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Antibacterial potential of indigenous red mangrove (*Rhizophora racemosa*) fungal endophytes and bioactive compounds identification

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Abstract

The antibacterial activity of indigenous red mangrove leaves- endophytic fungi against marine pathogenic bacteria and bioactive compounds identification and quantification were carried out. Healthy leaves of red mangrove (*Rhizophora racemosa)* were collected from Eagle Island mangrove forest in Port Harcourt, Nigeria. The endophytic fungi were isolated with acidified Potato dextrose agar plates after incubation at 28°C for 5 days. Isolated colonies were identified based on their colonial and microscopic morphology. The ethyl acetate and methanolic extracts of the mangrove leaves-endophytic fungal isolates were screened against marine pathogenic bacteria (*Staphylococcus aureus*, *Salmonella* sp., *Shigella* sp., *Vibrio cholerae* and *Vibrio parahaemolyticus*) using agar well diffusion assay at different concentrations. An isolate which was active against all the pathogens was further identified molecularly and the bioactive compounds in its methanolic extract identified and quantified using Gas chromatography – Mass spectrometry analysis. Six endophytic fungi of the genera *Rhizopus*, *Pestalotiopsis*, *Aspergillus*, *Penicillium*, *Geotrichum* and *Phomopsis* were isolated. Methanolic extract of *Aspergillus* sp., identified molecularly as *Aspergillus niger* KU350621.1, was active against all the tested pathogens. Eleven bioactive compounds were identified in its methanolic extract with 9, 12-Octadecadienoic acid (Z, Z) methyl ester ($C_{19}H_{34}O_2$) as the major compound at 39.896% peak area. These results reveal that red mangrove leaves- endophytic fungi have antibacterial effect against marine pathogenic bacteria. The identified bioactive compounds can be used for drug development.

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Introduction

Mangroves are forest wetlands located across the intertidal zones of the tropics and subtropics in the creeks and estuaries. They are salt tolerating, marshy plants, that are well adapted to their far beyond normal environmental conditions of high salinity, high temperature, anaerobic soil and changes in sea level through their pneumatophoric roots, salt-expelling leaves, and water diffusing seeds (propa-gules) (Silva *et al*., 2011). They are the most fragile but highly productive ecosystems along the coastal areas. They protect coastlines from wave action, prevent coastal erosion and reduce damages in inland areas during storms.

Red mangrove plants belong to the family of mangrove called *Rhizophoraceae*. They are trees but at times appear as shrubs with special root called "prop root". In Nigeria, red mangrove plants are predominantly found in the mangrove forest along the shores of coastal water. The unique mangrove ecosystem adjacent to the coastal waters provides a wide variety of organic substrates and a significant salinity gradient caused by daily changes in the sea level.

This constitutes an ideal environment for the bases of trunks and submerged aerating roots of mangrove plants, making mangrove forests important sources for unique endophytic fungi (Maria *et al*., 2005).

Endophytic fungi are fungi that inhabit the internal tissues of plants without causing any symptomatic negative effect on the plant. Every marine and terrestrial plant is known to be host to at least one endophytic micro organism. The host plant provides energy, nutrients and shelter for the endophytic fungi and also protects them from environmental stress. On the other hand, the endophytic fungi defend the plant biologically against foreign phytopathogens by releasing metabolites to attack any antagonist, or indirectly by inducing host defence mechanisms. They produce bioactive compounds and enzymes, which aid the ability of the host plants to adapt to abiotic stresses such as high salinity, excessive light, high temperature, and drought (Alvin *et al*., 2014).

Agricultural and aquatic pathogenic micro organisms have been discovered to have developed resistance to commonly used antimicrobial chemicals. Thus there is a need to discover novel bioactive compounds from mangrove endophytic fungi which can serve as raw materials for the production of agrochemicals, antibiotics and food preservatives with low toxicity and minor environmental impact. This development made researchers to suggest alternative methods of preve-nting diseases which include the use of non pathogenic micro organisms such as endophytes (Liu *et al*., 2007). Information on the antibacterial activity of indigenous red mangrove leaves- endophytic fungi against marine pathogenic bacteria and their bioactive compounds identification and quantification are lacking. Therefore, the present study aimed at evaluating the antibacterial potentials of ethyl acetate and methanolic extracts of indigenous red mangrove leaves- endophytic fungi against marine pathogenic bacteria. Molecular identification and bioactive compounds identification and quantification of the most active isolate were carried out.

Materials and methods

Source of marine pathogenic bacteria

Marine pathogenic bacteria (*Staphylococcus aureus*, *Salmonella* sp., *Shigella* sp., *Vibrio cholerae* and *Vibrio parahaemolyticus*) were obtained from culture collection in Environmental Microbiology Division, University of Port Harcourt. Port Harcourt, Nigeria.

Collection of mangrove leaves samples

Healthy red mangrove (*Rhizophora racemosa*) leaves were randomly collected from Eagle Island mangrove forest in Port Harcourt, Rivers State, Nigeria with ethanol- disinfected knife.

The leaves were placed in labelled sterile Ziploc bag to prevent moisture loss. The bag was transported to the laboratory, in ice box, within 3 hours and stored at 4°C until isolation procedure was completed (Chaeprasert *et al*., 2010).

Isolation and identification of mangrove leaves-endophytic fungi

The leaves were washed under running tap water to remove dust and debris. They were cut into 5 mm pieces using a sterile cutter under aseptic conditions and allowed to air dry. Surface sterilization was done by treating leaves with 75% ethanol for 3 minutes followed by immersion in 0.5% sodium hypochlorite solution for 1 minute and 75% ethanol for 30 seconds and finally rinsed with sterile distilled water for 1 minute to remove the sterilants and then air-dried on sterile filter paper (Kumaresan and Suryanarayanan, 2008).

The sterilized leaves fragments were placed aseptic-ally on acidified Potato dextrose agar (PDA) in Petri dishes in such a way that the freshly cut edges were in contact with the Potato dextrose agar surface. The plates were sealed with paraffin and then incubated at 28°C for 5 days. Fungal growth from the incubated plates were subcultured on separate plates under aseptic condition and incubated at 28°C for 5 days for purification. They were identified based on their colonial morphology such as shape, size, colour and surface texture, and microscopic features. The pure culture of endophytic fungal strains were cultured in sterile Potato dextrose agar slants and stored at 4°C for further studies (Jirayu *et al.,* 2011).

Extraction of bioactive compounds (metabolites)

Endophytic fungal isolates were collected from 5-day-old culture grown on Potato dextrose agar plates using a sterile cork borer 10 mm in diameter and transferred with a sterile glass rod into the 500 ml Erlenmeyer flask containing 300 ml of sterilized Potato dextrose broth. The inoculated broth was incubated at 28°C for 21 days under stationary condition (Chaeprasert *et al*., 2010).

The broth culture was filtered through a sterile cheese cloth to separate the filtrate and mycelium. The filtrate was labelled accordingly and 50 ml of 100% (v/v) ethyl acetate was added to the filtrate and was centrifuged at 1500 rpm for 10 minutes. The tube was allowed to settle for 5 minutes till two clear immiscible layers were formed. The upper layer containing the extracted compound was separated using a rotary funnel and collected into a sterile beaker. This procedure was repeated thrice. The extract was evaporated at 45°C to dryness using a rotary evaporator. The extracted residue was weighed and dissolved in Dimethyl sulfoxide (DMSO) and stored at-16°C for antibacterial assay and Gas chromatography – mass spectrometry (GC-MS) analysis (Kjer *et al*., 2010).

The fungal mycelium was soaked in 50 ml of 70 % (v/v) methanol for 2 days. Then the mixture was filtered with a sterile cheese cloth. The filtrate was collected and evaporated at 45°C to dryness using the rotary evaporator to obtain the methanolic extract. Extracts were weighed and dissolved in Dimethyl sulfoxide (DMSO) and stored at -16°C for antibacterial assay and GC-MS analysis (Kjer *et al*., 2010).

Antibacterial assay

A colony of each of the pathogenic bacteria was inoculated into nutrient broth and incubated for 24 hours at 37°C. The cell density of the pathogenic bacteria in the incubated nutrient broth culture was adjusted to 10^{-6} cfu/ml by serial dilution and inocu-lated on Mueller-Hinton agar surface respectively in separate labelled Petri dishes in a laminar flow cabinet. Agar wells were made equidistantly in the inoculated plates using a sterile cork borer of 6 mm diameter. Each well was loaded with 0.1ml of the fungal extracts at different concentrations (20, 40, 60, 80 and 100 mg/ml). Wells loaded with 0.1ml of standard antibacterial chloramphenicol solution (100 mg/ml) served as positive controls. Wells containing 0.1ml of sterile distilled water served as negative controls.

The plates were left on the work bench for 30 minutes to allow the extract to diffuse evenly into the agar. Then, the plates were incubated at 37°C and observed at an interval of 24 hours for growth inhibition around the wells. After the incubation, the inhibition zone around the well was recorded and expressed in millimetre (Bhimba *et al.*, 2012).

Molecular identification of mangrove leaves - endophytic fungi

Norgen's Fungi/Yeast Genomic DNA Isolation Kit was used for the DNA extraction. The kit is designed for the rapid preparation of genomic DNA from viable yeast cells, fungal spores or mycelium and Gram - positive bacteria. Genomic DNA was efficiently extracted from the cells by a combina-tion of the use of heat treatment, detergents and the use of bead tubes.

Principle of spin column chromatography was employed in the purification process. The purified genomic DNA was fully digestible with all restriction enzymes that have been tested, and was completely compatible with downstream applicati-ons such as Polymerase Chain Reaction (PCR), Restriction Fragment Length Polymorphism (RFLP) and Amplified Fragment Length Polymorphism (AFLP) (Zhang *et al*., 2010).

DNA was quantified by making serial dilution of known DNA standard and then measuring the absorbance at optical density of 450 nm. Serial dilution of 1 in 10 to 1 in 80 was plotted against their absorbance 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 Norgenbiotek Canada and test carried out as directed by the manufacturer.

The master mix and the primers (general purpose Pfastbact 1 and 2 and the template DNA were mixed in order to get 25 microns in the following, 0.2 microns of master mix, followed by 0.1 microns of forward and backward primers and the 0.2 microns of template DNA. This was then made to 25 microns by the addition of molecular grade water. PCR was performed on a thermocycler (hybaidomnigene) run-ning 35 cycles of standard programs.

After the PCR reaction, the PCR product was separated on a 1.5% agarose gel (Solis Biodyne, Estonia). One hundred base pair (100 bp) 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 Cluster W and phylogenetic tree was drawn with MEGA 6 software.

Phylogenetic analysis

Phylogenetic analysis was performed with Molecular Evolutionary Genetics Analysis (MEGA) version 6. Modification of the aligned sequences was carried out to ensure that each of them was of the same size. Gaps within the sequences which were common to all sequences were removed. Phylogenetic analysis was carried out with a neighbour-joining tree and bootst-rapping of 1000 replicates. The tree was drawn to scale and bootstrap values of greater than 50% are given in the nodes of the tree (Tamura *et al*., 2013).

Gas chromatography-mass spectrometry (GC-MS) analysis of bioactive compounds

Bioactive compounds of the endophytic fungal methanolic extract that exhibited maximum zones of inhibition were determined using Gas Chromatog-raphy–Mass Spectrometry analysis.

The analysis was carried out using Agilent 7890A-5975C GC-MS system as described by Tao *et al*. (2011). One millimetre of the extract was placed in a vial and injected into the Gas Chromatography–Mass Spectrometer system. Separation of compounds was conducted on a 60 m HP-INNOWAX capillary column of 0.25 mm, using nitrogen as a carrier gas.

The injector temperature was 250°C with the volume of 0.5µl. The carrier gas flow was 1 ml/min which has a split ratio of 10:1. The temperature of the oven initially was 110°C to 200°C at 10°C/min and then increased to 200°C to 280°C at 5°C/min and held for 9 minutes. Thereafter, mass spectra were taken at 70 eV (Tao *et al*., 2011).

Identification of mass spectrum of the Gas chroma-tography – mass spectrometry (GC-MS) analysis was conducted using the NIST (National Institute of Standards and Technology) Database. The spectrums of the unknown bioactive compounds were compared with spectrum of known compounds stored in the NIST library. The name, molecular weight and peak ratio in percentage of the bio-active compounds in the test fungal extracts were ascertained.

Results and discussion

In this study, six endophytic fungi of the genera *Rhizopus*, *Pestalotiopsis*, *Aspergillus*, *Penicillium*, *Geotrichum* and *Phomopsis* were isolated from the red mangrove leaves (Table 1). Endophytic fungi can be transmitted from one generation to the next through the tissue of the host, seed or vegetative propagules (Richardson *et al*., 2009). It is of great interest to know that in spite of the surface sterilization process the leaf samples were subjected to, the sporulation ability of the endophytic fungi were not affected. This can be attributed to their unique characteristics of being able to survive in adverse environmental setting. Isolation of most of these endophytic fungi such as *Aspergillus* sp., *Penicillium* sp., *Cladosporium* sp., *Fusarium* sp., *Colletotrichum* sp., *Phomopsis* sp., and *Xylaria* sp., from mangrove plants has also been reported by some authors (Chaeprasert *et al.,* 2010; Huang *et al.,* 2008; Yang *et al.,* 2010).

Table 1. Antibacterial activity of ethyl acetate extract of red mangrove -endophytic fungi against marine pathogenic bacteria.

Code	Isolate identity	Concentration of extract (mg/ml)	Inhibition zone (mm) ±S.D.				
			Marine pathogenic bacteria				
			Staphylococcus aureus	*Salmonella* sp.	*Shigella* sp.	*Vibrio cholerae*	*Vibrio parahaemolyticus*
RA1	*Rhizopus* sp.	20	-	-	-	-	-
		40	-	-	-	-	-
		60	-	-	-	-	-
		80	10.00 ± 1.00	8.33 ± 0.58	10. 00 ± 1.00	-	-
		100	12. 00 ± 1.00	10. 33 ± 0.58	11. 00 ± 1.00	-	-
	positive control	Chloramphenicol (100)	25. 00 ± 1.00	21. 00 ± 1.00	23. 00 ± 1.00	19. 00 ± 1.00	16. 00 ± 1.00
	negative control	Distilled water	-	-	-	-	-
RB2	*Pestalotiopsis* sp.	20	-	-	-	-	-
		40	-	-	-	-	-

		60	-	-	-	-	-
		80	9. 00 ± 1.00	-	-	-	-
		100	11. 00 ± 1.00	-	-	-	-
RC3	*Aspergillus* sp.	20	-	-	-	-	-
		40	-	-	-	-	-
		60	9. 00 ± 1.00	-	-	-	-
		80	11. 00 ± 1.00	9.00 ± 1.00	9.00 ± 1.00	-	-
		100	12.67 ± 0.58	11.00 ± 1.00	11.33 ± 0.58	-	-
RD4	*Penicillium.*sp.	20	-	-	-	-	-
		40	-	-	-	-	-
		60	-	-	-	-	-
		80	9.00 ± 1.00	-	-	-	-
		100	9.67 ± 0.58	-	-	-	-
RE5	*Geotrichum* sp.	20	-	-	-	-	-
		40	-	-	-	-	-
		60	8.33 ± 0.58	-	-	-	-
		80	10. 00 ± 1.00	-	9.00 ± 1.00	-	-
		100	12. 00 ± 1.00	-	10.67 ± 0.58	-	-
RF6	*Phomopsis* sp.	20	-	-	-	-	-
		40	-	-	-	-	-
		60	-	-	-	-	-
		80	10. 00 ± 1.00	8.33 ± 0.58	8.67 ± 0.58	-	-
		100	11.67 ± 0.58	10. 00 ± 1.00	10.67 ± 0.58	-	-

- = no zone of inhibition

The results of the antibacterial assay of ethyl acetate and methanolic extracts of red mangrove leaves-endophytic fungi against marine pathogenic bacteria are presented in Tables 1 and 2 respectively. Generally, *Staphylococcus aureus*, a gram positive bacterium was more susceptible to the fungal extracts than gram negative bacteria (*Salmonella* sp., *Shigella* sp., *Vibrio cholerae* and *Vibrio parahaemolyticus*).

Similar results have been reported by other authors (Jirayu *et al*., 2011, Chaeprasert *et al*., 2010 and Schulz *et al*., 2006). They reported that when 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, considerable zones of inhibition were recorded against gram positive bacteria than gram negative bacteria.

The higher resistance level of the Gram negative bacteria compared to Gram positive bacterium used in this study can be attributed to the presence of a distinctive double outer membrane surrounding their cells. Although all bacteria have an inner cell membrane, gram-negative bacteria have a unique outer membrane. This outer membrane prevents certain drugs and antimicrobial agents from penetrating the cell, thereby making them more resistant to the antibacterial activity of the fungal extracts than gram-positive bacteria.

The presence of this outer membrane in gram negative bacterial cells which act as a barrier against antimicrobial agents was also reported by Alias *et al*. (2010). Methanolic extract of *Aspergillus* sp. was active against all the pathogens employed at MIC of 60 mg/l (Table 2). Molecular identification revealed it as *Aspergillus niger* KU350621.1 (Fig. 1).

Table 2. Antibacterial activity of methanolic extract of red mangrove -endophytic fungi against marine pathogenic bacteria.

Code	Isolate identity	Concentration of extract (mg/ml)	Inhibition zone (mm) ± S.D.				
			Marine pathogenic bacteria				
			Staphylococcus aureus	*Salmonella* sp.	*Shigella* sp.	*Vibrio cholerae*	*Vibrio parahaemolyticus*
RA1	*Rhizopus* sp.	20	-	-	-	-	-
		40	-	-	-	-	-
		60	-	-	-	-	-
		80	-	-	-	-	-
		100	-	-	-	-	-
RB2	*Pestalotiopsis* sp.	20	-	-	-	-	-
		40	-	-	-	-	-
		60	-	-	-	-	-
		80	-	-	-	-	-
		100	-	-	-	-	-
RC3	*Aspergillus* sp.	20	10. 00 ± 1.00	-	8.67 ± 0.58	8.33 ± 0.58	-
		40	11. 00 ± 0.00	-	10. 00 ± 1.00	9.00 ± 1.00	-
		60	13. 00 ± 1.00	8.67 ± 0.58	11. 00 ± 1.00	9.67 ± 1.63	8.33 ± 0.58
		80	17. 33 ± 0.58	10. 00 ± 1.00	13. 00 ± 1.00	12.33 ± 0.58	9.00 ± 1.00
		100	21.00 ± 1.00	13. 00 ± 1.00	17. 33 ± 1.63	13. 00 ± 1.00	10.33 ± 0.58
RD4	*Penicillium.* sp.	20	-	-	-	-	-
		40	-	-	-	-	-
		60	-	-	-	-	-
		80	8.33 ± 0.58	-	8.33 ± 0.58	-	-
		100	9.00 ± 1.00	-	9.33 ± 0.58	-	-
RE5	*Geotrichum* sp.	20	-	-	-	-	-
		40	-	-	-	-	-
		60	8.67 ± 0.58	-	-	-	-
		80	10.33 ± 0.58	-	8.33 ± 0.58	-	8.33 ± 0.58
		100	11.67 ± 0.58	9.33 ± 0.58	9.33 ± 0.58	-	8.67 ± 0.58
RF6	*Phomopsis* sp.	20	-	-	-	-	-
		40	-	-	-	-	-
		60	-	9.33 ± 0.58	-	-	-
		80	8.67 ± 0.58	12. 00 ± 1.00	-	-	-
		100	10.33 ± 0.58	13.33 ± 0.58	-	-	-

- = no zone of inhibition

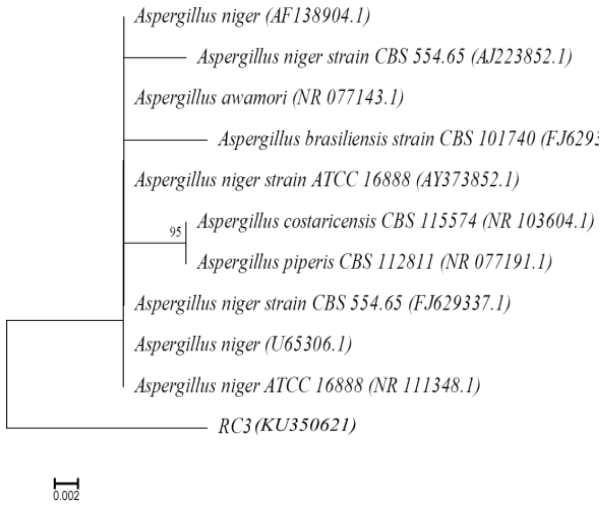

Fig. 1. Phylogenetic tree of isolate RC3 (NCBI accession numbers are given in parentheses).

Its antibacterial activity may be due to the presence of bioactive compounds that are capable of inhibiting the growth of the tested pathogenic bacteria and the solubility of those bioactive compounds in the organic solvent used. Similar result has been reported by Silva *et al*. (2011) in which the inhibitory activity of crude extract of *Aspergillus niger* among other endophytic fungi such as *Curvularia pallescens*, *Guignardia bidwelii*, *Paecilomyces variotii* isolated from leaves of Brazilian mangrove plant (*Laguncularia racemosa*) against some pathogenic bacteria were evaluated. Eleven bioactive compounds were detected in the methanolic extract of *Aspergillus niger* KU350621.1 (Table 3).

Table 3. Identification and quantification of bioactive compounds in the methanolic extract of *Aspergillus niger* KU350621.1.

S/N	Retention time (minutes)	Compound name	Molecular weight (g/mol)	Molecular formular	Chemical structure	Peak area (%)
1	5.151	4H-Pyran-4-one,5-hydroxy-2-hydroxymethyl	142.11	$C_6H_6O_4$		7.041
2	5.233	Pentadecanoic acid,14-methyl, methyl ester	270.45	$C_{17}H_{34}O_2$		8.148
3	7.812	n-Hexadecanoic acid	256.42	$C_{16}H_{32}O_2$		4.032
4	14.042	Undecanoic acid	186.29	$C_{11}H_{22}O_2$		9.709
5	14.470	9,12-Octadecadienoic acid, methyl ester	280.45	$C_{18}H_{32}O_2$		3.685
6	16.479	9,12-Octadecadienoic acid(z,z) methyl ester	294.47	$C_{19}H_{34}O_2$		39.896
7	16.554	9- Octadecenoic acid(Z)- methyl ester	296.49	$C_{19}H_{36}O_2$		14.617
8	16.989	Methyl-13-Octadecenoate	296.48	$C_{19}H_{36}O_2$		7.507
9	17.052	9,12- Octadecadienal	264.44	$C_{18}H_{32}O$		1.420
10	18.923	9,12 - Octadecadienal	264.44	$C_{18}H_{32}O$		1.400
11	25.393	E-7-Tetradecenol	212.37	$C_{14}H_{28}O$		2.545

Recent researches have proven that mangrove–endophytic fungi are potential sources of novel bioactive compounds for exploitation in modern medicine, agriculture and industry (Strobel and Daisy, 2003; Joseph and Priya, 2010). These bioactive compounds can be exploited in the production of agrochemicals, antimicrobial agents, immunosuppressant, antioxidants, food preservatives and some other industrial purposes (Schulz *et al*., 2006).

These include flavonoids, fatty acids, terpenoids, alkaloids, phenolic acids, benzopyr-anones, steroids, xanthones and quinines. Endophytic fungi also contribute to the rapid growth of their host plant through different mechanisms such as production of phytohormones, synthesis of siderop-hores, nitrogen fixation, solubilisation of minerals, ethylene suppression via assisting phytoremediation.

The GC-MS chromatogram of the methanolic extract of *Aspergillus niger* KU350621.1 is shown in Fgi. 2 while the mass spectrum of the major compound (9, 12–Octadecadienoic acid (Z, Z) methyl ester ($C_{19}H_{34}O_2$)) in the extract is shown in Fgi. 3.

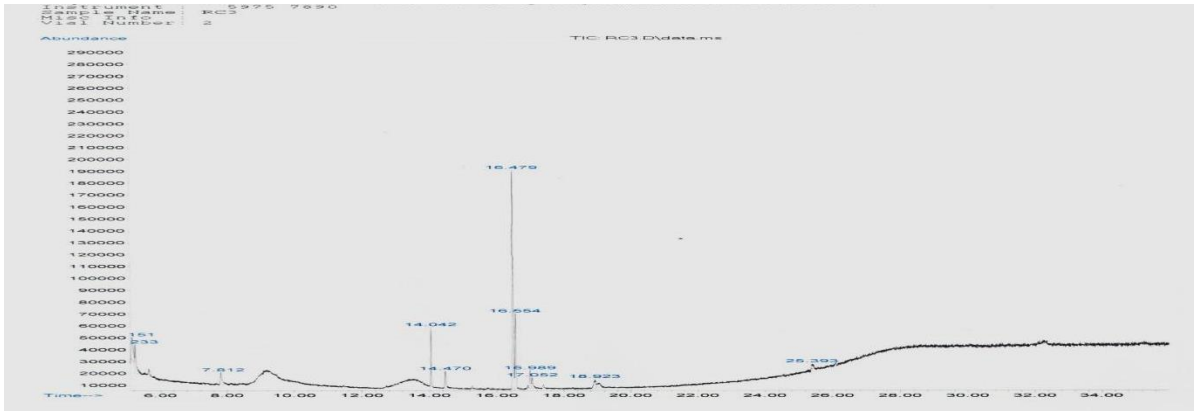

Fig. 2. Gas chromatogram of bioactive compounds in the methanolic extract of *Aspergillus niger* KU350621.1.

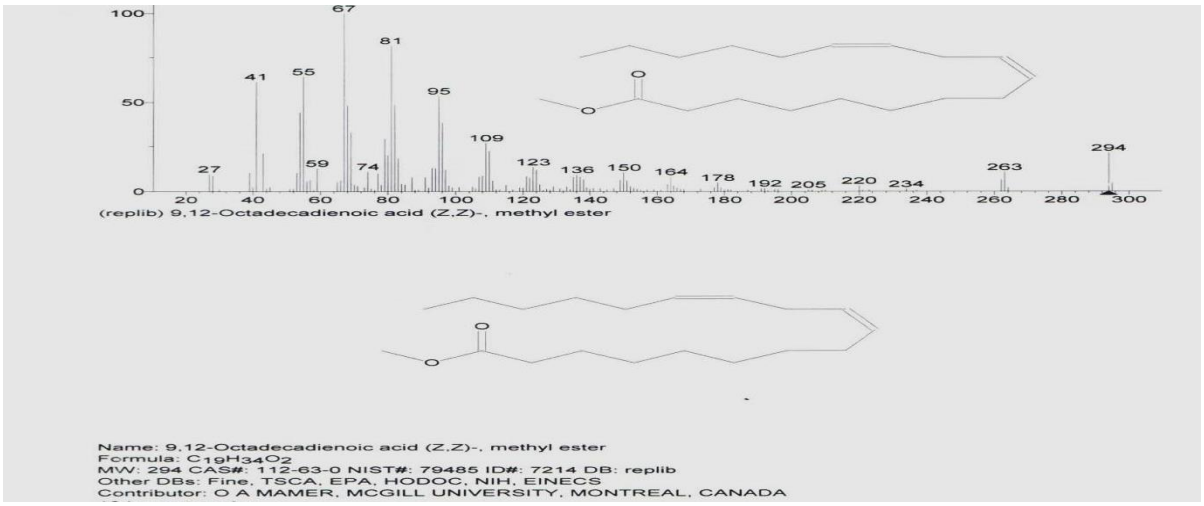

Fig. 3. Mass spectrum of the major bioactive compound (9, 12 – Octadecadienoic acid (Z, Z) - methyl ester) in the methanolic extract of *Aspergillus niger* KU350621.1.

The bioactive compound, 9, 12-Octadecadienoic acid (Z, Z), methyl ester, known as Linoleic acid, is a polyunsaturated fatty acid (produced mostly in plants). It acts as potent antibacterial and antifungal agent. It also has acne reductive, anti-inflammatory and moisture retentive properties when applied topically on the skin.

It is used in the production of drugs and food additives. It is also used industrially as a drying agent for protective coatings and manufacturing emulsifying agents, soaps, oil paints and varnishes (Gehan *et al.,* 2009). The antibacterial activities of the red mangrove leaves-endophytic fungal extract can be attributed to the presence of these unique bioactive compounds.

An important factor to consider in the isolation of endophytic fungi that are capable of producing 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 have occupied ancient land mass with unusual longevity in areas of high biodiversity.

Mangrove plant is a good example of plants with these unique properties (Abhiroop *et al*., 2014). Research has also shown that the mangrove-endophytic fungi have been able to adapt themselves to their special micro-environments gradually by genetic variation, including uptake of some plant DNA segments into their own genomes, as well as insertion of their own DNA segments into the host genomes. This could have led to their unique characteristics of biosynthesis of unique bioactive compounds that are originally from their host plants (Schulz *et al*. 2006).

Therefore, mangrove-endophytic fungi are outstan-ding sources of bioactive compounds (natural products) with diverse bio-prospecting potentials that need to be fully discovered.

Conclusion

The results of this study showed that the endophytic fungi from red mangrove leaves have antibacterial potentials. They have also proven to be rich sources of novel bio-active compounds with a wide-spectrum of biological activities. Therefore, culturing these micro organisms on large scale and broadening the biolog-ical screens used in bioactive compounds discovery will increase the possibility of discovering novel bioactive compounds, instead of harvesting the host plants which invariably might adversely affect enviro-nmental biodiversity.

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