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Antimicrobial and antiradical activities of ethyl acetate extracts from endophytic fungi isolated from Cameroonian medicinal plants

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Abstract

The antimicrobial activity of extracts from endophytic fungi were determined using microdilution method. The 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity was used for antiradical activity and the chemical profile of promising extracts was analyzed using HPLC-DAD, UPLC/MS-QTOF and HPLC/MSⁿ Electrospray Ion Trap. The Minimum Inhibitory Concentration on yeasts and bacteria ranged from 0.019 to 5 mg/mL. Extract from *T. atroviride* was most active against yeasts (MIC from 0.019 to 5mg/ml) and *P. chermesinum* (MIC from 0.625 to 0.019 mg/ml) was against bacteria. The best radical scavenging activity was found with *Diaporthe* sp extract (%I from 9.929 to 41.134%). The chemical composition of extracts from *T. atroviride*, *P. chermesinum* and *Diaporthe* sp showed the presence of 8, 12 and 10 compounds respectively amongst which Genipinic acid, Citreomantanol, Dermoglaucin, Alternariol-5-methylether, Ascochitine, Aflatoxicol, Ascorbic acid, 4-deoxybostrycin, Bionectriamide B, Nivalenol, and Trisdechlonornidulin were identified. These results indicate that metabolites produced by endophytic fungi found in Cameroonian medicinal plants are potent source of bioactive compounds.

Keywords: Endophytic fungi, antimicrobial, antiradical, chemical profile

1. Introduction

The management of infections in both the community and hospital settings has become increasingly challenging over the last decade because of the rapid emergence of microorganisms resistant to a variety of antibacterial and antifungal compounds and has become a major problem worldwide [1-4]. For his defense against infectious pathogens, a major antimicrobial defense mechanism of Human body mounted by phagocytes is the production of reactive oxygen species (ROS) through a process known as the respiratory burst [5]. Nevertheless, the uncontrolled production of ROS leads to oxidative stress. In fact, oxidative stress is a process involved in numerous pathologies where oxidative damage on biological macromolecules (DNA, lipids and proteins) can considerably disturb the cell machinery [6]. Thus, the investigation of new bioactive compounds that can act as antimicrobial and antioxidant is urgently needed. A current trend is opt for natural products.

Natural products are metabolites and/or by-products derived from living organisms. The most prominent producers of natural products can be found within different groups of organisms including plants, animals, marine macro-organisms, and microorganisms [7]. Amongst the microorganisms, fungi are one of the most important source of new natural drug structures. Metabolites of microbial origin are well known as a valuable supply of new compounds and lead structures in the quest for drug candidates against infectious diseases and many other illnesses [8, 9]. Realizing the capacity of fungi to produce bioactive metabolites, the investigation of fungal isolates from ecological niches may lead to investigating novel fungal groups with novel and diverse of secondary metabolites. One such unexplored group are the endophytic fungi.

Endophytic fungi can be defined as fungi residing inside healthy plant tissues without causing any negative impact on the host plants [10]. Endophytes are capable of synthesizing bioactive and novel compounds useful for medical, pharmaceutical and agricultural purposes [11-13]. Some endophytes have been reported to produce compounds similar to those of the host and this was confirmed when taxol was obtained from an endophytic fungus isolated from *Taxus brevifolia* [14]. Thus, an initiative to explore the endophytic fungi was carried out by selecting the medicinal plants, *Cananga odorata*, *Terminalia catappa* and *Terminalia mantaly*.

In fact, *Cananga odorata* (Annonaceae), commonly called Ylang ylang, is a tropical plant used to treat hepatitis and has a wide range of other medicinal applications. Flowers from this plant are the source of valued essential oils in the cosmetics and perfume industries [15]. Decoctions and infusions of the leaves, bark and wood of *Terminalia catappa* and *Terminalia mantaly* (Combretaceae) are used for the treatment of several diseases, including gastroenteritis, dysentery, hypertension, diarrhea, diabetes, headache, colic, intestinal parasites, oral and skin conditions [15, 16]. In spite of the wide medicinal uses of these three plants species, limited information about the antimicrobial and antiradical activities of associated endophytes are available.

Materials and Methods

Sources of endophytic fungi

Four hundred and eighty-three (483) isolates of endophytic fungi were obtained from the surface sterilized pieces of leaf blade, leaf vein, stem, root, root bark, flowers, and petal samples of the three healthy and mature plants organs of *T. catappa* (51244/HNC), *T. mantaly* (64212/HNC) and *C. odorata* (42250/HNC) in Yaoundé, Cameroon on September 2, 2014. Amongst these, 120 isolates were identified by sequencing of their ITS1-5.8S-ITS2 region as described by Sánchez Márquez *et al* [17]. Overall, the identified isolates were found to belong to the following genera: *Fusarium*, *Phomopsis*, *Cercospora*, *Xylaria*, *Diaporthe*, *Pestalotiopsis*, *Lasiodiplodia*, *Colletotrichum*, *Galactomyces*, *Hypoxylon*, *Ophioceras*, *Ampelomyces*, *Fusicoccum*, *Paraconiothyrium*, *Pleosporales*, *Sordariomycetes*, *Botryosphaeria*, *Corynespora*, *Nigrospora*, *Phoma*, *Trichoderma*, *Penicillium*, *Guignardia*, *Cladosporium*, *Septoria*, *Nectria* and *Chaetomium* (Toghueo *et al.* submitted). Amongst these isolates, *Fusarium oxysporum*, *Colletotrichum* sp, *Fusarium* sp isolated from *C. odorata*, *Trichoderma atroviride*, *Trichoderma* sp, *Penicillium chermesinum*, *Aspergillus niger* isolated from *T. catappa* and *Diaporthe* sp, *Phomopsis* sp, *Cryptococcus flavescens* isolated from *T. mantaly* were used in this study.

Preparation of fungal extracts

Each fungus was cultivated by placing agar blocks of actively growing pure culture (3 mm diameter) in a 250 mL erlenmeyer flask containing 100g of rice medium. Flasks were incubated at 25 ± 2 °C for 30 days. After incubation, the mouldy rice was macerated with ethyl acetate and filtered through three layers of muslin cloth. The organic phase was collected and the solvent was then removed at 70 °C using a rotary vacuum evaporator. The dry solid residue was kept for evaluation of its antimicrobial and antiradical activities.

Antimicrobial activity

Microorganisms

The microorganisms included pathogenic bacteria (*Escherichia coli* ATCC 25922, isolate of *Escherichia coli*, *Enterococcus faecalis* ATCC 51299, *Staphylococcus aureus* CIP 7625, *Staphylococcus aureus* ATCC BAA-977, *Salmonella enterica* NR13555, *Shigella flexneri* NR518, *Klebsiella pneumoniae* ATCC 13883, *Klebsiella pneumoniae* NR 41916) and yeasts, (*Candida albicans* NR-29450, *Candida krusei* ATCC 6258, and *Candida parapsilosis* ATCC 22019, and *Cryptococcus neoformans*). Isolates were obtained from the Yaoundé Central Hospital, Cameroon and the reference strains from BEI resources and the American Type Culture Collection. The microorganisms were maintained on agar slope at 4 °C and sub-cultured for 24h and 48 h before use.

Preparation of stock solutions of fungal extracts and reference drugs

The stock solutions of crude extracts were prepared at 20 mg/mL using 10% dimethyl sulfoxide (DMSO). Fluconazole and chloramphenicol (Sigma Aldrich) were used as reference drugs and they were prepared at 512µg/mL and 20 mg/mL respectively. The stock solutions were filter-sterilized with a 0.20 µm syringe filter and stored at -20 °C until use.

Anti-yeast activity

The minimum inhibitory concentration (MIC) was determined according to the Clinical Laboratory Standards Institute M27-A3 microdilution method [18] using 96-wells microtitre plates. 100 µL of two-fold diluted extracts and reference drugs in RPMI 1640 (Sigma Aldrich) were added to the wells, followed by addition of 100 µL of yeasts inoculum standardized at 2.50×10^3 cells/mL. A blank column was included for sterility control. The concentrations of extracts ranged from 4.88 10^{-3} mg/mL to 5.00 mg/mL and that of fluconazole ranged from 1.25µg/mL to 128µg/mL. After 48 hours of incubation at 37 °C, the turbidity was observed as an indication of growth. MIC was defined as the lowest concentration inhibiting the visible growth of yeasts. All tests were performed in triplicate.

Antibacterial activity

The MIC was determined according to the Clinical laboratory Standards Institute (CLSI) M7-A9 microdilution method [19] using the 96-wells microtitre plate format. 100µL of two-fold diluted extracts in Muller Hinton Broth (Lab M Limited Topley House) were introduced in the wells of the plate. Thereafter, 100µL of the bacterial inoculum standardized at 0.5Macfarland were added to each well containing the test substances except for the blank column for sterility control. The concentrations of tests substances ranged from 0.00488 to 5 mg/mL. Chloramphenicol was included as a positive control at 4.88 10^{-3} -5 mg/mL. After incubation for 24 hours at 37 °C, the turbidity was observed as an indication of growth, and the lowest concentration inhibiting the visible growth of bacteria was recorded as the MIC. All the experiments were performed in triplicate.

Antiradical activity

The free radical scavenging assay was performed to study the antioxidant potency of ethyl acetate extracts of endophytic fungi, based on the scavenging activity of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) using method described by Warjeet and Brajeshwari [20]. A sample (3mL) was mixed with a DPPH solution (Sigma) in HPLC grade methanol (Merck), vortexed at room temperature and left standing for 10 min. The UV/VIS absorbance was measured at $\lambda=517$ nm serving the methanol without DPPH solution as blank solution. A reference solution of butylated hydroxyl toluene (BHT, Sigma) in methanol was used taking 100% radical scavenging activity. The concentration of extracts were 0.1, 0.5, 2.5, 5.0 and 10.0 mg/mL. The scavenging percentage was calculated with the following equation:

$$\% \text{ Radical Scavenging activity} = (A_0 - A_5) \cdot 100 / A_0$$

where, A₀, A₅ are the absorbance values of DPPH + Sample solution at 0.0 min and after 0.5 min, respectively. The assay was performed in triplicate and two times.

Chemical analysis of extract of promising endophytic fungi HPLC –DAD

All the fungal extracts were weighed at 50mg and diluted in 10mL of MeOH/H₂O (50:50) with sonication until complete

dissolution. The homogenized solution was centrifuged at 3000rpm for 1minute. The supernatant was transferred into HPLC vials. The analysis was performed on an Agilent 1260 series HPLC system equipped with autosampler and diode array detector (DAD). Chromatographic separation was performed on an Agilent Eclipse XDB C8 column 2.1 x 150 mm, 5 µm. The mobile phase consisted of acetonitrile and aqueous formic acid 0.1% with a flow rate of 1 mL/min and the injection volume of 20ul. The column was thermostated at 25 °C. The selected wavelengths were 254, 280 and 350 nm.

UPLC/MS-QTOF

The apparatus used was an Agilent 1290 equipped with and ion source electrospray (ESI) and quadrupole Time Of Flight (qTOF), Mass spectrum (Waters XEVO G2) with the mobile phase being water in 0.1% formic acid (A) and acetonitrile (B).

HPLC/MSⁿ Electrospray Ion Trap

HPLC-MSⁿ analysis was performed on a Varian 212 chromatograph equipped with a Prostar 430 (Varian) autosampler and Ion trap Mass detector MS500 using both

Electrospray (ESI) and Atmospheric Pressure Chemical Ionization (APCI). Separations were obtained on an Agilent Eclipse XDB C-8 2.1 x 150 mm 5µm. The mobile phase was composed of aqueous formic acid 0.1% (A) and acetonitrile (B). Flow rate was 200 µL/min. ESI parameters were: capillary voltage 80 V, needle voltage 5000 V, RF loading 100%, nebulizing gas pressure 35 psi, drying gas pressure 10 psi, and drying gas temperature 350 °C. Mass range was 100-2000 Da, ionization in positive and negative ion mode. Fragmentation patterns of eluted compounds were obtained using the turbo detection data scanning (TDDS®) function of the instrument.

Identification of compounds

Interpretation on mass spectrum of UPLC/MS-QTOF and HPLC/MSⁿ was done using four databases including Metline library, Human metabolome databases (HMDB), SDDBS database and yeast metabolome databases (YMDB). The mass spectrum of the unknown component was compared with the spectrum of the known components stored in these databases.

Results

Antifungal activity of fungi extracts

Table 1: Minimal Inhibitory Concentration (MIC) of fungal extracts on different yeast species. Means ± SD (mg/mL)

Fungus name	<i>C. albicans</i> NR-29450	<i>C. albicans</i> NR-29445	<i>C. Krusei</i> ATCC 6258	<i>C. parapsilosis</i> ATCC 22019	<i>Cryptococcus neoformans</i>
<i>F. oxysporum</i>	5.0±0.00	5.0±0.00	5.0±0.00	5.0±0.00	2.50±0.00
<i>Colletotrichum</i> sp	1.250±0.00	5.0±0.00	5.0±0.00	5.0±0.00	1.25±0.00
<i>Fusarium</i> sp	0.625±0.00	0.625±0.00	0.625±0.00	0.625±0.00	5.0±0.00
<i>T. atroviride</i>	0.019±0.00	0.019±0.00	0.625±0.00	0.625±0.00	5.0±0.00
<i>P. chermesinum</i>	1.250±0.00	1.250±0.00	1.250±0.00	1.250±0.00	0.078±0.00
<i>Phomopsis</i> sp	0.625±0.00	0.625±0.00	5±0.00	5.0±0.00	1.25±0.00
<i>C. flavescens</i>	5.0±0.00	5.0±0.00	5.0±0.00	5.0±0.00	5.0±0.00
<i>Aspergillus niger</i>	1.250±0.00	1.250±0.00	1.250±0.00	1.250±0.00	0.625±0.00
<i>Diaporthe</i> sp	1.250±0.00	1.250±0.00	5±0.00	5.0±0.00	2.5±0.00
<i>Trichoderma</i> sp	2.5±0.00	2.5±0.00	2.5±0.00	5.0±0.00	2.5±0.00
<i>Fluconazole</i>	0.032±0.00	0.032±0.00	0.008±0.00	0.016±0.00	0.032±0.00

The Minimal Inhibitory Concentration (MIC) ranged from 0.0195 to 5.0 mg/mL depending to on the fungi extracts, and the target microorganism (Table 1). *C. albicans* NR-29450 and NR-29445 were the most sensitive strains. The antifungal activity profile of most extracts was similar on the tested yeasts. Overall, all extracts were active on the four yeasts species. *T. atroviride* (MIC=0.0195mg/ml) was the most active

on *C. albicans* NR-29450 and NR-29445, while *P. chermesinum* was most active on *C. neoformans* (MIC=0.078 mg/ml). Extracts from *T. atroviride* and *Fusarium* sp (MIC=0.625mg/ml) were the most active on *C. parapsilosis* and *C. krusei*.

Antibacterial activity of fungi extracts

Table 2: Minimal inhibitory concentration (MIC) of extracts on bacteria species (mg/ml). Means ±SD

Fungi name	<i>S. aureus</i> BAA-977	<i>S. aureus</i> CIP 7625	<i>S. flexneri</i> NR 518	<i>S. enterica</i> NR 4311	<i>E. coli</i> ATCC 25922	<i>E. coli</i>	<i>K. pneumoniae</i> NR 41916	<i>K. pneumoniae</i> ATCC 13883	<i>E. faecalis</i> ATCC 51299
<i>F. oxysporum</i>	0.039±0.00	0.156±0.00	0.156±0.00	2.5±0.00	2.5±0.00	2.5±0.00	1.875±0.883	1.25±0.00	1.25±0.00
<i>Colletotrichum</i> sp	0.039±0.00	0.625±0.00	1.25±0.00	2.5±0.00	2.5±0.00	2.5±0.00	1.25±0.00	1.25±0.00	1.25±0.00
<i>Fusarium</i> sp	5±0.00	2.5±0.00	2.5±0.00	5±0.00	5±0.00	5±0.00	5±0.00	5±0.00	5±0.00
<i>P. chermesinum</i>	0.078±0.00	0.019±0.00	0.039±0.00	0.312±0.00	0.625±0.00	0.156±0.00	0.625±0.00	0.156±0.00	0.156±0.00
<i>Phomopsis</i> sp	0.312±0.00	0.312±0.00	0.625±0.00	0.625±0.00	0.937±0.441	1.25±0.00	0.312±0.00	0.468±0.220	0.312±0.00
<i>C. flavescens</i>	0.312±0.00	2.5±0.00	2.5±0.00	5±0.00	0.312±0.00	5±0.00	2.5±0.00	2.5±0.00	2.5±0.00
<i>Aspergillus niger</i>	0.312±0.00	0.625±0.00	1.25±0.00	1.25±0.00	1.25±0.00	1.25±0.00	0.625±0.00	0.625±0.00	0.625±0.00
<i>Diaporthe</i> sp	0.156±0.00	0.625±0.00	1.25±0.00	1.25±0.00	1.875±0.883	1.25±0.00	1.25±0.00	1.25±0.00	0.937±0.00
<i>Trichoderma</i> sp	0.234±0.110	5±0.00	0.625±0.00	5±0.00	5±0.00	5±0.00	5±0.00	2.5±0.00	5±0.00
<i>T. atroviride</i>	0.625±0.00	/	/	/	0.625±0.00	/	/	/	/
Chloramphenicol	0.019±0.00	0.004±0.00	0.004±0.00	0.008±0.00	0.019±0.00	0.004±0.00	0.008±0.00	0.008±0.00	0.156±0.00

The MIC values ranged from 0.019 mg/ml to 5.0 mg/ml depending on the fungal extract and microorganism tested (Table 2). Thus, *F. oxysporum*, *Colletotrichum* sp and *P. chermesinum* were the most active on both *S. aureus* strains (MIC=0.078 – 0.156 mg/ml). *P. chermesinum* was the most active on both *E. coli* strains (MIC=0.156-0.625 mg/ml). Extract from *P. chermesinum* was the most active on *K. pneumoniae* ATCC 13883, *S. flexineri*, *S. enterica* and *E. faecalis*. In general, *P. chermesinum* followed by *Phomopsis*

sp, *F. oxysporum*, *Aspergillus* sp, *Colletotrichum* sp were the most active. Except *Fusarium* sp and *Trichoderma* sp, all endophytic fungi have showed broad spectrum antibacterial activity.

Antiradical activity of fungi extracts

The antiradical properties of extracts from endophytic fungi was assessed and the results are summarized in table 3 above.

Table 3: Radical Scavenging activity of fungi extracts (%). Means \pm SD

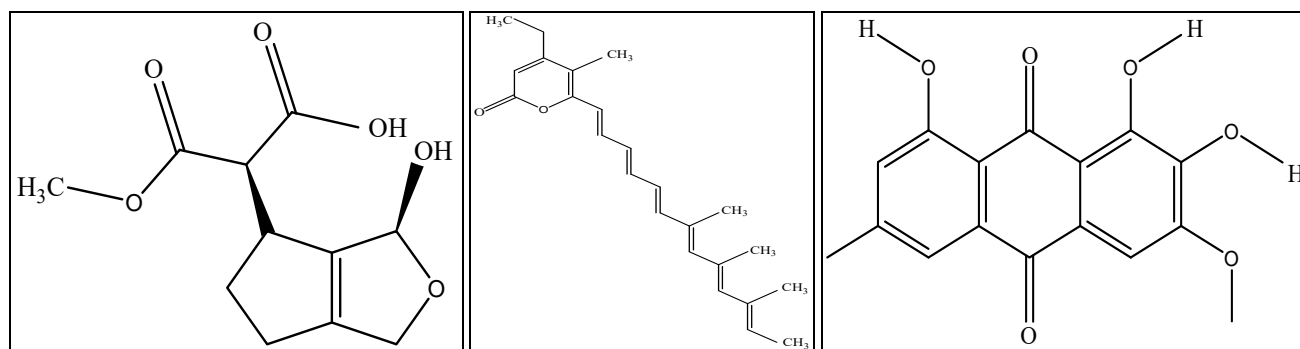
Fungi name	0.1mg/ml	0.5 mg/ml	2.5 mg/ml	5 mg/ml	10 mg/ml
<i>F. oxysporum</i>	5.99 \pm 0.32	9.73 \pm 0.32	12.36 \pm 0.56	17.41 \pm 0.00	23.59 \pm 0.00
<i>Colletotrichum</i> sp	5.20 \pm 0.20	7.80 \pm 0.00	13.47 \pm 0.00	19.86 \pm 0.00	26.24 \pm 0.00
<i>Fusarium</i> sp	8.80 \pm 0.32	10.67 \pm 0.00	15.17 \pm 0.00	24.34 \pm 0.32	41.10 \pm 0.00
<i>P. chermesinum</i>	6.74 \pm 0.00	10.11 \pm 0.00	17.78 \pm 0.32	22.47 \pm 0.00	35.58 \pm 0.00
<i>Phomopsis</i> sp	6.38 \pm 0.00	9.93 \pm 0.00	15.01 \pm 0.20	19.50 \pm 0.00	28.72 \pm 0.00
<i>C. flavescens</i>	7.09 \pm 0.00	9.22 \pm 0.00	13.83 \pm 0.00	15.13 \pm 0.00	19.03 \pm 0.20
<i>Aspergillus niger</i>	6.38 \pm 0.00	9.93 \pm 0.00	27.30 \pm 0.00	36.88 \pm 0.00	38.18 \pm 0.20
<i>Diaporthe</i> sp	9.93 \pm 0.00	15.25 \pm 0.00	20.80 \pm 0.20	31.20 \pm 0.00	41.13 \pm 0.00
<i>Trichoderma</i> sp	5.05 \pm 0.00	6.74 \pm 0.00	8.43 \pm 0.00	10.11 \pm 0.00	14.04 \pm 0.00
<i>T. atroviride</i>	8.80 \pm 0.32	11.05 \pm 0.32	16.48 \pm 0.32	17.04 \pm 0.32	28.65 \pm 0.00
BHT	28.84 \pm 0.32	47.94 \pm 0.32	50.56 \pm 0.97	51.68 \pm 0.00	50.75 \pm 0.32

From this table, inhibition percentage ranged from 5.056 to 51.685% depending on extracts and tested concentrations. It was found that the scavenging activity of extracts increased with the concentration of various extracts in the reaction medium. The best radical scavenging activity was found with *Diaporthe* sp with inhibition percentage ranged from 9.929 to 41.134% followed by *Fusarium* sp (8.800 to 41.107%), *Aspergillus niger* (6.382 to 38.179%) and *P. chermesinum* (6.741 to 35.584%). Butylated hydroxyl toluene was taken as standard showing from 28.838 to 51.685% antioxidant activity.

Chemical profile of promising extracts

The chemical investigation of promising extracts from *T. atroviride*, *P. chermesinum* and *Diaporthe* sp was done by

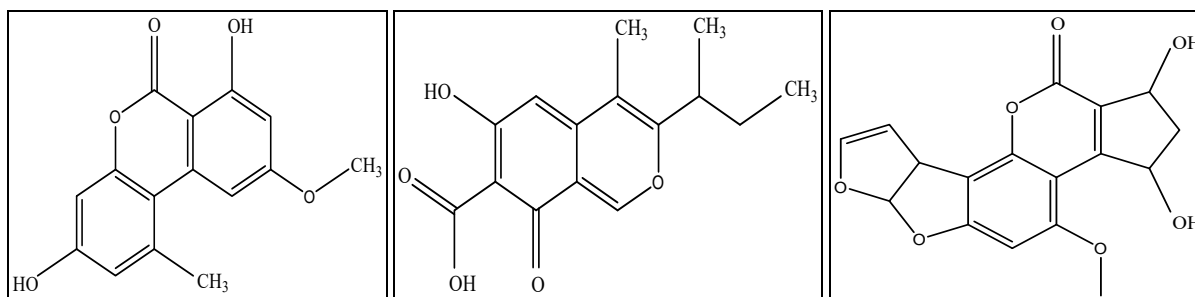
HPLC-DAD couple with UPLC/MS-QTOF and HPLC/MSⁿ Electropray Ion Trap. Structures and names of the compounds identified were ascertained below in Fig. 1. In fact, eight compounds were detected from *T. atroviride* extract amongst which Genipinic acid (1), and Citreomantananin (2). In *P. chermesinum* extract, 12 compounds were detected and Dermoglaucin (3), Alternariol-5-methylether (4), Ascochitine (5) and Aflatoxicol (6) were identified. In the *Diaporthe* sp extract, ten (10) compounds were detected and Ascorbic acid (7), Genipinic acid (1), 4-deoxybostrycin (8), Bionectriamide B (9), Nivalenol (10), Trisdechloronormidulin (11) were identified. This chemical screening showed the clear difference between *T. atroviride*, *P. chermesinum* and *Diaporthe* sp extracts justifying their different bioactive potential.



(1) Genipinic acid

(2) Citreomantananin

(3) Dermoglaucin



(4) Alternariol-5-O-methylether

(5) Ascochitine

(6) Aflatoxicol

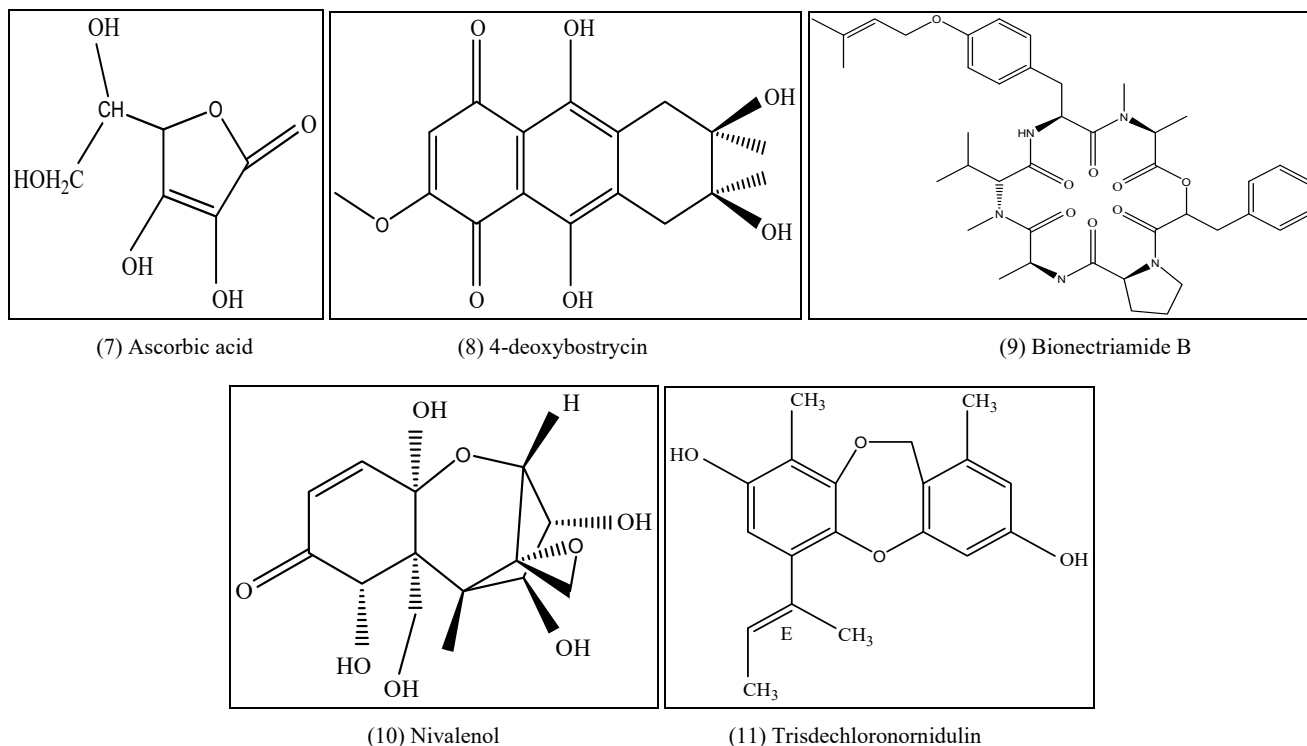


Fig 1: Compounds identified from *T. atroviride*, *P. chermesinum* and *Diaporthe* sp extracts

Discussion

The need for new antimicrobial agents, comes from the increasing rates of development resistance to existing antibiotics. Endophytic fungal species are now considered as exciting novel sources for obtaining new bioactive compounds and have been reported from several hosts [21]. In this study, an initial assessment was performed for the antibacterial, antifungal and antiradical activities of ethyl acetate extracts of some endophytic fungi isolated from *T. catappa*, *T. mantaly* and *C. odorata*.

The most active extracts against yeasts were respectively *T. atroviride*, *P. chermesinum*, *Fusarium* sp, *Aspergillus* sp and *Phomopsis* sp while that of bacteria, were *P. chermesinum* followed by *Phomopsis* sp, *F. oxysporum*, *Aspergillus* sp, *Colletotrichum* sp. Literature reveals that endophyte belonging to these genera possess antimicrobial activity [21-25]. From our results, it was clear that, ethyl acetate extracts of endophytic fungi obtained from the same plant showed different antimicrobial activity. These differences in susceptibility could be attributed to the type of isolates, nature and level of the antimicrobial agents present in their extracts as well as their mode of action on different test microorganisms. Many workers have demonstrated that the endophytes isolated from medicinal plants are excellent producers of strong fungicidal, bactericidal and cytotoxic metabolites [26]. In accordance, the results of this investigation showed that the most active fungi *T. atroviride* and *P. chermesinum* contain 8 and 12 compounds respectively amongst which Genipinic acid, Citreomantanol, Dermoglaucin, Alternariol-5-methylether, Ascohitine, Aflatoxicol and 4-deoxybostrycin. These compounds were found to be produced by other fungi and exhibit antimicrobial activity [27-30].

To determine the ability of these endophytes extracts to fight against reactive oxygen species, the DPPH radical scavenging assay was assessed. It is evident from the results that the fungal extract contains promising radical scavenging compounds. The best radical scavenging activity was found with *Diaporthe* sp

followed by *Fusarium* sp, *Aspergillus* sp and *P. chermesinum*. This study showed that these extracts have proton donating ability and could serve as free radical scavenging, acting as primary antioxidant. The chemical analysis of our potent extract from *Diaporthe* sp showed the presence of ten compounds amongst which Ascorbic acid, a known natural antioxidant compounds. Some antioxidant compounds isolated from endophytic fungi have also been reported [31, 32] and generally, typical compounds that possess antioxidant activity have been characterized as phenolic compounds [33, 34]. One mechanism by which antioxidants inhibit oxidation is by quenching reactive species through hydrogen or electron donation [35].

Considering the results of the antimicrobial and the DPPH radical scavenging activity, these findings reveal the potential of the fungal extracts as a source for natural antimicrobials and antioxidants. It indicates that the metabolites of the endophytic fungi isolated from *T. catappa*, *T. mantaly* and *C. odorata* could be potential producers of compounds useful for scavenging free radicals, treating microbial infections and diseases related to free radical reactions. This work provides an insight into understanding some basis of therapeutic properties of fungal endophytes of *T. catappa*, *T. mantaly* and *C. odorata* in traditional medicine. Further, studies on the isolation and characterization of the endophytic fungal metabolites as well as *in vitro* assays will be necessary for the discovery of new bioactive compounds.

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