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(RESEARCH ARTICLE)

Antimicrobial and antioxidant potentials of an endophytic *cunninghamella* sp. isolated from the leaves of *Chrysophyllum albidum*

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Abstract

Background: Several studies have identified endophytic fungi associated with Nigerian plants as potential sources of new drug discovery.

Aim: This study was carried out to investigate the antimicrobial and antioxidant properties of secondary metabolites isolated from an endophytic *Cunninghamella* sp. associated with leaves of *Chrysophyllum albidum*, a Nigerian indigenous plant.

Method: The plant material was collected in Agulu, Anambra State, South-East Nigeria. Endophytic fungal isolation and identification, as well as fermentation and extraction of fungal secondary metabolites, were carried out using standard methods. The antimicrobial and antioxidant activities of the fungal extract were evaluated using the agar well diffusion method and the 1,1-diphenyl-2-picryl-hydraxyl (DPPH) free radical assay, respectively.

Result: An endophytic fungus was isolated from the leaves of C. albidum. Following a molecular identification protocol of DNA amplification and sequencing of the fungal ITS region, the fungus was identified as *Cunninghamella* sp. The fungal extract exhibited antimicrobial activity against the test bacteria and fungus. At concentrations ranging from 2.5 - 20 mg/mL, the extract produced inhibition zone diameters (IZD) ranging from 2 - 11 mm against *Pseudomonas aeruginosa.* Antibacterial activity was also observed against *Staphylococcus aureus* at concentrations ranging from 10 - 20 mg/mL, with IZD ranging from 3 - 6 mm. Antifungal activity was observed against *Candida albicans* at concentrations ranging from 1.25 - 20 mg/mL, with IZD ranging from 2 - 7 mm. The fungal extract demonstrated significant antioxidant activity in the DPPH assay when compared to the standard control, quercetin. The extract showed a percentage inhibition range of 95 - 98% at concentrations ranging from 20 to 100 µg/mL.

Conclusion: The findings of this study highlight the potential of endophytic *Cunninghamella* sp. and other endophytic microorganisms of indigenous Nigerian plants as sources of bioactive compounds with antimicrobial and antioxidant properties for pharmaceutical and industrial applications.

Keywords: Antimicrobial; Antioxidant; Secondary Metabolites; Endophytic Fungus; *Cunninghamella Sp.; Chrysophyllum albidum*

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1. Introduction

Because of the enormous health problems caused by drug-resistant microorganisms, there is a greater need to expand the search for new therapeutic agents. As a result, the need to screen many medicinal plants and microorganisms for potential bioactive agents that could be useful in the development of novel therapeutic agents has grown [1, 2]. *Chyrysophyllum albidum* (Sapotaceae), also known as white star apple, is a tropical fruit-tree found in tropical Africa and other parts of the world [3]. *C. albidum* is used in Nigerian folk medicine to treat a variety of diseases, and ethnopharmacology research has revealed that the plant's parts have antioxidant, hypoglycemic, antimicrobial, anti-plasmodial, analgesic, and anti-inflammatory properties [4,5,6,7,8.9,10,11]. Endophytic microorganisms associated with indigenous Nigerian medicinal plants, on the other hand, have been shown in several studies to have enormous potential as sources of biologically active compounds with potential applications in the pharmaceutical, industrial, and agricultural sectors [1, 12,13,14,15,16,17, 18, 19,20,21,22]. In line with ongoing research for new therapeutic compounds from endophytic microorganisms associated with Nigerian medicinal plants, the current study seeks to investigate the antimicrobial and antioxidant potentials of secondary metabolites fungus isolated from the leaves of *C. albidum.*

2. Materials and Methods

2.1. Plant Collection

Healthy leaves of *C. albidum* were collected in March 2020 from Nneogidi, Agulu, Anaocha Local Government Area of Anambra State, South-Eastern Nigeria.

2.2. Isolation of endophytic fungus

Isolation of endophytic fungi from the plant leaves was carried out using the method described by Okezie *et al*., [19]. Healthy plant leaves were thoroughly washed in running tap water to remove dust particles before being rinsed with sterile water. The leaves were then cut into 1 - 2 mm segments and surface-sterilized by washing in 2% sodium hypochlorite for 2 min, followed by 70% ethanol for 2 min, followed by a final rinse in sterilized distilled water for 3-5 min. The leaf segments were selected and placed in Petri dishes with malt extract agar (MEA) supplemented with chloramphenicol. The plates were then incubated at 25-27ºC. Fungal growth from leaf segments was monitored, and hyphal tips from distinct colonies emerging from leaf segments were sub-cultured on fresh MEA plates to obtain pure colonies.

2.3. Identification of endophytic fungus

Following molecular identification involving DNA amplification and sequencing of the fungal ITS region [1], an endophytic fungus isolated from the leaves of *C. albidum* was identified as *Cunninghamella* sp. The fungal DNA sequence data were deposited in the NCBI database (GenBank) under the accession number OP846522.

Figure 1 Leaves of *Chyrysophyllum albidum* (A); endophytic *Cunninghamella* sp in malt extract agar (B)

2.4. Fermentation and extraction of secondary metabolites

The fungus was solid-state fermented in an Erlenmeyer flask containing sterilized rice medium (100 g of rice + 100 mL of distilled water, autoclaved at 121ºC at 15 psi for 30 min). The flasks were inoculated with 3 mm diameter agar blocks

containing the fungus and incubated at 25-27ºC for 30 days. Following fermentation, the fungal secondary metabolites were extracted with ethyl acetate and concentrated under vacuum at 40ºC using a rotary evaporator [19].

2.5. Antimicrobial assay

The antimicrobial screening of the endophytic fungal extract was performed using the agar well diffusion method [19, 23]. Two-fold serial dilutions of the fungal extract ranging from 20 - 1.25 mg/mL were prepared in dimethyl sulfoxide (DMSO, 100% v/v). Using sterile cotton swabs, standardized broth cultures of test bacterial isolates (*Pseudomonas aeruginosa* and *Staphylococcus aureus*) and fungal isolate (*Candida albicans*) were spread aseptically onto the surfaces of Mueller Hinton agar (MHA) and Sabouraud dextrose agar (SDA) plates, respectively. The plates were allowed to dry for about 5 min before making agar wells of 6 mm with a sterile cork-borer. The wells were filled with 20 µL of the fungal extract and controls, respectively. The plates were left at room temperature for 1 hour to allow the agents to diffuse into the agar medium. The MHA plates were then incubated at 37ºC for 24 h, while the SDA plates were incubated at 25-27ºC for 2-3 days. The inhibition zone diameters (IZDs) were measured and recorded. Ciprofloxacin (5 µg/mL) and miconazole (50 µg/mL) were used as positive controls in the antibacterial and antifungal tests, respectively, while DMSO (100% v/v) was used as the negative control. To calculate the actual IZD values, the cork borer size (6 mm) was subtracted from the measured IZDs. This procedure was repeated three times, and the mean IZDs were calculated.

2.6. Antioxidant assay

The antioxidant activity of the fungal extract was evaluated using the 2, 2-diphenyl-1-picryl-hydraxyl (DPPH) free-radical assay described by Okezie *et al*., [21]. Concentrations (20, 40, 60, 80, and 100 µg/mL) of the fungal extract and control (quercetin) were prepared in methanol (100% v/v). In addition, 0.1 mol/L of DPPH was prepared in methanol. The samples were exposed to the DPPH solution in a 96-well microtiter plate and incubated in dark at 27 ºC for 30 min. The reaction mixture comprised of 25 µL of sample, 150 µL of methanol, and 25 µL of 0.1 mM DPPH solution. A mixture of methanol (175 µL) and DPPH solution (25 µL) was used as blank. Absorbance was measured at 490 nm using a UV/VIS spectrophotometer. The DPPH free-radical scavenging activity of each sample was expressed as percentage inhibition, which was calculated using the formula: DPPH free radical scavenging activity = $[(Ao - A_1)/Ao] \times 100/1$; where: Ao = absorbance of the blank solution; A_1 = absorbance of the positive control.

3. Results

The results of the antimicrobial screening of the fungal extract are shown in Table 1. The fungal extract exhibited antimicrobial activity against the test bacteria and fungus. At concentrations ranging from 2.5 - 20 mg/mL, the extract produced inhibition zone diameters (IZD) ranging from 2 - 11 mm against *P. aeruginosa.* Antibacterial activity was also observed against *S. aureus* at concentrations ranging from 10 - 20 mg/mL, with IZD ranging from 3 - 6 mm. Antifungal activity was observed against *C. albicans* at concentrations ranging from 1.25 - 20 mg/mL, with IZD ranging from 2 - 7 mm. The fungal extract demonstrated significant antioxidant activity in the DPPH assay when compared to the standard control, quercetin (Figure 2). The extract showed a percentage inhibition range of 95 - 98% at concentrations ranging from 20 to 100 µg/mL.

Table 1 Result of antimicrobial assay of *Cunninghamella sp.* extract

Concentration(mg)	**Inhibition Zone Diameter (mm)**		
	P. aeruginosa	*S. aureus*	*C. albicans*
20 (mg/mL)	11	6	7
10 (mg/mL)	7	3	3
5 (mg/mL)	3	0	3
2.5 (mg/mL)	2	0	2
1.25 (mg/mL)	0	0	1
Ciprofloxacin (5ug/mL)	9	10	-
Miconazole (50 ug/mL)	-	-	16
DMSO (100% v/v)	0	0	0

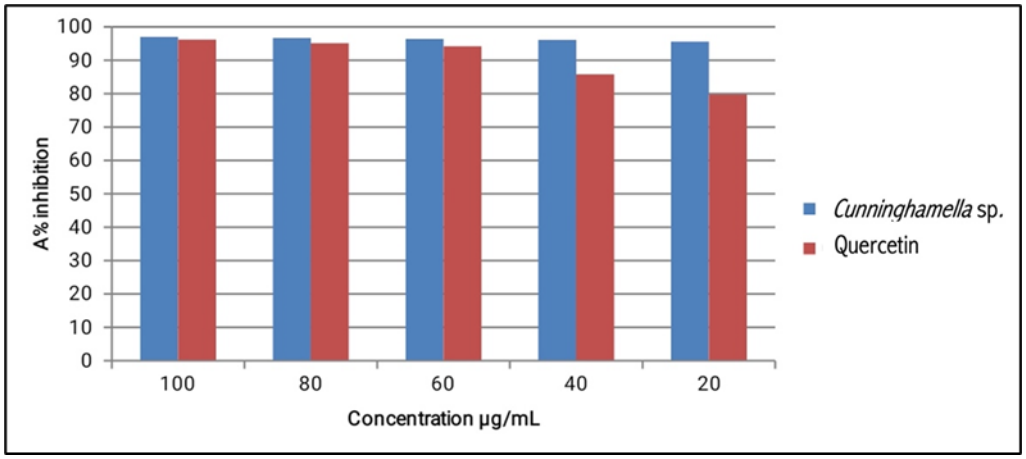

Figure 2 Result of antioxidant assay of *Cunninghamella* sp. extract

4. Discussion

The *in vitro* antimicrobial activity assay has been described as the first step in the discovery and development of new antimicrobial chemotherapeutic agents. Researchers have reported the antimicrobial and antioxidant properties of endophytic fungi from various plants [1, 13, 20, 22]. Such bioprospecting research is required to contribute to the long-term discovery and development of new antibiotics.

In this current study, secondary metabolites of an endophytic *Cunninghamella* sp. isolated from *C. albidum* leaves demonstrated broad-range antimicrobial activity against Gram-negative (*P. aeruginosa*) and Gram-positive (*S. aureus*) test bacteria, as well as against the test fungus, *C. albicans* (Table 1). The broad-spectrum antimicrobial activity of the endophytic fungal extract indicates that it can be explored to yield antibacterial and antifungal molecules that could be developed for the treatment of bacterial and fungal infections.

The extract's antimicrobial activity against the Gram-negative, *P. aeruginosa,* indicates that the fungal secondary metabolites contain potent antimicrobial compounds capable of disrupting the outer membrane and penetrating the Gram-negative cell, which contains lipopolysaccharides that serve as a mechanical barrier to macromolecules and hydrophobic compounds, allowing Gram-negative bacteria to be more resistant to antimicrobial agents [24]. The broad-spectrum antimicrobial activity recorded by the endophytic fungal extract is an indication that it could have potentials to be developed as antibiotics and antifungal agents against bacterial and fungal infections.

In addition to antimicrobial activity, the fungal extract demonstrated significant antioxidant activity in the DPPH antioxidant assay, with a considerably higher percentage inhibition than the standard control (quercetin) (Figure 2). Endophytic fungi associated with Nigerian plants have been found to produce a variety of antioxidant compounds, including ferulic acid, protocatechuic acid, and indole-3-acetic acid [13, 15]. As the number of diseases caused by free radicals continues to rise, researchers are turning to natural products with antioxidant properties to supplement the existing synthetic antioxidants, which are gradually losing potency [25].

The extract of *Cunninghamella* sp. displayed antimicrobial and antioxidant properties, indicating that it contains antimicrobial and antioxidant compounds or constituents. These qualities emphasizes the drug development potential of the fungal secondary metabolites and highlights the need for further purification and characterization of the extract's active principles.

5. Conclusion

The findings of this study highlight the potential of endophytic *Cunninghamella* sp. as a source of antimicrobial and antioxidant compounds. This study also confirms endophytic microorganisms associated with indigenous Nigerian plants as potential sources of bioactive compounds for pharmaceutical and industrial applications.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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