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Endophytic Fungi of Tropical Forests: A Promising Source of Bioactive Prototype Molecules for the Treatment of Neglected Diseases

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1. Introduction

Endophytic fungi are ubiquitous fungi that reside inter- or intracellularly in plant parts for at least a portion of their lives without causing apparent symptoms of infection (Petrini, 1991) and represent a large portion of fungal species. Endophytic fungi can be biotrophic mutualists, benign commensals, decomposers or latent pathogens (Promputtha et al., 2007). According to Rodriguez et al. (2009), all plants in the natural environment can shelter endophytic fungi, including algae, mosses, ferns, conifers and angiosperms. This fungal group appears to significantly influence the lifestyle of its host. Taxonomically, most of the endophytic fungi belong to the phylum *Ascomycota* and its associated anamorphs, while some species belong to the phyla *Basidiomycota* and *Zygomycota* (Huang et al., 2001). There have been many studies on the diversity, ecology and biotechnological applications of endophytic fungi in grasses and wood plants in temperate environments. However, there is limited information about the diversity of endophytic fungal communities in tropical forests, which are endowed with a rich biodiversity of flora. Dreyfuss & Chapela (1994) have estimated that approximately 1.3 million species of endophytic fungi remain to be discovered. This diverse fungal group could impact the ecology, fitness and shape of plant communities, conferring resistance to abiotic (temperature, pH, osmotic pressure) and biotic (from bacteria, fungi, nematodes and insects) stresses (Rodriguez et al., 2001). Endophytic fungi are an important source of bioactive molecules. These bioactive metabolites have a broad range of biological activities and could be the starting materials for pharmaceuticals or lead structures for the development of pharmaceutical or agrochemical products. The substances produced by endophytic fungi originate from different biosynthetic pathways, including isoprenoid, polyketide and amino acid and belong to diverse structural groups, such as terpenoids, steroids, xanthones, quinones, phenols, isocoumarins, benzopyranones, tetralones, cytochalasins and enniatins (Schulz et al., 2002). Indeed, these bioactive

molecules represent a chemical reservoir for discovering new compounds, such as antibiotic, antioxidant, immunomodulating, anticancer and antiparasitic compounds, for use in the pharmaceutical and agrochemical industries.

2. Neglected tropical diseases

The neglected tropical diseases (NTDs), a group of chronic, debilitating and poverty-promoting parasitic, bacterial, viral and fungal infections, are among the most common causes of illness in the poorest people living in developing countries (Hotez et al., 2008). NTDs cause over 500,000 deaths annually and are estimated to result in a greater number of lost disability-adjusted life years than malaria and tuberculosis (Hotez et al., 2006, 2009). These diseases flourish in areas where water supply and sanitation are inadequate, nutrition is poor, literacy rates are low, health systems are rudimentary, and insects and other disease vectors are constant household and occupational companions. Not surprisingly, these diseases cluster together and frequently overlap where these conditions occur. In fact, the link with poverty is so strong that the prevalence of these diseases serves as an indicator of the level of a country's socioeconomic development (WHO, 2006).

The NTDs have some common features. They occur in impoverished settings and are chronic conditions; victims can harbour NTDs for years or decades, frequently resulting in disability, disfigurement and stigmatisation. Not only are these diseases of poverty, but they also promote poverty because of their effects on child development, cognition and education as well as on adult agricultural worker productivity (Hotez & Yamey, 2009). These diseases have also been neglected by research and development. There is little incentive for industries to develop drugs and vaccines for markets that cannot pay. When inexpensive and effective drugs already exist and are available, delivery fails because patients cannot pay and health systems are weak or non-existent (WHO, 2006).

The most important bacterial NTDs are trachoma, leprosy, and some of the bacterial zoonoses, especially leptospirosis (Hotez et al., 2008). Interruption and default of therapies against bacterial NTDs are still important obstacles to disease control in many endemic countries, with consequences for both patients and control programs; low adherence results in potential remaining sources of infection, incomplete curing and irreversible complications and may lead to multidrug resistance (Heukelbach et al., 2011).

Several mycoses, such as paracoccidioidomycosis (PCM), are also responsible for major public health and economic hardships in Latin America (Hotez et al., 2008). The drugs most commonly used for treating patients with paracoccidioidomycosis (PCM) are sulphonamides, ketoconazole, itraconazole and amphotericin B. Extended periods of treatment are necessary, and there are increasing concerns about drug toxicity, the cost of treatment, and unacceptable rates of noncompliance with these therapies (Travassos et al., 2008).

The most important viral NTDs are dengue and yellow fevers (Hotez et al., 2008). Tropical climates have experienced a great resurgence in dengue fever in recent years, and it appears to be spreading to new areas (Carroll et al., 2007). The WHO reports that two-fifths of the world's population is at risk of dengue infection, with an increase in the annual number of cases (Murrell et al., 2011). There is no specific treatment for dengue fever. Dengue fever is an increasing concern because of the lack of a licensed vaccine that protects against all four dengue serotypes (WHO, 2006). The increase in dengue infections and the prevalence of all four circulating dengue serotypes has contributed to a rise in the incidence of dengue haemorrhagic fever (Murrell et al., 2011).

Yellow fever originated in Africa and was imported to Europe and the Americas as a consequence of the slave trade between these continents (Gardner & Ryman, 2010). An inexpensive live attenuated vaccine against yellow fever (the 17D vaccine) has been effectively used to prevent yellow fever for more than 70 years. Interest in developing new inactivated vaccines has been spurred by the recognition of rare but serious and sometimes fatal adverse events following live virus vaccination (Hayes, 2010).

Leishmania (*Trypanosomatidae*) are protozoan parasites that cause high morbidity and mortality levels and are recognised by the WHO as a major tropical public health problem (Asford, 1997). There are currently no vaccines for leishmaniasis; although the drugs available for leishmaniasis treatment are toxic, expensive and sometimes ineffective, they are the only effective way to treat all forms of the disease (Croft & Coombs, 2003). Chagas disease (American *Trypanosomiasis*) is caused by the haemoflagellate protozoan *Trypanosoma cruzi* and is transmitted to humans either by blood-sucking triatomine vectors, blood transfusion or congenital transmission. The geographical distribution of human *T. cruzi* infection extends from the southern United States and Mexico to southern Argentina (WHO, 1991). According to Reyes & Vallejo (2005), there is evidence that trypanocidal drug treatment with nitrofuran and imidazole compounds can treat acute *T. cruzi* infection, but further studies are needed to develop new trypanocidal drugs.

Helminths are parasitic worms that are the most common agents of human infection in developing countries and include the NTDs schistosomiasis, cysticercosis and onchocerciasis. There are two major phyla of helminths, which include the major intestinal worms, filarial worms that cause lymphatic filariasis and onchocerciasis and platyhelminthes such as the schistosomes and the agent of cysticercosis (Hotez, 2008). Only the drugs albendazole, oxamniquine, praziquantel and ivermectin are available to treat helminthiasis (Hotez, 2008). New advances in helminth biology, particularly molecular techniques, have led to the identification of new targets for the discovery and development of anthelmintic drugs.

3. Diversity of tropical endophytic fungi

Dreyfuss & Chapela (1994) have estimated that there are 1.3 million species of endophytic fungi alone, the majority of which are likely found in tropical ecosystems. This estimate is supported by various studies that have sought to characterise the fungal communities associated with tropical plants. Fungal endophytic communities are divided into two basic groups: generalists (which are found in high abundance among different plant species) and singletons (which are found in low abundance and in a specific plant host). Tropical plants are expected to shelter a highly diverse population of endophytic fungi, but few tropical plants have been screened for their presence. Studies have shown that tropical plants shelter a great diversity of singleton species.

According to Hawksworth (2004), the magnitude of fungal diversity in tropical forests is unclear, and new species remain to be described. The greatest fungal diversity probably occurs in tropical forests, where a highly diverse population of angiosperms is present (Arnold et al., 2000). In support of this proposal, a large number of fungal endophytic species have been described in association with plants in Asia, Australia, Africa, Central and South America, Mexico and some Pacific and Atlantic Islands. However, the diversity of endophytic fungi can vary across different biomes of a tropical forest. Suryanarayanam et al. (2002) showed that the endophytic fungal assemblage of a dry tropical forest had much less

endophyte diversity than a wet tropical forest. Endophytic fungi can be passive residents or act as an assemblage of latent pathogens in their host (Ganley et al., 2004). Arnold et al. (2000) suggested that endophytic fungi are hyperdiverse and that 1.5 million species may be an underestimate of their magnitude. In addition, the taxonomic placement of tropical fungi has been confounded by misidentifications made in comparison with temperate fungal communities, including the endophytic fungal community present in the leaves of tropical plants (Arnold et al., 2001).

In general, endophytic fungi have been categorised into two main groups based on differences in evolution, taxonomy, plant hosts and ecological functions: clavicipitaceous, which are able to infect only some species of grasses, and nonclavicipitaceous, which are found in the asymptomatic tissues of bryophytes, ferns, gymnosperms and angiosperms (Rodriguez et al., 2001). Clavicipitaceous endophytes belong to the family *Clavicipitaceae* (*Hypocreales*; *Ascomycota*), many species of which are known to produce bioactive molecules (mainly of the genera *Cordyceps*, *Balansia*, *Epichloë/Neotyphodium*, *Claviceps* and *Myriogenospora*). In contrast, nonclavicipitaceous endophytes are a large group that have not been well-defined taxonomically, but the majority of the species belong to the phyla *Ascomycota* and *Basidiomycota*, represented by the genera *Alternaria*, *Arthrobotrys*, *Aspergillus*, *Cladosporium*, *Colletotrichum*, *Coprinellus*, *Curvularia*, *Fusarium*, *Paecilomyces*, *Penicillium*, *Phanerochaete*, *Phoma*, among others. Different species of these two endophytic groups have been investigated for their ability to produce various molecules, and species living in association with tropical plants have been shown to be significant producers of bioactive metabolites.

4. Hosts of tropical bioactive endophytic fungal communities

Tropical and temperate forests are considered to be the most diverse terrestrial ecosystems, with the greatest number and diversity of endophytic fungi (Strobel, 2002). The constant innovation present in ecosystems where the evolutionary race to survive is the most active may result in the production of a plethora of chemical molecules (Strobel, 2006). Tropical rainforests are an important example of this type of environment: there is great competition, resources are limited, and selection pressure is at its peak. Consequently, there is a high probability that fungi associated with tropical hosts may be a source of novel molecular structures and compounds that are active against neglected diseases.

Endophytic relationships may have begun from the time that higher plants first appeared hundreds of millions of years ago. Evidence of plant-associated fungi has been discovered in fossilised tissues of stems and leaves (Taylor et al., 1999). As a consequence of these long-term associations, some of these microorganisms may have developed genetic systems that allow the exchange of information between themselves and the higher plant. This exchange would allow the fungi to more efficiently cope with the environmental conditions and perhaps increase compatibility with the plant host. Moreover, the dependent evolution of endophytic fungi may have allowed them to better adapt to the plant such that the fungi could contribute to the relationship by performing protective functions against pathogens and insects (Petrini et al., 1992; Strobel & Daisy, 2003; Gunatilaka, 2005). To make these contributions to their plant hosts, endophytic fungi may produce secondary metabolites that have potential uses in agriculture, medicine and industry (Strobel & Daisy, 2003).

Each of the approximately 300,000 known plant species may host at least one endophytic fungus. As tropical and subtropical regions harbour most of the world's plant diversity,

endophytic fungal diversity in this climatic zone is also higher, and all vascular plant species examined to date possess an endophytic fungus. According to Strobel (2003), reasonable guidelines should govern the plant selection strategy for the discovery of bioactive endophytic fungi, which would include plants that are found in unique environmental settings, have ethnobotanical histories, or are endemic or growing in regions of high diversity. All of these selection strategies are applicable for the isolation of endophytic fungi from tropical and subtropical hosts, and these microorganisms can be obtained from the leaves, stems, petioles, barks and roots of many tropical angiosperms.

5. Techniques for the isolation and identification of endophytic fungi

The methods used to isolate endophytic fungi vary in the technique used for surface-disinfection of the host plant tissue (leaves, stems, roots, bark, flowers, fruits and seeds) and the choice of culture media. The disinfection process can influence the detection of endophytic fungi; in general, the plant surface is disinfected with a strong oxidant or disinfectant agent for a specific period of time. The most commonly used agents include 1-4% detergent, 3% H_2O_2, 2-10% NaOCl, or 70-95% ethanol. The culture medium is another important parameter. Commonly used media include potato dextrose agar, malt extract agar, yeast malt agar and Sabouraud agar, supplemented with antibacterial agents (chloramphenicol, penicillin, ampicillin, tetracycline and streptomycin, among others) to suppress contaminating bacteria and isolate endophytic fungi.
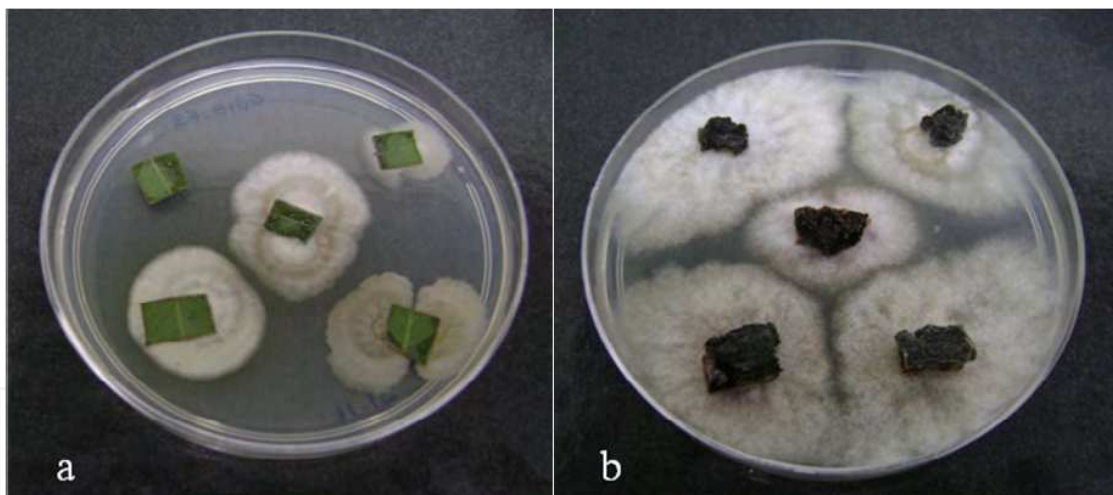

Fig. 1. Mycelium of endophytic fungi emerging from the (a) leaves and (b) bark of the medicinal tropical Brazilian plant *Stryphnodendron adstringens*.

After isolation, the endophytic fungi, including the bioactive species, must be identified correctly. Macro- and micromorphological cultural characteristics, molecular analyses and metabolite profiles are the main criteria that are used to identify endophyte fungal taxonomy. The identification of endophytic fungi relies significantly on the taxonomic expertise of the mycologist and frequently requires polyphasic taxonomy. In tropical regions, multiple endophytic fungal species are recovered and are commonly grouped based on similar culture characteristics into morphospecies (Figure 2), which represent a functional taxonomic unit for endophytic fungal species (Arnold et al., 2000). After characterisation as a morphospecies, endophytic fungi are submitted to molecular grouping

using microsatellite markers that are detected with $(GTG)_5$, M13 or EI primers based on PCR-fingerprinting methods that amplify genomic segments different from the repeat region itself (Lieckfeldt et al., 1993).

Fig. 2. Morphospecies of endophytic fungal isolates associated with the tropical plant *Vellozia graminea*.

However, most endophytic fungi (about 50%) do not produce conidia or spores when cultured on common mycological media. In these cases, endophytic fungi can frequently be identified based on the sequence of the Internal Transcribed Spacer (ITS) region of the large subunit of the rRNA gene. Molecular techniques are a powerful tool for identifying the endophytic genera and species of non-sporulating fungi. After sequencing the ITS1–5.8S–ITS2 region, the sequence of the endophytic fungus is compared with the sequences of other taxa deposited in public databases. The GenBank database is a major source of nucleotide sequences.

In addition, endophytic fungi produce a large number of metabolites, and certain molecules are very consistent at the species level in some genera when cultured under standardised conditions. According to Larsen et al. (2005), fungal isolates of different species have different chemotypes, which can be differentiated or grouped by modern methods for dereplication analysis. The chemotaxonomic analysis begins with the preparation of the fungal extract, which typically requires the media potato dextrose agar (PDA), Sabouraud agar, malt extract agar (MEA), yeast extract sucrose agar (YES) or Czapek yeast autolysate agar (CYA). The chemical analysis includes techniques such as Thin Layer Chromatography (TLC), Gas Chromatography (GC), High Performance Liquid Chromatography (HPLC), Mass Spectrometry (MS), and Nuclear Magnetic Resonance (NMR), alone or in combination, in association with informatics tools.

6. Fermentation techniques and crude extract production

Secondary metabolites are compounds with varied and sophisticated chemical structures that are usually produced only during the stationary phase of growth (Robinson et al., 2001). These compounds do not have a physiological role during exponential phase, and their production starts when a key nutrient source, such as carbon, nitrogen or phosphate, is

exhausted (Barrios-González & Mejia, 1996). The last two decades have been a period of rapid discovery of new biological activities of these compounds, and appropriate modern strategies for identifying metabolites are essential (Petrini et al., 1992).

Endophytic fungi must first be acquired in pure culture, and optimal media and growth conditions must be determined to begin microorganism fermentation and production of crude extracts. These extracts can then be separated by various chromatographic procedures to yield the substance of interest. Factors that can quantitatively and qualitatively affect the production of secondary metabolites include temperature, pH, medium composition, culture duration and the degree of aeration. These parameters can be manipulated and modified to improve the production of compounds of bioactive significance (Barrios-González & Mejia, 1996).

There are several media that can be used to cultivate endophytic fungi, such as potato dextrose agar (PDA), corn meal agar (CMA), oatmeal agar (OMA) and Czapek yeast autolysate agar (CYA). The medium is chosen based on the purpose and the species under investigation. Liquid media are usually used for physiological studies, but agar media are more convenient and practical for the rapid screening of plentiful isolates (Hölker et al., 2004). To isolate endophytic fungal secondary metabolites, fermentation techniques such as Submerged fermentation (SmF) (or Liquid fermentation) and Solid-state fermentation (SSF) have become widely used (Table 1) (Barrios-González & Mejia, 1996; Pandey, 2003; Hölker et al., 2005).

Characteristics	Solid-State	Submerged
Microorganism, substrate	Static	Agitated
Water usage	Limited	Unlimited
Oxygen supply by	Diffusion	Aeration
Volume of fermentation	Smaller	Larger
Energy requirement	Low	High
Capital investment	Low	High
Concentration of the end product	High	Low
Sterility demands	Low	High
Simulation of the natural environment	Better	Worse

Table 1. Comparison of the main characteristics of Solid-state fermentation and Submerged fermentation.

Although the SmF and SSF techniques differ, both can be used to identify secondary metabolites produced by endophytic fungi. Figure 3 illustrates examples of SmF and SSF processes for obtaining these substances for screening programs. However, the appropriateness of a given technique should be evaluated based on the aim of the study and the available resources. In addition, optimal parameters for both techniques, such as incubation conditions, medium composition, agitation, temperature and pH, must be standardised to improve process efficiency and maintain reproducibility.
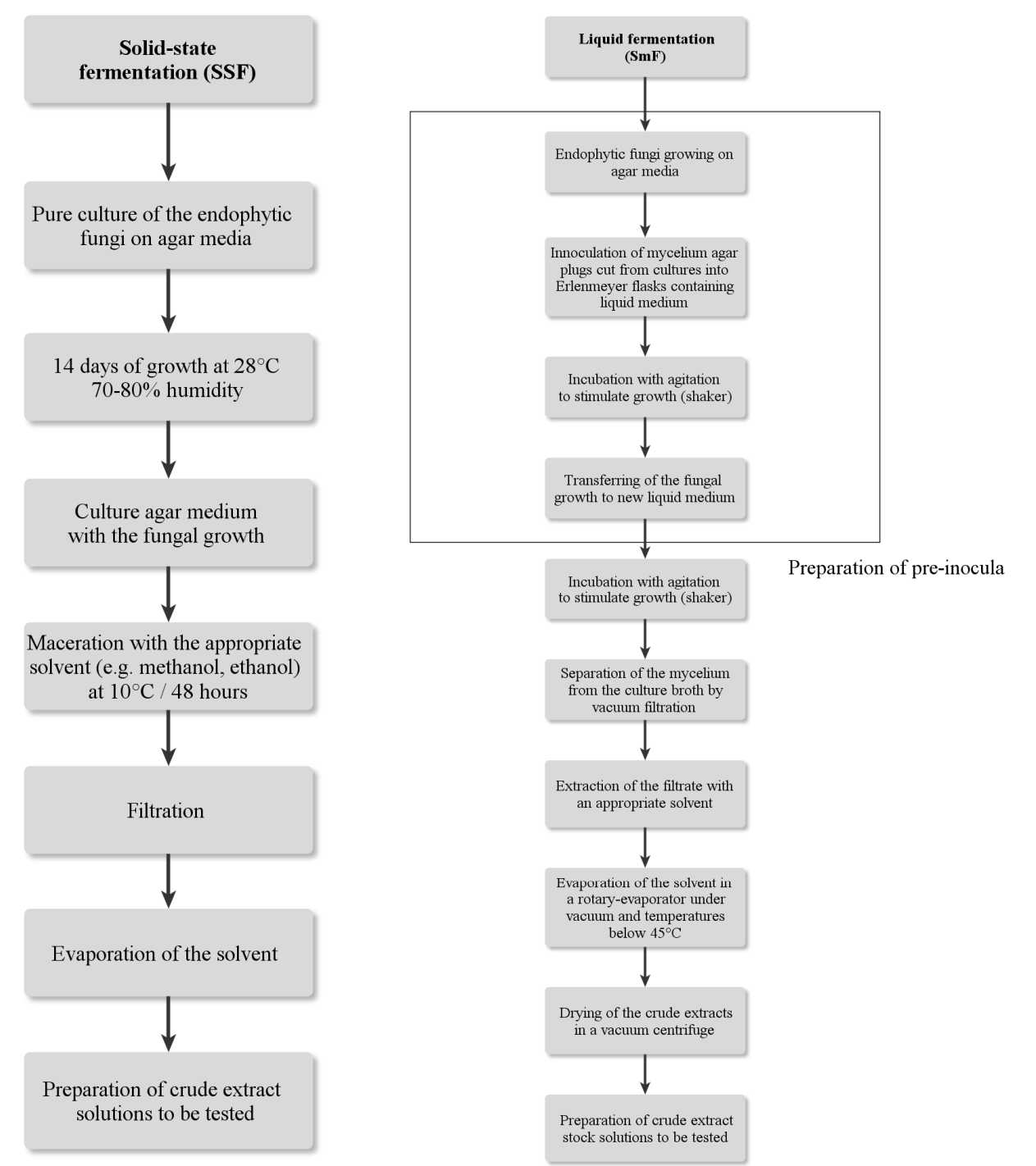
Fig. 3. Liquid fermentation and Solid-state fermentation processes for obtaining endophytic fungal secondary metabolites.

7. Bioactive compounds against neglected diseases

To date, fungal metabolites have primarily served as lead structures for the development of anticancer, antifungal and antibacterial agents. Although new drugs are needed to treat all aspects of leishmaniasis, the scientific literature on the bioprospecting of endophytic fungi of tropical rainforests is limited. Brazilian ecosystems are a potential source of endophytic

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fungi that are able to produce bioactive prototype molecules for developing drugs to combat NTDs. *Penicillium janthinellum* was isolated as an endophytic fungus from the fruit of *Melia azedarach* (*Meliaceae*), a plant collected in Brazil. Methanol extract fractionation furnished the known polyketide, citrinin (Fig. 4), which was previously found in *Penicillium citrinum* and several *Aspergillus* species (Vrabcheva et al., 2000) and inhibited 100% of *Leishmania mexicana* at a concentration of 40 μg/mL (Marinho et al., 2005).
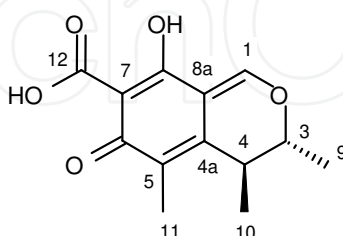
Fig. 4. Citrinin.

The endophytic fungus *Edenia* sp. was isolated from a mature leaf of *Petrea volubilis* (*Verbenaceae*), which was collected from the Coiba National Park in Panama. Bioassay-directed fractionation of organic extracts of *Edenia* sp. led to the isolation of the antileishmanial compounds preussomerin EG_1 [IC_{50} 0.12 μM (Fig.5, 2)], palmarumycin CP2 [IC_{50} 3.93 μM (Fig. 5, 3)], palmarumycin CP17 [IC_{50} 1.34 μM (Fig. 5, 4)], palmarumycin CP18 [IC_{50} 0.62 μM (Fig. 5, 5)], CJ-12,37 [IC_{50} 8.40 μM (Fig. 5, 6)], palmarumycin CP19 [IC_{50} 11.6 μM (Fig. 5, **7**)] and 5-methylochracin (IC_{50} 33.4 μM), which inhibited the growth of amastigote forms of *Leishmania donovani*. Preussomerin EG1 was the most active substance and inhibited growth of *L. donovani* with a potency similar to that of amphotericin B (IC_{50} 0.09 μM) (Martínez-Luis et al., 2009).
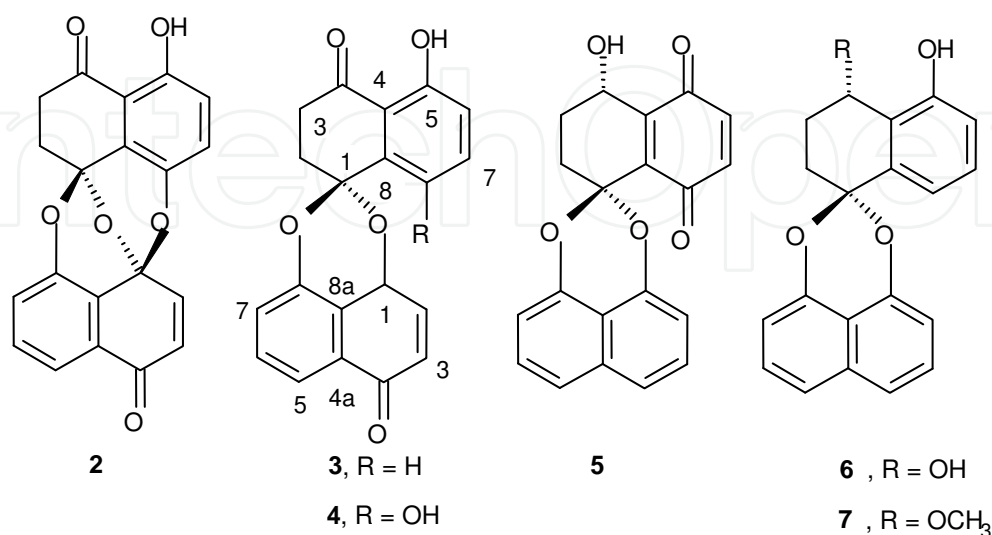

Fig. 5. Preussomerin EG_1 (2), palmarumycin CP2 (3), palmarumycin CP17 (4), palmarumycin CP18 (5), CJ-12,37 (6) and palmarumycin CP19 (7)

The isolate UFMGCB 555, obtained from the plant *Piptadenia adiantoides* and identified as *Cochliobolus* sp., produces cochlioquinone A (Fig.6, 8) and isocochlioquinone A (Fig. 6, 9). Both compounds were active in an assay against *L. amazonensis*, with EC_{50} values of 1.7 μM and 4.1 μM, respectively (Campos et al., 2008).
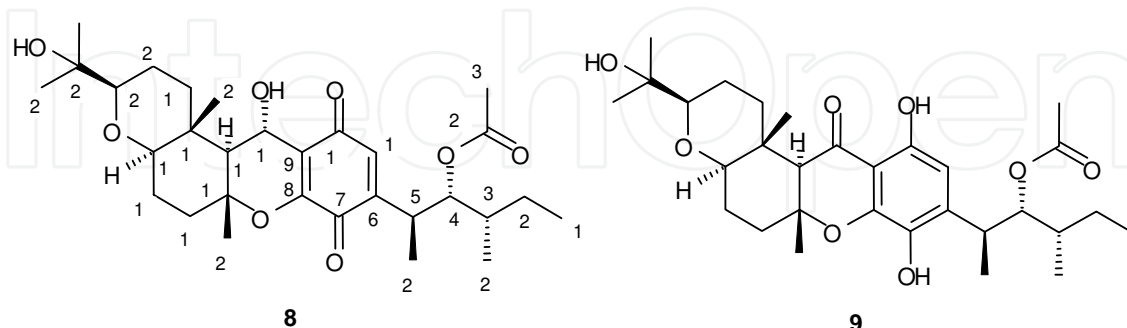

Fig. 6. Cochlioquinone A (8) and isocochlioquinone A (9)

Grandisin (Fig. 7, 10), a tetrahydrofuran lignan isolated from *Piper solmsianum* (*Piperaceae*) (Martins et al., 2003) and *Virola surinamensis* (*Myristicaeae*) (Lopes et al., 1996), has potent trypanocidal activity against the trypomastigote form of *T. cruzi* at 5 μg/mL (Lopes et al., 1998). Biotransformation of this compound by the endophytic fungus *Phomopsis* sp., obtained from *Viguiera arenaria*, yielded a new compound, 3,4-dimethyl-2-(4′-hydroxy-3′,5′-dimethoxyphenyl)-5-methoxy-tetrahydrofuran (Fig. 7, 11). The metabolite had trypanocidal activity (IC_{50} 9.8 μmol/mL) similar to the natural precursor (IC_{50} 3.7 μmol/mL) (Verza et al., 2009).
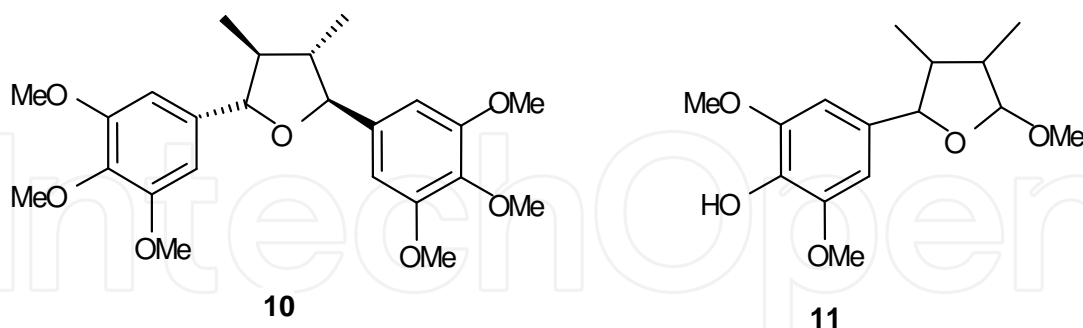
Fig. 7. Grandisin (10) and 3,4-dimethyl-2-(4′-hydroxy-3′,5′-dimethoxyphenyl)-5-methoxy-tetrahydrofuran (11).

Altenusin (Fig. 8) is a metabolite obtained from the organic extract of a broth culture of the endophytic fungus *Alternaria* sp. UFMGCB 55, which was isolated from a plant known to contain trypanocidal compounds, *Trixis vauthieri*. This fungus inhibited TryR enzymatic activity with an IC_{50} value of 4.3 mM (Cota et al., 2008). The endophytic fungus *Diaporthe phaseolorum*, recovered from *Viguiera arenaria*, displayed promising results by inhibiting the parasitic enzyme gGAPDH (95%) at 100 μg/mL (Guimarães et al., 2010).
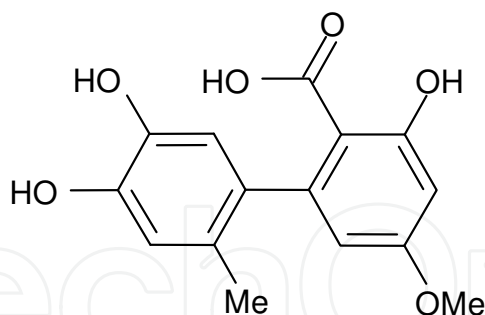
Fig. 8. Altenusin.

An organohalogen natural product (2-chloro-5-methoxy-3-methylcyclohexa-2,5-diene-1,4-dione) (Fig. 9, 13) and a quinone derivative (7-hydroxy-8-methoxy-3,6-dimethyldibenzofuran-1,4-dione) (Fig. 9, 14) were obtained from the organic extract of *Xylaria* sp. PBR-30. This endophytic fungus was isolated from healthy leaves of *Sandoricum koetjape* (*Meliaceae*). These natural products had *in vitro* activity against *P. falciparum* (K1, multidrug-resistant strain), with IC_{50} values of 1.84 and 6.68 µM (Tansuwan et al., 2007).
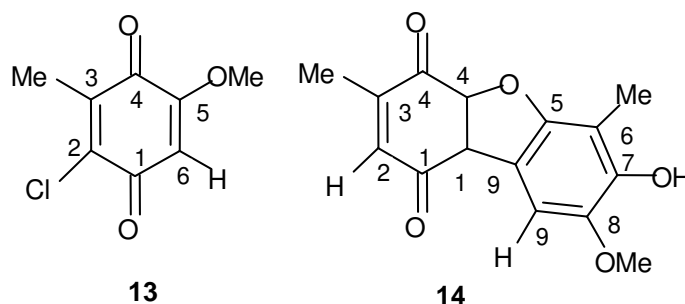
Fig. 9. 2-chloro-5-methoxy-3-methylcyclohexa-2,5-diene-1,4-dione (13) and 7-hydroxy-8-methoxy-3,6-dimethyldibenzofuran-1,4-dione (14).

The yeast-like fungus *Aureobasidium pullulans*, which was isolated from a leaf of *Culophyllum* sp. collected in Narathiwat Province, Thailand (Isaka et al., 2007), produces the cyclohexadepsipeptides pullularins A-D (Fig. 10, 16-19). Pullularin A exhibited antimalarial activity (IC_{50} 3.6 µg/mL) and moderate antituberculosis activity (MIC 25 µg/mL). Pullularin B exhibited considerable antimalarial activity (IC_{50} 3.3 µg/mL), but this substance and pullularin C exhibited weaker activities in other assays when compared with pullularin A. The low lipophilicity of a deprenyl analogue of pullularin A may explain the inactivity of this substance in all of the assays (Isaka et al., 2007).

Codinaeopsis gonytrichoides was isolated from *Vochysia guatemalensis* (*Vochysiaceae*), a white yemeri tree collected in Costa Rica. A new tryptophan-polyketide hybrid named codinaeopsin (Fig. 11, 20), which contains an unusual heterocyclic unit linking indole and decalin fragments, was isolated from the crude extract of this endophytic fungus. Codinaeopsin is active against the 3D7 strain of *P. falciparum* with an IC_{50} value of 2.3 µg/mL (4.7 µM). Codinaeopsin has the same scaffold as the HIV integrase inhibitor

equisetin (Fig. 11, 21), the antifungal agent cryptocin (Fig. 11, 22), and the telomerase inhibitor UCS1025A (Fig. 11, 23). These compounds have a linear fragment joined to amino acids or N-methyl amino acids (Kontnik & Clardy, 2008).

Stems of *Melaleuca quinquenervia* (*Myrtaceae*), collected from Toohey Forest, Australia, were examined for fungal content. Chemical investigations of a fermentation culture from the endophytic fungus *Pestalotiopsis* sp. yielded three caprolactams, which were named pestalactams A–C (Fig. 12, 24-26). Pestalactams A (Fig. 12, 24) and B (Fig. 12, 25) displayed modest *in vitro* selectivity against chloroquine-resistant (IC_{50} 41.3 and 36.3 μM, respectively) and chloroquine-sensitive (IC_{50} 16.2 and 20.7 μM, respectively) cell lines of the malaria-causing parasite *P. falciparum* versus neonatal foreskin fibroblasts (NFF, IC_{50} 20.2 and 12.8 μM, respectively), with both compounds yielding ~16–41% parasite growth inhibition at 25 mM and ~12–64% NFF inhibition at 100 mM (Davis et al., 2010).
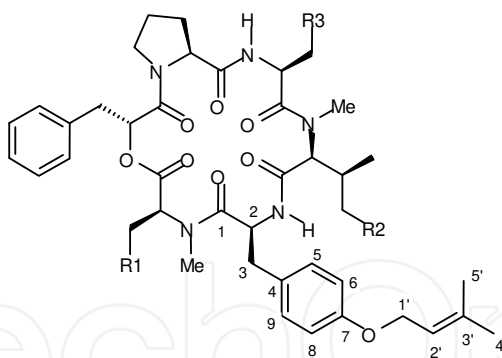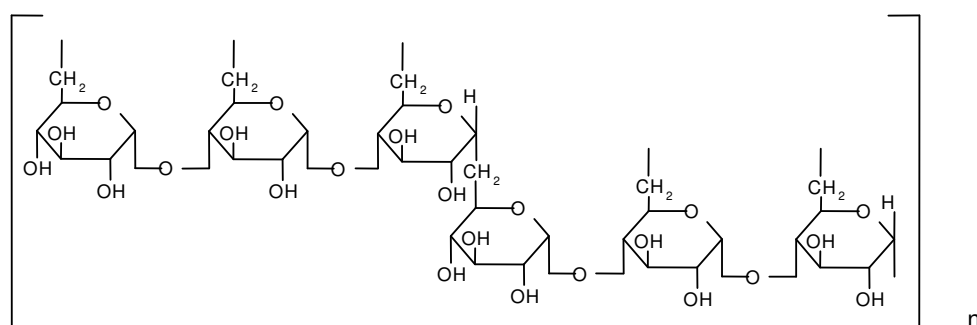
16, R1 = H, R2 = CH$_3$, R3 = OH

17, R1 = CH$_3$, R2 = CH$_3$, R3 = OH

18, R1 = H, R2 = H, R3 = OH

19, R1 = H, R2 = CH$_3$, R3 = OH

Fig. 10. Pullularins A-D (16-19)

Chalara alabamensis, an anamorphic fungus, was isolated from the host plant *Asterogyne martiana* (*Arecaceae*), which was collected in Costa Rica. The dichloromethane extract of this fungus inhibited PfHsp86, an essential protein-folding chaperone from *P. falciparum*, with an EC_{50} value of 24 μg/mL. The only active compound isolated from the extract was viridiol (Fig. 13), a steroidal furan with an EC_{50} value of 1.2 μg/mL (Cao et al., 2010).

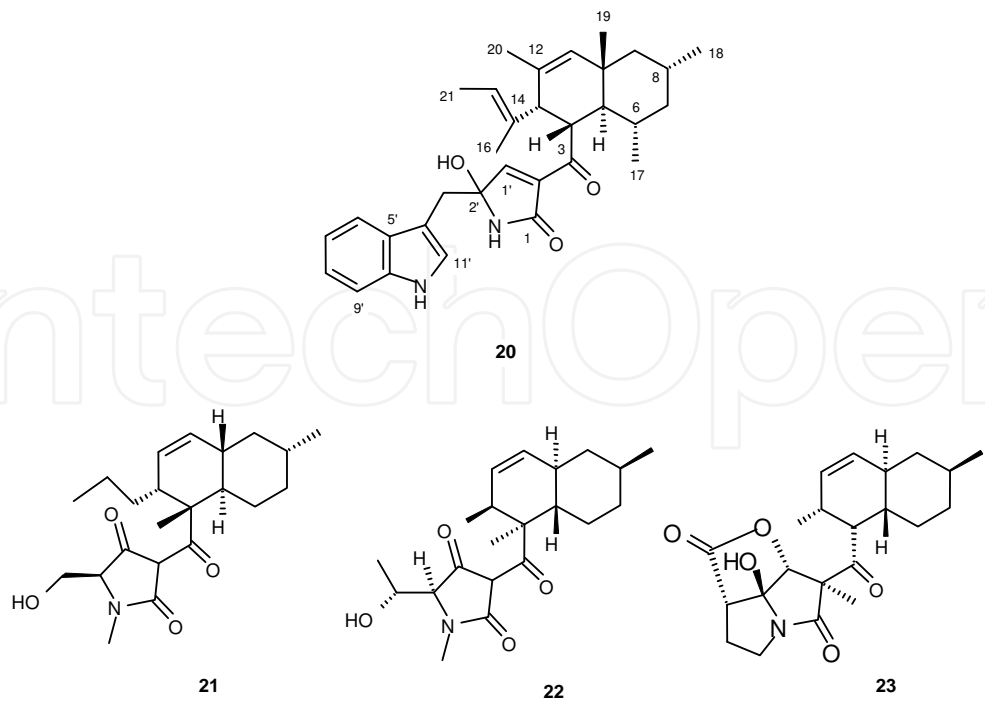Endophytic Fungi of Tropical Forests: A Promising
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Fig. 11. Codinaeopsin (20), equisetin (21), cryptocin (22) and UCS1025A (23).
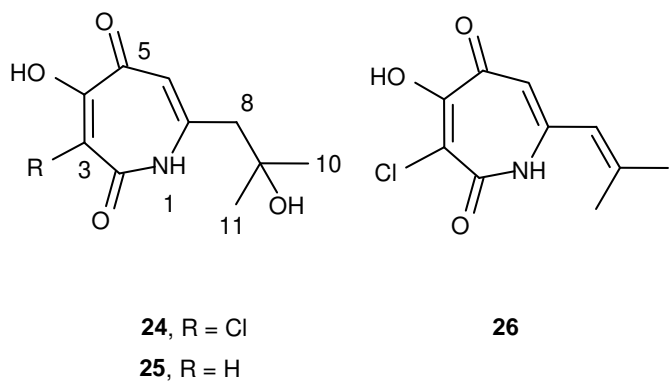
24, R = Cl
25, R = H
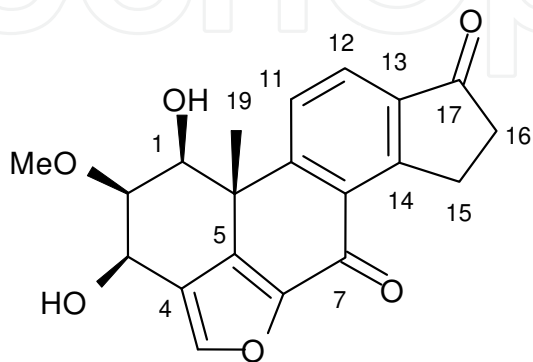
26

Fig. 12. Pestalactams A–C (24-26).

Fig. 13. Viridiol.

Structurally related aromatic sesquiterpenes named phomoarcherins A-C (Fig. 14, 28-30) were obtained from the ethyl acetate extract of the endophytic fungus *Phomopsis archeri*, which was isolated from the cortex stem of *Vanilla albidia* Blume (*Orchidaceae*). The most active compound in the series was amphotericin B, which had antimalarial activity against *P. falciparum* (IC$_{50}$ of 0.79 μg/mL) and contains a ketone function at C-3 and an aromatic lactone ring (Hemtasin et al., 2011).
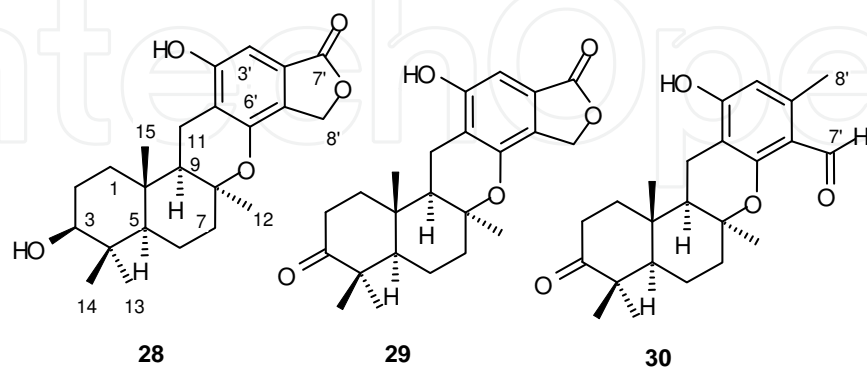

Fig. 14. Phomoarcherins A-C (28-30).

Pestalopyrone (Fig. 15), 6-(1'-methylprop-1'-enyl)-4-methoxy-2-pyrone, which was isolated from a Costa Rican endophytic fungus, *Phomatospora bellaminuta*, had activity against *P. falciparum* in an assay with an IC$_{50}$ value of 37 μM and is a promising candidate for a prototype molecule for antimalarial drugs (Cao & Clardy, 2011).
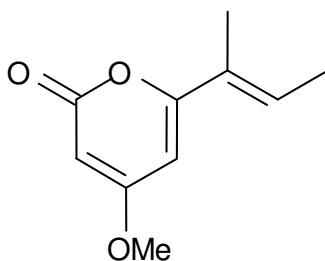

Fig. 15. Pestalopyrone.

8. Conclusion

Few bioactive molecules discovered from tropical endophytic fungi were included in studies for the development of new drugs. However, the compounds alternusin (anti-*T. cruzi*), cochlioquinone A and isocochlioquinone A (anti-leishmania) isolated from tropical fungal endophytes are *in vitro* and *in vivo* studies to maybe became new neglected disease drugs. Indeed, the use the tropical endophytic fungi as novel scaffolds for development of new drugs against neglected diseases represent a challenge to researchers of several scientific areas. The study of this fungal group offer some unique advantages: (i) endophytic fungi have a complex relationship with their host plant, which to survive produce a rich bioactive metabolic profile; (ii) fungi are eukaryotic organisms able to produce complex

molecules, which only few of them can be obtained by chemical synthesis; (iii) there are a lot of different isolates of same species of endophytic fungi, which also have differences in their capability to produce bioactive compounds; (iv) tropical endophytic fungi preserved in culture collections can be growth in different conditions of nutrients, temperature, pH, agitation and aeration to optimize the recover the high amount of crude extracts, as well as bioactive pure compounds; (v) if the crude extract and fractions produced by endophytic fungi do not display toxic activities, they can be used as "mycotherapic" agents. In addition, the metabolites described in this review also may be used against non-neglected diseases, because they are able to act against eukaryotic cells such as cancer cells, immune system cells, cells infected with virus, some human pathogenic fungi, among others. Unexplored natural environments are an excellent source of bioactive compounds that can act as the scaffold for commercial drugs. By taking advantage of new genomic, proteomic and drug design techniques, endophytic fungal communities associated with tropical forest plants, with their high diversity of species and their diverse genetic and metabolic pathways, may be resources for intelligent screening for discovering new drugs to treat neglected diseases.

9. Acknowledgments

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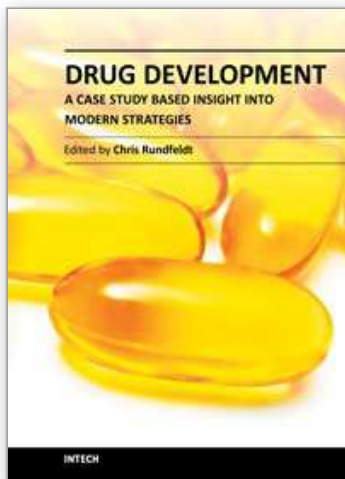
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