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# Screening of secondary metabolites produced by a mangroove-derived *Nigrospora species* an endophytic fungus isolated from *Rhizophora racemosa* for antioxidant and antimicrobial properties

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## Abstract

Marine fungi have shown large chemo-diversity of unharnessed important secondary metabolites required for drug development. In this study, endophytic fungi *Nigrospora species* (5S3) was isolated from the fresh stem of *Rhizophora racemosa* from a mangrove forest in Lagos Nigeria and axenic colonies were fermented on sterile rice medium for 21 days. The antimicrobial and antioxidant activities of the extract were evaluated. Chemical analyses such as High Performance Liquid Chromatography Diode Array Detector (HPLC-DAD) and Nuclear magnetic Resonance Imaging (NMR) analyses of selected Vacuum Liquid Chromatography (VLC) fractions revealed the presence of ten known compounds with established biological activities. Tested fractions of the extract exhibited antimicrobial activities against at least one Gram positive and Gram negative bacteria with MIC values that ranged between 0.13 to 1 mg/mL. FE2 and FE3 fractions of the extract demonstrated poor antioxidant activity of less than 10%. Septicine, Aureonitol, Papuamine, Di-iso-octylphtalat, 1-(2,4 Dihydroxy-3,5-Dimethylphenyl)-Ethanone, Cladosporin, Tetrabenzofuran, Dihydrophthalate, 9-Octadecaenoic acid and Eicosane were the compounds detected in the extracts. Our findings reveal that *Nigrospora spp* an endophytic fungi possesses unique chemo diversity of bioactive secondary metabolites that could be utilized for development of new drugs.

**Keywords:** Marine Fungi; *Nigrospora* species*; Rhizophora racemosa*; Anti-microbial activity; Anti-oxidant activity

## 1. Introduction

Plants have long served as a source of bioactive compounds for medicinal use against diverse forms of ailments. [1] It has also been recently found that microorganisms associated with plants can offer products with potentially high therapeutic effect. [2]. In particular, marine fungi have been recognized as a renewable source of secondary metabolites in the drug discovery process [3].

Marine fungi tend to grow in habitats with unique conditions, consequently leading to the activation of metabolic pathways and the synthesis of distinct unknown molecules [4] which help in supporting the adaptation and survival of the fungi in marine ecosystems [5].

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The genera, *Rhizophora* are salt-tolerant mangrove flora located in tropical and subtropical intertidal coastal regions which includes Nigeria.

It is worth noting that of the nearly 300,000 plant species that exist on Earth, any given plant is colonized by several to few hundreds of endophytic fungal species. Only a few of these plants have ever been completely studied as regards to their endophytic biology [6] The monophyletic *Nigrospora* genus belongs to the Apiosporaceae family and is a rich source of novel and diverse bioactive metabolites.

In previous studies, **195** metabolites were discovered in *Rhizophora*-derived endophytic fungi, and their structures are reported within a biogenetic context. Bioassays of previous studies showed antitumor, antimicrobial, as well as anti-H1N1 activities to be the most notable bioactivities of the secondary metabolites observed. [7]

According to Pub Med, two hundred and thirty one (231) compounds have been isolated from five known species and twenty-one unidentified species of *Nigrospora* from January 1991 to June 2022 [8] The structures of these compounds can be attributed to polyketides, terpenoids, steroids, N-containing compounds, and fatty acids.

Furthermore, it appears more feasible economically to produce secondary metabolites in large scale from microorganisms than from plants, and this makes Endophytes an interesting source of new valuable compounds [9].

Hence, this study was carried out in order to investigate the antimicrobial and antioxidant activities of secondary metabolites produced by a mangrove-derived endophytic fungi *Nigrospora species*, and also to identify the bioactive secondary metabolites present in the fungal extract using both High Performance Liquid Chromatography Diode Array Detetctor (HPLC-DAD) and Nuclear Magnetic Resonance Imaging spectroscopy (NMR).

## 2. Materials and Methods

### 2.1. Isolation of Endophytic Fungi, Fermentation and Extraction of Secondary Metabolites

The fresh stem of *Rhizophora racemosa* was harvested from a mangrove forest in Lagos Nigeria. Isolation of endophytic fungi from the plant root was carried out as described by [10]. Harvested healthy plant stem was washed under a running tap water and then cut into small fragments using a sterile knife blade. The fragments were surface-sterilized by soaking for 2 min in 2% sodium hypochlorite solution and then for additional 2 min in 70% ethanol before rinsing off in sterile double distilled water for about 5 min. The internal tissues of the sterilized stem segments were re-exposed by cutting the edges with a sterile knife blade and immediately inoculated into sterile malt extract agar (MEA) supplemented with 500 mg/L Chloramphenicol. The plates were properly sealed with paraffin and incubated at $28^0$C for 5 days. Periodic monitoring of endophytic fungal growth emerging from the cultured stem segment was done and hyphal tips emerging from the cultured stem segments were sub-cultured onto fresh MEA plate. This was done in order to obtain **axenic** colonies. Solid state fermentation of the axenic endophytic fungus was carried out in 1L Erlenmeyer flask containing sterile rice medium (100g of rice + 100 mL of sterile distilled water, autoclaved at $121^0$C at 15 psi for 30 mins). The flask was incubated at $28^0$C for 21 days. Upon completion of fermentation, the biosynthesized secondary metabolites in the fermentation medium were extracted with 500 mL Ethyl acetate, and the extract concentrated under vacuum at $40^0$C using a Rotary Evaporator (Stuart RE400, USA).

### 2.2. Fungal DNA Isolation, Purification, Amplification and Sequencing

The taxonomic identification of the isolated endophyte (5S3) was achieved by DNA amplification and sequencing of the fungal internal transcribed spacer (ITS) region. The fungal genomic DNA was extracted and purified directly from fresh, axenic mycelia using fungal DNA extraction and purification kit (ZR Fungal/Bacterial DNA MiniPrep™ Kit. Catalog No. D60C5. Zymo Research Corp, USA). The molecular identification was performed according to a molecular biologic protocol as described by [11]. The endophytic fungus was identified based on the analysis of the DNA sequences of the internal transcribed spacer region (ITS) of its ribosomal RNA gene

### 2.3. Antimicrobial assay

Preliminary antimicrobial screening of the endophytic fungal extract was carried out using the agar well diffusion assay as described by [12]. Five standardized broth culture of the test bacterial isolates (*S. aureus, B. subtilis, P. aeruginosa, Klebsiella pneumonia* and *E. coli*) and two fungal isolates (*Aspergillus niger,* and *Candida albicans*) were used. A 0.5 McFarland standard bacteria and fungi suspensions of each of the test isolates was applied on sterile Mueller-Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) (LS BIOTECH, USA) respectively, using sterile swab stick. Thereafter, a sterile cork borer was used to make five wells (8 mm in diameter) on each of the MHA and SDA plates. Aliquots of 80

µL of each extract dilution, reconstituted in DMSO at concentrations of 1, 0.5, 0.25, 0.125, 0.0625 mg/mL was applied in each of the wells in the culture plates previously inoculated with the test organisms. Ciprofloxacin (5 µg/mL) and Miconazole (50 µg/mL) served as the positive controls while DMSO served as negative control. The cultures in MHA plates and SDA plates were incubated at $37^0$C for 24 hr and $27^0$C for 48 hours respectively. The antimicrobial potential of each of the extract was determined by measuring the zone of inhibition around each well (excluding the diameter of the well). The Minimum Inhibitory Concentration (MIC) of the active endophytic fungal extract was determined for each of the test organisms that was sensitive in the preliminary screening. Agar dilution method was adopted.

Stock solutions of 2 mg/ml of the various fractions of the extracts were prepared thereafter specific volumes were diluted with specific volumes of sterile molten agar to the desired concentration. For a final concentration of 1mg/mL:

A stock of 10 mg/mL was prepared thereafter two fold serial dilution was done to get graded concentrations such as 5, 2.5, 1.25, 0.625 mg/mL. Then, 1 mL from each of this concentration was transferred into steril Petri dishes and 9 mL of molten agar cooled to 40-$50^0$C was added to it and rocked clock wisely and anticlockwise to ensure proper mixing. Thereafter, a loopful of each of the test organism previously standardized to McFarland turdity was streaked on their respective segment on the solidified agar. The addition of the agar (i:e specific volume) diluted the concentration of the extract to a final concentration of (1, 0.5, 0.25, 0.125, and 0.0625 mg/mL).

Also for negative control the organisms were streaked on the sterile molten agar not containing the extract. The culture plates were then incubated under optimum conditions. After incubation the plates were examined for microbial growth by checking for visible growth, using a plus sign (+) indicating growth (resistance) while a negative sign (-) indicates no growth (inhibition / susceptibility).

### 2.4. Antioxidant Assay

The antioxidant assay was carried out by testing both the free radical scavenging activity on 1,1-diphenyl-2-picryl-hydrazyl (DPPH), and the Ferric Reducing Antioxidant Power (FRAP).

#### *2.4.1. DPPH scavenging assay*

The free radical scavenging activity of the fungal extracts and fractions were evaluated using the method of [13] with some modifications. The free radical scavenging properties of the extracts against 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical were measured at 490 nm. The concentrations of the extracts and ascorbic acid used were 20, 40, 60, 80, and 100 µg/mL The reaction mixture consists of25 µl of the stock, 25 µl of DPPH (0.1 mol/L) and 150 µl of methanol solution. These were added into their respective wells in the microtiter. The plate was incubated at $27^o$C for 30 min**.** The absorbance of the mixtures was measured at 490 nm using a UV-vis spectrophotometer (06452; USA). The experiment was done in triplicate for each fungal extract.

Free radical scavenging activities were expressed as the percentage inhibition of each extract and calculated using the formula:

$$[(Ao - A_1)/ Ao] * 100$$

Ao is the absorbance of the positive control
$A_1$ is the absorbance of the sample solution

#### *2.4.2. Ferric Reducing Antioxidant Power (FRAP) Assay*

FRAP assay was carried out following the method described by [14] Two hundred and fifty micro-litre (0.25 ml) of various fold dilutions of samples as well as 7.82, 15.63, 31.25, 62.5, 125, 250, 500 and 1000 µg/ml ascorbic acid were mixed with 0.625 ml of phosphate buffer and 0.625 ml of 1% potassium ferricyanide [$K_3FeCN_6$]. The mixtures were heated at $50^0$C for twenty minutes. Then, 0.625 ml of 10% trichloroacetic acid (TCA) was added and the mixtures were centrifuged at 3000 rpm for 5 minutes. From the upper layer, 0.625 ml was pipette and mixed with 0.625 ml of distilled water and 0.125 ml of 0.1% (w/v) ferric chloride ($FeCl_3$) solution. Absorbance of the mixture were measured at 700 nm against air using a Spectrophotometer. Ascorbic acid was used as standards. Absorbance were performed in triplicates.

Percentage inhibition was calculated using the formula below.

$$\% \text{ Inhibition} = (\text{Absorbance of sample} - \text{Absorbance of blank}) \times 100$$

For ascorbic acid, a graph of percentage inhibition against concentration was plotted and the effective concentration ($EC_{50}$) was extrapolated using an equation.

High optical density (absorbance) values indicate high FRAP activity.

### 2.5. High Performance Liquid Chromatography (HPLC)

A 2mg of each fungal extract was reconstituted with 2 mL of HPLC grade methanol. The mixture was sonicated for 10 minutes, followed by centrifugation at 3000 rpm for 5 minutes. Then, a volume of 100 µL of the dissolved sample was transferred into HPLC vials containing 500 µL of the HPLC grade methanol. The HPLC analysis was carried out on the samples with a Dionex P 580 HPLC system coupled to a photodiode array detector (UVD340S, DionexSoftron GmbH, Germering, Germany). Detection was at 235 nm. The separation column (125 mm × 4 mm; length × internal diameter) was pre-filled with Eurospher-10 C18 (Knauer, Germany) and a linear gradient of nanopure water (adjusted to pH 2 by addition of formic acid) and methanol was used as eluent. The compounds were detected by comparing the retention times and UV spectral with inbuilt library.

### 2.6. Nuclear Magnetic Resonance Imaging (NMR) Analysis

The $^{1}H$- and $^{13}$ C NMR spectra were measured at room temperature using a Bruker 400 MHz spectrometer. $^{1}H$- 13C NMR data were recorded in $CDCl_3$, with a residual internal solvent signal of $CDCl_3$ as 7.26 and 77.00ppm, respectively.

## 3. Results

**Table 1** DNA sequence of the identified fungi

| Code | Fungal DNA Sequence | Fungal Name |
|---|---|---|
| 10DA10 | CCTCGCGCCCCGGGCGGCCCGCCGGCGGACAAACCAAACTCTGTTATCTTCGTTGATTATCTGA GTGTCTTATTTAATAAGTCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGA ACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAAC GCACATTGCGCCCATTAGTATTCTAGTGGGCATGCCTGTTCGAGCGTCATTTCAACCCCTAAGC ACAGCTTATTGTTGGGCGTCTACGTCTGTAGTGCCTCAAAGACATTGGCGGAGCGGCAGCAGT CCTCTGAGCGTAGTAATTCTTTATCTCGCTTCTGTTAGGCGCTGCCCCCCCGGCCGTAAAACCC CCAATTTTTTCTGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAAT | *Nigrospora sp*. |

**Table 2** Antimicrobial Assay

| Test organisms | Concentration (mg/mL) / Inhibition zone diameter (mm) | | | | | |
|---|---|---|---|---|---|---|
| | **FE2** | | | | | |
| | **1** | **0.5** | **0.25** | **0.13** | **0.06** | **Positive control** |
| *S. a* | 6.5±0.7 | 2.5±0.7 | 0±0 | 0±0 | 0±0 | 8 |
| *B. s* | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 15 |
| *E. c* | 3±0 | 2±0 | 0±0 | 0±0 | 0±0 | 4 |
| *P. a* | 2±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0 |
| *K.p* | 5±0 | 4±0 | 3±0 | 3±0 | 0±0 | 5 |
| *A.n* | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 13 |
| *C.a* | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 11 |
| | FE3 | | | | | |
| | 1 | 0.5 | 0.25 | 0.13 | 0.06 | Positive control |
| *S. a* | 4±0 | 3±0 | 0±0 | 0±0 | 0±0 | 8 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *B. s* | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 15 |
| *E. c* | 4±0 | 0±0 | 0±0 | 0±0 | 0±0 | 4 |
| *P. a* | 2±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0 |
| *K.p* | 3±0 | 2±0 | 3±0 | 3±0 | 0±0 | 5 |
| *A.n* | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 13 |
| *C.a* | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 11 |
| | FE4 | | | | | |
| | 1 | 0.5 | 0.25 | 0.13 | 0.06 | Positive control |
| *S. a* | 4±0 | 3±0 | 0±0 | 0±0 | 0±0 | 8 |
| *B. s* | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 15 |
| *E. c* | 5±0 | 2±0 | 0±0 | 0±0 | 0±0 | 4 |
| *P. a* | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0 |
| *K.p* | 5±0 | 4±0 | 4±0 | 0±0 | 0±0 | 5 |
| *A.n* | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 13 |
| *C.a* | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 11 |
| | FE5 | | | | | |
| | 1 | 0.5 | 0.25 | 0.13 | 0.06 | Positive control |
| *S. a* | 4.5±0.7 | 2±0 | 0±0 | 0±0 | 0±0 | 8 |
| *B. s* | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 15 |
| *E. c* | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 4 |
| *P. a* | 2±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0 |
| *K.p* | 7±0 | 4.5±0.7 | 4±0 | 0±0 | 0±0 | 5 |
| *A.n* | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 13 |
| *C.a* | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 11 |
| | FE6 | | | | | |
| | 1 | 0.5 | 0.25 | 0.13 | 0.06 | Positive control |
| *S. a* | 4.5±0.7 | 2±0 | 0±0 | 0±0 | 0±0 | 8 |
| *B. s* | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 15 |
| *E. c* | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 4 |
| *P. a* | 4±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0 |
| *K.p* | 5±0 | 0±0 | 0±0 | 0±0 | 0±0 | 5 |
| *A.n* | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 13 |
| *C.a* | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 11 |

**Table 3** Minimum Inhibitory Concentration (MIC)

| Extract/fractions | Test Organisms | MIC (mg/mL) |
|---|---|---|
| FE2 | *S.a* | 0.5 |
| | *B.s* | > 1 |
| | *E.c* | 1 |
| | *P.a* | 1 |
| | *K.P* | 0.13 |
| | *A.n* | > 1 |
| | *C.a* | > 1 |
| FE3 | *S.a* | 0.5 |
| | *B.s* | > 1 |
| | *E.c* | 1 |
| | *P.a* | 1 |
| | *K.p* | 1 |
| FE4 | *A.n* | >1 |
| | *C.a* | > 1 |
| | *S.a* | 0.5 |
| | *B.s* | > 1 |
| | *E.c* | 0.5 |
| | *P.a* | > 1 |
| FE5 | *K.p* | 0.5 |
| | *A.n* | > 1 |
| | *C.a* | > 1 |
| | *S.a* | 0.5 |
| | *B.s* | > 1 |
| | *E.c* | > 1 |
| | *P.a* | 1 |
| FE6 | *K.p* | 0.25 |
| | *A.n* | > 1 |
| | *C.a* | > 1 |
| | *S.a* | > 1 |
| | *B.s* | > 1 |
| | *E.c* | > 1 |
| | *P.a* | 1 |
| | *K.p* | 1 |
| | *A.n* | > 1 |
| | *C.a* | > 1 |

Key: *S. a*: *Staphylococcus aureus; B. s: Bacillus subtilis; E. c: Escherichial coli; P. a: Pseudomonas aeruginosa;Klebsiella pneumonia, K.p; A. n:Aspergillusniger; C. a: Candida albicans*

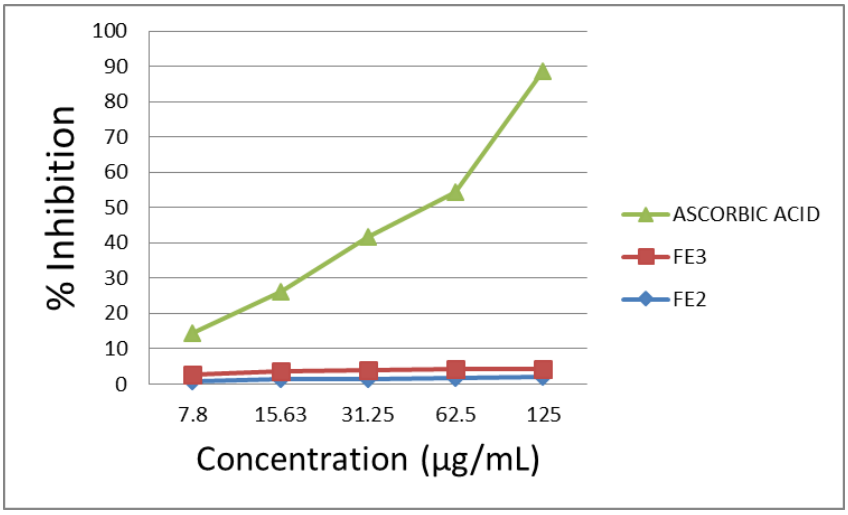



**Figure 1** Antioxidant assay (DPPH)

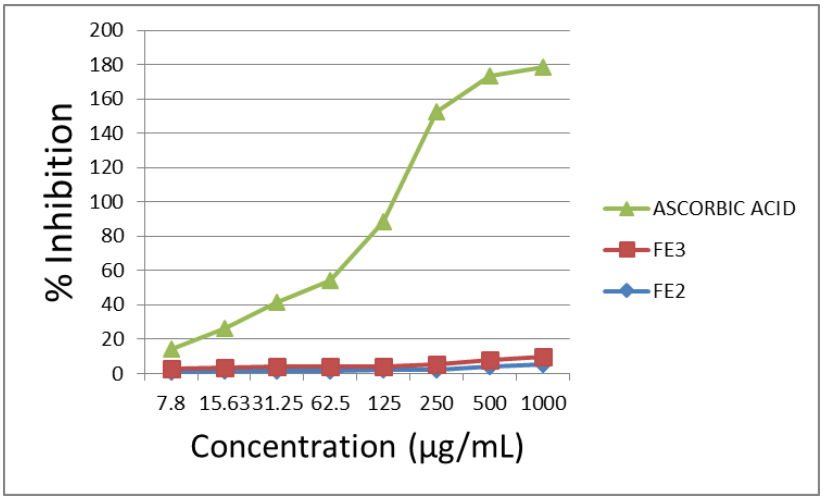
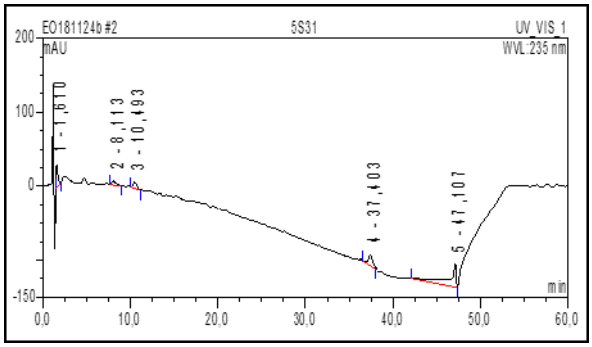
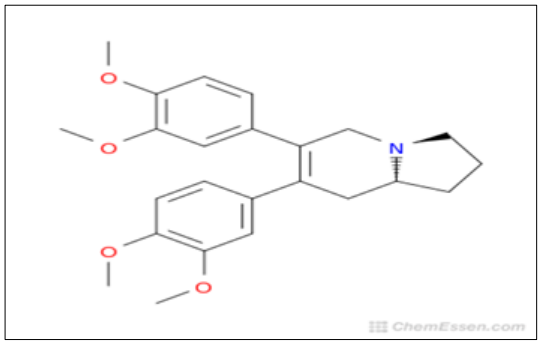
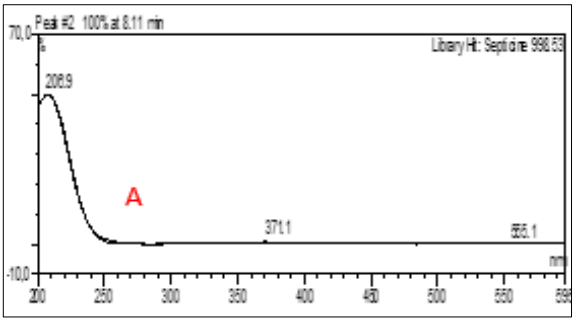



**Figure 2** Antioxidant assay (FRAP)

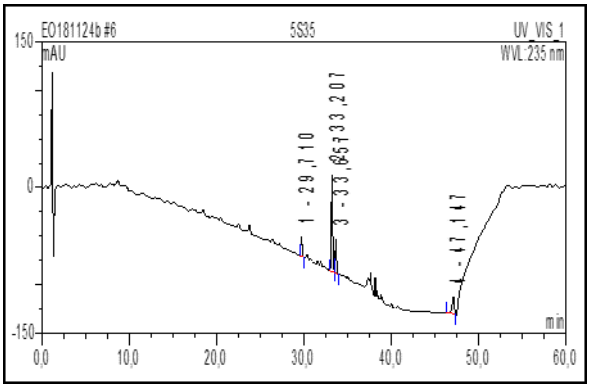
### 3.1. Chromatogram, Structures and UV-spectra of some identified Compounds from fractions of Nigrospora sp.

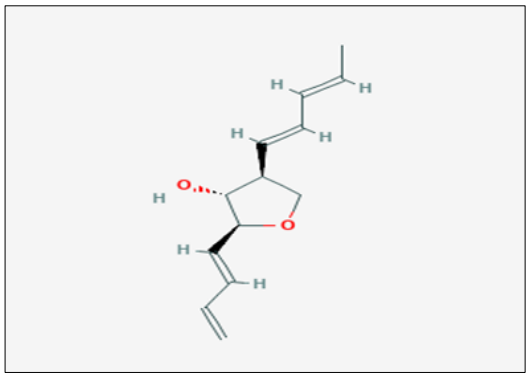


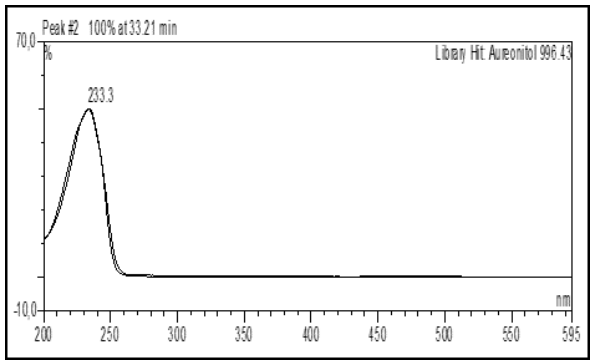





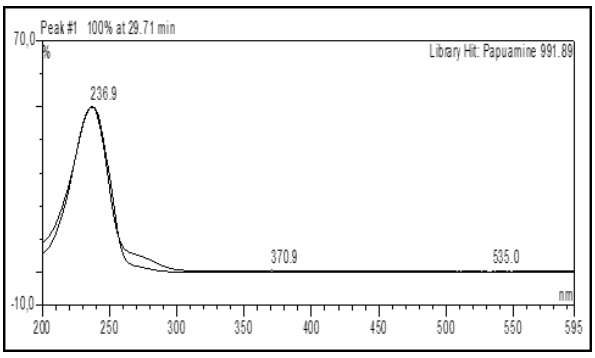

Septicine
Molecular formula: C24H29NO4
Molecular weight: 395.5g/mol
FRACTION: FE1

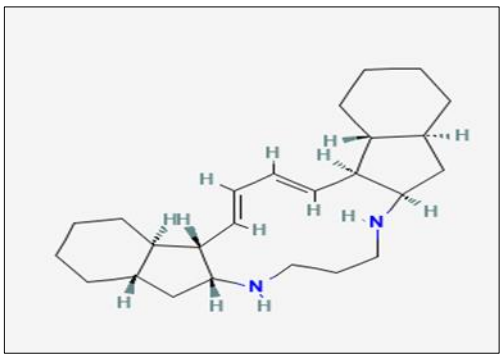


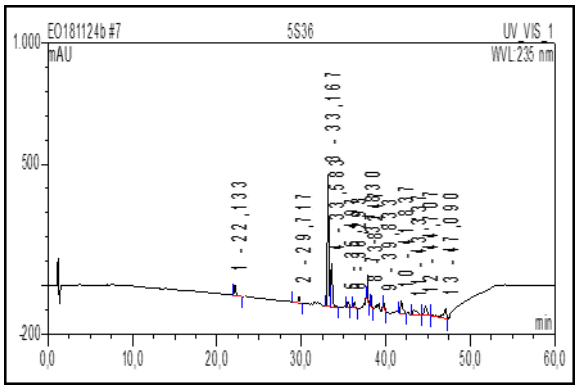


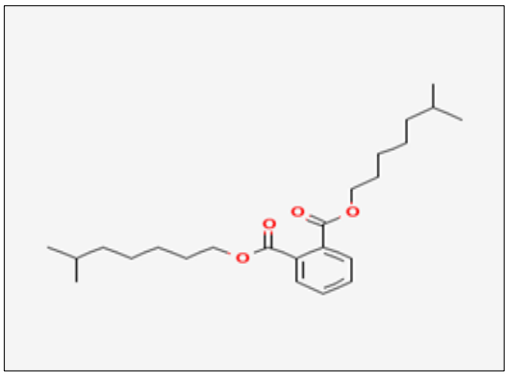

AUREONITOL
MOLECULAR FORMULAR: C13H18O2
MOLECULAR WEIGHT: 206.28g/mol
FE5

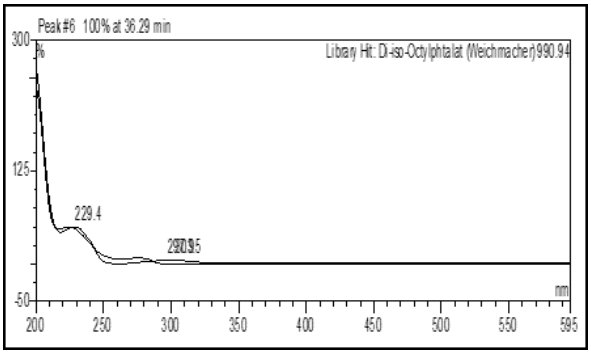


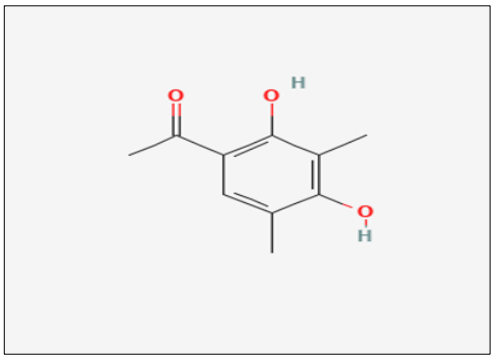



PAPUAMINE
MOLECULAR FORMULAR: C25H40N2
MOLECULAR WEIGHT: 368.6g/mol
FE5





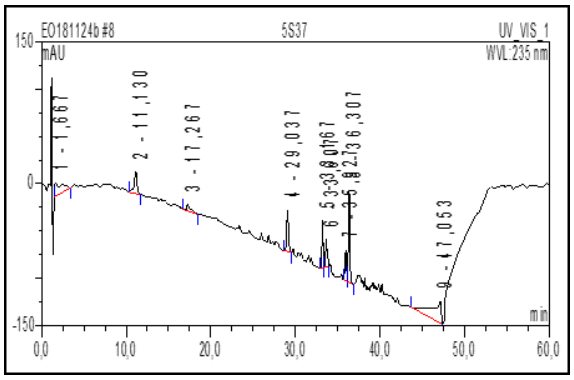



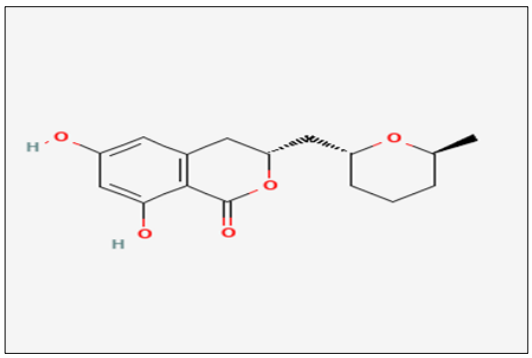
1-(2,4DIHYDROXY-3,5-DIMETHYL PHENYL)-ETHANONE

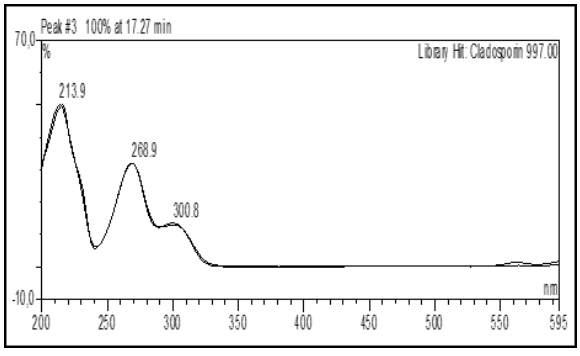
MOLECULAR FORMULAR: C10H12O3

MOLECULAR WEIGHT: 180.20g/mol

FE6






CLADOSPROIN

MOLECULAR FORMULAR: C16H20O5

MOLECULAR WEIGHT: 292.33 g/mol

FE 7

**Figure 3** HPLC Chromatogram and UV- Spectra of major compounds detected from Fractions of *Nigrospora spp*

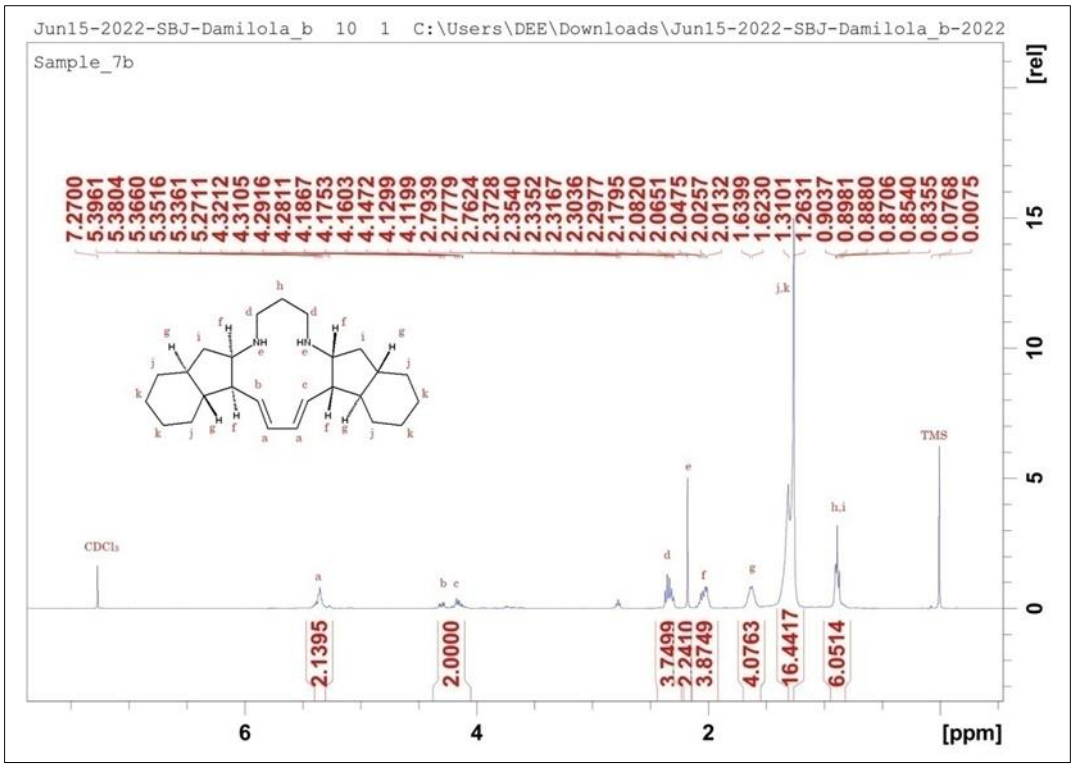



**Figure 4** Structure elucidation based on proton NMR spectra suggesting the presence of papuamine in the sample

Following further purification using Sephadex on Fraction FE4, NMR analysis was also carried out to obtain the following compounds:

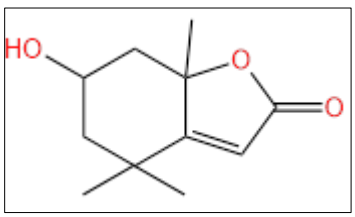
### 3.2. 2D-2 (compound H code)

*3.2.1. 1H-NMR*

Number of signals: 5; chemical shifts:H1 (5.3 ppm, d, J= 0.59Hz), H2 (5.7 ppm, s, J =0.18), H3(725ppm, s, J = 2.74Hz), H4(7.55 ppm, q, J = 0.7 Hz), H5(7.71ppm, q, J = 0.63Hz).

*3.2.2. 13C-NMR*

Nos of carbons: 13 mainly methine carbons at ppm 112.92 and 143.56, quaternary groups at ppm 167.82-18.94.




Compound 2: tetrabenzofuran, mol. wt 212.29

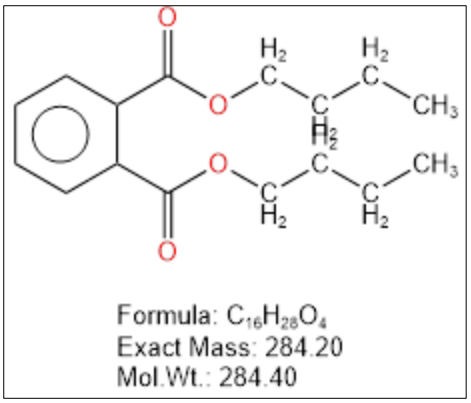
### 3.3. 2D-3

*3.3.1. 1H-NMR*

Number of signals: 5; chemical shifts:H1 (2.85 ppm, d, J= 0.25Hz), H2 (3.25 ppm, d, J =0.48), H3(3.67ppm, s, J = 1.84Hz), H4(3.99 ppm, q, J = 1.84 Hz), H5(4.3ppm, q, J = 2.24 Hz).

*3.3.2. 13C-NMR*

Nos of carbons: 8 mainly carbonyls carbons at ppm 76-79.




Compound 3: dihydrophthalate , mol. wt 284.40

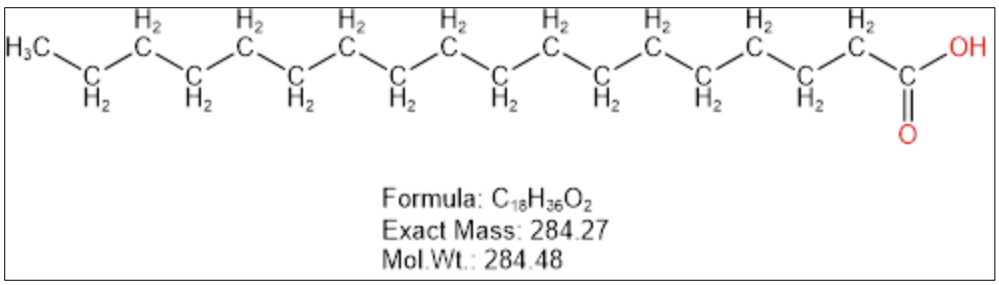
### 3.4. 2D-4

*3.4.1. 1H-NMR*

Number of signals: 6; chemical shifts:H1 (1.85,2.05 ppm, m, J= 1.92Hz), H2 (2.20 ppm, q, J =0.77), H3(2.35ppm, m, J = 1.24 Hz), H4(2.45 ppm, d, J = 0 Hz), H5(2.55 ppm, m, J = 0 Hz), H6 (2.60 ppm, s, J = 0).

*3.4.2. 13C-NMR*

Nos of carbons: 20; CH3 carbons at ppm 38.39-39.47, CH2 carbons at ppm 36.69-37.05, CH at ppm 41.01-41.96, and carbonyl carbons at ppm 45.55-47.94.




Compound 4:9-Octadecanoic acid

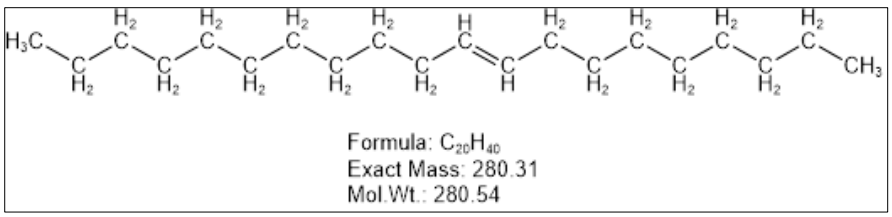
### 3.5. 2D-5

*3.5.1. 1H-NMR*

Number of signals: 8; chemical shifts:H1 (0.78 ppm, t, J= 2.39 Hz), H2 (0.8-1.0 ppm, m, J = 11.47), H3(1.08 ppm, s, J = 0.97 Hz), H4(1.13 ppm, s, J = 0.66 Hz), H5(1.2-1.3 ppm, m, J = 16.12 Hz), H6 (1.42-1.47 ppm, m, J = 10.34 Hz), H7 (1.70-1.73 ppm, m, J = 10.36Hz), H8(1.78 ppm, s, J = 10.36 Hz).

*3.5.2. 13C-NMR*

Nos of carbons: 29; CH2 carbons at ppm 23.73-28.14, CH carbons at ppm 30.19-31.95. No carbonyl carbons seen.




Compound 5: Eicosane, mol. wt 280.54

## 4. Discussion

In this study, the chemical analysis and bioassay of the secondary metabolite produced by the endophytic extract were evaluated.

From the antimicrobial test result, the various fractions demonstrated antimicrobial activity against at least one of the test isolates at concentrations of 1mg/ml. The activities of the various fractions of the endophytic extract against the different test organisms varied. This is evident by the varying inhibition zones produced which ranged from 4-6mm (*S. aureus*), 3-5mm (*E.coli*), 2-4mm (*P. aeruginosa*) and 3-7mm (*K. pneumonia*). However, non of the tested fractions had activity against *B. subtilis, C.Albican* and *A. niger* at the tested concentration. The activities demonstrated by FE2, FE3, FE4, FE5 and FE6 were observed to be broad spectrum. This can be attributed to the combined activities of the bioactive constituents present in each fraction.

DPPH (2,2-diphenyl-1-picrylhydrazyl) is considered an accurate, valid and easy method to evaluate radical scavenging activity of antioxidants [15]. The assay is based on the measurement of the scavenging capacity of antioxidants towards it. On mixing DPPH solution with a substance that can donate hydrogen atom, it gives rise to the reduced form with the loss of violet colour [16] From the results, weak scavenging activity was exhibited by FE2 and FE3 as depicted by their poor percentage inhibitions in both DPPH and FRAP analysis.

IC50 values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals. From this study, we observed that as the concentration increases, the absorbance value also increases. Previous studies showed that many bioactive compounds produced by endophytic fungi exhibit antioxidant, anticancer, anti-inflammatory, antimicrobial and other biological activities [17][18]

The DPPH scavenging and Reducing Powers (FRAP) were based on different mechanisms of antioxidant assay and results from both methods provided a basis for comparison. Furthermore, it provides more reason to believe the extract possessed weak antioxidant activity since it has been suggested that antioxidant activity should not be concluded based on a single test model [19].

As a result of the recorded antibacterial activity, the endophytic fungi isolated in this work, *Nigrospora spp* exhibited potential as source of bioactive agents needed for the development of new medicines therefore, it was expedient to assess the chemo diversity of this fungal extract. A preliminary investigation of the secondary metabolic profiles of some of the fractions of the isolated endophytic fungi isolated in this study using HPLC-DAD system was determined according to the clear peaks detected in the chromatogram. Also, further structural elucidation was carried out using the Nuclear magnetic Resonance Imaging (NMR) Spectroscopy to gain clarity on structural details. These compounds have also been previously isolated from both fungi and plant extract be several authors at sundry times and reported to possess various biological activities including antimicrobial activity.

Septicine (Library hit, 998) is a natural product with a molecular weight of 395.5g/mol and molecular formular of $C_{24}H_{29}NO_4$. It is a biogenetic precursor of the alkaloid tylophorine and indolizidine alkaloid, which has been reported to possess Antibacterial activity. [20] Aureonitol (Library hit 996) with molecular formula of $C_{13}H_{18}O_2$ molecular weight of 206.28g/mol is a fungi-derived tetrahydrofuran. It has been shown to inhibit influenza replication by targeting its surface glycoprotein hemaglutinin, hence its antiviral property [21]

Papuamine (Library hit, 991) is a natural product also found in *Neopestrosia chaliniformis*, *Neopetrosia exigua*, and Haliclona. Papuamine is apentacyclic alkaloid with antifungal and antimicrobial activitiy. The compound has a C2-symmetric structure and was originally isolated from marine sponges of the genus *Haliclona.* Papuamine has been shown to inhibit the growth of the dermatophyte *Tricophyton mentagrophytes* and can be screened with the use of *Mycobacterium marinum*.[22]

Di-iso-octylphtalat (Library hit, 990) is a phthalate ester and diester. Also referred to as Diop and is a natural product found in lythrumsalicaria and Streptomyces parvulus.It has a molecular mass of 390.27mg. Phthalic acid esters are broadly known to possess among other biological properties antimicrobial, insecticidal activity [23]. However, the synthesized Phthalic Acid Esters (PAE) are considered to pose potential hazards to ecosystem functioning hence requiring further studies to evaluate and improve its safety profile for human use.

Cladosporin (Library hit, 997) also known as (asperentin), 3,4-dihydroxy-3-(6-methyl) isocoumarin, is an important secondary metabolite isolated from *Cladosporium cladosporioides* in 1971. *Cladosporium chaetomiumglobosum* and

*Aspergillus flavus*. Cladosporin exhibited potent antibacterial and antifungal properties against *Cryptococcus neoformans* in an experiment carried out by [24].

Structures similar to 9-Octadecanoic acid has been showed to posses potent antimicrobial activity against Methicillin-resistant *Staphylococcus aureaus* (MRSA) [25]

In a study done by Zona Octarya *et al* 2021 [26], Eicosane was discovered among other compounds in a study to evaluate the antimicrobial activity of the bioactive constituents of *Aspergillus* fumigates 269 isolated from Sungai Pinang Hot Spring, Riau, Indonesia.

## 5. Conclusion

In conclusion, in this study we isolated and identified *Nigrospora spp,* an endophytic fungi from a Marine plant, *Rhizophora recemosa* and showed that it is a good source for exploration of secondary metabolites with antimicrobial effect. Also, with the aid of a standard chemical (HPLC) analytic protocol and NMR Spectroscopy, several classes of bioactive secondary metabolites with varying biological activities were detected in the endophytic extract.

The present work has shown that the VLC fractions from the ethyl acetate extract of *Nigrospora spp* showed good antimicrobial properties and exhibited weak antioxidant activity when assayed with DPPH model and FRAP models.

The secondary metabolites present in this endophytic fungi have potential for pharmaceutical application given that they have been previously reported to possess antimicrobial properties. However, large-scale harvesting of these plants could untowardly affect climate change. Alternatively, endophytic fungi from these plant leaves could serve as an eco-friendly sustainable source of natural antimicrobials for pharmaceutical industries.

## Compliance with ethical standards

### *Acknowledgments*

The authors are grateful to Prof. Dr. Peter Proksch of the Institute of Pharmaceutical Biology and Biotechnology, Heinrich Heine University, Düsseldorf in whose laboratory the HPLC-DAD was carried out.

### *Disclosure of conflict of interest*

The authors declare no conflict of interest

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