
Biologically active phenolic acids produced by *Aspergillus* sp., an endophyte of *Moringa oleifera*

Dominic O. Abonyi¹, Peter M. Eze^{1*}, Chika C. Abba², Nonye T. Ujam³, Peter Proksch⁴, Festus B. C. Okoye², Charles O. Esimone¹

¹ Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria

² Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria

³ Department of Pharmaceutical Microbiology and Biotechnology, Enugu State University of Science and Technology, Enugu, Nigeria

⁴ Institute of Pharmaceutical Biology and Biotechnology, Heinrich Heine University, Düsseldorf, Germany

*Corresponding author: Peter M. Eze; E-mail: ezep2004@hotmail.com

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ABSTRACT

This study investigates the secondary metabolites of an endophytic *Aspergillus* sp. isolated from leaves of *M. oleifera* growing in Anambra State, South-Eastern Nigeria. Antimicrobial and antioxidant screening of the fungal extract and isolated compounds, as well as cytotoxicity assay of the extract against cisplatin-sensitive A2780 (sens) and cisplatin-resistant A2780 (cisR) ovarian cancer cell lines were carried out using standard methods. Chemical investigations of the fungal extract involving a combination of different chromatographic methods and spectroscopic techniques were carried out to isolate and characterize the constituents of the extract. At a concentration range of 1-4 mg/ml, the crude extract of *Aspergillus* sp. showed mild antimicrobial activity against *Bacillus subtilis*, *Klebsiella pneumoniae*, and *Candida albicans*. The fungal extract showed good antioxidant activity at 500 µg/ml, with an inhibition of 72.1%. Also, at 100 µg/ml, the extract showed excellent cytotoxic activity against A2780 (sens)

and A2780 (cisR), with growth inhibitions of 105.1% and 105.5% respectively. Two known pharmacologically active phenolic compounds (*p*-hydroxyphenyl acetic acid and ferulic acid) were isolated from the fermentation extract of the endophytic fungus. At 250 µg/ml, ferulic acid exhibited an excellent antioxidant activity with an inhibition of 90.4%, while an inhibition of 35.4% was recorded for *p*-hydroxyphenyl acetic acid. Ferulic acid also showed a mild antifungal activity at 500 µg/ml against *A. niger* with an IZD of 2 mm. *p*-Hydroxyphenyl acetic acid showed no antimicrobial activity. These results further confirm the potentials of endophytic fungi associated with Nigerian plants as source of bioactive compounds with pharmaceutical or industrial applications.

Keywords: Phenolic acids; *Aspergillus* sp.; Endophytic fungus; *Moringa oleifera*; Secondary metabolites.

1. INTRODUCTION

Endophytes are potential sources of biologically active natural products for exploitation in medicine, agriculture and industries. The discovery of novel drug molecules from endophytes is an important alternative to overcome the increasing threats of cancer and drug resistance by plant and human pathogens.

Moringa oleifera, also known as moringa, drumstick tree, ben oil tree, benzoil tree, or horse-radish tree, is the most widely cultivated species of the genus *Moringa*, the only genus in the family Moringaceae. *M. oleifera* is a fast-growing, drought-resistant tree, widely cultivated in tropical and subtropical areas of the world, where its leaves and seed pods are used as vegetables and in herbal medicine [1].

M. oleifera leaves have been reported to be a rich source of calcium, potassium, protein, β -carotene, and natural antioxidants like vitamin C [2]. Traditionally, the roots, barks, pods, and leaves of *M. oleifera* are used in medicine for the treatment of a variety of human ailments such as inflammation, cardiovascular, hematological, hepatic and renal disorders [3, 4]. The plant is reported to show various biological activities, including antihypertensive and cholesterol lowering [5-7], diuretic [8-9], antispasmodic [10-12], anti-ulcer [13], hepatoprotective [14], antibacterial and antifungal activities [15-18], and anticancer [19] activities.

There are several reports of endophytic fungi associated with *M. oleifera*. Dhanalakshmi et al. [20] isolated endophytic *Alternaria*, *Aspergillus*, *Bipolaris*, *Exosphaeria*, *Nigrospora* and *Penicillium* species from *M. oleifera* growing in the Yercaud hills of India. Secondary metabolites of some endophytic fungi of *M. oleifera* were evaluated for their antimicrobial and antioxidants properties [21-23]. Zhao et al. [22] reported the isolation of four bioactive compounds (griseofulvin, dechlorogriseofulvin, 8-dihydramulosin, and mullein) from *Nigrospora* sp. associated with *M. oleifera*.

Nigeria is rich with enormous and resourceful plant biodiversity, and these plants are hosts to millions of endophytic microbial communities that can be explored as renewable source of natural products and present the opportunity to discover a plethora of compounds [24]. With the poten-

tials reportedly possessed by the Nigerian plant *M. oleifera* and its associated endophytes, our study was aimed at investigating the secondary metabolites of an endophytic fungus isolated from the leaves of *M. oleifera* growing in Anambra State, South-Eastern Nigeria.

2. MATERIALS AND METHODS

2.1. Isolation, identification and fermentation of endophytic fungus

Fresh healthy leaves of *M. oleifera* were collected from Agulu, Anambra State, South-Eastern Nigeria. The leaves were washed thoroughly in running tap water and cut into 1 cm fragments. The leaf fragments were surface-sterilized by immersion in 2% sodium hypochlorite solution for 2 min, and then in 70% ethanol for nearly 2 min, before a final rinse in sterile water for 5 min. The leaf fragments were then placed on Petri plates containing freshly prepared malt extract agar [(MEA) Oxoid, UK] supplemented with chloramphenicol. The Petri plates were incubated at 28°C, and fungal growths from the leaves were monitored. Hyphal tips from distinct colonies emerging from the leaves were sub-cultured onto fresh MEA plates to obtain pure colonies. Identification of the isolated fungus was carried out based on its cultural, morphological and microscopic characteristics as described by Barnett and Hunter [25] and Ainsworth et al. [26]. Morphological identification, according to the standard taxonomic key, included colony diameter, texture, colour and the dimensions and morphology of hyphae and conidia.

2.2. Fermentation, extraction, and isolation of metabolites

The endophytic fungus was subjected to solid state fermentation in a 1L Erlenmeyer flask containing autoclaved rice medium (100 g of rice and 200 ml of distilled water). The flask was inoculated with about 3 mm diameter agar blocks containing the fungus, and then incubated at 28°C for 21 days. At the completion of fermentation, the fungal secondary metabolites were extracted with EtOAc and the crude extract was concentrated under reduced pressure. Fractionation of the extract was

done using vacuum liquid chromatography on silica gel 60 (Merck, Germany) packed to a hard cake up to a height of 15 cm. Stepwise gradient elution was done using non-polar:moderately polar solvent system (DCM:MeOH). Fractions were further separated on Sephadex LH-20 (Sigma-Aldrich, Germany) using DCM:MeOH in the ratio of 1:1 (V/V) as mobile phase. Metabolites-containing fractions were further purified by semi-preparative HPLC. Analytical HPLC was used to identify important peaks in the extract and fractions, as well as to evaluate the purity of isolated compounds.

2.3. Antimicrobial assay

Preliminary antimicrobial screening of the endophytic fungal extract was carried out using the agar well diffusion assay method described by Akpotu et al. [27]. A stock concentration of 4 mg/ml of the fungal extract was prepared by dissolving the extract in dimethyl sulphoxide [(DMSO) 100% v/v]. The stock solution was further diluted in a 2-fold serial dilution process to obtain 2, 1, and 0.5 mg/ml. Using sterile cotton swabs, standardized broth cultures of test bacterial isolates - *S. aureus*, *B. subtilis*, *S. pneumoniae*, *P. aeruginosa*, *E. coli*, and *K. pneumoniae*, and fungal isolates - *Aspergillus niger* and *Candida albicans* were spread aseptically onto the surface of Mueller Hinton Agar [(MHA) Oxoid, UK] and Sabouraud Dextrose Agar [(SDA) Oxoid, UK] plates respectively. The culture plates were allowed to dry for about 5 min, and wells were made in the agar using a sterile 6 mm cork-borer. These wells were respectively filled with 20 µl of the different dilutions of the fungal extract and controls. Ciprofloxacin (5 µg/ml) and miconazole (50 µg/ml) were used as positive controls in the antibacterial and antifungal evaluations respectively; while DMSO (100% v/v) was used as the negative control. The culture plates were kept at room temperature for 1 h to allow the agents to diffuse into the agar medium. The MHA plates were then incubated at 37°C for 24 h, and the SDA plates were incubated at 25-27°C for 2-3 days. The plates were observed for inhibition zones diameters (IZDs) which were measured and recorded. The diameter of the well (6 mm) was deducted from the measured values to get the actual IZDs. For each test isolate, this procedure was conducted in triplicate

and the mean IZDs calculated.

2.4. Cytotoxicity assay against ovarian cancer cell line A2780

The cytotoxic property of the fungal extract was determined using the MTT assay method described by Mueller et al. [28] and Engelke et al. [29]. Human ovarian cancer cells (A2780) were cultivated in RPMI-1640 medium supplemented with FBS (10%), streptomycin (120 µg/ml), and penicillin (120 U/ml), and incubated at 37°C in a humidified atmosphere containing 5% CO₂. The cisplatin (cDDP)-resistant subclone A2780CisR was obtained by intermittent treatment of A2780 cells with cDDP for 24 weekly cycles. In the MTT assay, cells were plated into 96-well microtiter plates (about 9,000 cells/well) containing growth medium, and pre-incubated overnight. The cells were then incubated with appropriate concentrations of test sample for 72 h, followed by the addition of 25 µl of a solution of MTT to each well. With the formation of formazan crystals after about 10 min, the medium was removed. The formazan crystals were then dissolved in 75 µl DMSO, and using the BMG FLUOstar (BMG Labtechnologies, Offenburg, Germany), absorption was measured at 544 nm (test wavelength) and 690 nm (reference wavelength). Absorption of the reference wavelength was subtracted from that of the test wavelength.

2.5. Antioxidant assay (DPPH free radical assay)

The free radical scavenging activity of the endophytic fungal extract was evaluated using the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay method previously reported by Shen et al. [30], but with modification. Here, the percentage inhibition of the samples and positive control were determined at a concentration of 500 µg/ml from UV absorbance values recorded at 517 nm. A solution of 0.2 mM DPPH was prepared by adding 3.94 mg of DPPH (Sigma-Aldrich, Germany) in 50 ml of MeOH. A volume of 2 ml of the 0.2 mM DPPH solution was then added to 2 ml of the samples dissolved in MeOH (1 mg/ml, 1000 µg/ml). These final reaction mixtures resulted in a 2-fold dilution of the DPPH solution and samples, resulting in final concentration of 0.1 mM for the DPPH solution, and 500 µg/ml

for the samples. Quercetin was used as the positive control and 0.1 mM DPPH solution was used as blank. The mixtures were shaken vigorously and incubated at room temperature for 30 min, after which the absorbance (Abs) was measured at 517 nm using a UV-VIS spectrophotometer. The free radical scavenging activity of the samples was calculated using the following formula:

$$\text{DPPH scavenging effect (\% inhibition)} = \frac{\text{Abs of blank } (A_0) - \text{Abs of sample } (A_1) \times 100}{\text{Abs of blank } (A_0)}$$

2.6. Bioassay of isolated compounds

The antimicrobial and antioxidant activities of the compounds isolated from the endophytic fungal extract were determined using the methods described above.

2.7. General procedures

¹H-NMR spectra were recorded using Bruker 300 and 600 spectrometers (Bruker BioSpin, Germany), and the spectra were referenced relative to the residual solvent signals. Mass spectra were recorded with a LC-MS HP1100 Agilent Finnigan LCQ Deca XP Thermoquest spectrometer (Thermo Electron, Germany). Analytical HPLC analysis was performed using a Dionex P580 system coupled to a P580A LPG pump and a photodiode array detector UVD340s (Dionex Softron, Germany). The separation column (125 x 4 mm) was prefilled with Eurosphere-10 C18 (Knauer, Germany), and the

following gradient solvent system was used: 0 min (10% MeOH), 5 min (10% MeOH), 35 min (100% MeOH), and 45 min (100% MeOH). Semi-preparative HPLC was performed using a Merck/Hitachi HPLC System (UV detector L-7400; pump L-7100), with a Eurosphere column (100 C18, 300 x 8 mm, Knauer, Germany). Gradient MeOH-H₂O mixtures were used as mobile phase at a flow rate of 5.0 ml/min. Vacuum liquid and open column chromatography were applied for fractionation using Silica gel 60 (70-230 mesh, Merck, Germany) and Sephadex LH-20 (Sigma-Aldrich, Germany) respectively. TLC analysis on pre-coated silica gel plates (Kiesel-gel 60 F₂₅₄, 20x20 cm, 0.25 mm thick, Merck, Germany) was used to monitor and collect fractions under UV detection (Camag UV cabinet 4, Germany) at wave length of 254 and 366 nm. Distilled and spectral-grade solvents were used for column chromatography and spectroscopic measurements respectively.

3. RESULTS

The results of the preliminary antimicrobial screening revealed that at a concentration range of 1-4 mg/ml, crude extract of *Aspergillus* sp. showed antibacterial activity against one Gram positive bacteria *B. subtilis* and one Gram negative bacteria *K. pneumoniae* with inhibition zone diameters (IZDs) ranging from 1-5 mm. At concentrations of 2 and 4 mg/ml, antifungal activity was recorded against *C. albicans* with IZDs of 3 and 5 mm respectively (Table 1).

Table 1. Result of antimicrobial assay of *Aspergillus* sp. crude extract.

Test organisms	Mean Inhibition Zone Diameters (IZDs)(mm)					
	Concentration (mg/ml)				Positive control	Negative control
	4	2	1	0.5	Ciprofloxacin (5 µg/mL)	DMSO
<i>S. aureus</i>	0	0	0	0	6	0
<i>B. subtilis</i>	3	1	0	0	8	0
<i>S. pneumoniae</i>	0	0	0	0	10	0
<i>P. aeruginosa</i>	0	0	0	0	4	0
<i>E. coli</i>	0	0	0	0	24	0
<i>K. pneumoniae</i>	5	3	1	0	8	0
					Miconazole (50 µg/ml)	DMSO
<i>C. albicans</i>	5	3	0	0	16	0
<i>A. niger</i>	0	0	0	0	8	0

In the DPPH antioxidant assay, at a concentration of 500 µg/ml, the crude extract of *Aspergillus* sp. showed a good antioxidant activity with an inhibition of 72.1% (Table 2). The results of the cytotoxicity assay revealed that at a concentration of 100 µg/ml, crude extract of *Aspergillus* sp. showed an excellent cytotoxic activity against cisplatin-sensitive ovarian cancer cell line A2780 (sens) and cisplatin-resistant ovarian cancer cell line A2780 (cisR) with growth inhibitions of 105.1% and 105.5% respectively (Table 3). At 10 µg/ml, the extract exhibited poor cytotoxic activity against the cell lines with growth inhibitions of 8.69% and 3.04%, respectively.

The fungal crude extract was subjected to several chromatographic separations (vacuum liquid and open column chromatography) and semi-preparative HPLC for the isolation of the bioactive compounds, as well as spectroscopic analyses (LC-MS and NMR), for structural elucidation of the isolated compounds. Two phenolic compounds

(Compounds 1 and 2) were isolated (Figure 1). All these steps were monitored by subjecting the crude fractions and isolated pure compounds to HPLC analysis.

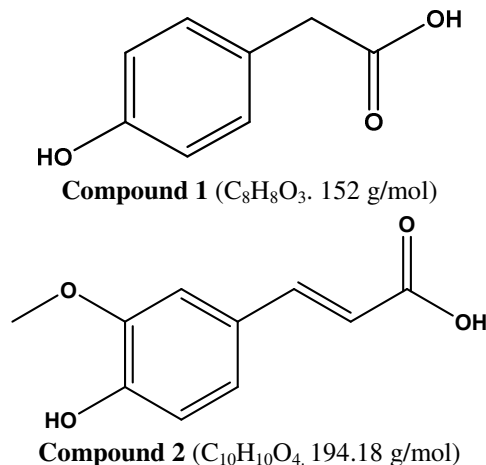


Figure 1. Phenolic compounds isolated from *Aspergillus* sp.: compounds 1 (*p*-hydroxyphenyl acetic acid) and 2 (ferulic acid).

Table 2. Antioxidant assay of *Aspergillus* sp. crude extract.

Fungal extract	Concentration (µg/ml)	% Inhibition
<i>Aspergillus</i> sp. crude extract	500	72.1
Quercetin (control)	500	91.7

Table 3. Cytotoxicity assay of *Aspergillus* sp. extract on ovarian cancer cell lines.

Ovarian cancer cell lines	Concentration (µg/ml)	Growth inhibition (%)
2780 sens	100	105.1±1.41
2780 CisR	100	105.5±2.06

The antimicrobial and antioxidant activities of the isolated compounds isolated from extract of *Aspergillus* sp. were also determined. Results of the bioassay carried out on the isolated compounds showed that *p*-hydroxyphenyl acetic acid exhibited a mild antioxidant activity at a concentration of 250 µg/ml with an inhibition of 35.4% (Table 5). At a concentration of 500 µg/ml, the compound showed no antibacterial or antifungal activities (Table 4). Ferulic acid exhibited an excellent antioxidant activity at a concentration of 250 µg/ml with an inhibition of 90.4% higher than that recorded for the positive control Quercetin (83.9%) (Table 5). At a concentration of 500 µg/ml, the compound showed

mild antifungal activities against *A. niger* with an IZD of 2 mm. No antibacterial activity was recorded (Table 4).

3.1. Compound 1

The compound was isolated as an off-white crystalline solid. It exhibited U-maxima at λ_{max} 222.4 and 276.3 nm, which is characteristic of phenol derivatives. The LC-MS showed peak at m/z 151.1 [M-1]⁻ in the negative mode, which is consistent with molar mass of 152 g/mol. The ¹H-NMR spectrum (500MHz, MeOH-*d*₄) showed 4 proton signals of the AA'BB' coupling pattern at

δ H 7.11 (d, 2H) and 6.75(d, 2H) assigned to H-2/6 and H-3/5 respectively. An aliphatic proton signal at δ H 3.50 (s, 2H), which integrated to 2 protons were assigned to H-2'A/A of the acetic acid moiety. The compound was thus unequivocally identified

as 2-(4-hydroxyphenyl) acetic acid, also known as *p*-hydroxyphenyl acetic acid or 4-hydroxyphenyl-acetic acid. Spectroscopic data of the isolated compound is confirmed by the reports of Ohtani et al. [31] and Abe et al. [32].

Table 4. Antimicrobial assay of isolated compounds.

Test organisms	Mean Inhibition Zone Diameters (mm)			
	Compound 1 (<i>p</i> -HPA) (500 μ g/ml)	Compound 2 (FA) (500 μ g/ml)	Ciprofloxacin (5 μ g/ml)	DMSO
<i>E. coli</i>	0	0	5	0
<i>S. aureus</i>	0	0	8	0
<i>S. typhi</i>	0	0	7	0
<i>B. subtilis</i>	0	0	8	0
	0		Miconazole (50 μ g/ml)	DMSO
<i>A. niger</i>	0	2	12	0
<i>C. albicans</i>	0	0	14	0

Table 5. Antioxidant assay of the isolated compounds.

Isolated compounds	Concentration (μ g/ml)	% Inhibition
Compound 1 (<i>p</i> -HPA)	250	35.4
Compound 2 (FA)	250	90.4
Quercetin (control)	250	83.9

3.2. Compound 2

The compound was isolated as a light brown solid. It exhibited UV-maxima at λ_{\max} 217.0, 235.4 and 323.1 nm. The UV also showed a shoulder around 300 nm. This UV features are characteristic of cinnamic acid derivatives. The LC-MS showed peaks at *m/z* 195.0 [M+1]⁺, 413.2 [2M+23]⁺, and 177.1 [M-18]⁺ in the positive mode and 193.2 [M-1]⁻ in the negative mode. Analysis of these MS fragments indicated a molar mass of 194 g/mol. The ¹H-NMR spectrum (300 MHz, MeOH-*d*₄) showed signals of 3 aromatic proton of the ABX coupling pattern at δ H 7.18 (d, J=1.9, 1H), 7.07 (dd, J=2.0, 8.2, 1H) and 6.81 (d, J=8.2, 1H) assigned to H-2, H-6 and H-5 respectively. The ¹H-NMR spectrum also showed two oleaginous proton signals at δ H 7.60 (d, J=15.9, 1H) and 6.31 (d, J=15.9, 1H) assigned to H-2' and H-3' respectively. The high coupling constant (15.9 Hz) was an indication that the two protons are in trans-configuration. The NMR also showed

a methoxy signal at δ H 3.90 (s, 3H) assigned to MeO-3. The compound was thus elucidated as 4-hydroxy-3-methoxycinnamic acid (ferulic acid). Spectroscopic data of the isolated compound is confirmed by the reports of Sajjadi et al. [33] and El-gizawy and Hussein [34].

4. DISCUSSION

The genus *Aspergillus* (Moniliaceae), with over 180 species, is a diverse genus with high economic and social impact. Species occur worldwide in various habitats and they are known to spoil food, produce mycotoxins and are often reported as human and animal pathogens. The genus *Aspergillus* is one of the significant contributors to the secondary metabolites of fungal origin. They produce a broad range of structurally heterogeneous secondary metabolites and are known to be a rich source of alkaloids, terpenoids, xanthenes, steroids, and polyketides, some of which showed antimicro-

bial, antifouling, antifeedant, phytotoxic, or other interesting bioactivities [35-37]. Even after investigations spanning over several decades, this genus nevertheless continues to yield metabolites with new structures and interesting biological activities [38].

As in our study, there are reports of the isolation of endophytic *Aspergillus* species from *M. oleifera* [20, 23, 39, 40]. Endophytic *Aspergillus* species have also been isolated from several other plants including *Cynodon dactylon* [38], *Gloriosa superba* [41], *Ipomoea batatas* [42], *Nymphoides peltata* [43], *Zingiber officinale* [44], *Carica papaya* [45], and *Loranthus micranthus* [46].

In our study, the crude EtOAc extract of *Aspergillus* sp. was tested for antimicrobial, cytotoxic and antioxidant activities. From the results of the bioassay, it was observed that *Aspergillus* sp. extract exhibited both antibacterial and antifungal activities (Table 1). The extract showed good antioxidant activity in the DPPH assay with an inhibition of 71.2% at a concentration of 500 µg/ml (Table 2). At a concentration of 100 µg/ml, the crude extract of *Aspergillus* sp. showed excellent cytotoxic activity against cisplatin-sensitive ovarian cancer cell line (2780 sens) and cisplatin-resistant ovarian cancer cell line (2780 CisR) with a growth inhibition of 105.1% and 105.5%, respectively (Table 3).

Chemical investigations of *Aspergillus* sp. crude extract yielded two phenolic compounds *p*-hydroxyphenyl acetic acid (*p*-HPA) and ferulic acid (FA). The compounds were characterized and screened for both antioxidant and antimicrobial activities. FA only showed mild antifungal activity against *A. niger* (at 500 µg/ml), but displayed an excellent antioxidant activity (at 250 µg/ml) with an inhibition of 90.4% higher than that recorded for the positive control quercetin (83.9%). *p*-HPA exhibited a mild antioxidant activity (at 250 µg/ml) with an inhibition of 35.4%. The compound showed no antimicrobial activity against any of the tested organisms (at 500 µg/ml) (Tables 4 and 5).

p-HPA is an important intermediate which is used for the synthesis of substances useful for pharmaceuticals. Many plants contain *p*-HPA and this compound is reported to be present in olive oil and beer [47, 48]. *p*-HPA has been isolated from several endophytic fungi including *C. gloeosporioides* [49] and *Oidiodendron* sp. [31]. The

nematicidal, antimicrobial and plant growth promoting activities of *p*-HPA have been reported [31, 32, 49, 50].

FA is a ubiquitous natural phenolic phytochemical present in plant seeds and leaves, and was first isolated from the plant *Ferula foetida* [51]. The compound is an enormously copious and almost ubiquitous phytochemical phenolic derivative of cinnamic acid, present in plant cell wall components as covalent side chains [52]. Collectively with dihydroferulic acid, FA acid is the component of lignocelluloses, where it confers rigidity to the cell wall by making the crosslink between polysaccharides and lignin [51].

There are some reports on the isolation of FA from fungi. Cheng et al. [53] also reported expression of the compound by the endophytic fungi *Annulohyphoxylon stygium*. A significant yield of FA and other phenolic compounds was reported to be achieved when rice bran, containing low levels of the compound, was fermented by the fungus *Rizhopus oryzae* [54]. FA has also been reported as a secondary metabolite of endophytic *Aspergillus* species. Danagoudar et al. [44] reported the presence of FA together with other phenolic compounds in culture extract of *A. austroafricanus* isolated from *Zingiber officinale* rhizomes.

FA has been reported to possess several pharmacological activities including antioxidant [51, 55, 56], anti-diabetic [51, 57, 58], antihypertensive [51, 59, 60], anticancer [33, 61, 62], anti-inflammatory [33, 63], hepatoprotective [34], and antimicrobial [51, 63] activities. Other reported biological activities of FA include increase of sperm viability, modulation of enzyme activity, activation of transcriptional factors, gene expression, signal transduction, metal chelation, anti-allergic, antithrombotic, antiviral, and vasodilatory activities [51].

The excellent antioxidant activity showed by FA in this study confirms the reports of several authors on the antioxidant activity of the compound [51, 55, 56]. This compound may be responsible for the antioxidant activity showed by the fungal crude extract (Table 1). Because of these properties and its low toxicity, FA is now widely used in the food and cosmetic industries. It is used as the raw material for the production of vanillin and preservatives, and as a cross-linking agent for the preparation of food gels and edible films. It has been approved in some

countries as food additive to prevent lipid peroxidation [33, 63, 64].

The results of this study, together with other similar findings [24, 27, 45, 46, 65-71], confirms the many potentials possessed by Nigerian plants as hosts of endophytes that could be reservoirs for excellent sources of pharmacologically active compounds.

5. CONCLUSION

The fungus *Aspergillus* sp. from *M. oleifera* produced two phenolic acids *p*-hydroxyphenyl acetic acid and ferulic acid whose biological activities are well known, are diverse, and are being explored for their pharmaceutical and industrial importance.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. COE, FBCO and PP designed and supervised the study. DOA, PME, CCA, and NTU managed the laboratory analyses. DOA and PME managed the data analysis and literature searches, and prepared the first draft of the manuscript. All authors read and approved the final manuscript.

TRANSPARENCY DECLARATION

The authors declare that there is no conflict of interest regarding the publication of this article.

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