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*Original Research Article*

# Bioactive Compounds Characterization and Antimicrobial Potentials of Crude Extract of *Curvularia lunata,* a Fungal Endophyte from *Elaeis guineensis*.

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ABSTRACT

Bioactive compounds of microbial origin remain the frontline for novel drug discovery and development in the pharmaceutical industry. Endophytic fungi are gaining much attention as reservoirs of bioactive compounds with beneficial therapeutic activities. The present study is focused on evaluating the chemical compositions and antimicrobial activity of secondary metabolites produced by *Curvularia lunata*, an endophytic fungus of *Elaeis guineensis*. The endophytic fungus was isolated from healthy leaves of *E. guineensis* using standard methods and identified using internal transcribed spacer (ITS-rDNA) sequence analysis. The fungus was subjected to solid-state fermentation and secondary metabolites extracted using ethyl acetate and concentrated under a vacuum. The crude extracts were screened for antimicrobial activity against selected pathogenic bacteria and *Candida albicans* using the agar diffusion method. The bioactive components of the fungal extracts were identified using gas chromatography-mass spectrometry (GC-MS) analysis. Crude extract of *C. lunata* at 1mg/mL displayed potent antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa, and Candida albicans*, with inhibition zones of 8 ± 0.6 mm, 2 ± 1.0 mm, and 10 ± 1.5 mm respectively. GC-MS analysis revealed the presence of compounds, such as 2,4-di-tert-butylphenol, γ-terpinene, heptadecane, 2,6,10,14-tetramethyl, tetradecanoic acid, 2-hydroxy-, methyl ester, p-cymene, oxirane (chloromethyl) among others in the fungal extracts. These compounds are known to possess several beneficial biological properties. Hence from the results of this study, the endophytic fungus *C. lunata*, isolated from *E. guineensis* produce interesting bioactive compounds that can be explored in the development of effective antimicrobials and other pharmaceutical agents.

***Keywords***: *Curvularia lunata*, Antimicrobial activity, *Elaeis guineensis*, GC-MS analysis, Bioactive metabolites.

## Introduction

There is increased urgency in the search for new therapeutic agents, as one of the biggest health problems in the world relates to diseases caused by drug-resistant pathogenic microorganisms. This has necessitated the screening of several sources for potential bioactive agents that can be applied in the development of novel therapeutic agents. Natural products, including secondary metabolites produced by plants and microorganisms, have long been studied for their antimicrobial activity in the search for eco-friendly substitutes for synthesized chemicals drugs.[1,2] Microorganisms, especially endophytic fungi are important sources of bioactive natural products with enormous potential for the discovery of new molecules for drug discovery, industrial use, and agricultural applications.[3]

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Natural products remain a consistent source of drug leads with more than 40% of new chemical entities (NCEs) reported to be derived from microorganisms.[4] The emergence of new diseases, development of drug-resistant pathogenic microorganisms, management of post-operative complications in patients with organ transplantation, and the tremendous evolvement of general health disorders in the worlds' population are some of the challenges confronting scientists today. These situations have forced scientists to explore different natural sources for safe and potent agents to meet these challenges.[5]

It is estimated that at least 80% of the world's population especially in developing countries use plant materials as their source of primary health care.[6] The perennial plant *Elaeis guineensis* belonging to the family *Palmae* and tribe *Cocoinea* is widely used in traditional medicine in West Africa for treating various ailments, due to its numerous pharmacological properties.[7,8] Plants and fungi engage in diverse intimate relationships leading to both harmful and beneficial activities. *Curvularia lunata* is an ascomycete fungus that belongs in the class Dothideomycetes, and order Pleosporales.[9] Notwithstanding their ability to cause diseases, *C. lunata* exist as endophytes in the tissues of healthy plants.[10-12]

Endophytes are microorganisms that grow intercellularly and asymptomatically within living tissues establishing a mutual relationship with the host plant.[13] Until recently, these microbial entities have been generally overlooked as a component of ecosystems, which is why they have been regarded as a trove of

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unexplored biodiversity.[14] A large number of bioactive compounds isolated from endophytes, belonging to several structural classes like alkaloids, peptides, steroids, terpenoids, phenols, quinines, and flavonoids have been reported.[15] The selective pressure on fungal growth exerted by other organisms, predators, competitors, and pathogens, induces the production of fungal metabolites to defend their growth,[16] however, these metabolites often have other bioactivities.[17] Endophytic fungi are regarded as one of the most creative groups of secondary metabolites that play important biological roles and are potential sources of novel natural pharmaceutical agents.[18] This study aimed to evaluate the chemical compositions and antimicrobial activity of secondary metabolites produced by *C. lunata*, an endophytic fungus of *E. guineensis.*

## Materials and Methods

*Collection of plant sample*

Fresh and healthy leaves of *E. guineensis* were collected from mature plants from Agbani, Enugu State located in South-Eastern Nigeria in February 2021. The plant material was authenticated and a voucher specimen (PCG499/A/040) was deposited in the herbarium of the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Anambra State, Nigeria.

*Isolation and identification of the endophytic fungus*

Isolation of endophytic fungi from the leaves of *E. guineensis* was carried out as described by Nwobodo *et al.*[19] The leaves were washed thoroughly in running tap water, and then cut into small fragments (about 1 cm2). The leaf fragments were surface sterilized by immersion in 2% sodium hypochlorite solution for 2 min., 70% ethanol for 2 min., before a final rinse in sterile water for 5 min. These leaf fragments were transferred into malt extract agar (MEA) plates, supplemented with chloramphenicol (500 mg/L). The Petri plates were then incubated at 27°C for 7 days. Hyphal tips of fungal colonies emerging from the leaf segments were sub-cultured on fresh MEA plates, and then purified using single spore technique.

The molecular identification of the endophytic fungus was carried out using DNA amplification and sequencing of the fungal ITS region.[20] Extraction of the fungus deoxyribonucleic acid (DNA) was done using Zymo fungal / bacteria DNA extraction kit (Zymo Research Corp., South Africa) according to the manufacturer's instructions. Polymerase chain reaction was carried out to amplify the ITS gene of specific DNA of the fungus using the primer pair ITS-1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS-4 (5′-TCCTCCGCTTATTGATATGC-3′). The amplified products were purified with ExoSAP and Sanger sequencing carried out using Nimagen, BrilliantDye™ Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000. Identification of endophytic fungus was performed on the basis of similarity of amplified sequence with ITS sequence data from strains available in the US National Centre for Biotechnology Information (NCBI) database using Basic Local Alignment Search Tool (BLAST) N sequence match routines.

*Fermentation and extraction of secondary metabolites.*

Solid-state fermentation was carried out as previously described Okoye *et al.*,[21] in 1 L Erlenmeyer flasks containing 100 g of rice media and 200 mL of water, which was then autoclaved at 121°C at 15 psi for 30 min. The flasks were inoculated with about 3 mm diameter agar blocks containing the axenic fungal culture and incubated at 28°C for 21 days. The secondary metabolites were extracted using ethyl acetate. The organic phase was vacuum-concentrated at 40°C under reduced pressure using a rotary vacuum evaporator to obtain the crude extract.

*Antimicrobial assay*

The potential antimicrobial activity of the fungal extract was assessed *in vitro* using the agar well diffusion assay method described by Eze *et al.*[22] A working concentration (1 mg/mL) of the fungal extract was prepared by dissolving the extract in dimethyl sulphoxide (DMSO 100% v/v). Standardized broth cultures ($1\times 10^5$ cells/ mL) of test bacterial isolates (*Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa,* and *Escherichia coli*) and the fungus *Candida albicans* were spread aseptically onto the surface of Mueller Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) plates respectively using sterile cotton swabs. All culture plates were allowed to dry for about 5 min and wells were made using a sterile cork-borer (6 mm in diameter). These wells were respectively filled with 60 µL of the fungal extracts and controls. Gentamicin (10 µg/mL) and ketoconazole (50 µg/mL) were used as positive controls in the antibacterial and antifungal evaluations respectively; while DMSO (100% v/v) was used as the negative control. The plates were then kept at room temperature for 1 h to allow the agents to diffuse into the agar medium and incubated accordingly. The MHA plates were incubated at 37°C for 24 h, and the SDA plates were incubated at 25°C for 2 days. The inhibition zones diameters (IZDs) were measured and the size of the well (6 mm) was deducted from the values obtained to get the actual IZDs. This was conducted in triplicate and the mean IZDs were calculated and recorded.

*Gas Chromatography-Mass Spectroscopy (GC-MS)*

GC-MS analysis of the fungal extract was carried out as described by Ibrahim *et al.*,[20]and Buss and Butler,[23]but with modifications. The analysis was performed using an Agilent 7820A gas chromatograph coupled to an Agilent 5975C inert mass selective detector (MSD) with a triple-axis detector operated in an electron impact (EI) mode with ionization energy of 70 eV. An HP-5 capillary column coated with 5% phenyl methyl siloxane (30m × 250 µm diameter × 0.25 µm film thickness) was used for the separation. The sample (1 µL, diluted 1: 100 in dichloromethane) was injected in splitless mode at an injection temperature of 300°C. Purge flow to split vent was 15 mL/min at 0.75 min with a total flow of 16.654 mL/ min. Helium was used as the carrier gas at the flow rate of 1 mL/min with an initial nominal pressure of 1.4902 psi and an average velocity of 44.22 cm/sec. The oven temperature was initially programmed at 50°C for 1 min then ramped at 3°C / min to 300°C for 10min. Run time was 43 min with a hold time of 5°C / min. The relative quantity of the chemical compounds present in the extract was expressed as a percentage based on the peak area produced in the chromatogram. The constituents of the extract were identified by their GC retention time (RT) and comparison of their mass spectra with those of the National Institute for Standard Technology (NIST) mass spectral library.

*Statistical analysis*

Data were expressed as mean ± standard deviation (SD) for three parallel experiments. The mean inhibition zones diameter of the fungal extracts against the various isolates were compared using one way ANOVA. Statistical significance was considered at $p \leq 0.05$. Analysis of data and graph were made using Microsoft Excels 2016 software.

## Results and Discussion

*Isolation and identification of Curvularia lunata*

In this study, *Curvularia lunata* was isolated and identified from healthy leaves of *E. guineensis* based on its DNA sequence of the ITS region. The fungus was identified as *C. lunata* and the DNA sequence data was deposited in the NCBI database (GenBank) with accession number: OL347929. The BLAST phylogenetic tree produced using BLAST pairwise alignments is presented in Figure 1. This genus is isolated as an endophyte from *E. guineensis* healthy plants for the first time in this study. However, Khiralla *et al.*[24] reported that most *Curvularia* species are found in tropical and subtropical areas as endophytes on selected plant species. Despite being pathogenic to selected plants, *C. lunata* is well known as a common endophytic fungus associated with different plant parts, isolated from different plants, and has been reported in several studies.[11,12,25]

*Antimicrobial activity*

The preliminary antimicrobial activity result of the crude extract of *C. lunata* is presented in Figure 2. The extract only displayed antibacterial activity against *S. aureus* and *P. aeruginosa* with IZD of 8 ± 0.6 mm and 2 ± 1.0 mm respectively.

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No activity was observed against *E. coli* and *B. subtilis*. It also showed a good antifungal activity against *C. albicans* with an IZD of 10 ± 1.5 mm. Antimicrobial bioactive metabolites from microorganisms have several benefits when compared to other sources such as their host plants. Some of these advantages include less destruction of resources, sustainable use, large-scale industrial productions, and quality control.[26,27] In this study, the fungal crude extracts from *C. lunata* displayed antibacterial activity against *S. aureus* and *P. aeruginosa* with good antifungal activity against *C. albicans* (Figure 2). However, no activity was observed against *E. coli* and *B. subtilis.* This suggests that the fungal crude extracts contain compounds with strong and specific antimicrobial activity. According to Kaczorowski *et al.*,[28] and Sharma *et al.*,[29] specific bioactivities, defined as high inhibition of growth of one type of target organism with little or no activity against others, is of particular interest in drug discovery: it suggests the presence of compounds that have specific modes of action as opposed to highly toxic compounds that are often of little use as medication.

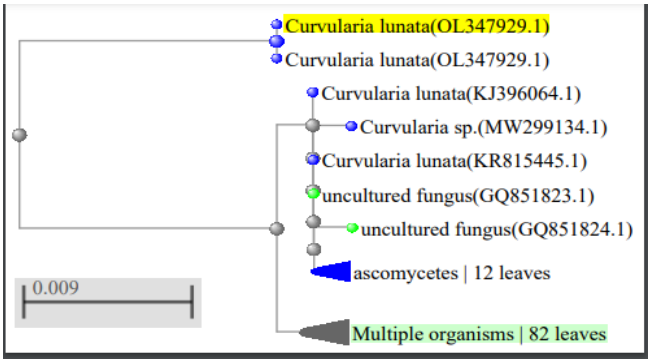
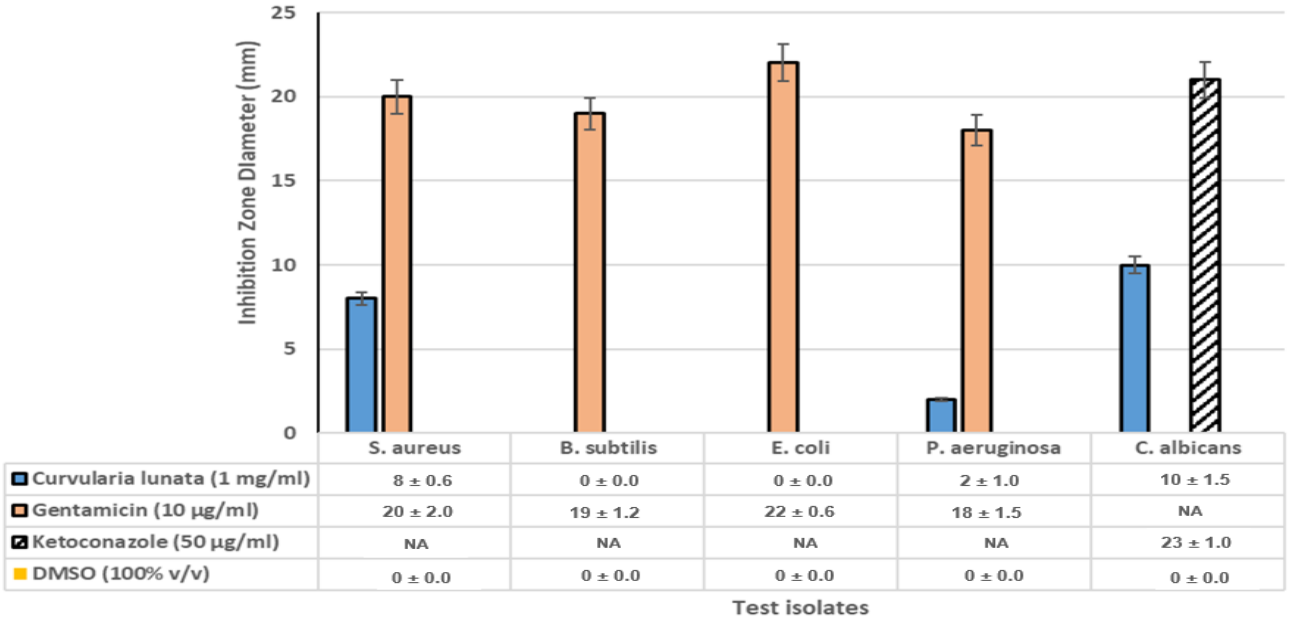



**Figure 1:** Guiding phylogenetic tree for *Curvularia lunata* isolated from *E. guineensis*




| | S. aureus | B. subtilis | E. coli | P. aeruginosa | C. albicans |
|---|---|---|---|---|---|
| Curvularia lunata (1 mg/ml) | 8 ± 0.6 | 0 ± 0.0 | 0 ± 0.0 | 2 ± 1.0 | 10 ± 1.5 |
| Gentamicin (10 µg/ml) | 20 ± 2.0 | 19 ± 1.2 | 22 ± 0.6 | 18 ± 1.5 | NA |
| Ketoconazole (50 µg/ml) | NA | NA | NA | NA | 23 ± 1.0 |
| DMSO (100% v/v) | 0 ± 0.0 | 0 ± 0.0 | 0 ± 0.0 | 0 ± 0.0 | 0 ± 0.0 |

**Figure 2**: Antimicrobial activity of the crude extract of *C. lunata* against selected microbial pathogens. Key: NA; not applicable

The crude extract of *C. lunata* displayed better antimicrobial activity against the Gram-positive organism *S. aureus.* This is not surprising since Gram-negative bacteria are generally less susceptible than Gram-positive bacteria.[30] Structurally, the outer membrane of Gram-negative bacteria contains hydrophilic lipopolysaccharides (LPS) molecules, which acts as a mechanical barrier to macromolecules and hydrophobic compounds, providing Gram-negative bacteria with higher tolerance toward hydrophobic antimicrobial compounds like those found in essential oils identified in the endophytic fungal extract in this study.[31]On the other hand, Ugwu *et al*. [32], also reported the antifungal activity of endophytic fungal extract isolated from *Dacryodes edulis* against *C. albicans*. The good antifungal activity exhibited by the extract of *C. lunata* against *C. albicans* could be as a result of antifungal bioactive compounds such as 2,4-di-tert-butylphenol (Table 1) produced by the fungus.

There are other proven reports of the antimicrobial activity of fungi crude extract. Some extracts are recorded to be effective against all the bacterial pathogens used in this study, whereas other endophytic fungal extracts showed low or no antimicrobial activity.[29,32,33] The antimicrobial outcome of each endophytic fungal extract is attributed either to the potency of the constituents of the extract, or the concentration of the active compounds in the extracts. On the other hand, according to Idris *et al.*,[32] endophytic fungal crude extracts with low or no activity could yield more potent compounds once they had undergone some purification. This means that the low activity of fungal crude extract does not indicate that this fungus does not have any activity.[29] Similarly, Chong *et al.*,[34] reported the inhibition of *S. aureus*, *P. aeruginosa*, and *C. albicans* by the leaf extract of *E. guineensis*. This indicates that the endophytic fungus *C. lunata* isolated from the leaves of *E. guineensis* possibly produced the same bioactive compounds as the host plant. This also further strengthens the argument that endophytic fungi may produce the same or better bioactive metabolites as their host plants.

*GC-MS analysis of the fungal secondary metabolites*

Over the years, endophytic fungi have been shown to produce very potent bioactive secondary metabolites for effective pharmaceutical, industrial and agricultural applications. The GC-MS analysis of the endophytic fungal crude extract showed the presence of twenty-seven (27) important compounds. The identified compounds, retention time (RT), peak area (%), molecular weight, nature of compound and biological activities are presented in Table 1. The result revealed the presence of major compounds such as heptadecane, 2,6,10,14-tetramethyl (14.6%), 2,4-di-tert-butylphenol (9.2%), undecane (8.1%), 1-octadecene (7.0%), tetradecane (5.1%), tridecane (4.4%), *γ*-terpinene (4.0%), dodecane, 2,6,11-trimethyl (3.6%), decane, 2,3,5,8-tetramethyl (3.4%), Z-8-hexadecene (3.2%), cetene (1.6%), tetradecanoic acid, 2-hydroxy-, methyl ester (1.1%), p-cymene (1.3%), oxirane, (chloromethyl) (1.0%), 1,3-cyclohexadiene, 1-methyl-4-(1-methylethyl) (0.7%), ethyl oleate (0.5%), *β*-myrcene (0.3%). The chromatogram showing the peaks for the most abundant compounds is illustrated in Figure 3, while Figure 4 shows the structures and molecular formula of some compounds.

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**Table 1:** Bioactive compounds identified in the extract of *C. lunata* by GC-MS, and reported biological activities

| S/n | Compound | RT | MW (g/mol) | Area (%) | Nature of compound | Biological activities |
|---|---|---|---|---|---|---|
| 1 | *β*-Myrcene | 6.301 | 136 | 0.33 | Monoterpene | Antioxidant, anti-inflammatory, antibacterial,[47] and anticancer [48] |
| 2 | Oxirane, (chloromethyl)- | 6.496 | 92.5 | 1.01 | Ether | Antibacterial [54] |
| 3 | Benzene, 1,4-dichloro- | 6.849 | 147 | 1.57 | Aromatic hydrocarbon | Pesticide [44] |
| 4 | 1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)- | 6.956 | 136 | 0.70 | Monoterpene | Antibacterial [45] |
| 5 | *p*-Cymene | 7.201 | 134 | 1.27 | Monoterpene | Antioxidant, anti-inflammatory, antiparasitic, antiviral, antitumor, antibacterial, and antifungal [55,56] |
| 6 | *γ*-Terpinene | 8.160 | 136 | 4.02 | Monoterpene | Antioxidant[57] and anti-inflammatory[58] |
| 7 | Dodecane, 2,6,11-trimethyl- | 8.382 | 212 | 3.58 | Alkane | Antifungal and antibacterial [59] |
| 8 | Undecane, 3,7-dimethyl- | 8.700 | 184 | 1.55 | Alkane | Mild sex attractant for various types of moths & cockroaches, ants [59,60] |
| 9 | Heptadecane, 2,6,10,14-tetramethyl | 8.958 | 296.6 | **14.57** | Alkane | Antibacterial [60] |
| 10 | Octane, 3,5-dimethyl- | 9.120 | 142 | 2.12 | Alkane | Antimicrobial [60] |
| 11 | Decane, 2,3,5,8-tetramethyl- | 9.178 | 198 | 3.39 | Alkane | Antimicrobial [60] |
| 12 | Tetradecane | 9.339 | 198 | **5.13** | Alkane | Antimicrobial, cytotoxicity, antipyretic, anthelmintic, tumor, tuberculosis, dyspepsia, anemia, elephantiasis, anti-diabetic, anti-inflammatory, anti-diarrhoeal [59,60] |
| 13 | Undecane | 9.806 | 156 | 8.12 | Alkane | Mild sex attractant for various types of moths and cockroaches, ants [59,60] |
| 14 | Decane, 2,4-dimethyl- | 10.101 | 170 | 2.64 | Alkane | Antimicrobial [60] |
| 15 | Dodecane | 12.262 | 170 | 1.86 | Alkane | Antibacterial and antifungal [59,60] |
| 16 | Tridecane | 15.109 | 184 | 4.44 | Alkane | Antimicrobial [60] |
| 17 | Cetene (1-Hexadecene) | 17.625 | 224 | 1.62 | Alkene | Antimicrobial [60] |
| 18 | Pentadecane | 20.413 | 212 | 1.11 | Alkane | Antibacterial [29,59] |
| 19 | 2,4-Di-tert-butylphenol | 20.974 | 206 | **9.22** | Phenol | Antifungal, antimalarial,[49]antioxidant,[51]antibacterial, anti-inflammatory, cytotoxicity, antiviral, insecticidal, and nematicidal [52] |
| 20 | Z-8-Hexadecene | 22.690 | 224 | 3.16 | Alkene | Antimicrobial [60,61] |
| 21 | 1-Octadecene | 30.257 | 252 | **7.00** | Alkene | Antimicrobial [60,61] |
| 22 | 6-(Trifluoromethoxy)-N-(trimethylsilyl)-1,3-benzothiazol-2-amine | 30.433 | 234 | 0.60 | Benzothiazole | Antimicrobial, anti-inflammatory, anticancer, antidiabetic, and anti-HIV [62] |
| 23 | Ethyl Oleate | 31.666 | 310.5 | 0.50 | Fatty acid ester | Antimicrobial,[63] pesticide activity, [64] and Anti-inflammatory [65] |
| 24 | 1-Docosene | 31.814 | 308.6 | 2.17 | Alkene | Antimicrobial [45,58] |
| 25 | Tetradecanoic acid, 2-hydroxy-, methyl ester | 34.216 | 258 | 1.11 | Fatty acid ester | Antifungal, Antioxidant, cancer preventive [45,66] |

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|---|---|---|---|---|---|---|
| 26 | 9,19-Cyclolanost-24-en-3-ol, (3.beta.)- | 35.582 | 468.8 | 2.11 | Alcohol | Antifungal and antibacterial [39] |
| 27 | 5.alpha.-Cholest-8-en-3-one, 14-methyl- | 36.021 | 398.7 | 2.23 | Ketone | Antifungal [40,61] |

Compounds were identified by comparing the mass spectra obtained in the GC-MS run with available NIST and Wiley spectra libraries. The identities of the constituents were confirmed by comparing the computed linear retention index of each compound to corresponding indices in literature and available web-based sources.[35]In this study, the majority of the active compounds identified by GC-MS are essential oils, which may donate the antimicrobial activity observed against the susceptible bacteria (*S. aureus* and *P. aeruginosa*) and fungi (*C. albicans*). Osuntokun and Cristina,[36] reported that the role of essential oils in the discovery of new drugs cannot be overemphasized, especially in an era of antimicrobial resistance. This is in agreement with studies reporting that endophytic fungi produce secondary metabolites with various levels of antimicrobial activity.[37,38]

Alkane compounds (38%) such as heptadecane, 2,6,10,14-tetramethyl, undecane, tetradecane, tridecane, dodecane, pentadecane, octane, 3,5-dimethyl) and monoterpenes (14%) such as *γ*-terpinene, 1,3-cyclohexadiene, p-cymene, and *β*-myrcene constituted the major group of compounds identified in the fungal extract (Table 1). This alkane group of compounds has been reported to be produced by other endophytic fungi including *C. cladosporioides* and *C. tenuissimum* and are also known to possess potential biological activities.[39,40] Dodecane,2,6,11-trimethyl-, is an alkane compound that has been reported to exhibit antifungal and antibacterial activities.[41] Monoterpene essential oils are hydrophobic molecules, and perhaps move from the aqueous phase into cell membranes, causing toxic effects to the bacterial membrane structure and function,[42] which may ultimately lead to bacterial cell death.[43]*p*-Cymene [1-methyl-4-(1-methylethyl)-benzene] is a consistent monoterpene reported in numerous plant species. Several studies have confirmed the pharmacological properties of the monoterpenes p-cymene, including antioxidant, anti-inflammatory, antiparasitic, antidiabetic, antiviral, antitumor, antibacterial, and antifungal activities.[44,45,46] Similarly, myrcene (*β*-myrcene), an abundant monoterpene that occurs as a major constituent in diverse plant species is attributed with various biological activities including analgesic, sedative, antidiabetic, antioxidant, anti-inflammatory, antibacterial,[47] and anticancer effects.[48]

The compound 2,4-di-tert-butylphenol (2,4-DTBP) (Figure 5B) is a phenolic known to possess various biological activities. It has been reported to have antifungal activities,[49] and *in vitro* antimalarial activity.[49,50] 2,4-DTBP has been reported to be present in fruits as well as seeds and exert antioxidant properties.[51] Varsha et al.,[49] also reported that 2,4-DTBP has been shown to exhibit fungicidal potential at higher concentrations where fluconazole failed to act completely. Other reported biological activities of 2,4-DTBP include antibacterial, anti-inflammatory, cytotoxicity, antiviral, insecticidal, and nematocidal activities.[52] A high amount of phenolic components in essential oils is known to contribute to their antimicrobial activity against fungi and Gram-positive bacteria.[53] 2,4-DTBP being one of the most abundant compounds of the fungal extract in this study could account for the reasonable antimicrobial activity recorded against the test fungi and Gram-positive bacteria.

Oxirane, (chloromethyl), also known as alpha-epichlorohydrin or g-chloropropylene oxide, belongs to the class of organic compounds known as epoxides. Husain and Shaharyar[54] report that epoxides derived from chalcones increase antibacterial activity against Gram-positive and Gram-negative bacteria.

Thus, the endophytic fungus *C. lunata* in this study was observed to possess great bioactive potentials and can be applied in the development of pharmaceutical agents. However, Further studies on the isolation, characterization, and understanding of the mechanism of action of the individual bioactive compounds, as well as subjecting them to various biological activities and ascertaining their toxicity profile, will yield more fruitful results. This will lead to an alternative approach to drug discovery which could be natural, reliable, economical, and environmentally safe.

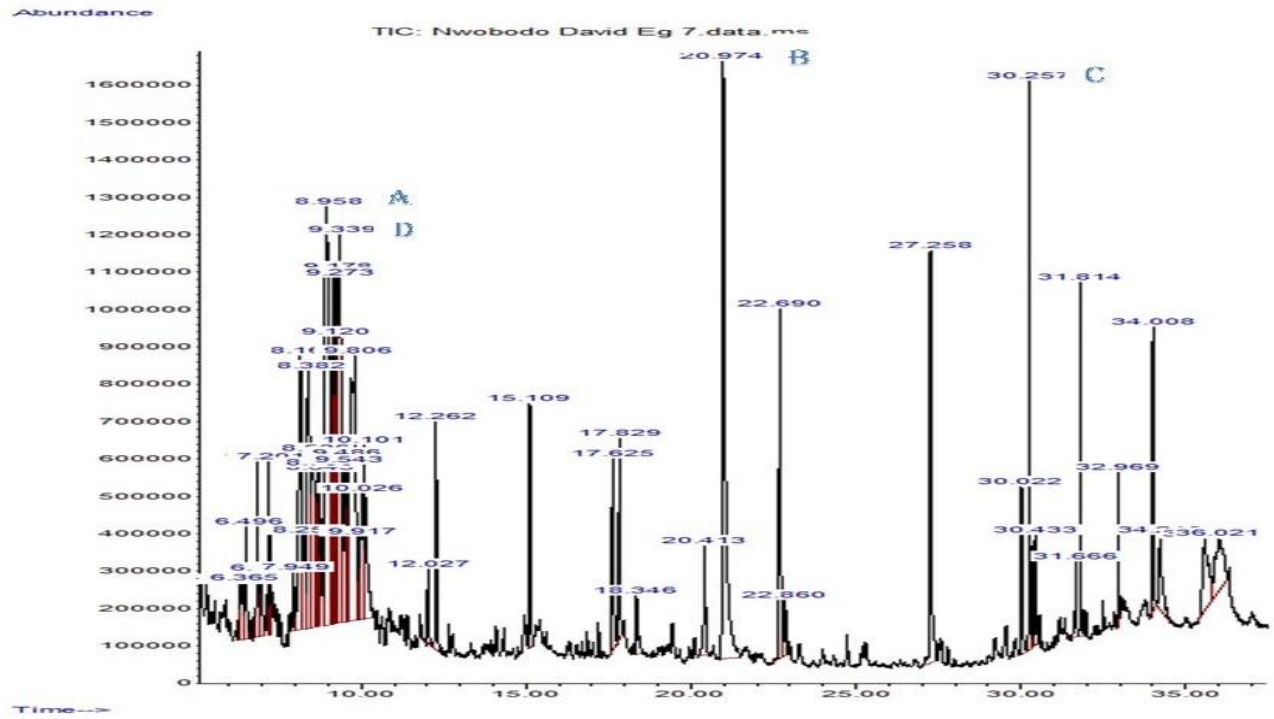


**Figure 3:** GC Chromatogram of *C. lunata* crude extract. A; Heptadecane, 2,6,10,14-tetramethyl, B; 2,4-Di-tert-butylphenol, C; 1-Octadecene and D; Tetradecane

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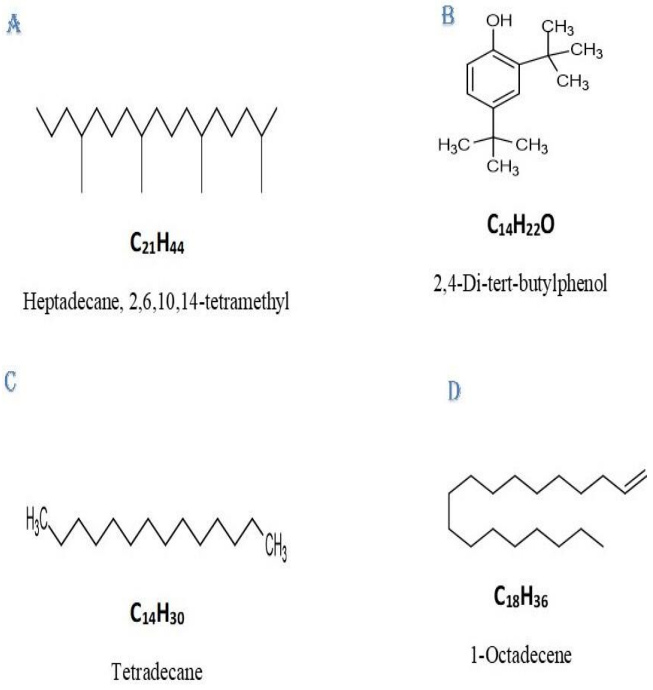


**Figure 4:** Structure and molecular formula of selected bioactive compounds with highest percentage area identified in the crude extract of *C. lunata*

## Conclusion

The endophytic fungus *C. lunata* isolated from the leaves of *E. guineensis* is a unique organism with the ability to produce various useful bioactive compounds. In this work, the crude extracts of *C. lunata* showed antimicrobial activity against common human pathogens such as *S. aureus*, *P. aeruginosa* and *C. albicans.* GC-MS analysis revealed the presence of different biologically active compounds, majorly antimicrobial compounds. This study illustrates that the endophytic fungus *C. lunata* can produce different bioactive compounds that may serve as lead molecules in the development of novel and cost-effective drugs against pathogenic microorganisms.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

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

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