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Original Research Article

Bioactive constituents and antimicrobial activity of essential oil of ginger (*Zingiber officinale*) on microorganisms isolated from smoke-dried catfish (*Clarias gariepinus*)

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Essential oils are gaining prominence because of their antimicrobial and antioxidative properties. This study aims to investigate the bioactive constituents and antimicrobial activity of essential oil of Ginger (*Z. officinale*) on microorganisms isolated from smoke-dried Catfish (*Clarias gariepinus*). Dried *Z. officinale* rhizomes were obtained from Oba market in Edo State. Essential oil was extracted using steam distillation and the chemical composition of oil was determined using the gas chromatography-mass spectroscopy (GC-MS) technique. The antimicrobial activity *Z. officinale* was evaluated using agar well diffusion method. The results showed that *Z. officinale* contained thirty-three compounds which consisted of 4.94% monoterpene hydrocarbons, 58.45% sesquiterpene hydrocarbons, 5.28% oxygenated sesquiterpenes and 28.34% oxygenated monoterpenes. The most abundant compound were beta.-Sesquiphellandrene (16.53%), Cyclohexene,1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-, (*S*)- (13.87%), alpha.-Curcumene (8.34%) and (-)-Zingiberene (8.02%). *Z. officinale* oil elicited antibacterial activity against both Gram positive and negative bacterial isolates. The essential oil had a higher antibacterial activity than antifungal activity. The MIC, MBC and MFC studies revealed that *Z. officinale* was biocidal at the minimal concentration of 150 mg/mL against five bacterialisolates; *B. lichenformis* DSM 13, *P. aeruginosa* AAU2, *S. epidermidis* PM221, *S. aureus* NCTC 8325, *P. aeruginosa* PB112 165 and three fungal isolates; *Aspergillus flavus, Mucor sp*. and *Rhizopus sp*.

Key words: Bioactive components, essential oils, antimicrobial activity, *Zingiber officinale*, well diffusion method, *Clarias gariepinus.*

INTRODUCTION

Ginger (*Zingiber officinale*) belongs to the family Zingiberaceae. It is aherbaceous rhizomatous perennial plant that is widely grown in warm climatic areas of the world such as Bangladesh, Taiwan, India, Jamaica, and the United States of America and Nigeria and exported. Ginger can grow to about 2 - 4 foot with linear, grass-like leaves

and oblong yellowish green flowers in a few scarious bracts (Ogbuewu et al., 2014). The presence of volatile essential oils (1-3%) and oleoresins (4-7.5%) has broad antioxidant activity as well as a pungent flavor which is a distinctive characteristic flavor (Balanchandarn et al., 2006). The oil may vary in color from pale yellow to darker amber colour and the viscosity also ranges from medium to watery. The most predominant important reported components are α-zingiberene, geranial, geraniol, β-bisabolene, nerol, 1,8-cineol, α-terpineol, borneol, p-cineole, α-terpineol β-phellandrene, α-curcumene, α-farnesene, β-sesquiphellandrene, camphene, neral, geranyl acetate, α-terpinene; cadina-1,4-diene, 6-gingerol and 6-shogaol (El-Ghorab et al., 2010; El-Baroty et al., 2010; Moshafi et al., 2009; Malek et al., 2005; Sultan et al., 2005; Pino et al., 2004; Zancan et al., 2002). Ginger is a common herb widely utilized as spice universally and over the years, has been used for the treatment of few illnesses in traditionalmedicine (Bartley and Jacobs, 2000).

It possess antimicrobial, antioxidant, cardiovascular protection, anti-inflammatory, glucose lowering and anti-cancer activities as a result of the presence several therapeutically active plant-derived secondary metabolites such as oleoresin, phenolics, zingiberene and gingerols which are of great pharmacological importance (Shukla and Singh, 2007; Yang et al., 2011). Ginger has been successfully used in the treatment of gastrointestinal infections, dizziness, vomiting, nausea, rheumatic diseases and to relieve headache (Singh et al., 2016). Currently, essential oils and their constituents and products of secondary metabolites have been used in food industry because of their antimicrobial and antioxidative properties instead of the commonly used synthetic preservatives such as butylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT), propyl gallate (PG) and tertbutylhydroquinone (TBHQ). These synthetic preservatives have elevated fears as a consequence of their noxious and carcinogenic effects. Several studies on the antimicrobial activity of essential oil from Ginger have been reported (Lopez et al., 2017; Nanasembat and Lohasupthawee, 2005; Gupta and Ravishankar, 2005; Ficker et al., 2003; Agarwal et al., 2001; Martins et al., 2001). However, there is little information about the constituents and antimicrobial activity of ginger essential oils from Nigeria. The objective of our study was to investigate the bioactive constituents and antimicrobial activity of essential oils of Ginger (*Zingiber officinale*) on microorganisms isolated from smoke-dried Catfish (*Clarias gariepinus*) .

MATERIAL AND METHODS

Collection of materials

Dried Ginger (*Zingiber officinale*) rhizomes 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 rhizomes were then ground into fine powder and stored in an air tight plastic container and placed at room temperature (28 ± 2 ˚C) for 48 hours before use.

Extraction of the essential oils

3000 g of the ground dried powder was weighed into distillation flask fitted with condensers. Steam was supplied to the flask through a steam generator at constant flow. The essential oil which vaporizes with the steam was condensed into a collecting separating funnel. The oil was separated by gravity and dried over anhydrous sodium sulphate, filtered and stored at 4°C until analysed (Hussain et al., 2008).

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The essential oil was analysed using a QP2010Plus 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 x 0.32 mm, film thickness of 0.25µm) was used.The carrier gas used was Helium at a flow rate of 36.2 cm/sec and at a constant pressure of 90 k Pa. 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 per minute to 180°C and held for 3 minutes. This was then increased at 12°C per minute to 280°C which was finally held for 2 minutes, while maintaining the injection temperature at 250 °C.

Identification of constituents

The identification of components was based on comparison of their mass spectra with those present in the National Institute for Standard Technology computer data bank (NIST:2009s. LIB) (Adams, 2001).

Antimicrobial Assay of Essential oils

Collection and identification of Bacterial and Fungal isolates

The isolates used in this study were obtained from fish samples already infected with microorganisms in Oba Market, Benin City, Edo State. The bacteria isolates were maintained on Nutrient agar (Oxoid Ltd., Basingstoke, Hampshire, UK) agar slant at 37°C for 24 hrs while fungal isolates were cultivated on Potato Dextrose Agar (Oxoid Ltd., Basingstoke, Hampshire, UK) slant at 25˚C for 3 days. Following incubation, bacterial isolates were identified by morphological and biochemical methods according to (Cowen and Steel, 2003). While fungal isolates were identified on the basis of cultural and morphological characteristics as described by (Oyeleke. and Okusanmi,

2008). The isolates 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) for further analyses.

Extraction of DNA from Bacteria Isolates

Genomic DNA was isolated from the bacteria isolates using Sigma Aldrich DNA extraction Kit. Further 16S rDNA was amplified by PCR from the above isolated genomic DNA. 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.

Polymerase Chain Reaction

16S rDNA region was amplified by PCR from bacterial genomic DNA using PCR universal pimers: 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. 3µl of PCR product was loaded with 3µl 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 centimeters from the wells. Further the gel was viewed on Gel documentation system.

Purification of Bacteria Isolates

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 BLAST (Basic Local Alignment Search Tool) 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 were determined by 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 hours. A bacterial colony was obtained 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 stand for 15 minutes and 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 hour and then incubated at 37˚C for 24 hours. A suspension of 0.5% w/v ciprofloxacin (Fidson Healthcare Ltd., Nigeria) was used as positive control while the solvent only was used as negative control. This experiment was performed in triplicate plates for each isolate per dissolution solvent. The diameter (mm) of zone of inhibition was recorded for the different concentrations of essential oil samples.

Antifungal Screening of Essential oils

The fungal isolates were sub cultured on Potato Dextrose Agar at (28 ± 2˚C) for 3-5 days. Equally spaced 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 added to the wells as described above. A 4 mm diameter fungal mycelial disc 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 and the different dissolution solvent systems 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 protocol 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 achieve 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 the respective test organism. All test plates were incubated at 37˚C for 24 hrs for bacteria and (28±2°C) for 72 hrs 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 derived 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 incubated at 37 °C for 24 hours for bacteria and (28 ± 2°C) for 3-5 days for fungi were observed for growth. The absence of growth at the end of incubation period signifies 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 using statistical package for social science (SPSS) version 21.0. Duncan Multiple Range Test at 95% confidence level was used to separate significant.

RESULTS

Bioactive constituents of Ginger (*Zingiber officinale*)

The detected components of the essential oil of *Zingiber officinale*, chemical formular and their relative percentages are presented in Table 1. Thirty-three components were identified representing 100.00% of the oil. In general, the essential consisted of 3.28% monoterpene hydrocarbons, 58.89% sesquiterpene hydrocarbons, 27.01% oxygenated monoterpenes, 12.92% oxygenated sesquiterpenes and 1.22% aromatic compound. The main constituents of the oil were beta.-Sesquiphellandrene (16.53%), Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-,(*S*)- (13.87%), alpha.-Curcumene (8.34%),(-)-Zingiberene (8.02%), alpha.-Citral (5.54%), D-nerolidol (4.61%), beta.-Citral (4.15%), 1,6,10-Dodecatriene,7,11-dimethyl-3-methylene- (3.40%), Bicyclo[7.2.0]undec-4-ene,4,11,11-trimethyl-8-methylene-, [1R (1R*,4Z, 9S*)] (2.80%), (Z,E)-Farnesol (2.76%), alpha.-Phellandrene (2.57%), Cyclopropanecarboxaldehyde,2-methyl-2-(4-methyl-3-pentenyl)-,trans-(.+-.) (2.23%), Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)- (2.04%), Eudesm-4(14)-en-11-ol and 2-Cyclohexen-1-ol,2-methyl-5-(1-methylethenyl)-,trans- (1.94%), Isoborneol (1.71%), Copaene (1.65%), Geraniol acetate (1.63%), Bicyclo[3.1.0]hexan-2-ol,2-methyl-5-(1-methylethyl)-,(1.alpha.,2.beta.,5.alpha.)- (1.61%), alpha.-Bisabolol (1.56%), 6,10-Dodecadien-1-yn-3-ol, 3,7,11-trimethyl- (1.51%), gamma Elemene (1.39%), p-menth-1-en-8-ol (1.34%), Linalool (1.32%), Benzene, 1-methyl-2-(1-methylethyl)-(1.22%). While minor constituents were 2-Undecanone (0.94%), Citronellol (0.84%), Bergamotol, Z-.alpha.-trans- (0.51%), Cyclopropanemethanol,.alpha.,2-dimethyl-2-(4-methyl-3-pentenyl)-,[1.alpha.(R*), 2.alpha.]- (0.48%), beta.-Farnesene and alpha.Curcumene (0.44%), 1HCycloprop[e]azulene,1a,2,3,4,-4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-,[1aR-(1a.alpha.,4.alpha.,4a.beta.,7b.alpha.)]- (0.41%), beta.-Myrcene (0.37%) and 2,6-Octadiene, 2,6-dimethyl- (0.34%).

Antibacterial activity of *Zingiber officinale* Essential oil

The bacteria isolates identified are shown in Table 2. They include *B. lichenformis* DSM 13, *C. pseudodiphtheriticum* DSM 44287, *S. aureus* NCTC 8325, *S. epidermidis* PM221, *M. luteus* NCTC 2665, *B. subtilis* 6051-HGW, *S. saprophyticus* ATCC 15305, *B. subtilis* KCTC 1028, *P. aeruginosa* AAU2 and *P. aeruginosa* PB112 165. The antibacterial activity of *Zingiber officinale* against isolates is shown on Table 3. The zone of inhibition against all isolates increased with concentration of the essential oil. The essential oil of *Zingiber officinale* had no activity at 6.25 mg/mL. *B. lichenformis* DSM 13 and *P. aeruginosa* PB112 165 were the only organisms inhibited at 12.5 mg/mL with a zone of 2.67 mm and 2 mm respectively. *C. pseudodiphtheriticum* DSM 44287*, S. epidermdis* PM221*, B. subtilis* KCTC 1028 and *B. subtilis* 6051-HGW, *P. aeruginosa* AAU2 and *P. aeruginosa* PB112 165 were inhibited at the concentration of 50 mg/mL. All test organisms were inhibited at 100 mg/mL with *C. pseudodiphtheriticum* DSM 44287 having the lowest zone (4 mm) and *B. lichenformis* DSM 13 is having the highest zone (22 mm). At 150 mg/mL, the lowest zone was observed against *C. pseudodiphtheriticum* DSM 44287 (12.33 mm) while the highest was observed against *B. lichenformis* DSM 13 (27 mm). There was a significant difference ($p<0.05$) between all concentrations and the Ciprofloxacin (positive control) except at 150 mg/mL.

The antifungal activity of *Zingiber officinale* Essential oil

The fungal isolates identified are shown in Table 4. They include *A. niger, A. flavus, Aspergillus sp*., *Mucor sp., Rhizopus sp.* and *Trichoderma sp.* The antifungal activity of *Zingiber officinale* against isolates is shown on Table 5. The essential oil had no activity against the fungal isolates at 6.25, 12.5 and 25 mg/mL respectively. With the exception of *Rhizopus sp* (4.33 mm), no activity was recorded at 50 mg/mL of the oil against the other organisms. At 100 mg/mL and 150 mg/mL, the lowest inhibition zone was observed against *Trichoderma sp* (6.67 mm and 12 mm) while the highest was observed against *Mucor sp* (12.67mm and 18mm) respectively. There was a significant difference ($p<0.05$) between all concentrations and the Nystasin (positive control) except at 100 mg/mL. The antifungal activity of *Z. officinale* essential oil, at a concentration of 150 mg/mL against the exposed fungal cultures in comparison to the activity of Nystatin was impressive as only *Trichoderma sp*.

Table 1. Chemical composition of essential oil from Ginger (*Z. Officinale*)

S/N	Compound	Chemical formula	RT	Area (%)
1	beta.-Myrcene	$C_{10}H_{16}$	6.117	0.37
2	alpha.-Phellandrene	$C_{10}H_{16}$	6.408	2.57
3	Benzene, 1-methyl-2-(1-methylethyl)-	$C_{10}H_{14}$	6.725	1.22
4	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-	$C_{10}H_{18}O$	6.817	1.61
5	Linalool	$C_{10}H_{18}O$	7.842	1.32
6	Isoborneol	$C_{10}H_{18}O$	9.025	1.71
7	p-menth-1-en-8-ol	$C_{10}H_{18}O$	9.350	1.34
8	Citronellol	$C_{10}H_{20}O$	9.742	0.84
9	beta.-Citral	$C_{10}H_{16}O$	9.958	4.15
10	alpha.-Citral	$C_{10}H_{16}O$	10.375	5.54
11	2-Undecanone	$C_{11}H_{22}O$	10.575	0.94
12	2,6-Octadiene, 2,6-dimethyl-	$C_{10}H_{18}$	11.333	0.34
13	Geraniol acetate	$C_{12}H_{20}O_2$	11.767	1.63
14	Copaene	$C_{15}H_{24}$	11.833	1.65
15	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-	$C_{15}H_{24}$	12.025	2.04
16	1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.alpha.,4a.beta.,7b.alpha.)]-	$C_{15}H_{24}$	12.308	0.41
17	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-	$C_{15}H_{24}$	12.492	2.80
18	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-	$C_{15}H_{24}$	12.717	3.40
19	alpha.-Curcumene	$C_{15}H_{22}$	13.208	8.34
20	(-)-Zingiberene	$C_{15}H_{24}$	13.433	8.02
21	Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-, (S)-	$C_{15}H_{24}$	13.558	13.87
22	.beta.-Sesquiphellandrene	$C_{15}H_{24}$	13.808	16.53
23	D-nerolidol	$C_{10}H_{26}O$	14.275	4.61
24	gamma.-Elemene	$C_{15}H_{24}$	14.492	1.39
25	alpha.-Bisabolol	$C_{15}H_{26}O$	14.850	1.56
26	(Z,E)-Farnesol	$C_{15}H_{26}O$	15.242	2.76
27	Cyclopropanecarboxaldehyde, 2-methyl-2-(4-methyl-3-pentenyl)-, trans-(.+-.)-	$C_{11}H_{18}O$	15.567	2.23
28	Eudesm-4(14)-en-11-ol	$C_{15}H_{26}O$	16.300	1.94
29	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, trans-	$C_{10}H_{16}O$	16.675	1.94
30	6,10-Dodecadien-1-yn-3-ol, 3,7,11-trimethyl-	$C_{15}H_{24}O$	16.833	1.51
31	:Bergamotol, Z-.alpha.-trans-	$C_{15}H_{24}O$	17.750	0.51
32	Cyclopropanemethanol, .alpha., 2-dimethyl-2-(4-methyl-3-pentenyl)-, [1.alpha.(R*),2.alpha.]-	$C_{12}H_{22}O$	18.725	0.48
33	beta.-Farnesene	$C_{15}H_{24}$	19.183	0.44
	Total identified (%)			**100.00%**
	Monoterpene hydrocarbons			4.94%
	Oxygenated monoterpenes			28.34%
	Monoterpenoids			**33.28%**
	Sesquiterpene hydrocarbons			58.45%
	Oxygenated sesquiterpenes			8.28%
	Sesquiterpenoids			**66.73%**

elicited a mean inhibitory zone that was lower than that exhibited by the same isolate exposed to Nystatin.

Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) of *Z. officinale* Essential oil

The MIC and MBC of the essential oils against bacterial isolates are shown in Table 6. The MIC and MBC of *Z. officinale* varied from 150 mg/mL to 600 mg/mL against the test organisms, with the lowest MIC and MBC values of 150 mg/mL against *B. lichenformis* DSM 13, *P. aeruginosa* AAU2, *S. epidermidis* PM221, *S. aureus* NCTC 8325 and *P. aeruginosa* PB112 165. The MIC and MFC of the essential oils against the fungal isolates are shown in Table 7. The MIC and MFC values ranged between 150 to 300 mg/mL with the lowest values of 150 mg/mL against *A. flavus*, *Mucor sp* and *Rhizopus sp*.

Table 2. Biochemical characterization of bacterial isolates from smoke-dried Catfish (*Clarias gariepinus*)

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 sp.*
3	GFRC	GNB	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	-	-	*Pseudomonas aureginosa*
4	B/GFRC	GPC	-	+	-	+	-	+	+	-	-	-	-	+	+	+	+	-	-	*S. epidermidis*
5	SFRC	GPC	+	+	-	-	-	-	+	-	-	-	-	-	-	-	A+	-	A+	*Micrococcus sp.*
6	LRFC	GPR	+	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	*Bacillus subtilis*
7	PROCC	GPC	-	+	-	+	-	-	+	-	+	-	-	-	+	+	+	-	+	*Staphylococcus aureus*
8	PROCC	GPC	-	+	-	+	-	-	+	-	-	-	-	-	+	+	+	-	+	*S. saprophyticus*
9	LRFC	GPR	+	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	*Bacillus subtilis*
10	GFRC	GNB	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	-	-	*Pseudomonas aureginosa*
11	PROCC	GPC	-	+	-	+	-	-	+	-	+	-	-	-	+	+	+	-	+	*Staphylococcus aureus*

KEY: 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. Zone of inhibition (mm) for antibacterial activity of Ginger (*Zingiber officinale*) essential oils by the disc diffusion method

Bacterial isolates	Zone of inhibition (mm)						Positive control	Negative control
	150mg/mL	100mg/mL	50mg/mL	25.5mg/mL	12.5mg/mL	6.25mg/mL	(Ciprofloxacin)	(DMSO)
Gram positive								
B. lichenformis DSM 13	$27.00^{a}\pm0.58$	$22.00^{c}\pm0.58$	$17.33^{d}\pm0.88$	$9.00^{e}\pm 1.15$	$2.67^{f}\pm1.45$	$0.00^{e}\pm0.00$	$25.00^{a}\pm0.40$	$0.00^{e}\pm0.00$
C. pseudodiphtheriticum DSM 44287	$12.33^{b}\pm1.20$	$4.00^{c}\pm0.58$	$0.00^{d}\pm0.00$	$0.00^{d}\pm 0.00$	$0.00^{d}\pm 0.00$	$0.00^{d}\pm0.00$	$21.67^{a}\pm0.11$	$0.00^{d}\pm0.00$
S. aureus NCTC 8325	$20.67^{a}\pm1.20$	$15.33^{c}\pm0.88$	$9.00^{d}\pm 0.58$	$0.00^{e}\pm 0.00$	$0.00^{e}\pm 0.00$	$0.00^{e}\pm0.00$	$17.67^{b}\pm0.30$	$0.00^{e}\pm0.00$
S. epidermidis PM221	$20.33^{a}\pm0.88$	$17.00^{c}\pm1.15$	$7.67^{d}\pm 0.88$	$4.00^{e}\pm 1.15$	$0.00^{f}\pm 0.00$	$0.00^{f}\pm0.00$	$19.00^{b}\pm0.20$	$0.00^{f}\pm0.00$
M. luteus NCTC 2665	$16.67^{b}\pm0.88$	$6.33^{c}\pm0.88$	$0.00^{d}\pm 0.00$	$0.00^{d}\pm 0.00$	$0.00^{d}\pm 0.00$	$0.00^{d}\pm0.00$	$27.33^{a}\pm0.41$	$0.00^{d}\pm0.00$
B. subtilis 6051-HGW	$20.67^{b}\pm1.76$	$12.33^{c}\pm0.88$	$9.67^{d}\pm 0.88$	$4.67^{e}\pm 1.20$	$0.00^{f}\pm 0.00$	$0.00^{f}\pm0.00$	$25.00^{a}\pm0.20$	$0.00^{f}\pm0.00$
S. saprophyticus ATCC 15305	$14.33^{b}\pm0.88$	$9.00^{c}\pm0.58$	$5.00^{d}\pm 0.58$	$0.00^{d}\pm 0.00$	$0.00^{d}\pm 0.00$	$0.00^{d}\pm0.00$	$19.67^{a}\pm0.30$	$0.00^{d}\pm0.00$
B. subtilis KCTC 1028	$17.00^{d}\pm1.15$	$12.33^{d}\pm0.88$	$6.67^{d}\pm0.88$	$1.67^{c}\pm 0.88$	$0.00^{b}\pm 0.00$	0.00±0.00	$21.67^{a}\pm0.30$	0.00±0.00
Gram negative								
P. aeruginosa AAU2	$26.33^{a}\pm0.88$	$21.67^{b}\pm0.88$	$16.00^{c}\pm 0.58$	$9.67^{d}\pm 1.20$	$0.00^{e}\pm 0.00$	$0.00^{e}\pm0.00$	$17.00^{c}\pm0.20$	$0.00^{e}\pm0.00$
P. aeruginosa PB112 165	$23.00^{a}\pm1.15$	$16.67^{b}\pm1.20$	$10.67^{c}\pm 1.20$	$5.00^{d}\pm 0.58$	$2.00^{e}\pm 1.15$	$0.00^{f}\pm0.00$	$17.67^{b}\pm0.50$	$0.00^{f}\pm0.00$

*****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 (lower case) are not significantly different (P>0.05) from the positive control.

Table 4. Biochemical characterization of fungal isolates from smoke-dried Catfish (*Clarias gariepinus*)

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
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 sp*	*Mucor sp.*	*Rhizopus sp.*	*Trichoderma sp*

DISCUSSION

The constituents of *Z. officinale* essential oil recorded in this study indicatethat it is of good quality.The oil displayed great chemical homogeneity typified by relatively high amounts of Sesquiterpene hydrocarbons (58.89%) which comprised of beta.-Sesquiphellandrene (16.53%), Cyclohexene,1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-(S)- (13.87%), (-)-Zingiberene (8.02%), alpha.-Curcumene (7.90%) and alpha.-Citral (5.54%). These agrees with studies reported by other authors that the oil of *Z. officinale* essential oil is characterized by high percentages of sesquiterpene hydrocarbons López et al., (2017), Mesomo et al., (2013), Yeh et al., (2014), Sasidharan, and Menon, (2010), who obtained similar results. Also the proportion of zingiberene and beta-sesquiphellandrene to arcurcumene which has a ratio of 2:3 agrees with recommended ratio for a good quality of ginger oil (Zachariah, 2008).

There have been several variations in the chemical composition of ginger essential oil. El-baroty et al. (2013) identified beta.-Sesquiphellandrene as the most abundant compound in ginger oil while Sasidharan and Menon (2010), Saberi and Alimohammadi (2016) and Zhan et al. (2008) identified zingiberene as the most abundant compound. Other studies by Sa-Nguanpuag et al., (2011) identified camphene when extracted by hydro distillation whereas Lopez et al., (2017) reported eudesmol as the most abundant compound. These variations are due to the diversity in the climatic, seasonal and geographical conditions of the regions in which the speciesis grown, the maturity of the plant, the time of harvest, the physical conditions of the plant material (e.g. dry or fresh) and the extraction methods used in obtaining the oil (Yamamoto-

Table 5. Zone of inhibition (mm) for antifungal activity of Ginger (*Zingiber officinale*) essential oils by the disc diffusion method

Fungal isolates	Zone of inhibition (mm)						Positive control (Nystasin)	Negative control (DMSO)
	150mg/mL	100mg/mL	50mg/mL	25.5mg/mL	12.5mg/mL	6.25 mg/mL		
A. niger	13.33^{a}±0.88	10.00^{b}±1.15	0.00^{c}±0.00	0.00^{c}±0.00	0.00^{c}±0.00	0.00^{c}±0.00	11.00^{b}±0.20	0.00^{c}±0.00
A. flavus	16.67^{a}±0.88	10.00^{c}±1.15	0.00^{d}±0.00	0.00^{d}±0.00	0.00^{d}±0.00	0.00±0.00	12.00^{b}±0.20	0.00^{d}±0.00
Aspergillus sp.	11.33^{a}±0.88	6.67^{c}±0.88	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 sp.	18.00^{a}±0.88	12.67^{b}±1.20	0.00^{c}±0.00	0.00^{c}±0.00	0.00^{c}±0.00	0.00^{c}±0.00	13.67^{b}±0.30	0.00^{c}±0.00
Rhizopus sp.	16.67^{a}±0.88	13.33^{b}±0.88	4.33^{d}±0.88	0.00^{e}±0.00	0.00^{e}±0.00	0.00^{e}±0.00	10.33^{c}±0.11	0.00^{e}±0.00
Trichoderma sp.	12.00^{b}±1.15	6.67^{c}±1.20	0.00^{d}±0.00	0.00^{d}±0.00	0.00^{d}±0.00	0.00^{d}±0.00	13.33^{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 (lower case) are not significantly different (P>0.05) from the positive control.

Table 6 Minimum Inhibitory Concentration (mg/mL) and Minimum Bactericidal Concentration (mg/mL) of the bacterial isolates exposed to varying concentrations of Z. *officinale* essential oils

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

Ribeiro et al., 2013; Anwar et al., 2009;, Nicoll and Henein, 2009; Wohlmuth et al., 2006).

Z. officinale essential oil elicited antibacterial activity against both Gram positive and Gram negative bacterial isolates. Gram positive bacteria have been reported to be more susceptible to oil due to the cell wall which is composed of a thick layer of peptidogly can surrounding the cytoplasmic membrane (Burt, 2004). However, the susceptibility of Gram negative bacteria isolates is comparable to reports by Mesomo et al., (2013); Karuppiah and Kajarum (2012); Sivasothy et al., (2011); Singh et al., (2008); Jane et al., (1999) for *P. aeruginosa, Enterobacter sp., E. coli, K. pneumoniae* and *Proteus vulgaris*. This has been reported to be as a result of their lipophilic properties, which interact with the membranes by altering their fluidity and permeability (Berger, 2007). *B. lichenformis* DSM 13 and *P. aeruginosa* PB112 165 were the most susceptible to the essential oil as visible inhibitory growth zones were detected in respect to these isolates exposed to 12.5 mg/mL concentration of the Z. *officinale* oil extract. This trend could be attributed to the presence of active phytochemicals with

Table 5: Minimum Inhibitory Concentration (mg/mL) and Minimum Fungicidal Concentration (mg/mL) of the fungal isolates exposed to varying concentrations of Z*. officinale* essential oil

Fungal isolates	*Z. officinale*	
	MIC	MFC
Aspergillus flavus	150	150
Aspergillus niger	300	300
Aspergillus sp.	300	300
Mucor sp.	150	150
Rhizopus sp.	150	150
Trichoderma sp.	300	300

increased antibacterial activity in the *Z. officinale* oil extract (Idu et al., 2014). The expressed antimicrobial activity was concentration dependent as higher concentrations elicited a corresponding maximal antimicrobial activity. This is in agreement 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. Comparatively, the antibacterial activity of the essential oils was greater than the recorded antifungal activity of the essential oil extract. This could indicate the inability of the antimicrobial compounds to gain access into the cytoplasmic cellular matrix of the eukaryotic fungal isolates. The observation could also suggest that the degree of myco-toxicity of the essential oils was low in comparison with the antibacterial activity of the examined essential oil (Aderibigbe, 2012). Aside from *B. lichenformis* DSM 13, *S. aureus* NCTC 8325, *S. epidermidis* PM221, *P. aeruginosa* AAU2 and *P. aeruginosa* PB112 165 the elicited mean inhibitory zones elaborated by the bacterial isolates exposed to discs impregnated with *Z. officinale* essential oil were lower than the inhibitory growth zones elicited upon exposure to the positive control (ciprofloxacin). This finding indicates that Z*. officinale* oil confers a comparable antibacterial activity to the control drug in inhibiting the growth of the bacterial isolates. The mean inhibitory zone of Z. *officinale* against the fungal isolates werehigher than that of the antibiotic. The smaller zones of inhibition recorded for the fungi isolates suggests that they are more resistant which an indication of the potential of Z. *officinale* essential oil as a source of potent antifungal metabolites. This confirms with previous reportsby that *Z. officinale* possess antifungal activity against *Penicillium spp., Rhizopus sp., A. flavus, A. solani, A. oryzae, A. niger, F. moniliforme, F. verticillioides* (Bellik, 2014; Yamamoto-Ribeiro et al., 2013; Silva et al., 2012; Sasidharan and Menon, 2010; Singh et al., 2008). A major proponent of oils with antifungal properties is their ability to diffuse into the cell membrane and cause an expansion, thereby increasing their fluidity or disordering membrane embedded enzymes (Mendoza et al., 1997). The observed low MIC and MBC/MFC values against these microorganisms suggests that the plant has the potential to inhibit or kill the growth of these isolates and treat any ailments associated with these pathogens effectively.

CONCLUSION

The constituents of essential oil of *Z. officinale* in this study were characterized by high percentages of sesquiterpene hydrocarbons. The bioactive components were beta.-Sesquiphellandrene, Cyclohexene, 1- methyl- 4- (5-methyl-1-methylene-4-hexenyl)-(S)-, (-)-Zingiberene, alpha.-Curcumene and alpha.-Citral. *Z. officinale* showed the greatest *Z. officinale* exhibited a greatest bactericidal at MIC/MBC of 150 mg/ml against *B. lichenformis* DSM 13, *P. aeruginosa* AAU2, *S. epidermidis* PM221, *S. aureus* NCTC 8325 and *P. aeruginosa* PB112 165 and the greatest fungicidal activity MIC/MFC of 150 mg/mL against *A. flavus*, *Mucor sp* and *Rhizopus sp*. The oil showed an antibacterial activity comparable to that of Ciprofloxacin but had antifungal activity greater than that of Nystasin. The study reveals that *Z. officinale* essential oil can be used against these organisms to inhibit their growth as an ecofriendly alternative to the synthetic chemicals.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of the paper.

REFERENCES

Adams RP (2001). Identification of Essential Oil by Gas Chromatography Quadrupole/Mass Spectroscopy. Allured Publishing Corporation, Illinois.

Aderibigbe SA (2012). Antimicrobial activities of *Garcinia kola* seed oil against some clinical microbial isolates. International Research Journal of Pharmaceuticals. 2(3): 68-72.

Agarwal M, Walia S, Dhingra S, Bhupinder PS, Khamba Y (2001). Insect growth inhibition, antifeedant and antifungal activity of compounds isolated /derived from *Zingiber officinale* Roscoe (ginger) rhizomes. *Pest Manag Sci*. 57(3):289-300.

Ajaiyeoba EO, Onocha PA, Nwozo SO, Sama W (2003).

Antimicrobial and cytotoxicity evaluation of *Buchholzi acoriacea* stem bark. Fitoterapia. 74: 706-709.

Anwar F, Ali M, Hussain AI, Shahid M (2009) Antioxidant and Antimicrobial Activities of Essential Oil and Extracts of Fennel (*Foeniculum vulgare* Mill.) Seeds from Pakistan. Flavour and Fragance J. 24: 170-176.

Balanchandarn S, Kentish Se, Mawson R (2006). The effect of both the preparation method and season on the super critical extraction of ginger.*Sep. Purif. Tech.* 48: 94-105.

Bartley J, Jacobs A (2000). Effects of drying on flavour compounds in Australian grown ginger (*Zingiber officinale*). J. Sci. Food and Agric. 80: 209-215.

Bellik Y (2014). Total Antioxidant Activity and Antimicrobial Potency of the Essential Oil and Oleoresin of *Zingiber officinale* Roscoe.Asian Pacific J. Tropical Disease. 4: 40-44.

Berger RG (2007) Bioactivity of Essential Oils and Their Components. In: Flavors and Fragrances: Chemistry, Bioprocessing, and Sustainability, Springer, Berlin. 88-90.

Burt S (2004) Essential Oils: Their Antibacterial Properties and Potential Applications in Foods—A Review. Int. J. Food Microbiol.94: 223- 253.

El-Baroty GS, Abd El-Baky HH, Farag RS, Saleh MA (2013).Characterization of antioxidant and antimicrobial compounds of cinnamon and ginger essential oils. Advanced Res. J. Biochem.1(4): 078-085.

El-Baroty GS, Abd El-Baky HH, Farag RS, Saleh MA, (2010).Characterization of antioxidantand antimicrobial compounds of cinnamon and ginger essential oils. Afr. J. Biochem. Res. 4(6):167-174.

El-Ghorab AH, Nauman M, Anjum FM, Hussan S, Nadeem M (2010). A comparative study on chemical composition and antioxidant activity of Ginger (*Zingiber officinale*) and cumin (*Cumunum cyminum*). J. Agric. Food. Chem. 58(14): 8231-8237.

Ficker C, Smith Ml, Akpagana K, Gbeassor M, Zhang J, Durst TA,Ssabgui A, ArnasonJT (2003). Bioassay-guided isolation and identification of antifungal compounds from ginger. Phytother Res. 17:897-902.

Gupth S, Ravishanker S (2005). A comparison of the antimicrobial activity of garlic, ginger, carrot and turmeric pastes against
E.coli in laboratory buffer and ground beef. Foodborne Pa thog Dis. 2(4):330-340.

Hussain AI, Anwar F, Sherazi STH, Przybylski R (2008). Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. Food Chemistry. 108: 986-995.

Janes ME, Nannapaneni R, Johnson MG (1999). Identification and Characterisation of Two Bacterioc in Producing Bacteria Isolated from Garlic and Ginger Root. J. Food Protection. 62: 899-904.

Karuppiah P, Rajaram S (2012).Antibacterial Effect of *Allium sativum* Cloves and *Zingiber officinale* Rhizomes against Multiple-Drug Resistant Clinical Pathogens.Asian Pacific J. Tropical Biomed. 2: 597-601.

López EIC, Balcázar, MFH, Mendoza JMR, Ortiz ADR, Melo MTO, Parrales RS, Delgado TH(2017). Antimicrobial activity of Essential oil of *Zingiber officinale Roscoe* (*Zingiberaceae*). American J. Plant Sci. 8:1511-1524.

Malek SRA, Ibrahim H, Hong SL, Lee GS, Chan KS,Ali NAM (2005).The essential oils of *Zingiber officinale* variants. Malaysian J. Sci. 24(2): 37–43.

Mandoza L, Wilkens M, Urzua A (1997). Antimicrobial study of the resinous exudates and of diterpenoids and flavonoids isolated from some Chilean *Pseudographatiumasteracae*. J. Pharmacol. 58:85-88.

Martins AP, Salgueiro L, Goncalves MJ, Proenca DA Cunha A, Vila R, Canigueral S, Mazzoni V, Tomi F, Casanova J (2001). Essential oil composition and antimicrobial activity of three Zingiberaceous from S. Tome Principe. Planta Med. 67: 580-584.

Mesomo MC, Corazza ML, Ndiaye, PM, Dalla Santa OR, Cardozo L, Scheer AP (2013). Supercritical CO_2 extracts and essential oil of Ginger (*Zingiber officinale R.*): Chemical Composition and Antibacterial Activity. The J. Supercritical Fluids. 80:44-49.

Moshafi MH, Fariba S, Dehghan RG, Ameri Ali EH (2009). Bioassay screening of the essential oil and various extracts of fruits of Heracleum persicumDesf. and Rhizomes of *Zingiber officinale* Rocs. using Brine Shrimpcytotoxicity assay. Iranian J. Pharma. Res. 8(1): 59-63.

Nanasembat S, Lohasupthawee R (2005). Antibacterial activity of crude ethanolic extracts and essential oils ofspices against Salmonellae and other Enterobacteria. KMITL Sci. Tech J. 5(3): 527-538.

Nicoll R, Henein MY (2009) Ginger (*Zingiber officinale* Roscoe). A hot Remedy for Cardiovascular Disease? Int. J. Cardiol.131: 408- 409.

Oboh IE, Akerele OJ, Obasuyi O (2007). Antimicrobial activity of the ethanol extract of the aerial parts of *Sida acuta* burm. f. (malvaceae). Trop. J. Pharm. Res. 6: 809-813.

Ogbuewu IP, Jiwuba PD, Ezeokeke CT (2014). Evaluation of phytochemical and nutritional composition of ginger rhizome powder. Int'l J. Agric. and Rural Dev. 17(1):1663-1670.

Okeke MI, Iroegbu CU, Eze EN, Okoli AS, Esimone CO (2001). Evaluation of extracts of the root of *Landolphia owerrience* for antibacterial activity. J. Ethnopharmacol. 78: 119-127.

Pino JA, Marbot R (2004). Rosado A and Batista A. Chemical compositionofthe essential oil of *Zingiber officinale* Roscoe L. from Cuba. J. Essential Oil Res. 16(3): 186–188.

Saberi A, Alimohammadi M (2016). Separation identification and antioxidant evaluation of *Zingiber officinale* essential oil. Iranian Chemical Communication. 5: 278-285.

Sa-Nguanpang K, Kanlayanarat S, Srilaong V, Tanprasert K, Techavuthiporn C (2011). Ginger (*Zingiber officinale*) oil as an antimicrobial agent for minimally processed produce: a case study in shredded green papaya. Int. J. Agric. Biol. 13: 895-901.

Sasidharan I, Menon AN (2010). Comparative chemical an antimicrobial activity fresh & dry Ginger oils (*Zingiberofficinale Roscoe*). Int. J. Current Pharm. Res. 2: 40-43.

Shukla Y, Singh M. (2007). Cancer preventive properties of Ginger: A brief review. Food and Chemical Toxicology. 45(5): 683-690.

Silva CF, Chalfoun MS, Siqueira MV, Botelho DMS, Lima N, Batista RL (2012). Evaluation of antifungal activity of essential oils against potentially mycotoxigenic *Aspergillus flavus* and *Aspergillus parasiticus*. Rev. bras. farmacogn. 22(5):1002-1010.

Singh G, Kapoor IP, Singh P, Heluani CS, Lampasona MP, Catalan CA (2008). Chemistry, Antioxidant and Antimicrobial Investigations on Essential Oil and Oleoresins of *Zingiber officinale*. Food and Chem. Toxicol. 46: 3295-3302.

Singh M, Khan MMA, Naeem M(2016). Effect of Nitrogen on Growth, Nutrient Assimilation, Essential oil Content, Yield and Quality Attributes in *Zingiber officinale* Rosc. J. Saudi Society of Agric. Sci.. 15: 171-178.

Sivasothy Y, Chong WK, Hamid A, Eldeen IM, Sulaiman SF, Awang K (2011).Essential Oils of *Zingiber officinale* var. Rubrum Theilade and Their Antibacterial Activities.Food Chemistry. 124: 514-517.

Sultan M, Bhatti HN, Iqbal Z (2005). Chemical analysis of essential oil of ginger (*Zingiber officinale*). Pakistan J. Biol. Sci. 8(11): 1576–1578.

Wohlmuth H, Smith MK, Brooks LO, Myer SP, Leach DN (2006). Essential oil composition of diploid and tetraploid clones of ginger (*Zingiber officinale Roscose*) grown in Australia. J. Agric. Food Chemistry.54: 1414-1419.

Yamamoto-Ribeiro MM, Grespan R, Kohiyama CY, Ferreira FD, Mossini SA, Silva EL, Filho BA, Mikcha JM, MachinskiJr M (2013) Effect of *Zingiber officinale* Essential Oil on *Fusarium verticillioides* and Fumonis in Production. Food Chemistry. 141: 3147-3152.

Yang ZB, Yang WR, Wang Y, Jiang SZ, Zhang GG (2011). Effects of ginger root on laying performance and antioxidant status of laying hens and on dietary oxidation stability. Poult. Sci. 90: 720–1727.

Yeh H, Chuang C, Chen H, Wan C, Chen T, Lin L (2014). Bioactive components analysis of two various Gingers (*Zingiber officinale Roscoe*) and antioxidant effect of Ginger extracts. LWT- Food Science and Technol. 55: 329-334.

Zancan KC, Marques M, Petenate AJ, Meireles M (2002). Extraction of ginger (*Zingiber officinale* Roscoe) oleoresin with CO_2 and co-solvents: a study of the antioxidant action of the extracts. J. Supercritical Fluids. 24 (1): 57–76.

Zhan K, Wang C, Xu K (2008). Analysis of volatile and non-volatile compositions in ginger oleoresin by gas chromatography-mass spectrometry. China J. Chromatograph. 26: 692-696.