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Natural Sources of Taxol

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Author's contribution

The sole author studied literature, interpreted generated information, designed, analyzed and prepared the manuscript.

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Review Article

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ABSTRACT

Taxol® is the most successful anticancer agent developed in the last 50 years. The alkaloid taxol and other taxoids were at first isolated from *T. brevifolia*. Because of the small yield that threatened existence of the natural population due to exploitation for taxol, a search for alternative sources were made among *Taxus* species. As a result, isolation of taxol and other taxanes were achieved in all *Taxus* species. The success encouraged exploration of other members of Taxaceae, the gymnosperms, and angiosperms. In order to address the supply crisis, microbial endophytes sources in known taxol and non-taxol-producing plants were further explored with resultant successes. Production of taxol in endophytes is yet to translate into industrial production. Application of biotechnology through metabolic engineering is hoped to address the supply of taxol to meet demand for the production of the anticancer drug, considering the increasing prevalence of cancer around the world.

Keywords: Natural products; anticancer drugs; Taxus; taxol; endophytes; biotechnology.

ABBREVIATIONS

BMS - Bristol-Myers Squibb, RTI – Research Triangle Institute, NCI – National Cancer Institute, FDA – Food and Drug Administration.

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1. INTRODUCTION

The chemical name taxol was given to the compound now known as paclitaxel (Fig. 1a) by Drs. Wall and Wani in their original 1971 publication, and was used in the scientific literature for 20 years before Bristol-Myers Squibb trademarked it for their formulation of the drug [1]. Because of the historical nature of this review, the name Taxol® or paclitaxel refers to the anticancer drug while taxol for the chemical compound (derived from source genus with alcohol ending–ol), no infringement of the Bristol-Myers Squibb trademark is intended [2]. The mitotic inhibitor was discovered and isolated from the bark, roots and branches of *Taxus brevifolia* in an extensive screening program on natural compounds from plant sources with anticancer activities at RTI [1]. Along with docetaxel (Fig. 1b), Taxol® is regarded the most successful developed anticancer drug in the past five decades and represents taxane family of drugs with worldwide sales of $1.5 billion in 1999. It decreased by 24 % between 2006–2007 due to patent expiry, increased generic competition in Europe and Japan in the quarter of the year 2006 but, total market remained well above $1billion per year with continual expansion anticipated due to new clinical uses [3]. Paclitaxel is approved for use in treatment of patients with lung, ovarian, breast, neck, head cancers, advanced forms of Kaposi`s sarcoma, many cancer types and also as an antiproliferative agent for prevention of restenosis of coronary stents [4]. Mechanism of action of the drug involves stabilization of microtubules, leading to interference with their normal breakdown during cell division with resultant inhibited cell proliferation that occurs through specific binding of the drug to β subunit of the tubulin heterodimers, thereby, promote cytotoxicity [5-7]. The tumor cells show defects in mitotic spindle assembly, chromosomes segregation and cell division with inability to achieve metaphase spindle configuration and a resultant blockage of mitosis progression along with prolonged activation of mitotic checkpoint. The effect triggers apoptosis or a reversion to G-phase of cell cycle without cell division [8-9].

Several challenges were encountered during development of paclitaxel as an anticancer drug including material supply problem, formulation of taxol into accepted delivery form, preclinical toxic effects in tissue with high cell turnover such as gastrointestinal, lymphatic, hematopoietic and reproductive tissues. Earlier, bark of *T. brevifolia* was the only source of taxol before needles and roots were discovered as alternative natural sources [10]. Because of the small taxol yield of the species requiring 10,000 kg of bark to produce 1 kg of taxol, concern was raised about the environmental impact of the sourcing from Pacific Yew, leading to search for alternative sources of the alkaloid first from other *Taxus* species before semisynthesis and total synthesis were achieved [11-13]. The search extended to microbes with resultant discovery of taxol-producing endophytic fungus but, production of the alkaloid by fermentation gave small and variable yield [14]. In the end, an alternative environment-sustainable approach using plant cell culture was developed. The method employed by Phyton Biotech, the largest paclitaxel producer, uses plant tissue culture using large-scale fermenters with increased productivity of taxanes through application of several strategies. In this article, progresses on plant and endophytes natural sources of taxol are discussed. Role of biotechnology in addressing supply crisis of taxol for production of the drug and future applications are highlighted.

2. HISTORICAL DEVELOPMENT

In 1962, first collection of plant samples including *T. brevifolia* bark was made by group of researchers employed by U.S. Department of Agriculture. The researchers were contracted by the NCI to find a natural product for the cure of cancer. A part of their plant collections got shipped to the natural product laboratory of Monroe and Wani and coworkers at RTI. After two years of research, the team discovered an extract from samples in one of the several collections of the plants showed cytotoxicity and identified the sample as *T. brevifolia*. This lead to collection of more of *T. brevifolia* bark, the purification and identification of the most active part in the bark extract after several years of work and was named taxol [1]. Separation of taxol from bark samples of *T. brevifolia* was difficult and slow, research on the extracted chemical proved a difficult task as every chemical showed toxicity to cancerous and non-cancerous cells [15-16]. After successful purification, studies on taxol production in *Taxus* species, identification of the greatest yielding species and plant part (through bioactivity testing), the effect of genotype and environment to yield was made, leading to the decision on *T. brevifolia* as best source of the alkaloid.The obstacles of antitumor activity, potency, acceptable formulation, cost of obtaining taxol

due to low yield, difficulty in the purification from the best source of taxol (bark extract), the high dose of the drug required to treat cancer, affected biological effect testing in clinical trial. In 1977, NCI confirmed the antitumor effect of taxol in the mouse melanoma B16 model and the extract showed antitumor effect in animal model MX-1 mammary, CX-1 colon and LX-1 lung tumors in 1978. During mid-1977, NCI advertised to companies for supply of taxol quantities needed for advanced studies. Polysciences Inc. became the first to achieve production of the amount of taxol requested by the NCI in 1979, and as a result, became the main contractor for large-scale isolation and supply of the alkaloid to NCI.The supplied taxol was distributed to select laboratories for further studies, leading to the discovery that taxol inhibited the proliferation of cancer cells at G2-M phase in the cell cycle with resultant blockage of mitosis. The studies indicated taxol among the naturally occurring spindle poisons that stabilized microtubules and inhibited their polymerisation [17]. Further investigations established taxol as mitotic inhibitor that stabilized microtubules and inhibited their depolymerization back to tubulin during cell division in cancerous cells, leading to encouraging recognition of taxol as worthy alkaloid for drug development [18-19]. Studies on clinical trial began in tumor cells in animal models, at first in mouse model implanted with tumor cells with resultant discouraging results using i.p implantation of the tumor. However, regression of tumor cells occurred using kidney capsule subcutaneous administration and as a consequence, anti-tumor effect got secured in 1978 and further development of the drug became assured [20]. In 1984, NCI began Phase I clinical trial of taxol against number of cancer types, followed by Phase II trial in 1985. The trials achieved success with first reported positive response in melanoma followed by ovarian and breast cancer [21-23]. Report by researchers at John Hopkins that taxol drug produced partial or complete response in 30 % of patients with advanced ovarian cancer led to the expansion of supply and trials as well as marketing of the drug [22]. Due to difficulties in the harvest of Pacific Yew bark for taxol and complexities in synthesis of the compound, development to clinical drug was slow; yield of taxol in *T. brevifolia* was 100 mg/kg bark, NCI clinical trial required 27,000 kg of the bark [19]. Typical 100-year-old *T. brevifolia* tree yields 3 kg of the bark, which means the trial required 9,000 trees [24]. Because the collection of bark kills the trees, population of *T. brevifolia* became threatened with extinction [25]. However, BMS ensured further supplies of taxol to NCI, leading to the successful marketing approval of paclitaxel in treatment of refractory ovarian cancer by the FDA in December 1992. Clinical studies and several successes on alternative methods of obtaining taxol followed in later years. The report on clinical patients and a search for formulation acceptable in the human body were the first stages. The discovery that cremophore formulation seemed to work and proved active against B16 paved ways for toxicological studies [26]. Further discovery of 10-deacetylbaccatin III from needles of *Taxus* species in greater yield quantity provided renewable source for semi-synthesis to meet demand for production of the drug and assured continuous supply without threat to the species' population [27-28]. Paclitaxel got tested for effectiveness in the treatment of advanced breast cancer; clinical trials showed the effectiveness and, as a result, approved by the FDA in 1994. The encouraging results pointed to test for effectiveness of the drug against other cancer types and in combination with other therapies. It further stimulated search for other natural sources of taxol to complement the plant source. The search extended to microbes and the first report of taxol production from endophytes isolated from Pacific Yew appeared [29]. Since then, several endophytes from *Taxus* and other plants are reported to produce taxol. Recent and most successful used alternative method for obtaining taxol from natural sources is developed by Phyton Catalytic. The company is utilized by paclitaxel commercial suppliers (BMS) for production of the drug. In the present, progress is made on angiosperms bioprospecting for production of taxol, use of plant cell/tissue culture, genetic engineering of microbes for taxol production. Developing more effective analogs, chemotherapy using paclitaxel for treatment of other cancer types and combinatorial therapy with other treatments are the clinical uses.

3. PLANT SOURCES OF TAXOL

In an attempt to address the supply problem of taxol, alternative plant sources besides *Taxus brevifolia* were explored. The genus *Taxus* contains nine extant and one extinct species. *T. wallichiana, T. globosa, T. floridana, T. chinensis, T. canadensis, T. baccata, T. cuspidata, T. fauna* and *T. sumatrana* are the other *Taxus* species. The hybrid species *T. media* (cross between *T. baccata* and *T. cuspidata)* and *T. hunnewelliana* (cross between *T. cuspidate* and *T. canadensis)*

are also recognized species. At a point, the extant species were considered subspecies of *T. baccata* but, molecular evidences showed existence of many distinct and closely related species, although others still recognized the nine species [29]. The most distinct of the species are *T. sumatrana* and *T. globosa*. After remarkable discovery of antitumour properties of the extract of the bark of *T. brevifolia*, screening for taxol in different parts of the tree was made with resultant isolation of several taxoids [1,30]. The mixture of taxoids produced is variable among species and tissues of the same species, but taxoids biosynthesis pathway is believed to be universal [30]. Important finding was the discovery of 10-deacetylbaccatin III (Fig. 1c) from needles and leaves of *T. baccata* that provided precursor for semisynthesis of taxol and synthesis of docetaxel from the precursor [5]. The encouraging findings lead to screening of *Taxus* species for taxol and prospecting of high-yielding species across ecological and geographical diverse sites in the U.S.A and around the world. The search extended to varieties, cultivars, organs and tissues of the species. The ecological and genetic factors to taxol yield were explored leading to discovery of taxol in all *Taxus* species with highest average yield in bark followed by roots, leaves and wood, seedlings then twigs [10,31-32]. In Irish Yew (*T. baccata* var. *fastigiata*) greatest taxol yield was in April and least in February but, "*aurea*" varieties yield traces of taxol and shoot growth occurs between May and July [33]. The roots of *T. wallichiana* gave nine taxoids; taxol, pentaacetoxytaxadiene, baccatin III and IV, 1-hydroxybaccatin, a C-oxygenated taxoid, taxusin, 7-xylosyl-10-deacetyltaxol, and a rearranged taxoid [34]. Concentration of taxanes in bark and foliage showed decreasing gradient from the stem base to tip of branches and the decreased concentration was due to higher taxanes concentration in the phloem tissues and thickness of the inner bark from the base to branch tip. The concentration in bark and needles showed variation over a growth season; it increased from May to August in bark but showed little change in needles over the period. The yield of most taxanes was lower in needles than bark but baccatin III yield reached levels comparable to that of bark in the summer; brevifoliol yield increased from March to August and reached levels nine times greater than in bark [35].Survey of needles from *T. baccata*, *T. cuspidata*, *T. canadensis*, *T. globosa*, *T.sumatriensis* obtained from various parts of Europe, Asia and USA showed taxol yield comparable to that of *T. brevifolia* dried bark [36]. The concentration of taxol and related compounds in bar and needle of *T. cuspidata* varied with location and plant part. The taxol content in bark was higher than the reported for *T. brevifolia* raised in the same area. Bark and needles of *T. cuspidata* var. *latifolia* contained higher concentration of 10-deacetylbaccatin III, possibly due to environmental factors [37]. Analysis of 750 needle samples collected from many *Taxus* species and cultivars in the Netherlands and UK for taxol, 10-deacetyltaxol, cephalomannine (Fig. 1d), baccatin III, 10-deacetylbaccatin III and brevifoliol showed substantial variation in taxane content. High yield of brevifoliol was recovered in *T. brevifolia*, taxol and 10-deacetylbaccatin III yield varied (0-500 μg^{-1} and 0-4500 μg^{-1}) in the dried needles; baccatin III, 10-deacetyltaxol and cephalomannine yield was from 0 to 500 μg^{-1} in the dried needles [38]. In seeds, variable content of taxol and taxanes similarly exists; taxane content of *T. cuspidata* dried seeds and seeds at different maturation stages depends on individual trees within species and among species. The average taxol and 10-deacetylbaccatin III yields in mature seeds were similar but mature seed part of *T. cuspidata* var. *latifolia* contained highest level of taxol compared to endosperm and testa; taxol yields per seed were highest in testa followed by endosperm and embryo. The content was greatest in new seed and seeds at middle stage of maturation but decreased with further maturation [39]. Survey of 6 *Taxus* cultivars out of 35 from different locations in the USA showed taxol yields from new needles comparable or higher to the dried bark of *T. brevifolia*. These led to initiation of nursery cultivar *Taxus* x *media Hicksii* as potential renewable source for the large-scale production of taxol [40]. *In vitro* cultures of the cuttings could be a reliable source of taxol and related taxanes because cuttings maintained in B5 liquid medium accumulated 10-deacetylbaccatin III, baccatin III, 10-deacetyltaxol, cephalomannine, 7-epi-10-deacetyltaxol and taxol over time. Higher concentration of taxanes from plant material than culture medium was recovered but, the overall taxol recovered in culture medium and harvested cuttings was equivalent to taxol yield of new plant cuttings [41]. Taxol and 10-deacetylbaccatin III yields in needles of *T. marei* giant tree of over 1,000 years were relatively high. The yield was elevated in the rooted cuttings ramets and mature needles due to genetic factors. For this reason, vegetative propagation and harvest of young needles of the elite Yew trees could be an

economic tissue source to increase taxol production. Further, *in vitro* propagation of the mature Yews was achieved using bud explants from field grown trees and might be an important strategy for mass propagation of high yielding Yews to produce plantlets for nursery establishment [42]. Besides genetic and environmental factors, storage condition affects taxol yields in *Taxus* species. Taxol showed variable yield due to instability in needles and extent of the instability was determined by drying and handling conditions [10,43]. Das et al. [44] observed decline in taxol yield from plant material and extracts when stored for one year without a care. However, when stored in the freezer and under sunlight, taxol-rich fraction in the extract was quite stable. Recovery of taxol and related compounds under different drying conditions and based on a projection of fresh biomass analysis gave near total recoveries of expected levels of taxol. Clippings dried under tobacco barn; oven, freeze-dried and greenhouse conditions gave 75-85% of expected yield for 10-deacetyltaxol and 10-deacetylbaccatin III. However, extending drying duration up to 10–15 days had an adverse effect on taxanes yield, but storage condition did not affected the yield in many needles samples [45-46]. Besides genus *Taxus,* few members of the family Taxaceae were explored for possible production of taxol and other taxanes. Simpler taxanes were produced by single member of *Autotaxus* (*Autotaxus spicata*) [47] and *Pseudotaxus* [48]. *Podocarpus gracilior* was the first species outside Taxaceae reported to produce the taxol, with a yield of 0.54 mg/Kg recovered [48]. The claim is deficient in evidence because of instrumentation techniques of HPLC and MS used for the study [49]. The alkaloid and homologs are produced in stems and leaves of *Cephalotaxus manii*; *C. fortunei*, *C. hainannensis* and *P. forrestii* [48]. Furthermore, 10-deacetylbaccatin III were isolated in needles of *Pinus massoniana* and *C. sinensis* [50].

Taxol and taxoids occurrence in other gymnosperms was adequately explored and seems produced by few species. So a search for taxol in plants was extended to angiosperms with first reported recovery in hazelnut tree (*Corylus avellana* cv. “Gasaway”). The taxanes were recovered from defatted leaves; green and brown shell extract of the tree and the yield in different tissues of hazel was similar to many *Taxus* species [51]. Using HPLC and electrospray mass spectrometry, taxol and other taxanes were detected from stem and branches of hazelnut. The detected taxol and other taxanes production were attributed to the role of the endophytic fungus in the hazel [52]. Because of the fast growth in Hazel when compared to *Taxus* species, the species could offer an alternative for the supply of taxol. So production of taxol in cell and tissue cultures of hazel was achieved using different explants. The alkaloid was detected in cell suspension cultures, callus, somatic embryos and production was not due to contamination from endophytic fungi as earlier noted. Moreover, addition of methyl jasmonate and chitosan elicitors in the culture medium enhanced yield of the taxol in established cultures [53]. Therefore establishing culture condition for optimized production of taxanes, studies on biosynthetic pathway of taxanes as well as the relationship between taxanes in Hazel nut and *Taxus* species needs to be examined. Another hazelnut, *C. mandshurica* was reported to produce taxol [54]. Possible taxol biosynthesis genes were detected for the first time in the species and close relationship found between the differentiated genes and various adaptations to fungal infection as well as cold in *C. mandshurica* and *C. avellana* [55]. In the course of screening for chemical constituents, taxanes were detected in *Yunnanopilia longistaminata* from China. The detected taxanes were isolated from tender burgeon of *Y. longistaminata*, the structures identified as taxayunnansin B, L-iditol and taxumairol E on the basis of NMR and MS spectrum. Besides these compounds, possibility of taxol production from the species exists but so far was not investigated [56].

By far, *T. brevifolia* is the best known plant source of taxol but, due to environmental concern, cost and improvements in technology, significant taxol supplies are nowadays made by isolation of 10-deacetylbaccatin III from the needles of *T. wallichiana* and *T. baccata*, taxol is then semisynthesized from the precursor.

4. TAXOL FROM ENDOPHYTES

Overwhelming pharmaceutical success of paclitaxel as anticancer agent, demand due to increasing cancer cases and many challenges on sourcing of taxol such as slow growth of *Taxus* species, the small yield, lead to search for alternative ways to obtain the alkaloid. The search continued to endophytes besides plant sources. Endophytes are omnipresent in most of the plants studied for the presence but, nature of the relationship between plant and the endophytes depends on many factors. They

inhabit plant, many of which show the symbiotic relationship. Because of a number of compounds produced by these organisms, they could enhance growth characteristics of plant, increase adaptation to the environment and in many instances produce alkaloids of host plant [57]. The search for taxol-producing endophytes was at first made within *Taxus* species before extended to other species. Up to early 1990s there wasn't any report of endophytic fungi from *Taxus* species that produce taxol, but, after several years of research, novel endophytic fungus that produce taxol was isolated from earliest known source of the alkaloid, *T. brevifolia. Taxomyces andreanae* was isolated from the phloem of the inner bark of Pacific Yew. The fungus produces taxol when grown in semi-synthetic liquid medium and identity of taxol confirmed using spectrometry, chromatography and reactivity with monoclonal antibodies specific for taxol [14]. Three years later, another endophytes, *Pestalotiopsis microspore* isolated from the inner bark of *T. wallichiana*, was reported to produce taxol when cultivated in semisynthetic medium and accumulates in mycelia culture at the level of 50 ng to 50 µg [58]. The findings generated interest on the search for possible and alternative endophytes sources of taxol in other *Taxus* species, leading to isolation of several endophytes [e.g 59-61, ., ., .,…..]. Besides the success, search for taxol-producing endophytes continued to non-*Taxus* species. The first surprising finding was the isolation of *P. microspore* from *Taxodium distichum* [62]. Since then, several reports on the production of taxol in endophytes have been reported (Table 1). In the present, about 200 endophytic fungi belonging to over 40 genera and several orders of Ascomycota and Deuteromycota, few Basidiomycota and Zygomycota were reported to produce taxol and the list continues to grow but is yet to translate into industrial production from microbes [63]. However, with an increase in a number of isolated endophytes, several strategies including biotechnological tools are employed to maximize production and yield for industrial application of taxol production from the endophytes. Among the strategies includes strain improvement and media optimization [64] biotechnological and industrial processes of fermentation condition optimization, genetic engineering including mutagenesis and breeding of protoplasts among others [65-67]. In the recent, *Escherichia coli* was genetically engineered to produce taxadiene, first committed taxol intermediate in taxol biosynthesis, with a yield of 1 g/L. The conversion of taxadiene to taxadiene-5-α-ol was achieved for the first time in microbe [68]. So far, least reported yield of taxol from endophytes is 0.025 µg/L in *T. andreanae* [14] while the greatest is from *Metarhizium anisoplias* with 846.1 µg/L [69]. In the recent, [70] isolated 81 endophytic fungi from *T. media* and grouped them into eight genera based on morphological and molecular evidences. *Guignardia* and *Colletotrichum* were the dominant genera, and maximal yield of 720 ng/L was from *G. mangiferae*. Bacteria occur in large numbers in rhizosphere than any other parts of the plant. Many fungal endophytes produce taxol but, few reports on bacterial production of taxol are known. Majority of known taxol-producing fungal microbes are Ascomycetes and Deuteromycetes, and the bacterial belongs to *Kitasatospora* and *Streptomyces*. Two recent publications addressed the issue of taxol production in endophytes and the evolution of taxol biosynthesis genes through horizontal genes transfer between host plant and endophytes. Heinig et al. [71] addressed the issue of taxol biosynthesis in endophytic fungi. They assessed the 'inconsistencies' generated through proliferation of reported production of taxol by endophytes for two decades and the evidences provided in the publications following earlier reported breakthrough work of Stierle et al. [14]. Through experimental evidences, they showed non-existence of homology in terpene biosynthetic genes in *T. andreanae* and *Taxus* species and horizontal gene transfer previously speculated as the origin of taxol biosynthetic genes in endophytes does not apply [71]. Their experimental evidences showed taxol detected from endophytes was a "carryover" of taxol from host plant and, therefore, was not biosynthesized by the endophytes. The evidences provided explanations on independent taxane biosynthesis in endophytic fungi and others lacking evidences. However, the argument on horizontal genes transfer was supported by Yang et al. [72]. They found potential genes involved in taxol biosynthesis in *T. baccata* and *Penicillium aurantiogriseum* NRRL 62431 different but showed 19-65% identical amino acid sequences. By Comparing the genes in host hazel and *P. aurantiogriseum* NRRL 62431, the experimental results showed are not highly conserved but shared 21-62% sequence identities. The argument taxol-producing endophytes do not biosynthesize taxol, instead was a "carryover" from host was, therefore, refuted, because taxol-producing endophytes were isolated from non-*Taxus* species [72]. Evolution of molecular studies in the 21st century will give a sounding

knowledge on taxol biosynthesis in endophytes and fill the 'loopholes' of knowledge soon. Over 20 years since the discovery of taxol production in microbes, yet, industrial application is not near sight. Genomic instability due to prolonged culture of endophytes with the resultant effect on the reduction in production of taxol is one of the challenges affecting microbial production of the alkaloid at industrial scale.

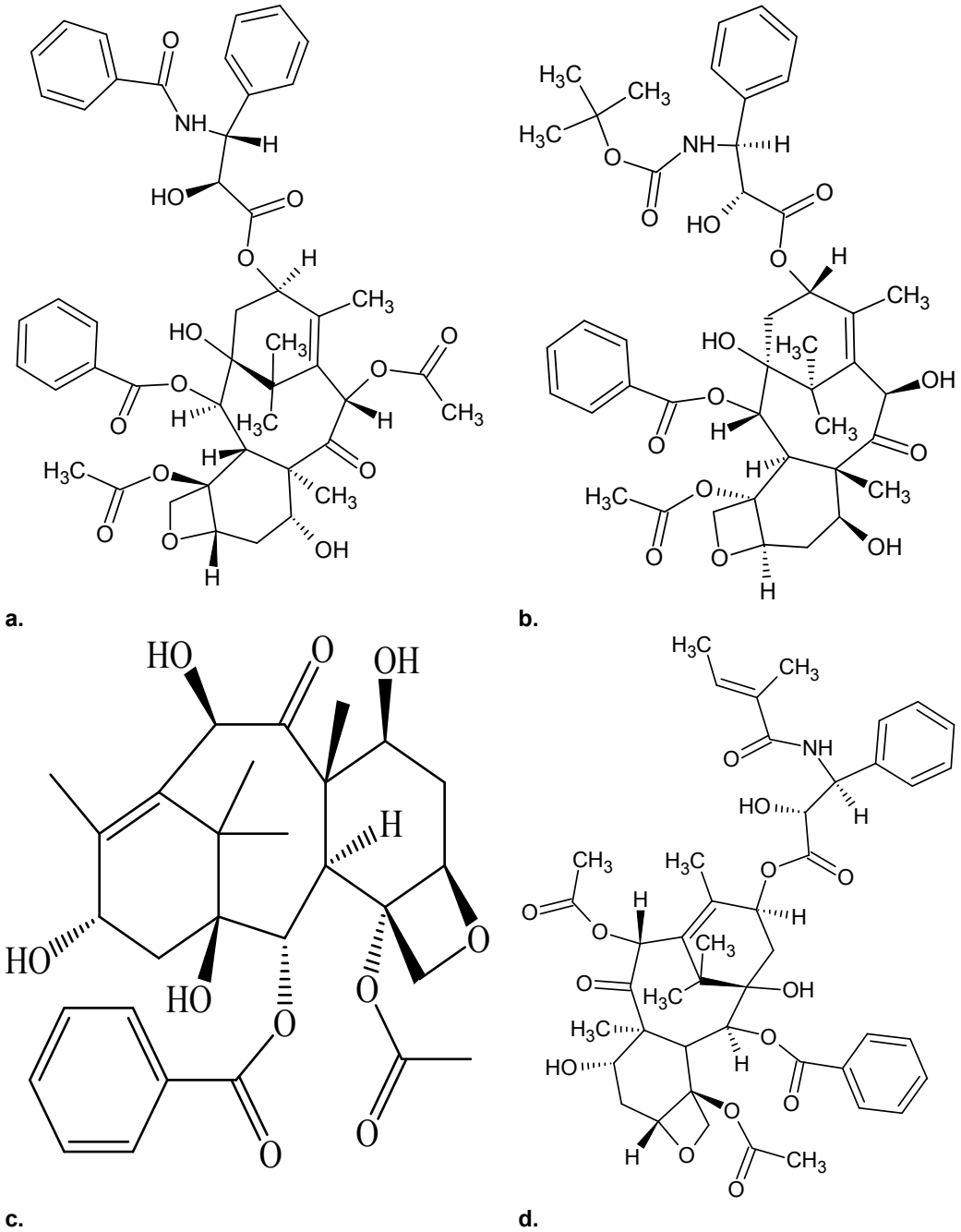

Fig. 1. Chemical structure of Taxanes: a. Paclitaxel, b. Docetaxel, c. 10-deacetylbaccatin III, d. Cephalomannine

Table 1. Some isolated taxol-producing endophytes

Endophyte	Host Plant	Reference
Taxomyces andreanae	*Taxus brevifolia*	[14]
Alternaria sp.	*T. cuspidata*	[88]
Fusarium lateritium	*T. baccata*	[88]
Monochaetia sp.	*T. baccata*	[88]
Pestalotia bicilia	*T. baccata*	[88]
Pestalotiopsis microspora (Ja-73)	*T. cuspidata*	[88]
P. microspora (Ne-32)	*T. wallichiana*	[85]
P. microspora (No 1040)	*T. wallichiana*	[85]
P. microspore (Cp-4)	*Taxodium distichum*	[61, 63]
Pithomyces sp.	*T. sumatrana*	[57]
P. guepinii	*Wollemia nobilis*	[57]
Pestalotiopsis sp. *(W-X-3)*	*W. nobilis*	[57]
Pestalotiopsis sp. *(W-IF-1)*	*W. nobilis*	[57]
P. microspore (Ne 32)	*T. wallichiana*	[85]
Periconia sp.	*Torreya grandifolia*	[86]
Alternaria sp.	*Ginkgo biloba*	[87]
Tubercularia sp.	*T. chinensis* var *mairie*	[88]
Seimatoantlerium nepalense	*T. wallichiana*	[89]
Fusarium solani (Tax-3)	*T. chinensis*	[90]
Sporormia minima	*T. wallichiana*	[91]
Trichothecium sp.	*T. wallichiana*	[91]
Nodulisporium sylviforme	*T. cuspidate*	[75, 78]
Taxomyces sp.	*T. yunnanensis*	[92]
Alternaria alternate	*T. chinensis* var *mairei*	[93]
Botrytis sp.	*T. chinensis* var *mairei*	[94]
Ectostroma sp.	*T. chinensis* var *mairei*	[95]
Ozonium sp.	*T. chinensis* var *mairei*	[96]
Papulaspora sp.	*T. chinensis* var *mairei*	[92]
Fusarium mairei (Y1117)	*T. chinensis* var *mairei*	[96]
Aspergillus fumigatus	*Podocarpus* sp.	[97]
Botryodiplodia theobromae	*T. baccata*	[98]
Botrytis sp.	*T. cuspidata*	[99]
Fusarium arthrosporioides	*T. cuspidata*	[100]
F. mairei (UH23)	*T. chinensis*	[101]
F. solani	*T. celebica*	[102]
Pestalotiopsis pauciseta	*Cardiospermum helicacabum*	[103]
Phyllosticta citricarpa (No. 598)	*Citrus media*	[104]
P. spinarum (No. 625)	*Cupressus* sp.	[105]
P. dioscoreae (No. 605)	*Hibiscus rosa-sinensis*	[106]
Pestalotiopsis terminaliae	*Terminalia arjuna*	[107]
Mucor rouxianus	*T. chinensis*	[108]
Metarhizium anisopliae	*T. chinensis*	[69]
Cladosporium cladosporioides	*T. media*	[109]
Aspergillus niger var *taxi*	*T. cuspidate*	[110]
Guignardia mangiferae	*T. media*	[70]
S. tepuiense	*Maguireothamnus speciousus*	[111]

5. APPLICATION OF BIOTECHNOLOGY IN TAXOL PRODUCTION

Studies on biotechnology application in taxol production started in the early 1990s. Considering the market value of taxol and other taxanes, biotechnological production offers economical, sustainable and alternative compared to extraction from natural plant sources and production by plant cell cultures most appropriate approach [73]. Plant cell culture is the most promising strategy for the sustainable production at industrial level and most employed technique nowadays in supply of taxol, related taxoids along with semisynthesis and microbial fermentation technology. Production using *in*

vitro techniques depends on explant, culture medium and growth condition among other factors. Somaclonal variation accompanied with small taxol yield due to prolonged subculture, unstable release into culture medium, slow growth of cultures and medium browning are limitations in use of *in vitro* techniques in production of taxol in *Taxus* species. Fortunately, because of the ease at which tissues are harvested for taxol recovery from *in vitro* cultures, when compared to extraction from bark or needles, use of *in vitro* method for taxol production is more favorable. Molecular studies have aided understanding paclitaxel metabolism and role of processes optimization for enhanced production of taxol by using the techniques, but, knowledge on paclitaxel global metabolism including regulation, transportation and degradation is limiting [74]. Metabolic engineering for enhanced taxol yield along with the application of *in vitro* techniques are gaining more research attention in the present, and various strategies are used to enhance taxol and related taxoids production in cell and tissue cultures. Among the *in vitro* strategies include culture medium optimization, high-yielding cell lines selection, addition of precursors into culture medium and elicitation. Because of the amenability in suspension culture to scale-up and environmental optimization, the method is most appropriate for optimized taxol production. Elicitation of *Taxus* species suspension cultures is the most efficient strategy for enhanced taxol and other taxanes production [75].

During the decades following discovery of taxol production in endophytes, endophytic fungal diversity and isolation for production of taxol received comprehensive scientific exploration. In the last decade, a shift to study relationship between endophytes and host plants, evolution of the relationship to improve productivity using the techniques of genetic engineering, fermentation technology and microbial breeding received considerable research interest [76,77]. The identity of endophytes, biological properties and understanding biochemistry of taxol production to culture condition are areas that needs to be studied adequately, so as to understand the factors involved in species-specific production of taxol. Further, quantification of taxol yield from endophytes is met with 'bottle necks' due to variable recovery of the alkaloid from microbe, depending on the efficiency of extraction method employed, the solvent used and evaporation temperature. However, the technique devised extracted all taxol produced in fungal culture [76]. Among several methods used to detect taxol from fungal fermentation product, HPLC is the most employed method. Therefore, there is a need to devise efficient extraction and detection technique. Application of mutation breeding by exposing endophytes to single or combination of mutagens to develop high yielding mutants followed by cumbersome screening of mutants and protoplast fusion are recent strategies to produce high yielding endophytes for production of taxol at industrial scale [78-81]. Modern biotechnology approaches such as transformation to produce high yielding endophytes is a difficult task due in part to the difficulty to introduce and express taxol biosynthetic genes in endophytes. The introduction of taxol biosynthesis genes and expression up to the conversion of taxadiene to taxadiene-5-α-ol in *E. coli* was a great achievement towards genetic engineering of endophytes for taxol production [68]. Although the transformation and genetic engineering of endophytes for production of taxol remains a significant challenge, poor understanding of the regulation of the biosynthesis that is prerequisite to enhanced production in microbes is among limitations to the achievement. Present evolution and understanding of microbial genomics opens up possibility to develop high yielding genetically engineered microbes for production of taxol at industrial scale [68,82-84]. Because of the simplicity and less cost of endophytes culture media, production of taxol in endophytes seems of economic advantage to the plant cell culture but, small production of the alkaloid is an issue to solve. Improving culture condition of endophytes through supplementing culture media with elicitors (biotic and abiotic), taxol precursor feeding into culture media, inhibitors appending, and other strategies will address the problem. Fermentation of endophytes is the prospective plan for industrial production of taxol from microbes in the present.

6. CONCLUSIONS

Bioprospecting of plant kingdom and microbes for taxol production to address supply crisis showed many biota alternatives. The alternative sourcing range from extraction from wild and cultivated *Taxus* species, few gymnosperms and angiosperms, partial and total synthesis, application of *in vitro* techniques, fungal and bacterial endophytes to genetic engineering. Discovery of taxanes production in angiosperms open up more possibility to address supply crisis

from plant sources due to the high number of species and fast growth of the plants compared to gymnosperm sources. Total and semisynthesis, genetic engineering and tissue culture are promise strategies for the supply of taxol despite economic cost but, in the present, taxol supply is met to a greater extent using plant cell culture techniques. Enhancement of production through application of plant tissue culture, metabolic engineering along with efficient extraction and separation methods are given more priority in the present.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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