Antimicrobial and Antioxidant Compounds Produced by Fungal Endophytes Isolated from Selected Nigerian Medicinal Plants

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ABSTRACT

Endophytic fungi can inherently establish mutual relationships with host plants while also synthesizing similar and enhanced bioactive compounds as the host. The focus of this study was to investigate the exploitable antibacterial and antioxidant bioactive compounds produced by fungal endophytes from selected medicinal plants. According to standard procedures, 15 fungi were isolated from leaf segments of *Cola acuminata*, *Bambusa vulgaris*, and *Elaeis guineensis*. By solid-state fermentation using a rice medium, fungal secondary metabolites were extracted using ethyl acetate. About six human pathogens, including *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Aspergillus niger*, and *Candida albicans*, the antibacterial properties of the crude extracts were evaluated using the agar diffusion assay. Except for *E. coli*, all endophytic fungal extracts showed promising inhibitory effects against all test isolates. The free radical scavenging activity was estimated using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical assay, and the bioactive constituents were analyzed using gas chromatography-mass spectrometry (GC-MS). The metabolites of the endophytic fungi also showed good antioxidant activity. Three of the fungi with the best activities were identified as *L. theobromae* (Ca1), *C. lunata* (Bv4), and *C. lunata* (Eg7), using molecular techniques (ITS region). GC-MS analysis of these fungal extracts revealed the presence of 17 antimicrobial and antioxidant compounds such as 2,4-di-tert-butylphenol, p-Cymene, γ-Terpinene, β-Bisabolene, and hexadecanoic acid. Endophytic fungi associated with Nigerian medicinal plants are potentially rich sources of antibacterial and antioxidant compounds that could be exploited for drug discovery.

Key Words: *Endophyte, Antimicrobial, Antioxidant, Cola acuminata, Bambusa vulgaris, Elaeis guineensis*

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INTRODUCTION

According to the World Health Organization (WHO), microbial diseases are included as the leading causes of mortality in the world and still increase significantly [1]. Similarly, other diseases, including cancer, are constantly developing resistance to existing drugs. Antioxidants, which are compounds that inhibit oxidation, are considered promising treatments and preventatives for illnesses like cancer, diabetes, Alzheimer's disease, hypertension, atherosclerosis, Parkinson's disease, and ischemia that are triggered by reactive oxygen species [2]. Nature continues to be a rich supply of essential natural ingredients that have helped humanity overcome numerous life-threatening

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issues. Plants and microorganisms make up the great bulk of nature's chemical diversity, and they continue to be a vital source for the pharmaceutical industry's search for new lead compounds [3]. Endophytes, and in particular endophytic fungi, have been demonstrated to be abundant sources of novel natural chemicals with a broad range of biological activity and a high degree of structural variety [4]. These endophytes are simple alternatives to plants because they produce both distinctive compounds and compounds that are comparable to those produced by their host plants [5]. This reduces ecological distortion.

The biochemical diversity of endophytic fungi's secondary metabolites suggests that these organisms are useful for discovering new drugs. Numerous endophytic fungi have been found to create novel alkaloids, steroids, flavonoids, and terpenoid derivatives, as well as other compounds with antibacterial, antifungal, antiviral, anti-inflammatory, and anticancer properties [6, 7]. This is understandable given that microbes have long been a significant source of bioactive natural compounds with considerable promise for the synthesis of novel molecules for pharmacological, industrial, and agricultural uses [8, 9]. To treat and manage severe multidrug infections and other life-threatening consequences while protecting our ecosystem, novel medications with distinct and focused mechanisms of action are urgently required.

Bioprospecting of endophytic fungi holds great promise for the discovery of bioactive secondary metabolites and innovative lead compounds for modification with therapeutic applications. It is believed that plants growing in unique ecological settings and having ethnobotanical uses are associated with endophytic microorganisms with the ability to produce unique secondary metabolites that may have applicability in medicine [10]. Therefore, the present study focused on investigating the antimicrobial and antioxidant compounds produced by endophytic fungi isolated from three chosen ethnomedicinal plants growing in Nigeria, which had not been previously screened for the presence of fungal endophytes.

MATERIALS AND METHODS

Plant samples

Healthy and Fresh leaves of *Elaeis guineensis* (*E. guineensis*) (PCG499/A/040), *Cola acuminata* (*C. acuminate*) (PCG499/A/041), and *Bambusa vulgaris* (*B. vulgaris*) (PCG499/A/042) were collected in Agbani, Enugu State, authenticated by a taxonomist, and deposited in Nnamdi Azikiwe University's Department of Pharmacognosy and Traditional Medicine's herbarium.

Isolation and identification of endophytic fungi

Endophytic fungi were isolated aseptically from plant leaves, as described by Nwobodo *et al.* [11]. DNA amplification and sequencing of the fungal internal transcribed spacer (ITS) region were used to identify isolated endophytic fungi [12]. The endophytic fungi were identified by using BLAST N sequence match algorithms to compare the amplified sequence ITS sequence data from strains available in the US National Center for Biotechnology Information (NCBI) database.

Calculation of colonization frequency (CF)

To assess the endophytic fungus richness of each plant species, the colonization frequency was calculated as stated by Suryanarayanan *et al.* [13]:

$$\text{Percentage CF (\%)} = \frac{\text{Number of segments colonized by fungi}}{\text{Total number of segments sampled}} \times 100 \quad (1)$$

Fermentation and extraction of secondary metabolites

Each isolated endophytic fungal strain was cultivated in 1000 mL Erlenmeyer flasks at 27 °C for 21 days on a solid rice medium made of 100 g rice and 200 mL distilled water. A rotary vacuum evaporator (R000101564, ST15, OSA, UK) was used to concentrate the crude extracts at a lowered temperature of 50 °C after which the secondary metabolites were extracted using ethyl acetate.

Screening for preliminary antimicrobial activity

The obtained extracts were tested for antibacterial activity using the agar diffusion method previously published by Nwobodo *et al.* [11]. In brief, the extracts were prepared in dimethyl sulfoxide (100% v/v) at a concentration of 1 mg/ml and tested against two gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), two gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*), and two fungi (*Candida albicans* and *Aspergillus niger*). In the antibacterial and antifungal assays, gentamicin (10 g/mL) and ketoconazole (50 g/mL) were employed as positive controls, while DMSO (100% v/v) was utilized as the negative control. Finally, the antibacterial plates were incubated at 37 °C for 24 hours and the antifungal plates at 25 °C for 48–72 hours. The inhibition zone diameters (IZDs) around each well were measured and noted after the incubation.

DPPH (1, 1-Diphenyl-2-picrylhydrazyl) free radical scavenging assay

The endophytic fungal extracts' ability to scavenge DPPH radicals was tested using a method previously described by [14]. Using a spectrophotometer, the absorbance of DPPH at 517 nm was measured to estimate the samples' capacity to scavenge free radicals at various concentrations (250,

125, 62.5, 31.3, 15.6, and 7.8 µg/mL). The standard, ascorbic acid was prepared following the identical steps as the test samples. Using the following formula, the percentage (%) of DPPH scavenging activity was determined:

$$\text{scavenging} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \times 100 \quad (2)$$

Where:
Abs of control = Absorbance of DPPH solution without extract/standard
Abs of sample = Absorbance of DPPH solution with extract/standard

Gas chromatography-mass spectroscopy (GC-MS) analysis of endophytic fungal extracts

The fungal extract was GC-MS analyzed as previously described by Nwobodo *et al*. [15]. An Agilent 7820A gas chromatograph was used in conjunction with an Agilent 5975C inert mass selective detector (MSD) with a triple-axis detector operating in electron impact (EI) mode with an ionization energy of 70 eV. For the separation, an HP-5 capillary column coated with 5% phenyl methyl siloxane (30 m × 250 µm diameter × 0.25 µm film thickness) was utilized. The sample (1 µL, diluted 1: 100 in dichloromethane) was injected splitlessly at a temperature of 300 °C. With a total flow of 16.654 mL/min, the purge flow to the split vent was 15 mL/min at 0.75 minutes. At a flow rate of 1 mL/min, helium was utilized as the carrier gas, with an initial nominal pressure of 1.4902 psi and an average velocity of 44.22 cm/sec. The oven's temperature was first set at 50 °C for 1 minute before ramping up to 300 °C for 10 minutes at a rate of 3 °C per minute. Run with a hold time of 5 °C/min, the run time was 43 minutes. Based on the peak area created in the chromatogram, the relative quantity of the chemical components present in the extract was expressed as a percentage. By comparing the mass spectra of the extracts to those in the National Institute for Standard Technology (NIST) mass spectral library, as well as by the GC retention time (RT), the contents of the extracts were identified.

Statistical analysis

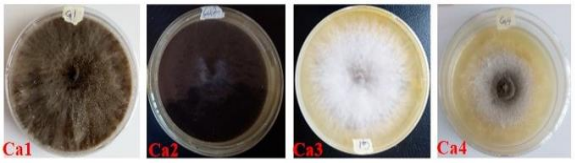
The analyses were performed in triplicate and mean IZDs of the various fungal extracts were compared using one-way ANOVA. Statistical significance was assessed at $P \leq 0.05$. Data and graphs were analyzed using Microsoft Excel 2013 and SPSS version 20.

RESULTS AND DISCUSSION

Isolation and identification of endophytic fungi

A total of 15 endophytic fungi were isolated from the leaf segments of the three medicinal plants used in this study **(Figure 1)**. Four from *C. acuminata* (27%), four from *B. vulgaris* (27%), and seven from *E. guineensis* (46%). The isolate with the highest colonization frequency (CF) was observed to be Ca1, isolated from *C. acuminata* leaves with a frequency of 56.7%, followed by Bv4 (36.7%), Ca2 (33.3%), and Eg7 (30%), isolated from *B. vulgaris*, *C. acuminata*, and *E. guineensis*, respectively **(Figure 2)**. This indicates that the isolation of these endophytic fungi did not happen by mere chance, as indicated by their frequency of isolation. Based on the observed antimicrobial and antioxidant activities, three endophytic fungi were identified; *Lasiodiplodia theobromae* (Ca1), *Curvularia lunata* (Bv4), and *Curvularia lunata* (Eg7) isolated from *C. acuminata*, *B. vulgaris*, and *E. guineensis*, respectively. The isolates were identified using molecular techniques (sequencing ITS region), with all three fungi having above 98% similarity to the reference strain in the NCBI nucleotide database. DNA sequencing data from *L. theobromae*, *C. lunata* Bv4, and *C. lunata* Eg7 were also deposited in the NCBI database (GenBank) under the accession codes OL342232, OL347861, and OL347929, respectively.

There have been multiple reports of endophytic fungi isolated from *C. acuminata*, *E. guineensis*, and *B. vulgaris* or similar species. Recently, 106 endophytic fungal isolates were isolated from various organs of *C. acuminata*, demonstrating that all organs of the plant host one or more endophytic fungi [16]. Similarly, endophytic fungi associated with *E. guineensis* have been attracting attention lately, with a few being isolated from various areas of the *E. guineensis* plant [17, 18]. Several studies had previously observed the association and isolation of endophytic fungi from several other bamboo species [19, 20]. However, studies on the isolation of fungal endophytes from *B. vulgaris* are scarce.



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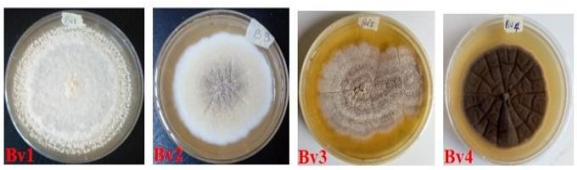

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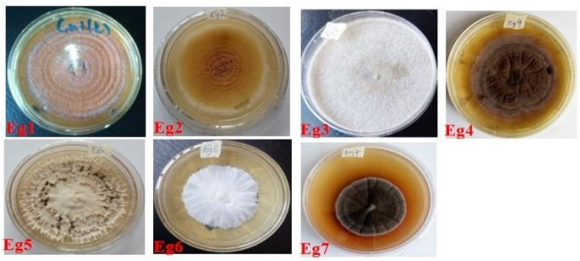

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Figure 1. Pictorial presentation of pure endophytic fungi isolated from the plant leaves. a) isolates from *C. acuminata*; b) isolates from *B. vulgaris*; and c) isolates from *E. guineensis*

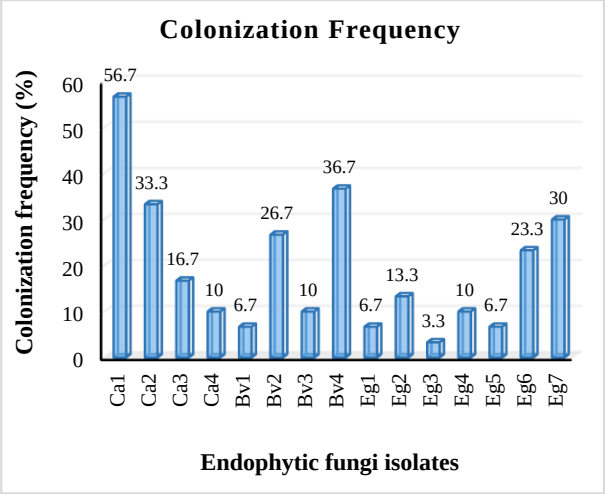


Figure 2. The colonization frequency of isolated endophytic fungi from the three medicinal plants studied. Key: Ca = *C. acuminata*; Bv = *B. vulgaris*; Eg = *E. guineensis*

Antibacterial activity

All endophytic fungal extracts displayed a varying degree of inhibitory activity against all test isolates at 1 mg/ml concentration, except against *E. coli* **(Table 1)**. Similar findings were made by Mwanga *et al.* [21], who noted that *E. coli* was resistant to the crude extracts of all four endophytic fungi isolated from *Moringa oleifera* leaves. *E. coli* is a frequent contributor to nosocomial and community-acquired infections, including those of the respiratory tract, enteric, and urinary tract [22]. The ability of *E. coli* to rapidly develop antibiotic resistance through several routes is largely responsible for the clinical hazard it poses. *S. aureus* appeared to be the most susceptible of all the bacterial isolates used in the study. It was susceptible to 93% of the endophytic fungal extracts, being resistant to only the extract of Eg1. Collaboratively, Charria-Girón *et al*. [23] reported that a reduced number of EF extracts of *Ochna gracilipes* showed only modest antibacterial activity against *S. aureus*, the majority were found to be efficient against the gram-negative bacterium *E. coli*. In general, it has been shown that endophytic fungi produce compounds that are similar to those of their host plants [5]. This seemed to be true, as the crude extracts from the plants *C. acuminata* [24], *B. vulgaris* [25], and *E. guineensis* [26] have all been reported to have antibacterial activity against *S. aureus.*

Figure 3 shows the clear zones of inhibitions produced by some of the fungal extracts against some of the selected test isolates. The best antibacterial activity was shown by the extract of Ca1 isolated from *C. acuminata*, with the highest IZD of 12 ± 1.4 mm against *S. aureus*. However, the extract of Bv4 isolated from *B. vulgaris* displayed the best broad-spectrum activity with IZD of 8 ± 0.0 mm against *S. aureus*, 6 ± 0.0 mm against *B. subtilis*, and 6 ± 0.7 mm against *P. aeruginosa*. Similarly, the extract of Eg7 isolated from *E. guineensis* displayed a promising broad-spectrum activity against *S. aureus* (8 ± 0.7 mm) and *P. aeruginosa* (2 ± 0.7 mm). The extract of Eg3 was observed to display the least activity, inhibiting only *P. aeruginosa* (4 ± 0.7). *P. aeruginosa* has, over the years, been shown to be a recalcitrant pathogen with a high degree of resistance to conventional antibiotics [27]. However, some other studies have reported the ability of the crude extracts produced by endophytic fungi to inhibit *P. aeruginosa* [11, 28].

Overall, this study found that the extracts of fungal endophytes were extremely resistant to *P. aeruginosa* and *E. coli*. The structural and physiological differences between gram-positive and gram-negative bacteria may be the cause of this. According to Powthong *et al*. [29], Gram-negative bacteria are significantly more resistant to most antibacterials because their cells are more complicated and protected than Gram-positive bacteria due to the presence of a lipopolysaccharide outer membrane that covers the peptidoglycan. Other research by Zhao *et al*. [30] and Souza *et al*. [31] reported similar findings.

Antifungal activity

Most of the extracts (80%) obtained in this study were observed to inhibit the growth of *C. albicans*. Although *C. albicans* is found as a member of the normal microbiota of around half of the population, it is responsible for 80–90 % of *Candida* infections [32]. *A. niger* was observed only to be sensitive to 33% of the fungal extracts, resisting 67% of the extracts. Similarly, it is known that *Aspergillus* species pose a major health risk to humans, causing a variety of invasive diseases with notable mortality in humans, particularly in immunocompromised people [33]. All fungal extracts displayed antifungal activity against at least one of the pathogenic fungi used in the study, except the extracts of Eg2, Eg3, and Eg6.

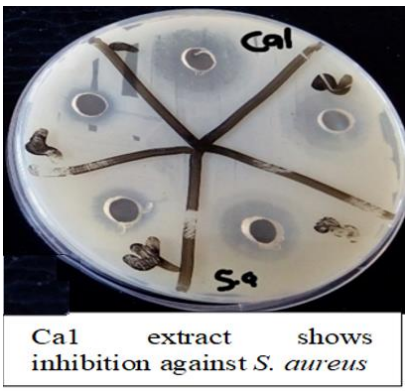
The best antifungal activity was displayed by the extract of Ca1 isolated from *C. acuminata*, with an IZD of 12 ± 1.4

and 8 ± 1.4 against *C. albicans* and *A. niger*, respectively, and followed by the extract of Eg7 isolated from *E. guineensis*, with IZDs of 10 ± 1.4 and 6 ± 1.4 against *C. albicans* and *A. niger*, respectively **(Table 1)**. Endophytic fungi produce bioactive substances with antifungal activity against *C. albicans* and *A. niger*, according to several other researchers. It was noteworthy to notice that fungal infections, particularly *C. albicans*, were shown to be more vulnerable to endophytic fungal extracts than bacterial test pathogens in the current study. This is interesting as fungi are believed to be more resistant than bacteria. This is owing to the glucosamine polymer chitin composition of the fungal cell wall, which is relatively resistant to external influences as well as microbial degradation [34]. In a related investigation by Ujam *et al*. [35], antifungal activity against both *C. albicans* and *A. niger* was observed for a crude extract of an endophytic fungus from *Ageratum conyzoides*. The ability of endophytic fungal extracts from *Dacryodes edulis* to inhibit the growth of fungal pathogens such as *C. albicans* was also reported by Ugwu *et al*. [28]. These endophytic fungal extracts are thought to have antimicrobial effects, presumably by inducing changes in the fungal pathogen's cell membranes. These alterations in the cell membrane would impede the cell's ability to regulate osmolality, which would ultimately result in cell death.

Table 1. Inhibition zone diameters (mm) of various extracts of isolated endophytic fungi

Fungal Extract (1 mg/mL)	**IZD = Mean ± SEM**					
	S. aureus	***B. subtilis***	***E. coli***	***P. aeruginosa***	***C. albicans***	***A. niger***
Ca1	12 ± 1.4*	-	-	-	12 ± 1.4*	8 ± 1.4
Ca2	4 ± 0.7	-	-	-	4 ± 1.4	4 ± 0.0
Ca3	6 ± 1.4	6 ± 0.0	-	-	8 ± 1.4	4 ± 1.0
Ca4	6 ± 0.0	6 ± 2.1	-	-	8 ± 1.4	-
Bv1	4 ± 0.0	6 ± 0.0	-	-	4 ± 0.7	-
Bv2	2 ± 0.0	-	-	-	8 ± 0.7*	-
Bv3	6 ± 0.0	8 ± 1.4	-	-	8 ± 1.4	-
Bv4	8 ± 0.0*	6 ± 0.0	-	6 ± 0.7*	4 ± 1.4	6 ± 1.4
Eg1	8 ± 0.7	-	-	-	4 ± 0.0	-
Eg2	8 ± 1.4	-	-	-	-	-
Eg3	-	-	-	4 ± 0.7*	-	-
Eg4	4 ± 0.0	-	-	-	4 ± 0.0	-
Eg5	4 ± 0.0	-	-	-	6 ± 0.0	-
Eg6	6 ± 2.1	-	-	-	-	-
Eg7	8 ± 0.7*	-	-	2 ± 0.7	10 ± 1.4*	6 ± 1.4
Gentamicin (10 µg/mL)	20 ± 0*	19 ± 0.7*	22 ± 0*	18 ± 1.4*	NA	NA
Ketoconazole (50 µg/mL)	NA	NA	NA	NA	23 ± 0*	21 ± 1.4*

Where (-) = absence of activity, Ca = *C. acuminata*, Bv = *B. vulgaris*, Eg = *E. guineensis*
* Significant differences were observed between the inhibitory values of the different extracts and the control against the test microorganisms ($P < 0.05$).



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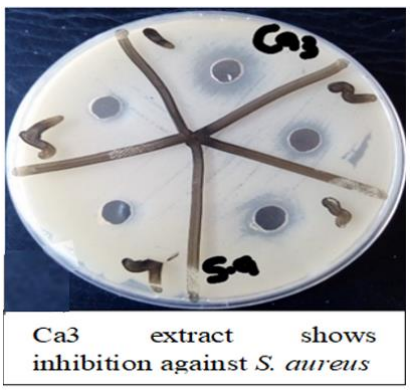

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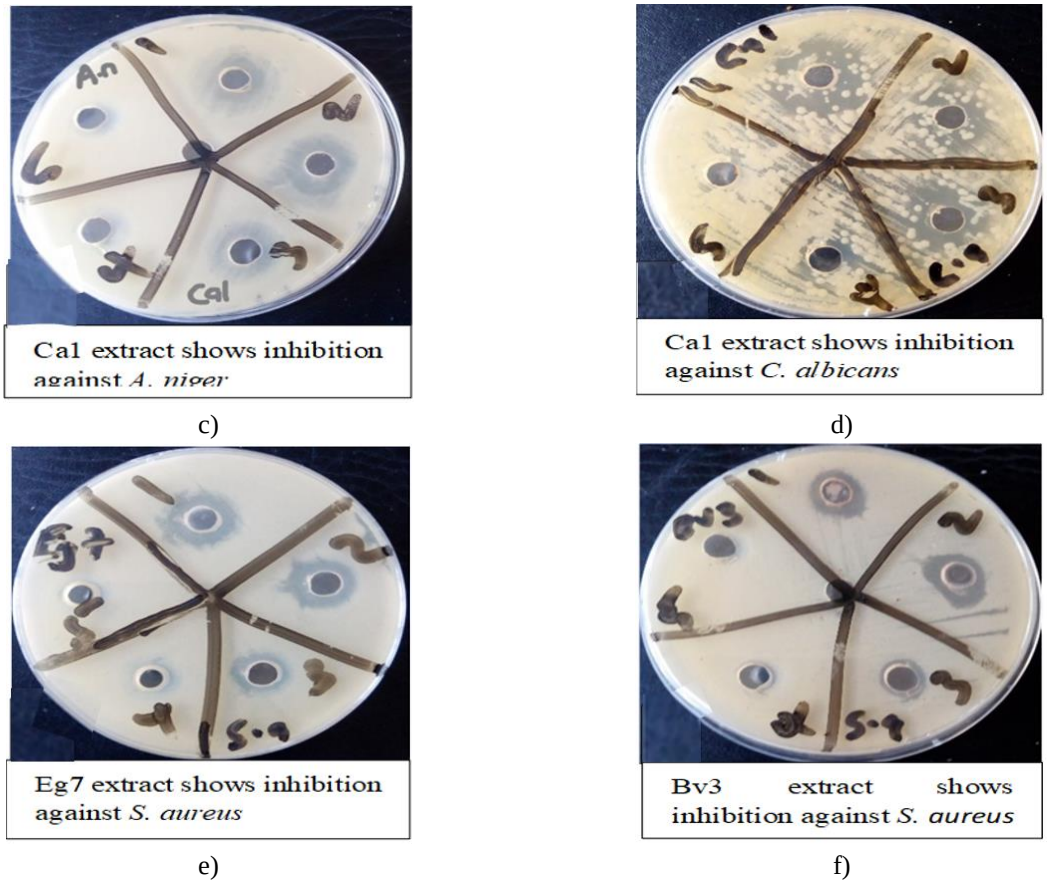

Figure 3. Clear zones of inhibition as exhibited by some of the fungal extracts against the test bacterial isolates.

DPPH radical scavenging activity

The percentage of DPPH scavenging activity of the endophytic fungal extracts is presented in **Figure 4**. The fungal extracts in this study displayed a concentration-dependent antioxidant activity, with percentage inhibition values directly proportional to the concentration of the extract. The extract of Eg1 demonstrated the highest antioxidant activity that can scavenge free radicals, with an inhibition of 93.5% at 300 g/mL, followed by the extracts of Ca1 (93.8%) and Ca4 (90.9%).

Statistically, ANOVA indicated a great significant difference ($P < 0.01$, $n = 3$) between the mean percentage inhibition values of the experimental group and the standard drug ascorbic acid. The post hoc test revealed that the extracts of Eg1 had the best percentage of scavenging, followed by the extract of Ca1. However, when compared with ascorbic acid, no significant difference was observed in the DPPH scavenging activity of Eg1 and ascorbic acid with $P = 0.0888$. Similarly, no significant difference was observed between the percentage scavenging values of Eg1 and Ca1 when compared using the Student T-test. This invariably means that the extracts of Eg1 and Ca1 contain compounds with good antioxidant activity equivalent to ascorbic acid. This is not surprising as substantial evidence suggests that endophytic fungi produce several antioxidant compounds that are responsible for host plant stress tolerance [36]. According to Suryanarayanan *et al*. [37], Graphislactone A, a phenolic compound produced by the endophyte *Cephalosporium* species, was found to exhibit better antioxidant activity than ascorbic acid and butylated hydroxytoluene (BHT). Similarly, Nwobodo *et al*. [14], using the same DPPH assay coupled with different other assays, reported that fungal endophytes isolated from two medicinal plants, *Cola nitida*, and *Garcinia kola*, displayed good antioxidant activities. The endophytic *Fusarium oxysporum* isolated from *Otoba gracilipes* leaves exhibited antioxidant activity, scavenging 51.5% of DPPH [38], which is lesser than the percentage inhibition obtained in the majority of the endophytic fungal extracts in this study. Similarly, the extract of the endophytic fungus *Alternaria alternata* AE1 isolated from *Azadirachta indica* also showed antioxidant capability, with IC50 values of 38.0 in the DPPH free radical scavenging assay [39]. This is comparable with the IC_{50} of Eg1 (37.8 µg/mL), further indicating that endophytic fungi are a promising target for the development of novel antioxidant agents.

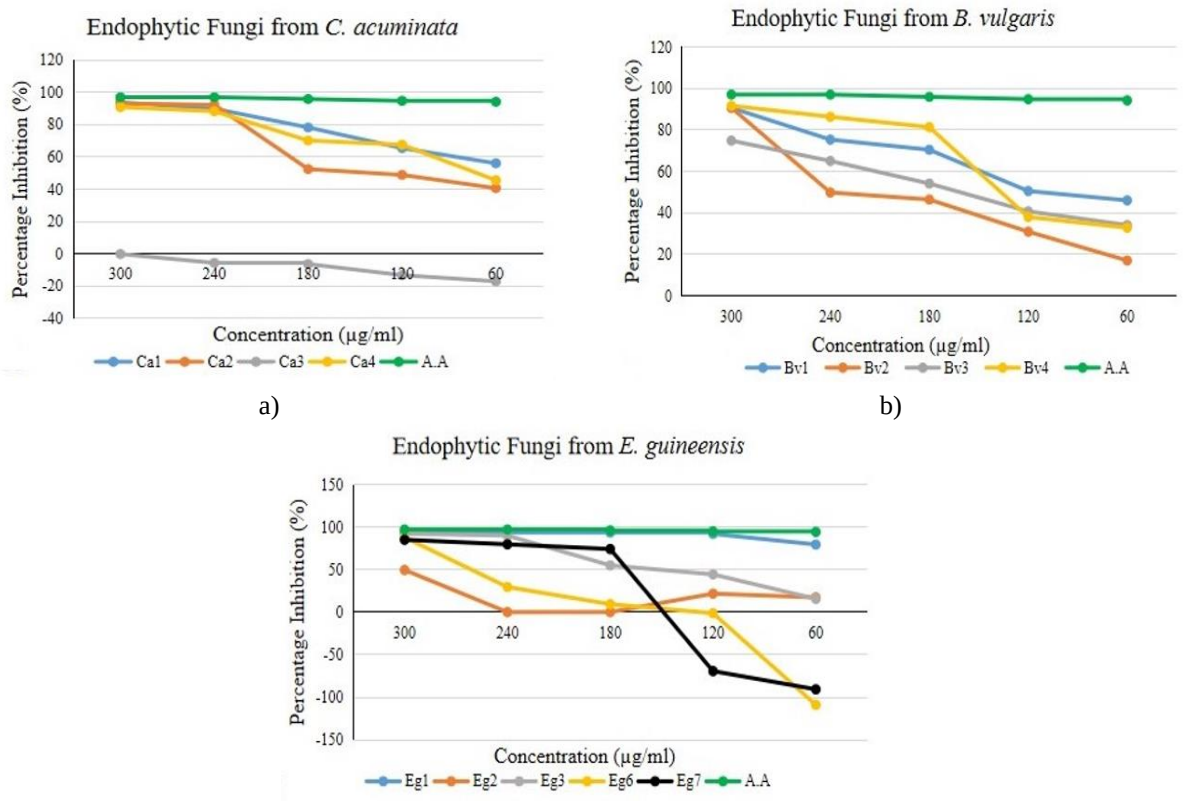

Figure 4. Percentage inhibition of DPPH scavenging abilities of the crude extracts of EF isolated from the three medicinal plants. NB: the extracts of Eg 4 and Eg5 did not display any activity and were omitted from the graph above.
Where A. A = ascorbic acid

Identification of bioactive constituents by GC-MS technique

The endophytic fungi Ca1, Bv4, and Eg7 were among the fungal extracts that showed effective action and were therefore chosen for chemo-diversity investigation based on the observed antibacterial and antioxidant capabilities. This was done to identify any active components in the fungal crude extracts that might be behind the observed biological activities. The secondary metabolic profiles of the endophytic fungal isolates were preliminarily investigated using GC-MS, and the identified compounds are shown in **Table 2**.

Table 2. Antimicrobial and antioxidant compounds identified in the crude extract of *L. theobromae* (Ca1), *C. lunata* (Bv4), and *C. lunata* (Eg7) by GC-MS

S/n	Name of compound	RT (min)	Biological activity	Fungal isolate
1	p-Cymene	7.201	Antioxidant and antimicrobial	*C. lunata* (Bv4), *C. lunata* (Eg7)
2	γ-Terpinene	8.160	Antioxidant	*C. lunata* (Bv4), *C. lunata* (Eg7)
3	Dodecane, 2,6,11-trimethyl-	8.382	Antifungal and antibacterial	*C. lunata* (Eg7)
4	Heptadecane, 2,6,10,14-tetramethyl	8.958	Antibacterial	*L. theobromae* (Ca1), *C. lunata* (Eg7)
5	Decane, 2,4-dimethyl-	10.101	Antimicrobial	*L. theobromae* (Ca1), *C. lunata* (Bv4), *C. lunata* (Eg7)
6	Naphthalene	14.950	Antimicrobial, antioxidant	*L. theobromae* (Ca1), *C. lunata* (Bv4)
7	Tridecane	15.109	Antimicrobial	*L. theobromae* (Ca1), *C. lunata* (Bv4), *C. lunata* (Eg7)
8	β-Bisabolene	20.678	Antioxidant and antimicrobial	*C. lunata* (Bv4)

9	2,4-Di-tert-butyl phenol	20.974	Antioxidant and antimicrobial	*L. theobromae* (Ca1), *C. lunata* (Bv4), *C. lunata* (Eg7)
10	Z-8-Hexadecene	22.690	Antimicrobial	*L. theobromae* (Ca1), *C. lunata* (Eg7)
11	Piperine	28.803	Antioxidant and antimicrobial	*C. lunata* (Bv4)
12	Octadecene	30.257	Antimicrobial	*L. theobromae* (Ca1), *C. lunata* (Eg7)
13	Hexadecanoic acid, ethyl ester	30.283	Antioxidant	*C. lunata* (Bv4)
14	9-Octadecenoic acid, ethyl ester	31.662	Antibacterial	*C. lunata* (Bv4)
15	Tetradecanoic acid, 2-hydroxy-, methyl ester	34.216	Antifungal and antioxidant	*C. lunata* (Eg7)
16	Handianol	35.582	Antifungal and antibacterial	*C. lunata* (Eg7)

An extract from Nwobodo *et al*. [15]

In the three fungal crude extracts examined, a total of sixteen recognized compounds with antibacterial and antioxidant activities from various classes were found. These compounds include p-Cymene, γ-Terpinene, Dodecane, 2,6,11-trimethyl-, Heptadecane, 2,6,10,14-tetramethyl, Decane, 2,4-dimethyl-, Naphthalene, Tridecane, β-Bisabolene, 2,4-Di-tert-butylphenol, Z-8-Hexadecene, Piperine, Octadecene, Hexadecanoic acid, ethyl ester, 9-Octadecenoic acid, ethyl ester, Tetradecanoic acid, 2-hydroxy-, methyl ester, Handianol. The majority of the chemicals described as being produced by the fungal endophytes in this investigation have also been found to be produced by other endophytic fungi species in earlier studies [40-42]. Endophytic fungi that were isolated from the three medicinal plants under study were shown to have significant bioactive potential and can aid in the development of antibacterial and antioxidant drugs.

CONCLUSION

The findings of this study suggest that endophytic fungi of the Nigerian ethnomedicinal plants *C. acuminata*, *B. vulgaris*, and *E. guineensis* are promising sources of bioactive chemicals for the development of antibacterial and antioxidant agents. The existence of many classes of bioactive secondary metabolites was also discovered by GC-MS analysis, suggesting that they may have contributed to the reported biological activities. The isolated endophytes may be valuable as natural microbial cell resources for the production of antimicrobials and antioxidants.

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Ethics statement: None

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