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Abstract:
Podophyllotoxin is derived from plant sources and exhibits strong anticancer activity. However, limited natural availability and environmental impacts from traditional extraction methods drive the search for alternative production approaches. This review explores diverse strategies for sustainable podophyllotoxin synthesis, including biosynthesis, semi-synthesis, and biotransformation. Biosynthetic methods involve metabolic pathway engineering in plant or microbial cells, enabling increased yields by manipulating precursor availability and gene expression. Semi-synthetic approaches modify podophyllotoxin precursors or intermediates to enhance therapeutic effects, with derivatives like etoposide and teniposide showing clinical efficacy. Biotransformation, utilizing organisms such as endophytic fungi or human hepatic enzymes, enables the transformation of substrates like deoxypodophyllotoxin into podophyllotoxin or its derivatives, yielding compounds with reduced environmental impact and improved purity. The anticancer efficacy of podophyllotoxin and its derivatives stems from multiple mechanisms. These compounds disrupt cell mitosis by inhibiting microtubule assembly, impairing nucleoside transport, and blocking topoisomerase II activity, leading to DNA cleavage and cancer cell apoptosis. Podophyllotoxin and its derivatives also exhibit anti-angiogenesis and anti-metastatic effects through signalling pathway modulation. Notably, derivatives like deoxypodophyllotoxin utilize advanced delivery systems, enhancing targeted efficacy and reducing side effects. Given the varied mechanisms and growing therapeutic applications, optimizing biotransformation and delivery techniques remains essential for advancing podophyllotoxin-based therapies. This comprehensive review underscores the compound's potential as a robust anticancer agent and the need for continued research to maximize its production and clinical effectiveness.

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Conceptualization, critical analysis, interpretation of the data, critical revision of the review. M S Reddy

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Comprehensive Biotechnological Strategies for Podophyllotoxin Production from Plant and Microbial Sources

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Abstract

Podophyllotoxin is derived from plant sources and exhibits strong anticancer activity. However, limited natural availability and environmental impacts from traditional extraction methods drive the search for alternative production approaches. This review explores diverse strategies for sustainable podophyllotoxin synthesis, including biosynthesis, semi-synthesis, and biotransformation. Biosynthetic methods involve metabolic pathway engineering in plant or microbial cells, enabling increased yields by manipulating precursor availability and gene expression. Semi-synthetic approaches modify podophyllotoxin precursors or intermediates to enhance therapeutic effects, with derivatives like etoposide and teniposide showing clinical efficacy. Biotransformation, utilizing organisms such as endophytic fungi or human hepatic enzymes, enables the transformation of substrates like deoxypodophyllotoxin into podophyllotoxin or its derivatives, yielding compounds with reduced environmental impact and improved purity. The anticancer efficacy of podophyllotoxin and its derivatives stems from multiple mechanisms. These compounds disrupt cell mitosis by inhibiting microtubule assembly, impairing nucleoside transport, and blocking topoisomerase II activity, leading to DNA cleavage and cancer cell apoptosis. Podophyllotoxin and its derivatives also exhibit anti-angiogenesis and anti-metastatic effects through signalling pathway modulation. Notably, derivatives like deoxypodophyllotoxin utilize advanced delivery systems, enhancing targeted efficacy and reducing side effects. Given the varied mechanisms and growing therapeutic applications, optimizing biotransformation and delivery techniques remains essential for advancing podophyllotoxin-based therapies. This comprehensive review underscores the compound's potential as a robust anticancer agent and the need for continued research to maximize its production and clinical effectiveness.

Keywords: Podophyllotoxin, *Podophyllum hexandrum,* Berberidaceae, Natural product, Endophytic fungi, Bioproduction

Introduction

Plants have been playing an important role in human lives since the onset of civilizations for food, shelter, and healing. In ancient times, the discovery of medicinal plants resulted from trial-and-error methods to find their biological activities [1]. The therapeutic capability of medicinal plants has been widely recognized in recent times, with increasing experimental proofs. Since plants are easily accessible and affordable, there is an increased interest in bioprospecting, consumption, and drug discovery of medically important plants.

In the present times, numerous plant-derived drugs have been discovered, and due to their high demand, over-extraction has led to a biodiversity deficit and environmental degradation [2]. Many endophytic fungi isolated from medicinal plants help combat the decline of medicinal plants; thus, alternative sources like endophytes capable of producing compounds need to be explored.

Endophytes are microorganisms, often fungi or bacteria, that reside in a plants inter and intracellular location and have an endosymbiotic relationship with the plant. Endophytes inhabit the plant for all or a part of their life cycle and usually do not cause any harm to the host. They are abundant in nature and demonstrate complex relationships with their hosts, namely mutualism, antagonism, and sometimes parasitism. These microorganisms improve resistance to pests and insects and augment the ability of the host to withstand various types of stress factors, such as increasing drought tolerance, high salinity, high/low temperature, low pH, and heavy metals stress in the soil [3]. Studies have ascertained the existence of one or more endophytes in all the plants analysed [4].

Plant endophytic fungi have been known to be an essential part of the plant micro-ecosystems. Plant and endophytic fungi live in a symbiotic relationship. The host supplies nutrition and habitat to the fungi, and in return, fungi help the host resist various types of stress by producing biologically active metabolites. Since 1993, with the discovery of Taxol from *Taxomyces andreanae*, researchers have been interested in fungal endophytes as a source for novel bioactive products. In the last 20 years, a plethora of biologically active compounds have been isolated from endophytic fungi and have been classified as quinones, alkaloids, lactones, lignans, terpenoids, phenols, and steroids [5]. These compounds have diverse biological activities like anticancer, antidiabetic, immunosuppressant, antioxidant, antimicrobial, antiproliferative, and insecticidal properties, and they are also employed in biofuel production.

Extract of the plants of the genus *Podophyllum* (Berberidaceae) have been extensively used in traditional medicine and modern medicine for a range of diseases like periodontitis, perianal and venereal warts, skin disorders, coughs, certain tumours, lymphadenopathy, snake bites, and several intestinal worm diseases. The genus *Podophyllum* contains two species that are mainly used for commercial production of podophyllotoxin: *Podophyllum peltatum* Linnaeus and *Podophyllum hexandrum* Royle. *P. hexandrum* is considered as better source due to its higher resin yield compared to *P. peltatum* [6]. The first account of the antiviral activity of podophyllin (resin that contains podophyllotoxin) was in 1942 when it was used to treat venereal wart (*Condyloma acuminatum*), which is caused by papilloma virus. Later, in several studies, researchers tested the antiviral activity of podophyllotoxin against HSV-1, Sindbis virus, the measles virus, human immunodeficiency virus (HIV), murine cytomegalovirus (a herpes DNA virus), and vesicular stomatitis virus and found that podophyllotoxin analogues exhibited antiviral activity because of their capability to bind tubulin, disrupting the cellular cytoskeleton and the viral replication [7]. Podophyllotoxin is also effective against psoriasis vulgaris and to treat anogenital warts caused by *Molluscum contagiosum* that affects children and HIV patients. Podophyllotoxin-related analogues, etoposide and teniposide, have been reported to have exceptional anticancer activity. These compounds are utilized to treat a variety of cancers, including genital tumours, Wilms tumours, lung cancer, and non-Hodgkin lymphomas, as well as other lymphatic malignancies [8]. Anticancer activity of podophyllotoxin is accredited to either podophyllotoxin binding to tubulin during mitosis leading to inhibited microtubule assembly, or to its ability to inhibit the activity of DNA-topoisomerase II [9]. Podophyllotoxin derivatives have also exhibited immunosuppressive activity. They also have been tested for phytogrowth inhibitory activity, insecticidal activity, and ichthyotoxic activity [10]. The diversity in the biological and medicinal applications demonstrated by podophyllotoxin and its derivatives is remarkable. Further research on the compound may lead to new information and medicinal applications.

Methods

The literature review on *Podophyllum* species and the antiproliferative drug podophyllotoxin was conducted using multiple online databases, including PubMed, Scopus, and Google Scholar. A comprehensive search strategy employed keywords such as *Podophyllum hexandrum*, podophyllotoxin, podophyllotoxin derivatives, anticancer activity of podophyllotoxin, podophyllotoxin mode of action, endophytic fungi producing podophyllotoxin, co-cultivation fermentation of podophyllotoxin, and bioprocess optimization

for podophyllotoxin. Articles published approximately within the last 25 years were prioritized, with older studies included when deemed relevant. The inclusion criteria prioritized primary research articles and reviews that explored the chemistry, pharmacology, and production methods of podophyllotoxin and its derivatives. Studies addressing bioproduction advancements, including tissue culture, endophytic fungi, genome mining, and bioprocess optimization, were also considered. Exclusion criteria involved non-English articles and papers lacking full-text access. The review aimed to extract and synthesize data on the historical development, mechanisms of action, production techniques, and anticancer efficacy of podophyllotoxin to provide a detailed understanding of its potential and advancements in cancer treatment research.

Chronology of Podophyllotoxin as an Antiproliferative Drug

Historical Development

Podophyllin, a bitter-tasting resin made from the rhizomes and roots of the *Podophyllum* plants (*Podophyllum peltatum*, *Podophyllum hexandrum* and *Podophyllum emodi*), first came into light in 1753 when Carl Linnaeus described *Podophyllum* as a genus. Different parts of the *Podophyllum*, like roots, rhizomes, and fruits, treat ulcers, minor cuts/ wounds, constipation, tuberculosis, and anticancer agents. The resin content in the roots or rhizomes of the source plant is 7-15%. Podophyllin can be collected from the leaves with content varying from 7.8 to 9.7%, which is the primary source of the resin at the time of slow growth or overuse of the roots and rhizome. Podophyllin, collected freshly, has a higher amount of active compounds and a longer shelf life. It is used to treat some ailments like lesions in the skin or neoplasms arising in the body where topical therapy can be executed. The resin is also used against dermatological infections and gives symptomatic relief to skin inflammation and some allergies. It is also a vermifuge used against large roundworms (*Ascaris lumbricoides*); it first stimulates, paralyzes, and finally kills the worm [11].

In the search for alternative plant sources, some recent studies reported the isolation of podophyllotoxin from some other species, such as *Linum*, *Juniperus*, *Nepeta*, and *Dysosma* [7]. The primary plant source of podophyllotoxin is roots or rhizomes; however, its presence has been reported in these plants' seeds, fruits, leaves, and stems. Podophyllotoxin content in the Indian *Podophyllum* is more than double that of the American *Podophyllum*. However, α- β-peltatins isolated from *P. peltatum* are absent in *P. hexandrum* [7]. Valerian Podwyssotzki [12]

was the first to isolate and study podophyllotoxin (Figure 1a). Kaplan [13] reported the use of podophyllin to treat Condyloma acuminatum (genital wart), which is the medicine of choice for this disease. Podophyllotoxin has reportedly been used as a laxative and treatment for various ailments, such as tuberculosis, psoriasis, cough, syphilis, gonorrhoea, and menstrual disorders [12]. Since the 1980s and 1990s, the U.S. FDA approved the podophyllin drug "etoposide" for the treatment of small cell lung cancer (VP-16), “teniposide” (VM-26) and etopophos. Ammonium salts have been developed successively since the launch of etopophos [14].

Evolution of Podophyllotoxin Derivatives

Podophyllotoxin became the compound of interest as an antimitotic agent when the preliminary studies on podophyllotoxin reported distinct activity against cancer cells. However, further clinical research showed that podophyllotoxin had detrimental side effects such as bone marrow suppression, gastrointestinal toxicity, hair loss, and neurotoxicity, which led the research towards the discovery of less-toxic analogues.

In 1967, a derivative of podophyllotoxin, etoposide (Figure 1b), was discovered; in 1971, teniposide (Figure 1c) was found. The interaction between this drug and DNA came to light 20 years later, and the mediation by topoisomerase II was identified [15,16]. From 1984 to 2000, multiple promising chemotherapeutic drugs have been developed, like etopophos (Figure 1d), GL-331(Figure 1e), NK611(Figure 1f), NPF (Figure 1g), and TOP53 (Figure 1h) [17]. Etoposide has an IC_{50} value of 30.16 μM and 0.051 μM against HepG2 and MOLT-3 cancer cells, respectively [18]. It also exhibits an IC_{50} value of 43.74 ± 5.13, 209.90 ± 13.42, and 139.54 ± 7.05 μM against tumour cell lines of BGC-823, HeLa, and A549, respectively [19]. As per the Genomics of Drug Sensitivity in Cancer database, Teniposide has demonstrated IC_{50} values of 3.024 and 0.407 μM against tumour cell lines of HeLa and A549, respectively. The standard dose of etoposide is 300–600 mg/m^2, which is administered intravenously over 3-5 days and repeated every 3-4 weeks. Teniposide has a similar mode of administration and schedule for adults with a standard dose of 300 mg/m^{2,} and for children, the dose is 150-200 mg/m^2 weekly or 100 mg/m^2 twice every week [20].

From 1984 to 2000, multiple compounds were developed, like NK611, GL-331, Azatoxin, TOP53, and Tafluposide [17]. Early clinical investigations of these compounds indicated a lack of efficacy or dose-limiting toxicities and, as a result of continued interest in this class of compounds, led to the identification of three newer compounds, namely QS-ZYX-1-61,

F14512, and Adva-27a, that have shown better anticancer efficacy in comparison to etoposide [21].

The diversity in the biological and medicinal applications demonstrated by podophyllotoxin and its derivatives is remarkable. Extracts of the *Podophyllum* plant have been extensively used in traditional medicine and modern medicine for a range of diseases like periodontitis, perianal and venereal warts, skin disorders, coughs, certain tumours, lymphadenopathy, snake bites, and several intestinal worm diseases [6]. Podophyllotoxin has dermatological significance as it is effective against psoriasis vulgaris and treats anogenital warts that affect children and HIV patients. It has also been tested for phytogrowth inhibitory, insecticidal, and ichthyotoxic activity. Podophyllin derivatives have also exhibited immunosuppressive activity and are used to treat malaria [10].

In 1942, podophyllin was used to treat papilloma virus-caused genital warts, which is when the first report of its use against antiviral activity was made [17]. Subsequently, podophyllotoxin's antiviral activity was examined in a number of studies against a variety of viruses, including HIV, vesicular stomatitis virus, measles virus, Sindbis virus, and HSV-1. It was discovered that podophyllotoxin analogues exhibited antiviral activity because of their ability to bind tubulin, which caused podophyllotoxin to disrupt the cellular cytoskeleton and thereby inhibited viral replication [17].

Etoposide was recently redeveloped as a drug to treat the cytokine storm in critical complications patients of the coronavirus disease (COVID-19) in phase II of the clinical trials by Boston Medical Centre (i.e., Clinical Trials NCT04356690) by conventional drug new use strategy [22]. Current research on the compound has shown promising results and may lead to new information and medicinal applications. Several attempts have been made to overcome poor biodistribution and its natural dose-limiting toxicity by producing derivatives or compositions of podophyllotoxin [17]. The resultant compounds have been found to have extensive applications as chemotherapeutic agents. Institutional researchers and pharmaceutical companies have shown great interest in podophyllotoxin studies, which can be corroborated by the increased number of patents filed to protect various inventions involving podophyllotoxin or its derivatives [17].

Recent research has focused on improving the podophyllotoxin's bioactivity and minimizing toxicity. Chemical modifications, commonly on the C-ring (mainly at the C4 and C5 positions),

have led to the development of hybrid molecules that exhibit better selectivity and reduced side effects, making them more viable for cancer treatment [14]. Hybridization with other natural compounds or synthetic molecules has shown promise in creating more potent derivatives with enhanced anticancer properties. These innovations are particularly valuable as they target drug-resistant cancer cells and can be combined with other anticancer drugs for more effective therapies [23].

Another area of progress is the nano-delivery systems, which represent the main strategies for enhancing the clinical viability of podophyllotoxin. Nano-carriers, including lipid- and polymer-based systems, have been extensively explored. These systems improve podophyllotoxin's water solubility, stability, and cellular uptake while enabling targeted delivery to tumor cells. For instance, encapsulating podophyllotoxin in nanocarriers improves its pharmacokinetic profile by allowing environment-responsive release and combination therapies. Nanocarriers like lipid-based and polymeric nanoparticles (NPs) offer high biocompatibility and degradability. Polymer-based systems, in particular, demonstrate superior stability, higher drug-loading capacity, and extended circulation time [24].

Mode of Action

Podophyllotoxin and its derivatives have been shown to have anticancer properties through their inhibition of tubulin and DNA topoisomerase II. Furthermore, an increasing interest in research on podophyllotoxin derivatives has demonstrated that a number of signalling pathways linked to cancer may be closely related to their anticancer properties.

Tubulin Inhibitor

With its strong anticancer action, podophyllotoxin has been demonstrated to impede microtubule assembly by inhibiting the colchicine binding site, which inhibits microtubule protein polymerization and induces G2/M blockage, as seen in Figure 2 [25]. It has also been demonstrated that podophyllotoxin derivatives possess the same capability [26]. Following the synthesis of a series of conjugates of 4-aza-2,3-didehydropodophyllotoxins, it demonstrated a considerable reduction in tubulin units by fluorescence tubulin polymerization study in which the podophyllotoxin-based conjugates induced caspase-3-dependent death in A549 cancer cells [27]. Similarly, Kandil et al. [28] developed a derivative of 4-azapodophyllotoxin that causes sub-micromolar disruption of the microtubule cytoskeleton in MCF-7 cancer cells. Comparably, 4β-[(5-substituted)-1,2,3,4-tetrazolyl] podophyllotoxin derivatives were created,

and at a mere 5μM concentration, they were able to suppress approximately 90% of tubulin polymerization [29]. Comparable in structure to podophyllotoxin, 6-methoxypodophyllotoxin was the subject of an investigation by Sadeghi et al. [30] on its anticancer impact. Applying 6-methoxypodophyllotoxin to human bladder cancer (5637) and myeloid leukaemia (K562) cell lines resulted in a notable decrease in tumour cell viability and the induction of programmed cell death. Furthermore, following 6-methoxypodophyllotoxin treatment, the expression of TOPIIA, a crucial nuclease in DNA replication, and TUBB3, a vital component of β-tubulin, was suppressed in tumour cells. Additionally, the carbon-sulphur bond at the 4-position (C-4) of the carbon ring of podophyllotoxin decreased the inhibitory effect of dosage on tubulin polymerization, boosting its therapeutic effect [31,32]. X-ray crystallographic research on compound 4β-NH-(6-aminoindole)-4-deoxy-podophyllotoxin indicated it could target the colchicine binding domain and the α-tubulin binding site. Notably, these podophyllotoxin compounds showed no appreciable toxicity in vivo and nanomolar anticancer effectiveness in vitro [31].

DNA Topoisomerase II inhibitor

Podophyllotoxin usage in cancer treatment has been restricted because of its significant side effects, which include gastrointestinal disturbance and high toxicity. Widespread use in cancer treatment has resulted from extensive structural alterations of podophyllotoxin, which have produced less toxic and more effective anticancer drugs like teniposide and etoposide [33,34]. They prevent topoisomerase II from degrading and eventually cause cell death by accumulating chromosomal breakage by stabilising the cleavage complex between the enzyme and its DNA substrate, as shown in Figure 3. Topoisomerases, specifically topo-I and topo-II, are a class of potentially effective anticancer targets involved in transcription and DNA replication. γ-H2AX is a traditional marker of double-stranded DNA breaks during DNA damage, and researchers generally consider that the bulky motif at the C-4 position of podophyllotoxin may be responsible for the suppression of topo II [35,36]. The novel podophyllotoxin analogue 4β-acrylamidopodophyllotoxin congeners were created by Kamal et al. [37] as a possible anticancer agent. Their findings demonstrated that whilst double-stranded DNA breaks were barely noticeable in podophyllotoxin-treated cells, a significant number of γ-H2AX foci were seen in cells treated with etoposide and this podophyllotoxin derivative. The compound synthesized by Shankaraiah et al. [38], using a simple one-pot approach, demonstrates selective DNA topoisomerase II inhibitory activity and anticancer efficacy against human prostate

cancer (DU-145) cell line. Furthermore, Reddy et al.'s [39] synthesis of 4bβ-aminotriazole podophyllotoxin derivative demonstrated potent cytotoxicity on prostate cancer cell lines and effective inhibition of DNA topoisomerase II. Similarly, β-carboline podophyllotoxin congeners shown remarkable cytotoxicity against human prostate cancer (DU-145) cell lines, and comet analysis, DNA binding investigations, and docking studies verified these congeners' capacity to block DNA topoisomerase II [40].

Countering Multidrug Resistance (MDR) in Cancer

A significant obstacle in cancer chemotherapy is the emergence of multidrug resistance, where cancer cells develop resistance to a multitude of anticancer drugs. Podophyllotoxin derivatives offer a promising strategy to circumvent multidrug resistance by targeting ATP-binding cassette (ABC) transporters, particularly the well-characterized P-glycoprotein, as seen in Figure 4. P-glycoprotein acts as an efflux pump, actively removing chemotherapeutic agents from the cell, diminishing their efficacy. Studies by Chen et al. [41,42] demonstrate the potency of podophyllotoxin derivatives against multidrug resistance cancer cell lines. These derivatives not only exhibited cytotoxicity but also downregulated the MDR-1 gene and P-glycoprotein expression, potentially explaining their ability to overcome multidrug resistance. This downregulation could be due to transcriptional repression or accelerated protein degradation of P-glycoprotein.

Modulators of Glucose Metabolism in Cancer

Cancer cells exhibit a distinct metabolic profile characterized by aerobic glycolysis, also known as the Warburg effect. Cancer cells generate energy rapidly, owing to this metabolic switch, in order to support their high proliferation rates, even in the presence of oxygen. While the precise effects of podophyllotoxin derivatives on glucose metabolism remain fully elucidated, some studies suggest the potential for targeting the Warburg effect, as shown in Figure 5. Tailor et al. [43] reported a novel podophyllotoxin derivative that directly activates AMPK (AMP-activated protein kinase), a master regulator of cellular metabolism. AMPK activation promotes mitochondrial oxidative phosphorylation, a more efficient ATP production pathway compared to aerobic glycolysis. SU212 may regulate the Warburg effect in triple-negative breast cancer cells through the AMPK/hypoxia-inducible factor 1α (HIF-1α) pathway. HIF-1α is a transcription factor that upregulates genes involved in glycolysis under hypoxic conditions. By targeting AMPK and potentially inhibiting HIF-1α, SU212 offers a promising strategy to

disrupt the Warburg effect and starve cancer cells of energy. Further investigations are needed to explore the broader effects of SU212 on cellular metabolism and its potential therapeutic efficacy in vivo.

Epithelial-Mesenchymal Transition (EMT) inhibitor

EMT is a critical step in cancer progression, enabling epithelial cancer cells to acquire mesenchymal features, such as increased motility and invasiveness, and ultimately promoting metastasis. Certain podophyllotoxin derivatives demonstrate the potential to inhibit EMT, offering a potential strategy to impede cancer spread, as shown in Figure 6. The natural podophyllotoxin analogue, 40-demethyl-deoxypodophyllotoxin glucoside (4DPG), increases the expression of checkpoint kinase 2 (Chk2), a tumour suppressor that suppresses EMT and metastasis by regulating key EMT-associated proteins [44]. Similar effects on Chk2 and EMT-related proteins were detected in human colorectal cancer cells treated with 4DPG by Katoch et al. [45]. Furthermore, Li et al. [46,47] designed podophyllotoxin derivatives ($Ptox^{Pdp}$, $Ptox^{Dpt}$) that inhibited EMT in hepatocellular carcinoma cells. These derivatives may function by downregulating the PI3K/AKT/mTOR pathway, a well-established signalling cascade known to promote EMT. Further research is needed to confirm these findings and explore the precise mechanisms by which podophyllotoxin derivatives modulate EMT signalling pathways.

Inducer of Apoptosis in Cancer Cells

Beyond overcoming MDR, podophyllotoxin derivatives exhibit potent pro-apoptotic properties, triggering programmed cell death in cancer cells, as shown in Figure 7. Their mechanism of action involves caspase activation, a well-established executioner pathway in apoptosis. Research has identified that podophyllotoxin derivatives can target various caspases with varying degrees of selectivity. For instance, DPMA specifically activates caspase-3 [48], while 4β-amidopodophyllotoxins target caspase-9 [49]. Interestingly, some podophyllotoxin derivatives, like OAMDP [50] and spin-labelled podophyllotoxin, can trigger the activation of multiple caspases (caspase-3 and -9) simultaneously, leading to a more robust and amplified cell death signal [51]. Additionally, podophyllotoxin derivatives may induce apoptosis through other signalling pathways beyond caspase activation. For example, Han et al. [52] reported that podophyllotoxin -norcantharidin hybrids induced cell cycle arrest and apoptosis in MCF-7 cancer cells, potentially via upregulation of cell cycle regulator CDK1 and downregulation of

cyclin B1, a protein essential for mitosis. Further studies are required to elucidate the complete spectrum of signalling pathways influenced by different podophyllotoxin derivatives.

Source and Production Methods of Podophyllotoxin

Extraction from Plant Species

Because podophyllotoxin has therapeutic value, research on its bioprospection across various plant and fungal species has been conducted. Both conventional and contemporary medical systems have documented the usage of *Podophyllum* extracts [7]. The family Berberidaceaceae, which includes *Podophyllum*, has been shown to contain podophyllotoxin [53]. In the past, the genus *Podophyllum* consisted of several species, now divided into three genera: *Sinopodophyllum*, *Dysosma*, and *Podophyllum*. The Chinese species is classified in the genus *Dysosma*, while the *Podophyllum* genus contains *P. peltatum* or American Mayapple, and the *Sinopodophyllum* genus has *Sinopodophyllum hexandrum*, or Indian Mayapple [54]. Because of its high podophyllotoxin concentration, Indian species are commercially sought and exploited more than others.

Furthermore, based on DNA fingerprinting, the Indian species verifies the existence of authentic genetic communities regardless of geographic dispersion [55]. According to data, Indian species adapted well to their changing surroundings. The selection of top germplasm and conservation studies was made possible by the availability of DNA fingerprinting data. It should be emphasised that *Podophyllum* and allied species differ not only in terms of their podophyllotoxin content but also in terms of their physical characteristics [56]. These variances impact both conservation tactics and species identification. In addition to Berberidaceae, Cupressaceae, Lamiaceae, Linaceae, Podophyllaceae, and Polygalaceae members have also been observed to contain podophyllotoxin, as can be seen in Table 1. The current study thoroughly analysed the family and parts used by these species. Even though these species contained lower levels of podophyllotoxin, they might still be used in industry through biotechnological techniques.

Since podophyllotoxin cannot be produced commercially through organic synthesis, it is instead isolated from the roots and rhizomes of the *Sinopodophyllum* and *Podophyllum* species [11]. However, the age, location, season, biotic, abiotic, and genotype of the species under study were all found to have an impact on the variation in podophyllotoxin content [79–81]. In addition to concentrating efforts on the conservation and propagation of high podophyllotoxin-

producing species, it may be anticipated that knowledge of the impact of diverse signals on podophyllotoxin accumulation and alteration will enable purposefully enhanced metabolite production.

Tissue and Cell Culture

Plant cell and culture techniques offer a controlled environment to grow plant cells and manipulate them to produce desired compounds like podophyllotoxin. This approach has several advantages, such as sustainability, scalability and control over optimizing the culture conditions, which can potentially help researchers increase podophyllotoxin yield. Table 2 describes podophyllotoxin production in the in-vitro culture system of different plant species.

According to reports, seed germination is the primary factor restricting a species' ability to spread. Nonetheless, it was said that the main obstacles to *S. hexandrum*'s propagation were seed dormancy, hypocotyl dormancy, endosperm limitation, and delayed seed germination [105,106]. To overcome these natural obstacles in *S. hexandrum*, a variety of strategies were used, including seed pretreatment, storage conditions, temperature, light and dark conditions, application of growth regulators, and seed scarification [107–114]. GA_3 was shown to be the most promising growth regulator in terms of improving seed germination and having a beneficial impact on the appearance of true leaves [105]. Hydroquinone treatment shortened the growth and developmental cycle of *S. hexandrum* seeds [96]. It was discovered that *P. peltatum* seeds consumed by seed dispersal devices germinated more quickly than those not consumed [115]. In several rhizomatous species, vegetative propagation is an effective method of reproduction. Practical strategies for vegetative multiplication of Indian Mayapple were reported, involving the treatment of rhizome segments with IBA, IAA, and NAA. In *S. hexandrum*, the GA3 treatment was the most effective in causing sprouting and flowering [81,105]. Rhizome cutting was another method of vegetative growth in *P. peltatum* that was reported to have occurred [116]. The American Mayapple's dormancy was reported to be overcome by low-temperature treatment, although the type of propagule and planting timing were found to influence the shoot emergence [117,118]. Plant tissue culture has also shown promise in getting beyond obstacles that have been put in the way. It is a valuable substitute for raising the status of endangered medicinal species. The first report of podophyllotoxin synthesis in vitro was found in *P. peltatum* [82]. In *S. hexandrum*, callus culture was established in 1989, and in 1990, a methodology for somatic embryogenesis, in vitro multiplication, and plant development was published [119,120]. Afterwards, several in vitro regeneration

protocols were created utilising explants such as buds, rhizomes, leaves, and roots [97,121]. An effective procedure for improving the rooting, hardening, and transplanting of specific species was also created [122]. Various research groups reported effective methods for *P. peltatum* and *L. album* in vitro experiments [65,123,124]. B5 and Murashige & Skoog media were widely utilised among the tried media. Podophyllotoxin is present in *S. hexandrum* callus, suspension, and cell culture [125,126].

Podophyllotoxin-Producing Endophytic Fungi

In addition to plant species, several endophytic fungi, including those isolated from *D. sinensis, S. hexandrum*, and *D. veitchii*, have also been found to produce podophyllotoxin [127]. Furthermore, it was discovered that two strains of *Phialocephala fortinii*, PPE5 and PPE7, isolated from the rhizomes of *P. peltatum* and *Alternaria* sp. from its host *Sabina vulgaris* both produced podophyllotoxin [128]. Furthermore, it was demonstrated that podophyllotoxin could be produced by *Trametes hirsuta*, *Fusarium solani*, and *Mucor fragilis* that were separated from *S. hexandrum* and *F. oxysporum* that was taken from *J. recurva* [129–132]. The research above indicated that there may be room for bioprospection of species not currently recognised as podophyllotoxin suppliers. Endophytic fungi's ability to produce secondary metabolites has drawn much interest. Notably, endophytic fungi produce anticancer chemicals such as taxol, camptothecin, rohitukine, and hypericin [133–136], which have created new avenues for the investigation of items obtained from plants. A list of podophyllotoxin-producing fungi, their host plants, and their yields are presented in Table 3. Since plant-based podophyllotoxin production has some drawbacks, the fungal endophytes have profound significance as fungal culture can be scaled up to enhance metabolite production.

However, the processes by which endophytic fungi produce secondary metabolites remain a mystery. There have been suggestions that the genes generating secondary metabolites can be found in plasmids, endophytic hypha, or endohyphal bacteria [144]. It is impossible to completely disregard the likelihood of horizontal gene transfer of secondary metabolic pathway genes from host plant species to endophytic fungus [145]. Therefore, future research must improve our understanding of the host-endophyte relationship, the conditions for metabolite production, the selection of suitable host plants, and transgenic strategies to facilitate the commercial exploitation of secondary metabolites producing endophytes. Further research will likely face challenges in identifying and characterising unknown metabolites endophytes produce.

Maximizing Bio-Production Strategies

However, to boost the production of metabolites from these species, it is crucial to carefully evaluate factors, including the extraction process, scaling-up techniques, and biological approaches, without compromising the quality of podophyllotoxin.

Molecular Manipulation of the Microbial Strain

Microbes play a vital role in various industries, from producing biofuels and pharmaceuticals to cleaning pollutants. However, their natural production capacity often falls short of industrial demands. Here, molecular manipulation emerges as a powerful tool for unlocking the full potential of microbes. By precisely altering their genetic makeup and metabolic pathways, scientists can engineer microbial strains for significantly enhanced production of desired compounds. This approach holds immense promise for revolutionizing various sectors and creating sustainable solutions for a growing world.

Outside of the plant system, metabolic engineering can effectively produce anticancer compounds. An approach utilizes hairy root cultures derived from *Agrobacterium rhizogenes* infection of *L. album*. This study compared cell suspension cultures and hairy root cultures for podophyllotoxin production. Transformed cultures with confirmed integration of specific *Agrobacterium* genes (ags but not rol) displayed superior growth and podophyllotoxin content. Notably, the maximum volumetric productivity achieved was 4.40 mg/L per day in cell suspension cultures and 2.75 mg/L per day in hairy root cultures [146].

Another study explored the use of *A. rhizogenes* for *P. hexandrum* transformation. Embryos of this plant were transformed with different *A. rhizogenes* strains, resulting in callus cultures with confirmed transformed status. These transformed cultures exhibited a three-fold increase in podophyllotoxin content compared to untransformed controls, demonstrating the potential of this method for enhancing podophyllotoxin production [147].

A study evaluated molecular manipulation of the microbial strain to create anticancer medications [148]. Through the heterologous expression of three hepatic P450 enzymes (CYP1A2, CYPC9, and CYP3A4) in *E. coli* DH5-alpha, the microbial transformation of deoxypodophyllotoxin into epipodophyllotoxin was confirmed. According to the experiment, in an unoptimized system, CYP3A4 could catalyse the hydroxylation of deoxypodophyllotoxin into epipodophyllotoxin with a yield of almost 90%. A stereoisomer of podophyllotoxin is

called epipodophyllotoxin. CYP1A2 and CYPC9, the other two enzymes, did not detect epipodophyllotoxin or podophyllotoxin. It is known that human CYP3A4 uses 3'-Odemethylation to biotransform the podophyllotoxin derivatives etoposide and teniposide [149]. In another study, an efficient method was described to convert (+)-pinoresinol into (-)-pluviatolide using the genes from different plants, FiPLR (from *F. intermedia*) encoding pinoresinol-lariciresinol reductase, PpSDH (from *P. pleianthum*) encoding secoisolariciresinol dehydrogenase, CYP719A23 (from *S. hexandrum*) encoding a cytochrome P450 monooxygenase. The entire process was performed in a strain of *E. coli* bacteria co-expressing all genes mentioned above, along with an appropriate NADPH-dependent reductase to guarantee P450 activity. This method successfully produced highly pure (enantiopure) (−)-pluviatolide at 76% isolated yield [150]. Thus, the microbial transformation of epipodophyllotoxin into etoposide and teniposide provides a new and different approach to producing these two powerful anticancer medications.

Bioprocess Optimization Strategy

According to studies, five main factors affect the biosynthesis of podophyllotoxin: pH and nutrient availability of the soil, luminance, chilling units/hours, macro- and micronutrients, and soil. Red light exposure, for instance, can dramatically increase podophyllotoxin content in *L. album* cells, reaching a staggering 256 mg/g dry weight [151]. Conversely, *S. hexandrum* seems to be light-sensitive, with UV-B exposure leading to a decrease in yield [152]. This highlights the importance of tailoring light conditions for each plant species. It also has been studied that precursor feeding with coniferin can increase yield by 4.5-fold [153]. Podophyllotoxin yield also appears to be positively correlated with elevation, with studies showing higher yields at higher altitudes [154,155]. Studies also describe the influence of cultivation time. *P. hexandrum* shows the highest podophyllotoxin yield (16 ± 4 mg/g dry weight) after 20 days, though interestingly, the difference between harvest times might not be statistically significant [156].

Additionally, in the case of plants that produce podophyllotoxin, the soil's acidity or alkalinity levels show changes in yield [17]. Studies suggest that using cells from older plants (2-year-old *P. hexandrum*) can significantly increase podophyllotoxin production. Another research unveils a surprising influence of cold temperatures. Exposing *P. hexandrum* cells to chilling temperatures (4°C and 10°C) can dramatically enhance podophyllotoxin yield by up to 5-fold [157]. This finding suggests the potential for manipulating temperature regimes to optimize

production. Interestingly, similar positive effects of cold temperatures are observed in *Dysosma versipellis* [158].

Media composition plays a critical role in influencing podophyllotoxin yield. For instance, specific macro-nutrients like optimal phosphate concentration (1.25 mM) and a balanced glucose level (around 60 g/L) are crucial for *P. hexandrum* cultures [159,160]. Micronutrients also play a part, with a positive correlation observed between elements like iron and manganese and podophyllotoxin production [155]. Table 4 lists different factors that have been reported to affect podophyllotoxin yield.

Co-Cultivation and Mixed Fermentation

One other approach for producing a variety of phytochemicals is the use of plant cells. However, the low content of the target metabolite, recalcitrant nature and sluggish growth rate, genotypic variability, chemical instability, and unfeasible downstream processing continue to hinder the commercial viability of this technology. For the biotechnological production of plant-based chemicals in plant cell cultures, techniques such as elicitation, medium renewal, biogenetic precursor addition, and optimisation of culture medium composition and environmental conditions are typically used, either alone or in combination, as yield enhancement strategies. For plants, a substance that can cause morphological, physiological, or phytoalexin accumulation is called an "elicitor." It contains biotic elicitors from bacteria, fungi, or herbivores, as well as chemicals released at the assault site by plants in response to pathogen or herbivore attacks. Abiotic elicitors include metal ions and inorganic substances. It is commonly recognised that treating plants with elicitors or allowing an incompatible pathogen to assault them results in a variety of defence responses, one of which is the accumulation of a variety of secondary metabolites that are defensive to plants in intact plants or plant cells [164,165].

One study investigated the effect of arbuscular mycorrhizae-like fungi (AMF) on podophyllotoxin production in *L. album* cell cultures. Culture filtrates from *Piriformospora indica* and *Sebacina vermifera*, both AMF species, significantly increased podophyllotoxin and its derivative 6-methoxypodophyllotoxin compared to control cultures. This study suggests that fungal metabolites or signalling molecules stimulate the plant's phenylpropanoid pathway, ultimately leading to lignan biosynthesis. Further co-cultivation experiments with these fungi and *L. album* cells resulted in even greater podophyllotoxin and 6-methoxypodophyllotoxin

accumulation. Interestingly, phenylalanine ammonia-lyase activity, a key enzyme in the phenylpropanoid pathway, also increased with co-cultivation, highlighting its role in promoting lignan production. This study achieved a remarkable total lignan yield of 745.6 mg/l with a high podophyllotoxin productivity of 628.9 mg/L [166].

These studies collectively highlight the promise of co-cultivation and mixed fermentation strategies for boosting podophyllotoxin production. Co-cultivation with AMF stimulates the plant's natural metabolic pathways for lignan biosynthesis, while Agrobacterium-mediated transformation offers an alternative approach for establishing high-yielding cell lines. Further research optimizing these techniques, including exploring different fungal partners, *Agrobacterium* strains, and fermentation parameters, holds immense potential for large-scale, sustainable production of this valuable anticancer agent.

Genome Mining

Several studies have employed genome mining techniques to unravel the mysteries of podophyllotoxin biosynthesis in *P. hexandrum*. A research study has focused on ATP-binding cassette (ABC) transporters, which are vital in transporting secondary metabolites within plants. Studies have identified several ABC transporter genes in *P. hexandrum*, with two candidates (PhABC6 and PhABCIII) showing a positive correlation with podophyllotoxin content, suggesting their possible involvement in podophyllotoxin accumulation [167]. Another study analysed the complete transcriptome of *P. hexandrum* tissues. It revealed differential gene expression patterns across various tissues, with rhizome exhibiting upregulation of genes specific to podophyllotoxin biosynthesis. At the same time, leaves showed enrichment of genes in precursor pathways (shikimate and phenylalanine). This knowledge allows researchers to target specific tissues or manipulate genes for optimized podophyllotoxin yield [168]. Furthermore, research on *L. album*, another podophyllotoxin-producing plant, demonstrates the effectiveness of polyploidy induction in enhancing podophyllotoxin content [169].

Understanding the regulatory mechanisms controlling podophyllotoxin biosynthesis is also crucial. Studies have demonstrated that methyl jasmonate (MeJA) treatment significantly increases podophyllotoxin accumulation in *P. hexandrum* cell cultures [170]. This effect appears mediated by MeJA-induced reactive oxygen species (ROS) production. ROS, in turn, upregulates the expression of specific genes involved in the podophyllotoxin biosynthetic

pathway, such as PhCAD3, PhCAD4, and NAC3. Additionally, MeJA downregulates specific miRNAs, upregulating other podophyllotoxin biosynthetic genes not directly affected by ROS [170]. These findings suggest a complex interplay between MeJA signalling, ROS generation, and gene regulation in controlling podophyllotoxin production.

Further research has identified essential genes within the podophyllotoxin biosynthetic pathway itself. Transcriptome analysis revealed nine genes, including those encoding enzymes like shikimate dehydrogenase, phenylalanine deaminase, and cinnamyl alcohol dehydrogenase, that exhibited significantly higher transcript abundance in *P. hexandrum* roots and rhizomes, which naturally accumulate higher levels of podophyllotoxin [171]. The presence of conventional regulatory elements in the promoter regions of these genes suggests their co-regulation, potentially offering targets for manipulating podophyllotoxin production. Transcription factors also play an imperative role in regulating secondary metabolite biosynthesis. Studies have identified transcripts encoding various transcription factor families, such as bZIP, MYB, WRKY, and bHLH, in *P. hexandrum*. Some of these transcripts strongly correlated with podophyllotoxin content, suggesting their involvement in regulating podophyllotoxin biosynthesis. Further in silico analysis revealed promoter regions for these transcription factors binding sites in the known podophyllotoxin biosynthetic genes, providing additional evidence for their regulatory role [172].

Transcriptome sequencing of *P. hexandrum* cell cultures has revealed a wealth of information. One study generated over 40,000 assembled transcripts, including putative members of the phenylpropanoid pathway, which is known to be involved in podophyllotoxin biosynthesis [173]. This data provides valuable leads for identifying key genes responsible for podophyllotoxin production.

In conclusion, genome mining has emerged as a powerful tool for unravelling the complexities of podophyllotoxin biosynthesis in *P. hexandrum*. By integrating transcriptome data, gene expression analysis, and studies on regulatory mechanisms, researchers gain a deeper understanding of the factors influencing podophyllotoxin production. This knowledge can be channelled to develop strategies for enhancing podophyllotoxin yield through genetic engineering-optimized cultivation practices. Genome mining holds immense promise as research progresses to ensure a sustainable source of this valuable anticancer agent.

Biotransformation

Biotransformation offers a sustainable and potentially more efficient alternative for podophyllotoxin production. This approach utilizes living organisms such as endophytic fungi or microbial cultures to convert readily available substrates into podophyllotoxin. Biotransformation holds promise for increased yields, reduced environmental impact, and potentially improved purity of podophyllotoxin compared to traditional methods. This introduction emphasizes the growing importance of biotransformation for the sustainable production of podophyllotoxin, a valuable compound in the fight against cancer.

A study explores the application of human hepatic enzymes for podophyllotoxin biotransformation. The study investigated the activity of CYP1A2, CYP2C9, and CYP3A4 enzymes on deoxypodophyllotoxin, a podophyllotoxin precursor. They found that only CYP3A4 effectively converted deoxypodophyllotoxin to epipodophyllotoxin, highlighting the specific role of this enzyme in podophyllotoxin metabolism [174]. Another report utilized *Hordeum vulgare* cell suspension cultures to modify podophyllotoxin. They identified isopicropodophyllone as the significant biotransformation product. The study optimized factors like podophyllotoxin concentration, cell cycle stage, and addition time to maximize the yield of this derivative [175]. Another study investigated the potential of endophytic fungi for podophyllotoxin production. They isolated a fungal strain, TQN5T (identified as *Fusarium proliferatum*), from *D. versipellis* that was confirmed to host plant extract or phenylalanine (a precursor for plant podophyllotoxin biosynthesis) and significantly increased podophyllotoxin yield [143].

These studies showcase the diverse approaches for podophyllotoxin biotransformation. Endophytic fungi offer a promising avenue for sustainable podophyllotoxin production, potentially surpassing yields achieved through traditional methods. Plant cell cultures can be employed for targeted podophyllotoxin modification. Finally, human enzymes provide valuable insights into podophyllotoxin metabolism and potentially aid in developing novel therapeutic agents. Future research efforts should focus on optimizing these biotransformation techniques for large-scale production and exploring the anticancer properties of biotransformed podophyllotoxin derivatives.

Anticancer Effects and Pharmacological Mechanisms of Podophyllotoxin and its derivatives

Podophyllotoxin’s molecular action against cancer is mainly demonstrated by how well it induces autophagy and death in cancer cells. Podophyllotoxin first impedes cell mitosis by interfering with microtubule assembly and nucleoside transport. Podophyllotoxin and etoposide can prevent cells from absorbing thymine and uracil, which prevents the creation of DNA, RNA, and proteins [176]. These two substances, however, affect microtubule assembly in different ways. Because of its spindle poisons, podophyllotoxin inhibits polymerization, but etoposide, which includes glycosyl, does not. According to additional research, podophyllotoxin may disrupt spindle organisation and modify chromosomal layout during the middle of the first mitosis [177]. Joseph compiled an earlier study and concluded that the inhibition of the mitotic cycle, particularly during the late S and G2 stages, is the cause of etoposide's inhibition of DNA synthesis activity [178]. Second, podophyllotoxin inhibits type II topoisomerases (TOP2s), raising the steady-state level of drug-TOP2s-DNA cleavage complexes to encourage the formation of cytotoxic DNA lesions, causing double-strand breaks and ultimately slowing down the rapid growth of cancer cell which is how podophyllotoxin kill cancerous cells [179]. However, the podophyllotoxin is not a TOP2 inhibitor by itself; its action only happens following conformational changes and adding sugar groups. Concurrently, the enhanced podophyllotoxin's cytotoxicity is significantly decreased due to the complexes' degradation upon the medicines' departure. Researchers determined the high-resolution crystal structure, which revealed the intricate interaction between the enzyme, the DNA, and the etoposide. This information helped to further explain the three-dimensional structural basis of the drug actions and resistance. It is possible that the drug stabilises the cleavage complex by, among other things, discouraging the relegation of cleaved DNA ends through the decoupling of the key catalytic residues. This finding offers methods for creating isoform-specific TOP2-targeting medications as well as mechanistic explanations for structure-activity connections that have been identified [180].

Naturally produced deoxypodophyllotoxin differs from podophyllotoxin solely in the C-4 location. The semi-synthesis of the cytostatic medications teniposide and etoposide phosphate uses deoxypodophyllotoxin as a precursor. It was also discovered that deoxypodophyllotoxin exhibits vascular disruption as well as anti-angiogenesis properties, which makes it a potentially effective new anticancer medication and in 2017, the National Medical Products Administration in China approved it for use in phase I–III drug clinical research [181]. The same year, another discovery was made that deoxypodophyllotoxin lowered phosphorylated Akt and mTORC1 levels, inhibiting the development of the cells [182]. Further investigations

revealed that the deoxypodophyllotoxin inhibits cell survival pathways controlled by the MAPK/ERK and NF-kB signalling pathways, making it a potent inducer of caspase-dependent programmed cell death (apoptosis) in malignant MB231 breast cancer cells [183]. It was also found that it increased apoptotic cell death and caused G2/M cell cycle arrest, which hindered the proliferation of gastric cancer cells. At the same time, caspase pathways acted as mediators [184].

The practical usage of podophyllotoxin derivatives as medications with chemotherapeutic properties, such as teniposide, etoposide (VP-16), etoposide phosphate (etopofos), GL331, NK-611, and TOP53, has been confirmed by several scientific research, during the S-terminal stage and the first G-stage of the cell cycle, etoposide, teniposide, and etopophos block topoisomerase II by stabilising the enzyme-DNA complex [185,186]. GL-331, a paminoaniline derivative, was initially produced by Lee et al. DNA strands are broken by GL-331, which also ends the cell cycle's G-2 phase [187]. Etoposide derivative NK-611 functions by inhibiting topoisomerase II and has a dimethylamine group at the 2" position of ethylidene glycoside [188]. The TOP-53 variant was created and designed at Taiho Pharmaceutical Co, Ltd. Compared to VP-16, TOP-53 is more toxic and inhibits topoisomerase II. Studies have shown that using it to treat lung tissue metastases of tumours is successful [189,190]. Another inhibitor of topoisomerase II, azatoxin, was created using molecular modelling of a pharmacophore model [191,192].

Studies with podophyllotoxin derivative Ching001 and podophyllotoxin acetate showed a decrease in microtubule polymerization, which halted cell division and cell death. Additionally, these compounds trigger a rise in proteins like Aurora kinase B, survivin, and p-PMPM2, which are involved in cell division regulation. Furthermore, it promotes DNA damage, as indicated by increased γ-H2AX, and triggers ER stress, putting further pressure on the cancer cells and contributing to cell death [193,194]. DPMA (2,6-dimethoxy-4-(6-oxo-(5R,5aR,6,8,8aR,9-hexahydrofuro [3',4':6,7] naphtho[2,3-d] [1,3] dioxol-5-yl) phenyl ((R)-1-amino-4-(methylthio)-1-oxobutan-2-yl) carbamate) and HY-1 (4β-[benzoyl-thioureido]-4-deoxypodophyllotoxin), observed cell cycle arrest, particularly at the G2/M phase, a critical stage for cell division. Additionally, HY-1 increases the levels of P-cdc2, P-cdc25c, Weel, Chk1, and ATR, which are all proteins that regulate the cell cycle at the G2/M checkpoint [48,195]. Studies with *All-Trans*-Retinoic Acid-podophyllotoxin conjugate (ATRA-podophyllotoxin conjugate) suggest it downregulates CDK1 and CDK2, which are protein

kinases essential for driving the cell cycle through various phases. By inhibiting these kinases, it disrupts the orderly progression of the cell cycle and halts cell growth. Furthermore, it activates caspases, a group of enzyme executioners dismantling cellular components during apoptosis. It also downregulates Bcl-2, an anti-apoptotic protein, and increases Bax, a pro-apoptotic protein. This combined effect promotes the dismantling of cancer cells through apoptosis [196].

Similarly, studies with 2-pyridinealdehyde hydrazone dithiocarbamate S-propionate podophyllotoxin ester (Podophyllotoxin^{Pdp}) and di-2-pyridineketone hydrazone dithiocarbamate S-propionate podophyllotoxin ester (Podophyllotoxin^{Dpt}) suggest these derivatives may target the PI3K/AKT/mTOR pathway, another critical pathway for cell growth and proliferation [46,47]. Another derivative, 4DPG (4′-demethyl-deoxypodophyllotoxin glucoside), has been shown to upregulate p53 and Chk-2 in HCT-116, SW-620 and HT-29 cell lines. It suppresses EMT and vimentin expression, making it harder for cancer cells to detach from the tumour and metastasize [45]. The study involving polyamidoamine-conjugated with podophyllotoxin dendrimer (DPODO) investigated a nanoparticle delivery system for podophyllotoxin using polyamidoamine dendrimers, which reduces IL-6 and NF-κB, both of which are involved in inflammation and can promote cancer cell survival. Additionally, it disrupts tubulin depolymerization, hindering microtubule formation and cell division [197].

6-methoxypodophyllotoxin derivative shows promise against bladder cancer cells (5637 cells) by halting the cell cycle at the G2/M phase [30]. Derivative XWL-1-48 was tested against breast cancer cells (MCF-7 and MDA-MB-231 cells), and it has been shown to downregulate Topoisomerase II, cause cell cycle arrest and promote apoptosis [198]. 4ß-amidopodophyllotoxin conjugates target MCF-7 and MDA-MB-231 breast cancer cells with impressive potency. They inhibit proteins like CDK1 and cyclin B1, which disrupts the orderly progression of the cell cycle, leading to uncontrolled growth arrest [49]. Studies on podophyllotoxin-norcantaridin have been shown to disrupt the G2/M checkpoint by inhibiting proteins like CDK1 and cyclin B1, which halts the cell cycle progression, preventing cells from entering mitosis.

Additionally, it inhibits tubulin polymerization, which hinders mitosis and significantly reduces cancer cell proliferation [52]. Podophyllotoxin-indirubin hybrid (Da-1) has shown activity against leukaemia K562/VCR cells resistant to the anticancer drug vincristine. It disrupts the cell cycle at the G2 phase by inhibiting proteins like CDKs. It stands out for its

ability to induce autophagy. In vitro studies suggest it converts LC3-I, an autophagy marker protein, into its active form, LC3-II. Additionally, it seems to target microtubules, potentially hindering proper cell division [199].

Podophyllotoxin and its derivatives are promising as novel cancer therapeutics targeting these different mechanisms. However, research is ongoing to improve their effectiveness, and further research on the compound may lead to new information and medicinal applications.

Conclusions and future prospect

Summary of Research Progress

Podophyllotoxin, a potent lignan extracted from *Podophyllum* species like *P. hexandrum* and *P. peltatum*, has captivated researchers for decades due to its remarkable anticancer properties [17]. While its medicinal use dates back centuries, recent advancements delve deeper into its fascinating journey, shedding light on its intricate biosynthesis and diverse mechanisms of action against cancer cells.

The natural production of podophyllotoxin within *Podophyllum* plants is an active research area. While the biosynthetic pathway remains under investigation, evidence suggests a potential role played by endophytic fungi within these plants [17]. These microscopic fungi, living symbiotically inside the plant tissues, might contribute specific enzymes crucial for podophyllotoxin biosynthesis. This intriguing relationship opens exciting avenues for further exploration, potentially leading to the development of novel strategies for enhancing podophyllotoxin production.

The limited natural availability of podophyllotoxin necessitates alternative production methods. Semi-synthetic derivatives like etoposide and teniposide have revolutionized cancer treatment, surpassing podophyllotoxin in potency and offering more excellent clinical utility [200]. However, the future lies in metabolic engineering and biotransformation techniques. By manipulating the metabolic machinery of microorganisms to induce podophyllotoxin production, researchers are paving the way for sustainable and scalable production systems, potentially surpassing the limitations of traditional plant extraction.

Podophyllotoxin's primary weapon against cancer lies in its ability to inhibit topoisomerase II, an enzyme essential for DNA replication and repair. The formation of a stable complex of

podophyllotoxin and this enzyme disrupts the DNA replication process, leading to cell cycle arrest and ultimately triggering apoptosis [201]. Recent research suggests that podophyllotoxin possesses additional anticancer properties beyond topoisomerase II inhibition. Studies have revealed its ability to suppress inflammation and angiogenesis, two processes crucial for tumour growth and metastasis. This multifaceted approach potentially contributes to the overall therapeutic efficacy of podophyllotoxin against various cancers [202].

Current research on podophyllotoxin is focused on several key areas. Researchers are developing innovative methods to deliver podophyllotoxin and its derivatives, aiming to improve their pharmacokinetic profiles, enhance their efficacy, and minimize side effects, which includes exploring liposomal formulations, nanoparticle delivery systems, and antibody-drug conjugates for targeted therapy. Combining podophyllotoxin with other anticancer agents holds immense promise for achieving synergistic effects, potentially overcoming drug resistance mechanisms employed by cancer cells. Exploring combinations with targeted therapies, immunotherapies, and other chemotherapeutic agents is actively being investigated.

It is imperative to understand the mechanism of cancer cell resistance against podophyllotoxin to develop strategies to circumvent the issue, which involves identifying specific mutations and signalling pathways that contribute to resistance, allowing researchers to design novel therapeutic approaches to overcome these challenges and maximize the clinical effectiveness of podophyllotoxin.

In conclusion, the ongoing research on podophyllotoxin promises to further elucidate its biosynthesis, unveil its diverse anticancer mechanisms and pave the way for its enhanced production and clinical application. Advancements in metabolic engineering, biotransformation, drug delivery, and combination therapies hold immense potential for solidifying podophyllotoxin's position as a valuable weapon in the fight against cancer. Moreover, exploring its additional biological activities might lead to the discovery of novel therapeutic avenues, further expanding the possibilities for this potent natural product.

Future Directions in Podophyllotoxin Research

Podophyllotoxin, a promising anticancer compound, faces limitations in production. While heterologous expression and chassis cell development offer alternatives, the underlying biosynthetic pathway in endophytic fungi remains unclear. Additionally, complete elucidation

of the pathway in plants and microorganisms is still lacking and further investigation is needed to determine whether peptide biosynthesis applies to podophyllotoxin.

However, advancements in genetic sequencing technology are lowering costs, paving the way for biocomponent development and pathway elucidation. Nevertheless, significant technical hurdles persist in efficient podophyllotoxin production, regardless of the chosen host (tobacco or yeast).

Recent breakthroughs in artificial intelligence offer a fresh perspective through synthetic biology. By employing deep learning algorithms, researchers can intelligently design podophyllotoxin biosynthetic pathways. This approach can screen suitable chassis cells, potentially overcoming technical limitations and enabling smarter podophyllotoxin production. Podophyllotoxin exemplifies the potential of natural products for drug discovery. Several podophyllin-based anticancer drugs are nearing clinical use, but most rely on chemical synthesis. Biological synthesis remains elusive. The "reverse biosynthesis" method offers a promising solution. This approach flips the traditional logic, starting from the known structure of podophyllin drugs. By "reverse-engineering" these structures into building blocks readily available in biological systems, researchers can design efficient biosynthetic pathways. This strategy bypasses the need for complete pathway elucidation in natural sources.

Targeted modification or selection of biosynthetic components can accelerate drug development. This approach, rooted in synthetic biology principles, represents a future trend in drug discovery. Researchers can rationally design and optimize podophyllotoxin biosynthesis pathways by employing reverse thinking, which paves the way for constructing cell factories capable of efficient podophyllotoxin synthesis. This strategy is expected to become a viable drug development and production method, enabling the synthesis of podophyllotoxins and potentially other lignan compounds.

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Conflicts of Interest

The authors declare no conflicts of interest.

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Legends for Figures

Fig. 1. Structure of Podophyllotoxin (a) Etoposide (b), Teniposide (c), Etopophos (d), GL331 (e), NK611 (f), NPF (g), and TOP53 (h)

Fig. 2. Schematic diagram of the mode of action of podophyllotoxin derivatives on cancer cell cycle as inhibitors of tubulin to induce G_2/M arrest in tumour cells

Fig. 3. Schematic diagram of the mechanism of podophyllotoxin derivatives as inhibitor of the degradation of topoisomerase II to cause DNA damage cleavage

Fig. 4. Schematic diagram of the mechanism of podophyllotoxin derivatives as inhibitor of tumour cell growth by affecting drug-resistant tumour cells by downregulating P-glycoprotein and MDR-1 expression

Fig. 5. Schematic diagram of the mechanism of podophyllotoxin derivatives by affecting gluconeogenesis-related molecules by upregulating AMPK (AMP-activated protein kinase) and disrupting the Warburg affect.

Fig. 6. Schematic diagram of the mechanism of podophyllotoxin derivatives by upregulating Chk2 and inhibiting EMT (epethelial to mesenchymal transition) related molecules vimentin, E-cadherin and MMP-2 (matrix metalloproteinase-2)

Fig. 7. Schematic diagram of the mechanism of podophyllotoxin derivatives by upregulating caspases and CDK-1 (Cyclin-dependent kinase 1) leading to apoptosis

Table 1. Podophyllotoxin-producing plant species and their yield

Plant species	Plant part	Bioactive compounds and their yield	Reference
Podophyllum peltatum	Leaf blades, roots and rhizomes	Podophyllotoxin (0.75 mg/g dry weight), 4'-Demethylpodophyllotoxin (0.02 mg/g dry weight), Desoxypodophyllotoxin (0.07 mg/g dry weight), 4'-Demethyldesoxypodophyllotoxin (0.02 mg/g dry weight)	[60]
Podophyllum pleianthum	Rhizome and roots	Podophyllotoxin (1.35 mg/g dry weight), 4'-Demethylpodophyllotoxin (0.41 mg/g dry weight), Desoxypodophyllotoxin (0.1 mg/g dry weight), 4'-Demethyldesoxypodophyllotoxin (0.03 mg/g dry weight)	[60]
Sinopodophyllum hexandrum	Rhizome, root, leaf and stem	Podophyllotoxin (12.8 mg/g dry weight), 4'-Demethylpodophyllotoxin (1.35 mg/g dry weight), Desoxypodophyllotoxin (0.05 mg/g dry weight), 4'-Demethyldesoxypodophyllotoxin (0.03 mg/g dry weight)	[60]
Dysosma aurantiocaulis	Rhizome and roots	No data	[61]
Callitris drummondii	Needles		[62]
Diphylleia cymosa	Roots and leaves	No data	[63]
Diphylleia grayi	Roots and leaves	No data	[63]
Dysosma versipellis var. tomentosa,	Aerial part	No data	[64]
Diphylleia sinensis	Rhizome	No data	[65]
Hyptis verticillata	Aerial part	No data	[66]
Nepeta. nuda ssp. glandulifera	Aerial parts	Podophyllotoxin (0.11 % of dry weight)	[67]
Phlomis nissoli	Aerial parts	Podophyllotoxin (0.05 % of dry weight)	[67]
Salvia cilicica	Aerial parts	Podophyllotoxin (0.08 % of dry weight)	[67]
Teucrium chamaedrys	Aerial parts	Podophyllotoxin (0.09 % of dry weight)	[67]
Thymus capitatus	Aerial parts	Podophyllotoxin (0.05 % of dry weight)	[67]
Juniperus chinensis	Leaves	Podophyllotoxin (0.0025% of dry weight)	[68]
Hernandia sonora	Seeds	No data	[69]
Dysosma tsayuensis	Rhizome and roots		[70]
Linum rigidum	Leaves	No data	[71]
Linum lewisii	Leaves	No data	[71]
Nepeta cataria	Leaves	No data	[71]
Thymus sp.	Leaves	No data	[71]
Dysosma pleiantha	Leaves	Podophyllotoxin (3.17 mg/g dry weight)	[71]
Juniperus sabina	Needles, stem, and roots	Podophyllotoxin (0.14 mg/g dry weight)	[71]
Juniperus virginiana	Needles	Podophyllotoxin (4.7 mg/g dry weight)	[71]
Linum flavum	Leaves	Podophyllotoxin (0.16 mg/g dry weight)	[71]
Linum hirusutum	Leaves	Podophyllotoxin (0.15 mg/g dry weight)	[71]
Linum usitatissimum	Leaves	Podophyllotoxin (0.05 mg/g dry weight)	[71]
Teucrium polium	Leaves	Podophyllotoxin (2.6 mg/g dry weight)	[71]
Thuja occidentalis	Needles, stem, and roots	Podophyllotoxin (0.08 mg/g dry weight)	[71]
Linum mucronatum	Roots, leaves, and buds	Podophyllotoxin (0.595% g/g dry weight) 6-methoxypodophyllotoxi (1.491% g/g dry weight) 5-demethoxy-6-methoxypodophyllotoxi (0.994% g/g dry weight)	[72]
Hyptis suaveolens	Aerial parts and roots	No data	[73]
Dysosma versipellis	Rhizome and roots	Podophyllotoxin (3.84±1.31 µmol/g) Deoxypodophyllotoxin (16.00±3.70 µmol/g) 4′-podophyllotoxin-glucoside (3.84±1.31 µmol/g)	[74]
Juniperus squamata	Stem and leaves	Podophyllotoxin (47 µg/g dry weight) Deoxypodophyllotoxin (1670 µg/g dry weight) Podophyllotoxone (0.02 µg/ g dry weight)	[75]
Juniperus communis	Stem and leaves	Podophyllotoxin (15 µg/g dry weight) Deoxypodophyllotoxin (2109 µg/ g dry weight) Podophyllotoxone (0.03 µg/ g dry weight)	[75]
Juniperus recurva	Stem and leaves	Podophyllotoxin (60 µg/g dry weight) Deoxypodophyllotoxin (33 µg/ g dry weight) Podophyllotoxone (1.41 µg/ g dry weight)	[75]

Juniperus blaaws	Stem and leaves	Deoxypodophyllotoxin (5 µg/ g dry weight) Podophyllotoxone (0.02 µg/ g dry weight)	[75]
Juniperus procumbens	Stem and leaves	Deoxypodophyllotoxin (20.5 µg/ g dry weight) Podophyllotoxone (0.02 µg/ g dry weight)	[75]
Juniperus x-media	Stem and leaves	Podophyllotoxin (1000 µg/g dry weight) Deoxypodophyllotoxin (3315 µg/ g dry weight) Podophyllotoxone (5.55 µg/ g dry weight)	[75]
Juniperus bermudiana	Leaves	Podophyllotoxin (22.6 ± 0.5 mg/g dry weight) Deoxypodophyllotoxin (4.7 ± 0.3 µg/ g dry weight)	[76]
Juniperus phoenicea	Leaves	Podophyllotoxin (1.8 ± 0.1mg/g dry weight) Deoxypodophyllotoxin (0.4 ± 0.1µg/ g dry weight)	[76]
Juniperus scopulorum	Leaves	Podophyllotoxin (0.263 % of dry weight)	[77]
Juniperus horizontalis	Leaves	Podophyllotoxin (0.477 % of dry weight)	[77]
S. sikkimensis	Rhizome and roots	Podophyllotoxin (0.13-0.73 % of dry weight)	[78]
Dysosma difformis	Rhizome and roots	Podophyllotoxin (21.36 ± 1.07 mg/g dry weight)	[79]
Callitris rhomboidea	Aerial parts	Deoxypodophyllotoxin (3.50 ± 0.19 mg/g dry weight)	[80]
Callitris endlicheri	Aerial parts	Deoxypodophyllotoxin (5.77 ± 0.80 mg/g dry weight)	[80]
Callitris preissii	Aerial parts	Deoxypodophyllotoxin (0.15 ± 0.05 mg/g dry weight)	[80]
J. virginiana	Leaves	Podophyllotoxin (2.09 mg/g dry weight)	[81]

Table 2. Podophyllotoxin production in in-vitro culture system of different plant species and its yield

Plant species	Explant	In-vitro culture system	Bioactive compounds and their yield	References
Podophyllum peltatum	Embryos	Callus	No data	[97]
Sinopodophyllum hexandrum	Roots	Callus	Podophyllotoxin (0.095 % cell dry weight) 4'-demethylpodophyllotoxin (0.053% cell dry weight) Podophyllotoxin 4-O-glucoside (0.018% cell dry weight)	[107]
S. hexandrum	Roots	Suspension	Podophyllotoxin (0.056 % cell dry weight)	[108]
Dysosma pleianthum, P. peltatum, S. hexandrum	Stem	Callus	No data	[109]
Juniperis chinensis	Stem	Callus	Podophyllotoxin (0.005% of dry weight)	[68]
Linum album	Shoot culture	Suspension	Podophyllotoxin 5-methoxypodophyllotoxin deoxypodophyllotoxin 5′-demethoxy-5-methoxypodophyllotoxin	[110]
P. peltatum	Rhizome tips	Rooted bud and plantlet cultures	Podophyllotoxin (6.761 mg/g dry weight) Epipodophyllotoxin (0.15 mg/g dry weight) 4'O-demethylpodophyllotoxin (2.42 mg/g dry weight)	[111]
S. hexandrum	Embryos	Embryogenic callus	Podophyllotoxin (0.393% cell dry weight)	[112]
S. hexandrum	In vitro germinated seedlings	Suspension	Podophyllotoxin (556.82 µg/g dry weight)	[113]
S. hexandrum	Roots	Suspension	Podophyllotoxin (0.655 mg/g dry weight)	[114]
S. hexandrum	Roots	Suspension	Podophyllotoxin (0.9 mg/g dry weight)	[115]
J. chinensis	Leaves	Callus	Podophyllotoxin (4 mg/g dry weight)	[116]
L. album	Excised plant part of in vitro germinated seedling	Suspension	Podophyllotoxin (0.24% cell dry weight)	[117]
S. hexandrum	Embryos	Callus	Podophyllotoxin (0.79% cell dry weight)	[118]
P. peltatum	Embryo	Cell and adventitious root cultures	Podophyllotoxin (0.588 mg/g dry weight)	[119]
S. hexandrum Royle	Roots	Root culture	Podophyllotoxin (151.50 µg/g dry weight)	[92]
S. hexandrum	Leaf and rhizome	Callus	No data	[101]
Hyptis suaveolens	Aerial parts, roots, and seeds	Root cultures	Podophyllotoxin (0.013% of dry weight)	[120]
L. album		Cell culture, callus, and in vitro propagated plantlets	Podophyllotoxin	[121]
Linum mucronatum	Hypocotyl and root explants	Hairy roots	Podophyllotoxin (5.78 mg/g dry weight) 6-methoxy podophyllotoxin (49.19 mg/g dry weight)	[122]
Linum persicum	Excised plant part of in vitro germinated seedling	Shoot culture	Podophyllotoxin (1.62±0.21% dry weight)	[123]
Juniperus virginiana	Leaves	Callus	Podophyllotoxin (0.56 mg/g of dry weight)	[124]
J. virginiana		Suspension	Podophyllotoxin (1.47 mg/g of dry weight)	[125]
Dysosma pleiantha	Leaves	Callus	Podophyllotoxin	[126]

Table 3.: Podophyllotoxin-producing endophytic fungi and their host plants

Endophytic fungi	Host plant	Bioactive compounds and their yield	Reference
Monilia sp.	*Dysoma veitchii*	No data	[127]
Phialocephala fortinii	*Podophyllum peltatum*	Podophyllotoxin (0.5-189 µg/l)	[128]
Trametes hirsuta	*Podophyllum hexandrum*	Podophyllotoxin (30 µg/g dry weight), Demethoxypodophyllotoxin (11 µg/g dry weight), and Podophyllotoxin glycoside (20 µg/g dry weight)	[132]
Alternaria neesex	*Sinopodophyllum hexandrum*	Podophyllotoxin (2.418 µg/l with 85 g/l dry cell weight)	[137]
Fusarium oxysporum	*Juniperus recurva*	Podophyllotoxin (28 µg/g dry mass)	[130]
Aspergillus fumigatus	*Juniperous communis*	Deoxypodophyllotoxin (4 mg/100 g dry mycelia, 3 mg/l spent broth)	[138]
Alternaria sp.	*Sabina vulgaris*	No data	[5]
Alternaria sp.	*P. hexandrum*	No data	[5]
Monilia sp.	*D. veitchii*	No data	[5]
Penicillium sp.			
Penicillium sp.	*S. hexandrum*	No data	[5]
Penicillium implicatum	*Diphylleia sinensis, D. veitchii*	No data	[5]
Fusarium solani	*P. hexandrum*	Podophyllotoxin (29.0 µg/g dry weight)	[131]
Mucor fragilis	*S. hexandrum*	Podophyllotoxin (49.3 µg/g of mycelial dry weight)	[129]
Alternaria tenuissima	*Sinopodophyllum emodi*	Podophyllotoxin (chloroform extract: 50.5 mg, butanol extract: 348 mg, methanol extract: 1139.9 mg)	[139]
Phialocephala podophylli	*P. peltatum*	No data	[140]
Chaetomium globosum, Pseudallescheria sp.	*S. hexandrum*	Podophyllotoxin	[141]
Fusarium sp.	*Dysosma versipellis*	Podophyllotoxin (277 µg/g and 1.25 µg/g wet weight)	[142]
Fusarium proliferatum	*D. versipellis*	Podophyllotoxin (314 µg/g dry weight)	[143]

Table 4. Podophyllotoxin yield from plant source on changing various parameters

Plant Source	Parameters	Sub-Parameter	Podophyllotoxin Yield	Reference
Linum album	Light	Red light	256 mg/g dry weight	[151]
Sinopodophyllum hexandrum		UV-B	0.86-fold decrease	[152]
Podophyllum hexandrum Royle	Precursor feeding	Coniferin (2.1 mM)	4.5-fold increase	[153]
P. hexandrum	Soil Nutrients	pH (4.82)	6.62 % dry weight	[154]
P. hexandrum		Carbon (3.32%)	6.86 % dry weight	[154]
P. hexandrum		Phosphorus (above 0.149%)	Production inhibition	[154]
P. hexandrum	Micro Nutrients	Nitrogen (2.7%)	6.86 % dry weight	[154]
P. hexandrum		SO42−, K+	Negative correlation	[155]
P. hexandrum		Mg2+, Ca2+, Cu, Zn	No correlation	[155]
P. hexandrum	Soil type	Peat-perlite	16 ± 4 mg/g dry weight	[156]
P. hexandrum	Cultivation time	20 days	16 ± 4 mg/g dry weight	[156]
P. hexandrum Royle	Chilling Temperature	4°C	5-folds increase	[157]
P. hexandrum Royle		10°C	3.33-folds increase	[157]
Dysosma versipellis		(4∼6°C)	3.49-folds increase	[158]
P. hexandrum	Macro Nutrients	Glucose (60 g/L)	0.63 mg/g dry weight	[159]
P. hexandrum		Phosphate (1.25 mM)	Optimum production	[159]
P. hexandrum		Nitrogen (60 mM)	0.59 mg/g dry weight	[159]
P. hexandrum	Plant age	2-year-old	Significant increase	[160]
P. hexandrum	Growth period	Late growth period	Highest, but insignificant	[160]
P. hexandrum	Elevation	4300 m	9.533±0.484 (% dry weight)	[154]
P. hexandrum		3621 m	58.29 mg/g dry weight	[155]
P. Hexandrum		3300 m	2.2- 5.3-fold increase	[161]
Leptohyptis macrostachys	Culture condition	IBA (3 mg/L)	4.1 ± 0.06 mg/g dry weight	[162]
L. macrostachys		IAA (3 mg/L)	3.9 ± 0.04 mg/g dry weight	[162]
Juniperus virginiana		NAA (3 mg/L)	3.2 ± 0.03 mg/g dry weight	[162]
J. virginiana		2,4-D (2 mg/L)	2.9 ± 0.04 mg/g dry weight	[162]
J. virginiana		Picloram (2 mg/L)	2.2 ± 0.02 mg/g dry weight	[162]
P. hexandrum		Methyl jasmonate treatment	17 ± 3 mg/g dry weight	[156]
P. hexandrum Royle		8.88 µM BAP + 5.37 µM ANA	4.0871 ± 0.0509 mg/g of dry weight	[163]
P. hexandrum Royle		Salt: 0.5 Murashige and Skoog medium Sucrose: 1.5%	5.8309 mg/g dry weight	[163]
P. hexandrum Royle		Cinnamic acid (10 mmol/L)	1.47 mg/g dry weight	[125]
P. hexandrum Royle		Salicylic acid (10 mmol/L)	0.52 mg/g dry weight	[125]
P. hexandrum Royle		Jasmonic acid (5 mmol/L)	0.67 mg/g dry weight	[125]
L. macrostachys	Photoperiod	16 hours	0.4015 mg/g dry weight	[163]