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The Pharmaceutical and Chemical Journal, 2018, 5(6):72-77

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ISSN: 2349-7092
CODEN(USA): PCJHBA

Investigation of Secondary Metabolites of an Endophytic Fungus Isolated from the Leaves of *Chromolaena odorata* for Possible Antimicrobial and Antioxidant Activities

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Abstract Nigeria's rich plant biodiversity play host to endophytic fungal populations that possess enormous potentials as sources of novel bioactive molecules. This study was aimed at investigating the metabolites of an endophytic fungus isolated from *Chromolaena odorata*, a weedy plant commonly found in Nigeria, for antimicrobial and antioxidant activities and also identifying some of its constituents. An endophytic fungus was isolated from *C. odorata* and fermentation extract of the fungus was tested for antimicrobial and antioxidant activities using the agar diffusion and DPPH assay methods respectively. Some of the bioactive components of the endophytic fungal extract were identified using high performance liquid chromatography (HPLC). The fungal extract at 1 mg/mL showed only antibacterial activity with inhibition zone diameters of 2, 2, and 6 mm produced against *S. aureus*, *B. subtilis*, and *S. typhi* respectively. Also, at 100 ug/mL, the fungal extract showed average antioxidant activity with an inhibition of 46%. HPLC analysis of the extract suggested the presence of indole-3-acetic acid and acropyrone which may be responsible for the biological activities exhibited by the fungal extract. The results of this study showed that the endophytic fungus isolated from leaves of *C. odorata* produced secondary metabolites with antimicrobial and antioxidant properties. This implies that endophytic fungi associated with leaves of *C. odorata* could be a promising source of biologically active compounds.

Keyword: *Chromolaena odorata*, endophytes, HPLC analysis, secondary metabolites, antimicrobial and antioxidant activities

Introduction

Nigeria is rich in plant biodiversity, and these plants which are hosts to millions of endophytic microbial communities, present the opportunity to discover a plethora of biologically important compounds and offer a renewable source of natural products [1]. Studies on the endophytic fungal populations of Nigerian medicinal plants have highlighted the enormous potentials possessed by these organisms as sources of novel bioactive molecules, and the need to further explore Nigeria's plant biodiversity for endophytes producing biologically important molecules [1-12].

Chromolaena odorata is a shrub, belonging to the family Asteraceae. It originates from the Neotropics and has spread to many tropical parts of the world, where it exists mainly as a weed of plantation crops and pastures of southern Asia and western Africa [13,14]. The plant was introduced into Nigeria from Sri Lanka and it has become one of the worst weeds in the country [15].

C. odorata have been reported to possess antioxidant, anti-inflammatory, analgesic, antimicrobial, cytoprotective properties [14-19]. In Nigeria, *C. odorata* have been used traditionally for the treatment of several disease conditions including soft-tissue wounds, burns, skin infections, malaria, and measles [20].

In the search for bioactive molecules from endophytes associated with Nigerian plants, this present study was aimed at investigating the metabolites of an endophytic fungus isolated from *C. odorata* for antimicrobial and antioxidant activities, and also identifying some of its constituents.

Materials and Methods

Isolation of endophytic fungus, fermentation and extraction of metabolites

Fresh healthy leaves of *C. odorata* were collected from Agulu, Anambra state, Nigeria. Isolation of the endophytic fungus from the plant leaves, solid state fermentation of the fungus and extraction of the fungal metabolites were carried out using methods previously described by Eze *et al.* [1].

Antimicrobial Assay

Antibacterial and antifungal screening of the fungal extract was carried out using the agar well diffusion method [6,7]. A concentration of 1 mg/mL of the extract was tested against laboratory strains of *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Salmonella typhi, Candida albicans,* and *Aspergillus fumigatus.* Gentamicin (10 µg/mL) and ketoconazole (50 µg/mL) were used as positive controls in the antibacterial and antifungal tests respectively, while DMSO was used as the negative control in both tests. The inhibition zone diameters (IZDs) produced against the test isolates were measured and recorded.

Antioxidant Assay

The antioxidant activity of the fungal extract was carried out using the 1, 1-diphenyl-2-picryl-hydraxyl (DPPH) free radical assay as described by Brand-Williams *et al.* [21], but with modification. A concentration of 100 µg/mL of the fungal extract and control (ascorbic acid) were prepared in methanol. A 0.1 mM solution of DPPH was also prepared in methanol. The samples of the fungal metabolites were reacted with the stable DPPH free radical in a methanol solution in a 96-well micro-titer plate. The reaction mixture consisted of 25 µL of sample, 150 µL of methanol and 25 µL of 0.1 mM DPPH radical solution. Absorbance was measured at 490 nm wave length after 30 min of incubation in the dark, using a UV/VIS spectrophotometer The mixture of methanol (175 µL) and DPPH radical solution (25 µL) was used as blank.

The DPPH free radical scavenging effect of the samples was calculated using the following formula:

$$\text{DPPH scavenging effect (\% inhibition)} = \frac{\text{Abs of blank } (A_0) - \text{Absorbance of sample } (A_0)}{\text{Abs of blank } (A_0)} X\ 100 \qquad (1)$$

High performance liquid chromatography (HPLC)

HPLC analysis of the fungal extract was carried out using a Dionex P580 HPLC system coupled to a photodiode array detector (UVD340S, Dionex Softron GmbH, Germering, Germany) [1]. The separation column (125 × 4 mm; length × internal diameter) was prefilled with Eurospher-10 C18 (Knauer, Germany) and a linear gradient of nanopure water (adjusted to pH 2 by addition of formic acid) and methanol was used as eluent. Detection was at 235 nm, and the absorption peaks of the fungal extract were analyzed by comparing with those in the HPLC-UV/Vis database.

RESULTS

An endophytic fungus (CO-2AL3) was isolated from the leaves of *C. odorata*. The crude ethyl acetate extract from solid state fermentation of the endophytic fungus was subjected to antimicrobial and antioxidant assays. The result of the antimicrobial assay of the fungal extract (Table 1) revealed that at 1 mg/mL, the extract showed antibacterial activity only against *S. aureus B. subtilis*, and *S. typhi*, with IZD of 2, 2, and 6 mm respectively. The extract showed no antifungal activity against the test fungi *C. albicans* and *A. fumigatus*. In the DPPH antioxidant assay, at 100 µg/mL, the fungal extract displayed an average antioxidant activity with an inhibition of 46.4% (Table 2).

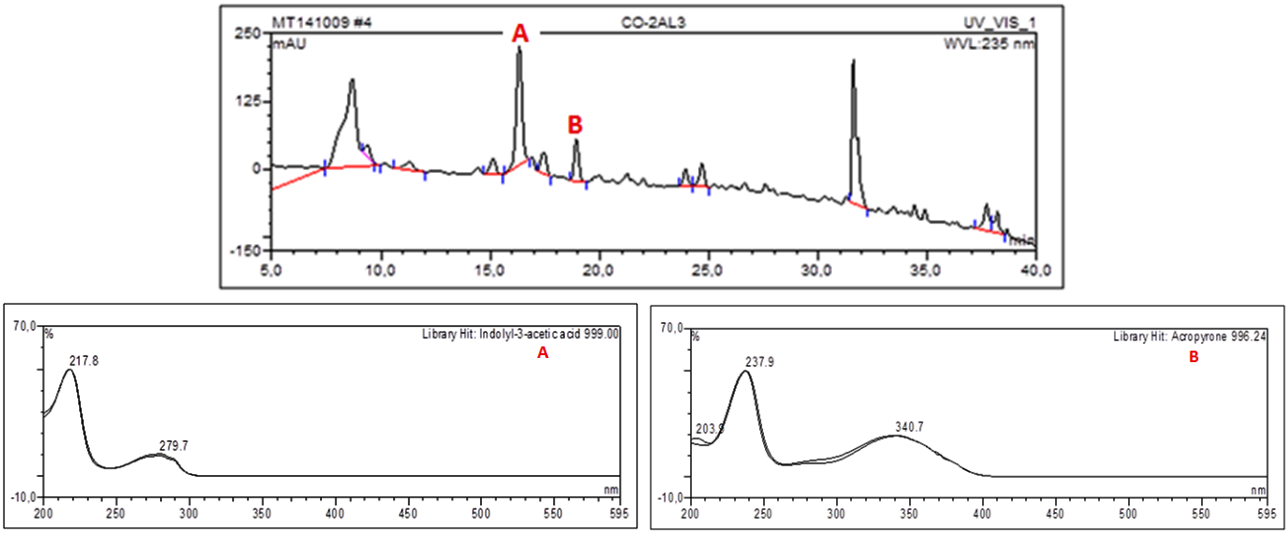
Identification of the constituents of the fungal extract was achieved using HPLC analysis. Two compounds (acropyrone and indole-3-acetic acid) were detected in the fungal extract. The HPLC chromatogram of the fungal extract, as well as the UV-spectra and chemical structures of detected compounds are presented in Figure 1.

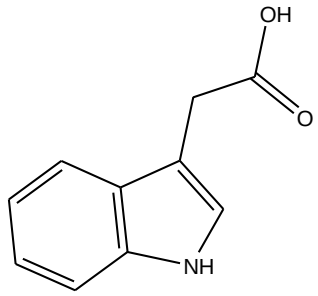
Table 1: Antimicrobial assay of the endophytic fungal extract showing the Inhibition zones diameters (IZDs) produced against test organisms

Test Organisms	Fungal Extract (1 mg/mL)	Positive control	Negative control
		Gentamicin (10 µg/ml)	DMSO
E. coli	0	16	0
S. aureus	2	17	0
B. subtilis	2	22	0
S. typhi	6	21	0
		Ketoconazole (50 µg/ml)	DMSO
C. albicans	0	17	0
A. fumigatus	0	4	0

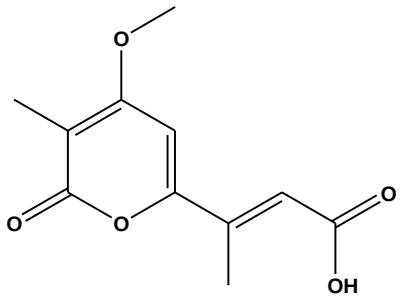
Table 2: DPPH scavenging activity of the endophytic fungal extract

Concentration	% Inhibition	
	Endophytic fungus (CO-2AL3)	Ascorbic acid
100 µg/mL	46.4	59.6





Indole-3-acetic acid (A)
$C_{10}H_9NO_2$. 175.19 g·mol^{-1}



Acropyrone (B)
$C_{11}H_{12}O_5$, 224 g.mol^{-1}

Figure 1: HPLC chromatogram of the fungal extract, UV Spectra and chemical structures of the detected compounds: A (indole-3-acetic acid) and B (acropyrone)

Discussion

The two detected compounds possess biological activities that are either cytotoxic, anti-inflammatory or antioxidant, and may be responsible for the antimicrobial and antioxidant activities exhibited by the fungal crude extract. Acropyrone has been previously isolated from *Acronychia pedunculata* and has been reported to show cytotoxic activity [22,23]. Indole-3-acetic acid has been has been reported to show cytotoxic/anticancer, antioxidant, and anti-inflammatory activities [24-26].

These two compounds - acropyrone and indole-3-acetic acid have also been previously reported as major secondary metabolites produced by fungal endophytes of Nigerian plants [1,5-7]. These organisms can be a ready source for large scale production of these compounds for pharmaceutical or industrial applications.

Nigeria's rich plant biodiversity presents an enormous platform for researchers to explore in the search biologically active molecules without the destructive harvesting of plants, but by exploring their associated endophytic organisms for pharmaceutically and industrially important molecules [1]. This approach of bioprospecting from endophytes will also help in securing and conserving plant biodiversity.

Conclusion

The endophytic fungus isolated from leaves of *C. odorata* produced secondary metabolites which showed antimicrobial and antioxidant activities. This further confirms that endophytes associated with Nigerian plants hold key of possibilities to the discovery of novel molecules for pharmaceutical, agricultural, and industrial applications.

Conflict of Interests

The authors declare no conflict of interest.

Acknowledgements

The authors are grateful to Prof. Dr. Peter Proksch of the Institute of Pharmaceutical Biology and Biotechnology, Heinrich-Heine-University, Düsseldorf, for his express permission to use his facilities for the HPLC analysis.

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