Production of valuable chemical compounds isolated from plants by endophytic fungi

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Abstract. Natural products have always been an integral part of medical therapy. Many drugs have been developed from natural products mostly derived from plants. For example, the most effective anticancer compounds such as anthracyclines, vinblastine, vincristine, paclitaxel and camptothecins are produced by plants. However, productions of these highly valuable compounds have threatened the existence of the plants as it requires a large number of materials from plants. This paper highlights the potential and importance of endophytic fungi as the producers of highly valuable compounds as it needs only a small amount of material from plants. For example, anticancer drugs like paclitaxel, vincristine and vinblastine were more commonly found in endophytic fungi than in plants.

1. Introduction
Plants have been widely known as the major sources of bioactive agents for treatment of cancers. Some of the important anticancer drugs from plants include vinca alkaloids, vinblastine and vincristine produced by Catharanthus roseus [1, 2]. Etoposide and teniposide are also clinical drugs used for the treatment of cancer which was developed from natural product epipodophyllotoxin [3]. Epipodophyllotoxin is an isomer of podophyllotoxin found in the roots of various species of the genus Podophyllum [4]. These plants have been long traditionally used for the treatment of skin cancers and warts in the American and Asian communities [5].

The most valuable anti-cancer drug, paclitaxel (Taxol®), was originally from the bark of Pacific Yew (Taxus brevifolia) [6]. Paclitaxel became the most exciting plant-derived anticancer drug with average sales approximately over $1 billion [7]. The annual demand for paclitaxel was estimated at around 100-200 kg per year. On the other hand, paclitaxel is present in limited quantities from its natural sources as 1 kg of paclitaxel requires 10,000 kg of Taxus bark [7]. Thus, it is estimated thousands of Pacific Yew trees will cut down and this will bring serious consequences on the ecological perspective of this valuable and slow-growing tree. Therefore, alternatives production of drugs should be developed. Endophytic fungi have attracted great attention for drug development [8-10] since the discovery of endophytic fungus Taxomyces andreanae from Taxus brevifolia which was
also able to produce paclitaxel [11]. This paper highlights and discusses the production of drugs originally from plants by endophytic fungi.

2. Endophytic Fungi as Producer of Valuable Chemical Compounds Originally from Plants

The co-evolution of endophytic fungi with their host plant has been recognized to shape the production of chemical metabolites by the endophytic fungi. Host plant provides nutrition and habitat for endophytic fungi while endophytic fungi produce bioactive metabolites to help the adaptation of their host plant against biotic and abiotic stress. Several reports have shown the biosyntheses of valuable chemical compounds derived from plants by endophytic fungi especially the anticancer drugs and drug leads from plants.

2.1 Paclitaxel from Endophytic Fungi

Paclitaxel produced by Yew tree has broadband of antitumor activities including ovarian, neck, breast, lung and head cancers as well as Kaposi’s sarcoma. The first report of the production paclitaxel from endophytes was from the endophytic fungus Taxomyces andreanae [11] isolated from the plant Pacific yew Taxus brevifolia (Taxaceae). Many other endophytic fungi have been known to produce paclitaxel. These endophytic fungi were isolated not only from the plant of Taxus species but also from other species such as hazelnut producing plants [12], Citrus medica (Rutaceae), Podocarpus sp. (Podocarpaceae), Torreya grandifolia (Taxaceae), Cupressus sp. (Cupressaceae), Hibiscus rosa-sinensis (Malvaceae), Taxodium distichum (Taxodiaceae), Cardiospermum halicacabum (Sapindaceae), Terminalia arjuna (Combretaceae), Ginkgo biloba (Ginkgoaceae) and Wollemia nobilis (Araucariaceae) [13].

There are more than 20 genera of endophytic fungi that are able to produce paclitaxel and its analogs including Botryodiplodia, Tubercularia, Alternaria, Botrytis, Cladosporium, Ectostroma, Fusarium, Metarhizium, Monochaetia, Pithomyces, Pestalotiopsis, Phyllosticta, Pestalotta, Taxonomyces and Aspergillus) [14]. Among these fungi, fungal strains Metarhizium anisopliae was able to produce 846.1 μg/L of paclitaxel [15] while fungal strain Cladosporium cladosporioides isolated from Taxus media produced around 800 μg/L [16].

Of special interest, there are several interesting reports regarding the production of paclitaxel from fungi inhabiting the plants other than Taxus species such as the fungal strain Phyllossticta citricarpa residing in Citrus medica and Phyllossticta dioscoreae isolated from Hibiscus rosa-sinensis [17] produced 265 and 298 μg/L taxol, respectively.

![Figure 1. Chemical structure of left: vinblastine; middle: vincristine; right: paclitaxel](image)

2.2 Vinca alkaloid: Vincristine and vinblastine from Endophytic Fungi

Vinblastine and vincristine are dimeric indole alkaloids produced by the plant Catharanthus roseus. They are clinically used for the treatment of breast, lung, leukemia and lymphoma cancers. Alternaria sp from C. roseus was first reported to produce vinblastine [18]. Moreover, endophytic fungi
Fusarium oxysporum has been known to produce both drugs. Endophytic fungus *F. oxysporum* isolated from *C. roseus* was reported to produce vincristine [19]. In 2013, *F. oxysporum* isolated from *C. roseus* collected in India was known to produce both vinblastine and vincristine around 76 µg/L and 67 µg/L, respectively. In addition, endophytic fungus *Talaromyces radicus* isolated from *Catharanthus roseus* was also observed to produce both vincristine and vinblastine [20].

![Chemical structure of (left to right) camptothecin (R1=R2=H) or 9-methoxycamptothecin (R1=OMe, R2=H) or 10-hydroxycamptothecin (R1=R2=OH); middle: topotecan; right: irinotecan](image)

**Figure 2.** Chemical structure of left: camptothecin (R1=R2=H) or 9-methoxycamptothecin (R1=OMe, R2=H) or 10-hydroxycamptothecin (R1=R2=OH); middle: topotecan; right: irinotecan

### 2.3 Camptothecin and its analogue from Endophytic Fungi

Camptothecin was originally isolated from the plant *Camptotheca acuminata* in 1966 [21]. It has become the lead compound for drugs irinotecan and topotecan that were clinically used for the treatment of small lung, ovarian and refractory ovarian cancers [22]. Camptothecin was still supplied from the wild tree of *Camptotheca acuminata* (Nyssaceae) and *Nothapodytes nimmoniana* (Icacinaceae). Another source of camptothecin was first reported from an endophytic fungus *Entrophospora infrequens* associated with the plant *Nothapodytes foetida* [23]. The endophytic fungus *Neurospora* sp isolated from the seeds of *Nothapodytes foetida* was also reported to produce camptothecin [24]. From the plant of *Camptotheca acuminata*, the endophytic fungus *Fusarium solani* produced camptothecin and its analogues 9-methoxy camptothecin together with 10-hydroxy camptothecin [25]. Two fungal strain endophytic *Fusarium solani* MTCC9667 and MTCC9668 obtained from the plant *Apodytes dimidiate* (Icacinaceae) were also known to produce camptothecin, 9-methoxy camptothecin and 10-hydroxy camptothecin [26]. Shweta et al in 2013 [27] reported the production of camptothecin from three endophytic fungi *Alternaria alternata*, and *Phomopsis* sp isolated from the seeds and fruits of plant *Miqueliadentata* Bedd which is known to accumulate very high concentrations of camptothecin in its seeds.

### 2.4 Podophyllotoxins from Endophytic Fungi

Podophyllotoxin is the lead compound of clinically anti-cancer drugs etoposide and teniposide which can inhibit the topoisomerase by preventing the formation of mitotic-spindle of microtubules [28]. Podophyllotoxin is currently supplied from the plant *Podophyllum* species [14]. The species of *podophyllum* plants have been categorized as endangered species due to overexploitation.
Figure 3. Chemical structure of left: podophyllotoxin (R=OH) or deoxypodophyllotoxin (R=H); middle: etoposide; right: teniposide

Other sources of podophyllotoxins have been discovered from several endophytic fungi such as *Phialocephala fortiniii* and *Trametes hirsuta* obtained from the plants of *Sinopodium peltatum* and *S. hexandrum* [29, 30], *Alternaria* sp from *Sinopodium hexandrum* [31] and *Fusarium oxysporum* obtained from *Sabina recurva* (Kour et al., 2008) and *Fusarium solani* isolated from the root of *S. hexandrum* [32] together with *Aspergillus fumigatus* obtained from *Juniperus communis*. Ran et al. in 2017 [33] has also reported the accumulation of camptothecin from the endophytic *Fusarium solani* obtained from the host *Camphotheca acuminate*.

3. Conclusion
Recent development has shown that the occurrence of anticancer drugs originally derived from plants was found more common in endophytic fungi. Of special interest, the endophytic fungi producing the valuable drugs were also isolated from the host plants which were not known to produce the drugs. As isolation of endophytic fungi required only a small material of plants, this provides an alternative source for future direction in drug discovery programs.

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