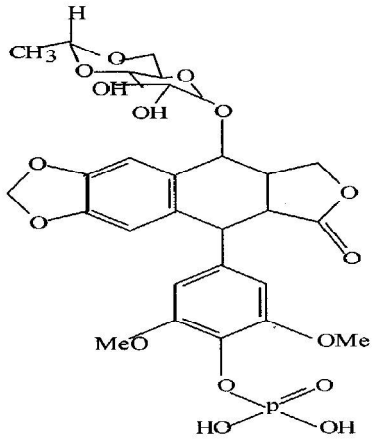


TENIPOSIDE

ETOPOSIDE PHOSPHATE

It is also known as etophos and is a water-soluble prodrug of etoposide which completely and easily gets converted into etoposide after intravenous injection. It has been found that the pharmacokinetics profile

of drug after treatment with either etoposide or etoposide phosphate is same. The main advantages of etoposide phosphate are its ability to be administered as a bolus and its easy water solubility. It can be made up to a concentration of 20 mg ml−1 which meams high doses in small volumes and as continuous infusion without any incidence of hypotension or other acute toxicity problems [25]. Therefore, it is easier to use and as such represents an improved formulation of the parent compound etoposide. Etoposide phosphate or etophos is also sold by Bristol Myers Squibb Company of USA.



ETOPOSIDE PHOSPHATE

MEDICINAL USES

Indian May Apple has a long history of usage amongst the native people throughout the length and breadth of Himalayan region. It is as such a highly poisonous species due to the presence of a number of secondary metabolites of toxic nature. Since the metabolites are of toxic nature and present in copious amounts it should not be used without the supervision of a qualified practitioner. Nevertheless it is a highly medicinal plant known for its podophyllotoxin alkaloid which itself or through its derivatives very effective against many human health disorders and diseases. There are only two species of plants which are at present the commercially exploitable sources of podophyllotoxin and they are *Sinopodophyllum hexandrum* and *Podophyllum peltatum*. The later which is an American Podophyllum species contains 4–5% podophyllotoxin resin whereas the Indian May Apple has a content of 7–16%. The rhizome and roots which are the only source of podophyllotoxin bear 4–16% resin with podophyllotoxin concentration of 40%. The variation in percentage of resin in Indian species is chiefly attributed to multiple factors like its own unique metabolism, different geographical placement, seasonal differences and also age of plant at the time of harvest [26]. However maximum percentage of resin is usually obtained at flowering stage during the month of May and June. If the harvesting is done at proper time yield of about 3 times more than American species can be obtained. Moreover the quantitative comparative analysis of yields have revealed that the resin of Indian species bears double to triple the amount of podophyllotoxin [27,28,29,30,31]. Therefore, Himalayan *Sinopodophyllum hexandrum* seems to be superior to the American *Podophyllum peltatum* with respect to the yields of active ingredient in its rhizomes and roots.

The traditional Ayurvedic system of medicine in India finds its mention in the treatment of several health disorders like tuberculosis, cough, constipation, syphilis, gonorrhoea, rheumatism and skin diseases [32]. The ripe fruits are not toxic in nature rather edible and often used by local people as a cough remedy [33]. Phytochemical studies on fruits mention the presence of 16 prenylated flavonoids and 8 known analogues. These prenylated flavonoids exhibit various pharmacological actions such as antiviral, antimicrobial, anticancer, antioxidant, anticoagulant, anti-inflammatory, antigenotoxic, antiplasmodial, and also activity of estrogen regulation [34].

The rhizomes are also used to cure typhoid, dysentery, fever, scofula, kidney problems and hepatic disorders like jaundice and chronic hepatitis [35]. The whole plant, but especially the rhizome is also cholagogue, cytostatic and purgative [36]. The roots are used as purgative in the form of pills in case of chronic constipation. It is a drastic but a slow acting purgative. An aqueous extract of the roots of the plant acts as common cathartic. The root paste is applied on ulcers, cuts and wounds. It has been used as a remedy in ophthalmia. Its resin has been used by diverse cultures since ancient as antidote against poisons, antihelminthic, vesicant and even as suicidal agent [37].

The major active constituents of rhizome and roots are podophyllotoxin, quercetin and kampherol which exhibit various properties such as anticancerous, antimicrobial, antirheumatic, radioprotective, and anthelminthic [38,39,40]. However, among these lignans podophyllotoxin is most important for its use in the preparation of semisynthetic derivatives that are clinically applied as cytostatic drugs in the treatment of several types of cancer. Successful development of highly marketed anticancer drugs etoposide, teniposide and etophos from natural podophyllotoxin has focused attention on *Sinopodophyllum hexandrum* as a natural economic source of lignans [41,42]. The antitumor activity is an outstanding property of podophyllotoxin and hence it is useful in the treatment of many kind of tumours like lung cancer, refractory testicular cancer, stomach and pancreatic cancers and myeloid leukemias [43,44,45,46]. It is also effective in the treatment of Wilms tumours, different types of genital tumours like carcinoma verrucosus and some other lymphomas [47]. It is also widely used across the world for treatment of ailments like Taenia capitis, monocytoid leukemia, Hodgkin's disease, non-Hodgkin's Lymphoma, cancer of brain, bladder and venereal warts [48,49]. Podophyllotoxin is also included in many pharmacopoeias since long and hence used as an antiviral agent in the treatment of disease condyloma acuminata caused by human papilloma virus [50]. It has been found that application of podophyllotoxin easily and completely cures different warts without any side effect which are otherwise difficult to cure. Podophyllotoxin also finds applications in dermatology for the cure of many skin related diseases particularly psoriasis vulgaris. Podophyllotoxin is also the precursor of a derivative CPH 82 that has been found efficacious for treatment of rheumatoid arthritis [51]. Keeping in view its high medicinal importance particularly in the preparation of highly potent anticancer drugs it is not improper to call this scarce plant resource as the green gold of Himalayan region.

CONSERVATION EFFORTS

Sinopodophyllum hexandrum can thrive well under low temperature conditions due to its tolerance to freezing temperature. Nevertheless it is a hardy plant but it cannot withstand long dry spells. It likes to grow in humus rich soils of moist meadows preferably shaded places of Himalayan scrub and alpine forests. It is commonly seen as an under growth on forest floor along with other herbaceous flora. In natural conditions its propagation occurs through both the seeds and rhizomes [52]. The availability of its resin from underground parts has limitations due to scarcity of the plant resource because of its rarity as well as over exploitation by the poor forest dwellers [53,54]. Reproductive biology of species reveal that it has a long juvenile phase and due to that many young individuals of the populations get destroyed either due to grazing pressure or anthropogenic activities in its diminishing habitat. It is pertinent to mention that it was intensely used by the British physicians in India during their regime due to its medicinal importance. It was widely collected during that period from the Himalayan region for local consumption as well as to export England. Therefore, British Colonialism marks the beginning of its over-exploitation in the region. Even nowadays due to its continued medicinal importance, ever increasing demand and trade in the domestic and international markets it has drawn considerable attention of pharmaceutical sector. In India it is procured at rates 80-100 rupees per kg. Since the demand is largely met by harvesting in inaccessible hilly areas of Himalayan states & UT of Ladakh, Jammu and Kashmir, Himachal Pardesh, Uttarakhand and parts of north-eastern states the cost of collection is high. However, the existing return of the plantation per hectare is estimated to 1.5 to 2.0 lac rupees. The present supply of its raw material is low against high demand not only in Indian but in international markets as well. The massive harvesting of its rhizome and roots for the extraction of podophyllotoxin and consistent over exploitation since the centuries has resulted in drastic dwindling of its populations and extreme depletion of this natural plant resource. It has been placed in rare and endangered species category under IUCN criteria. The export of *Sinopodophyllum hexandrum* and its parts from the wild except some of its formulations from India is prohibited under the CITES. The Govt. of India allows only cultured or artificially propagated plant species for export under the cover of CITES export permit or legal procurement certificate and certificate of cultivation from designated authorities of forest department (Lakhanpal N.L, 1998). It is high time to genuinely initiate both ex-situ and in-situ conservation efforts especially in the Himalayan states and union territories of its natural habitat. In India some conservation efforts have been initiated for the cultivation and propagation of its elite stocks in specially designed nurseries in the vicinity of its natural habitats [55]. The commercial cultivation and propagation of species lays emphasis on the selection of superior genetic stock in order to get maximum profits of farming and boost the cultivation [56]. Therefore, it is of paramount importance to give immediate thrust to artificial breaking of seed dormancy, cultivation of elite stocks near its natural habitat and generate other conventional protocols of mass cultivation of *Sinopodophyllum hexandrum*. Keeping in view the present scenario of its endangered state and high medicinal value efforts are to be made to conserve it by involving in-vitro propagation also. The process of successful generation of plantlets through in-vitro

culture technique has been standardized. It can also be exploited for elite clone selection, propagation and cultivation. Efforts have been made to collect and maintain its germplasm by National Medicinal Plant Board of India. It is worthwhile to mention that National Medicinal Plant Board of India has also taken up the responsibility of conservation of endangered medicinal plants including *Sinopodophyllum hexandrum* in the country.

Plate-1, Flower of *Sinopodophyllum hexandrum*

Plate-2, Fruit of *Sinopodophyllum hexandrum*

Table-1 List of Chemical Compounds obtained from *Sinopodophyllum hexandrum*.

Chemical Compounds present in Rhizome Resin	
Lignans	**Flavinoids**
Podophyllotoxin α-peltatin β-peltatin Desoxypodophyllotoxin Dehydropodophyllotoxin 4′-demethylpodophyllotoxin Sikkimotoxin Picropodophyllinglucoside Podophyllotoxin glucoside α-peltatinglucoside β-peltatinglucoside 4′-demethylpodophyllotoxinglucoside	Quercetin Kaempferol Isorhamnetin Quercetin 3-galactoside

FUTURE PROSPECTS

The yield of podophyllotoxin by conventional extraction methods is low and therefore, it is an expensive starting compound for the synthesis of anticancer drugs. The effective availability of these drugs will ultimately depend upon the supply of raw material and it has been an important issue to pharmaceutical companies particularly engaged in the manufacture of anticancer drugs. Alternatively the chemical synthesis of podophyllotoxin is not easy rather it is a complicated and very costly adventure to begin with due to its complex chemistry of having four chiral centers, a rigid trans lactone and an axially locked α-aryl substituent [57,58]. Apart from its lower yield there are some other problems associated with the extraction of podophyllotoxin for production of pharmaceuticals from biomass collected from wild populations of plants. The destruction of plant populations due to grazing pressure and over exploitation or natural calamities also affect the supply of raw materials to the pharmaceutical industry. The wild populations are represented by different genotypes growing under different environmental conditions which can affect the purity of the product and dilute the drug profile. Nevertheless the biotechnological means of production of podophyllotoxin using plant cell and tissue culture has also been considered as an attractive and viable alternative. However, high yielding genetically stable cell lines may provide a suitable means for the large-scale production of podophyllotoxin. The commercial cultivation of high

yielding clones can increase the production and also supply of raw material that too with consistent alkaloid quality. There is also the need to give immediate thrust to generate reliable conventional protocols of in-vitro culturing of cells and tissues of *Sinopodophyllum hexandrum*. The in-vitro suspension culture often results spontaneous somaclonal variations whereas the genetic basis of somaclonal variation has not yet been extensively studied. During early attempts the callus cultures of *S. hexandrum* were difficult to initiate. Van Uden et al. (1989) tested basal medium with many phytohormone combinations and concentrations and found BS medium supplemented with 2% coconut milk, 4 mg l^{-1} naphthalene acetic acid and 4% sucrose to be the best. The earlier attempts of cell suspension cultures of *S. hexandrum* did not secrete podophylloyoxin in the culture medium. Whereas appropriate alterations in the culture conditions and some other complex factors involving ingredients of culture medium lead to in-vitro biosynthesis of podophyllotoxin [59,60]. As early as 1990, Woerdenbag et al. reported increased podophyllotoxin content in *S. hexandrum* cell cultures supplemented with coniferyl alcohol and β-cyclodextrin [61]. However, there is now a paradigm shift in identifying, mapping and understanding the genes and regulatory sequences involved in the synthesis of podophyllotoxin using molecular markers. The secondary metabolite production in the targeted tissue can be analyzed and improved by a proper understanding of physiological characteristics, plant cell differentiation and inherent regulatory mechanisms. Moreover, expressed sequence tag based investigations are in progress to identify the genes involved in this pathway. Genes encoding essential plant enzymes can be introduced and expressed in-vitro in fast growing microorganisms. Possibly non plant genes and enzymes can be used for the construction of successful in-vitro production with a view to engineer a better production system of podophyllotoxin and related drugs. The increased understanding of in-vitro metabolic pathways may lead to an improvement in the alkaloid accumulation during cell culture through biotechnological methods. Thus, it seems attractive to establish a biotechnological production system outside the plant not only to enhance the product generation related to cancer drugs but also to usher in the conservation of the depleting plant resource.

CONCLUSION

It can be concluded that currently there are only two plant species which are providing the raw material for podophyllotoxin but the most promising one is *Sinopodophyllum hexandrum*. However, due to overexploitation and limitations in its reproductive biology and restricted habitat this Himalayan species is in the list of rare and endangered category. Podophyllotoxin is the only starting compound for the synthesis of many drugs including the much sought after anticancer drugs. The semisynthetic derivatives of podophyllotoxin etoposide, tenuposide and etophos are of particular significance due to their high potency in anticancer drugs. However there is a huge demand and supply gap of plant based raw material from pharmaceutical companies and production. The plant can be grown successfully in-vitro by somatic embryogenesis provided media and other culture conditions are optimum as per standardized protocol. The traditional culturing techniques do not tend to increase the podophyllotoxin production whereas production can be increased by altering the culture conditions. There is research gaps regarding genetic data about the plant genes involved in metabolite production. There is also the need to further develop multiple approaches like commercial cultivation in the vicinity of its habitats, germplasm conservation and upgradation of genetic engineering and biotechnological techniques for in-vitro production of podophyllotoxin.in order to get sustainable supply of raw material. Therefore, there are many research gaps which need to be addressed through further research and development in order to achieve the sustainable development of this valuable medicinal resource for the betterment of human kind as well as conservation of species.

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