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Synthesis of Endophytic Fungi Metabolites, Antimicrobial Potentials, and Detection of their Bioactive Molecules Using Gas Chromatography-Mass Spectrometry

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ABSTRACT

Endophytes are recognized for their potential as producers of natural bioactive metabolites because of the diversity of products naturally synthesized and the ease with which they grow. Endophytes have a promising perspective on drug development to reduce the challenges of antimicrobial resistance in conventional drugs. This study concentrated on isolating fungal endophytes from five herbal plants, namely: *Carica papaya, Jatropha gossypifolia, Moringa oleifera, Mangifera indica,* and *Ocimum gratissimum*. Two strains of *Aspergillus niger, A. amstelodami, and Rhodotorula mucilaginosa* were the fungal endophytes isolated. Each of the fungi endophytes was submerged in a fermentation setup containing Potato dextrose broth (PDB) for the synthesis of their crude metabolites. Different bioactive molecules in the crude metabolites were identified using the Gas chromatography-mass spectrometry (GC-MS) method. The antimicrobial potential of the synthesized four crude metabolites were further determined against three (3) clinical isolates: *Staphylococcus aureus, Enterobacter cloacae,* and *Candida albicans*. Inhibition zones ranged between 20±0.32 mm and 35±1.23 mm were observed and recorded for all the four crude metabolites tested against the clinical isolates mentioned above. All the crude metabolites showed to have possessed quantifiable bioactive molecules, which can be applied further in pharmaceutical processes.

Keywords: Antimicrobial, Bioactive molecules, Endophytes, Fermentation, Medicinal Plants, Metabolites.

Introduction

The antimicrobial resistance potential of pathogenic microorganisms to conventional drugs is of grim concern to the public's health today. Many factors have contributed to this challenge, including improper use of antimicrobials, poor hygiene, malnutrition, continuous flow of travelers in and out of a community, increasing rate of immune-compromised people, and delayed infection diagnosis.[1-2] This has triggered the need to explore and develop new drugs that will curtail the problem, especially from natural sources, by exploring new microbial niches. One of the under-explored groups of microbes is fungal endophytes, which are found within the plant's tissues and are believed to be harmless to the plant hosting them. Dreyfuss *et al.* suggested that approximately one million fungal species live within plants tissues.[3-4]
Extensive reports have been documented about the significance of this bio-product in pharmaceutical processes.[5] the import of natural biomolecules as means for novel analeptic promoters can be demonstrated via antimicrobials application in infectious diseases management.[6-7]

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It is imperative to note that biological diversity often not explored or underexplored may be rich sources for novel biochemicals. Endophytes are microbes comprising fungi and bacteria adapted to live within plant tissues with no known pathology on the host. Examination of the metabolites of endophytes isolated from many plant species has proven that endophytes could be sources for many potential bio-products with novel uses in modern medicine, agriculture, and industrial procedures.[8-9] Reports show that many endophytes synthesize bioactive compounds with antimicrobial potential against human pathogens.[10-11] Many research works suggest that fungal endophytes confer a degree of protection over their plant hosts from contagious and parasitic pathogens and help them withstand adverse conditions by discharging active metabolites.[13] Metabolites from fungi endophytes also have great beneficial applications in pharmaceuticals; the active agent in the anticancer drug taxol is synthesized by the fungi *Taxus brevifolia*. This discovery prompted many researchers to focus on fungal endophytes, searching for other beneficial biochemical compounds.[14-15] Most medicinal herbs are believed to possess fungal endophytes. These fungi are assumed to produce bioactive molecules with pharmaceutical properties because of their association with known medicinal plants. These fungi are suggested to produce biochemical with similar properties as produced by the plants that harbor them.[16]
Studies have proposed that endophytic fungi exist as symbiotic or mutualistic organisms within their host. This could explain the shared protection and provision both the host and fungi enjoy from the relationship.[17-20] The research on endophytic fungi started over 30 years ago, and more efforts have been put into understanding them to identify and extract various bioactive metabolites.[21-25] Employing fungal endophytes are advantageous because of their ease of

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fermentation and up-scaling for the industrial production process, reproducibility, and economic feasibility.[26]
Endophytic fungi inhabiting plants of medicinal importance[27-28] are therefore a possible source of biologically active compounds that can treat many diseases.[29-31] This research focused on synthesizing and extracting bioactive metabolites of fungal endophytes and studying their antimicrobial potentials and other biological features.

Materials and Methods

Collection of plant parts
Five (5) freshly collected whole plant samples, namely: *Carica papaya, Jatropha gossypifoli, Moringa oleifera, Mangifera indica,* and *Ocimum gratissimum*, were obtained from Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria Botanical Garden on 23 March 2019(Plate 1). The plant authentication was done at the Plant Biology Division of the Department with Voucher numbers (VN); VN005, VN007, VN012, VN017, and VN019, respectively. They were instantly placed in sterile polythene packs and taken straight to the Microbial Resources Research Laboratory for processing.

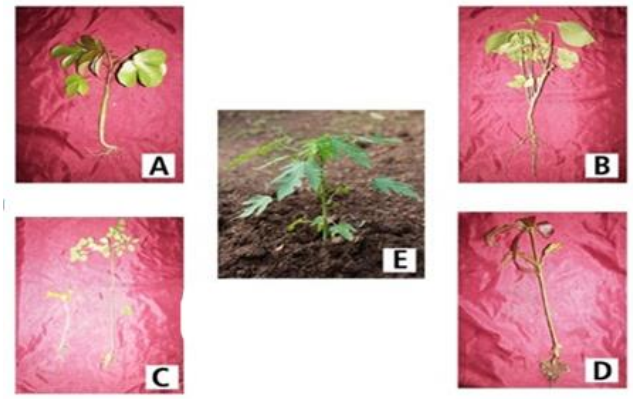

Plate 1: *Plants samples where the endophytic fungi were isolated.*

KEY:
A: *Jatropha gossypifolia,*
B: *Ocimum gratissimum*
C: *Moringa oleifera,*
D: *Mangifera indica,*
E: *Carica papaya*

Collection of test microbial isolates
Three (3) clinical isolates: *Candida albicans, Enterobacter cloaca,* and *Staphylococcus aureus*, were collected from the culture bank of the Medical Microbiology Laboratory at Ladoke Akintola University of Technology (LTH) Teaching Hospital, Ogbomoso, Nigeria. The organisms were isolated from patients with different disease conditions and were used to assess the antimicrobial potentials of the synthesized metabolites.

Isolation of fungi endophytes
The plants were washed thoroughly under running water to wash off the sand and other impurities. The leaves, stems, or roots were cut separately to 0.5 – 0.7 cm in sterile Petri dishes using a sterile razor blade under a laminar airflow chamber. The leaf, stem, and root were surfaces-sterilized for 30 seconds in 50 ml of 70% ethanol to eliminate epiphytic microorganisms. The ethanol was neutralized with 65 ml of 3.5% sodium hypochlorite treatment for 2 minutes. Furthermore, the samples were washed thrice with sterilized water and placed on Potato Dextrose Agar (PDA) medium treated with chloramphenicol as an antibacterial at 0.25 mg/L.[32]Incubation was done at 28ºC for the period of7 days. The plates were observed daily for growth. Fungal colonies observed on the plates were collected and sub-cultured to achieve pure colonies maintained on PDA slants. All pure colonies were transferred and maintained for identification and characterization on PDA slants.

Synthesis and extraction of the fungal metabolite by Submerged Fermentation (SMF)
Fresh mycelium of the isolated endophytic fungi was excised using a cork-borer and transferred into 500 mL of aseptically prepared (PDB)contained in Erlenmeyer flask (1 L). Media were maintained at 28^{o}C. Growth was monitored for fourteen (14) days, and biomass production rate was observed daily and recorded. On the 14th day of incubation, the biomasses were harvested using sterilized muslin cloth under an aseptic condition. The biomass was crushed to release their intracellular metabolite, and the leftover was separated from the intracellular product using a sterilized muslin cloth. The metabolite extraction were achieved using ethanol as an organic solvent in the ratio of 1:2 left overnight for total separation to occur. Each of the four (4) crude metabolites were then later separated from the solvents by centrifugation at 10,000 rpm.[16,29]

Antimicrobial susceptibility test (AST)
Aseptically prepared Muller Hilton agar (MHA) was poured into Petri dishes into which the microbial isolates were inoculated by evenly streaking the surface of the MHA media. Sterilized filter paper discs were impregnated separately with 100 µL of each of the four crude metabolites. The paper discs were carefully laid at the center of the inoculated plates; the setups were incubated at 37ºC for 18 – 24 hours. The bacterial inhibitory potential of each metabolite were estimated by measuring the observed zones of inhibition in millimeters.[33]

Gas Chromatographic-Mass Spectrometry
The five fungi metabolites were analyzed via GC-MS analysis to detect probable biologically active molecules present in them.[32] A triple axis detector Mass Spectrophotometer (5975C) furnished with a 10 µL syringe auto-injector hyphenated to an Agilent USA Gas Chromatography which uses Helium gas as a carrier gas was used in this process. Capillary column (0.2 µm diameter, 30 m in length, and 250 µm thickness) for all chromatographic separation was pre-treated using phenyl methyl siloxane.GC-MS conditions were maintained as thus: temperature (EI) of ion source was maintained at 250^{o}C, interface temperature was maintained at 300^{o}C, the pressure was set at 16.2 psi, out time was set at 1.8 mm, 1 µL injector in Split mode with split ratio 1:50 with injection temperature of 300^{o}C.
The column start temperature was fixed at 35^{o}C for 5min, then increased at 4^{o}C/min to 150^{o}C. The temperature was later raised at a rate of 20^{o}C/min to 250^{o}C and was maintained for 5mins while total elution was 47 minutes.[34]System control and data retrieval were done with MS Solution software. Compound identification was established by comparing with the standard mass spectra readings.

Molecular characterization and identification of fungal endophytes
Fungal genomic DNA extraction
Endophytic fungal isolates for genomic DNA isolation were grown and incubated at room temperature on a cellophane membrane placed on Potato dextrose agar (PDA). 50 to100 mg (wet weight) of the fungal isolates were collected in sterile ZR Bashing Lysis tubes and mixed with 750 µL of lysis buffer for complete crushing. This was held on a bead fitted on a tube measuring 2 mL in a holder setup and treated at maximum speed for $\geq$ 5 minutes, swirled briefly, and centrifuged with ZR Bashing Bead^{TM} Lysis Tube at 10,000 rpm for one minute. About 400 µL supernatant was decanted into a Zymo-Spin^{TM} IV Spin Filter placed within a Collection Tube, and further centrifugation at 7,000 rpm for 1 min, 1200 µL of fungal DNA binding buffer was introduced into the filtrate contained in the collection tube, 800 µL of the mixture was decanted into a Zymo-Spin^{TM} IIC Column in a Collection Tube and further centrifuged at 10,000 rpm for 1 minute. The flow-through after centrifugation was discarded, and 200 µL DNA pre-wash buffer was introduced into the Zymo-Spin ^{TM} IIC Column in fresh Collection Tube; this was centrifuge at 10,000 rpm for 1 minute. Fungal DNA wash buffer measuring about five hundred (500) µL was introduced to the Zymo-Spin^{TM} IIC Column a second time; this was also centrifuged at 10,000

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rpm for 1 minute. Zymo-Spin ™ IIC Column was transferred to a clean 1.5 mL micro-centrifuge tube within which 100 µL (35 µL minimum) DNA elution buffer was introduced and centrifuged at 10,000 rpm for 30 seconds to elude the fungal DNA for PCR and other downstream applications.[35]

Polymerase chain reaction

Amplification of extracted fungal DNA was achieved utilizing a thermal cycler (Gene Amp PCR system 9700). The PCR reaction mixture consisted of 10 µL extracted fungal DNA, 2.5 µL 10X PCR loading buffer, 2.0 µL 2.5Mm dNTPs mix, 2.5 µL H_2O, 1.0 µL 25Mm $MgCl_2$, 1 µL DMSO, 5 µL of Taq Polymerase and 1 µL each of the forward and reverse primer which made a total reaction volume of 25 µL.

The forward primer sequence was ITS1- F (5'CTTGGTCATTTAGAGGAAGTAA3'), while the reverse primer sequence was ITS4- R (5'TCCTCCGCCGCTTATTGATAGC3').[35]

Agarose gel electrophoresis

Agarose salt (1.5 g) was dissolved in TBE buffer (100 mL). In preparing Agarose gel electrophoresis using a magnetic stirrer, 2 µL of ethidium bromide was added to this mixture using a Pasteur pipette and allowed to cool for some minutes. The mixture was pipetted into the electrophoretic gel tray. The amplicon from the PCR reaction was homogenized with the loading buffer/dye and electrophoresed for about 1 hr at 50 V in TBE buffer. The DNA was visualized using a UV transilluminator. The standard base pair of the amplicons was 650bp.

DNA sequencing and identification of fungal isolates

Genetic analyzer ABI 3500 was used for sequencing. Recovery of PCR amplicons was made using a DNA isolation Test kit. Isolates with sequence similarity of >99% were accepted as the same species. FASTA Algorithms and EMBL/Gen-bank database for fungal nucleotide sequences were used in Sequence-based identifications. That with sequence similarity less than 99% was considered as unidentified.[36-37]

Results and Discussion

Isolation and identification of endophytic fungi

Four (4) fungi endophytes strains obtained from within the tissues of leaf, stem, and root of five medicinal plants obtained from LAUTECH Botanical Garden, Ogbomoso, Oyo State, Nigeria are shown in Table 1. Initially, each fungus was coded after isolation (Table 1) for easy identification before further work on their cellular morphology and molecular characterization. The fungal endophytes found in each of the excised plants parts are as shown in Table 2 where all the plants parts showed the presence of fungal endophytes except the stem of *C. Papaya*. Under submerged fermentation, the growth rate of each of the fungi was monitored. There was consistency in the growth rate of all the strains at 72 hours interval as shown in Table 3.

Antimicrobial susceptibility test

Fungal endophytes have been poorly explored as a source for natural products; hence it is necessary to include them in research involving new bioactive metabolites. [38]The significance of bioactive compounds in the process of drug discovery and development has been extensively reported.[39] It is only appropriate to isolate and identify endophytic mycobiota since the therapeutic properties may be related to the product of the endophytes within its tissue.[40-41] The case of taxol produced by *Taxus brevifolia* as an anticancer agent has also been reported many times.[41]

In this work, each fungal endophyte was cultured to synthesize their metabolites at optimum pH, although this varies from one fungal endophyte to another, as seen in Figure 1. The pH was between 8.6 and 9.7. The antimicrobial activities of the five (5) fungal metabolites were studied by challenging three (3) pathogenic organisms of clinical origins with the metabolite. These were: *Staphylococcus aureus, Enterobacter cloaca, and Candida albicans.*

Table 1: Common/Scientific names of plants used and their Endophytic fungi codes

Common Name	Scientific Name	Fungi Code Used
Moringa	*Moringa oleifera*	*MOL-MAJ-LAU*
Pawpaw	*Carica papaya*	*CPR-MAJ-LAU*
Saint leaf	*Ocimum gratissimum*	*OGR-MAJ-LAU*
Mango	*Mangifera indica*	*MIL-MAJ-LAU*
Cotton-leaf	*Jatropha gossypifolia*	*JGR-MAJ-LAU*

Table 2: Occurrence of Fungi Endophytes in Different Plant Parts

Plant	Leaf	Stem	Root
Moringaoleifera	+	+	+
Carica papaya	+	-	+
Ocimum gratissimum	+	+	+
Mangifera indica	+	+	+
Jatropha gossypifolia	+	+	+

Present (+); Absent (-)

Table 3: Qualitative Growth Pattern of Cell Biomass of the Endophytic Fungi

	DAYS				
Organisms/Day	**3rd**	**6th**	**9th**	**12th**	**15th**
A. niger	++	+++	++++	+++++	++++++
R. mucilaginosa	+	++	+++	++++	+++++
A. niger	++	+++	++++	+++++	++++++
A. amstelodami	++	+++	++++	+++++	++++++

Growth (+); No growth (-)

Table 4: Zones of inhibition observed by metabolites from endophytic fungi against clinical isolates

Source of metabolite	Zone of inhibition (mm)		
	S. aureus	***E. coli***	***C. albicans***
A. niger(CPR)	-	-	-
R. mucilaginosa	25±3.31	-	-
A. niger(OGR)	26±0.45	35±1.23	-
A. amstelodami	-	-	20±2.47

(-) resistant

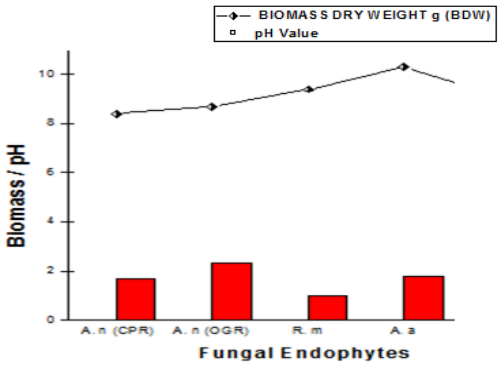

Figure 1: Biomass dry weight of the fungal endophytes at different pH values**.** *A.n (Aspergillus niger); R.m (Rhodotorula mucilaginosa); A.a (Aspergillus amstelodami)*

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A zone of inhibition (ZI) of 26±0.45mm was observed with the metabolite of *A. niger* isolated from *O. gratissimum* root against *S. aureus;* it was observed to diffuse the entire plate of *E. cloaca* with *ZI of* 35±1.23mm and no inhibition against *C. albicans*. The metabolite of *R. mucilaginosa* isolated from *M. indica* leaf had a zone of inhibition of 25±3.31mm against *S. aureus* and none against *E. cloaca* and *C. albicans.* There was no zone of inhibition with the metabolite of *A. amstelodami* isolated from *J. gossypifolia* root against *S. aureus* and *E. cloaca* but had 20±2.47mm against *C. albicans.* The clinical isolates grew resistant to the metabolite of *A. niger* isolated from *C. papaya* root as represented in Table 4. This agrees with Wiyakrutta *et al* (2004),[41] who suggested that the proficiency of endophytes as a prolific producer of antimicrobial compounds has already been reported.[7,43] Studies have suggested that several factors such as medium composition, incubation temperature, and aeration can influence the quantity, quality and type of metabolite synthesized.[44-46]
In addition, the endophytic fungi produced active metabolites due to the medicinal properties of the plants from which they were isolated. As can be seen from the GC-MS analysis (Figure 4), multiple peaks indicated the presence of several biomolecules that could be responsible for the antimicrobial activities of the synthesized metabolite.

Molecular identification and phylogenic relationships

Four (4) isolates nucleotide sequenced were related to two strains of *Aspergillus niger*, *Rhodotorula mucilaginosa*, and *A. amstelodami*. The nucleotide sequence of the fifth isolate had no similarity with sequences already deposited at the Gene bank. Phylogenetic relationships of each of the isolates were established using MEGA X.[47] Multiple Sequence Alignment (MSA) using ClustalW Omega was performed (Figures 2a-d), while fifteen (15) retrieved sequences from the Gene bank was used for plotting the phylogenetic tree (Figures3a-d). A Phylogenetic tree was constructed, with branch lengths equivalent to the evolutionary distances. The method "maximum composite likelihood" was used in computing the evolutionary distances; this calculated the base substitutions units per site.[48]In all the plant samples used in the research, fungal endophytes was found domiciled in the root and leaf parts of the plants. This indicates that the bioactive molecules of the plants that support the survival and growth conditions of the endophytes are prevalent in the leaf and roof parts of the plants studied. *Aspergillus* spp had the highest prevalence among all the isolated fungi located in the root part of the plants studied (Table 5). This indicates a more significant plant-microbe interaction that favors the growth of *Aspergillus* spp. Although there were variations in the nucleotide base pair (bp) in each fungal endophyte as follows: *Aspergillus niger* isolated from *Carica papaya* and *Ocimum gratissimum* had 563bp and 566bp, respectively. *A. amstelodami* isolated from the root of *Jatropha gossypifolia* has 571bp, while *Rhodotorula mucilaginosa* isolated from the leaf of *Mangifera indica* had 585bp. According to Masumi *et al* (2015), *Aspergillus* spp were among the colonizing fungal endophyte that was isolated from *Thymus* plants among other organisms.[49-50]

GC-MS analysis of the metabolites for their biologically active molecules

GC-MS analysis shows the active ingredients present in the crude metabolites of all the fungi metabolites. The GC-MS chromatogram results of the metabolites are shown in Figure 4, showing multiple peaks. Many bioactive molecules were detected in the metabolite, which possesses antimicrobial properties. Ethylene was found present in the metabolite of *Aspergillus niger* obtained from OGR and had applications in agriculture as an agent for causing the ripening of fruits. Hydrocinnamic acid present in the metabolite of *Aspergillus niger* obtained from CPR has a role as an antifungal agent. L-arginine present in the metabolite of *Aspergillus niger* (OGR) is α-amino acid used in the biosynthesis of proteins. It is also used to treat cardiac, and blood vessel diseases conditions and high blood pressure. Further investigations on the isolation and purity of these molecules and many more are still under study.
The diverse ways by which endophytes have been applied have been extensively discussed by many researchers, including anticancer, antiviral, and precursor for manufacturing many essential chemicals.[44-45]

Figure 2a: Multiple Sequence Alignment (MSA) of the query sequence of Aspergillus niger with a subject sequence using ClustalW Omega

Figure 2b: MSA of query sequence of Aspergillus amstelodami with a subject sequence using ClustalW Omega.

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Figure 2c: MSA of query sequence of Rhodotorula mucilaginosa with a subject sequence using ClustalW Omega.

Figure 2d: MSA of query sequence of Aspergillus niger with a subject sequence using ClustalW Omega.

Table 5: Plant Site of Each Fungal Endophytes and Their Molecular Identity Search

Fungi Code	Plant	Plant Parts	No. of Nucleotide Bases	Molecular Identity Using NCBI BLAST
CPR-MAJ-LAU	*Carica papaya*	Root	563	*Aspergillus niger*
JGR-MAJ-LAU	*Jatropha gossypifolia*	Root	571	*Aspergillus amstelodami*
OGR-MAJ-LAU	*Occimum gratissimum*	Root	566	*Aspergillus niger*
MIL-MAJ-LAU	Mangifera *indica*	Leaf	585	*Rhodotorula mucilaginosa*

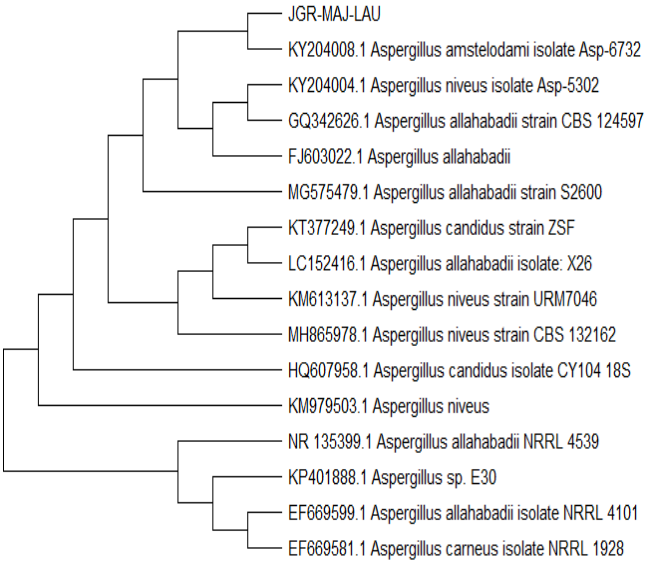

Figure 3a: Phylogenetic tree showing evolutionary relationships between JGR-MAJ-LAU and Aspergillus species retrieved at Gene bank Evolutionary history inferred by the neighbor-joining method [43]

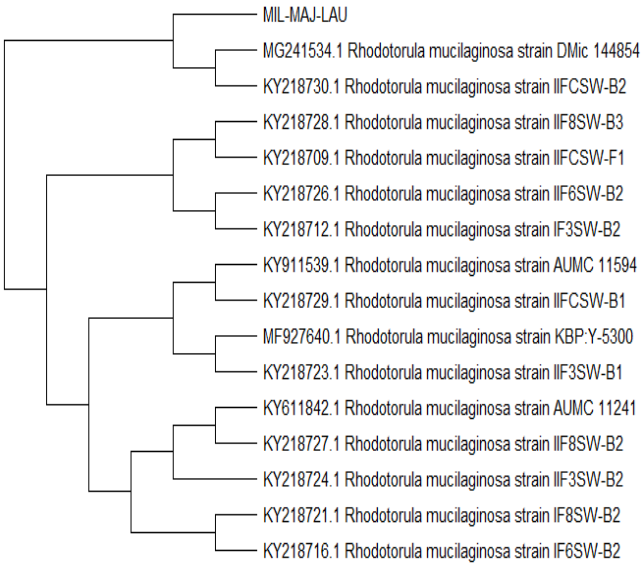

Figure 3b: Phylogenetic tree showing evolutionary relationships between MIL-MAJ-LAU and Aspergillus species retrieved at Gene bank.

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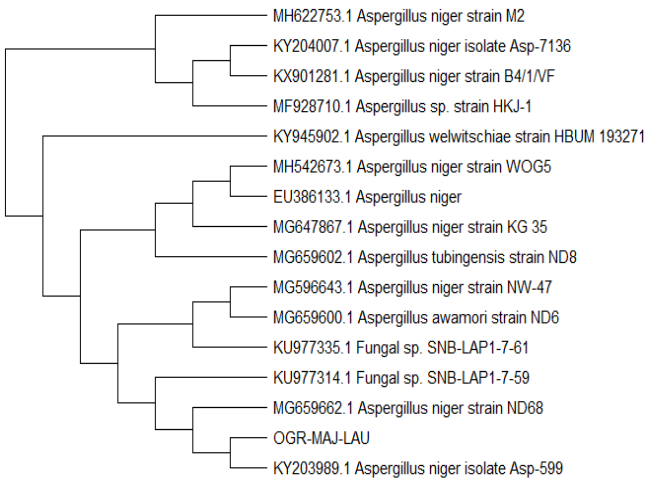

Figure 3c: Phylogenetic tree showing evolutionary relationships between OGR-MAJ-LAU and Aspergillus species retrieved at Gene bank.

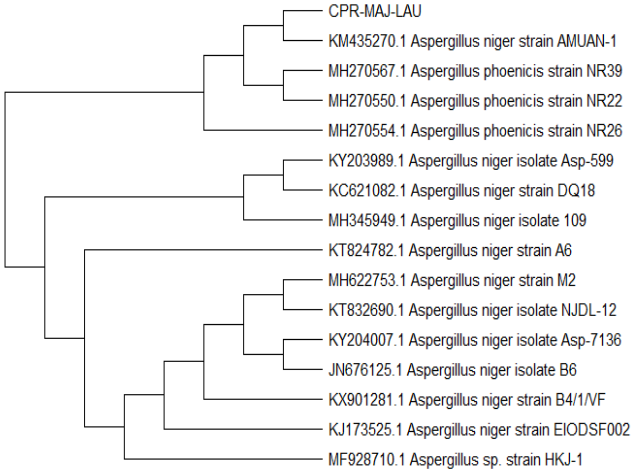

Figure 3d: Phylogenetic tree showing evolutionary relationships between CPR-MAJ-LAU and Aspergillus species retrieved at Gene bank

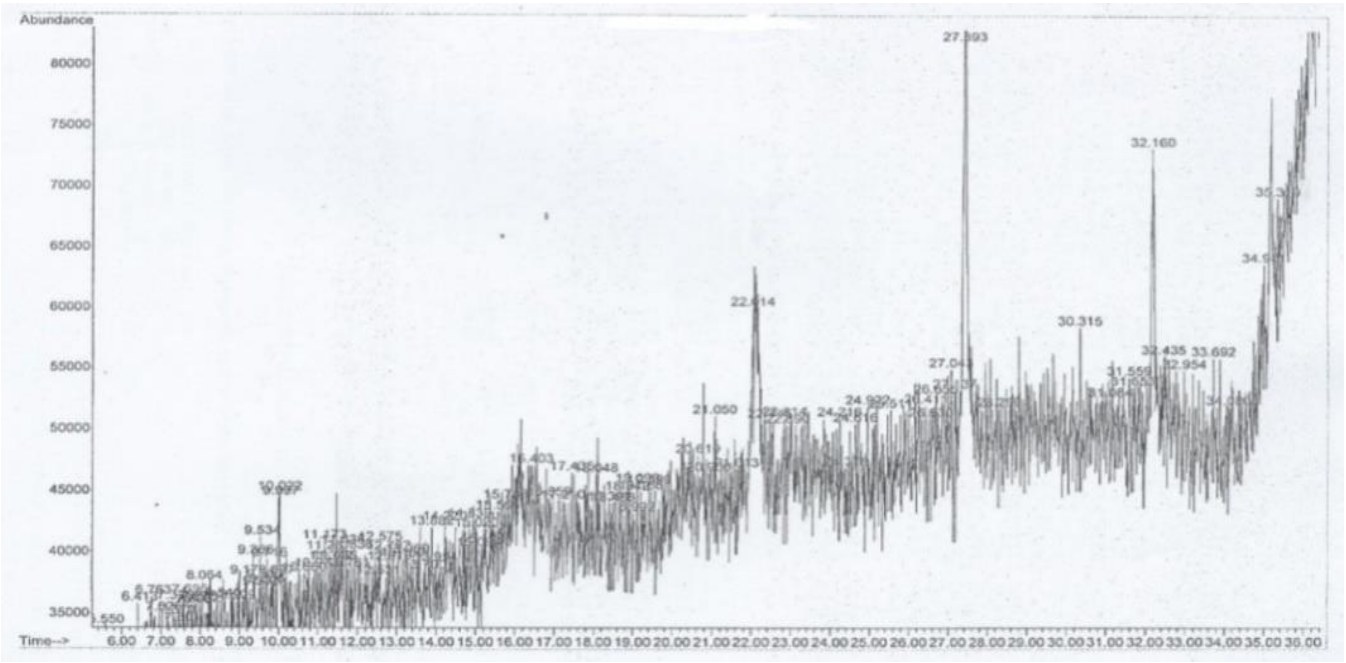

Figure 4: GC-MS Chromatogram of Metabolite Showing Multiple Peaks of Bio-molecules Present.

Conclusion

This research has shown that secondary endophytic fungi metabolites of fungi are reservoirs of valuable biomolecules which can be explored for applications in antimicrobial study. These metabolites have demonstrated inhibition against microbial pathogens which are of clinical origin. The arrays of these bioactive molecules were seen in the GC-MS Chromatogram peaks. They can be purified further to obtain pure compounds, which can later be scaled up by pharmaceutical industries and harnessed to manufacture drugs. It is highly recommended that bioactive molecules deposited in fungi be explored to detect new and novel substances for combating infectious agents that threaten lives.

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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References

1. Von Nussbaum F, Brands M, Hinzen B, Weig S, Häbich D. Antibacterial natural products in medicinal chemistry-exodus or revival? Angew Chem. 2006; 45:5072-5129.
2. Akpotu MO, Eze PM, Abba CC, Umeokoli BO, Nwachukwu CU, Okoye FBC, Simone CO. Antimicrobial

activities of secondary metabolites of endophytic fungi isolated from *Catharanthus roseus.* J Health Sci. 2017; 7(1):15-22.
3. Dreyfuss MM and Chapela IH. Potential of fungi in the discovery of novel, low-molecular-weight pharmaceuticals. The discovery of Natural Products with Therapeutic Potential (ed. V.P. Gullo). Butterworth-Heinemann, London, UK. 1994; 49-80p.
4. Gokhale MS, Gupta D, Gupta U, Fara R, Sandhu SS. Patents on Endophytic Fungi. Rec Pat Biotech.2017; 11(2):120-140.
5. Koehn FE and Carter GT. The evolving role of natural products in drug discovery. Nature Rev Drug Discov.2005; 4:206-220.
6. Clardy J and Walsh C. Lessons from natural molecules. Nature.2004; 432:829-837.
7. Deepthi VC, Sumathi S, Faisal M, Elyas KK. Isolation and identification of the endophytic fungi with antimicrobial activities from the leaves of *Elaeocarpus sphaericus* (gaertn)Int. J Pharm Sci Res. 2018; 9(7):2783-2791.
8. Meena J and Anchana D. Isolation, Characterisation and Antimicrobial Activity of Pigment Isolated from *Monascus* sp. Curr Res Microbiol Biotechnol.2015; 3:713-717.
9. Strobel G and Daisy B. Bioprospecting for microbial endophytes and their natural products. MicrobiolMolBiol Rev. 2013; 34:491-502.
10. Refaz AD, Shabir AR, Saleem M, Parvaiz HQ. Purification and characterization of endophytic fungal strains from four different high-value medicinal plants of Kashmir valley.Int J Latest Res Sci Technol. 2015; 4(1):75-80.
11. Gunatilaka AA. Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity, and implications of their occurrence. J Nat Prod.2006; 69:509-526.
12. Bramki A, Ghorri S, Jaouani A, Dehimat L, Kacem CN. Antibacterial activity of *Aspergillus* isolated from different Algerian ecosystems. Afr J Biotechnol.2017; 16(32):1699-1704.
13. Chiang HM, Chen HC, Wu CS, Wu PY, Wen KC. Rhodiola plants: chemistry and biological activity. J Food Drug Anal.2015; 23:359-369.
14. Cui J, Guo T, Chao J, Wang M, Wang J. Potential of the endophytic fungus *Phialocephalafortinii*Rac56 found in Rhodiola plants to produce salidroside and p-tyrosol. Mol. 2016; 21:502.
15. Chun-Yan SU, Qian-Liang MING, Rahman K, Ting HAN, Lu-Ping QIN. *Salvia miltiorrhiza*: Traditional medicinal uses, chemistry, and pharmacology. Chin J Nat Med. 2015; 13:163-182.
16. Pandi M, SenthilKumaran R, Choi Kim HJ, Muthumary J. Isolation and detection of taxol, an anticancer drug produced from *Lasiodiplodia theobromae*, an endophytic fungus of the medicinal plant *Morindacitrifolia*. Afr J Biotechnol. 2011; 10:1428-1435.
17. Card SD, Johnson LJ, Teasdale S. Deciphering the microbial behavior-the link between endophyte biology and efficacious biological control agents. FEMS Microbiol Ecol. 2016; 92(8):1-19
18. Strobel GA, Daisy B, Castillo U, Harper J. Natural products from endophytic microorganisms J Nat Prod. 2004; 67(2):257-268.
19. Arnold AE and Herre EA. Canopy Cover and leaf age affect colonization by tropical fungal endophytes, ecological pattern, and process in *Theobroma cacao.* Mycologia.2000; 95:88-398.
20. Saikkonen K, Wali P, Helender M, Faeth SH. Evolution of endophyte plant symbiosis. Trends Plant Sci. 2004; 9:275-280.
21. Aly AH, Edrada-Ebel R, IndrianiID, WrayV, Muller WEG, Totzke F, Zirrglebel F, Schachtele C, Kubbutat MHG, Lin WH, Proksch P, Ebel R. Cytotoxic metabolites from the fungal endophyte *Alternaria* sp. and their subsequent detection in its host plant *Polygonum senegalense.*J Nat Prod. 2010; 71(6):972-980.
22. Liu X, Dong M, Chen X, Jiang M, Lv X, Zhou. Antimicrobial activity of an endophytic Xylaria sp YX-28 and identification of its antimicrobial compound 7-amino-4 methyl coumarin. Appl Microbiol Biotechnol. 2008; 78 (2):241-247.
23. Arnold AE and Lutzoni F. Diversity and host range of foliar fungal endophytes. Ecol. 2007; 881:541-549.
24. Hyde KD and Soytong K. The fungal endophyte dilemma.Fungal Divers.2008; 33:163-173.
25. Panaccione DG, Beaulieu WT, Cook D. Bioactive alkaloids in vertically transmitted fungal endophytes. Funct Ecol. 2014; 28:299-314.
26. Na R, Jiajia L, Dongliang L, Yingzi P, Juan H, Xiong L, Nana Z, Jing Z. Yitian, L. Identification of vincamine indole alkaloids producing endophytic fungi isolated from *Neriumindicum*, Apocynaceae. Microbiol Res. 2016; 192:114-121.
27. Cui JL, Guo TT, Ren ZX, Zhang NS, Wang ML.Diversity and antioxidant activity of culturable endophytic fungi from alpine plants of *Rhodiolacrenulata*, *R. angusta*, and *R. sachalinensis.*PloS One. 2015; 10:e0118204.
28. Tejesvi MV, Kini KR, Prakash HS, VenSubbiah HS. Genetic diversity and antifungal activity of species of Pestalotiopis isolated as endophytes from medicinal plants. Fungal Divers.2007; 24:37-54.
29. Kumar S, Agarwal RP, Shukla H, Rajak RC, Sandhu SS. Endophytic fungi: as a source of antimicrobial bioactive compounds. World J Pharm Pharm Sci. 2014; 3:1179-1197.
30. Singh R and Dubey AK. Endophytic actinomycetes as an emerging source for therapeutic compounds.Ind Global J Pharm Sci. 2015; 5:106-116.
31. Gorgani L, Mohammadi M, Najafpour GD, Nikzad M. Piperine-The bioactive compound of black pepper: from isolation to medicinal formulations. Comp Rev Food Sci Food Saf. 2017; 16:124-140.
32. Majolagbe ON, Aina DA, Omomowo IO, Thomas A. Antimicrobial Potentials of Soil Fungi Metabolites, Molecular Dynamics and Their 3D- Protein Structural Prediction Using Bioinformatics Tool. Int J Curr Pharm Res. 2020; 12(5):20-26.
33. Aina AD, Owolo O, Ginikachukwu O, Aina FO, Majolagbe ON, Olukanni OD, Stephen MC, Adewumi AG.Biosynthesis of Silver Nanoparticles using Almond Plant leaf extract and their Antibacterial Activity.Int J Eng Sci Comput. 2018; 8(10):19227-19231.
34. Gurleen K and Sahil S. Gas Chromatography – A Brief Review. Int J Info Comput Sci. 2018; 5(7):125-131.
35. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, PCR Protocols: A Guide to Methods and Applications Academic Press, Innis, M.A., Gelfand, D.H., Sninsk, J.J., and White, T.J., Eds., New York. 1990; 315-322p.
36. Vilgalys, R and Hester M. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species.J Bacteriol.1990; 172:4238–4246.
37. Chithra S, Jasim B, AnishaC, Mathew J, Radhakrishnan EK. LC-MS/MS-based identification of piperine production by endophytic Mycosphaerella sp. PF13 from *Piper nigrum*. Appl Biochem Biotechnol.2014; 173:30–35.
38. El-Hawary SS, Mohammed R, AbouZid SF, Bakeer W, Ebel R, Sayed AM, Rateb ME. Solamargine production by a fungal endophyte of *Solanum nigrum.* J Appl Microbiol. 2016; 1201:143–150.
39. Kaul S, Gupta S, Ahmed M, Dhar MK. Endophytic fungi from medicinal plants: a treasure hunt for bioactive metabolites. Rev. 2012; 11:487–505.
40. Kusari S, Pandey SP, Spiteller M. Untapped mutualistic paradigms link host plant and endophytic fungal production

of similar bioactive secondary metabolites. Phytochem. 2013; 91:81–87.
41. El-Sayed AS, Abdel-Ghany SE. AliG S. Genome editing approaches: manipulating lovastatin and taxol synthesis of filamentous fungi by CRISPR/Cas9 system. Appl Microbiol Biotechnol. 2017; 101:3953-3976.
42. Stierle A, Strobel GA, Stierle D. Taxol and taxane production by Taxomyces andreanae an endophytic fungus of Pacific yew. Sci. 1993; 260:214–216.
43. Wiyakrutta S, Sriubolmas N, Panphut W, Thongon N. Danwisetkanjana K. Ruangrungsi N. Meevootisom V. Endophytic fungi with antimicrobial, anticancer and antimalarial activities isolated from Thai medicinal plants. World J MicrobBiot. 2004; 20:265-272.
44. Zhao K,Penttinen P, Guan T, Xiao J. Chen Q, Xu J. Endophytic actinomycetes' diversity and antimicrobial activity isolated from medicinal plants in Panxi Plateau. China Curr Microbiol. 2011b; 62:182–190.
45. Kuriakose GC, Palem PP, Jayabaskaran C. Fungal vincristine from EutypellasppCrP14 isolated from *Catharanthus roseus induces* apoptosis in human squamous carcinoma cell line-A431. BMC Compl Altern Med. 2016; 16:302.
46. Pan F, Su TJ, Cai SM, Wu W. Fungal endophyte-derived *Fritillariaunibracteatavarwabuensis*: diversity, antioxidant capacities in vitro and relations to phenolic, flavonoid or saponin compounds. Sci Rep. 2017; 6(7):420-428
47. Kumar S, Steecher G, Li M, Knyaz C, Tamura K. MEGA X, Molecular Evolutionary Genetics Analysis across computing platforms. Mol Biol Evol. 2018; 35(6):1547-1549.
48. Saitou N and Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol BiolEvol. 2017; 4:406.
49. Masumi S, Mirzaei S, Zafari D, Kalvandi R. Isolation, identification and biodiversity of endophytic fungi of Thymus. Progr Biol Sci. 2015; 5:43-50.
50. Ramirez-Estrada K, Vidal-Limon H. Hidalgo D, Moyano E, Golenioswki M, Cusidó RM, Palazon J. Elicitation: an effective strategy for the biotechnological production of bioactive high-added value compounds in plant cell factories. Molecules. 2016; 21:116.