Chapter 7

Endophytic Fungi as Alternative and Reliable Sources for Potent Anticancer Agents

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Abstract

In comparison with other natural sources like plants, highly diverse microorganisms are narrowly explored, especially with respect to their limitless potentials as repositories of exceptionally bioactive natural products. Of these organisms, fungi inhabiting tissues of plant in a noninvasive relationship (endophytic fungi) have proven undeniably useful and unmatchable as sources of potent bioactive molecules against several diseases such as cancer and related ailments. In general terms, endophytic fungi are highly prevalent organisms found in the tissue (intracellular or intercellular) of plants and at least for reasonable portion of their lives. It has been proven that virtually every plant, irrespective of habitat and climate, plays host to wide varieties of endophytes. Endophytic fungi produce metabolites produced by different biosynthetic pathways to that of the host plant, and this robustness equips them to synthesize unlimited structural entities and scaffolds of diverse classes. Interestingly too, the cohabitation/culture of these fungi with certain bacteria offers even stronger hopes for anticancer drug discovery. The endless need for potent drugs has necessitated the search of bioactive molecules from several sources, and endophytic fungi appear to be a recipe. This chapter is an attempt to present the current trend of research with these very promising organisms.

Keywords: microorganism, plants, sources, endophytes, fungi, anticancer, drug discovery

1. Introduction: the emergence of interest in endophytic fungi as sources of bioactive metabolites

Despite the fact that plant natural products (secondary metabolites) have been recognized as the most successful source of potential drug leads [1, 2], their recent implementation in drug discovery and development efforts have somewhat demonstrated a decline in interest [1]. Consequently, the need for new compounds to provide mitigation and relief in diverse human clinical conditions is ever-growing. In addition, the challenges of bacterial drug resistance, the emergence of highly virulent viruses and bacteria with organ transplant-associated complications demand very drastic drug discovery approaches in order to address them [3]. From the earliest times, when man directly tested plant materials for their potentials as medicines or foods to today when structured research approach has led to advancements in the discovery of bioactive natural product, it is evident that so much has been achieved in terms of the number of such agents that have great clinical importance. Notwithstanding, less than 10% of the world's biodiversity has been evaluated for potential biological activity, and therefore, many more useful natural lead compounds await discovery with the challenge being how to access this natural chemical diversity [4]. The search for bioactive natural products with potentials of addressing these identified challenges is as old as man's existence and has always relied heavily on the study of whole plant tissues (morphological parts) such as the roots (bark and inner tissues), leaves, stem (bark and inner tissues) and animal sources [5]. Unfortunately, the use of whole plant or animal tissues for the isolation of bioactive natural products has not been without its serious challenges. Fundamentally, the process leads to destruction of whole plant over time as the tissues are repeatedly collected without replenishment; most times, the existence of these plant species becomes threatened (**endangered**) and they are even lost after some years. Closely associated with this remarkable challenge are the problems of environmental degradation, land spoilage and the usually limited yield of the identified bioactive natural products. Secondly, the use of whole plant tissues in search of bioactive natural products has comparatively witnessed great wastage of research resources as a result of dereplication. Dereplication, which describes the process of fastly identifying or re-isolating already established biomolecules, is not only time and resources wasting but also discourages research zeal and excitement (reduction in interest). As at today, the probability of finding a novel bioactive molecule or chemical scaffold from whole tissues has further reduced because of dereplication. There are usually cross-family interactions among plant species in different biotopes complicated by most times poorly identified plant species, and as such, dereplication becomes a common challenge. Besides, the plant material has fixed biogenetic pathways which are tightly controlled by nature to produce predetermined secondary metabolites. As such and apart from the herculean plant tissue technology, man has little or no control over what a natural plant material produces as secondary metabolites. These identified problems led to loss of popularity in the natural product research efforts and, consequently, the development of combinatorial chemistry. Despite the great chemistry and huge investments witnessed in this era, not much breakthroughs have been recorded and, besides, combinatorial chemistry should act as a compliment to the natural product chemistry [6]. At this point, there arose a dire need for paradigm shift in the search for bioactive natural products. Accordingly,

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over a decade ago and based on accumulated successful drug discovery story, it appeared that the search for novel secondary metabolites should be refocused and research efforts consequently centered on the organisms that inhabit unique biotopes [1]. Of these organisms, endophytes were quickly recognized as veritable sources of novel bioactive natural products. Endophytes have been viewed as outstanding sources of novel products because there are so many of them occupying literally millions of unique biological niches (higher plant) growing in so many unusual environments. In all these, the endophytic fungi have been shown to be exceptionally useful in the drug discovery process, especially in the western world. An intensive literature search revealed that endophytic populations of the plants in rain forests of the African continent have not been significantly explored. Regrettably, sketchy research data have largely been documented on few usually unharnessed and abridged studies with whole plant tissues. This apparent low interest in researches with microbes as sources of antimetabolites is much more worrisome in trans-Saharan Africa. The reasons are not farfetched, mainly owed to weak infrastructural framework and systems. In Nigeria, for example, which is the acclaimed most populous black nation in Africa (approximately 160 million people—2006 National Population Census data), there are large rainforest zones in strategic locations of the country. Unfortunately, despite this robust biodiversity in Nigeria, bioprospecting for active molecules from these interesting microbes has remained largely unharnessed, and even the few research efforts have relied heavily on the direct plant tissues, with little work on the potentialities of fungal endophytes as veritable sources of novel bioactive compounds. This is a serious aberration and a major affront against any meaningful drug discovery effort of not only in Nigeria but also in several other African Countries. Consequently, there is need to awaken and boost research interest in the use of these specialized microbes (endophytes) for the discovery of potent bioactive molecules against the ever-increasing disease burden globally. In addition, given the rapidly increasing population of Nigeria and accompanying demand for drugs, it is critically important to identify and develop renewable sources of pharmaceuticals and their precursors. It is in line with this thought that we proposed this book chapter on endophytes as alternative and reliable sources of potent anticancer agents. It is our modest expectation that, through concerted research efforts in this promising area of drug discovery process, several strongly potent and safe molecules will be identified, isolated, assayed, characterized and hopefully progress into the several stages of clinical trial program as potential part of the armamentarium against cancer and associated conditions.

2. Endophytic fungi and search for active metabolites

The use of microbial biotopes as reliable sources of bioactive natural products has received significant attention so far [7, 8]. Current available data reveal that more than 40% of novel potent bioactive molecules obtained in a period of nearly two and half decades and half were microorganism-derived. Furthermore, over 60% of the anticancer and 70% of the antimicrobial drugs currently in clinical use are natural products or natural product derivatives. This is not surprising in the light of their evolution over millions of years in diverse ecological niches and natural habitats. The avalanche of microbial diversity with exciting metabolic complexes

in plant tissues has been established in the last two decades and is continuing [8]. It is expedient to emphasize that the discovery of an endophytic fungus, from *Taxus brevifolia*, which produces highly selling anticancer agent called ***taxol***, precipitated a surge in interest to researches with endophytes [9]. Accordingly, about a decade ago, it appeared that the search for novel secondary metabolites should be refocused and research efforts consequently centered on the organisms that inhabit unique biotopes [1]. Of these organisms, endophytes were recognized as veritable sources of novel bioactive natural products. Endophytes have been viewed as outstanding sources of novel products because there are so many of them occupying literally millions of unique biological niches (higher plant) growing in so many unusual environment. One very interesting feature of the endophytic fungi is that they are renewable, readily available and environmentally friendly sources of biologically active natural products. An intensive literature search revealed that endophytic populations of the plant in rain forests of the African continent have not been studied apart from few unharnessed studies with whole plant tissues. Despite this ugly and unacceptable picture, it is heartening to note that this trend is gradually changing as current research scholars of African origin, especially those from Nigeria and Cameroun with thrust in natural product chemistry focus more on endophytic fungi nowadays as compared to the use of whole plant tissues. In summary, the inadequacy of systematic exploitation of ecosystems for the discovery of novel microbial compounds had resulted in random sampling and has missed the true potential of many regions [10]. Numerous bioactive molecules have been isolated from endophytic fungi since this ground-breaking discovery [11–13]. It is known that these endophytic fungi are embedded within plant for a substantial part of their life cycle and, as well earlier stated, they are devoid of any established potential to cause diseases in the host [14, 15]. This attribute makes this class of microbes a unique resource base for the discovery and development of potent anticancer molecules without having to destroy whole plant tissues. Additionally, they have been found to be more active when compared to other types of fungi somewhat existing outside the host in terms of metabolic virility [6, 16]. It was previously thought that metabolic products are transferred between host plant and the endophyte, the theory of horizontal transfer from the host plant to its microbial symbiont [9, 11, 17, 18]. This belief has been disproved following the successful sequencing of the *taxadiene synthase* gene from the taxol-producing endophyte which established that the metabolic pathways of both the hosts and the endophytes are independent of each other. The implication of this is that there are ample opportunities available for the manipulation of the endophyte biosynthetic pathways [19] to yield wide varieties of molecules and scaffolds for drug discovery process. 'Endophytism' is, thus, a unique cost–benefit plant microbe association defined by 'location' (not 'function') that is transiently symptomless, unobstructive, and established entirely inside the living host plant tissues [20, 21]. Evidence of plant-associated microorganisms found in the fossilized tissues has revealed that endophyte-plant associations may have evolved from the time higher plants first appeared on the earth surface [22]. The existence of fungi inside the organs of asymptomatic plants has been known since the end of the nineteenth century [23], and the term, 'endophyte' was first proposed in 1866 [24]. Since their discovery and description, they have been isolated from various organs of different plant species, from aboveground tissues of liverworts, hornworts, mosses, lycophytes, equisetopsides, ferns and spermatophytes from the tropics to the arctic, and from the wild to agricultural ecosystems [25]. Interestingly, all plant species studied till date have been found

to harbor at least one endophyte. The most frequently encountered endophytes are fungi or bacteria (including actinomycetes), but future projections suggest that there could be revelation of nonendophytic microorganisms [3]. Endophytic fungi are a very diverse polyphyletic group of microorganisms; they can thrive asymptomatically in the tissues of plants aboveground as well as belowground, including stems, leaves and/or roots [26]. Many endophytes have the potential to synthesize various bioactive metabolites that may directly or indirectly be used as therapeutic agents against numerous diseases [3, 19, 21, 27–29]. Occasionally and usually hitting major buck, endophytes that produce host plant secondary metabolites with therapeutic value or potentials have been discovered; some examples include paciltaxel (also known as taxol) [9] and podophyllotoxin [17, 30]. The production of bioactive compounds by endophytes, especially those exclusive to their host plant, is not only important from an ecological perspective but also from a biochemical and molecular standpoint. In contrast to the direct bioprospecting with known medicinal plants (the common problem being that of dereplication), exciting possibilities exist for exploiting endophytic fungi for the production of a plethora of known and novel biologically active secondary metabolites. The potential of microorganisms is further limited by the presence of orphan biosynthetic pathways that remain unexpressed under general laboratory conditions [31]. However, the vast choice of techniques pertaining to the growth and manipulation of microorganisms such as media engineering, coculture, chemical induction, epigenetic modulation and metabolite remodeling, coupled with the fermentation technology for scale-up, make them suitable for production of useful natural products, both known and novel [16]. Of the myriads of ecosystems on earth, those having the greatest biodiversity seem to be ones also having endophytes with the greatest number and the most biodiverse microorganisms. Tropical and temperate rain forests are the most biologically diverse terrestrial ecosystems on earth. As such, one would expect that areas of high plant endemicity also possess specific endophytes that may have evolved with the endemic plant species. Ultimately, biological biodiversity implies chemical biodiversity because of constant chemical innovation that exists in ecosystems where the evolutionary race to survive is most active. Comparatively, tropical rainforests are a remarkable example of this type of environment because competition is great; resources are limited, and selection pressure is at its peak. This gives rise to a high probability that rainforests are excellent source of novel molecular structures and biologically active compounds [32]. Early researches in endophyte bioprospecting showed that a significantly higher number of tropical endophytes produced a larger number of active secondary metabolites than did fungi from temperate endophytes or other tropical substrata [33].

3. Isolation process and characterization of bioactive metabolites from endophytic fungi

The choice of plants for the explorative study of endophytes in search of potent bioactive molecules is a very crucial issue that requires the right information. Since the biochemical pathways of both endophytes and their host are strongly correlated, it is expedient that medicinally useful plants in different cultures are selected for bioprospecting of endophytes. The isolation of

endophytic fungi is carried out using the most probable morphological part of the properly identified and document host plant, most times the leaves. The wholesome leaves of the selected plant are collected in sterile plastic bags from designated area with the aid of the geographic position system (GPS) and stored at refrigeration temperature (4°C) in preparation for the isolation of endophytic fungi. There are times during which samples might have to be transported over long distances, and it is required that contingent transport and sterile cold chain arrangements should be made to ensure the integrity of the plant part on arrival. In all the processes, it is required that strict sterility is maintained so as to ensure that no external organisms are introduced as contaminants to the culture. The following procedures are then followed:

(i) The leaves or any other morphological part of interest is washed under flowing tap water for a minimum time of 10 min and dried under sterile dry air.

(ii) With the aid of a sterilized scalpel (use of Bunsen flame) or other techniques, pieces of the leaves are cut out and sterilized further using these agents; 95% ethanol (1–2 min), 3.5% (v/v) sodium hypochlorite (3–4 min), 70% (v/v) ethanol (30–40 s) in that order.

(iii) The sterilized samples will be washed trice in sterile distilled water and allowed to dry on filter papers under aseptic conditions. Subsequently, the samples are placed on the appropriate culture media of interest supplemented with an antibiotic concentration to prevent the growth of bacteria and depending on the substrate that will act as the primary carbon source in the medium. Some of the natural carbon sources include maltose, rice, beans, etc. The plating is usually done in replicates and inside air laminar flow hood of appropriate level of biosafety.

(iv) The plates will then be incubated at room temperature (30–37°C). The fungal mycelium growing out from leaf discs were subsequently transferred to fresh MEA plates by hyphal tip transfers and incubated further at room temperature for 1–2 weeks. The purity of isolated endophytic fungi was checked and their antimicrobial and anticancer activity was determined. The endophytic fungal isolates were maintained in MEA for future studies. This is referred to as the primary culture. When the pure fungal species is identified and fully characterized (after affirmation of interesting activities in this instance, anticancer property using different robust *in vitro* techniques), large-scale production of the fungi will then involve the use of secondary culture technique in larger vessels for the harvest of larger quantity in preparation for isolation of the fungal metabolites. Sometimes, selected bacteria are cocultured with the fungi as a way of stimulating and manipulating and exploiting the metabolic pathways of these organisms. Modifications of these procedures vary from laboratory to laboratory.

4. Identification of endophytic fungal isolates

Essentially, isolated endophytic fungi are identified based on both their macroscopic and microscopic structures [34]. Further identification sequel to establishment of satisfactory biological activities involves the use of biotechnological procedures. There are several taxonomical

classification guides available such as found in these references [35–38]. The nonspores are termed and classified as mycelia isolates. The fungal isolates with very interesting biological activity spectrum are further identified based on the analysis of nucleotide sequences of the internal transcribed spacer (ITS) regions of rDNA following the method described by earlier authors [39]. The confirmation of similarity of gene properties of the synthesized nucleotide rDNA sequence of ITS is usually compared with sequences from available GenBank using BLAST program of the National Institutes of Health, United States of America.

5. Large-scale endophytic fungal cultivation and extraction

The large-scale cultivation of the endophytic fungi is also carried out on the selected media as described above and then transferred (inoculated) into a 500-ml or larger Erlenmeyer flasks containing appropriate nutrients depending on target. The setup will be incubated at room temperature for one month standard conditions. The broth is then filtered and extracted with an appropriate solvent such as ethyl acetate. The extract was dried over anhydrous sodium sulfate and then evaporated under vacuum in a rotary evaporator, to yield ethyl acetate extracts. The dried extract will be partitioned into several solvent fractions and recovered for further testing of biological activities. Subsequently, the fractions are subjected to several processes of chromatography and purifications steps to isolate pure metabolites which are tested and fully characterized using physicochemical and spectroscopic methods. In order to maximize the unlimited potentials of the endophytic fungi as reliable repository for anticancer bioactive metabolites and other drug discovery, modern research should target the selection and isolation of samples from diverse ecosystems, manipulating microbial physiology to activate microbial natural-product biosynthetic machinery, and genetically modifying strains for production of unnatural microbial natural products. By manipulating all three of these approaches, the diversity of an extract collection can be maximized, and in doing so, the chance of finding a 'hit' can be increased.

6. An overview of anticancer agents from endophytic fungi

Although some battles have been won since the declaration of the 'war on cancer' in 1971 in the United Sates, the war is ongoing [40]. Furthermore, the much expected breakthrough in anticancer chemotherapy has been seriously challenged. Malignant tumors are one of the most serious diseases that damage human health in the modern world and the second largest deadly disease just after heart diseases [41], and as such, the search for newer anticancer agents remains endless. This search has, in recent times, shifted to the endophytic fungi. Interesting secondary metabolites are derived from endophytes which make them unmatchable synthesizers of very useful complex chemical scaffolds inside their hosts [42, 43]. In comparison, most of these metabolites have been engaged in the fight against diverse diseases of man and animal [44]. Since the advent of taxol from an endophytic fungus, efforts have relied on the manipulation and optimization of the culture conditions and this approach has produced several chemical constituents and novel analogues with quite

unique biochemistry [3]. For the purpose of this chapter, we limited our discussion to few selected important categories of bioactive metabolites produced by fungal endophytes of medicinal plants (**Figure 1**). Several authors in the past have reported excellent reviews on endophytes as potential sources of bioactive metabolites. One of such interesting reviews was published by Sanjana et al. [45] in 2012, covering their unmatched potentials as sources for anticancer, antioxidant, immunomodulatory, antiparasitic, antitubercular and insecticidal agents. In the current review, we attempt to present an updated chapter on endophytes as reliable sources of anticancer metabolites. Following the discovery of the multimillion dollar anticancer agent, taxol from the endophytic fungus *Taxomyces andreanae* [9], several others have been studied as potential repositories of anticancer agents. Taxol (paclitaxel) belongs to the diterpenoid class of natural products and is an exceptionally potent anticancer agent. Before, being isolated from the endophytic fungus *Taxomyces andreanae*, it was isolated for the first time, from the bark of *Taxus brevifolia*, commonly called yew plant from the South American pacific. The drug was subsequently approved by the Food and Drug Administration (FDA), USA, for the treatment of selected cancers [46]. The diverse sources of taxol from endophytic fungi are shown in **Table 1**. Lee et al. [47] reported the isolation of a dimeric quinone Torreyanic acid from the endophytic fungus, *Pestalotiopsis microsporum* growing in the plant *Torreya taxifolia*. This compound was shown to possess potent cytotoxic activity through apoptotic mechanisms [47]. Camptothecin, a potent antineoplastic agent from endophytic *Entrophospora infrequens* isolated from the host plant, *Nothapodytes foetida*, was also reported by Puri et al. [48], as having pronounced activity against lung cancer and ovarian cancer cell lines. Several authors attempted the derivatization of analogues of Campothecin, and a successful effort led to the discovery of two clinically useful anticancer drugs: topotecan and irinotecan. These potent anticancer compounds were extracted from the endophytic Fusariumsolani inhabiting *Camptotheca acuminata* [18]. Podophyllotoxin, a nonalkaloid lignin and its analogues are clinically relevant mainly due to their antiviral and anticancer activities; further, they are the precursors of many other useful anticancer drugs including etoposide, Teniposide and etopophos phosphate [49]. Podophyllotoxin and other related aryl tetralin lignans have also been reported to be produced by another novel endophytic fungus, Trametes hirsute with anticancer potential [17]. The different strains of endophytic fungi producing Podophyllotoxin are represented in **Table 2**. Various novel microbial sources of podophyllotoxin include *Aspergillus fumigatus* isolated from *Juniperus communis* [50], *Phialocephala fortinii* isolated from *Podophyllum peltatum* [30] and *Fusarium oxysporum* isolated from *Juniperus recurva* [49]. Ergoflavin, a novel anticancer agent, was isolated from the leaf endophytes of an Indian medicinal plant *Mimusops elengi*, belonging to family Sapotaceae. Ergoflavin is a dimeric xanthene linked at position-2, belonging to the ergochrome class of compounds [51]. Another compound from ergochrome class, i.e., secalonic acid D, a mycotoxin, isolated from the mangrove endophytic fungus also exhibits a good cytotoxic activity on HL60 and K562 cells by inducing leukemia cell apoptosis [52]. Wagenaar et al. [53] studied Rhinocladiella sp. inhabiting *Tripterygium wilfordii* and reported three novel cytochalasins: cytochalasin H, cytochalasin J and epoxycytochalasin H along with a known compound cytochalasin E. These compounds have been identified as 22-oxa-12-cytochalasins and have antitumor activity.

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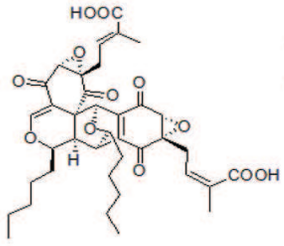
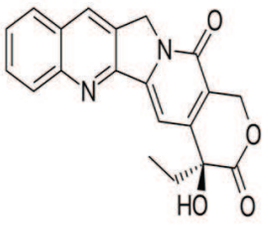
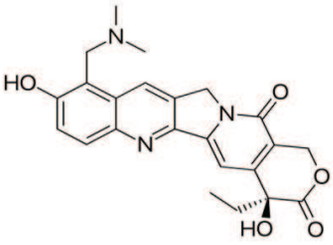


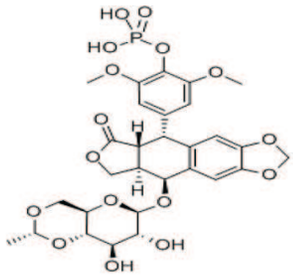
Torreyanic acid



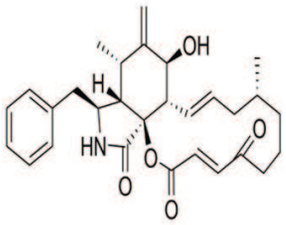
Camptothecin



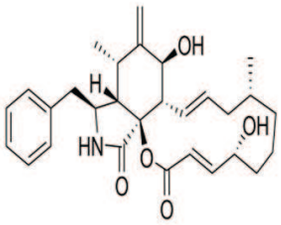
Topothecan



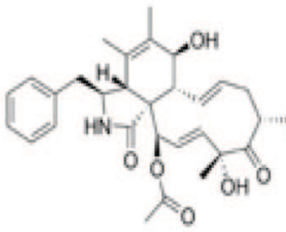
Etopophos



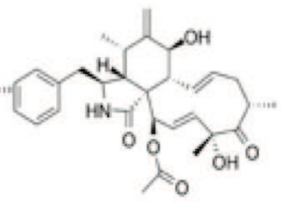
Cytochalasin A



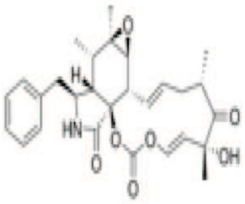
Cytochalasin **B**



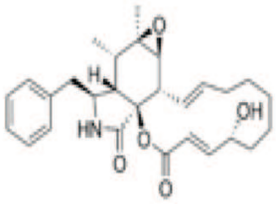
Cytochalasin C



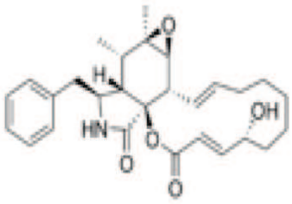
Cytochalasin D



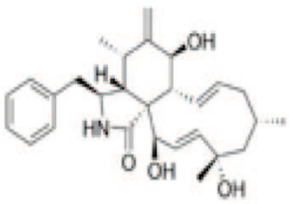
Cytochalasin E



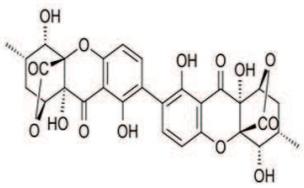
Cytochalasin F



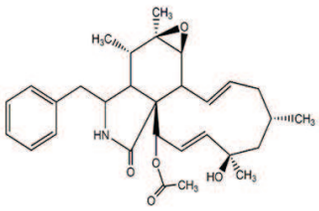
Cytochalasin H



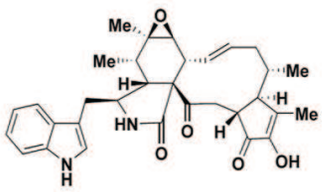
Cytochalasin J



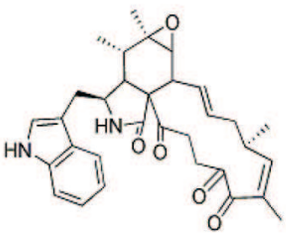
Ergoflavin



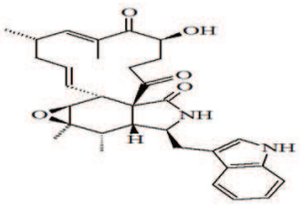
Epoxycytochalasin H



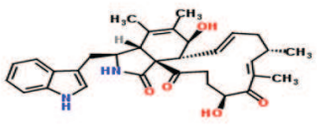
Chaetoglobosin U



Chaetoglobosin C



Chaetoglobosin F



Chaetoglobosin E

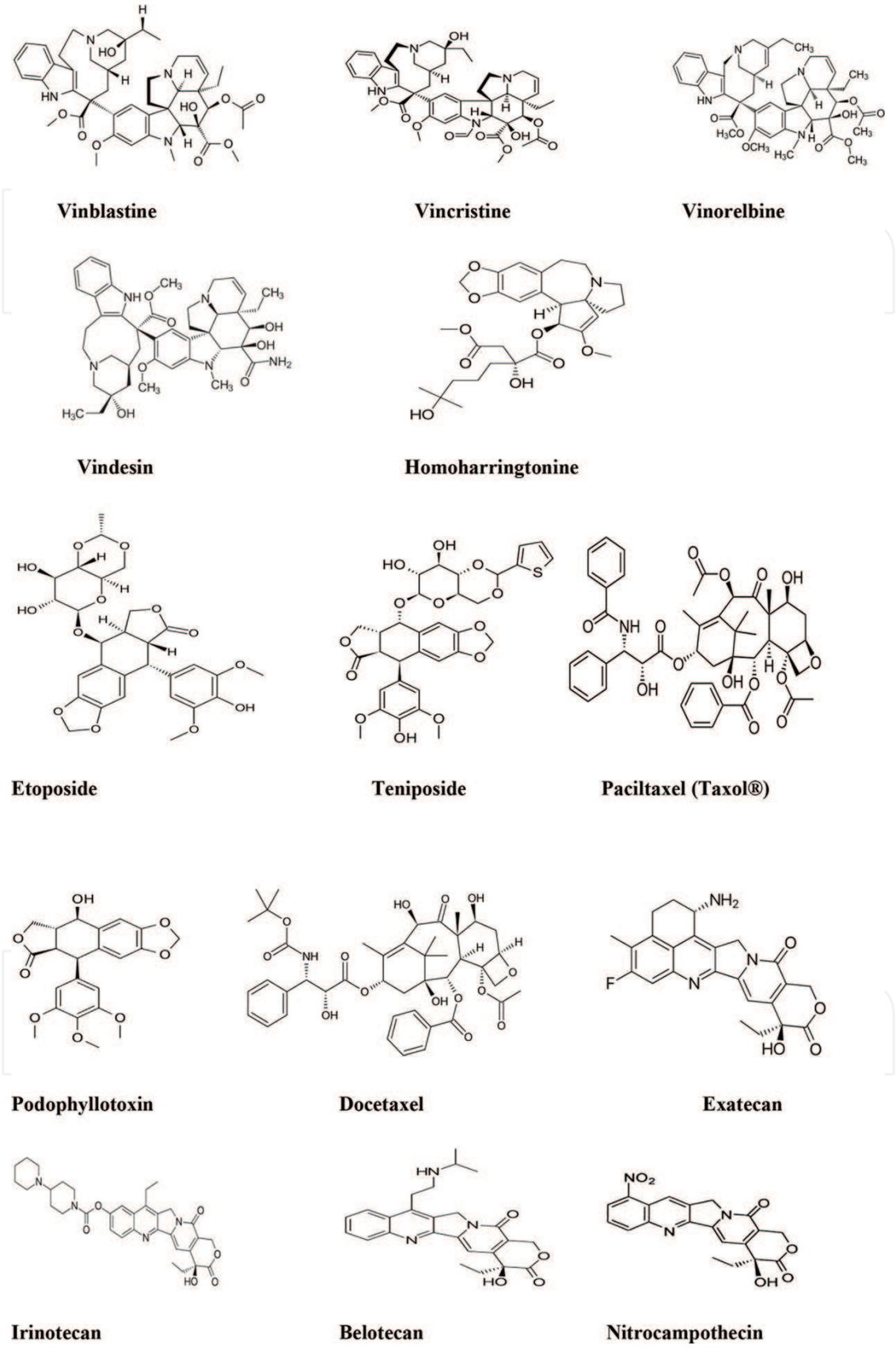


Figure 1. Selected structures of anticancer metabolites from endophytic fungi.

Endophytic fungus	Fungal strain	Host plant	Paclitaxel yield (μg/L)
Alternaria sp.	Ja-69	*Taxus cuspidata*	0.16
Alternaria sp.	–	*Ginkgo biloba*	0.12–0.26
Alternaria alternata	TPF6	*Taxus chinensis* var. *mairei*	84.5
Aspergillus fumigatus	EPTP-1	*Podocarpus* sp.	557.8
Aspergillus niger var. *taxi*	HD86-9	*Taxus cuspidata*	273.6
Botryodiplodia theobromae	BT115	*Taxus baccata*	280.5
Botrytis sp.	XT2	*Taxus chinensis* var. *mairei*	161.24
Botrytis sp.	HD181-23	*Taxus cuspidata*	206.34
Cladosporium cladosporioides	MD2	*Taxus media*	800
Ectostroma sp.	XT5	*Taxus chinensis* var. *mairei*	276.75
Fusarium arthrosporioides	F-40	*Taxus cuspidate*	131
Fusarium lateritium	Tbp-9	*Taxus baccata*	0.13
Fusarium mairei	Y1117	*Taxus chinensis* var. *mairei*	2.7
Fusarium mairei	UH23	*Taxus chinensis* var. *mairei*	286.4
Fusarium solani	–	*Taxus celebica*	1.6
Fusarium solani	Tax-3	*Taxus chinensis*	163.35
Metarhizium anisopliae	H-27	*Taxus chinensis*	846.1
Monochaetia sp.	Tbp-2	*Taxus baccata*	0.10
Mucor rouxianus	DA10	*Taxus chinensis*	–
Ozonium sp.	BT2	*Taxus chinensis* var. *mairei*	4–18
Papulaspora sp.	XT17	*Taxus chinensis* var. *mairei*	10.25
Periconia sp.	No. 2026	*Torreya grandifolia*	0.03–0.83
Pestalotia bicilia	Tbx-2	*Taxus baccata*	1.08
Pestalotiopsis guepinii	W-1f-2	*Wollemia nobilis*	0.49
Pestalotiopsis microspora	Ja-73	*Taxus cuspidata*	0.27
Pestalotiopsis microspora	Ne-32	*Taxus wallachiana*	0.5
Pestalotiopsis microspora	No. 1040	*Taxus wallachiana*	0.06–0.07
Pestalotiopsis microspora	Cp-4	*Taxodium distichum*	0.05–1.49
Pestalotiopsis microspora	Ne 32	*Taxus wallichiana*	0.34–1.83

Adapted from Zhao et al. (2010), *Endophytic* fungi for producing bioactive compounds originally from their host plants [62].

Table 1. Paclitaxel-producing endophytic fungi and their host plants.

A novel cytotoxic cytochalasan-based alkaloid chaetoglobosin U along with four known analogues, chaetoglobosin C, chaetoglobosin F, chaetoglobosin E and ponochalasin A, have been

Endophytic fungus	Fungal strain	Host plant	Podophyllotoxin content or yield
Alternaria sp.	–	*Sinopodophyllum hexandrum* (=*Podophyllum hexandrum*)	–
Alternaria neesex	Ty	*Sinopodophyllum hexandrum*	2.4 μg/L
Fusarium oxysporum	JRE1	*Sabina recurva* (=*Juniperus recurva*)	28 μg/g
Monilia sp.	–	*Dysosma veitchii*	–
Penicillium sp.	–	*Sinopodophyllum hexandrum*	–
Penicillium sp.	–	*Diphylleia sinensis*	–
Penicillium sp.	–	*Dysosma veitchii*	–
Penicillium implicatum	SJ21	*Diphylleia sinensis*	–
Penicillium implication	2BNO1	*Dysosma veitchii*	–
Phialocephala fortinii	PPE5, PPE7	*Sinopodophyllum peltatum*	0.5–189 μg/L
Trametes hirsuta	–	*Sinopodophyllum hexandrum*	30 μg/g

Adapted from Zhao et al. (2010), Endophytic fungi for producing bioactive compounds originally from their host plants.

Table 2. Podophyllotoxin-producing endophytic fungi and their host plants.

produced by the fungal endophyte *Chaetomium globosum* IFB-E019 isolated from *Imperata cylindrica*. Cheatoglobosin U exhibits cytotoxic activity against nasopharyngeal epidermoid tumor KB cell [54]. Chen et al. [55] reported Gliocladicillins A and B as effective antitumor agents in vitro and in vivo. They induced tumor cell apoptosis and also showed a significant inhibition on proliferation of melanoma B16 cells implanted into immunodeficient mice. Vincristine, an alkaloid with cytotoxic activity, was isolated from the endophytic mycelia sterilia inhabiting *Catharanthus roseus* [56]. This drug is mainly used as a chemotherapy regimen in acute lymphoblastic leukemia and nephroblastoma. Likewise, there are large numbers of anticancer agents produced by fungal endophytes inhabiting different medicinal plants. Several of such molecules are presently at different levels of clinical trials, and there are putative hopes that some of them will be approved for use in no distant time. Furthermore, the list of metabolites obtained diverse endophytic fungi with proven potent activity against several cancers is endless, thus indicating that these organisms hold the future in the attempt to conquer cancer therapeutically.

7. Conclusion

No doubt, endophytes remain an unmatchable biodiversity and repository for novel natural compounds with useful biological activities and form simple to complex scaffolds for the generation of more potent of compound. Because of the diversity of potential of advantages including the application of modern biotechnological techniques, metabolic technology and microbial fermentation process, we can better understand and manipulate this important

microorganism resource and make it more beneficial for the mankind [33, 57–60]. We can conclude that the endophytic fungi are a novel and important microbial resource for producing bioactive compounds and have attracted attention of many researchers on their theoretical study as well as their potential applications [61]. The future of discovering anticancer agents from endophytic fungi is undoubtedly bright.

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