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Original Research Article

Antibacterial Potential of Endophytic Fungi from *Xylopia aethiopica* and Metabolites Profiling of *Penicillium* sp. XAFac2 and *Aspergillus* sp. XAFac4 by GC-MS

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ABSTRACT

Endophytes exist without harming the tissue of plants. They manufacture a lot of chemicals for use in pharmaceuticals. *Xylopia aethiopica* (Dunal) A. Rich. is a member Annonaceae family, an indigenous medicinal plant in Nigeria. The variety and antibacterial properties of the fungal endophytes that inhabit its fruit are poorly understood. The isolation, molecular description, phylogenetic analysis, and antibacterial effectiveness of its fungal endophytes were thus the main objectives of this study. Standard methods were used to isolate the endophytes, and partial sequencing of the internal transcribed spacer (ITS) region of the rDNA was used to identify them before phylogenetic analysis. Using version X of the Molecular Evolutionary and Genetic Analysis (MEGA) tool, the phylogenetic tree was created using the neighbour-joining method. The tree was further annotated using the interactive tree of life (iTOL) version 6.0. The antibacterial activity of the fungal endophytes was studied using both perpendicular streaking and agar well diffusion assays. The Gas Chromatography-Mass Spectrometry (GC-MS) assay was used to identify some of the chemicals in the extracts. A total of 4 fungal endophytes were recovered. They were identified as *Scopulariopsis* sp. XAFac1, *Penicillium* sp. XAFac2, *Aspergillus* sp. XAFac3 and *Aspergillus* sp. XAFac4. Only *Penicillium* sp. XAFac2 and *Aspergillus* sp. XAFac4 exhibited good antibacterial activity. The most prominent compounds obtained from their extracts included 9-octadecanoic acid, methyltetradecanoate, dodecanoic acid, pentadecanoic acid, 5-tetradecene, 5-octadecene, trichloroacetic acid, and trifluoroacetoxyhexadecane. These results provided important knowledge on the diversity of endophytic fungi inhabiting *X. aethiopica* fruit and could be exploited as a novel source of bioactive compounds.

Keywords: Fungal endophytes, Molecular identification, Antibacterial, GC-MS profiling, *Penicillium* sp. XAFac2, *Aspergillus* sp. XAFac4

Introduction

Both industrialized and developing countries have relied mostly on medicinal plants as a source of medications for many centuries.[1] Many nations do not formally recognize the products of traditional medicine, however, the European Union is now establishing regulatory regulations for better traditional medicines. At least 25% of all currently prescribed medications are thought to be derived directly or indirectly from medicinal plants.[2] Traditional medical practices are used to treat a variety of illnesses all throughout the world. The existence of multiple complex chemical compounds in herbs known as secondary metabolites, which are only accumulated in specific areas of the plant, is what gives them their therapeutic properties. A common cause of the rising demand for novel antimicrobial drugs is antibiotic resistance.[3]

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Due to the fact that agricultural bacteria are known to have developed resistance to frequently used antibiotics, this issue goes beyond the clinical application of antimicrobial medications.

Most fungi are endophytic, which are widespread fungi that live at least part of their life inside plant tissues without showing any overt signs of infection.[4] Endophytes are organisms that inhabit the tissue below the plant's layers of epidermal cells and do not obviously affect their hosts. Examples of endophytic fungi include latent pathogens, benign commensals, mutualists, biotrophic organisms, or decomposers. All Plants can host endophytic fungi in their natural environment.[5] The endophytic fungus family appears to have a big impact on the host's way of life. Taxonomically, the Ascomycota phylum and its related anamorphs comprise the majority of endophytic fungi, while Basidiomycota and Zygomycota are phyla to a few species.[6] Endophytic fungi are thought to have about 1.3 million unknown species.[7] With its ability to confer resistance to biotic (bacteria, fungus, nematodes, and insects) and abiotic (temperature, pH, osmotic pressure) challenges, the endophytic fungal group may have an impact on the ecophysiology, resilience, and form of the host plant. A significant source of bioactive compounds is endophytic fungus. These biologically active secondary metabolites display a diverse range of biological functions and may serve as the basis for new pharmacological compounds or as a source for new agrochemicals.[8] By secreting beneficial secondary metabolites, these endophytes shield their hosts against pathogens and harmful circumstances.[9] Putative fungal endophytes can generate a wide range

of substances through fermentation, including terpenoids, steroids, xanthones, quinones, phenols, isocoumarins, benzopyranones, tetralones, cytochalasins, and enniatins, that come from various biosynthetic pathways, including the isoprenoid, polyketide, and amino acid pathways.[10] In actuality, these secondary metabolite biomolecules serve as a repository for the discovery of novel substances such as antibiotics, antioxidants, immune-modulating, anticancer, antiparasitic, and antidiabetic substances.[11]

Xylopia aethiopica (Dunal) A. Rich. is an evergreen, fragrance-producing, drought-resistant, succulent plant belonging to the family of Annonaceae and can grow up to a height of 20 meters. Greek term (xylon pikron) that means "bitter wood" is the root of the word *Xylopia*. Although it currently grows most significantly as a crop in Ghana, Togo, and other parts of West Africa, including Nigeria,[12] the second part of the plant's scientific name, *aethiopica*, refers to the tree's origin in Ethiopia. The fruit of *X. aethiopica* has been tentatively screened for its phytochemical components, and the results revealed the presence of several compounds of pharmaceutical interest.[13] More so, several bioactive compounds including carboxylic acids, esters, phenolic compounds and fatty acids have been associated with the extracts of this plant.[14] Reports from several investigations have shown that *X. aethiopica* possesses many biological activities including antimicrobial, anticancer, free-radical scavaging, glucose-modulatory, ulcer protective, liver repairing, and immune boosting.[15-17] Investigation on fungi endophytes associated with *X. aethiopica* fruit is scarce. This study hypothesizes that the antimicrobial activity attributed to this fruit could be because of fungi endophytes inhabiting them. Hence it tried to study the diversity of antagonistic fungi endophytes inhabiting this fruit. These fungal endophytes may be a promising source of fresh, physiologically active metabolites with potential for use in pharmacy and agrochemical industries.

Materials and Methods

Sample collection

Fruits that were mature and in good health were carefully chosen for sampling. They were bought from Olosha herbal market, Mushin Lagos on the 25th of May, 2022. The laboratory received the plant material in a zip-lock bag and they were processed within 24 hours after sampling. Fresh fruits of *X. aethiopica* were selected to minimize the contamination rate during the isolation of endophytes. The fruit was identified, assigned voucher number (LUH: 9050), and deposited in the Herbarium of the Department of Botany, University of Lagos, Nigeria.

Sample processing

To get rid of physical contaminants like sand, the fruits were washed for ten minutes under running water. Using a sterile scalpel, the fruits were spliced into tiny fragments measuring about 1 cm in length. Surface sterilization procedures were performed in the following order: ethanol (70% for 3 minutes), mercuric chloride (4% for 3 minutes), and a final surface cleansing with ethanol (70% for 10 seconds).[18] Fruits that had been surface-sterilized were rinsed by being run three times through sterile distilled water. Using sterilized glassware and mechanical tools, the entire experiment was carried out in laminar airflow. The sterile fruit segments were aseptically plated on Potato Dextrose Agar (PDA) medium mixed with 200 mg/L of streptomycin antibiotic. The Petri dishes tightly wrapped with parafilm tape were kept at 28°C pending the fungal mycelia growth. For further research purposes, the endophytic fungi were preserved in a freshly prepared agar slant at 4 °C. The media and antibiotics used were of Himedia source.

Morphological identification of endophytic fungal isolates

The fungal Endophytic isolates were initially analyzed based on their shapes and colours. Four separate species were described in terms of their microscopic traits, such as the structure of hyphae, conidia, and conidiophores, as well as their macroscopic characteristics, such as the colour, form, and growth of cultured colonies. Lacto-phenol cotton blue (LPCB) needle mounts of the fungi samples were used to morphologically identify the fungi and the features of the spore when seen at a magnification of 40X. After that, the information was matched against a widely used fungal identification manual and any similarity was documented.[19] Preliminary antibacterial activity investigation was carried out on all species identified.

DNA extraction, PCR amplification, and sequencing

The Quick-DNA™ fungal/bacterial kit from Zymo Research with Catalogue No. D6005 was employed for the extraction of fungal genomic DNA from the four fungal endophytic isolates. Utilizing the primers created by Inqaba Biotech, South Africa, and the OneTaq® Quick-Load® 2X master mix (NEB, catalog number: M0486), the ITS target area was amplified. The PCR products were run on a gel and then enzymatically cleaned using the well-known technique called EXOSAP.[20] The retrieved fragments were purified using Zymo Research's ZR-96 DNA sequencing clean-up kitTM and were sequenced in both forward and reverse direction with the help of Nimagen's BrilliantDyeTM Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000. The purified fragments were examined using the ABI 3500XL Genetic Analyzer (Applied Biosystems, ThermoFisher Scientific). Results from a BLAST search in the NCBI database were obtained when DNASTAR was used to evaluate the.ab1 files produced by the ABI 3500XL Genetic Analyzer.[21]

Phylogenetic analysis

In order to identify their most likely closely related species, the sequencing information gathered in the course of the work were subjected to nucleotide BLAST in the GenBank database (http://blast.ncbi.nlm.nih.gov/) and with the help of the MEGA X software's MUSCLE application, sequence alignments were performed.[22] The phylogenetic tree was drawn using Neighbour-joining method and it was annotated using Interactive Tree of Life (iTOL) version 6.0 (https://itol.embl.de).[22]

Preliminary antibacterial assay

A slight modification of the Singh *et al*., (2016) approach was employed to assess the antibacterial characteristics of isolated endophytic fungi.[23] The fungi endophytes were grown in a single straight line on soya casein dextrose agar for 3 days at 28 ℃. The bacterial pathogens were co-cultured perpendicularly to the fungi endophytes using a sterile loop and were allowed to be incubated at a room temperature of 37 ℃ for a day. Zones of inhibition were noted enabling the selection of endophytic isolates for the secondary antimicrobial screening. The choice of the media used was based on the preliminary investigation of Soy Casein Dextrose agar (SCDA) to support the growth of bacterial target pathogens as well as the endophytic fungi isolates.

Secondary metabolite extraction

The Sharma et al., (2016) procedure was used to extract secondary metabolites from the fungal broth culture.[24] A 1000 ml volume conical flask containing 500 ml of potato dextrose broth was used to inoculate positive endophytic fungal isolates and they were kept in the incubator at 37 ℃ for 10 days in a stationary position with intermittent shaking. The broth culture was filtered to separate the fungal mycelia from the filtrate. The collected filtrate was treated with an equal volume of ethyl acetate, thoroughly agitated for 15 minutes, and then left to stand for two hours until two distinct immiscible layers had formed. The part of the ethyl acetate containing the extracted chemicals was separated using a separating funnel. The mycelia were thoroughly ground using a mortar and pestle with ethyl acetate as the solvent and then filtered using silk cloth.

A rotary evaporator was used to dry the filtrate. To be utilized as a stock solution for an antibacterial experiment, the extracted residue was dissolved in 10% v/v dimethyl sulfoxide (DMSO) and kept at 4 °C.

Preparation of test organism

Several bacteria pathogens were examined for their susceptibility to endophytic fungal extracts obtained from *X. aethiopica*. The Nigerian Institute of Medical Research (NIMR) and the Microbiology Department of the University of Lagos Teaching Hospital (LUTH), both in Nigeria, provided the *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633, and *Klebsiella pneumonia* ATCC 100324 strains. Until they are required for the antibacterial assay, these

bacterial cultures were kept in sterile agar slants and kept in a refrigerator at 4 °C. Before conducting the susceptibility testing, each of the bacteria was reactivated by being transferred into a different test tube with nutrient broth and cultured there for an entire night at 37 °C.[25] Colonies obtained from nutrient agar plates were used to prepare the McFarland concentration of the organism.

Antibacterial secondary metabolite test

The antibacterial capabilities of the secondary metabolites were assessed by the agar diffusion method.[26] Antimicrobial experimental assay was conducted using Muller Hinton agar (MHA) and a sterile swab was used to inoculate the test organism into the sterile growth media. Using a sterile 6 mm cork borer, wells were dug onto the media and 150 µL of each crude extract was introduced into each well with the help of a micropipette. In the bioassays, ceftriaxone (0.01%, 100 µL) was utilized as a positive control while ten percent DMSO (10% DMSO) was employed as a negative control.

MIC (Minimum Inhibitory Concentration) determination

The minimum inhibitory concentration was conducted via broth dilution as earlier reported.[27] Double-strength broth was prepared and dispensed (9 mL) into different sterile test tubes, and dilutions of various strengths of extracts that showed sensitivity against the test organisms were made. The test tubes were incubated for 24 hours at 37 °C after being infected with (0.2 ml) of the standardized inoculum suspension. MICs were noted as the extract concentration at which no growth could be seen in the broth.

Analysis of fungal endophytic extracts by Gas Chromatography-Mass Spectrometry

At the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos, GC-MS analyses of the putative endophytic fungi extracts were performed using the Perkin-Elmer Clarus 680 system (Perkin-Elmer, Inc. U.S.A.) outfitted with a fused silica column, packed with Elite-5MS) capillary column (30 m in length 250 µm in diameter 0.25 µm in thickness). The carrier gas was 1 mL/min of pure helium gas (99.99%) flowing continuously. An electron ionization energy approach with a high ionization energy of 70 eV (electron Volts), 0.2 s of scan duration, and fragments spanning from 40 to 600 m/z was selected for GC-MS spectrum detection. The initial temperature of 250 °C was kept constant, and a 1 µL initial volume was used (split ratio: 10:1). The column oven's temperature was initially set at 50 °C (3 minutes), then increased by 10 °C per minute until 280 °C is achieved before elevating to 300 °C (10 minutes). The volatile bioactive compounds that were contained in the fungal extracts were identified using a comparison of the retention time (min), peak area, peak height, and mass spectral patterns of the test samples with the spectral database of standard compounds kept in the National Institute of Standards and Technology (NIST) library.[28]

Statistical analysis

Each experimental reading was performed three times, and the results are shown as X±SD, where X represents the Inhibition zone diameter (IZD) and SD stands for standard deviation.

Results and discussion

New medications have been earlier reported from a wide range of natural sources. Due to their numerous uses, endophytes from medicinal plants are currently attracting a lot of attention.[29] It has been acknowledged that endophytic fungi are a significant and new source of naturally occurring bioactive chemicals with potential medicinal value.[30] Efforts were made to isolate fungal endophytes from *X. aethiopica* fruit (Figure 1)

Endophytic fungi isolation and morphological characterization

Four different endophytic fungi were isolated showing distinct morphological features on potato dextrose agar (PDA). Their microscopical features were ascertained through lacto-phenol cotton blue staining technique. Isolate XAFac1 (a), showed a greyish coloured fungus, isolate XAFac2 (c) was whitish but later turned green while isolates XAFac3 (e) and XAFac4 (g) were greenish-brown and purple respectively (Figure 2).

Figure 1: Fruit of *X. aethiopica* used for the isolation of endophytic fungi.

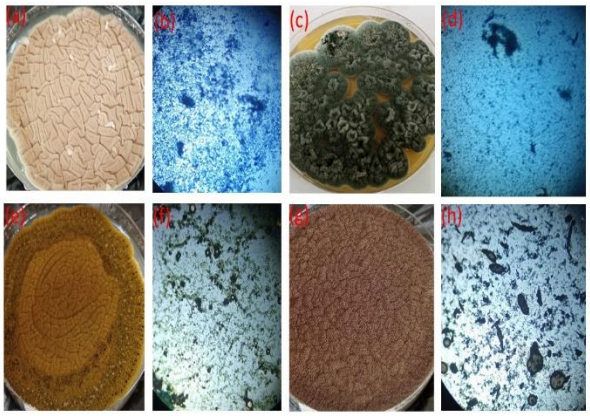

Figure 2: Morphology of different endophytic fungi isolated from *X. aethiopica*. The labels a, c, e, and g represent 10day old fungi cultures on PDA incubated at 28℃ while b, d, f and h represent their respective microscopic field view with Olympus microscope version X23 at 40X magnification.

Table 1: ITS primer sequences

Primer	Target	Sequence (5' to 3')
ITS-1	ITS rDNA	CCGTAGGTGAACCTGCGG
ITS-4	ITS rDNA	TCCTCCGCTTATTGATATGC

Table 2: Molecular identification of fungal endophytes

Isolates	E-value	Query coverage (%)	% Identity	Most similar hit	Accession no
XAFac1	0.0	99	97.34%	*Scopulariopsis cordiae*	NR_132958.1
XAFac2	0.0	99	99.61%	*Penicillium citrinum*	NR_121224.1
XAFac3	0.0	98	99.82%	*Aspergillus tamarii*	NR_135325.1
XAFac4	0.0	98	99.42%	*Aspergillus_uvarum*	NR_135330.1

E = value stands for Expect value, % identity = percentage identity, Accession no = Accession number of the most similar hits

Molecular identification

The fungal endophytic isolates were characterized through molecular sequencing of the ITS regions of the ribosomal deoxynucleic acid. The ITS-1 and ITS-4 universal primers were used to target the internal transcribed spacer of the fungi rDNA (Table 1). The sequences were blasted against those in the NCBI site. The results for the query cover, percentage identity and the accession number of the most similar hit were recorded (Table 2).

Sequences obtained were tendered to the NCBI GenBank and were assigned their submission numbers and subsequently their accession numbers (Table 3).

Preliminary antibacterial assay of the isolated fungi

A preliminary antibacterial assay was carried out to ascertain the biosynthetic potential of the isolates to produce antibiotics. The result showed that two out of the four isolates were found to inhibit the growth of bacterial pathogens. Endophytic fungi isolate XAFac2 was observed to inhibit the growth of all the targeted bacterial pathogens while isolate XAFac4 inhibited all pathogens except *B. subtilis* (Figure 3).

In the current study, *Penicillium citrinum* (XAFac2, accession no. OQ568946) and *Aspergillus uvarum* (XAFac4, accession no. OQ568959) fungal endophyte isolated from *X. aethiopica* were evaluated for inhibitory effect against multidrug-resistant bacterial pathogens. A total of four morphologically distinct fungal endophytes were found to inhabit the fruit of *X. aethiopica*. Two fungal endophytes demonstrated good antibacterial activity against tested pathogens. *Penicillium* and *Aspergillus* genera of endophytic had been reported to demonstrate antimicrobial activity.[31] Endophytic *penicillium* species have been cited to greatly inhibit several human pathogens and produced inhibition zone diameters up to 30mm against strains of *Salmonella typhi*.[32] This is comparable with our findings which showed the genus *Penicillium* produced the highest proportion of pharmacologically active biomolecules and exhibited inhibitory effect against all tested bacterial pathogens. The report by Somwanshi and Bodhankar (2015) is consistent with ours indicating the antimicrobial potential of endophytic *Penicillium* species when tested against several multidrug-resistant (MDR) pathogens.[33]

Table 3: Submission to the NCBI GenBank

Sample code	Submission number	Accession number
XAFac1	SUB12933587	OQ568951
XAFac2	SUB12933577	OQ568946
XAFac3	SUB12933591	OQ568958
XAFac4	SUB12933593	OQ568959

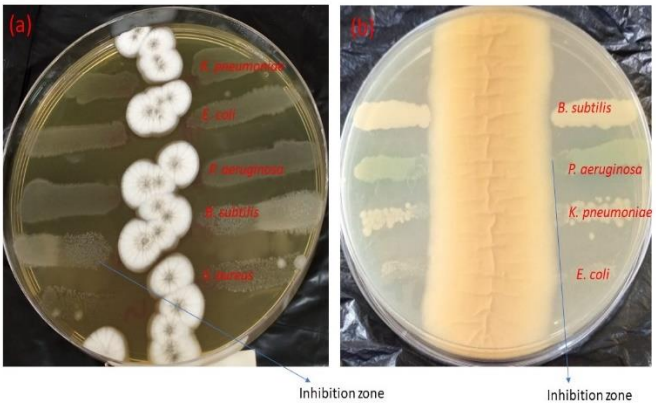

Figure 3: Preliminary antibacterial activity of endophytic fungi isolates by perpendicular streaking assay method. (a) Shows antibacterial activity of endophytic fungi XAFac2 against bacterial pathogens. (b) Shows antibacterial activity of endophytic fungi XAFac4 against bacterial pathogens. The endophytic fungi are at an angle of 180° on the agar plates while the bacterial pathogens were co-cultured perpendicularly from the fungi endophytes.

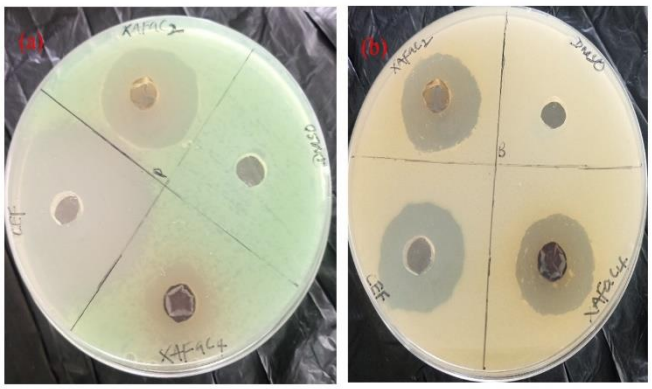

Figure 4: Some plates showing the inhibition zone diameters exhibited by our endophytic fungi strains against pathogenic bacteria. (a) represents the inhibition activity of our endophytic extracts against Pseudomonas aeruginosa while (b) represents the same against Bacillus subtilis. CEF stands for ceftriaxone while DMSO stands for dimethyl sulphoxide.

Table 4: Antibacterial activity of endophytic fungal extracts by disc diffusion method

Endophytic fungi strain	Inhibition zone diameter (mm)				
	K	E	P	B	S
Penicillium citrinum XAFac2	15 ± 0.2	13 ± 1.2	22 ± 0.8	20 ± 0.3	12 ± 0.2
Aspergillus uvarum XAFac4	08 ± 0.1	08 ± 0.5	10 ± 0.2	18 ± 0.8	0
Ceftriaxone (CEF)	20 ± 0.2	15 ± 0.6	30 ± 0.4	20 ± 0.5	16 ± 0.1
Dimethyl sulphoxide (DMSO)	0	0	0	0	0

K = *K. pneumoniae*, E = *E. coli*, P = *P. aeruginosa*, B = *B. subtilis*, S = *S. aureus*

Table 5: Minimum inhibitory concentration of endophytic fungal extracts by agar dilution

Endophytic fungi strain	Minimum inhibitory concentration (g/mL)				
	K	E	P	B	S
Penicillium citrinum XAFac2	0.05	0.05	0.05	0.1	0.1
Aspergillus uvarum XAFac4	0.1	0.1	0.05	0.05	0

K = *K. pneumoniae*, E = *E. coli*, P = *P. aeruginosa*, B = *B. subtilis*, S = *S. aureus*

The preliminary and secondary assay for antibacterial effect showed that the endophytic fungi XAFac2 had inhibitory action against all the tested bacteria pathogens while XAFac4 did not show an inhibitory effect against *Staphylococcus aureus*. This is in tandem with the antibacterial activity of *Talaromyces trachyspermus* fungus which demonstrated activity against several known pathogens.[34]

The antibacterial efficacy of the putative endophytic fungi strains revealed *Penicillium* sp. XAFac2 to have inhibited all the tested pathogens at inhibitory diameter zone range of 12-22 mm while that of *Aspergillus* sp. XAFac4 is 8-18 mm (Table 4). More so, their minimum inhibitory concentration (MIC) ranges between 0.05-0.1 g/mL for both (Table 5).

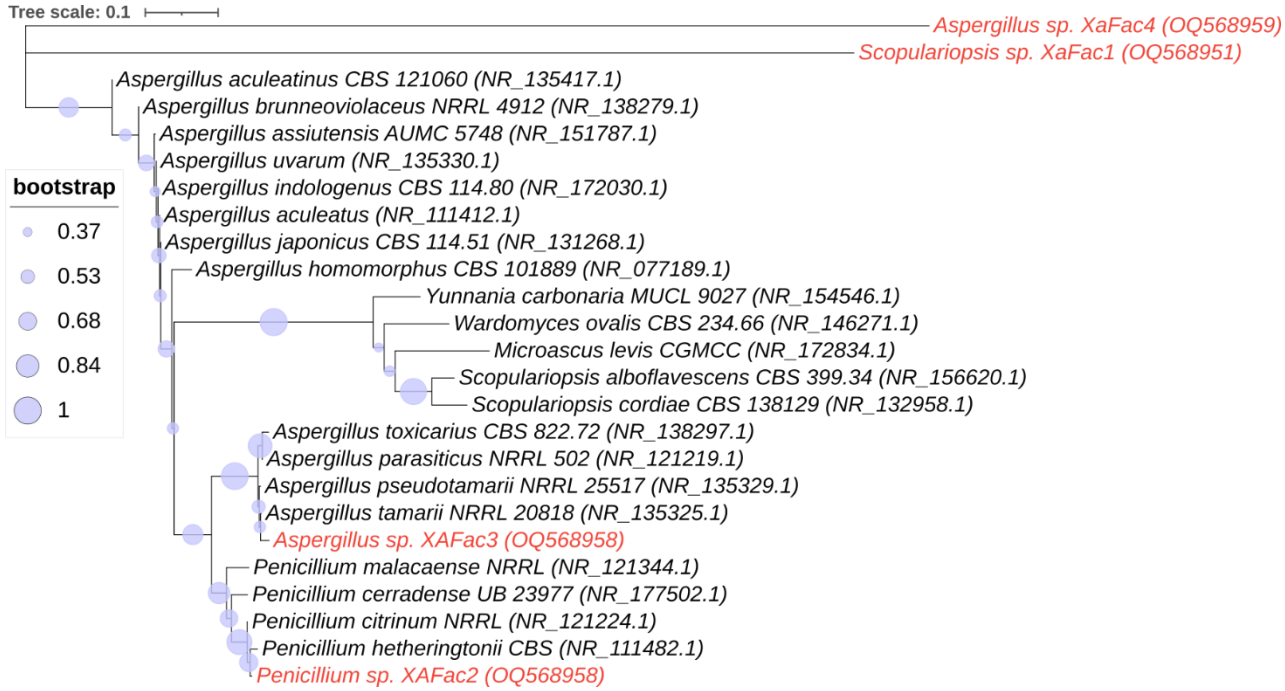

Figure 5: Maximum likelihood tree of the isolated fungal endophytes and their closely associated taxa obtained from GenBank based on ITS gene sequence. The trees were constructed based on the ITS region sequences using the maximum composite likelihood method. The GenBank taxa and our isolates are designated by species name with their accession numbers in parenthesis. Our isolates are highlighted in red.

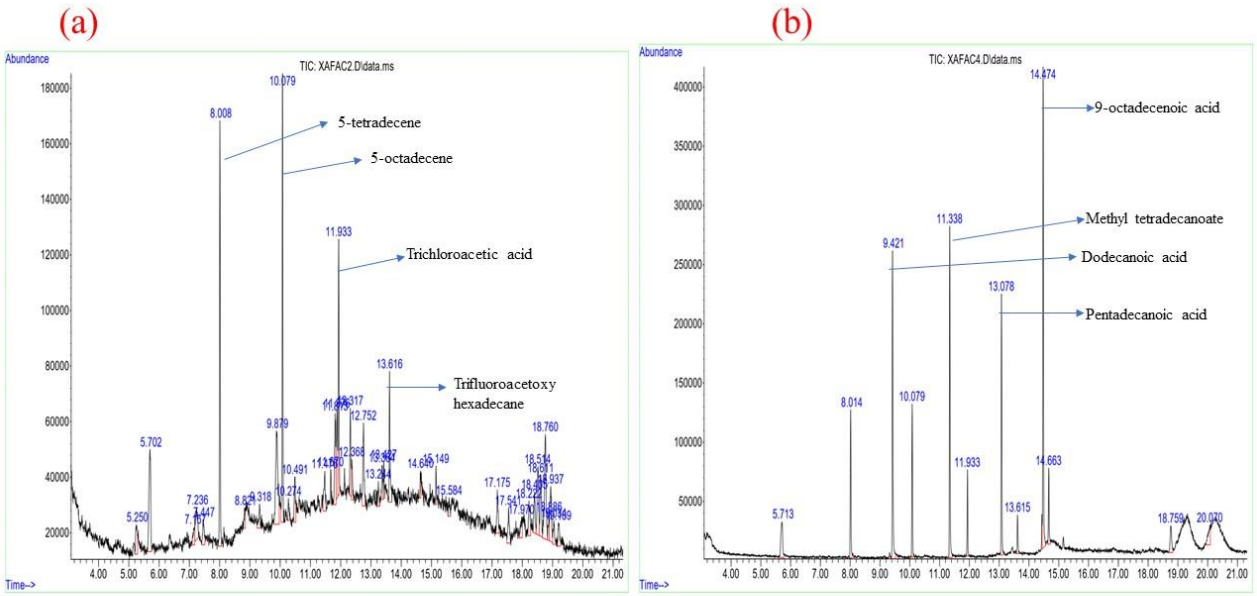

Figure 6: GC-MS Chromatogram of volatile compounds from endophytic fungal extracts. (a) shows the chromatogram of volatile compounds from *Penicillium citrinum* XAFac2 (OQ568946) (b) shows the chromatogram of volatile compounds from *Aspergillus uvarum* XAFac4 (OQ568959)

The endophytic crude extracts showed clear zones of inhibition against bacterial pathogens on agar well diffusion assay (Figure 4).

The phylogenetic tree analysis revealed that *Scopulariopsis* sp. XAFac1 and *Aspergillus* sp. XAFac4 had a common ancestral relationship and are separated from other fungal isolates and those from the GenBank. *Penicillium* sp. XAFac2 was shown to have a common ancestral link with *Penicillium citrinum* NRRL NR_121224.1 and *Penicillium hetheringtonii* CBS NR_111482.1 while *Aspergillus* sp. XAFac3 had a common relationship with *Aspergillus tamarii* NRRL 20818 NR_135325.1 and *Aspergillus Pseudotamarii* NRRL 25517 NR_135329.1. Furthermore, the phylogenetic tree revealed that *Aspergillus sp.* XAFac4 and *Scopulariopsis* sp. XAFac1 were in a monophyletic clade while *Aspergillus* sp. XAFac3 were in recent ancestral relationship with certain *Aspergillus* species from the GenBank. *Penicillium* sp. XAFac2 had 84% bootstrap value with *Penicillium hetheringtonii* CBS (NR_111482.1) (Figure 5)

Most of the biomolecules revealed by GC-MS analysis are documented to be useful in the field of pharmacy. The prominent bioactive molecules, oxacyclododecan-2-one, 5-tetradecene, 5-octadecene, trichloroacetic acid, Trifluoroacetoxyhexadecane, 9-octadecenoic acid, methyl tetradecanoate, dodecanoic acid and pentadecanoic acid identified from *Aspergillus sp. XAFac4 and Penicillium sp. XAFac2* had been reported to possess activities against bacteria, fungi, helminths and other pathogenic protozoa.[35] There is need to further exploit the bio-constituents of these putative fungal endophytes for therapeutic purposes.

Chromatograms were obtained for the putative endophytic fungi extracts using GC/MS analysis (Figure 6). Prominent compounds with higher percentage peaks were indicated. The predominant compounds from extract of *Aspergillus uvarum* XAFac4 were 9-octadecanoic acid, methyl tetradecanoate, dodecanoic acid, and pentadecanoic acid (Figure 6b) while that of *Penicillium citrinum* XAFac2 were 5-tetradecene, 5-octadecene, trichloroacetic acid, and trifluoro acetoxyhexadecane (Figure 6a).

Several compounds were identified through GC-MS profiling from the endophytic fungi extracts. Extracts from *Aspergillus uvarum* XAFac4 pulled only twelve compounds (Table 6) while a greater number of compounds up to thirty-three were identified from *Penicillium citrinum* XAFac2 extract (Table 7).

Table 6: GC-MS profiling of compounds from *Aspergillus uvarum* XAFac4

S/N	Retention time (min)	Area (%)	Compound	Molecular formular	Molecular weight (g/mol)
1.	5.713	4.76	6-Dodecene	$C_{12}H_{24}$	168.32
2.	8.014	7.70	1-tridecene	$C_{13}H_{26}$	182.35
3.	9.421	15.48	Dodecanoic acid	$C_{12}H_{24}O_2$	201.31
4.	10.079	6.77	9-octadecene	$C_{18}H_{36}$	252.50
5.	11.338	15.70	Methyl tetradecanoate	$C_{12}H_{24}O_2$	242.40
6.	11.933	3.70	1-Docosene	$C_{22}H_{44}$	308.60
7.	13.078	11.55	Pentadecanoic acid	$C_{15}H_{30}O_2$	242.40
8.	13.615	2.10	11-tricosene	$C_{23}H_{46}$	322.60
9.	14.474	22.58	9-octadecanoic acid	$C_{18}H_{34}O_2$	282.50
10.	14.663	3.87	Methyl stearate	$C_{19}H_{38}O_2$	298.50
11.	18.759	2.87	Phthalic acid	$C_8H_6O_4$	166.14
12.	20.070	2.91	Azulene	$C_{10}H_8$	128.17

Table 7: GC-MS profiling of compounds from *Penicillium citrinum* XAFac2

S/N	Retention time (min)	Area (%)	Compound	Molecular formular	Molecular weight (g/mol)
1.	5.250	1.67	Carbonic acid	H_2CO_3	62.03
2.	5.702	5.87	Cyclooctane	C_8H_{16}	112.21
3.	7.167	0.96	11-tricosene	$C_{23}H_{46}$	322.60
4.	7.236	2.11	2-tridecanol	$C_{13}H_{28}O$	200.36
5.	7.447	1.30	Hexadienyl angelate	$C_{11}H_{16}O_2$	180.24
6.	8.008	9.87	5-tetradecene	$C_{14}H_{28}$	196.37
7.	8.821	0.75	Benzoic acid	$C_7H_6O_2$	122.12
8.	9.313	1.11	6-Nitrocoumarin	C_9H_5NO	191.14
9.	9.879	7.04	Phthalic acid	$C_8H_6O_4$	166.14
10.	10.079	9.78	5-octadecene	$C_{18}H_{36}$	252.50
11.	10.274	0.72	Decanedioyl dichloride	$C_{10}H_{16}Cl_2O_2$	239.14
12.	10.491	1.78	2-pentadecanol	$C_{15}H_{32}O$	228.41
13.	11.476	1.83	4-Hepten-3-one	$C_7H_{12}O$	112.17
14.	11.670	0.70	Phosphine	PH_3	33.99
15.	11.933	5.70	Trichloroacetic acid	$C_2HCl_3O_2$	163.38

16.	12.317	2.81	1-Docosene	$C_{22}H_{44}$	308.60
17.	12.752	3.20	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.40
18.	13.244	0.76	Ethene sulfonyl chloride	$C_2H_5ClO_2S$	128.58
19.	13.364	1.12	Dodecyl propyl ester	$C_{15}H_{32}O_3S$	292.50
20.	13.616	3.72	Trifluoro acetoxy hexadecane	$C_{18}H_{33}F_3O_2$	338.40
21.	14.640	0.70	2-Chloroethyl thiocyanate	C_3H_4ClNS	121.59
22.	15.149	0.86	Acetic acid	CH_3COOH	60.052
23.	15.584	0.72	Bicyclo [2,2,1] heptan-2-one	$C_7H_{10}O$	110.15
24.	17.175	1.78	Nonaethylene glycol	$C_2H_6O_2$	62.07
25.	17.541	1.66	Benzene propanoic acid	$C_9H_{12}O_2$	152.19
26.	17.970	1.06	2-Bromo-4-chloroaniline	C_6H_5BrClN	206.47
27.	18.222	1.63	Oxa-cyclododecan-2-one	$C_{11}H_{20}O_2$	184.27
28.	18.405	1.74	3,5-Octadiene	C_8H_{14}	110.20
29.	18.611	2.66	Thiocarbamic acid	CH_3NS_2	93.18
30.	18.760	5.97	1,4-benzenedicaboxylic acid	$C_8H_6O_4$	166.13
31.	18.886	0.81	1H-indene-4-carboxylic acid	$C_{10}H_8O_2$	160.17
32.	18.937	2.88	5-methyl-2-phenylindolizine	$C_{15}H_{13}N$	207.27
33.	19.189	1.25	3-Pyridinamine	$C_5H_6N_2$	94.115

Conclusion

Our research indicates that endophytic fungi from the Nigerian native *X. aethiopica* (Dunal) A. Rich. produced secondary metabolites that are active against multi-drug resistant bacterial pathogens, speculating that they might be a source for new antibiotic discoveries. We believe that this is the first report on secondary metabolites produced by fungal endophytic strains *Penicillium* sp. XAFac2, accession no. OQ568946 and *Aspergillus* sp. XAFac4, accession no. OQ568959 exclusively isolated from *X. aethiopica* fruit.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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