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The Himalayan May Apple *(Podophyllum hexandrum):* A Review

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ABSTRACT: Plant based therapeuticals have been in use from time immemorial. The evolution of human race on this planet must have been closely followed by the advent of several diseases. The process took several million of years before he could identify the natural resources including plants for alleviating diseases by a process of trial and error. About 1,100 plants species are frequently used in Indian medicinal system and out of these, 500 plants are commonly used in preparation of different drugs. One of those plants is *Podophyllum hexandrum* Royle, commonly known as the Himalayan May Apple. The herb grows in the Himalayan alpine and subalpine zones. Underground part of the plant yield podophyllotoxin, an active ingredient used as a starting compound for the chemical synthesis of etoposide and teniposide, compound that are effective in treatment of lung cancer, a variety of leukemias, and other solid tumors. Though podophyllotoxin is present in different plant species, but in sufficient amounts it is present only in some species of genus *Podophyllum*. *Podophyllum hexandrum* of Indian origin contains three times more podophyllotoxin than its American counterpart *Podophyllum peltatum.* There has been massive extraction of its rootstock over the last several decades leading to destructive harvesting. This has led to severe reduction in its population density and the species is now listed in endangered plants species category. Any further ecological change and disturbance can cause its extinction. So it is a matter of urgency, considering the medicinal importance of this species to protect it in its natural population.

Keywords: Cromatography; HPLC; *Podophyllum hexandrum*; Podophyllotoxin and TLC.

INTRODUCTION: Plant based therapeuticals have been in use from time immemorial. The evolution of human race on this planet must have been closely followed by the advent of several diseases. The basic instinct of any biological species is survival and procreation. For survival, the early man used various tools, for not only taming nature, but also for fighting diseases. The process took several million of years before he could identify the natural resources including plants for alleviating diseases by a process of trial and error. Due to such efforts, we now have a pool of information regarding the specific therapeutical use of particular herbs. Gradually such information got codified into several different forms of traditional medicine systems worldwide. However, the inquisitive nature of humankind, further explored and identified the specific substances in plants, which are actually useful for treating various diseases. As a result of organized scientific research, we now know the specific organic compounds, which are quite useful either as such or in their derivatized forms for treating specific diseases. In this regard several examples like use of taxol in cancer treatment, quinine in malaria, atropine in eye diseases, digoxin in heart ailments, reserpine in high blood pressure, etc. (Singh, 1982; Liberti, 1993; Das and Anjani, 1998).

The herbal drugs are prepared from medicinal plants only; while the traditional medicines are derived from medicinal plants, minerals, and organic matter. India is the traditional most medico-culturally diverse country in the world where the uses of medicinal plants is part of a time-honored tradition that is in regular uses and respected even today by various indigenous healthcare systems of medicine include ayurveda, unani and siddha (Kirtikar and Basu, 1918). According to the World Health Organization (WHO), approximately 25% of modern drugs used in the United States have been derived from medicinal plants in practice today. At least 7,000 medical compounds used in drug industry in the modern pharmacopoeia are derived from plants and widely used in modern medicine today, 80% show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived (Fabricant and Farnsworth, 2001).

India has been identified as one of the top twelve mega diversity centers in the world having 45,000 species of floral diversity and 6,500 species of faunal diversity; out of these approximately 70% of India's medicinal plants have been found to be in tropical regions, whereas less than 30% in temperate and alpine areas their occurrence, but they include species

of high medicinal value in drug industry (Nautiyal and Nautiyal, 2003). Recently ministry of environment through a co-ordination research project on ethno botany has succeeded in getting the local uses of about 7,500 species documented. About 1,100 plants species are frequently used in Indian medicinal system and out of these, 500 plants are commonly used in preparation of different drugs (Rasbid and Anand, 2008).

The Himalayan region is home of numerous highly valued medicinal plants including *Podophyllum hexandrum* Royle which is a herbaceous, rhizomatous species of great medicinal importance, now endangered in India (Alam *et al*. 2009).*Podophyllum hexandrum* Royle belonging to family Berberidaceae, is a valuable medicinal herb commonly known as the Himalayan May Apple, grows in the Himalayan alpine and subalpine zones. Underground part i.e. rhizome and roots of the plant yield podophyllotoxin, an active ingredient used as a starting compound for the chemical synthesis of etoposide and teniposide, compound that are effective in treatment of lung cancer, a variety of leukemias, and other solid tumors(Sharma *et al*. 2000).

Though podophyllotoxin is present in different plant species, but in sufficient amounts it is present only in some species of genus *Podophyllum*. *Podophyllum* comprises about 22 species (Airi *et al.,* 1997) and out of the different species screened for podophyllotoxin and related lignans, Indian *Podophyllum* (*Podophyllum hexandrum* syn. *P. emodii*) and American *Podophyllum* (*Podophyllum peltatum*) have been found promising with reference to podophyllotoxin contents. *Podophyllum hexandrum* of Indian origin contains three times more podophyllotoxin than its American counterpart *Podophyllum peltatum* (Alam *et al*. 2009). There has been massive extraction of its rootstock (official part) over the last several decades leading to destructive harvesting. This has led to severe reduction in its population density and the species is now listed in endangered plant species category.

A species without enough genetic diversity is thought to be unable to cope with changing environments or evolving competitors and parasites. Considering the importance, threat perception and need for sustainable supply of its rootstock, there is need of not only multiplying its stock (by organized cultivation) but also assessing the relative active content concentration in its populations in different agroclimatic regions and also in different morphotypes. Such studies shall hopefully identify the best stocks/morphotypes, which can be further multiplied (through cultivation). Further, studies on population genetic diversity and the structure of population within a species may not only illustrate the evolutionary process and mechanism but also information useful for biological conservation of *Podophyllum hexandrum.*

MORPHOLOGY AND DISTRIBUTION: *Podophyllum* is a small, herbaceous, monotypic genus of family Podophyllaceae, predominantly occurring in the Northern temperate zone and discontinuously distributed in the Eastern North America and in bolt continental and insular East Asia. The genus comprises of about 22 species distributed mainly in different areas of China, Yunnan, Himalayas, USA, Bhutan, Indo-China, India etc. Four species i.e. *Podophyllum hexandrum, Podophyllum versipelli, Podophyllum aurantiocaule* and *Podophyllum sikkimensis* are reported from Indian Himalayas (Airi *et al.*, 1997).

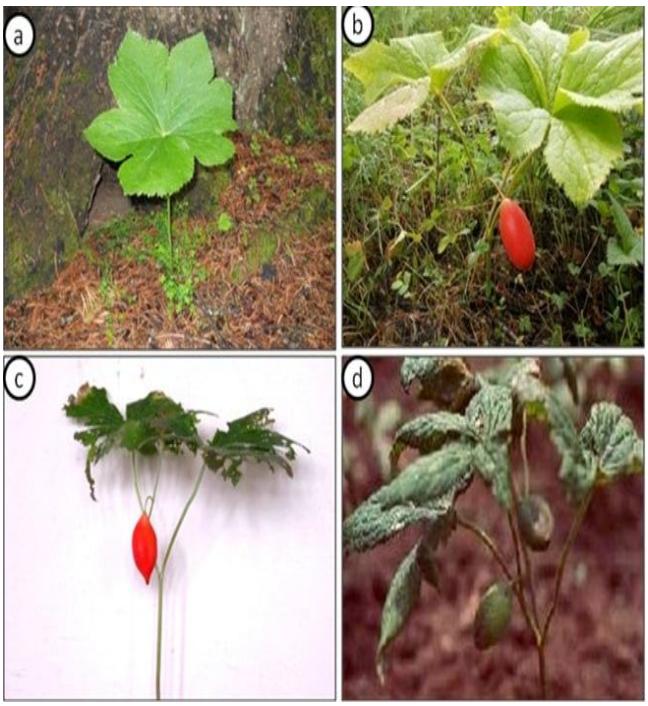

Figure 1: Different types of plants of *Podophyllum hexandrum* having single leaf, two leaves, three leaves and four leaves per plant.

Podophyllum hexandrum Royle syn. *P. emodi* Wall is an erect, glabrous, succulent, 15-60 cm tall herb with creeping rootstock. It bear 1-3 leaves (usually 2) which are alternate, long stalked, often purple spotted, round, 6-10 inch in diameter, deeply divided to the middle or base into 3-5 lobes, which are sharply toothed and often with deep incision (Figure 1). Flower large, 1.5to 2 inch in diameter, white or rose coloured, cup shaped, bisexual, actinomorphic and gamosepalous appearing at the same time as the leaves (Figure 2). Sepals 3-6, petaloid, petals 6-9 (rarely 4), stamen 6, anther opening by lateral slits, ovules many (Blatter, 1984; Hooker 1875; Airi *et al*., 1997).

The fruit is an ovoid berry 1-2.5 inch long, scarlet when ripe, with numerous seeds (Figure 3). Root system is perennial, which bear one aerial reproductive shoot and 4-5 vegetative shoots (Figure 4). Reproductive shoots generally have two exceptionally 3 leaves, whereas vegetative shoots bear a single leaf (Airi *et al.*, 1997). Chatterjee (1952), reported fruit of *Podophyllum hexandrum* as berry, orange or red 2.5 to 5 cm in diameter, with a short style, and is elliptic or oblong.

Figure 2: *Podophyllum hexandrum* plant at flowering stage.

Figure 3: Immature and Mature Berry of *Podophyllum hexandrum.*

The phenology of *Podophyllum hexandrum* is similar to that of other alpine and sub-alpine plants, where leaf emergence is at the onset of snow melt and dispersal of seed at the onset of winter. During winter, the plant is in its dormant condition and perenniates in the rhizomatous form. In spring, the plant has to produce flowers, successfully pollinate and reproduce/disperse seeds within a short span of 4 months (Rajkumar and Ahuja, 2010).

In India, *Podophyllum hexandrum* has been reported to be distributed in all the Himalayan states like Jammu & Kashmir, Himachal Pradesh, Uttrakhand and Sikkim. In Jammu & Kashmir it is reported to occur at, Daitwas forest; Gilgit Gulmarg (2,700-3,000 m); Jagran river bank between Kundi & Shikar (3,000-3,600 m) Kishenganga valley; Kanasar, Jhelum basin (2,400-2,700 m); Khelanmarg (2,700-3,000 m); Lidwas; Muzafarabad range forest (2,400 m); Sind Valley; Tanmarg forest (2,200-2,600 m); Zaskar (3,500 m); Mechigaon, Zozila pass (3,500 m). Trumba, Dagoum, Chandanwadi, Seshnag, Kargil, Pissughile, Pahalgam, Tanmarga. In Himachal Pradesh it is reported from, Chamba, Chulkot forest (3,000 m), Killar, Pangi, Kilar pass and Pangi, Sach valley and Pass (Chamba); Pulga, Haranghati pass (3,600 m), Pandrabis (2,400 m) (Bashar); Kala Tope forest (2,438 m), Keylong, Kullu, Lahaul, Pulga (2,400 m) (Kangra); Matian, in Shali hills, Narkunda, Dencho, Sissoo, Koksar, Dalhousie, Shimla. In Uttarakand, it is reported from, Deoban (2700 m), Kanjatra (2,600 m), Konain (Dt. Dehra Dun); Rudgaria Gar (4,000-4,300 m), Bhillangana, Panwali (Dt. Tehri); Jamnotri, Jamunachatti, Barkot (in Yamuna valley), Dodital, Gaumukh, KedarKanta (3,000-3,300 m) (Uttarkashi); Dasoli, Mundali (2,300 m), Bhyander, Hemkund (Dt. Chamoli); Madhya Maheshwar, Tunganath (Dt. Rudraprayag); Pindari glacier (Almora); Kuti, Yankti river valley (3,700-4,000 m), Bogudiar (2,400 m) (Pithoragarh). In Sikkim it is reported to occurs at Chamnaga (3,600 m), Thangu, Tsomgo, Chanaga, Thangu (3000-4200 m) (Shah, 2006).

Figure 4: Rootstock of *Podophyllum hexandrum* showing roots and rhizome.

CHEMICAL CONSTITUENT OF PODOPHYLLUM: Lignans are an important group of natural products occurring in different plants species. Podophyllotoxin is the most important lignan due to its therapeutic value. Plants belonging to family Berberidaceae containing podophyllotoxin and other related lignans arelisted in table 1.

Table 1: Podophyllotoxin and other related lignans isolated from plants of family berberidaceae.

Species	Plant part	DOP (%)	PT (%)	α-PEL (%)	Reference(s)
Diphylleiacymosa	Leaf		0.54	*Diphyl-*	Broomhead and Dewick, 1990
Diphylleiagrayi	Root		1.27	0.126	Broomhead and Dewick, 1990
Podophyllum hexandrum	Root/ Rhizome	0.017	4.27	0.01	Broomhead and Dewick, 1990
Podophyllum peltatum	Root/ Rhizome	0.023	0.25	0.33	Broomhead and Dewick, 1990
Podophyllum pleianthum	Root/ Rhizome	0.01	0.135		Broomhead and Dewick, 1990
Podophyllum/Dysosmaversipellis	Root/ Rhizome	DA	0.32	1.35	Broomhead and Dewick, 1990 Yu *et al.*, 1991

DOP = Desoxypodophyllotoxin
PT = Podophyllotoxin
α-PEL = α-Paltatin

Though *Podophyllum* lignans are present in several species of *Podophyllum* but the commercially exploitable species include only *Podophyllum hexandrum* and *Podophyllum peltatum*. Between these two species *Podophyllum hexandrum* is commercially more important due to high podophyllotoxin content present in its roots and rhizomes.

The Indian species *Podophyllum hexandrum* contains three times more podophyllotoxin than its American counterpart *Podophyllum peltatum*, which contains other lignansviz α and β peltatins (Bhattacharyya *et al.*, 2012). The Indian *Podophyllum* yields 7-15 % resin as compared to American *Podophyllum* which yields only 4-8 % resin (Thakur *et al*., 2010 and Qazi*et al*., 2011). The different lignans isolated from roots and rhizomes of *P. hexandrum* are: 1) Podophyllotoxin; 2) 4'-Demethylpodophyllotoxin 3) Desoxypodophyllotoxin; 4) 4'-Demethyldesoxypodophyllotoxin; 5) β-Peltatin; 6) α-Peltatin; 7) Podophyllotoxone; 8) 4'-Demethylpodophyllotoxone; 9) Isopicropodophyllone and 10) 4'- Demethylisopicropodophyllone (Anonymous, 1969; Dewick and Jackson, 1981; Husain, 1993). In addition to lignans other compounds like quercetin, kaempferol, astragalin, essential oil, waxes and mineral salts have also been reported to be present in *P. hexandrum* (Anonymous, 1969; Husain, 1993).

MORPHO-CHEMICAL VARIABILITY: In *Podophyllum hexandrum*, considerable morphological variation in plant height, leaf characteristics, fruit weight, seed weight and colour and other traits has been observed in Garhwal Himalayas by Bhadula *et al*. (1996). They identified six morphological variants in the Kedarnath population, five each in the Chopta and Ghangaria population, four each in Valley of Flowers and Dayara and two in the population from Tungnath on the basis of shape, size, lobing, number of leaves and seed colour. They further found that within each population, individual plant differed in leaf characteristics and seed colour ranging from black, brown to pink.

Plants of *Podophyllum hexandrum* bearing one, two, three and four leaves were observed in Uttarakhand region by Purohit *et al*. (1998). They observed variability in germination behavior of seed of two leaved, three leaved and four leaved plants. The germination in seeds of two leaved plants was epigeal whereas seed germination was hypogeal in three and four leaved plants. They further observed variation in number of seeds per berry in two leaved, three leaved and four leaved plant.

Mahajan (2004), observed six broad morphotype based on number of leaves and berry position. The identified morphotype are: single leaf plants (with berry and without berry), two leaved plants (with berry position axillary and extra axillary) and three leaved plants (with berry position axillary and extra axillary).

Alam*et al*. (2008) studied the genetic diversity among and within 28 populations of *Podophyllum hexandrum* from North-West region of Himalayas using ISSR-PCR markers and found high genetic diversity at the population level but low within individual study population. They further observed that 48% of genetic diversity among the study population was attributed to

geographical locations while 29% was attributed to differences in their habitat.

Alam *et al*. (2009) found morphological variation on the basis of number of leaves per plant in *Podophyllum hexandrum* samples collected from 11 forest division of Himachal Pradesh. They observed that 39.5% plants had single leaves, 30% has two leaves, 20% had three leaves and 10.5% has four leaved. The plant bearing single leaf did not bear fruit.

Purohit*et al*. (1999) found variation in resin and podophyllotoxin content in different population of *Podophyllum hexandrum* from Central Himalayas at altitude ranging from 1800 m to 3800 m amsl. They observed that resin and the toxin content were highest in plant with one leaf and lowest with those in four leaves. They further reported podophyllotoxin content in rhizome ranging from 5.18% to 8.26% in different morphological variants. Singh (1964), found resin content in roots of *Podophyllum hexandrum* ranging from 5.1% to 17% in plants collected from different locations.

Resin yield has been found to depend on the locality as well as on season of collection. Resin content has been found highest (about 14%) in May (flowering stage), which decreases to eight per cent in September and to about seven per cent in November, when the plants bear fruits (Chatterjee, 1952).

Considerable variation in Podophyllotoxin content has been observed in plants of *Podophyllum hexandrum* collected from different geographical locations by various workers. Sharma (2013) reported variation in podophyllotoxin content in leaves and rhizome from different sites of Himachal Pradesh as Marhi (3300 m) 0.30 %, Rohtang (3978 m) 0.22%, Koksar (3160 m) 0.05% and Kukumsari (2730 m) 0.097% in leaves and Marhi (3300 m) 5.87%, Rohtang (3978 m) 3.44%, Koksar (3160 m) 4.6% and Kukumsari (2730 m) 4.7% in rhizome.

Populations of *Podophyllum hexandrum*, collected from alpine region, have been observed with highest toxin content (Purohit*et al*., 1999). Substantial decrease in podophyllotoxin and resin contents was observed when plants collected from higher altitudes were planted at low altitudes (Sharma *et al*., 2000). However, only minor difference in podophyllotoxin content of *P. hexandrum* was observed between wild plants and those cultivated in the region of natural habitat (Prasad, 2000).

Genetic diversity among *Podophyllum hexandrum* genotype of North-Western Himalayas region for podophyllotoxin content was studied by Alam*et al*. (2009). In their study, they studied 28 genotypes from 11 forest division of Himachal Pradesh and found highest podophyllotoxin content (8.86 to 9.53% on dry wt basis) in the roots collected from Lahaul forest division (altitude 4300 m) and lowest (3.02 to 4.75% on dry wt basis) from Parvati forest division (altitude 1300 m). Further they observed change in Podophyllotoxin content with the change in altitude, being maximum in higher altitude and minimum in lower altitude.

Purohit *et al*. (1998) reported variation in podophyllotoxin content in the different morphotype of *Podophyllum hexandrum* as 8.26% in single leaf plant, 7.86% in two leaved plant and 6.20% in three leaved plant.

Shah (2006), cited that *Podophyllum hexandrum* is a very good substitute of American *Podophyllum* and contains resin three times higher. He cited concentration of podophyllotoxin from various site of Himachal Pradesh as: 2.8% from Kullu, 2.9 % from Hazra, 3.5 % from Bashar and 4.7 % from Chamba.

EXTRACTION, ISOLATION AND PURIFICATION OF PODOPHYLLOTOXIN AND OTHER LIGNANS: Active constituents of *Podophyllum* are present in the underground parts in the form of resin called podophyllin (Anonymous, 1955; Husain, 1993). Podophyllin was included for the first time in U.S. pharmacopoeia in 1820 as a cathartic and cholagogue (Ayres and Loike, 1990). The most important constituent present in *Podophyllum* resin is the cytotoxic lignin podophyllotoxin (Anonymous, 1969; Hussain, 1993). Hartwell *et al.* (1953) reported the presence of podophyllotoxin in the needles of *Juniperus sp.* all belonging to section *sabina*. Prior to the isolation of podophyllotoxin from the needles of *Juniperus sp.*, the extract of needles had been found to cause tumour hemorrhage and necrosis.

Podophyllotoxin in the impure form was isolated for the first time by Podwyssotski (1880). Podophyllin is prepared by pouring an alcoholic extract (in semi-solid form) of the drug in acidulated water. The precipitated mass on filtration followed by drying gives podophyllin (Anonymous, 1955). Podophyllotoxin can be extracted from podophyllin with solvents like chloroform and alcohol. Removal of the solvents gives podophyllotoxin, which can be crystallized from solvents like methanol and benzene (Chatterjee, 1952; Hokanson, 1978).

Podophyllin is amorphous powder varying in colour from light brown to greenish yellow or greenish grey mass. It possesses characteristic odour with bitter and acrid taste and becomes darker above 25^{o}C and on exposure to light (Anonymous, 1955; Anonymous,

1973; Anonymous, 1989). Podophyllin is practically insoluble in cold water but partly soluble in hot water, solvent ether, chloroform, dilute ammonia solution and completely soluble in alcohol (Anonymous,1966; Anonymous; 1989).

Lignans can be separated by TLC using silica gel as a stationary phase and chloroform: methanol (9:1, 10:1, 25:1) or toulene: ethyl acetate (5:7) as the mobile phases (Dewick and Jackson, 1981; Broomhead and Dewick, 1990; Van-Uden *et al*., 1990; Anonymous, 1999). Better resolution of different lignans on TLC plates can also be achieved by carrying out double development in solvent systems of chloroform: methanol (90:10) for 6 cm and then in toulene: acetone (65:35) for 15 cm (Anonymous, 1999).

The lignans can be detected on fluorescent silica gel coated TLC plates by UV light at 254 nm. Detection on TLC plates can also be made by using sulfuric acid: methanol/ethanol (1:1) as the spraying reagent, followed by heating at 110^0C for 10 min. when characteristic dark blue or purple coloured spots appear for lignans (Woerdenberg *et al*., 1990; Wichers *et al*., 1991). Spraying of TLC plates with a mixture of conc. nitric acid and acetic acid (3:10) immediately gives red coloured spots for some lignans (4'-demethylpodophyllotoxin, 4'-demethyldesoxypodophyllotoxin, α-peltain, 4'-demethylpodophyllotoxone and 4'-demethylisopicropodophyllone whereas, other lignans (podophyllotoxin, desoxypodophyllotoxin, β-peltatin, podophyllotoxone and isopicropodophyllone) give red spots after heating. Peltatins (α-and β-) give brown coloured spots (Jackson and Dewick, 1985). Peltatins can also be detected on TLC plates by spraying with fast blue reagent (Anonymous, 1999).

High performance liquid chromatography (HPLC) is the most common and widely applied method for quantitative analysis of lignans. Commonly used columns in HPLC system for the analysis of podophyllotoxin and other lignans are Silicon packed C-18 column or Nova pack C-18 Cartridge column or lichrosorb RP-18 (Chrompack) column with solvent systems for mobile phase as acetonitrile: water: methanol (37:58:15) or methanol: water (62:38; 40:60) or methanol alone (Purohit*et al*., 1998; Sharma *et al*., 2000). Podophyllotoxin is detected at wavelengths of 290, 286, 280 and 230 nm.

Separation of podophyllotoxin from roots of *P. hexandrum* has also been carried out by using supercritical CO_2 gas. It was found that podophyllotoxin was not completely extracted by pure super critical CO_2, however, use of methanol as a modifier has been found to enhance the extraction of podophyllotoxin (Choi *et al*., 1998).

High performance liquid chromatography (HPLC) is widely applied method for the quantitative and qualitative analysis of lignans. Methods use a reversed phase (RP-18) stationary phase. Use of straight phase columns, which involved silica 60 columns with aeluens consisting of heptane, $CHCl_3$, and MeOH. The routinely applied reversed phase columns; e.g. Lichrosorb or Nucleosil are combined with an isocratic mobile phase consisting of water and MeOH or MeCN. In some cases a gradient is used to enable changes in the H_2O / MeOH or H_2O / MeCN ratios **(**Rijksuniversiteit Groningen, 2003).

Sharma (2013), carried out HPLC analysis of *Podophyllum hexandrum* samples with Waters HPLC System 600 gradient pump; Waters 717 plus autosampler; 996 PDA detector; Empower Version 2 Software. Separation was done on RP-18e (LiCrosphere,5µm,250 x 4.0 nm) Merck made column. The mobile phase consisted of acetonitrile : water (40:60 v/v) in isocratic elution with flow rate 1ml/ min. injection volume of standard and samples was 10µL and run time was 20 min.

CULTIVATION TECHNIQUES: *Podophyllum hexandrum* grows well in organic rich black soils with sufficient moisture. Partially shaded places favour survival and growth of the plants at lower altitudes. *Podophyllum hexandrum* can be propagated by seeds as well as from sections of rhizomes (Nautiyal and Nautiyal, 2003, Qazi *et al*., 2011, Sreenivasulu*et al*., 2009, Chatterjee, 1952). Under natural conditions seeds show erratic and poor germination. The seeds germinate after remaining dormant for one or two years. The main reason for poor seed germination seems to be postharvest care of seeds. Nautiyal *et al.* (1987) observed no germination in seeds extracted from fresh berries, however, germination was recorded when fresh berries were used as such for germination. Seeds washed with water also showed better germination than unwashed seeds (Bhadula *et al*., 1996).

Purohit and Nautiyal (1988) found inhibitory effect of cotyledons on plumule development in *P. hexandrum*. Bhadula*et al.* (1996) observed inhibitory effect of fruit pulp on seed germination and removal of seed coat was found helpful in enhancing seed germination.

Nadeem *et al.* (2000) observed five times more seed germination of *P. hexandrum* when seeds were treated with sodium hypochlorite and two times more germination by treating the seeds with a combination of GA_3 and IBA.

Due to delayed and poor seed germination, multiplication of *P. hexandrum* is preferred through rhizome cuttings. The youngest top portion of the rhizome cuttings of 1.0-2.5 cm in length, bearing leaf primordium lead to better sprouting in *Podophyllum hexandrum* when planted in June-July in well prepared soil at a spacing of 30-30 cm (Nautiyal and Nautiyal, 2003).

Treatment of apical segments with IBA or NAA increases rooting percentage and also results in multiple root formation (Nadeem *et al.*, 2000). Roush and Russell (1976) observed that treatment of dormant rhizomes of *Podophyllum peltatum* with various concentrations of GA_3, IBA separately or in combination helps to induce bud and aerial stem formation and also in establishment of new root system. GA_3 also shows marked effect in inducing uniform sprouting and flowering in rhizomes of *Podophyllum hexandrum* grown at lower altitudes (Pandey *et al.*, 2001). Chaurasia*et al.* (2012) reported that vegetative propagation of May Apple is by rhizome cuttings or by micropropagation using the terminal buds as the source of explants-including adventitious buds with 70-90% success rate in soil acclimatization.

MEDICINAL USES: *Podophyllum hexandrum* is an important pharmacopoeial drug plant (Anonymous, 1966) that is highly sought after by the pharmaceutical industry for its anti-cancer, anti-fungal and immune-modulatory properties. Ripe fruit of *Podophyllum* are edible and used as a cough remedy (Chatterjee, 1952). Tea prepared from roots is effective in controlling constipation. Root paste is applied on ulcer, cuts and wounds. Roots are also used in jaundice, syphilis, fever, liver ailments and cancer (Bhattacharjee, 2001).

Rhizomes of *Podophyllum hexandrum* yield podophyllotoxin, an active ingredient used as a starting compound for the chemical synthesis of etoposide and tenioposide, compounds that are effective in the treatment of lung cancer, a variety of leukemias, and other solid tumours. The rhizomes, administered through suitable forms exhibit antioxidative and radioprotective properties in several animal experimental systems. Rhizome/root preparations of *P. hexandrum* are commonly used by tribal people of the Indian Himalayan Region to cure a range of ailments, such as hepatic disorders, gastric ulcer, gangrene, and constipation (Sharma *et al.*, 2010).

Podophyllotoxin and other lignans from Podophyllum species have been found to be clinically unsuitable as anticancer drugs due to their high toxicity. However, semisynthetic derivatives of podophyllotoxin namely etoposide, teniposide, and etopophos (etoposide phosphate) have proved excellent antitumour agents. Etoposide has been found useful in treatment of small cell lung cancer, testicular cancer, kaposis, sarcoma, lymphoma and leukemia, whereas teniposide has been used to treat acute lymphatic leukemia, neuroblastoma in children, non Hodgkin's lymphoma brain tumour in children (Husain,1993).

The resin is a slow but active purgative, producing copious liquid discharge, often with much griping. The activity is due to podophylloresin. Podophyllotoxin is more toxic, while podophylloresin is more purgative than toxic. In small doses the resin from the rhizomes is used in chronic constipation, especially mixed with aloe and cascara. (Chatterjee, 1952)

Podophyllum hexandrum extract have been found to offer radioprotection by modulating free radicals flux involving the role of lignans present. (Sultan *et al.*, 2010)

Kumar and Goel (2000), found anti-oxidant properties of *Podophyllum* which are seen due to chelation and modulation of redox state of iron ions and these may contribute towards its radioprotective manifestation.

The roots are used for diseases of liver and as purgative in the form of pills in case of chronic constipation. It is a drastic but a slow acting purgative. Active principle contained in it is being experimented upon by foreign concerns for production of a medicine to treat cancer (Singh, 1964).

Podophyllum resin has been used by diverse cultures since remote times as antidotes against poisons, or as cathartic, purgative, antihelminthic, vesicant, and suicidal agents. Antitumor activity is another outstanding property of Podophyllotoxin. It is effective in the treatment of Wilms tumours, different types of genital tumors (*Carcinoma verrucosus*) and in non-Hodgkin and other lymphomas and lung Cancer.Podophyllotoxin and some of its isomers have been tested for other activities such as insecticidal, phytogrowth inhibitory, antitoxic and antiparasitic activities (Shri ram, 2010).

The importance of podophyllotoxin as a potent antitumour agent when affixed to a glucopyranose moiety is well known. Beside many derivatives of podophyllotoxin have been investigated for antitumor properties. The lignans also posses anti-fungal and anti-viral activities. The resinous mixture (podophyllin) is effective in the treatment of genital warts (Anonymous 1999).

Fruits of *Podophyllum hexandrum* are used for cough remedies. Beside its use in curing cancer, *podophyllum* resin is also used as blood purifier, antibile medicine and hepatic stimulant for relief for constipation, skin diseases and tumors and as a tonic. Like colchi-

cines it is used in cytological work for affecting spindle formation and dispersing chromosomes (Purohit*et al*., 1998).

Nadeem *et al*. (2000) reported that the human veneral warts (*Condylomaacuminatum*) could be cured with topical application of podophyllin oil, the root extract of this plant has long been used by the Himalayan natives and the American Indians as a cathartic cholague.

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