# Antibacterial Activity and Characterization of Bioactive Compounds of Endophytic Fungi Isolated from Nipa Palm Leaves

**Akinduyite,*A.E and Ariole, C.N.**
Department of Microbiology, University of Port Harcourt, P.M.B. 5323
Port Harcourt, Rivers State, Nigeria.
Corresponding author: Email:adukeakinduyite@yahoo.com

**Abstract:** Microbial bioactive compounds are biologically active microbial compounds that are synthesized from microorganisms which exhibit antimicrobial, antitumor, antiviral activities and so on. This study aimed at determining the antibacterial activity, quantification and characterisation of bioactive compounds in the methanolic extract of endophytic fungi isolated from Nipa palm (*Nypa fructicans* Wurmb.) leaves. Healthy leaves of Nipa palm were collected randomly from Gbalajam Mangrove Swamp in Port Harcourt, Rivers state, Nigeria. The leaves were washed, cut, surface sterilized and plated on acidified Potato dextrose agar for 5 days at 30$^{o}$C. Fungal colonies were identified based on their morphological and microscopic characteristics. Bioactive compounds production was carried out by sub-merged fermentation of fungi at 30$^{o}$C for 21days and extracted with methanol. Extracts were screened against some pathogenic bacteria using agar well diffusion assay at different concentrations. Molecular identification of isolate with maximum zone of inhibition was done using PCR (Polymerase Chain Reaction) and 18S rRNA sequencing. The bioactive compounds in its extract were characterised and quantified using GC-MS (Gas chromatography – Mass spectrometry) analysis. Seven endophytic fungi were isolated namely; *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., *Pestalotiopsis* sp., *Phomopsis* sp., *Nigrospora* sp. and*Rhizopus* sp. Methanolic extract of *Aspergillus* sp. that was identified as *Aspergillus fumigatus* KU350620.1 exhibited maximum zones of inhibition. Eight bioactive compounds were characterised and quantified from the extract. Hexadecanoic acid, methyl ester ($C_{17}H_{34}O$) at 30.823% peak area was identified as the most active compound in the extract. The results of this study showed that Nipa palm leaves – endophytic fungal extract have antibacterial potential against pathogenic bacteria and the bioactive compounds from the extract can be used for drug development, industrial, agricultural and other biotechnological purposes.

**Keywords:** Bioactive compounds, Endophytic fungi, Gas Chromatography, Molecular identification

## INTRODUCTION

Nipa palm also known as *Nypa fruticans* Wurmb. is a special kind of palm tree and one of the oldest angiosperm plants that strive well and has the ability to survive and grow luxuriantly in mangrove wetlands (Hossain and Islam, 2015). It is a stem-less palm with tall erect fronds and underground rhizomatous stem possessing an extensive root system, well suited to resist swift running water. The flowers produce woody nuts which are arranged in globular cluster across their stalks (Kathiresan and Bingham, 2001). They can dominate in a simple water channel or complex tributaries, bays, tidal flats and creeks, as long as there is a tide and a freshwater outflow action (Udofia and Udo, 2005). In Nigeria, Nipa palm has become an invasive species, it has propagates aggressively and colonizing vast parts of our large indigenous mangrove ecosystem (Chukwuebuka *et al*., 2020).

Nipa palm - endophytic fungi are fungi that inhabit the internal tissues of Nipa palm without causing any symptomatic negative effect on the host plant (Ariole and Akinduyite, 2016). Every plant is known to be a host to at least one endophytic microorganism (Gianluca *et al*., 2020). The plant provides energy, nutrients and shelter for the endophytic fungi and also protects them from environmental stress (Dhanya and Padmavathy, 2014). On the other hand, the endophytic fungi defend the plant biologically against foreign phytopathogens by releasing metabolites to attack any antagonists, or indirectly by inducing host defence mechanisms through the production of phytohormones (Handayani *et al*., 2017). They also produce bioactive compounds and enzymes, which aid the plants adaptation to abiotic stresses such as high salinity, excessive light, high temperature and drought (Sadeer *et al.*, 2020).

Bioactive compounds are products of secondary metabolism that are synthesize through fermentation process (Jolanta *et al*., 2012). The search for novel bioactive compounds emanate generally as a result of pathogenic microorganisms resistance to most chemically synthesized antimicrobial drugs (Hema *et al*., 2011). Bioactive compounds and antibacterial activity of Black mangrove leaves' endophytic fungi has been reported by Akinduyite and Ariole, (2018). However, the bio-prospecting of endophytic fungi from Nipa-palm for the production of bioactive compounds has been found to be at the stage of infancy and adequate efforts should be made for its utilization thereby necessitating the need for this study. The objectives of this study are to determine the antibacterial potentials of Nipa palm-endophytic fungal extracts against some pathogenic bacteria, identify the most active fungal isolate through molecular identification, characterise and quantify the bioactive compounds in its methanolic extract through Gas chromatography- mass spectrometry analysis. It is noteworthy that an endophytic fungus, if well explored, may produce not only one but numerous unique bioactive compounds in large quantity with various agrochemicals, pharmaceutical, industrial and environmental importance (Akinduyite and Ariole, 2018).

## MATERIALS AND METHODS

### Collection of samples

#### i. Leaf samples

Healthy leaves of Nipa palm (*Nypa fructicans* Wurmb.) were collected randomly from Gbalajam Mangrove Swamp in Port Harcourt, River State, Nigeria and transported to the University of Port Harcourt Microbiology laboratory in labelled sterile Ziploc bags placed in ice box for processing.

#### ii. Marine pathogenic bacteria

Pure cultures of pathogenic bacteria (*Staphylococcus aureus, Salmonella typhi, Shigella dysentriae*, *Vibrio cholerae* and *Vibrio parahaemolyticus*) were obtained from the culture collection room of the Environmental Microbiology Laboratory of the Microbiology Department of University of Port Harcourt, Nigeria.

### Isolation and identification of Endophytic fungi

The leaves were washed, cut into 5 mm pieces and allowed to air dry. The air dried leaves samples were surface sterilized by immersion into100ml of 75% ethanol for 5 minutes, 0.5% sodium hypochlorite solution for 1 minute, rinsed in sterile distilled water twice for 5minutes and air-dried on sterile filter paper. The surface- sterilized leaves fragments were placed on Potato Dextrose Agar (PDA) seeded with lactic acid and incubated at 30$^{o}$C for 5 days. Fungal growth from the incubated plates were sub-cultured to obtain pure endophytic fungal isolates, transferred onto freshly prepared potato dextrose agar slants and stored at 4°C for further studies. The pure endophytic fungal isolates were identified based on their morphological and microscopic characteristics.

### Production of bioactive compounds through submerged fermentation

Five-day old culture of the isolated endophytic fungi was prepared and the surface was washed with sterile saline water. The cell turbidity was adjusted to $3\times 10^{-8}$cfu/ml. 10ml of the cell solution was transferred into 500 ml Erlenmeyer conical flask containing 300 ml of sterilized Potato dextrose broth and incubated at 30˚C for 21 days under a stationary condition. The cultured broth was filtered through a sterile Whatmann filter paper. The fungal mycelium was soaked in 100 ml of 70 % (v/v) methanol for 2 days, the mixture was filtered and the filtrate was collected and evaporated at 45$^{o}$C to dryness using the rotary evaporator to obtain the Methanolic extract. Extracts were weighed, dissolved in Dimethylsulfoxide (DMSO) and stored at -16$^{o}$C for Antibacterial assay and GC-MS analysis.

**Bioactivity (Antibacterial) Assay**

Fresh cultures of the pathogenic bacteria (*Staphylococcus aureus, Salmonella typhi, Shigella dysentriae, Vibrio cholerae* and *Vibrio parahaemolyticus*) were inoculated into nutrient broth and incubated for 24 hours at 37°C. Muller Hinton agar was also prepared and pour-plated for antibacterial sensitivity test. The cell density of the pathogenic bacteria in the incubated nutrient broth culture was adjusted to $10^{-8}$cfu/ml by serial dilution equivalent to a 0.5 McFarland turbidity standard and inoculated on Mueller-Hinton agar surface in a laminar flow cabinet.

Agar wells were made equidistantly on the inoculated Mueller-Hinton agar surfaces using a sterile cork borer of 6 mm diameter. Each well was loaded with 0.1ml of the fungal extracts at different concentration (20, 40, 60, 80 and 100 mg/ml) respectively. Wells loaded with 0.1ml of a standard antibiotic (Chloramphenicol solution at 100 mg/ml) and 0.1ml of sterile distilled water served as positive and negative controls respectively. The plates were incubated at 37$^{o}$C for 24 hours, inhibition zone around the well was recorded and expressed in millimetre.

**Molecular identification of Nipa palm - endophytic fungi**

Fungal isolate with maximum zones of inhibition was further identified through molecular identification using Polymerase Chain Reaction (PCR).

DNA extraction of the isolated endophytic fungi was done using Norgen's Fungi/Yeast Genomic DNA Isolation Kit. Genomic DNA was extracted and purified from the fungal cells by a combination of the use of heat treatment, detergents, use of bead tubes and spin column chromatography. The purified genomic DNA was digested with restriction enzymes and quantified by making serial dilution of known DNA standard. Serial dilution of 1 in 10 to 1 in 80 was plotted against their absorbance at optical density of 450nm to have standard concentrations at pico moles. The analysis was done using the Myassays software. Absorbance was measured in microplate reader thermomax Molecular devices.

Polymerase chain reaction (PCR) was carried out using the PCR master mix from Norgen biotek Canada according to the manufacturer's instruction. The PCR product was separated on a 1.5 % agarose gel (Solis Biodyne, Estonia). One hundred base pair DNA ladder (Solis Biodyne, Estonia) was used as DNA molecular weight marker. Electrophoresis was done at 80V for 1 hour and 30 minutes, and the gel was viewed under UV light after staining with ethidium bromide (Solis Biodyne, Estonia).

The sequence generated by the sequencer was visualized using Chromaslite for base calling. Bio Edit was used for sequence editing, before performing a Basic Local Alignment Search Tool (BLAST) using NCBI (National Centre for Biotechnology Information) database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Similar sequences were downloaded and aligned with ClusterW and phylogenetic tree was drawn with MEGA 6 software.

**Gas chromatography-mass spectrometry (GC-MS) analysis**

Bioactive compounds in the endophytic fungal extract that exhibited maximum zones of inhibition were characterised using Gas Chromatography – Mass Spectrometry analysis using Agilent 7890A-5975C GC-MS system.

One millimetre of the fungal extract was placed in a vial and injected into the Gas Chromatography – Mass Spectrometer system. Separation of compounds was conducted on a 60m HP-INNOWAX capillary column of 0.25 mm, using nitrogen as a carrier gas. The injector temperature was 250$^{o}$C with the volume of 0.5 ul. The carrier gas flow was 1ml/min which has a split ratio of 10:1. The temperature of the oven initially was 110$^{o}$C to 200$^{o}$C at 10$^{o}$C /min and then increased to 200$^{o}$C to 280$^{o}$C at 5$^{o}$C/minute and held for 9 minutes. Thereafter, mass spectra were taken at 70 eV.

Identification of mass spectrum of the Gas chromatography – mass spectrometry (GC-MS) analysis was conducted using the NIST (National Institute of Standards and Technology) Database.

## RESULTS

### Isolation and identification of endophytic fungi

Seven endophytic fungal isolates were isolated from the Nipa palm (*Nypa fructicans* Wurmb.) leaves used in this study and were identified as: *Fusarium* sp., *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp., *Phomopsis* sp., *Pestaloptiopsis* sp. and *Nigrospora* sp. respectively

### Antibacterial assay

Seven methanolic extracts obtained from the endophytic fungal isolates were screened against the five pathogenic bacteria (*Staphylococcus aureus, Salmonella typhi, Shigella dysentriae, Vibrio cholerae* and *Vibrio parahaemolyticus*) at different concentrations (20, 40, 60, 80 and 100 mg/ml) with Chloramphenicol (100 mg/ml) and sterile distilled water as positive and negative controls respectively and the diameter of inhibition around the wells were measured in millimetre and were presented in Table 1 to Table 4.

**Table 1**: **Antimicrobial activity of methanolic extract of Nipa palm-endophytic fungi against pathogenic bacteria**

| Isolate code | Isolate identity | Fungal extracts' Concentration (mg/ml) | Inhibition zone (mm) ±S.D. Pathogenic Bacteria | | | | |
|---|---|---|---|---|---|---|---|
| | | | ***Staphylococcus aureus*** | ***Salmonella typhi*** | ***Shigella dysentriae*** | ***Vibrio cholerae*** | ***Vibrio parahaemolyticus*** |
| **NA1** | *Phomopsis* sp. | 20 | 6.00±0.00 | 3.00±0.00 | 2.00±0.00 | - | 1.00±0.00 |
| | | 40 | 8.33±0.58 | 6.67±0.58 | 3.67±0.58 | 1.33±0.58 | 3.67±0.58 |
| | | 60 | 9.00±1.00 | 8.00±0.00 | 5.00±0.00 | 3.00±0.00 | 4.00±1.00 |
| | | 80 | 10.67±0.58 | 8.33±0.58 | 8.00±0.00 | 5.00±0.00 | 6.00±0.00 |
| | | 100 | 12.33±0.58 | 9.00±1.00 | 8.33±0.58 | 8.33±0.58 | 8.00±0.00 |
| **NB2** | *Pestalotiopsis* sp. | 20 | 3.67±0.58 | 2.00±0.00 | 1.33±0.58 | 1.00±0.00 | - |
| | | 40 | 6.67±0.58 | 5.00±0.00 | 5.33±0.58 | 4.00±1.00 | 2.00±1.00 |
| | | 60 | 8.33±0.58 | 8.33±0.58 | 8.00±0.00 | 6.67±0.58 | 5.00±0.00 |
| | | 80 | 9.00±1.00 | 10.67±0.58 | 9.00±1.00 | 8.00±0.00 | 8.00±0.00 |
| | | 100 | 10.67±0.58 | 11.00±0.00 | 10.67±0.58 | 8.33±0.58 | 8.33±0.58 |

KEY: - = no zone of inhibition +ve = Positive Control -ve = Negative Control
mm = millimetre S.D = Standard deviation.

**Table 2**: **Antimicrobial activity of methanolic extract of Nipa palm-endophytic fungi against pathogenic bacteria**

| Isolate code | Isolate identity | Fungal extracts' Concentration (mg/ml) | Inhibition zone (mm) ±S.D. Pathogenic Bacteria | | | | |
|---|---|---|---|---|---|---|---|
| | | | ***Staphylococcus aureus*** | ***Salmonella typhi*** | ***Shigella dysentriae*** | ***Vibrio cholerae*** | ***Vibrio parahaemolyticus*** |
| **NC3** | *Fusarium* sp. | 20 | 8.00±0.00 | 5.00±0.00 | 4.00±1.00 | - | - |
| | | 40 | 9.00±1.00 | 8.00±0.00 | 6.67±0.58 | 1.33±0.58 | 2.00±0.00 |
| | | 60 | 10.00±1.00 | 8.33±0.58 | 8.33±0.58 | 3.67±0.58 | 5.00±0.00 |
| | | 80 | 10.67±0.58 | 9.00±1.00 | 10.00±0.00 | 5.00±0.00 | 6.67±0.58 |
| | | 100 | 11.00±0.00 | 10.00±1.00 | 10.67±0.58 | 8.33±0.58 | 8.00±0.00 |
| **ND4** | *Penicillium* sp. | 20 | 6.00±0.00 | 2.00±0.00 | 1.00±0.00 | 1.00±0.00 | 1.00±0.00 |
| | | 40 | 8.00±0.00 | 3.00±0.00 | 3.67±0.58 | 2.00±0.00 | 3.00±0.00 |
| | | 60 | 12.33±0.58 | 6.67±0.58 | 6.00±0.00 | 3.67±0.58 | 5.00±0.00 |
| | | 80 | 14.67±0.58 | 8.33±0.58 | 9.00±1.00 | 6.00±0.00 | 6.67±0.58 |
| | | 100 | 16.00±1.00 | 10.33±0.58 | 11.00±1.0 | 8.00±0.00 | 8.33±0.58 |

**Table 3**: **Antimicrobial activity of methanolic extract of Nipa palm-endophytic fungi against pathogenic bacteria**

| Isolate code | Isolate identity | Fungal extracts' Concentration (mg/ml) | Inhibition zone (mm) ±S.D. Pathogenic Bacteria | | | | |
|---|---|---|---|---|---|---|---|
| | | | ***Staphylococcus aureus*** | ***Salmonella typhi*** | ***Shigella dysentriae*** | ***Vibrio cholerae*** | ***Vibrio parahaemolyticus*** |
| **NE5** | *Aspergillus* sp. | 20 | 12.00±1.00 | 6.67±0.58 | 8.00±0.00 | 5.00±0.00 | 3.67±0.58 |
| | | 40 | 15.00±0.00 | 8.00±0.00 | 8.33±0.58 | 6.67±0.58 | 4.00±1.00 |
| | | 60 | 18.67±0.58 | 10.33±0.58 | 9.00±1.00 | 8.33±0.58 | 6.00±0.00 |
| | | 80 | 20.00±1.00 | 11.00±0.00 | 10.67±0.58 | 9.00±1.00 | 8.00±0.00 |
| | | 100 | 23.33±0.58 | 14.67±0.58 | 12.33±0.58 | 11.00±0.0 | 10.00±1.0 |
| **NF6** | *Rhizopus* sp. | 20 | 3.67±0.58 | 1.33±0.58 | 2.00±0.00 | 1.00±0.00 | 1.33±0.58 |
| | | 40 | 5.00±0.00 | 4.00±1.00 | 3.67±0.58 | 1.33±0.58 | 3.00±0.00 |
| | | 60 | 8.00±0.00 | 6.67±0.58 | 6.00±0.00 | 4.00±1.00 | 5.00±0.00 |
| | | 80 | 11.00±0.00 | 8.33±0.58 | 8.00±0.00 | 6.00±0.00 | 6.67±0.58 |
| | | 100 | 14.67±0.58 | 10.33±0.58 | 11.00±0.0 | 9.00±1.00 | 10.67±0.58 |

**Table 4**: **Antimicrobial activity of methanolic extract of Nipa palm-endophytic fungi against pathogenic bacteria**

| Isolate code | Isolate identity | Fungal extracts' Concentration (mg/ml) | Inhibition zone (mm) ±S.D. Pathogenic Bacteria | | | | |
|---|---|---|---|---|---|---|---|
| | | | ***Staphylococcus aureus*** | ***Salmonella Typhi*** | ***Shigella dysentriae*** | ***Vibrio cholerae*** | ***Vibrio parahaemolyticus*** |
| **NG7** | *Nigrospora* sp. | 20 | 2.00±0.00 | 1.33±0.58 | 1.00±0.00 | - | - |
| | | 40 | 3.67±0.58 | 2.00±0.00 | 2.00±0.00 | 1.33±0.58 | 1.00±0.00 |
| | | 60 | 6.00±0.00 | 4.00±1.00 | 3.67±0.58 | 3.00±0.00 | 3.67±0.58 |
| | | 80 | 8.33±0.58 | 5.00±0.00 | 6.67±0.58 | 4.00±1.00 | 5.00±0.00 |
| | | 100 | 9.67±0.58 | 8.00±0.00 | 8.33±0.58 | 6.00±0.00 | 6.67±0.58 |
| **+VE** | Chloramphenicol | 100 | 25. 00±1.00 | 23.00±1.00 | 20.00±1.00 | 18.00±1.0 | 19.00±1.00 |
| **-VE** | Sterile distilled water | 0 | - | - | - | - | - |

**KEY: - = no zone of inhibition +ve = Positive Control -ve = Negative Control**
**mm = millimetre S.D = Standard deviation.**

**Molecular Identification**

The fungal isolate with the maximum zone of inhibition was identified as *Aspergillus fumigatus* KU350620.1 (NE5) with percentage identity of 18S rDNA of the isolates as reported by Tamura *et al.,* (2013). 98% as presented in Figure 1. The evolutionary distances computed using the Jukes-Cantor method aligned with the phylogenetic placement of the

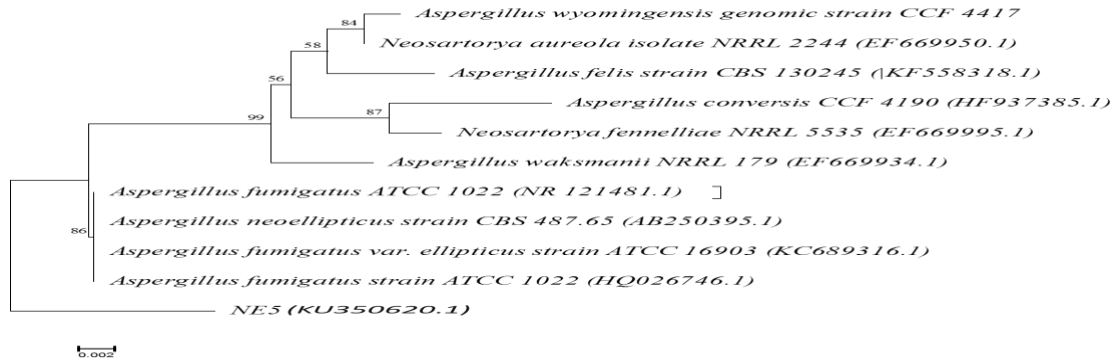


**Figure 1: Neighbour joining phylogenetic tree of isolate NE5 made by MEGA 6.0. Bootstrap values of >50% (based on 1000 replicates) are given in the nodes of the tree. NCBI accession numbers are given in parentheses**

## Gas Chromatography – mass spectrometric analysis

Eight bioactive compounds were characterised from the methanolic extract of NE5 (*Aspergillus fumigatus* KU350620.1) as shown in the gas chromatogram in Figure 2. However, there are some bioactive compounds that are similar but were eluted at different retention time as presented in Table 5.

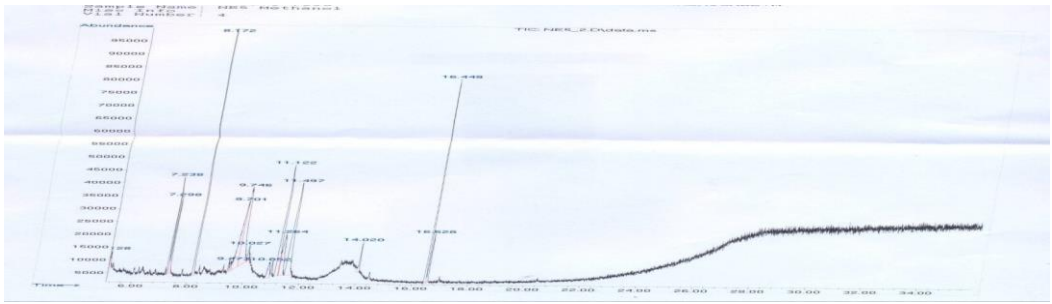

**Figure 2: Gas chromatogram of bioactive compounds in the methanolic extract of NE5 (*Aspergillus fumigatus* KU350620.1) from Nipa palm leaves**

**Table 5: Identification and quantification of bioactive compounds in the methanolic extract of *Aspergillus fumigatus* (KU350620.1)**

| S/N | Retention time (min) | Compound name | Molecular formula | Molecular weight | Peak area (%) |
|---|---|---|---|---|---|
| 1 | 5.128 | 2H-Tetrazol-2-ethanal,5-methyl- | $C_4H_6N_4O$ | 126.17 | 1.132 |
| 2 | 7.238 | Trimethyl(n-pentyl)silane | $C_8H_{20}Si$ | 144.33 | 4.683 |
| 3 | 7.298 | Trimethyl(n-pentyl)silane | $C_8H_{20}Si$ | 144.33 | 4.683 |
| 4 | 8.172 | Hexadecanoic acid acid,methyl ester | $C_{17}H_{34}O_2$ | 270.45 | 30.823 |
| 5 | 9.473 | Methyl β-d-galactopyranoside | $C_7H_{14}O_6$ | 194.18 | 0.781 |
| 6 | 9.701 | Methyl β-d-galactopyranoside | $C_7H_{14}O_6$ | 194.18 | 9.656 |
| 7 | 9.746 | Methyl β-d-galactopyranoside | $C_7H_{14}O_6$ | 194.18 | 15.010 |
| 8 | 10.027 | α-L-Galactopyranoside,methyl-6-deoxy- | $C_7H_{14}O_5$ | 178 | 1.397 |
| 9 | 10.852 | α-L-Galactopyranoside,methyl-6-deoxy- | $C_7H_{14}O_5$ | 178 | 2.211 |
| 10 | 11.122 | d-Glycero-d-tallo-heptose | $C_7H_{14}O_7$ | 210.18 | 6.097 |
| 11 | 11.264 | d-Glycero-d-tallo-heptose | $C_7H_{14}O_7$ | 210.18 | 4.985 |
| 12 | 11.497 | d-Glycero-d-tallo-heptose | $C_7H_{14}O_7$ | 210.18 | 3.873 |
| 13 | 14.020 | Patulin | $C_7H_6O_4$ | 154.12 | 1.733 |
| 14 | 16.449 | 9- Octadecenoic acid (z)- methyl ester | $C_{19}H_{36}O_2$ | 296.48 | 11.159 |
| 15 | 16.528 | 9- Octadecenoic acid(z)- methyl ester | $C_{19}H_{36}O_2$ | 296.48 | 2.728 |

Hexadecanoic acid, methyl ester at 30.823%, which was eluted at 16.449 seconds, was identified as the major compound in the extract. Its mass spectrum was presented in Figure 3.

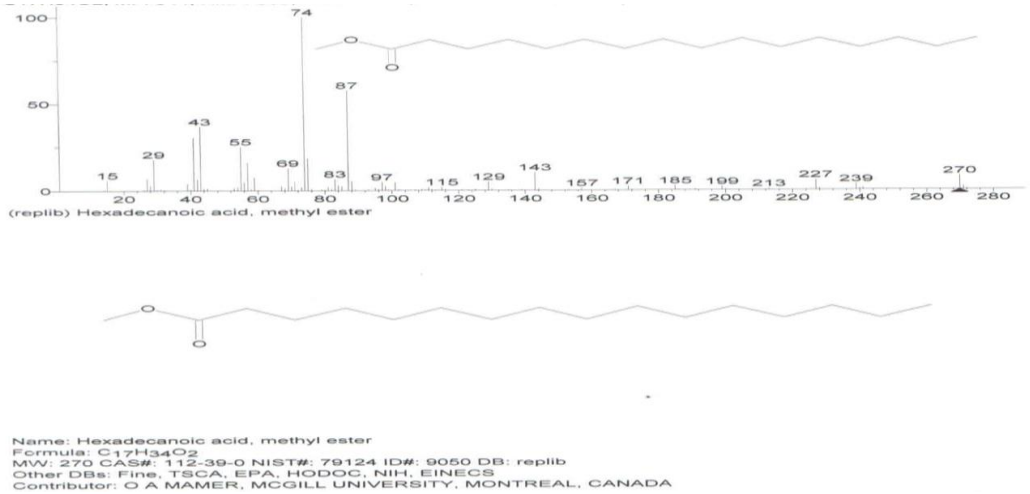


**Figure 3: Mass spectrum of the major bioactive compound (Hexadecanoic acid, methyl ester) in the methanolic extract of *Aspergillus fumigatus* KU350622.1 from Nipa palm leaves**

## DISCUSSION

It is of great interest to know that the surface sterilization of the Nipa palm leaves did not hinder the viability and survival of the endophytic fungal isolates obtained from this study. This can be attributed to their unique characteristics of being able to survive in extreme environmental conditions. Isolation of most of these endophytic fungi such as *Aspergillus* sp., *Penicillium* sp., *Cladosporium* sp., *Fusarium* sp., *Colletotrichum* sp., *Xylaria* sp. and *Phomopsis* sp. from mangrove environment have also been reported by some authors (Chaeprasert *et al.,* 2011; Wacira *et al.,* 2020). Chaeprasert *et al.,* (2011) and Wacira *et al*., (2020) stated that the most prominent fungal endophytes from mangrove leaves are *Phomopsis, Collectotrichum, Aspergillus, Cladosporium* and *Xylaria* and they varied from host to host.

The results of the antibacterial assay revealed that the methanolic extract from *Aspergillus fumigatus* (KU350620.1) exhibited maximum antibacterial activities with highest diameter of inhibition on all the pathogenic bacteria and *Staphylococcus aureus*, a gram positive bacteria show the highest zones of inhibition when compared with others that are gram negative (*Salmonella typhi*, *Shigella dysenteriae*, *Vibrio cholerae* and *Vibrio parahaemolyticus*). Similar results have been reported by other authors (Ariole and Akinduyite, 2016; Jirayu *et al*., 2011).They reported that antimicrobial activities of ethyl acetate, methanolic and hexane extracts of some endophytic fungi (*Fusarium* sp., *Aspergillus* sp., *Phomopsis* sp., *Penicillium* sp. and *Collectotrichum* sp.) were carried out against some pathogenic gram positive and gram negative bacteria in which higher zones of inhibition were recorded against gram positive bacteria than gram negative bacteria. The higher resistance potential of the Gram negative bacteria compared to Gram positive bacteria used in this study can be attributed to the presence of a distinctive double outer membrane containing lipopolysaccharides surrounding their cells (Zeinab *et al.,* 2020). Although all bacteria have an inner cell membrane, however, gram-negative bacteria have a unique outer membrane which prevents certain drugs and antimicrobial agents from penetrating the cell (Enrique *et al.*, 2018), thereby making them more resistant to the antibacterial activity of the fungal extracts than gram-positive bacteria.

Anteneh, (2020) has reported that endophytic fungi are able to adapt themselves to their special micro-environments gradually by genetic variation; their uptake of some plant DNA segments into their own genomes, as well as inserting their own DNA segments into the host genomes (Pragya and Hanhong, 2020). This could have resulted to their ability to synthesize unique bioactive compounds that are originally from their host plants.

Hexadecanoic acid, methyl ester, the major bioactive compound in the extract is a fatty acid ester that exhibits antibacterial, antifungal, antioxidant, nematicidal, pesticidal, hypocholesterolemic, haemolytic and 5-alpha-reductase inhibitor activity (Siswadi and Grace, 2021). It is used as raw material of emulsifiers or oiling agents for foods spin finishes and textiles, lubricants for plastics, paint and ink additives, surfactants and base materials for perfumery (Anand Gideon, 2015). It is also used as solvents or co-solvents; oil carrier in agricultural industry (Hema *et al*., 2011).Therefore, hexadecanoic acid, methyl ester has a wide-spectrum of biological activities with promising pharmaceutical, agrochemical and industrial importance.

An important factor to consider in the isolation of endophytic fungi for the biosynthesis of novel bioactive compounds is the choice of host plant. Such plant species should include those that can survive in adverse environmental conditions, have unique strategies for survival, have ethno-botanical history and can occupy ancient land mass with unusual longevity in areas of high biodiversity. Nipa palm plant is a good example of plants with these unique properties.

## CONCLUSION

The results of this study show that the endophytic fungus from Nipa palm leaves have antibacterial potentials and its methanolic extract has also proven to be a rich source of novel bioactive compounds of various biotechnological importances. We are thus confident that in the near future, microorganisms will continue to provide an abundant harvest of novel bioactive compounds.

## Availability of Data and Materials

All data that are relevant to the study are reported within this manuscript.

## Consent for Publication

The authors approved the consent for publishing this manuscript.

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