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GC-MS Analysis, Antibacterial and Antibiofilm Activities of Extracts of *Zingiber Officinalis* against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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ABSTRACT

Background and Purpose: *Zingiber officinalis* Roscoe (Zingiberaceae) is used in ethnomedicine for conditions related to bacterial infections. This present study was carried out to determine the chemical composition of *Zingiber officinalis* extract and evaluate its antibacterial and antibiofilm activities.
Methods: Extracts were obtained using ethanol, methanol and water as solvents and the phytochemical composition of the ethanol extract was determined using GC-MS analysis. The antibacterial and antibiofilm activities were done using the tetrazolium microplate assay method.
Results: Twenty-seven bioactive phytochemical compounds were identified in the ethanol portion. The dominant compounds were gingerol (10.61%); 2-butanone, 4-(4-hydroxy-3-methoxyphenyl) (9.35 %); 9,12-octadecadienoic acid (8.55 %); 1,3-cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl (7.74%). The extracts exhibited significant antibacterial activity but stimulated biofilm formation when compared with ciprofloxacin.
Conclusion: The results of this study show that *Zingiber officinalis* possesses some antibacterial properties and can be used to prevent and treat infections caused by sensitive bacteria.

INTRODUCTION

Ginger (*Zingiber officinalis*), is a slender perennial herb that reaches two feet in height and has greenish flowers that resemble orchids. The rhizomes are aromatic, thick lobbed, white to yellowish brown, irregularly branched, annulated and compressed with smooth surface (Warrier *et al.,* 1997). The dried rhizome of ginger contains approximately 1-4% of volatile oils which are the medicinally active ingredients that are responsible for the characteristic odour and taste (Shareef *et al.,* 2016). It originated from tropical Asia but it is now cultivated as a commercial crop in India, China, Australia, America, Africa as well as South East Asia (Zachariah, 2008).

It has a long history of medicinal use dating back 2500 years in China and India for conditions such as nausea and vomiting, diarrhea, dyspepsia, rheumatism, and colds (Njobdi *et al.,* 2018). It is extensively utilized worldwide as a spice, flavoring agent and herbal remedy. In different traditional systems, it is used to cure a variety of diseases such as nausea, vomiting, asthma, palpitation, inflammation, dyspepsia, loss of appetite, constipation, digestion and pain (Pieters and Vlietinck, 2005; Balunas and Kinghorn, 2005).

The major pungent compounds of fresh ginger are active gingerols which are derivatives of phenols. The most abundant compound is 6-gingerol which appears to be responsible for characteristic taste of ginger and works synergistically with 6-shogaol in having antipyretic, analgesic, anti-inflammatory, anti-tussive and hypotensive effects (Khushtar *et al.,* 2009; Altameme *et al.,* 2015). Patients often seek alternative and complementary therapy, and ginger is one of the most popular herbal remedies for inflammatory conditions (Aryaeian and Tavakkoli, 2015). There is a growing interest in detecting natural substances with flavors for the preservation of food and pharmaceuticals (Shim *et al.,* 2011; Wang *et al.,* 2011). Interest in ginger has arisen mainly because of its nonvolatile pungent constituents that show remarkable biological activities including anti-inflammatory, antiplatelet aggregation (Nurtjahja-Tjendraputra *et al.,* 2003), antioxidant, immunomodulatory, anti-tumor, anti-apoptotic, hypoglycemic, hypolipidemic, anti-emetic actions (Ali *et al.,* 2008), antihypertensive, antimicrobial and others (Afzal *et al.,* 2001). This study is therefore aimed at determining the GC-MS analysis, antibacterial and antibiofilm activities of ethanol extract of *Zingiber officinalis* against two food borne pathogens.

MATERIALS AND METHODS

Collection and Identification of Plant Material

The rhizomes of the plant *Zingiber officinalis* (ginger) were randomly purchased from Ubaani Main Market in Umuahia metropolis, South East Nigeria. The identification of the plant material was done in the Forestry Department of Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Preparation of Plant Materials

The plant material was air-dried at room temperature for three days and ground to a fine powder in an electric blender (SONIK R, Japan).

Preparation of Aqueous Extracts

Aqueous extraction was done using the modified maceration technique (Remington, 2000). The powdered plant material (50 g) was macerated with 150 mL of distilled water for 3 h with intermittent stirring. This was followed by vacuum filtration using Whatman (No. 1) filter paper in a Buchner funnel. The extraction process was repeated twice on the residue using 100 mL and 50 mL of water at the second and third extraction, respectively. The portions of aqueous extracts obtained were pooled together into small conical flasks, sealed with parafilm and frozen at -4ºC before drying. The portions were concentrated to dryness in a hot air oven (SELLECTA CE 0505, USA) at 45ºC with constant monitoring. The extract was double-sealed with parafilm and stored in a desiccator to avoid the extracts picking up moisture. The percentage yield was obtained by dividing the weight of extract by the weight of the starting material. The extract was stored at 4ºC until used for experiments.

Preparation of Methanol and Ethanol Extracts

Both the ethanol and methanol extracts were prepared using the same process (Remington, 2000). The ground plant material (50 g) was extracted with 150 mL of the solvent in a foiled-sealed flask for 3 h with occasional shaking. Thereafter, the extracts were vacuum filtered using Whatman (No. 1) filter paper in a Buchner funnel. The residues were extracted twice with 100 mL and 50 mL of the solvent. The filtrates were concentrated in an oven at 50ºC, and then transferred to a glass petri dish for drying at 60ºC for 24 h in a vacuum oven. The extracts were weighed to obtain percentage yields and stored in sealed vials at 4ºC.

Preparation of Cultures

Typed bacterial strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa* were obtained from the Diagnostic Centre of National Veterinary Research Institute, Vom, Plateau State, Nigeria. The strains were *S. aureus* ATCC 12540 and *P. aeruginosa* ATCC 10200.

Glycerol stock cultures of each organism were prepared and kept at -4ºC prior to use. The strains were revived onto sterile Tryptone Soy Agar (Oxoid, UK) and incubated at 37ºC for 18 h. Selective media were used to confirm their identity. Following incubation, the organisms were inoculated into sterile Tryptone Soy Broth (TSB) and

incubated at 37ºC overnight. The overnight culture was standardized to a concentration of 1.0×10^6 CFU/mL. This was done by diluting the overnight cultures with TSB to obtain an absorbance (OD_{590} nm) of 0.02 for all bacteria (Sandasi *et al.*, 2008).

Biofilm Formation and Quantification

A sterile 96-well flat bottomed microtitre plates was filled with 230 µL TSB per well and thereafter and 200 µL of overnight bacterial culture standardized to a concentration of 1.0×10^6 CFU/mL was pipetted into each well and then incubated at 37ºC for 24 h. The negative control wells contained TSB only. After incubation, the contents of the plates were poured off and the wells were washed three times with 300 µL of sterile distilled water. The remaining attached cells were fixed with 250 µL of methanol per well and emptied after 15 min and allowed to dry at a temperature of 28 ± 2ºC for 3 h. The plates were stained with 250 µL of 0.1% (w/v) crystal violet per well for 5 min. Excess stain was rinsed by placing the microtitre plate under running water and air drying. The dye bound to adherent cells was re-solubilized with 250 µL of 90% ethanol per well. Absorbance was measured at 570 nm (Stepanovic *et al.*, 2007). Experiments were performed in triplicates.

Analysis of Plant Extracts Using Gas Chromatography-Mass Spectrometry (GC-MC)

The ethanol extract of the *Z. officinalis* was subjected to GC-MS analysis using the instrument GCMS-QP2010 PLUS (SHIMADZU, Japan). The oven temperature was programmed at 80ºC for 1.0 min, and was gradually increased to 200ºC at 10.0/4.0 min and then ending with 280ºC at 10.05 min. Four microlitre (4.0 µL) of sample was injected for analysis. Helium gas of 99.995% purity was used as a carrier gas as well as an eluent.

The flow rate of helium gas was set to 1.58 mL/min. The sample injector temperature was maintained at 250ºC and the split ratio was 1.0 throughout the experiment periods. The analysis was done with 70 eV for the ionization mass spectroscopy. The mass spectra were recorded for the mass range 40-600 *m/z* for about 25 min. Based on comparison of their mass spectra, the components were identified. On elution through the column, they were detected as electronic signals as the compounds were separated. As individual compounds eluted from the Gas chromatographic column, they entered the detector (electron ionization detector) where they were bombarded with a stream of electrons causing them to be fragmentized. With a certain mass, the fragments were charged ions. From the mass spectrum graph which was the fingerprint of the molecule, the mass to charge (m/z) ratio was calibrated. GC-MS interpretation was carried out using the National Institute of Standard and Technology (NIST) database, having over 62,000 patterns. The spectra of the known components stored in the NIST Library 2008 WILEY8, FAME were compared with the spectra of the unknown components. The components of the test materials were ascertained by noting their name, molecular weight and structure (Hussein *et al*., 2017).

Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

The Minimum Inhibitory Concentration (MIC) of each extract was determined using the tetrazolium microplate assay as described by Eloff (1998) with slight modifications. The assay was performed using the round-bottomed polystyrene 96-well clear microtitre plates with standard plate layout as proposed by Cos *et al.*, (2006). Briefly, each extract was dissolved in 0.5% dimethyl sulfoxide (DMSO) and identical two-fold serial dilutions were made to form 0.03125 – 4.0 mg/mL. Following the serial dilutions, 100 µL of the standard culture (1.0×10^6 CFU/ mL) was then added to all the wells. The plates were sterile-sealed and incubated at 37ºC for 24 h. The MIC was detected following the addition of 50 µL of 0.2 mg/mL of INT (2-4-iodophenyl-3-4-nitrophenyl-5-phenyl-2H-tetrazolium chloride) in all the wells and incubating for further 30 min at 37ºC. Bacterial growth was determined by observing the colour change of INT in the microplate wells. Biologically active bacterial cells reduce the colourless tetrazolium salt which act as an electron acceptor to a red-coloured formazan product (Berridge *et al.*, 2005). When the solution in the well became clear after incubation with INT, the inhibition of bacterial growth was observed. MIC is defined as the lowest extract concentration that completely inhibits the growth of microorganisms and it is indicated by the first clear well in the column. For the determination of minimum bactericidal concentration (MBC), 20 µL of culture medium from the microtitre plate wells that showed no changes in colour were re-inoculated on Mueller Hinton (MH) agar plates. After 24 h of incubation at 37ºC, MBC was determined as the lowest concentration that showed no bacterial growth on MH agar plates. The MIC and MBC determination were performed in duplicate. The positive and negative controls are ciprofloxacin (V.S. International Pvt Ltd. India) and TSB (Oxoid, UK) respectively.

Determination of Minimum Biofilm Inhibitory Concentration (MBIC)

The MBICs of each extract was determined using the methods described by Cernohorská and Votava (2004; 2008) with slight modifications. The experiments were done in a 96-well polystyrene microtitre plates with round bottoms (Sigma Aldrich, Costa, USA). A volume of 75 µL of an overnight standard culture of 1.0×10^6 CFU/mL was added to the wells and the plates incubated for 24 h at

37ºC. The wells were washed three times with phosphate buffered saline (PBS, pH 7.2) under aseptic conditions to remove unattached bacteria and then dried in an inverted position at a temperature of 60ºC for 1 h. Volumes of 100 µL of two-fold dilutions of the respective plant extract were transferred into the dried wells with established biofilms. The microtitre plates were incubated for 18–20 h at 37ºC. Following incubation, 50 µL of 0.2 mg/mL of INT was added in all the wells and incubated for further 30 min at 37ºC. The MBIC corresponded to the lowest concentration of the extracts which inhibited growth of biofilm cells as indicated by the first clear well. The positive and negative controls were ciprofloxacin (V. S. International Pvt Ltd. India) and Tryptone Soy Broth (TSB) (Oxoid, UK) respectively. The experiment was repeated twice.

Inhibition of Cell Attachment

The extracts were tested for their potential anti-adhesive properties at a concentration of 1 mg/mL. A volume of 200 µL was added to each of the 96-well microtitre plates, and equal volumes of TSB and ciprofloxacin (MIC value) were added as negative and positive controls, respectively. Another volume of 200 µL of standardized culture (1.0×10^6 CFU/mL) was then pipetted into each well to yield a final volume of 400 µL. In triplicate, the cultures were introduced into the wells. Without shaking, the plates were sterile-sealed and incubated at 37ºC for 8 h to allow biofilm development and cell attachment (Fairbanks *et al.*, 2014). The modified crystal violet assay following incubation, was performed to assess biofilm biomass, and the results presented as percentage inhibition (Sandasi *et al.*, 2008).

$$\%\,inhibition = \frac{OD\,(NC) - OD\,(E)}{OD\,(NC)} \times 100$$

Where OD is optical density, NC is negative control, and E is experimental.

Assessment of Biofilm Biomass (Crystal Violet Staining Assay)

Cell attachment was assessed using the modified crystal violet (CV) assay (Djordjevic *et al.*, 2002). Before incubation, with sterile distilled water to remove loosely attached cells, the plates were washed three times. The plates were first air-dried before drying for 45 min in an oven at 60°C. Following drying, the wells were stained with 100 µL of 1% crystal violet and incubated at room temperature for 15 min after which the plates were washed three times with sterile distilled water to remove unabsorbed stain. By adding 125 µL of ethanol to destain the wells, the semi-quantitative assessment of biofilm formation was performed. A volume of 100 µL of the destaining solution was then transferred to a cuvette, and the absorbance (OD) was determined at 590 nm using a spectrophotometer (Spectrumlab S23A, China). The mean OD at 590 nm of the samples was used to calculate the percentage inhibition as described above (Sandasi *et al.*, 2008).

Inhibition of Biofilm Growth and Development

A biofilm was allowed to preform by aliquoting 100 µL of a standardized ($1{\cdot}0 \times 10^6$ CFU/mL) culture of the test organisms into a 96-well microtitre plate. The plates were incubated at 37ºC for 4 h to allow cell attachment. A volume (100 µL) of each plant extract was added to yield a final concentration of 1 mg/mL in the wells. For positive and negative controls, an equal volume of ciprofloxacin and TSB were added respectively. The plates were further incubated for 24 h before the crystal violet assay was performed (Sandasi *et al.*, 2008).

Statistical Analysis

One way analysis of variance (ANOVA) test was used to evaluate significant differences between the mean absorbance values obtained without the extracts (controls) and those obtained in the presence of the different extracts used. Differences were considered statistically significant at $P<0.05$. The means were separated using the Duncan's New Multiple Range Test. The statistical variables were evaluated using the International Business Machines (IBM) Statistical Package for Social Sciences (SPSS) 16.0 (IBM, USA) for windows software.

RESULTS

Yields of Plant Extracts after Extraction with Different Solvents

Quantitatively, the aqueous extract had the highest yield of 16.58% w/w. This is followed by methanol (11.0% w/w) and ethanol (5.10% w/w) as shown in Table 1.

Table 1: Yield of Plant extracts after extraction with various solvents

Solvent	Yield (g)	Yield (%w/w)
Water	8.29	16.58
Ethanol	2.55	5.10
Methanol	5.50	11.00

Figure 1 shows the chromatogram of the ethanol rhizome extract of *Z. officinalis.* Twenty-seven (27) compounds were identified and the chromatogram shows 7 prominent peaks in the retention time range 11.292 - 27.492. The Fourth less prominent peak at 11.292 retention time with the peak area 7.74 denotes 1,3- cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl (Figure 2). The Second less prominent peak at 13.783 retention time having the peak area 9.35% is due to the presence of 2-butanone, 4-(4-

hydroxy-3-methoxyphenyl) (Figure 3). The third less significant peak at 21.350 retention time with the peak area 8.55% is 9, 12– octadecadienoic acid (Figure 4).

The largest peak at 23.317 retention time having the peak area 10.61% is gingerol (Figure 5). The other less prominent peaks at other retention times are given in Table 2.

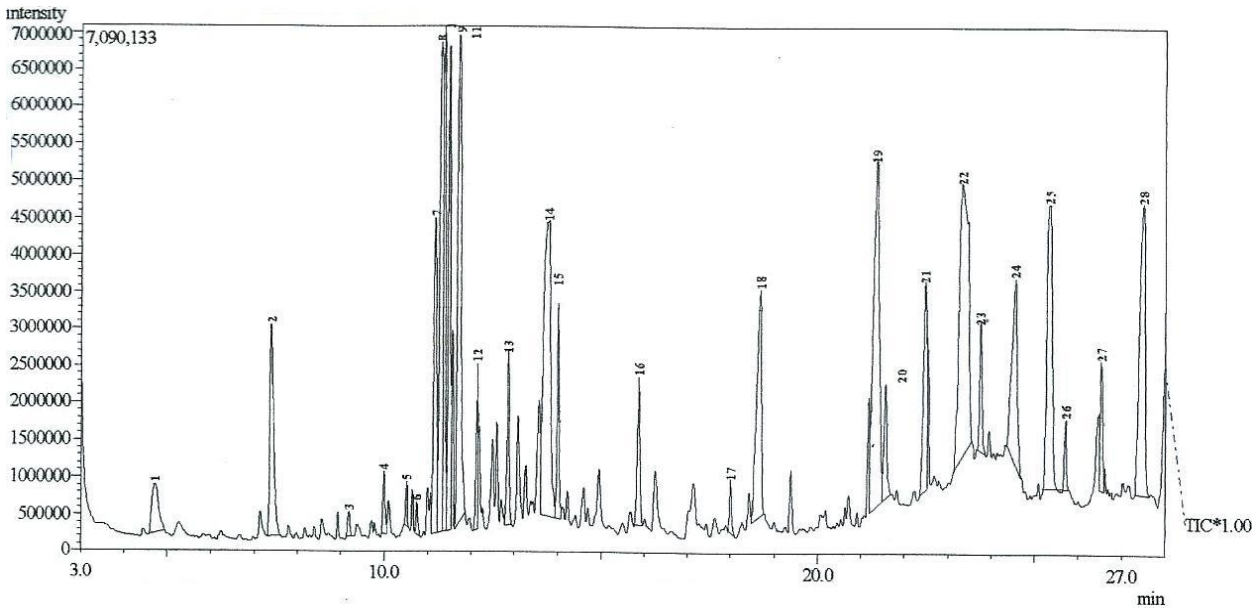

Figure 1: Chromatogram of ethanol rhizome extract of *Zingiber officinalis*

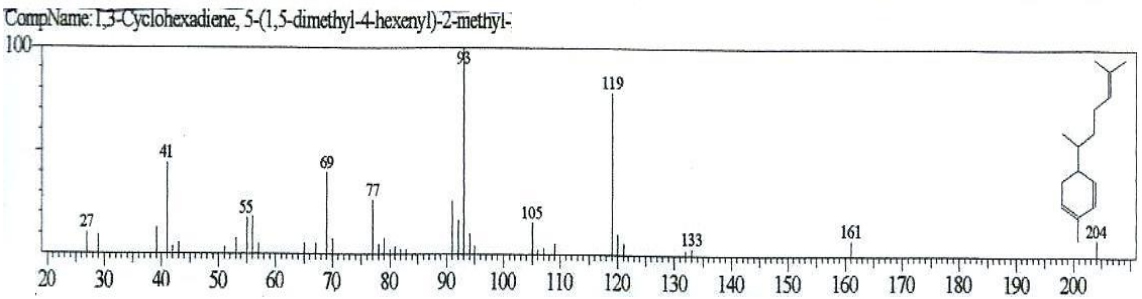

Figure 2: Mass spectrum of 1, 3-cyclohexadiene, 5-(1, 5-dimethyl-4-hexenyl)-2-methyl

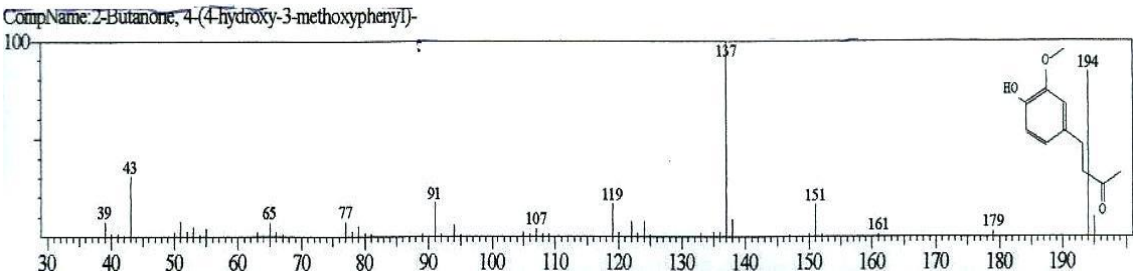

Figure 3: Mass spectrum of 2-butanone, 4-(4-hydroxy-3-methoxyphenyl)-methyl

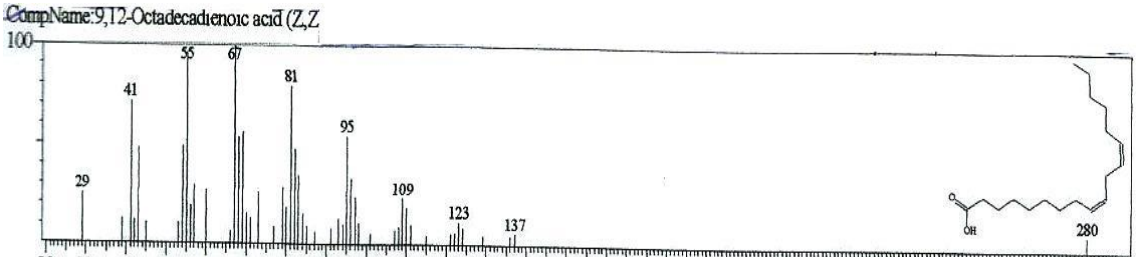

Figure 4: Mass spectrum of 9, 12-octadecadienoic acid (Z, Z)

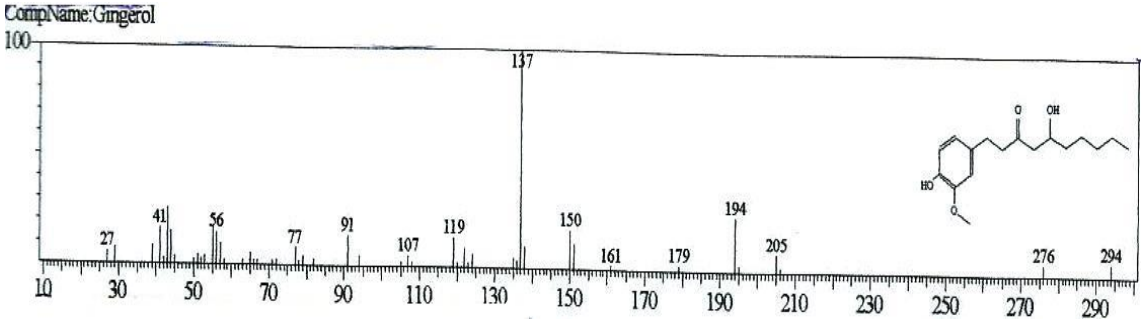

Figure 5: Mass spectrum of gingerol

Table 2a: Identified phytochemical constituents of *Zingiber officinalis* with their retention time, molecular weight and composition

	Retention Time (Min)	Name of Compound	Molecular Formula	MW	Peak Area (%)
1	4.708	Octanal	$C_8H_{16}O$	128	1.46
2	7.392	Decanal	$C_{10}H_{20}O$	156	3.70
3	9.183	Benzene methanol, 4-(1-methylethyl)	$C_{10}H_{14}O$	150	0.27
4	9.983	Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-, (S)	$C_{15}H_{24}$	204	0.63
5	10.517	Gamma-Elemene	$C_{15}H_{24}$	204	0.32
6	10.750	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene	$C_{15}H_{24}$	204	0.25
7	11.175	Benzene, 1-(1,5-dimethyl-4-hexenyl) 4-methyl	$C_{15}H_{22}$	202	3.95
8	11.292	1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl	$C_{15}H_{24}$	204	7.74
9	11.383	Alpha-Farnesene	$C_{15}H_{24}$	204	4.44
10	11.483	Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-(S)	$C_{15}H_{24}$	204	4.80
11	11.717	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene	$C_{15}H_{24}$	204	7.41
12	12.133	1,6,10-Dodecatrien-3-ol,3,7,11-trimethyl-[S-(Z)]	$C_{15}H_{26}O$	222	1.52
13	12.858	Alpha-Bisabolol	$C_{15}H_{26}O$	222	1.47

Table 2b: Identified phytochemical compounds in *Zingiber officinalis* with their retention time, molecular weight and composition

	Retention Time (Min)	Name of Compound	Molecular Formula	MW	Peak Area (%)
14	13.783	2-Butanone,4-(4-hydroxy-3-methoxyphenyl	$C_{11}H_{14}O_3$	194	9.35
15	14.000	6,10-Dodecadien-1-yn-3-ol, 3,7,11-trimethyl	$C_{15}H_{24}O$	220	2.05
16	15.867	Ledol	$C_{15}H_{26}O$	222	1.85
17	18.000	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl	$C_{15}H_{22}$	202	0.53
18	18.667	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	4.35
19	21.350	9,12-Octadecadienoic acid (Z, Z)	$C_{18}H_{32}O_2$	280	8.55
20	21.558	Octadecanoic acid	$C_{18}H_{36}O_2$	284	1.35
21	22.475	Phenol, 2-methoxy-4-propyl-	$C_{10}H_{14}O_2$	166	3.08
22	23.317	Gingerol	$C_{17}H_{26}O_4$	294	10.61
23	24.550	Capsaicin	$C_{18}H_{27}NO_3$	305	4.10
24	25.333	Cyclohexanone, 2-(2-nitro-2-propenyl)-	$C_9H_{13}NO_3$	183	6.18
25	25.700	4,5-Heptadien-2-one, 3,3,6-trimethyl	$C_{10}H_{16}O$	152	0.60
26	26.533	4-Methoxy-phenyl)-(2-nitrocyclohexyl)-methanol	$C_{14}H_{19}NO_4$	265	1.48
27	27.492	2,5-Octadiene, 3,4,5,6-tetramethyl-	$C_{12}H_{22}$	166	6.75

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

The type of solvent used for the extraction greatly influenced the antimicrobial activities of the extracts, with the ethanol extracts showing greater activities against all test organisms than the methanol and aqueous extract (Table 3). The ethanol extract was the most active against the test organisms with MIC values of 0.125 and 0.50 mg/mL against *S. aureus* ATCC 12540 and *P. aeruginosa* ATCC 10200 respectively. The positive control (ciprofloxacin) showed inhibitory activity with MIC values of 0.0625 and 0.05 against *S. aureus* and *P. aeruginosa* respectively. Generally, *S. aureus* (0.5 mg/mL) recorded a high susceptibility to the extracts than *P. aeruginosa* (1.0 mg/mL) as evidenced by its lower MBC values.

Also, the capability of the plant extracts to attenuate biofilm formation is also shown in Table 3. The potent biofilm inhibition activity was shown by ethanol extract with MBIC value of 2.0 mg/mL and 2.5 mg/mL against *S. aureus* and *P. aeruginosa* respectively. The remainder of the biofilms recorded the lowest resistance to the plant extracts with MBIC value of 4.0 for the methanol extract while the aqueous extract showed no activity within the range of concentrations tested. Within the tested MBIC range (0.03125 to 4 mg/mL), ciprofloxacin showed good biofilm inhibitory activity against the test organisms.

Table 3: Minimum and Bactericidal Inhibitory Concentration of *Zingiber officinalis* against *S. aureus* and *P. aeruginosa*

	MIC (mg/mL)		MBC (mg/mL)		MBIC (mg/mL)	
Solvent	*S. aureus*	*P. aeruginosa*	*S. aureus*	*P. aeruginosa*	*S. aureus*	*P. aeruginosa*
Aqueous	1.0	1.5	2.0	4.0	NA	NA
Ethanol	0.125	0.5	0.5	1.0	2.0	2.5
Methanol	0.5	1.0	1.0	2.0	4.0	4.0
Ciprofloxacin	0.0625	0.05	0.10	0.10	0.125	0.125

Values are means of duplicate experiments. NA: indicates no activity within the range of concentration tested (0.03125 – 4 mg/mL)

Inhibition of Cell Attachment

Table 4 shows that some of the extracts reduced biofilm biomass compared to the control. However, none of the extracts showed complete inhibition of cell attachment including the positive control ciprofloxacin. Inhibition was expressed as percentage values with the most active extract showing the highest percentage inhibition and *vice-versa*. The ethanol extract, showed selective inhibition against either *S. aureus* or *P. aeruginosa* but not both. The methanol extract seemed to promote attachment of both *S. aureus* and *P. aeruginosa* to the microtitre plate. This is shown by the increase in biofilm biomass of the extract compared to the control biofilm which was represented as 0% inhibition. *P. aeruginosa* (27.66 %) was more resistant than *S. aureus* (1.96 %) as evidenced by its lower percentage inhibition values compared to that of *S. aureus*.

Inhibition of growth of a preformed biofilm

The ability of the extracts to inhibit growth of a preformed biofilm is shown in Table 5. None of the extracts was able to inhibit the growth of a preformed biofilm. The methanol and ethanol extracts enhanced biofilm growth as evidenced by the increase in biomass compared to the control.

Table 4: Mean biofilm biomass of *S. aureus* and *P. aeruginosa* after exposure to plant extracts at a concentration of 1 mg/mL (inhibition of cell attachment)

Extracts	*S. aureus* Absorbance (OD_{590})	Inhibition of Cell Attachment (%)	*P. aeruginosa* Absorbance (OD_{590})	Inhibition of Cell Attachment (%)
Ethanol	0.34 ± 0.015^{c}	27.66	0.50 ± 0.050^{a}	1.96
Methanol	0.49 ± 0.020^{a}	-4.26	0.52 ± 0.007^{a}	-1.96
Aqueous	NA	-	NA	-
Ciprofloxacin	0.10 ± 0.021^{d}	78.72	0.17 ± 0.038^{b}	70.59
Control biofilm	0.47 ± 0.011^{b}	-	0.51 ± 0.042^{a}	-

Values in each column followed by different superscript within each column are significantly different from each other at $P< 0.05$. Values are mean ± standard error of three replicates. NA: no activity at 1 mg/mL concentration of the extract. Concentration of ciprofloxacin was 0.0625 mg/ml and 0.05 mg/mL for *S. aureus* and *P. aeruginosa* respectively.

Table 5: Mean Biofilm Biomass of *S. aureus* and *P. aeruginosa* after exposure to plant extracts at a concentration of 1 mg/mL

Extracts	*S. aureus* Absorbance (OD_{590})	Inhibition of Preformed Biofilm (%)	*P. aeruginosa* Absorbance (OD_{590})	Inhibition of Preformed Biofilm (%)
Ethanol	0.71 ± 0.045^{b}	-1.43	0.97 ± 0.012^{b}	-18.29
Methanol	0.76 ± 0.020^{a}	-8.57	1.12 ± 0.024^{a}	-36.59
Aqueous	NA	-	NA	-
Ciprofloxacin	0.31 ± 0.033^{c}	55.71	0.49 ± 0.015^{d}	40.24
Control biofilm	0.70 ± 0.014^{b}	-	0.82 ± 0.024^{c}	-

Values in each column followed by different superscript within each column are significantly different from each other at $P< 0.05$. Values are mean ± standard error of three replicates. NA: no activity at 1 mg/mL concentration of the extract. Ciprofloxacin was used at a concentration of 0.125 mg/mL for both *S. aureus* and *P. aeruginosa.*

DISCUSSION

Our present study which was designed to obtain preliminary information on the *in vitro* antibacterial and antibiofilm activities of *Zingiber officinalis* extracts on selected pathogens showed that the ethanol extract of the plant exhibited antibacterial activity as it was active against the two microorganisms.Studies have shown that spices are active against a wide range of microorganism, their efficacy depended on solvent used for extraction, the type of spice, composition and concentration of secondary metabolites, and the test organisms (Ali *et al.,* 2011; Sawant *et al.,* 2021). In this study, the ethanol extract of *Z. officinalis* tested showed superior antibacterial activity against both *S. aureus* and *P. aeruginosa* compared to the methanol and aqueous extracts. The antimicrobial activity of *Z. officinalis* extracts has been attributed to the presence of oxygenated mono- and sesquiterpene phenolic compounds such as gingerol and shogaol (Michielin *et al.,*

2009; Elaissi *et al.,* 2011; Hossain *et al.,* 2011; Singh *et al.,* 2008). Phenolic compounds are believed to significantly contribute to the overall antimicrobial activity of spices (Condò *et al*., 2020). Various compounds are believed to interact mainly with the cell membrane to bring about their antimicrobial effects. Firstly, plant metabolites are believed to bond with membrane proteins through hydrophobic and hydrogen bonds, resulting in the subsequent transport of the metabolites through the lipid bilayer. This has been proven for naturally occurring hydrophobic compounds such as essential oil constituents (e.g. carvacrol, eugenol and thymol) that partition into the cytoplasm, due to their hydrophobicity (Condò *et al*., 2020). A second mechanism involves the disturbance of membrane permeability, resulting in an increase in fluidity and subsequent inhibition of membrane embedded enzymes (Khameneh *et al.,* 2019). Other mechanisms include disruption of both membrane and electron transport systems, which may result in some cellular processes such as respiration being shut down (Khameneh *et al.,* 2019). Phenolic compounds, in particular, are believed to sensitize the cytoplasmic membranes, increasing membrane permeability and reducing the availability of essential intracellular components (Khameneh *et al.,* 2019).

In contrast to the growth inhibitory activity against planktonic cells, biofilms of *P. aeruginosa* and *S. aureus* were less susceptible to the extracts. Eradication of bacteria living as biofilm is often more difficult compared to planktonic cells that form biofilms by use of pili to adhere to each other strongly. In the present study, the results of MIC, MBC, and MBIC have highlighted the interesting activity of *Z. officinalis*. The phytochemicals present in the crude ethanol extracts could play an important role in antibacterial activity. Medicinal plants are rich in secondary metabolites some of which are directly involved in the defense mechanisms of the plant against microorganisms (Cowan, 1999). In the current investigation, GC-MS analysis of the ethanol extract of *Z. officinalis* was carried out to identify the phytochemical compounds that might be responsible for any observed antibacterial and antibiofilm activity. Among the compounds identified in the plant extracts, gingerol (5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-decan-3-one) is the most abundant constituent, which has been reported to possess various pharmacological and physiological effects (Bhattara *et al.,* 2001). However, the predominant pungent principles in dried ginger are dehydrated products of gingerols called shogaols (Bischoff-Kont and Furst, 2021). A number of commercial preparations of ginger containing gingerol and /or shogaols have also been developed for the treatment of travel sickness and chronic arthritis (Lete and Allue, 2016).

Inhibition of initial attachment or impeding the growth of an established biofilm is one of the approaches currently used to reduce the accumulation of microorganisms on the surface. Cerca *et al*. (2005), reported that prevention of cell attachment to a substrate is easier to achieve than inhibiting the growth of an established biofilm. Prevention of attachment has become an attractive target in the prevention of infection and contamination of equipment and environmental surfaces in food industries (Zohra *et al.,* 2019). However, the enhancement of cell attachment observed for *Z. officinalis* methanol extract (-1.96%) in this study may have been due to the presence of certain compounds that provided a conditioning film that promoted microbial adhesion (Sandasi *et al*., 2011). Meadows (1971) reported that substances such as albumin, gelatin, fibrinogen, and casein play an important role in promoting microbial adhesion. This enhancing effect confirms reports that some natural compounds promote the growth of microorganisms (Sandasi *et al*., 2008). Some essential oil components promote the growth and development of a preformed *L. monocytogenes* biofilm *in vitro* (Sandasi *et al*., 2008). It has been reported that plant lectins may enhance the adsorption of cells onto a surface by acting as receptors of bacterial glycans, thereby promoting cell attachment (Ofek *et al.,* 2003).

Natural products derived from medicinal plants have been sources of bioactive compounds for the development of novel drug molecules including antimicrobial agents (Cowan, 1999). The results of this work support the study on antibiofilm effects of phenolic compounds of plants on *P. aeruginosa*, which showed that increased biofilm formation are as a result of different plant phenolic compounds, at concentrations that did not or weakly inhibits bacterial growth (Plyuta *et al*., 2013). In an investigation on *Bacillus subtilis*, biofilm formation was increased under the effect chlorine dioxide, a biocide and it was interpreted that this phenomenon is a self-protective response to the presence of harmful agents in the environment at their sub-lethal concentrations (Shemesh *et al*., 2010). Similar results have been observed in biofilm studies with the aminoglycoside antibiotics (Ryan *et al*., 2008). In this study, increase of biofilm formation by *S. aureus* and *P. aeruginosa*, in presence of *Z. officinalis* extracts, could be a protective response by bacteria to evade the antimicrobial effects of the extracts. It has been shown that some medicinal plants that have antimicrobial activity against planktonic form of some bacteria can induce biofilm formation and consequently more resistance in other bacteria. Another crucial factor in biofilm inhibition is the use of inappropriate amount of plant extracts and the dosage of plant extract which could yield an opposite result i.e., increase in biofilm production (Shemesh *et al*., 2010).

CONCLUSION

Natural products as alternatives to chemical disinfectants could offer consumers safer alternatives and a broader range of choice. The findings from this study validate the traditional use of the rhizomes of *Zingiber officinalis* for the treatment of bacterial infections. Hence, we conclude that *Z. officinalis* possesses some antibacterial properties and so can be used as potential source of active preservative for food and pharmaceuticals.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors conceptualized the study. CGO and CAN were responsible for the methodology, analysis, data curation, and manuscript preparation. Review and editing of the manuscript were done by CGO and CVA but all authors agreed on the content before submission.

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