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Original Research Article

Antimicrobial Potentials of Secondary Metabolites of Endophytic Fungi Isolated from *Synclisia scabrida* Miers

Chizoba A. Ozioko[1*], Stephen C. Emencheta[1*], Chinelo C. Eze[1], Martina C. Agbo[1], Chukwuma E. Ogbuanya[1], Emmanuel C. Ibezim[2], Anthony A. Attama[2*]

[1]*Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, 410001, Enugu State, Nigeria.*
[2]*Department of Pharmaceutics, University of Nigeria, Nsukka, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, 410001, Enugu State, Nigeria.*

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ABSTRACT

The increase in the development of multi-drug resistance among pathogens calls for the development of novel therapeutic agents with better effectiveness and low toxicity. The plant-associated endophytic fungi are producers of bioactive secondary metabolites with enormous potential. The study aimed to isolate, identify, and characterize the secondary metabolites of endophytic fungi isolated from different non-diseased plant parts (leaf, stem, and root bark) of *Synclisia. scabrida* Miers. The isolation of endophytic fungi was carried out using standard protocols and identified by molecular techniques. The bioactive secondary metabolites were extracted using ethyl acetate. The quantitative phytochemical screening of the fungal metabolites was carried out using Gas chromatography. The antimicrobial activities of ethyl acetate extracts of the isolates were analyzed via agar well diffusion technique and the minimum inhibitory concentrations (MICs) were determined by broth dilution method. The different endophytic fungal metabolites exhibited a good presence of different phytochemical compounds. The fungal metabolites showed antimicrobial activities against several of the six tested human pathogenic bacteria or fungi. Among these metabolites, L1, R1 and R2 metabolites were the most potent, exhibiting significant activities against the test organisms. L1 and R1 metabolites showed the lowest MIC of 0.53±0.13 and 0.70±0.07 mg/ml against *A. niger* and *C. albicans* respectively. A total of five endophytic fungi were isolated from the leaf, stem, and root bark of *S. scabrida* Miers, and three different fungal species were identified using molecular techniques. The plant, *S. scabrida* Miers harbors endophytic fungi yielding bioactive metabolites with potent antimicrobial activities against pathogenic microorganisms.

***Keywords*:** Endophytic fungi, *Synclisia scabrida*, Antimicrobial, Molecular characterization.

Introduction

The development of drug resistance in human pathogenic bacteria has prompted a search for more and better antibiotics, especially as diseases caused by pathogenic microorganisms now have become a growing threat to world health.[1] Antimicrobial resistance occurs when viruses, bacteria, fungi, and parasites do not respond to antimicrobial treatments in humans and animals, thus allowing the survival of microorganisms within the host. This results in an increased risk of disease spread, severity of illness, and death.[2] The cause of antimicrobial resistance is attributed to misuse and overuse of antibiotics in clinical treatment.[3] AMR also constitutes a threat to the global economy, with an impact on international trade, health care, and productivity. If not properly addressed, could cost the world's economy US$ 100 trillion by 2050.[4]

*Corresponding author. Email: angela.ozioko@unn.edu.ng
stephen.emencheta@unn.edu.ng
anthony.attama@unn.edu.ng
Tel: +2348036573071, +2348140477129, +2348077488245

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Plants have served as a source of medicinal bioactive compounds against many ailments for centuries. Recently, microorganisms that associate with plants, called endophytes, have become an area of interest and have proven to offer materials and products with high therapeutic potential.[5] Endophytes are microorganisms that inhabit the living tissues of plants, symbiotically. Researchers have reported the presence of one or more types of endophytes in every single plant studied to date.[6] Endophytes can colonize in the stem, roots, petioles, leaf segments, inflorescences of weeds, fruit, buds, seeds, and also dead and hollow hyaline cells of plants.[7,8] The population of endophytes in a plant species is highly variable and depends on various components, such as host species, host developmental stage, inoculum density, and environmental conditions.[9] They are known to produce chemical compounds inside the healthy tissues of plants without causing apparent symptoms of disease in the host.[10] A large number of secondary metabolites have been extracted, isolated, and characterized from endophytic microbes from various types of plants throughout the world.[6,11] Many of these synthesized chemical compounds are bioactive, and they include alkaloids, steroids, terpenoids, peptides, polyketides, flavonoids, quinols, and phenols as well as some halogenated compounds.[12] Therefore, plant endophytic fungi have been recognized as an important and novel resource of natural bioactive products with potential applications in medicine, agriculture, and the food industry.[1] Many valuable bioactive compounds with cytotoxic, antimicrobial, antidiabetic, insecticidal,

and anticancer activities have been fruitfully discovered from the endophytic fungi during the past two decades.[6]

Synclisia scabrida Mier, commonly known as 'goat weed' is a common shrub of tropical Africa present in Southern Nigeria, Cameroun, Gabon, Democratic Republic of Congo, and Angola. It is a plant used in ethnomedicine for the treatment of various illnesses such as gastrointestinal disorders, dyspepsia, menstrual pains, sexually transmitted diseases, and gastric ulcers. The medicinal properties of the plant are claimed to reside in the roots, stem, and leaves.[13,14,15]

This study focused on the isolation of endophytic fungi from the leaves, stem, and root bark of *Synclisia scabrida* Mier (Figure 1). The bioactive metabolites were extracted with ethyl acetate and the antimicrobial properties against various microbial pathogens were determined. This study hopes to establish some potent antimicrobial agents that may provide potential solutions to the problem of multidrug resistance in microbial infection treatment as well as the high cost of the currently available antibacterial agents.

Materials and Methods

Isolation of endophytic fungi

The endophytic fungal strains were isolated from the leaf, stem, and root of mature *Synclisia scabrida* Miers (medicinal plant), collected from Orba in Udenu Local Government Area of Enugu State, Nigeria in November, 2022 and duly authenticated (Voucher number: InterCEDD/700) by Mr. Alfred O. Ozioko (a certified taxonomist) of the International Centre for Ethnomedicine and Drug Development (InterCEDD). The method described by Okezie *et al*.[16] was used for the isolation. The plant materials (root, stem, and leaves) were thoroughly rinsed under running tap water to remove dust, soil particles, and debris. They were immersed in 70 % ethanol for 3 minutes after which they were re-immersed in sodium hypochlorite (5 %) for 5 minutes. Finally, they were rinsed in sterile distilled water twice to remove the effect of the sterilant and dried in between folds of sterile filter paper.

The sterile plant materials were then cut with a sterile knife into smaller pieces of about 3 cm under aseptic conditions and about 3 – 4 pieces of each plant part were placed on the surface of a prepared Potato Dextrose Agar amended with chloramphenicol (40 µg/L) using sterile forceps. The plates were incubated at 25 ^{o}C for about seven days while observed daily for the hyphal growth of the endophytic fungi. The hyphal tips of actively growing fungi from the plant parts were sub-cultured until pure isolates were obtained for further studies.

Molecular identification of the fungal isolates

Fungal DNA extraction was done using a ZR fungal/bacterial DNA miniprep kit (USA) following the manufacturer's instructions. The ribosomal RNA (rRNA) of the endophytic fungal isolates was amplified using the Internal Transcribed Spacer (ITS) gene amplification method described by Maksun.[17] A pair of primers, forward primer (ITS 1: 5'TCC GTA GGT GAA CCT GCG G3' and reverse primer ((ITS: 5'TCC TCC GCT TAT TGA TAT G3') were used to amplify the highly specific nucleic acid for endophytic fungi targeting the gene encoding for 18S rRNA.[18] The PCR reaction mixture (25 µl) consisting of 12.5 µL of Taq 2X Master Mix (PCR buffer, 4 Mm $MgCl_2$, 0.4 Mm of each dNTP, 0.05 U/µl Taq polymerase); 1µL each of 10µM forward primer (ITS 1: TCC GTA GGT GAA CCT GCG G) and reverse primer (ITS4 TCCTCCGCTTATTGATATGS); 2 µL of DNA template was made up with 8.5µL Nuclease free water. The PCR cycling conditions were initial denaturation at 94 ˚C for 5mins, followed by 36 cycles of denaturation at 94˚C for 30sec; annealing at 50 ˚C for 30 secs, and elongation at 72 ˚C for 45 sec. Followed by a final elongation step at 72 ˚C for 7 minutes and hold temperature at 10 ˚C forever. The PCR amplicons were electrophoresed using agar rose gel electrophoresis.

The final extension was carried out at 72 ℃ for 5 min and held at 4 ℃. The PCR amplification of the ITS region was verified by electrophoresis (10 µL) and the rest of the PCR products (40 µl) were purified according to the requirement of Cambridge Genomic Services (The University of Cambridge, UK) for DNA sequencing. Multiple BLASTN searches against the sequence were made at the National Center for Biotechnology Information (NCBI). Consensus sequences were submitted to the GenBank. Primarily, Clustal Omega was employed for Multiple Sequence Alignment and further followed by trimming using the trimAI tool for later alignment.

Fermentation and Extraction of Fungal Metabolites

Fermentation of endophytic fungal isolates

The fermentation was done according to Kumar & Kaushik (2013), [19] It was carried out using a rice medium. The fermentation medium consisted of 200 g of local rice with 400 ml of distilled water contained in a 2 L Erlenmeyer flask which was sealed tightly and sterilized at 121 for 15 minutes. Thereafter, the flask was allowed to stand for 24 hours to cool down completely. It was homogenized thoroughly to break up the rice lumps from its congealed state. The endophytic fungal isolate was aseptically transferred into the flask mixed thoroughly by shaking and then incubated for 20 days at room temperature.

Extraction of the fungal metabolites from the fermentation medium

After the 20-day incubation period, a 400 ml volume of ethyl acetate was poured into the flask, homogenized, and shaken occasionally for 2 days for proper extraction of the fungal metabolite. Thereafter, the ethyl acetate was filtered using Whatman filter paper (size: 188 mm). the filtrate was concentrated at 50 ^{o}C under reduced pressure using a rotary evaporator. The corresponding metabolites were weighed and their respective percentage yields were recorded. The fungal metabolites were reconstituted in dimethyl sulphoxide (DMSO) and subjected to biological studies.

Antimicrobial assay

The antimicrobial activities of the endophytic fungal metabolite were carried out using the agar well diffusion method.[20] The activities were tested against six human pathogenic microorganisms namely *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumonia*, and two fungi: *Candida albicans* and *Aspergillus niger*. These were laboratory cultures whose susceptibility to commonly used antibiotics was already established. The bacterial suspensions were prepared from an overnight culture of the bacterial test organisms. The bacterial suspensions were standardized to 0.5 MacFarland turbidity standard while the fungal suspensions were standardized using the spectrophotometric (OD_{600}) technique. 0.1 ml of standardized bacterial suspension was spread on the surface of previously sterilized Mueller Hinton agar plates using the spread plate method while the standardized fungal cultures were spread onto sterile potato Dextrose agar plates following the same method. A total of 6 wells, 8 mm in diameter were made on each of the MHA and PDA plates using a sterile cork borer.

A 100 mg/ml concentration of each of the fungal metabolites reconstituted using dimethylsulfoxide (DMSO) was further subjected to two-fold serial dilution, with DMSO to obtain other graded concentrations of 50, 25, and 12.5 mg/ml. A 0.1 ml dilution was applied in each of the wells in culture plates previously inoculated with the test organisms. Ciprofloxacin (10 µg/ml) and ketoconazole (50 µg/ml) were used as the positive controls against the bacterial and fungal test organisms respectively. The culture plates were left for 30 minutes at room temperature for pre-diffusion, after which, they were incubated at 37 ^{0}C for 24 h (for bacteria) and 25 ^{o} C for 48 h (for fungi) respectively. The antimicrobial activity of each metabolite was determined by measuring the diameters of the zones of inhibition (IZD) at the end of the incubation period. The mean of triplicate determinations was taken for each organism.

Evaluation of minimum inhibitory concentration (MIC) of the fungal metabolites

The MIC of the active fungal metabolites in the preliminary evaluation was determined by the standard method described by Deeksha *et al*.[21] Mueller-Hinton broth (MHB) was prepared and sterilized in an autoclave at 121 ^{o}C for 15 min. Two milliliters of the prepared broth were aseptically dispensed into sterile test tubes labeled 1 to 8 using a sterile syringe and needle. A stock of MHB containing 25 mg/ml of

each metabolite was prepared. The sterile MHB with 25 mg/ml crude metabolite was serially diluted two-fold in sterile tubes aseptically. Thereafter, each tube was inoculated with 0.2 ml of standardized bacterial or fungal culture. Tube 8 was used as a control for sterility of the medium and tube 9 for viability of the organisms. The final concentration of the metabolite in each of the test tubes numbered after dilution 25, 12.5, 6.25, 3.125, 1.563, 0.78 0.39,0.2 mg/ml were incubated at 37 ^{o}C for 24 h or 25 ^{o}C for 48 h for bacteria or fungi respectively. Thereafter, the content of each tube was streaked on a sterile nutrient agar plate and incubated appropriately. The plates were examined for growth and the lowest dilution that inhibited the growth of the test organisms was taken as the MIC. The readings were taken in triplicates.

Statistical analysis
Results obtained in this study were presented as mean ± standard error of the mean (SEM) of sample replicates (n = 3).

Figure 1: *Synclisia scabrida* Miers (Leaves and stem)

Results and Discussion

Endophytic fungal isolated from the different parts of the plant
The fungal isolates from different parts of *Synclisia scabrida* Miers plated on potato dextrose agar are shown in Figure 2. A total of five endophytic fungi were obtained: two fungal isolates (R1 and R2) from the root, two isolates (S1 and S2) from the stem, and one fungal isolate (L1) from the leaf of *Synclisia scabrida* Miers. These are shown in table 1 below.

Percentage yield of the fungal metabolites
The percentage yields of the different fungal metabolites are shown in Table 4. Varying yields of the fungal metabolites were observed for the isolates. L1 produced the highest yield of 3.974 %, followed by S2 which gave a percentage yield of 3.714 %. The lowest yield was observed with the S1 which gave a yield of 2.033 % while R1 gave a yield of 2.38 %.

Molecular identification of the endophytic fungal isolates
The genomic DNA of all five endophytic fungi was extracted and amplified by ITS4 and ITS5 universal primers. After gel electrophoresis, the PCR amplified products of 600 bases were obtained are presented in Table 2. A base pair of approximately 600 bp sequences was obtained for all the isolates. DNA sequences obtained from the amplification of the ITS region were submitted to GenBank and accession numbers for each fungus were obtained. Table 2 represents the sequence data of isolated endophytic fungi from *S. scabrida* Miers where 98 to 100 % BLAST match sequences were obtained.
Figure 3 shows the electrophoregram of the polymerase chain reaction result of different fungal isolates from *Synclisia scabrida* Miers. The DNA extract from all the fungal isolates showed distinct bands with a band size of 600bp. This implies that the DNA extraction was successful. The molecular weight of the DNA ladder which served as the DNA marker was 50bp.

The various fungal identities that resulted from sequence blasting with the GenBank database are presented in Table 2. The use of molecular techniques in the identification of fungi has been reported by many scientists. Deepthi *et al.*[22] made use of molecular techniques in identifying eight endophytic fungi from *Elaeocarpus sphaericus*. Sequenced amplicons from the endophytic fungi extracted genomic DNA were compared against the GenBank database to identify the endophytic fungi isolated in this study. Hence, L1 fungal isolate was identified as *Colletotrichum gloeosporioides* strain LCM 938.01, S1 - *Fusarium oxysprum* strain PSD-2, S2 - *Penicillium citrinum* strain JB-GC-3-4, R1 - *Penicillium citrinum* strain XQ18 while R2 as *Fusarium oxysporum* strain SUF191. Nurumbin *et a*l reported the isolation of 520 to 602 nucleotide pair sequences from endophytic fungi isolated from the mangrove plant, *Sonneratia apetala* (Buch.-Ham). *Neopestalotiopsis chrysea*, reportedly, had the shortest length of ITS region of 520 base pair in contrast to *Aspergillus niger* which was 602 base pair.[23] Table 2 represents the sequence data of isolated endophytes from *S. scabrida Miers*, where 90 to 100% BLAST match sequences were obtained.
The evolutionary relationship of the identified fungal isolates and their closest Genbank relatives is shown in Figure 3. It can be seen that the S1 fungal isolate, *Fusarium oxysporum* strain PSD-2 has the closest relationship with *Fusarium oxysporum* PSD-3 in the GenBank. These are related to S1 (*Fusarium oxysporum* strain SUF191) and L1 (*Colletotrichum gloeosporioides* strain LCM 938.01) which was closely related to *Colletotrichum gleosporioides* strain C6 in the GenBank. S2 (*Penicillium citrinum* strain JB-GC-3-4) and R1 (*Penicillium citrinum* strain XQ18) exhibited a distant relationship.

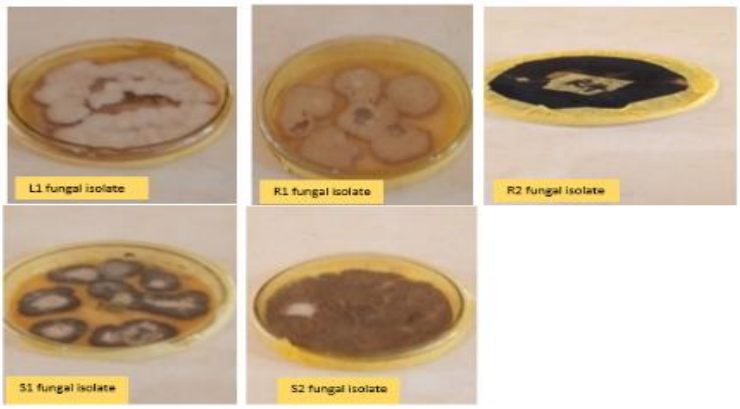
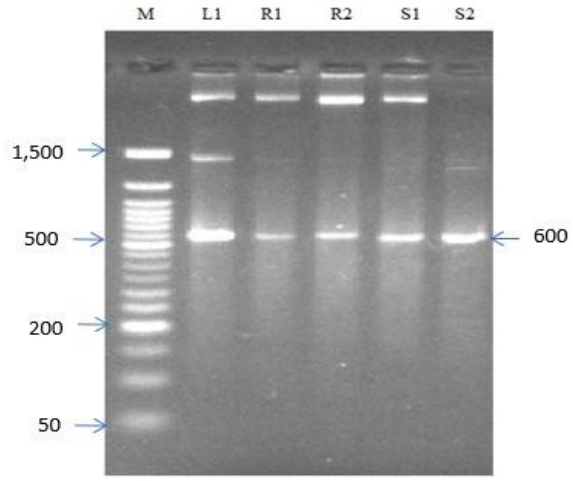


Figure 2: Pure cultures of endophytic fungi isolated from different parts of *Synclisia scabrida* Miers.



Gel image showing the amplification of the ITS region of the isolates. M is a 50bp DNA ladder

Figure 3: The molecular weight of the different endophytic fungal isolates identified from *Synclisia scabrida* Miers

Quantitative phytochemical screening of crude endophytic fungal metabolites from Synclsia scabrida Miers

Chemical analysis was carried out on fungal crude metabolites to determine the presence and type of chemical components that may serve as potential sources of drugs for medicinal and industrial use.[24] Their presence is an indication that they can be exploited as precursors in the development and advancement of synthetic drugs.

The quantitative phytochemical screening of crude metabolite from *Colletotrichum gloeosporioides* strain LCM 938.01 is shown in Table 3. It contains different classes of flavonoids with catechin (10.7512 %), kaempferol (10.8399 %), and flavone (8.4224 %) being the most abundant. Other phytochemicals present include different classes of alkaloids (linamarin, 7.1050 % and spartein, 16.0280 %), tannin (8.6868 %) and cyanogenic glycosides (11.1203 %). Sapogenin (1.4628 %), steroids (5.0560 %), and oxalates (2.4525%) were also present in small amounts. These phytochemicals have been observed to be responsible for the antimicrobial activities exhibited by crude metabolite from *Colletotrichum gloeosporioides* strain LCM 938.01.

Table 4 shows the quantitative phytochemical screening of crude metabolite from *Fusarium oxysporum* strain PSD-2 isolate. It can be seen that the metabolite contains different phytochemicals which are responsible for the activities shown by the metabolite. The metabolite contains different classes of flavonoids, saponins (2.8239 %), alkaloids %), oxalates (7.7642 %), resveratrol (7.1800 %) and phylates (7.0490 %). Flavonoid was the most abundant chemical detected in the fungal extract, with flavone (11.5813 %), kaempferol (8.4061 %), and flavanones (7.2275 %) being the most abundant class of flavonoid present in the fungal extract

The quantitative phytochemical screening of crude metabolite from *Penicillium citrinum* strain JB-GC-3-4 isolate is shown in Table 5. It contains different classes of flavonoids at varying concentrations, with flavone (13.695 %), kaempferol (8.245 %), catechin (7.128 %), and resveratrol (7.025 %) being the most abundant. Tannins (10.234 %) and oxalates (7. 470 %) also occurred in large amounts. Other phytochemicals present include sapogenin (2. 739 %), linamarin (3.474 %), steroids (3.582 %), etc. These phytochemicals may be responsible for the activities shown by the fungal metabolite. The qualitative and quantitative phytochemical analysis performed on the extract of *Aspergillus* sp.3 showed the presence of phenol, flavonoids, alkaloids, steroids, and terpenoids, 11.66 ± 0.577, 17.66 ± 0.577, 1.746 ± 0.0057, 0.446 ± 0.0057, and 9.746 ± 0.0057 (µg of standard/mg of extract), respectively.[25]

The different endophytic fungal metabolites have shown to be rich sources of different phytochemicals with good antimicrobial potentials against both the bacterial and fungal pathogens used in the study, and therefore, could be employed in the advancement of new drugs. Bhardwaj *et al.*[26] reported the presence of constituents such as flavonoids, alkaloids, phenols, saponins, steroids, tannins, and terpenoids in endophytic fungi from spikes of *Pinus rouxburghii*.[26] The phytochemical screening of ethyl acetate extract of *Penicillium sp*. Isolated from *Centella asiatica* has shown the presence of alkaloids, phenols, flavonoids, tannins, and glycosides.[27] The presence of alkaloids, flavonoids, phenolics, terpene, and saponins which belong to phenolics (natural antioxidant compounds) was reported in ethyl acetate extract of endophytic fungi associated with *Dillenia indica* L. These phytochemicals are reportedly responsible for the anti-oxidant activity exhibited by the extract.[28]

Phylogenetic analysis

Antimicrobial activity

From Figure 5, L1 fungal metabolite from the isolate from the leaf showed great activity against both the Gram-positive and Gram-negative bacterial test organisms and against, *Candida albicans* used in the study. However, the metabolite showed no activity against *Aspergillus niger*. Generally, the susceptibility of the test organisms to the active metabolites followed a concentration-dependent pattern. Among the test organisms, *K. pneumonia* and *Candida albicans* exhibited the highest susceptibility against L1 fungal metabolite at the different concentrations tested. The IZDs of the L1 metabolite against the bacterial test organisms were significantly higher than the control at 100 mg/ml and 50 mg/ml except for *E. coli* at 100 mg/ml (IZD = 18 mm) which was significantly lower than the control (25 mm). ciprofloxacin and ketoconazole used as the controls exerted different activities on the bacteria and fungi respectively. This indicates that the L1 fungal metabolite evaluated in this work possesses good antimicrobial activity. Generally, bioactive compounds in L1 metabolite had shown antimicrobial activity relative to the control, ciprofloxacin. The susceptibility of these Gram-positive and Gram-negative test organisms shows the broad-spectrum action of L1 fungal metabolite.

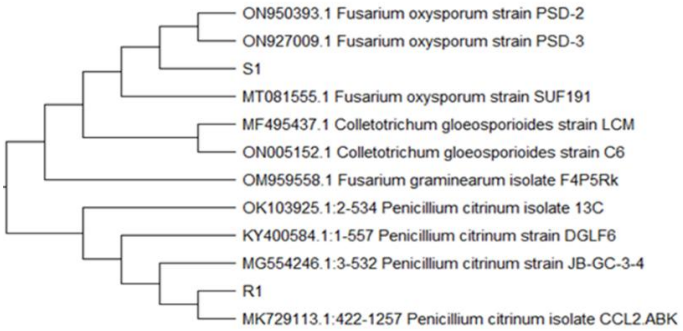


Figure 4: Evolutionary relationship of the identified fungal isolates and their closest GenBank relatives.

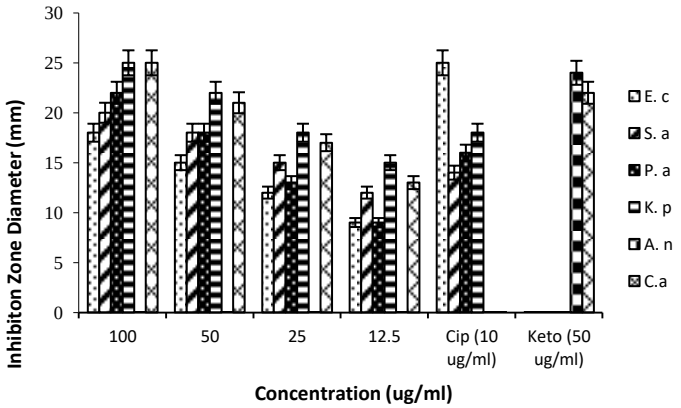


Figure 5: Antimicrobial activities of L1 metabolite against the test organisms. Where E. c = *Escherichia coli;* S. a = *Staphylococcus aureus*; P. a = *Pseudomonas aeruginosa;* K. p = *Klebsiella pneumoniae*; A. n = *Aspergillus niger and* C. a = *Candida albicans*.

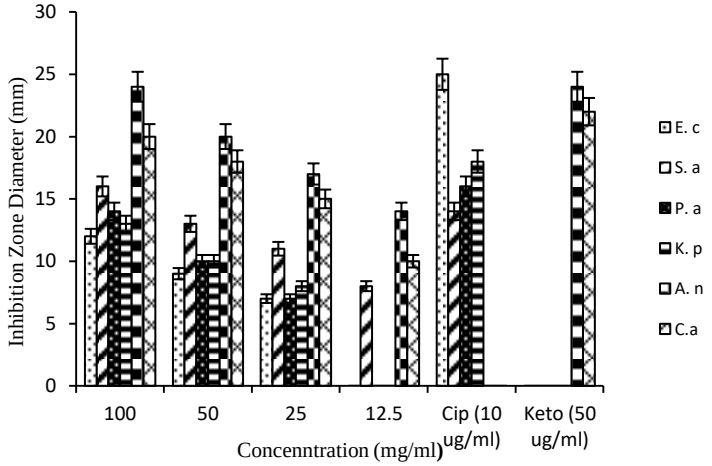


Figure 6: Antimicrobial activities of R1 metabolite on the test organisms. Where E. c = *Escherichia coli;* S. a = *Staphylococcus aureus*; P. a = *Pseudomonas aeruginosa*; K. p = *Klebsiella pneumoniae*; A. n = *Aspergillus niger and* C. a = *Candida albicans*.

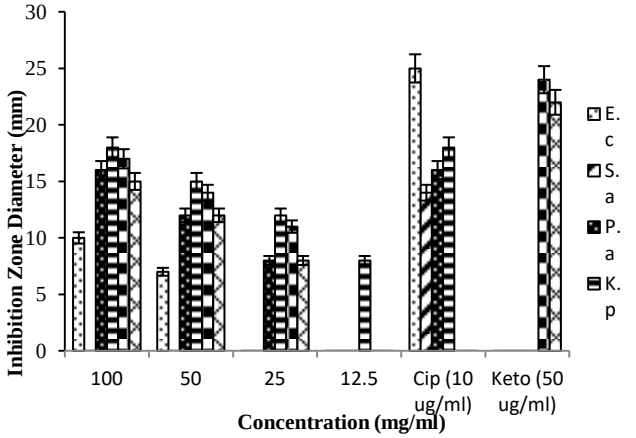


Figure 7: Antimicrobial activities of R2 metabolite against the test organisms. Where E. c = *Escherichia coli;* S. a = *Staphylococcus aureus*; P. a = *Pseudomonas aeruginosa;* K. p = *Klebsiella pneumoniae*; A. n = *Aspergillus niger and* C. a = *Candida albicans*

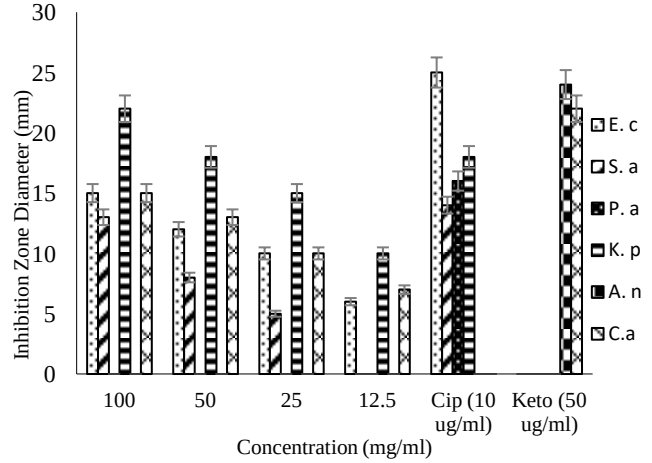


Figure 8: Antimicrobial activities of S1 metabolite against the test organism. Where E. c = *Escherichia coli;* S. a=*Staphylococcus aureus*; P. a = *Pseudomonas aeruginosa*; K. p = *Klebsiella pneumoniae*; A. n = *Aspergillus niger and* C. a = *Candida albicans*

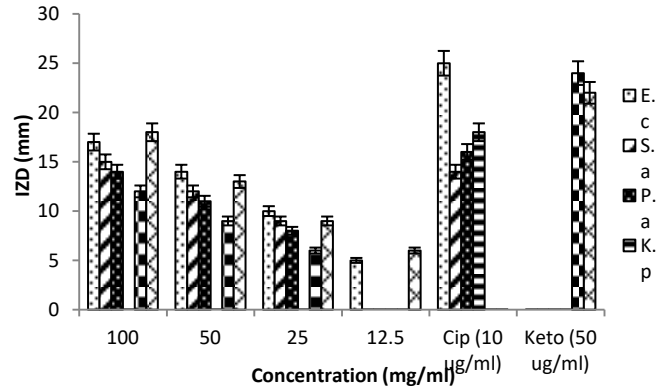


Figure 9: Antimicrobial activities of S2 metabolite against the test organisms. Where E. c = *Escherichia coli;* S. a = *Staphylococcus aureus*; P. a = *Pseudomonas aeruginosa*; K. p = *Klebsiella pneumoniae*; A. n = *Aspergillus niger and* C. a = *Candida albicans*

The antimicrobial susceptibility shown in Figure 6 indicated that metabolite from R1 endophytic fungal isolate has activity against all the test organisms. The fungal test organisms were the most susceptible. At a concentration of 12.5 mg/ml, however, only *S.. aureus* was susceptible among the bacterial test species (IZD = 8 mm). *A. niger* and *C. albicans* were also susceptible. There was no significant difference between the IZDs recorded against the fungi test organisms (*A. niger* (24 mm), *C. albicans* (20 mm) and the control (ketoconazole) (*A. niger* (24 mm), *C. albicans* (22 mm)) at the concentration of 100 mg/ml. The IZDs produced by the R1 fungal extract against the bacterial test organisms were significantly lower than the control values. Generally, R1 metabolite exhibited potent antimicrobial activity against the test organisms comparable with that of the controls.

Bioactive metabolite from R2 in Figure 7 demonstrated activity against the test organisms at varying degrees. *S. aureus* was not susceptible to the metabolite at the different concentrations tested. At the lowest concentration of 12.5 mg/ml, the metabolite showed activity against *K. pneumonia* (IZD = 8 mm). There was a significant difference ($p < 0.005$) in the antimicrobial activities of R2 fungal metabolite and the control (ciprofloxacin (50 µg/ml)) against the test bacteria (*E. coli, S. aureus, P. aeruginosa,* and *K. pneumonia*).

As shown in Figure 8, for the S1 metabolite, *Kleb pneumoniae* had the highest inhibition zone diameter at all the concentrations tested relative to other test organisms. The IZDs of S1 fungal metabolite against the bacteria and fungi test organisms were significantly lower ($p < 0.005$) than the control (ciprofloxacin 10µg/ml) at the various concentrations tested. There was no significant difference ($p < 0.005$) in the susceptibility of *E. coli* and *C. albicans* at the different concentrations of the S1 metabolite used. However, at a concentration of 12.5 mg/ml, only *E. coli* (6 mm), *Kleb pneumonia* (10 mm), and *C. albicans* (7 mm) were susceptible to S1 fungal metabolite. *Pseudomonas aeruginosa* and *Aspergillus niger* were not susceptible to the metabolite at the various concentrations used.

Bioactive compounds in S2 metabolite had no activity against *K. pneumoniae* but showed concentration-dependent activity against Gram-negatives, Gram-positive as well as fungi used in the study (Figure 9). However, *C. albicans* showed the highest susceptibility at all the concentrations used. At 12.5 mgml, only *E. coli* and *C. albicans* were susceptible. The susceptibility of the test organisms was significantly lower than the control, ciprofloxacin 10 µg/ml.

Minimum inhibitory concentration (MIC) of the crude endophytic fungi metabolites

The MICs of the *S. scabrida* Miers fungal metabolites are shown in Table 3. The MIC of R1 fungal metabolite against *E. coli* is higher than the MICs of S1 and S2 fungal metabolites. The MICs of S2 and R1 fungal metabolites against *P. aeruginosa* were higher than those of L1 and R2 fungal metabolites. Among the endophytic fungal species, R1 metabolite showed the highest MIC (16.67 ± 4.17 mg/ml) against E. coli followed by S2 and R1 against *P. aeruginosa* (8.33 ± 2.08 mg/ml) and R2 metabolite against *K. pneumonia* (8.33 ± 2.08 mg/ml). L1 fungal metabolite showed the least MIC (1.30 ± 0.26 mg/ml) *against s.* aureus and *K. pneumoniae* among the bacterial test organisms.

Table 1: Endophytic fungal isolates from *Synclisia scabrida* Miers

Plant parts	Unidentified fungus	Percentage (%) yield of metabolites
Leaf	L1	3.974

Stem	S1	2.033
Stem	S2	3.714
Root	R1	2.380
Root	R2	2.044

L1: fungal isolate from the leaf, S1:fungal isolate from the stem, S2:fungal isolate from the stem, R1: fungal isolate from the root, and R2: fungal isolates from the root of *Synclisia scabrida* Miers respectively.

The fungal metabolites showed the highest activities against the fungal test organisms, with the lowest MIC of 0.70 ± 0.07 mg/ml (L1) and 0.52 ± 0.13 mg/ml (R1) against *C. albicans* and *A. niger* respectively. S2 fungal metabolite produced the highest MIC (4.17 ± 1.04 mg/ml) against *A. niger* among the fungal test organisms. The high MIC values observed in some of the fungal metabolites can be attributed to the low production of antimicrobial compounds which contributed to the reduced antimicrobial activity observed in these metabolites. However, *S. aureus* and *Kleb*. *pneumoniae* with MICs of 1.30 ± 0.26 mg/ml for L1 fungal metabolite was the most susceptible among the bacterial test organisms to the fungal bioactive metabolites.

The bioactive compounds in plants potentially impact the development of new drugs.[25] The microbial diversity known as the endophytes are microorganisms that inhabit the living tissues of plants without causing any sign of infection.[26] Endophytic fungi have been reported to be the most predominant endophytes and they live in symbiotic association with their plant host thereby protecting the plant host from both biotic and abiotic stresses.[24] other researchers have worked on the antimicrobial potentials of endophytic fungi isolated from plants. Nurunnabi et al., 2020) reported the antimicrobial activity of extracts of 12 different endophytic fungal species isolated from the mangrove plant, *Sonneratia apetala* (Buch. -Ham) with their respective minimum inhibitory concentrations (MICs). These endophytic fungal extracts showed antimicrobial activity against more than one tested bacterium or fungus among 5 human pathogenic microbes, which included *Escherichia coli* NCTC 12241, *Staphylococcus aureus* NCTC 12981, *Micrococcus lutus* NCTC 7508, *Pseudomonas aeruginosa* NCTC 7508 and *Candida albicans* ATCC 90028. This showed that endophytic fungi from *S. apetala* species possess potential antimicrobial properties and in line with the findings of this work

The plant, *Synclisia scabrida* Miers is a plant used in ethnomedicine for the treatment of various ailments such as gastrointestinal disorders, dyspepsia, menstrual pains, sexually transmitted diseases, and gastric ulcers is rich in diversity of endophytic fungi. This study, reports for the first time, the isolation of five endophytic fungi from *Synclisia scabrida* Miers. These fungal endophytes have been molecularly identified as *Colletotrichum gloeosporioides* strain LCM 938.01 (L1), two different strains of *Fusarium oxysporum* (S1 and R2), and two different trains of *Penicillium citrinum* (R1 and S2).

The crude fungal metabolites exhibited good antimicrobial activities with varying inhibition zone diameters (IZDs) against Gram-positive and Gram-negative bacteria as well as fungi used in this study and therefore, could serve as lead materials in the development of antimicrobial drugs. Other researchers have reported that crude ethyl acetate extract of endophytic fungal metabolites exerted activity against test organisms. species of genus Epicoccum have been reported to produce active metabolites against Gram-positive and Gram-negative bacteria.[29]

Among the bacterial species, R1 metabolite showed the highest MIC (16.67 ± 4.17) against the bacterial test organisms followed by S2 and R1 against P. aeruginosa (8.33 ± 2.08) and R2 metabolite against *K. pneumonia* (8.33 ± 2.08). These higher MIC values were attributed to the reduced antimicrobial activity observed in these metabolites. However, *S. aureus* and *Kleb*. *pneumoniae* with MICs of 1.30 ± 0.26 mg/ml for L1 fungal metabolite was the most susceptible among the bacterial test organisms to the fungal bioactive metabolites. All the fungal metabolites were active against C. albicans with L1 being the most active (MIC = 0.70 ± 0.07). L1 and S1 metabolites were not active against *Aspergillus niger*. Similarly, Deepthi *et al.*[21] reported MIC range values of 0.625 to 10 µl/ml for extracted bioactive compounds from fungal isolates. The endophytic fungus, *Penicillium griseofulvum* MPR1 from the medicinal plant, *Mentha pulegium* L. was reported to have antibacterial activity against bacteria with a maximal inhibition zone of 45.5 mm against *Escherichia coli* and MIC of 50 µg / ml and 100 µg / ml on Gram-negative and Gram-positive bacteria respectively.[30] Munaganti *et al.*[31] evaluated the antimicrobial profile of *Arthrobacter kerguelensis* VL-RK_09 isolated from Mango orchards which exhibited antimicrobial activity against both Gram-positive and negative bacteria and fungi. These results support the finding of this study which showed that endophytic fungi can be promising sources of important antimicrobial bioactive molecules.

Table 2: Identified fungal species from different parts of *S. scabrida* Mier with their pairwise percentage similarities

Isolate code	Fungal species	NCBI Accession Number	Similarity (%)
L1	*Colletotrichum gloeosporioides* strain LCM 938.01	MF495437	99.47
S1	*Fusarium oxysporum* strain PSD-2	ON950393	98.79
S2	*Penicillium citrinum* strain JB-GC-3-4	MG554246	100
R1	*Penicillium citrinum* strain XQ18	KU216720	98.20
R2	*Fusarium oxysporum* strain SUF191	MT081555	90.71

Conclusion

This study, reports for the first time, the successful isolation of five different species of endophytic fungi belonging to three different genera from *Synclisia scabrida* Miers. These fungal endophytes have been molecularly identified as *Colletotrichum gloeosporioides* strain LCM 938.01 (L1), two different strains of *Fusarium oxysprum (S1 and R2)*, and two different strains of *Penicillium citrinum* (R1 and S2). Endophytic fungi are dependable sources of bioactive novel results showed promising activities. The findings of this study also suggest that endophytic fungi might be good sources for bio-prospecting of new antimicrobial compounds.

Conflict of Interest

compounds with potential applications in pharmacy and industry. The secondary metabolites produced by these endophytic fungi showed moderate activity against the bacterial and fungal pathogens due to the presence of different bioactive phytochemicals which, if well harnessed, could be exploited as precursors in the development and advancement of synthetic drugs. However, further studies should be initiated with the metabolites for potential bioactive compounds as the

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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Table 3: Quantitative phytochemical constituents of the crude metabolite from *Colletotrichum gloeosporioides* strain LCM 938.01 isolate.

S/N	Components	Subclass	Concentration (µg/ml)	% Concentration
1.	Flavonoids	Naringenin	5.6171	5.9352
		Catechin	10.1749	10.7512
		Flavone	8.4224	8.8994
		Rutin	0.8419	0.8895
		Flavanones	6.9145	7.3061
		Kaempferol	10.2588	10.8399
		Epicatechin	1.6295	1.7218
		Proanthocyanin	1.5198	1.6058
2.	Saponin	Sapogenin	1.3844	1.4628
3.	Tannin		8.2212	8.6868
4.	Alkaloids	Linamarin	6.7242	7.1050
		Spartein	15.1688	16.0280
5.	Steroids		4.7850	5.0560
6.	Cyanogenic glycosides		10.5242	11.1203
7.	Oxalate		2.4525	2.5914
	Total		94.6391	

Table 4: Quantitative phytochemical constituents of the crude metabolite from *Fusarium oxysporum* strain PSD-2 isolate.

S/N	Components	Subclass	Concentration (µg/ml)	% Concentration
1.	Flavonoids	Anthocyanin	3.2759	1.3286
		Kaempferol	20.7265	8.4061
		Rutin	7.9824	3.2374
		Catechin	15.7348	6.3816
		Flavone	28.5556	11.5813
		Epicatechin	3.4787	1.4108
		Naringenin	4.0270	1.6332
		Flavanones	17.8205	7.2275
		Proanthocyanin	1.6706	0.6776
2.	Alkaloid	Linamarin	8.8426	3.5863
3.	Saponin	Sapogenin	6.9629	2.8239
3.	Cardiac glycosides		3.8521	1.5623
4.	Steroids		9.1157	3.6971
5.	Tannin		31.2807	12.6865
6.	Ephedrine		8.9631	3.6351
7.	Resveratrol		17.7036	7.1800
8.	Oxalate		19.1439	7.7642
9.	Phytate		17.3805	7.0490

	Total	246.5649

Table 5: Quantitative phytochemical constituents of the crude metabolite from *Penicillium citrinum* strain JB-GC-3-4 isolate.

S/N	Components	Subclass	Concentration (µg/ml)	% Concentration
1	Flavonoids	Anthocyanin	2.8989	1.1519
		Catechin	17.9387	7.1283
		Rutin	7.8262	3.1110
		Kaempferol	20.7419	8.2451
		Flavone	34.4641	13.6950
		Naringenin	3.9682	1.5768
		Flavanones	22.0302	8.7572
		Epicatechin	3.3869	1.3463
		Proanthocyanin	1.6826	0.6688
		Resveratrol	17.6721	7.0248
2	Ephedrine		8.9142	3.5435
3	Saponin	Sapogenin	6.8935	2.7392
4	Tannin		25.7538	10.2337
5	Alkaloid	Linamarin	8.7431	3.4742
6	Steroids		9.0114	3.5821
7	Oxalate		18.7923	7.4701
8	Cardiac glycosides		3.8473	1.5288
9	Phytate		17.4633	6.9418
	Total		251.6546	

Table 6: Minimum inhibitory concentration (MIC) of the crude endophytic fungi metabolites

Fungal metabolites	MIC (mg/ml)					
	E. c	***S. a***	**P. a**	**K. p**	**C. a**	***A. n***
L1	2.083 ± 0.52	1.30 ± 0.26	2.60 ± 0.52	1.30 ± 0.26	0.70 ± 0.07	0.0 ± 0.0
S1	5.20 ± 1.04	2.60 ± 0.52	0.0 ± 0.0	4.16 ± 1.04	2.08 ± 0.52	0.0 ± 0.0
S2	4.16 ± 1.04	2.08 ± 0.52	8.33 ± 2.08	0.0 ± 0.0	1.04 ± 0.26	4.17 ± 1.04
R1	16.67 ± 4.17	2.60 ± 0.52	8.33 ± 2.08	4.16 ± 1.04	1.30 ± 0.26	0.52 ± 0.13
R2	0.0 ± 0.0	0.0 ± 0.0	4.16 ± 1.04	8.33 ± 2.08	2.08 ± 0.52	1.04 ± 0.26

Key: E. c = *Escherichia coli*; S. a =*Staphylococcus aureus*; P. a = *Pseudomonas aeruginosa;* K. p = *Klebsiella pneumoniae*; A. n = *Aspergillus niger and* C. a = *Candida albicans*

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