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UV-mediated enhancement of antibacterial secondary metabolites in endophytic *Lasiodiplodia theobromae*

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Abstract

In the science of drug discovery, ultraviolet (UV) irradiation has been applied to induce mutagenesis in fungi to provide possibilities for the stimulation or enhancement of fungal biosynthetic capabilities. This study was carried out to evaluate the effect of UV radiation on the biosynthesis of antibacterial secondary metabolites in an endophytic *Lasiodiplodia theobromae*. Using standard methods, the fungus was isolated from healthy leaves of *Cola acuminata* and identified based on PCR amplification and genomic sequencing of the internal transcribed spacer (ITS) region. Cultures of *L. theobromae* were exposed to UV radiation at different time intervals of 1, 2 and 5 min. The fungus was subjected to solid-state fermentation in rice medium before and after UV treatments. The fungal secondary metabolites were extracted and tested for antibacterial activity using the agar diffusion method. Compounds present in the obtained extracts were identified by HPLC and GC-MS analysis. At a concentration of 1 mg/ml, the extract of the wild type *L. theobromae* (untreated) was observed to only inhibit *Staphylococcus aureus*, with an IZD of 12 mm. However, the extract of UV-treated *L. theobromae* (2 min) inhibited *S. aureus, Escherichia coli* and *Pseudomonas aeruginosa* with an IZD of 10 and 4 mm respectively. A wide array of compounds in the phenolics, fatty acids, alkaloids and alkanes classes were identified in the UV-treated and untreated fungal extracts. Overall, UV treatments of *L. theobromae* stimulated the production of seventeen (17) new compounds that were not detected in the untreated strain. The study confirms UV irradiation as an effective method for stimulating microbial biosynthesis of new bioactive compounds, indicating a promising and potentially abundant source of new drug compounds from microorganisms.

Keywords: antibacterial; endophytic fungi; *Lasiodiplodia theobromae;* secondary metabolites; UV treatment

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Introduction

Endophytic fungi reside within different parts of plant tissues without causing any harm to their host (Vinu *et al*., 2021), rather they are said to protect them in hostile conditions by synthesizing secondary metabolites (Kaur *et al*., 2020). In recent years, the pursuit of new compounds from medicinal plants has become an enthralling area of study. Plants with ethno-pharmaceutical value are being exploited for their medicinal properties. Large-scale medicinal plant harvesting, on the other hand, has already become a major danger to biodiversity. Endophytes could serve as an alternative source of therapeutic compounds. Furthermore, the discovery of potent medicines with minimal side effects is a better alternative to conventional disease control and treatment methods. Endophytic fungi are potential sources of therapeutically important bioactive products, with ability to produce many unique natural compounds such as alkaloids, terpenoids, polyketides, flavonoids (Rustamova *et al*., 2020), antimicrobial metabolites (Nwobodo *et al*., 2020a), antioxidants (Nwobodo *et al*., 2017), anticancer, anti-diabetic and immunosuppressant compounds (Rai *et al*., 2014). Importantly, bioactive secondary metabolites from microorganisms are considered advantageous due to less destruction of resources, sustainable use, large scale industrial productions and quality control (Liang *et al*., 2012). They are endowed with a collection of enzymes that aid in the biosynthesis of structurally diverse and complex molecules that are often difficult to mimic (Deepika *et al*, 2016).

Notwithstanding, certain limitations continue to hamper the potential of using these fungi as alternative candidates for the production of a variety of pharmaceutically important secondary metabolites (Priti *et al*., 2009). The instability of the expression of genes involved in the biosynthesis of desired metabolite(s) is a major limitation in the production of bioactive SMs in current fermentation practices using fungal endophytes (Pandey *et al*., 2014). Endophytic fungi, on the other hand, exhibit a typical tendency to lose their ability to produce secondary metabolites after repeated sub-culturing in axenic medium (Deepika *et al*., 2016). As a result of these, there is a need to develop techniques that can be used to activate cryptic biosynthetic pathways in order to increase SMs biosynthesis in fungal endophytes. One of such techniques is the genetic modulation of endophytes by UV light treatment.

Genetic modulation of target strains is a classical strategy that can be exploited to activate cryptic biosynthetic gene clusters (BGCs) in microorganisms. UV treatment is a strong tool for cracking the chemical diversity of fungal endophytes by boosting the expression of cryptic or weakly expressed biosynthetic pathways. Geographically, *L. theobromae* can be found practically in all parts of the world, but it is most prevalent in tropical and subtropical areas, making it one of the most widely distributed Botryosphaeriaceae species (Mehl *et al*., 2017). The endophytic *Lasiodiplodia* is gaining attraction as a producer of biotechnologically relevant enzymes and secondary metabolites (Félix *et al*., 2019; Taufiq and Darah, 2020; Nwobodo *et al*., 2022). In this study, an attempt was made for the first time to activate silent genes and enhance bioactive compound production in *L. theobromae* using UV radiation.

Materials and Methods

Collection of plant sample

Fresh and healthy leaves of *Cola acumminata* were collected from mature plants from Agbani, in Enugu State South-Eastern Nigeria. The plant material was authenticated by a Taxonomist and deposited in the Herbarium of the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Nigeria.

Isolation and identification of endophytic fungus

Isolation of the endophytic fungus from plant leaves was carried out as described by Nwobodo *et al.* (2020b). Briefly, the leaves were washed thoroughly in running tap water, and then cut into small fragments (about 1 cm^2). 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 endophytic fungus was identified molecularly using DNA amplification and sequencing of the fungal ITS region (Ibrahim *et al*., 2021). Extraction of the fungal 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′). ExoSAP was used to purify the amplified product, and Sanger sequencing was performed with Nimagen's BrilliantDyeTM Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000. The endophytic fungus was identified using Basic Local Alignment Search Tool (BLAST) N sequence match procedures to compare the amplified sequence to ITS sequence data from strains in the US National Centre for Biotechnology Information (NCBI) database.

Fermentation and extraction of secondary metabolites.

Solid-state fermentation was carried out as previously described (Okoye *et al*. 2013) in 1000 mL 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 cultures and incubated at 27 °C for 21 days. The secondary metabolites were extracted using ethyl acetate. To obtain the crude extract, the organic phase was vacuum-concentrated at 40 °C under reduced pressure using a rotating vacuum evaporator.

Ultraviolent (UV) irradiation

Twenty-four (24) hours old subculture of *L. theobromae* was briefly exposed to UV light at 254 nm for 1, 2 and 5 min and then incubated for 7 days. After which, solid-state fermentation was carried out by inoculating the media with about 3 mm diameter agar blocks containing the mutated fungal culture and incubated at 27 °C for 21 days. The crude secondary metabolites were extracted using ethyl acetate concentrated at 40 °C under reduced pressure using a rotary vacuum evaporator. The obtained extracts were assayed for their antibacterial activities.

Antibacterial assay

The antibacterial activity of the fungal crude extracts was assessed *in vitro* using the agar well diffusion assay method described by Eze *et al*. (2019). The fungal extracts were dissolved in dimethyl sulphoxide (DMSO 100% v/v) to generate a working concentration of 1 mg/mL. Using sterile cotton swabs, standardized broth cultures of test bacterial isolates (*Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli*) were spread aseptically onto the surface of Mueller Hinton Agar (MHA) plates. All culture plates were allowed to dry for about 5 min and wells made using a sterile cork-borer (6 mm in diameter). These wells were respectively filled with 60 µL of the fungal extracts and control. After that, the plates were maintained at room temperature for 1 hour to allow the agents to diffuse into the agar medium before being incubated. The positive control was gentamicin (10 g/mL), while the negative control was DMSO (100 % v/v). After a 24-hour incubation period at 37 °C, the inhibition zone diameters (IZDs) were measured and values recorded.

Identification and characterization of bioactive compounds

High Performance Liquid Chromatography (HPLC) analysis

Each of the dried fungal extract (2 mg) was reconstituted with 2 mL of HPLC grade methanol. The mixture was sonicated for 10 min and thereafter centrifuged at 3000 rpm for 5 min. Then 100 µL of the dissolved samples were each transferred into HPLC vials containing 500 µl of the HPLC grade methanol. An Agilent 1100 system (Santa Clara, CA, USA) composed of quaternary pump, autosampler, diode array detector (DAD), and HP ChemStation Software was used for the HPLC analysis. Chromatographic separation was carried out on a Tskgel ODS C18 (150 x 4.6 mm i.d., 5 µm particle size) analytical column maintained at 40 °C, injection volume 10 µL and the wavelength monitored at 254 nm. The mobile phase [water in 0.1% Phosphoric acid (A) and methanol (B)] was set at a flow rate of 0.6 mL/min.

Gas Chromatography-Mass Spectroscopy (GC-MS)

The fungal extracts were subjected to GC-MS analysis as described by Nwobodo *et al*. (2022). An Agilent 7820A gas chromatograph was used in conjunction with an Agilent 5975C inert mass selective detector (MSD) with triple axis detector operating in electron impact (EI) mode with a 70eV ionization energy. For the separation, an HP-5 capillary column coated with 5% phenyl methyl siloxane (30m × 250 µm diameter × 0.25 µm film thickness) was utilized. 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. At a flow rate of 1 mL/min, helium was employed as the carrier gas, with an initial nominal pressure of 1.4902 psi and an average velocity of 44.22 cm/sec. The oven temperature was set to 50 °C for 1 minute, then ramped up to 300 °C at 3 °C/min for 10 minutes. The run time was 43 minutes, with a 5 °C/min hold time. Relative quantity of the chemical compounds present in each of the extracts of was expressed as percentage based on 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. Results obtained were statistically analysed using one way ANOVA, and their significance considered at $p \leq 0.05$.

Results and Discussion

Isolation and identification of endophytic fungi

The endophytic fungus *Lasiodiplodia theobromae* was isolated from healthy leaves of *C. acuminata*, and identified using molecular techniques and the DNA sequence data deposited in the NCBI database (GenBank) with accession numbers: OL342232 (Nwobodo *et al*., 2022). Aside from being a pathogen, *L. theobromae* have been reported as a non-host specific plant endophyte that can be found on a wide range of crops and trees and has been linked to over 500 distinct plant hosts (Chen *et al*., 2017; Salvatore *et al*., 2020). The ability of *L. theobromae* to produce biotechnologically relevant chemicals, such as enzymes (Félix *et al*., 2018), polysaccharides (Selbmann *et al*., 2003), and secondary metabolites (Félix *et al*., 2019), have attracted wide interest from researchers.

Antibacterial activity

Table 1 shows the comparison of the antibacterial activity results obtained from the wild type and UV treated *L. theobromae.* Wild type *L. theobromae* extract was observed to only inhibit *S. aureus*, with an IZD of 12 ±1.4 mm. However, the best antibacterial activity was displayed by the extract of UV treated *L. theobromae*

for 2 min, inhibiting *S. aureus* (10 ±0.7), *E. coli* (6 ±0.0) and *P. aeruginosa* (4 ±0.7). This is followed by extract of *L. theobromae* treated for 1 min, which inhibited *S. aureus* (12 ±0.7) and *E. coli* (6 ±0.7). This result demonstrates an enhancement in the antibacterial spectrum of extracts produced by *L. theobromae* after UV treatment. The extract of *L. theobromae* UV treated for 5 min was only able to inhibit *E. coli* (6 ±1.4). The antibacterial activity of wild type *L. theobromae* was significantly different ($P < 0.05$) when genetically modulated using UV treatment, but all exhibited antibacterial activities were weaker than that of the positive control. Interestingly, all extracts from the UV-treated *L. theobromae* inhibited *E. coli*, while only the extract from 2 min exposure inhibited *P. aeruginosa*. Recall that *E. coli* and *P. aeruginosa* were resistant to the crude extract of the wild type *L. theobromae*: implying that the UV treatment probably activated the production of new bioactive compounds with antibacterial activities against *E. coli* and *P. aeruginosa*. However, *B. subtilis* remained resistant to all extracts obtained from the UV treatment study. It is worthy to note that; the genus *Bacillus* is well known to produce several antimicrobial compounds and essential oils as secondary metabolites (Kaaria, 2018). Most of which they are tolerant to and are reported to be produced by the endophytic fungi in this study. For instance, in a study carried out by Kaaria (2018), several members of the genus *Bacillus* including *B. subtilis* produced various compounds such as phenolics, alkaloids, carboxylic acids, alkanes, alkenes, esters, alcohols, esters, and ketones. Still, other researchers have reported the susceptibility of *B. subtilis* to extracts produced by endophytic fungi (Chatterjee *et al*., 2019; Zhou *et al*., 2022). Nevertheless, it is pertinent to know that the classification of crude extract antimicrobial activities is not well established in the literature. As a result, comparing results of this study to previous results becomes challenging due to the numerous variables that can influence the final result.

Table 1. Antibacterial inhibition zones diameters (mm) obtained from the extracts of wild type and UV treated *L. theobromae*

Test isolates	Wild type (untreated)	UV-treated strains			Gentamicin (10 µg/ml)
		1 min	2 min	5 min	
Staphylococcus aureus	12	12	10	0	20
Bacillus subtilis	0	0	0	0	19
Escherichia coli	0	6	6	6	22
Pseudomonas aeruginosa	0	0	4	0	18

Given that the samples tested for antibacterial activity were crude extracts rather than pure compounds, the antibacterial activities demonstrated are noteworthy. In contrast to the antibacterial activity demonstrated by the wild type *L. theobromae*, an improvement in antibacterial activity was achieved in the current investigation by genetic modulation through the UV irradiation. These findings show that the same endophytic fungus might have diverse metabolic profiles in response to UV mutation, and could have resulted in the activation of cryptic genes, which would account for the mutants' enhanced antibacterial activity.

Identification of bioactive compounds

The HPLC chromatogram overlay of the wild type *L. theobromae*, UV-treated *L. theobromae* for 1, 2 and 5 min. comparing the peaks for different compounds in the crude extracts is presented in Figure 1. The UV treatment resulted in a variation of the number of compounds in each extract, evidenced by the observable number of peaks in chromatogram of each extract. Compound **A** was produced by the wild type *L. theobromae* and all mutants. However, the peak was higher in the extract of UV-treated *L. theobromae* for 5 min. It was observed that there was a loss of compound **B** in all *L. theobromae* mutants. Compound **C** was produced only by the UV-treated *L. theobromae* for 2 min. UV-treated *L. theobromae* for 5 min had more peaks (**D** and **E**

extra), implying the production of more compounds than UV-treated *L. theobromae* for 1 and 2 min. Unfortunately, these compounds were not identified using the HPLC analysis.

GC-MS analysis was carried out to identify the actual compounds produced by the different mutants of *L. theobromae* (Table 2). GC-MS analysis of the crude extracts of the wild type *L. theobromae*, and UV-treated *L. theobromae* showed the presence of thirty-four (34) important compounds with a significant percentage compositions and quality match ranging from 70 to 99%. The identified compounds, retention time (RT), peak area (%), molecular weight, and nature of compounds are presented in Table 2. Peak area is directly proportional to the concentration of the compound present in the solvent. Most of the listed compounds detected from the fungal endophytes in this study have been produced by other species of endophytic fungi in other studies, and observed to have varying biological properties (Guimarães *et al*., 2019; Jayaram *et al*., 2021; Khaled *et al*., 2021). The bulk of the active compounds identified by GC-MS in this study are essential oils, which may contribute to the antibacterial activity shown against pathogenic bacteria that are susceptible to them. Specifically, 2,4-di-tert-butylphenol is one of the abundant compounds produced by *L. theobromae*, and its UV-treated mutants, and has been recorded to have several biological activities (Nwobodo *et al*., 2022). This finding is in agreement with the report of Jayaram *et al*. (2021), that 2,4-di-tert-butylphenol is a major compound produced by different endophytes with several therapeutic applications.

Out of the sixteen (16) compounds produced by the wild type *L. theobromae*, all mutants of *L. theobromae* were only able to produce seven (7), which are benzene, 1,4-dichloro-, hexadecane, decane, 2,4-dimethyl-, dodecane, tridecane and 2,4-di-tert-butylphenol. Oxirane, (chloromethyl)- produced by the wild type fungus was not produced by any of the mutants. This means that the mutants must have lost the genes responsible for producing this compound at some time during their exposure to UV light. Notwithstanding, several other unique compounds were produced by each of the mutants as shown in Table 2.

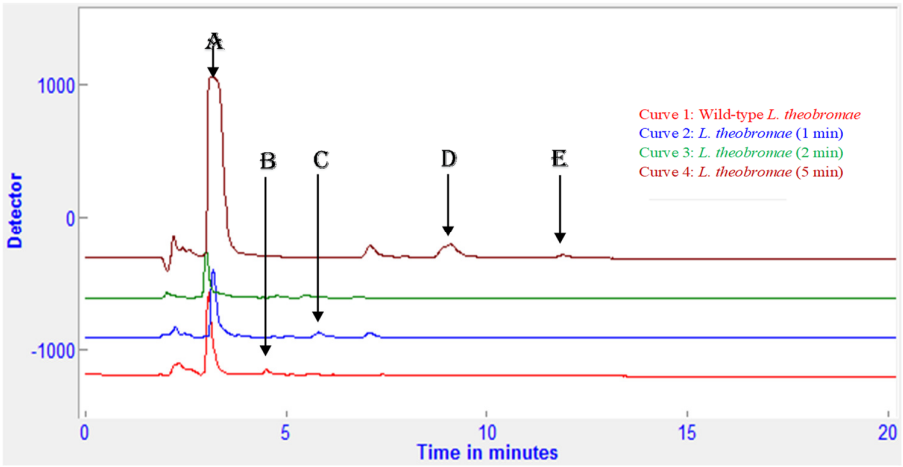

Figure 1. HPLC Chromatogram overlay of the wild type *L. theobromae* and UV-treated *L. theobromae* for 1, 2 and 5 min

Table 2. Comparison of the GC-MS identified compounds in the wild type *L. theobromae* and UV-treated *L. theobromae* extracts

S/n	Name of compound	RT (min)	MW (g/mol)	Nature of compound	Peak area (%)			
					Wild type (untreated)	UV-treated		
						1 min	2 min	5 min
1	Oxirane, (chloromethyl)-	6.496	92.5	Ether	0.39	ND	ND	ND
2	Benzene, 1,4-dichloro-	6.849	147	Aromatic hydrocarbon	1.79	1.50	1.20	0.30
3	M-Cymene	7.203	134	Monoterpenes	ND	ND	4.24	ND
4	Oxalic acid, isobutyl nonyl ester	8.113	272	Carboxylic acid ethyl ester	10.44	9.42	ND	0.45
5	γ-Terpinene	8.165	136	Monoterpenes	ND	ND	6.20	ND
6	Hexadecane	8.644	224	Alkane	1.68	0.64	0.45	0.71
7	Undecane, 3,7-dimethyl-	8.700	184	Alkane	4.80	1.30	ND	1.86
8	Heptadecane, 2,6,10,14-tetramethyl	8.958	296.6	Alkane	10.82	ND	4.53	0.54
9	Dodecane, 2,6,11-trimethyl-	8.958	212	Alkane	ND	7.12	ND	ND
10	4-Methylundecane	9.179	170	Alkane	ND	ND	4.58	ND
11	2-Methyldecane	9.341	156	Alkane	ND	ND	4.01	ND
12	Linalool	9.444	154	Monoterpenes	ND	ND	ND	2.47
13	Decane, 2,4-dimethyl-	10.101	170	Alkane	6.32	4.02	2.80	ND
14	Dodecane	12.262	170	Alkane	1.47	3.93	1.48	1.08
15	Naphthalene	14.950	128	Phenolic	1.51	0.51	ND	ND
16	Tridecane	15.109	184	Alkane	1.73	1.45	1.57	0.38
17	α-Selinene	18.741	204	Sesquiterpene	ND	ND	ND	3.86
18	Humulene	19.257	204	Sesquiterpene	ND	ND	ND	2.00
19	(E)-β-Famesene	19.372	204	Sesquiterpene	ND	ND	ND	4.33
20	β-Cubebene	19.991	204	Sesquiterpene	ND	ND	ND	3.91
21	β-Bisabolene	20.734	204	Sesquiterpene	ND	ND	ND	10.82
22	2,4-Di-tert-butylphenol	20.974	206	Phenolic	7.44	6.67	7.60	1.09
23	β- Sesquiphellandrene	21.104	204	Sesquiterpene	ND	ND	ND	10.58
24	Nerolidol	22.082	222	Sesquiterpene	ND	ND	ND	7.87
25	Cetene	22.690	224	Alkene	2.65	3.40	ND	ND
26	1-Nonadecene	22.690	266	Alkane	ND	ND	2.73	ND
27	Cyclododecane, ethyl-	22.693	196	Alkane	ND	ND	ND	2.98
28	Piperine	29.237	285	Alkaloid	ND	15.0	ND	ND
29	Pentadecanoic acid, 14-methyl-, methyl ester	29.561	256	Fatty acid	0.70	ND	ND	0.18
30	Cycloeicosane	30.256	280.5	Alkane	ND	2.01	2.67	ND
31	1-Octadecene	30.257	252	Alkene	3.18	2.74	2.78	ND
32	14-Octadecenoic acid, methyl ester	31.195	296.5	Fatty acid	ND	ND	6.81	ND
33	Ethyl Oleate	31.666	310.5	Fatty acid ester	0.56	ND	0.39	ND
34	1-Docosene	31.814	308.6	Alkene	2.05	1.85	2.17	ND
Total number of detected compounds					**16**	**15**	**17**	**18**

*ND indicates "no detection" of the particular compound in the fungal crude extract.

The spectra, chemical structures and molecular formulas of selected new compounds produced by the UV treated mutants of *L. theobromae* are presented in Figure 2. Two (2) new compounds dodecane, 2,6,11-trimethyl and piperine amounting to 22.1% of the total constituents were identified in the crude extract of *L.*

theobromae exposed to UV light for 1 min, with piperine (15%) the most abundant compound (Table 2). Both compounds have previously been reported to have antimicrobial activities against pathogenic bacteria. (Kawuri and Darmayasa, 2019). The relatively high concentration of the alkaloid piperine could account for the antibacterial activity exhibited against *E. coli*. This is probably because piperine was not produced by the wild type *L. theobromae*, and its crude extract did not inhibit *E. coli* in this study. This is in agreement with other researchers that, piperine exhibited antimicrobial activity when tested against *S. aureus* and *E. coli* (Amperayani *et al*., 2018; Maitra, 2017).

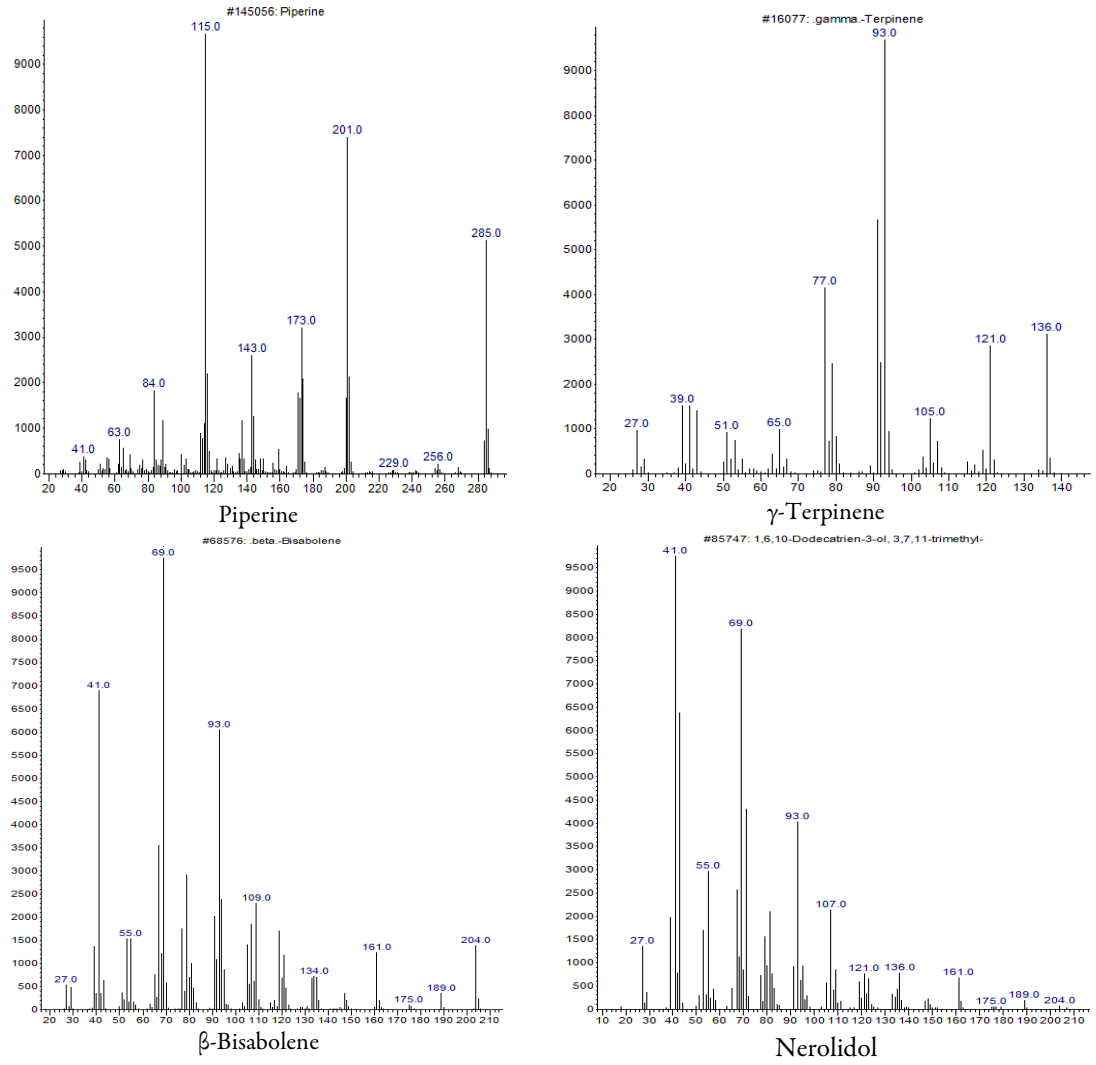

Figure 2. Spectra, chemical structures and molecular formulas of selected new bioactive compounds predominantly produced by UV treated *L. theobromae*

On the other hand, *L. theobromae* UV-treated for 2 min was observed to produce six (6) new compounds constituting 28.5% of the fungal crude extract. These compounds include: m-cymene, γ-terpinene, 4-methylundecane, 2-methyldecane, 1-nonadecene, cycloeicosane, and 14-octadecenoic acid, methyl ester, not identified in the extract of the wild type (Table 2). The fatty acid 14-octadecenoic acid, methyl ester was the most abundant with 6.81% composition, followed by the monoterpene γ-terpinene (6.20%). The extracts of *L. theobromae* UV-treated for 2 min was observed to display an enhanced activity against *E. coli* and *P.*

aeruginosa compared to the wild type fungus. The exposure to UV light no doubt had a positive effect for the production of new compounds, which could be responsible for the improved antibacterial activity observed. Octadecenoic acid have been reported to be produced by several endophytic fungi and possess antibacterial activities against wide range of bacterial species (Sharma *et al*., 2016; Ibrahim *et al*., 2021).

Furthermore, UV treatment of *L. theobromae* for 5 min led to the production of the greatest number of new compounds with the identification of nine (9) compounds. These compounds constitute 49% of the fungal crude extract and were identified as linalool, α-selinene, humulene, (E)-β-famesene, β-cubebene, β-bisabolene, β-sesquiphellandrene, nerolidol and cyclododecane, ethyl (Table 2). A similar outcome was reported by Andersen *et al*. (2013), that a higher number of new compounds (25) was produced by *A. nidulans* mutant, after genetic modulation. The sesquiterpenes β-bisabolene (10.82%), β-sesquiphellandrene (10.58%), were the most abundant compounds identified, followed by nerolidol (7.87%). Although, the extract of *L. theobromae* UV treated for 5 min was able to inhibit *E. coli*, however, it did not display inhibitory activity against any other bacteria, including *S. aureus*. The concentration of compounds such as benzene, 1,4-dichloro-, oxalic acid, isobutyl nonyl ester, dodecane, tridecane, and most importantly 2,4-di-tert-butylphenol, produced by the wild type fungus were low in the extract treated with UV for 5 min. Also, UV treated *L. theobromae* for 5 min did not produce any of decane, 2,4-dimethyl-, 1-octadecene and 1-docosene, that were produced by both the wild type and other mutants. Implying that the mutant treated with UV light for 5 min produced new compounds with antibacterial activity against *E. coli*, while also loosing genes for the biosynthesis of antibacterial compounds against *S. aureus*. Nonetheless, new compounds produced by this mutant are also known to possess several biological activities and can be useful as well.

Conclusions

Results obtained in this study suggest that these reported compounds produced by *L. theobromae* and its UV treated mutants could be responsible for the antibacterial activities demonstrated by their respective crude extracts. There was an increase in antibacterial spectrum after UV treatment of *L. theobromae*, compared to the wild type. The UV irradiation of the fungus yielded seventeen (17) new bioactive compounds that have been reported to be of useful biological and antimicrobial importance with applications in the medical, agricultural and industrial sectors. UV treatment of *L. theobromae* for 1, 2 and 5 min led to the production of two (2), six (6) and nine (9) new compounds respectively. More research into the isolation, characterization, and understanding of the mechanism of action of individual bioactive compounds, as well as subjecting them to various biological activities and assessing their toxicity profile, would surely generate more beneficial results. This will lead to a new approach to drug discovery that is more natural, dependable, cost-effective, and environmentally safe.

Authors' Contributions

Investigation, methodology, data curation and analysis: D.C.N., M.C.U., P.M.E. and U.M.O.; writing – original draft: D.C.N.; writing – review & editing, visualization and validation: D.C.N., M.C.U., P.M.E., U.M.O., and F.B.C.O; conceptualization, methodology, supervision, project administration: F.B.C.O and C.O.E. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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