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Chemical composition and *in-vitro* antimicrobial activity of essential oil of African nutmeg (*Monodora myristica* (Gaertn) Dunal on microorganisms isolated from smoke-dried catfish (*Clarias gariepinus*)

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Dried seeds of *Monodora myristica* were obtained from Oba market in Edo State, ground into fine powder and stored in an air tight plastic container at room temperature until when used. Essential oils were extracted using steam distillation and characterised using the gas chromatography-mass spectroscopy (GC-MS) technique. Microbial isolates obtained from smoke dried fish samples purchased from Oba Market, Benin City, Edo State. Fish samples were put in perforated polythene bags kept in plastic baskets and stored for four (4) weeks. Identification and characterisation of isolates were carried out following standard microbiological procedures. The antimicrobial activity was determined using agar well diffusion method. Data obtained were subjected to a one-way analysis of variance. Significant means were separated using Duncan Multiple Range Test at 95% confidence level. The result showed that *M. myristica* oil contained twenty-five components which were mainly monoterpene hydrocarbons constituents consisting of limonene (17.6%), α-phellandrene (16.3%), α-pinene (12.2%), β-myrcene (11.2%), 3-Thujene and β-Linalool (7.3%). The oil showed bacteriocidal activity at minimum ihhibitory concentration/minimum bactericidal concentration (MIC/MBC) of 150 mg/mL against *Staphylococcus epidermidis* PM221, *Micrococcus luteus* NCTC 2665, *Bacillus subtilis* 6051-HGW and *Pseudomonas aeruginosa* AAU2 and fungicidal activity at MIC/minimum fungicidal concentration (MFC) of 300 mg/mL against *Aspergillus flavus* and *Rhizopus* species. In view of the aforementioned, the expressed antimicrobial activity is a demonstration of the efficacy of the oil against fish spoilage organism and use in the fishery industry.

Key words: Chemical compositions essential oils, antimicrobial activity, *Monodora myristica*, agar well diffusion method, *Clarias gariepinus*.

INTRODUCTION

African nutmeg (*Monodora myristica*), is a perennial edible plant that belongs to the family Annonaceae. It is a berry commonly found in the evergreen forests of West Africa (Burubai et al., 2009). It can sometimes be referred to as Iwor (Itsekiri), Ikposa (Benin), Ehuru or Ehiri (Igbo), Ariwo (Yoruba), Uyengben (Edo), Guijiya dan miya (Hausa), Ehiriawosin (Ikale) and (Feyisayo and Oluokun, 2013; Enabulele et al., 2014). The fruit of is smooth,

green and spherical with a diameter of 20 cm which becomes woody at maturity. It is has a long stalk which is up to 60 cm long. It is composed of numerous oblong, pale brown seeds which are about 1.5 cm long and are enclosed by a whitish fragrant pulp. The seed extract contains important pharmacological compounds like alkaloids, flavonoids, and vitamins A and E as well as numerous significant lipids (George and Osioma, 2011). The seed of the plant is very popular as a result of its nutritional and medicinal qualities (Corbo et al., 2009). The seeds are used in Eastern Nigeria as condiment and have been shown to have anti-sickling properties (Uwakwe and Nwaoguikpe, 2005). Additionally, the seeds can be utilized for treating individuals with elevated cholesterol level in light of the fact that have the capacity to lower cholesterol (Onyenibe et al., 2015; Ekeanyanwu et al., 2010). The essential oil from the leaves has been shown to contain *β*-caryophyllene, *α*-humulene and *α*-pinene, whereas that from the seeds contains *α*-phellandrene, *α*-pinene, myrcene, limonene and pinene (Fournier et al., 1999). Owokotomo and Ekundayo (2012) reported that the essential oil of the seeds contained germacrene D-4-ol (25.48%), tricyclo[5.2.1(1,5)dec-2-ene (13.35%), δ-cadinene (11.09%) and linalool (15.10%) while the major constituents of the stems-bark oil were γ-cadinene (31.31%), α-elemene (17.98%), α-cubebene (6.70%) and γ-muurolene (5.94%). Recently, essential oil from this plant attains to researchers interest especially food industry because of their antimicrobial and anti-oxidative properties. However, literature report on the essential oils in terms of composition and antimicrobial activity from this plant is scarce especially in Nigeria. As a result, this study aims to evaluate the essential oil chemical composition and *in-vitro* antimicrobial activity of essential oil of African nutmeg (*M. myristica*) on microorganisms isolated from smoke-dried catfish (*Clarias gariepinus*).

MATERIALS AND METHODS

Collection of materials

The dried seeds of *M. myristica*, were obtained from Oba market in Edo State and were identified and authenticated in the Department of Plant Biology and Biotechnology, University of Benin, Benin City. The seeds were then ground into fine powder and stored in an air tight plastic container and placed at room temperature (28 ± 2°C) for 24 h before use.

Extraction of the essential oils

Ground dried powder (3000 g) was weighed into distillation flask fitted with a condenser. Steam was supplied to the flask through a steam generator at constant flow for about 45 to 50 min. The essential oil was condensed and collected by a separating funnel. The oil was separated by gravity and dried using anhydrous sodium sulphate, filtered and stored at 4°C for 24 h before being analysed (Hussain et al., 2008).

Gas chromatography-mass spectrometry (GC-MS) analysis

The essential oil was analysed using a QP2010 Plus Shimadzu, Japan-gas chromatography-mass spectroscopy (GC-MS) at the National Research Institute for Chemical Technology (NARICT), Zaria, Nigeria. A fused silica capillary column HP5-MS (30 m × 0.32 mm, film thickness of 0.25 µm) was used. The carrier gas used was Helium at a flow rate of 36.2 cm/s and at a constant pressure of 90 kPa. The samples were injected by splitting using a split ratio of 41.6. The column flow rate was 0.99 mL/min. The column oven initial temperature was 60°C held for 1 min. The temperature was then slowly increased at 10°C min^{-1} to 180°C and held for 3 min. This was then increased at 12°C min^{-1} to 280°C which was finally held for 2 min, while maintaining the injection temperature at 250°C.

Identification of constituents

The identification of components was based on comparison of their mass spectra and their retention index with those present in the National Institute for Standard Technology computer data bank (Adams, 2001).

Antimicrobial assay of essential oils

Collection of bacterial and fungal isolates from smoke dried fish samples

The micro-organisms used for the antimicrobial activity were obtained from smoke dried fish samples purchased from Oba Market, Benin City, Edo State. Fish samples were kept in plastic baskets and stored on open bench under laboratory conditions for a period of four (4) weeks. Ten grams of smoke dried fish samples were weighed each and homogenized in 90.0 ml of sterile distilled water to prepare a stock solution. 1.0 ml of the stock solution (homogenate) was transferred into a sterile test tube containing 9.0 ml of sterilized distilled water. This process was repeated for other sterilized test tubes so that at the end, dilution of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} were obtained. A liquid solution of 0.1 ml of each dilution was plated in a plate count agar (PCA) using pour plate technique. The PCA were treated with Fulcin (50 mg/20 ml NA) to discourage fungal growth. The plates were incubated aerobically at 37°C for 24 h. While for fungi a liquid of 10^{-5} dilutions was dropped upon sterile Petri dishes in triplicates and 0.2 ml of anti-bacterial mixture which comprised Penicillin (100 ml 5%) and Streptomycin (100 ml 7.5%) was added to discourage bacterial growth. Potato dextrose was poured into various dishes and allowed to solidify before incubating aerobically at 28 ± 2°C for 72 h (Emoghene, 1996).

Identification of bacterial and fungal isolates from smoke dried fish

All isolates were identified by standard microbiological and biochemical which includes: Gram stain, sugar fermentation, methyl red test, catalase, oxidase, coagulase, mannitol, Voges-proskaur

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test, indole test, citrate test, urease test and motility test (Cowen and Steel, 2003). While fungal isolates were identified on the basis of cultural and morphological characteristics as described by Oyeleke and Okusanmi (2008) (Tables 2 and 3). The organisms were then subcultured and preserved at -20°C in sterile McCartney's bottles containing either nutrient broth and 15% sterile glycerol (bacteria) or potato dextrose broth containing 15% sterile glycerol (fungi).

Extraction DNA from bacteria isolates

Genomic DNA was gotten from the bacteria isolated from smoke-dried fish sample using Sigma Aldrich DNA extraction kit. A single discrete band was observed when resolved on Agarose Gel. The PCR amplicon was purified by column purification in order to remove contaminants. DNA sequencing was carried out with PCR amplicon. The 16S rDNA sequence was used to carry out BLAST with the nrs database of NCBI genbank database (Table 4).

Polymerase chain reaction (PCR)

16S rDNA region was amplified by PCR from bacterial genomic DNA using PCR universal primers: 16S Forward Primer: 5'-AGAGTTTGATCMTGG -3'; 16S Reverse Primer: 5'-ACCTTGTTACGACTT-3'. PCR was carried out in a final reaction volume of 25 µl in 200 µl capacity thin wall PCR tube. PCR tubes containing the mixture were tapped gently and spin briefly at 10,000 rpm. The PCR tubes with all the components were transferred to thermal cycler. 3 µl of PCR product was loaded with 3 µl bromophenol blue (Loading Dye) in 1.5% agarose gel. The gel was ran at constant voltage of 100 V and current of 45 A for a period of 30 min till the bromophenol blue has travelled 6 cm from the wells. After amplification, the expected PCR product was verified by gel-electrophoresis.

Purification and DNA sequencing of samples

Amplified PCR product was purified using column purification as per manufacturer's guidelines, and further used for sequencing reaction. The concentration of the purified DNA was determined and was subjected to automated DNA sequencing on Genetic Analyzer.

Sequence analysis of 16S rDNA

Each nucleic acid sequence was edited manually to correct falsely identified bases and trimmed to remove unreadable sequence at the 3 'and 5' ends (considering peak and quality values for each base) using the sequence analysis tools. The edited sequences were then used for similarity searches using Basic Local Alignment Search Tool (BLAST) program in the NCBI GenBank which is a DNA database for identifying the bacterial strains.

Antibacterial screening of essential oils

The antimicrobial activity of plant essential oils was studied using agar well diffusion method (Okeke et al., 2001). Each bacterial isolate preserved in 15% glycerol broth was sub-cultured on freshly prepared Nutrient agar (Oxoid Ltd., Basingstoke, Hampshire, UK) plate and incubated at 37°C for 24 h. A bacterial colony was picked up with a sterile wire loop, and emulsified in 5 ml Mueller Hinton broth (Oxoid Ltd., Basingstoke, Hampshire, UK). The turbidity of the resulting mixtures was adjusted with sterile saline solution to 0.5 Mcfarland standard (1.5×10^8 cells·mL^{-1}). Fresh Mueller Hinton agar plates were labelled and seeded with the test bacterial suspension using a sterile swab steak. These plates were allowed to incubate for 15 min thereafter agar punched out with sterile cork borer to create wells of 4 mm diameter. The different concentrations of the essential oils extracts (150, 100, 50, 25, 12.5 and 6.25 mg/ml) were diluted with DMSO (0.5% v/v). 100 µl dilute essential oils of the different concentrations were dispensed into each agar well in a plate; plates were allowed to stand for 1 h and then incubated at 37°C for 24 h. A 0.5% w/v suspension of ciprofloxacin (Fidson Healthcare Ltd., Nigeria) was used as positive control while a sterile solvent was used as negative control. This experiment was performed in triplicate plates for each of the isolate per dissolution. The diameter (mm) of zone of inhibition was recorded for the different concentrations of essential oil.

Antifungal screening of essential oil

The fungal isolates were sub cultured on potato dextrose agar at (28 ± 2°C) for 3 to 5 days. Equidistant wells were bored radially on sterile potato dextrose agar plates using a 4 mm diameter sterile cork borer. 100 µl dilute essential oils of the different concentrations were poured into the wells as described earlier. A 4 mm diameter agar mycelial disc of the fungi was then placed at the centre of the plate. 0.05% w/v Nystatin suspension (Mutual Pharmaceutical Company, Inc., Philadelphia, PA, USA) was used as positive control while the different dissolution solvent, was used as negative control. Triplicate plates were used in each treatment. Plates were incubated at 28 ± 2°C for 3 days. The zone of inhibition was recorded to the nearest mm.

Determination of minimum inhibitory concentrations (MICs)

The standard agar dilution technique with doubling dilution was used to determine the MICs of the extracts (Oboh et al., 2007). Different concentrations of the oil was prepared in DSMO and then diluted to attain a decreasing concentration of 150, 100, 50, 25, 12.5 and 6.25 mg/ml, respectively. Each dilution was introduced into nutrient agar plates and potato dextrose agar plates already seeded with each test organism. All test plates were incubated at 37°C for 24 h for bacteria and 28 ± 2°C for 72 h for fungi. The minimum inhibitory concentration (MIC) of the extracts for each test organism was regarded as the agar plate with the lowest concentrations without growth.

Determination of minimum bactericidal/fungicidal concentrations (MBCs/MFCs)

The MBC/MFC of the plant extracts was obtained by sub culturing portions of the agar from plates that showed no growth in the tests for determination of MICs. These agar portions were transferred respectively into plates containing freshly prepared nutrient agar and potato dextrose agar. These plates were incubated at 37°C for 24 h for bacteria while fungi plates were incubated at 28±-2°C for 3 to 5 days while growth were observed. No growth at the end of incubation period was considered as total cell death. The minimum concentration of the plant extracts that produces total cell death is taken as the MBC/MFC (Ajaiyeoba et al., 2003).

Statistical analysis

All data obtained in this study were represented as mean ± standard error (SE) of triplicates values. The data were then subjected to a one-way analysis of variance laid in a completely randomized design replicated thrice. Duncan Multiple Range Test

at 95% confidence level was used to separate significant.

RESULTS AND DISCUSSION

Chemical composition of essential oil of African nutmeg (*M. myristica*)

The detected components, chemical formula and their relative percentages of the essential oil of *M. myristica*, are shown in Table 1. Twenty-five components were characterized which represents 100% of the oil. This value was higher than that obtained by Owokotemo and Ekundayo (2012) who identified 22 compounds in the seed oil and 20 compounds in the stem bark oil of *M. myristica.* The oil consisted of 70.9% monoterpene hydrocarbons. The major monoterpene hydrocarbons constituents were limonene (17.6%), α-phellandrene (16.3%), α-pinene (12.2%), β-myrcene (11.2%), 3-Thujene (7.3%), and β-Linalool (7.3%). This is in agreement with Koudou et al. (2007) who identified alpha-phellandrene (34.4%) and p-cymene (22.2%) as major constituents of the oil. Several studies regarding the constituents of the essential oils of *M. myristica* essential oils obtained in Nigeria have exhibited a few contrasts. Owolabi et al. (2009) reported geranial (40.1%), neral (29.74%) and myrcene (11.3%) as the major components of the oil while Onyenekwe et al. (1993) reported alpha-phellandrane (50.4%), alpha-pinene (5.5%), myrcene (4.35%) and germacrene-D-4-ol (9.0%) as the most abundant compounds of essential oil of *M. myristica*. The disparity in occurrence and concentration of compound may perhaps be due to the origin of the plant material (Lawrence et al., 1988), genetic factors, culture and environmental conditions (Charles and Simon, 1990). Some of the compounds identified in this study like, α-limonene, are used to promote weight reduction, prevent and treat cancer and bronchitis (Crowell, 1999). In foods, beverages, and chewing gum, limonene is used as a flavoring agent. In addition, in pharmaceuticals, limonene is added to help medicinal ointments and creams penetrate the skin (Tsuda et al., 2004). α-Phellandrene is a flavouring agent used for food products such as yoghurt and baked foods. While α-pinene has been widely used as a food flavoring ingredient (Limberger et al., 2007; Rivas et al., 2012) and has anti-microbial properties (Gomes-Carneiro et al., 2005).

Antimicrobial activity of African nutmeg (*M. myristica*) essential oil

The antibacterial activity of African nutmeg (*M. myristica*) essential oil against bacterial test microbes is shown in Table 5. No activity was recorded at 6.25, 12.5 and 25 mg/ml of the essential oil against all test microbes. There was also no activity observed for *Corynebacterium pseudodiphtheriticum* DSM 44287 and *Pseudomonas aeruginosa* PB112 165 at 50 mg/ml, the other organisms were inhibited with zones ranging from 1.33 mm for *Staphylococcus saprophyticus* ATCC 15305 to 9.33 mm for *P. aeruginosa* AAU2. At 100 mg/ml, all bacterial test organisms were inhibited with *Bacillus subtilis* KCTC 1028 having the lowest zone of inhibition (1.67 mm) and *Staphylococcus epidermidis* PM221 with the highest inhibition zone (12.33 mm). No activity was recorded for *P. aeruginosa* PB112 165 at this concentration. *P. aeruginosa* PB112 165 had the lowest inhibition zone (11.67 mm) while *P. aeruginosa* AAU2 had the highest zone of inhibition (23.33 mm) at 150 mg/ml. There were significant differences ($p<0.05$) between all concentrations and Ciprofloxacin (positive control) except 150 mg/ml for *Staphylococcus aureus* NCTC 8325 and *S. epidermidis* PM221. However, no significant differences ($p>0.05$) was recorded at concentrations below 100 mg/ml for *C. pseudodiphtheriticum* DSM 44287 and *P. aeruginosa* PB112 165, 50 mg/ml for *S. aureus* NCTC 8325, *S. epidermidis* PM221, *Micrococcus luteus* NCTC 2665, *B. subtilis* 6051-HGW, *Staphylococcus saprophyticus* ATCC 15305 and *B. subtilis* KCTC 1028, 25 mg/ml for *P. aeruginosa* AAU2 and DSMO (negative control). The essential oil inhibited the growth of the bacterial strains used in this study at different rates depending on the concentration and the type of tested organisms. These results are in agreement with Mohamed et al. (2016), Mardafkan et al. (2015) and Paster et al. (1990), who concluded that Gram positive and Gram negative organisms were both susceptible to the oil. The expressed antimicrobial activity was concentration dependent as higher concentrations of the respective oils elicited a corresponding maximal antimicrobial activity. This trend is consistent with earlier reports by Idu et al. (2014) and Karigar et al. (2010) which revealed concentration dependent antimicrobial activity of seed oil extracts of *Khaya senegalensis* and *Leucaena leucocephala*, respectively. The response of the positive control was significantly ($P>0.05$) greater than the essential oil at its maximum concentration of 150 mg/mL. Only *P. aeruginosa* AAU2 displayed inhibitory zones that were greater than zones elicited by exposure to ciprofloxacin. However, *S. epidermidis* PM221 elaborated a maximal mean inhibitory zone upon exposure to *M. myristica* essential oil that was identical to that elicited by the same culture exposed to the control antibiotic. The antifungal activity of African nutmeg (*M. myristica*) essential oil against test organisms is shown in Table 6. No activity was recorded at lower concentrations except for *Rhizopus* species (3.33 mm) at 50 mg/ml. Zone of inhibition was observed and recorded at 100 and 150 mg/ml with *Mucor* and *Tricoderma* species having the lowest zone of inhibition (3.67 and 9 mm), respectively. The highest zones for both concentrations were observed against *Rhizopus* spp. (9.00 mm) and *Aspergillus flavus* (13 mm), respectively. There were

Table 1. Chemical composition of essential oil of African Nutmeg (*M. myristica*).

S/N	Compound	Chemical formula	RT	Area (%)
1	3-Thujene	$C_{10}H_{16}$	5.258	7.3
2	α-pinene	$C_{10}H_{16}$	5.400	12.2
3	Camphene	$C_{10}H_{16}$	5.625	1.0
4	β-pinene	$C_{10}H_{16}$	6.025	3.5
5	β-myrcene	$C_{10}H_{16}$	6.142	11.2
6	α-phellandrene	$C_{10}H_{16}$	6.492	16.3
7	Limonene	$C_{10}H_{16}$	6.825	17.6
8	γ-terpinene	$C_{10}H_{16}$	7.208	0.8
9	Terpinolene	$C_{10}H_{16}$	7.650	1.0
10	beta.-Linalool	$C_{10}H_{18}O$	7.850	7.3
11	Terpinenol, cis-β	$C_{10}H_{18}O$	8.267	0.6
12	p-menth-1-en-8-ol	$C_{10}H_{18}O$	9.342	1.2
13	Cis-Sabinol	$C_{10}H_{16}O$	9.550	5.7
14	13-Oxabicyclo[10.1.0]tridecane	$C_{12}H_{22}O$	10.150	0.8
15	Copaene	$C_{15}H_{24}$	11.825	0.8
16	β-cis-Caryophyllene	$C_{15}H_{24}$	12.475	1.7
17	β-farnesene	$C_{15}H_{24}$	12.692	1.5
18	-Caryophyllene	$C_{15}H_{24}$	12.933	0.6
19	α-amorphene	$C_{15}H_{24}$	13.158	0.8
20	trans-.α-Bergamotene	$C_{15}H_{24}$	13.292	1.8
21	α- Bisabolene	$C_{15}H_{24}$	13.475	1.8
22	Aromadendrene	$C_{15}H_{24}$	13.71	1.8
23	δ-Cadinene, (+)-	$C_{15}H_{24}$	13.783	2.2
24	Germacrene D-4-ol	$C_{15}H_{26}O$	14.758	0.7
25	tau.-Muurolol	$C_{15}H_{26}O$	15.950	0.2
Total identified (%)				**100**
Monoterpene hydrocarbons				70.9
Oxygenated monoterpenes				15.6
Monoterpenoids				85.5
Sesquiterpene hydrocarbons				13.0
Oxygenated sesquiterpenes				0.9
Sesquiterpenoids				13.9

significant differences ($P<0.05$) between the highest concentration of 150 mg/ml for all organisms and the Nystasin (positive control) except for *Aspergillus niger* and *A. flavus*. No significant differences ($P>0.05$) were recorded at concentrations below 100 mg/ml for *A. niger, A. flavus, Aspergillus* species and *Mucor* spp. and 50 mg/ml for *Rhizopus* spp. and DMSO (negative control). *A. flavus*, *Aspergillus* spp. and *Rhizopus* spp. elicited mean inhibitory zones greater than the zones elaborated by the same respective isolates exposed to Nystatin. The antifungal activity of the tested oil varied. This may be as a result of a wide variety of secondary metabolites in the plants (Fleischer, 2003). The antifungal potency of the oil is indicated as a decrease in the radial mycelia growth with increase in the concentration of essential oil tested.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the essential oils against bacterial isolates

The minimum inhibitory concentration and minimum bactericidal concentration of the essential oils against bacterial isolates are shown in Table 7. The MIC and MBC of *M. myristica* varied from 150 to 600 mg/ml against the test organisms. The oil inhibited *Bacillus*

Table 2. Biochemical characterization of bacterial isolates from Catfish (*Clarias gariepinus*) at 4 weeks storage.

S/N	Colonial morphology	Gram Reaction	Oxidase	Catalase	Citrate	Urea	Methyl Red	MRVP Gas	Voges Proskauser	Indole	Coagulase	Motility	Dextrose	Lactose	Maltose	Fructose	Sucrose	Galactose	Mannitol	Bacterial identity
1	LRFC	GPR	+	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	A+	*Bacillus sp.*
2	GDRR	GPR	+	+	+	-	-	+	+	+	+	-	-	+	-	+	+A	+	-	*Cornyebacterium* spp.
3	GFRC	GNB	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	-	-	*P. aureginosa*
4	B/GFRC	GPC	-	+	-	+	-	+	+	-	-	-	-	+	+	+	+	-	-	*S. epidermidis*
5	SFRC	GPC	+	+	-	-	-	-	+	-	-	-	-	-	-	-	A+	-	A+	*Micrococcus* spp.
6	LRFC	GPR	+	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	*B. subtilis*
7	PROCC	GPC	-	+	-	+	-	-	+	-	+	-	-	-	+	+	+	-	+	*S. aureus*
8	PROCC	GPC	-	+	-	+	-	-	+	-	-	-	-	-	+	+	+	-	+	*S. saprophyticus*
9	LRFC	GPR	+	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	*B. subtilis*
10	GFRC	GNB	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	-	-	*P. aureginosa*
11	PROCC	GPC	-	+	-	+	-	-	+	-	+	-	-	-	+	+	+	-	+	*S. aureus*

A: Acid production, G: gas production, GPC: Gram positive cocci, GNB: Gram negative bacillus, GNR: Gram negative rods, SFRC: small flat round colonies, PROCC: pink raise, opaque, circular cocci, GFRC: green flat round colonies, B/GFRC: blue/green flat round colonies, LRFC: large round flat colonies, GDRR: golden diplocci raised round.

Table 3. Biochemical characterization of fungal isolates from Catfish (*Clarias gariepinus*) at 4 weeks storage.

1	Growth form	Black, woolly with profuse growth	Greenish, woolly with profuse growth	Yellowish, woolly with profuse growth	White extensive woolly cottony with coenocytic hyphae	Whitish, luxuriant with profuse growth fluffy	Greenish patches or cushion luxuriant growth
2	Colour of reverse plate	Black	Creamy	Creamy	Whitish	Creamy	Green
	Microscopy						
3	Hyphae	Septate	Septate	Septate	Non-septate (young) Septate (old)	Non-septate	Septate
4	Conidiophores	Non-seplate terminating in globose swelling	Non-septate terminating in clavate swelling	Non-seplate, terminating in globose swelling	Non-septate, long erect usually unbranch single from coenocytic hyphae	Non-septate, upright terminating in globose swelling	Hyaline, upright much branched
5	Conidia	Present one-celled globose in dry basipetal chain	Present, globose in dry basipetal chains	Present, one-celled globose in dry basipetal chain	Present, hyaline one-celled, globose non-motile	Present, one-celled globose in dry basi[eta; chain	Hyaline, one-celled ovoid borne in small terminal clusters
6	Stolen	Absent	Absent	Absent	Absent, presence of coenocytic hyphae	Present	Absent
7	Rhizoid	Absent	Absent	Absent	Absent	Present, multi-branched short rooted	Absent
8	Spore colour	Black	Greenish	Creamy	Whitish	Dark	Greenish

Table 3. Contd.

9	Spore attachment	Bear sterigmata at the apex with conidia attached at the tip	Radiate from the entire surface at the tip	Bear phialides at the apex with conidia at the tip	Tip of sporangiophore in the sporangia	Consist of terminal swelling of multinucleated hyphal branches with conidia at the tip	Phialids single with small terminal cluster at tip
10	Tentative Identity	*Aspergillus niger*	*Aspergillus flavus*	*Aspergillus* spp.	*Mucor* spp.	*Rhizopus* spp.	*Trichoderma* spp.

Table 4. DNA Sequence Blast for isolates obtained from spoilt catfish (*Clarias gariepinus*).

Isolate code	Sequence blast	Ascension no.	Sequence identity	Query coverage length	Score bits (%)
1	HG519617.1 *Bacillus licheniformis* DSM 13 GGGGACGTTACGGATTCGACAGGGACGGATCGAGCTTGAGCTGCGAGCCGAGAGGCGATCTCGTAAAAACGCACCTAAAT ATAACTGGCAAATCTAACCAGAACTTAGCACTAGCTGCCTAATTAGCGCAGCGAGCTCTTGCCCGCATCGCCTATGTGCCG GTTAAGAGCCCATAACGAAGTAGGCTACGCTTGCGCCCCCGTCTGAGGGCGCAGGAAGAGACTCATCAGACTAGCTCTCC TAGGGCCCGCCCGCAGGCACGAAGATGAGCGAAACTAAATATGCAGGGCTACGCTCGTAGACGCTGAAGCAATCGACGTT TCTGGACGTGGGTTCGACTCCCACCGTCTCCAT	HG519617.1	*B. licheniformis* DSM 13	399	319/366 (87)
2	TTTATGGAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGAAAGGCCTCTT CGGAGGTACTCGAGTGGCGAACGGGTGAGTAACACGTGGGTGATCTGCCCTGCACTCTGGGATAAGCCTGGGAAACTGG GTCTAATACCGGATAGGACCATGCTTTAGTGTGTGGTGGAAAGTTTTTTCGGTGTAGGATGAGCCCGCGGCCTATCAGC TTGTTGGTGGGGTAATGGCCTACCAAGGCGGCGACGGGTAGCCGGACTGAGAGGTTGGTCGGCCACATTGGGACTGAGA TACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCGACGCCGCGTG GGGGATGACGGCCTTCGGGTTGTAAACTCCTTTCGCCAGGGACGAAGCGTTTTGTGACGGTACCTGGAGAAGAAGCACCG GCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTGTCCGGAATTACTGGGCGTAAAGAGCTCGTAGG TGGTTTGTCACGTCGTCTGTGAAATTCCACAGCTTAACTGTGGGCGTGCAGGCGATACGGGCTGACTTGAGTACTGTAGGG GTAACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTTACTGGGCAG TTACTGACGCTGAGGAGCGAAAGCATGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGGTGGGCGC TAGGTGTAGGGACCTTTTGGGGTTTCTGTGCCGTAGCTAACGCATTAAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCT AAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGATTAATTCGATGCAACGCGAAGAACCTTACC TGGGCTTGACATACACTGGATCGCTGCAGAGATGTAGTTTCCCTTGTGGCTAGTGTACAGGTGGTGCATGGCTGTCGTCAG CTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCTTATGTTGCCAGCACGTTATGGTGGGGACT CATGAGAGACTGCCGGGGTTAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCTTCAC ACATGCTACAATGGCCGGTACAATGCGCAGCGAGCCTGTGAGGGTGAGCTAATCGCTTAAAGCTGGTCTTAGTTCGGATTG GGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAATGCTGCGGTGAATACGTTCCCGGGC CTTGTACACACCGCCCGTCACGTCATGAAAGTTGGTAACACCCGAAGCCGGTGGCTCAAACTCGTTTAGGGAGCCGTCGA AGGTGGGATCGGCGATTGGGACGAAGTCGTAACAAGGTAGCCGTACCGGAAGGTGCGGCTGGATCACCTCCTTTCTAAGG AGTTTTTA	JIAH01000003.1	*C. pseudodiphtheriticum* DSM 44287	1085	1265/1585 (80)

licheniformis DSM 13. *C. pseudodiphtheriticum* DSM 44287, *S. aureus* NCTC 8325 and *S. saprophyticus* ATCC 15305 were inhibited at 300 mg/ml. MIC/MBC of 600 mg/ml inhibited growth of *B. subtilis* KCTC 1028 and *P. aeruginosa* PB112 165. While *P. aeruginosa* AAU2, *subtilis* 6051-HGW were inhibited at the lowest concentration of 150 mg/ml.

The lowest MIC/MBC negative bacteria negates earlier reports that they are more resistant or less susceptible to antimicrobial activities because of their capacity form biofilm (Marwah et al., 2007;

Table 4. Contd.

Isolate code	Sequence blast	Ascension no.	*Sequence identity*	Query coverage length	Score bits (%)
3	AGTCGAGCGGATGAAGGGAGCTTGCTCCTGGATTCAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTG GGGGATAACGTCCGGAAACGGGCGCTAATACCGCATACGTCCTGAGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTAT CAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAACTGGTCTGAGAGGAT GATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAG CCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTA ATACCTTGCTGTTTTGACGTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCA AGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCAGCAAGTTGGATGTGAAATCCCCGGGCTCAACCTG GGAACTGCATCCAAAACTACTGAGCTAGAGTACGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATAT AGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGG ATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGCTAACG CGATAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGA GCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCTGAGAACTTTCCAGAGATGGATTGGTGC CTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGC GCAACCCTTGTCCTTAGTTACCAGCACCTCGGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGG GATGACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGCCAAGCCG CGAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAATCGCTA GTAATCGTGAATCAGAATGTCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTG CTCCAGAAGTAGCTAGTCTAACC	JQ904623.1	*P. aeruginosa* strain AAU2	2551	1383/1384 (99)
4	CGAAGAATTATTAGAATTAGTTGAAATGGAAGTTCGTGACTTATTAAGCGAATATGACTTCCCAGGTGACGATGTACCTGTA ATCGCTGGTTCTGCATTAAAAGCATTAGAAGGCGATGCTGAATACGAACAAAAAATCTTAGACTTAATGCAAGCAGTTGATG ATTACATTCCAACTCCAGAACGTGATTCTGACAAACCATTCATGATGCCAGTTGAGGACGTATTCTCAATCACTGGTCGTGG TACTGTTGCTACAGGCCGTGTTGAACGTGGTCAAATCAAAGTTGGTGAAGAAGTTGAAATCATCGGTATGCACGAAACTTCT AAAACAACTGTTACTGGTGTAGAAATGTTCCGTAAATTATTAGACTACGCTGAAGCTGGTGACAACATCGGTGCTTTATTAC GTGGTGTTGCACGTGAAGACGTACAACGTGGTCAAGTATTAGCTGCTCCTGGTTCTATTACACCACACACAAAATTCAAAGC TGAAGTATACGTATTATCTAAAGATGAAGGTGGACGTCACACTCCATTCTTCACTAACTATCGCCCACAATTCTATTTCCGTA CTACTGACGTAACTGGTGTTGTAAACTTACCAGAAGGTACAGAAATGGT	NZ_HG813242.1	*S. epidermidis* PM221	1153	624/624 (100)
5	GGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCTGTCGTGAAAGTCCGGGGCTTAACCCCGGATCTGCGG TGGGTACGGGCAGACTAGAGTGCAGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGA ACACCGATGGCGAAGGCAGGTCTCTGGGCTGTAACTGACGCTGAGGAGCGAAAGCATGGGGAGCGAACAGGATTAGATA CCCTGGTAGTCCATGCCGTAAACGTTGGGCACTAGGTGTGGGGACCATTCCACGGTTTCCGCGCCGCAGCTAACGCATTA AGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCAT GCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGTTCCCGATCGCCGTAGAGATACGATTTCCCCTT TGGGGCGGGTTCACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAA CCCTCGTTCCATGTTGCCAGCACGTAATGGTGGGGACTCATGGGAGACTGCCGGGGTCAACTCGGAGGAAGGTGAGGAC GACGTCAAATCATCATGCCCCTTATGTCTTGGGCTTCACGCATGCTACAATGGCCGGTACAATGGGTTGCGATACTGTGAG GTGGAGCTAATCCCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAA TCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGTCACGAAAGTCGGTAACAC CCGAAGCCGGTGGCCTAACC	NC_012803.1	*M. luteus* NCTC 2665	721	738/906 (81)
6	TTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGGACAGATGGGAGCTTGCTCCCTGA TGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATAC CGGATGGTTGTTTGAACCGCATGGTTCAAACATAAAAGGTGGCTTCGGCTACCACTTACAGATGGACCCGCGGCGCATTAG CTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAG ACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGT GAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGG TACCTAACCAGAAAGCC	NC_020507.1	*B. subtilis* strain 6051-HGW	894	493/497 (99)

Table 4. Contd.

Isolate code	Sequence blast	Ascension no.	*Sequence identity*	Query coverage length	Score bits (%)
7	GGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAACGGACGAGAAGCTTGCTTCTCTGATGTTAGCGGCGGACGGG TGAGTAACACGTGGATAACCTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCGGATAATATTTTGAACCGCATGG TTCAAAAGTGAAAGACGGTCTTGCTGTCACTTATAGATGGATCCGCGCTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCA ACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTAGGG AATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTCTTCGGATCGTAAAACTCTGTTATTAGGGA AGAACATATGTGTAAGTAACTGTGCACATCTTGACGGTACCTAATCAGAAAGCC	NC_007795.1	*S. aureus* NCTC 8325	767	460/483 (95)
8	GGCTTTCTGATTAGGTACCGTCAAGACGTGCACAGTTACTTACACGTTTGTTCTTCCCTAATAACAGAGTTTTACGAGCCGAAACCCT TCATCACTCACGCGGCGTTGCTCCGTCAGGCTTTCGCCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGACCGT GTCTCAGTTCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGTATCGTCGCCTTGGTAAGCCGTTACCTTACCAACTAGCTAATA CGGCGCGGGTCCATCTATAAGTGATAGCAAAACCATCTTTCACTTTAGAACCATGCGGTTCCAAATGTTATCCGGTATTAGCTCCGG TTTCCCGAAGTTATCCCAGTCTTATAGGTAGGTTACCCACGTGTTACTCACCCGTCCGCCGCTAACGTCAAAGGAGCAAGCTCCTTA TCTGTTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATCC	NC_007350.1	*S. saprophyticus* ATCC 15305	845	-
9	TTTGTATGCAATGAATGATTTAGGCATGACAAGTGACAAGCCTTATAAAAAATCCGCGCGTATCGTTGGAGAAGTTATCGGGAAATAC CACCCGCACGGTGATTCAGCGGTATATGAATCCATGGTCAGAATGGCTCAGGATTTCAACTACCGTTATATGCTCGTTGACGGTCAC GGAAACTTCGGTTCTGTTGACGGAGACTCAGCGGCGGCCATGCGTTATACAGAAGCACGAATGTCTAAAATCTCAATGGAGATTCTT CGCGACATCACAAAAGACACAATCGATTACCAGGATAACTATGACGGGTCAGAAAGAGAACCTGTCGTTATGCCTTCAAGGTTCCCG AATCTGCTCGTGAACGGTGCTGCCGGCATTGCGGTAGGTATGGCAACAAACATTCCTCCGCACCAGCTGGGAGAAATCATTGACGG TGTACTTGCTGTTAGTGAGAATCCGGACATTACAATTCCAGAGCTTATGGAAGTCATTCCAGGGCCTGATTTCCCGACCGCGGGTCA AATCTTGGGACGCAGCGGTATCCGGAAAGCATACGAATCAGGCCGAGGCTCTATCACGATCCGGGCAAAAGCTGAGATCGAACAA ACATCTTCGGGTAAAGAAAGAATTATCGTTACAGAGTTACCTTACCAAGTAAATAAGGCGAAATTAATTGAGAAAATTGCTGATCTCG TAAGGGACAAAAAGATAGAGGGTATCACAGATCTGCGTGATGAGTCAGATCGTACAGGTATGAGAATTGTCATTGAAATCAGACGCG ATGCCAATGCGAATGTTATCTTAAACAATCTGTACAAACAAACTGCTCTACAAAC	NZ_CP011115.1	*B. subtilis* KCTC 1028	1519	832/837 (99)
10	AGTCGAGCGGATGAAGGGAGCTTGCTCCTGGATTCAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGAT AACGTCCGGAAACGGGCGCTAATACCGCATACGTCCTGAGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCT AGGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGA ACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGT GTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGACGTTACCAAC AGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCG CGCGTAGGTGGTTCAGCAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTACTGAGCTAGAGTACGGTA GAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGAT ACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGT TGGGATCCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAA TTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCTGAGA ACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGT TAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACCTCGGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGG AGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGCC AAGCCGCGAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAATCGCTA GTAATCGTGAATCAGAATGTCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCTCCAG AAGTAGCTAGTCTAACC	JN996498.1	*P. aeruginosa* strain PB112 16S	1446	1383/1384 (99)
11	GGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAACGGACGAGAAGCTTGCTTCTCTGATGTTAGCGGCGGACGGG TGAGTAACACGTGGATAACCTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCGGATAATATTTTGAACCGCATGG TTCAAAAGTGAAAGACGGTCTTGCTGTCACTTATAGATGGATCCGCGCTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCA ACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTAGGG AATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTCTTCGGATCGTAAAACTCTGTTATTAGGGA AGAACATATGTGTAAGTAACTGTGCACATCTTGACGGTACCTAATCAGAAAGCC	NC_007795.1	*S. aureus* NCTC 8325	767	460/483 (95)

Table 5. Zone of inhibition (mm) for antibacterial activity of African Nutmeg (*M. myristica*) essential oil by the disc diffusion method.

Bacterial isolate	Zone of inhibition (mm)						Positive control (Ciprofloxacin)	Negative control (DMSO)
	150 mg/mL	100 mg/mL	50 mg/mL	25.0 mg/mL	12.5 mg/mL	6.25 mg/ mL		
Gram positive								
B. lichenformis DSM 13	13.00^{b}±0.58	8.33^{c}±0.88	3.33^{d}±0.33	0.00^{e}±0.00	0.00^{e}±0.00	0.00^{e}±0.00	25.00^{a}±0.40	0.00 ^{e}±0.00
C. pseudodiphtheriticum DSM 44287	12.67^{b}±1.200	5.00^{c}±0.58	0.00^{d}±0.00	0.00^{d}±0.00	0.00^{d}±0.00	0.00^{d}±0.00	21.67^{a}±0.11	0.00^{d}±0.00
S. aureus NCTC 8325	16.33^{a}±0.88	9.33^{b}±0.88	2.67^{c}±1.76	0.00^{d}±0.00	0.00^{d}±0.00	0.00^{d}±0.00	17.67^{a}±0.30	0.00^{d}±0.00
S. epidermidis PM221	19.00^{a}±0.00	12.33^{b}±0.88	2.67^{c}±1.45	0.00^{d}±0.00	0.00^{d}±0.00	0.00^{d}±0.00	19.00^{a}±0.20	0.00^{d}±0.00
M. luteus NCTC 2665	16.67^{b}±0.088	8.00^{c}±0.58	4.33^{d}±0.33	0.00^{e}±0.00	0.00^{e}±0.00	0.00^{e}±0.00	27.33^{a}±0.41	0.00^{e}±0.00
B. subtilis 6051-HGW	16.00^{b}±1.53	9.00^{c}±2.08	3.33^{d}±0.88	0.00^{e}±0.00	0.00^{e}±0.00	0.00^{e}±0.00	25.0^{a}±0.20	0.00^{e}±0.00
S. saprophyticus ATCC 15305	16.00^{b}±0.00	9.00^{c}±0.88	1.33^{d}±0.88	0.00^{d}± 0.00	0.00^{d}±0.00	0.00^{d}±0.00	19.67^{a}±0.30	0.00^{d}±0.00
B. subtilis KCTC 1028	8.33^{b}±0.88	1.67^{c}±0.88	1.33^{c}±0.88	0.00^{c}±0.00	0.00^{c}±0.00	0.00±0.00	21.67^{a}±0.11	0.00^{c}±0.00
Gram negative								
P. aeruginosa AAU2	23.33^{a}±0.88	20.00^{b}±0.58	9.33^{d}±0.88	3.67^{e}±0.88	0.00^{f}±0.00	0.00^{f}±0.00	17.00^{c}±0.20	0.00^{c}±0.00
P. aeruginosa PB112 165	11.67^{b}±1.20	5.00^{c}±0.58	0.00^{d}±0.00	0.00^{d}±0.00	0.00^{d}±0.00	0.00^{d}±0.00	17.67^{a}±0.50	0.00^{d}±0.00

*****Values are expressed as mean ± standard error of mean; values are exclude diameter of agar well (4 mm). *Means in the same row with same superscripts are not significantly different (P>0.05) from the positive control.

Table 6. Zone of inhibition (mm) for antifungal activity of Nutmeg (*Monodora myristica*) essential oil by the disc diffusion method.

Fungal isolate	Zone of inhibition (mm)						Positive control (Nystasin)	Negative control (DMSO)
	150 mg/mL	100 mg/mL	50 mg/mL	25.0 mg/mL	12.5 mg/mL	6.25 mg/mL		
A. niger	10.00^{a}±1.15	5.33^{b}±1.33	0.00^{c}±0.00	0.00^{c}±0.00	0.00^{c}±0.00	0.00^{c}±0.00	11.00^{a}±0.20	0.00^{c}±0.00
A. flavus	13.00^{a}±0.58	7.33^{b}±1.20	0.00^{c}±0.00	0.00^{c}±0.00	0.00^{c}±0.00	0.00^{c}±0.00	12.00^{a}±0.20	0.00^{c}±0.00
Aspergillus spp.	10.00^{a}±0.58	5.00^{c}±1.15	0.00^{d}±0.00	0.00^{d}±0.00	0.00^{d}±0.00	0.00^{d}±0.00	8.00^{b}±0.20	0.00^{d}±0.00
Mucor spp.	10.33^{b}±0.88	3.67^{c}±1.20	0.00^{d}±0.00	0.00^{d}±0.00	0.00^{d}±0.00	0.00^{d}±0.00	13.67^{a}±0.30	0.00^{d}±0.00
Rhizopus spp.	12.33^{a}±0.88	9.00^{c}±0.58	3.33^{d}±0.88	0.00^{e}±0.00	0.00^{e}±0.00	0.00^{e}±0.00	10.33^{b}±0.11	0.00^{e}±0.00
Trichoderma spp.	9.00^{b}±0.58	4.67^{c}±0.88	0.00^{d}±0.00	0.00^{d}±0.00	0.00^{d}±0.00	0.00^{d}±0.00	13.33^{a}±0.11	0.00^{d}±0.00

*****Values are expressed as mean ± standard error of mean; values are exclude diameter of agar well (4 mm). *Means in the same row with same superscripts are not significantly different (P>0.05) from the positive control.

Wannissorn et al., 2009; Bouhdid et al., 2009; Park and Lee, 2011; Gabriel et al., 2013). However, the variation observed between the two strains of *P. aeruginosa* AAU2 and *P. aeruginosa* PB112 165 at MIC and MBC of 150 and 600 mg/ml, respectively may be as a result of the cell wall composition of the organisms and their physiological properties. The MIC and MFC of the essential oils against the fungal isolates are shown in Table 8. *M. myristica*, MIC and MFC ranged between 300 and 600 mg/ml. *A. flavus* and *Rhizopus* spp. were inhibited minimally at 300

Table 7. Minimum inhibitory concentration (mg/ml) and minimum bactericidal concentration (mg/ml) of the bacterial isolates exposed to varying concentrations of *M. myristica* essential oil.

Isolate	MIC	MBC
Gram positive		
B. lichenformis DSM 13	300	300
C. pseudodiphtheriticum DSM 44287	300	300
S. epidermidis PM221	150	150
M. luteus NCTC 2665	150	150
B. subtilis 6051-HGW	150	150
S. aureus NCTC 8325	300	300
S. saprophyticus ATCC 15305	300	300
B. subtilis KCTC 1028	600	600
Gram negative		
P. aeruginosa AAU2	150	150
P. aeruginosa PB112 165	600	600

Table 8. Minimum inhibitory concentration (mg/ml) and minimum fungicidal concentration (mg/ml) of the fungal isolates exposed to varying concentrations of *M. myristica* essential oil.

Isolate	MIC	MFC
Aspergillus flavus	300	300
Aspergillus niger	600	600
Aspergillus spp.	600	600
Mucor spp.	600	600
Rhizopus spp.	300	300
Trichoderma spp.	600	600

mg/ml. All other fungal isolates were inhibited at 600 mg/ml. The higher MIC/MFC values among fugal isolates suggest that active compounds in *M. myristica* were less effective against *A. niger, Aspergillus* spp., *Mucor* spp. and *Trichoderma* spp.

Conclusion

In this study, *M. myristica* essential oil contained twenty-five components which were mainly monoterpene hydrocarbons constituents consisting of limonene (17.6%), α-phellandrene (16.3%), α-pinene (12.2%), β-myrcene (11.2%), 3-Thujene and β-Linalool (7.3%). The oil showed a lesser antimicrobial activity than the positive control (Ciprofloxacin), but showed antifungal activity comparable to Nystatin at maximum concentration of 150 mg/mL. However, the oil had the greatest bacteriocidal activity at MIC/MBC of 150 mg/mL against *S. epidermidis* PM221, *M. luteus* NCTC 2665, *B. subtilis* 6051-HGW and *P. aeruginosa* AAU2 and the greatest MIC/MFC of 300 mg/mL against *A. flavus* and *Rhizopus* spp. In view of the aforementioned, the expressed antimicrobial activity is a demonstration of the efficacy of the oil against fish spoilage organism and use in the fishery industry.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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