

Evaluation of secondary Metabolites Profiling of Ginger (*Zingiber officinale* Roscoe) rhizome using GC-MS and its Antibacterial potential on *Staphylococcus aureus* and *Escherichia coli*

Authors' contributions

This work was carried out in collaboration among both authors. Both authors read and approved the final manuscript.

ABSTRACT

Objective: Ginger (*Zingiber officinale* Roscoe) rhizome is a well-known food spice and flavoring ingredient with wide range of medicinal properties. The rhizome of ginger consists of a unique secondary metabolites compounds. The study evaluates the secondary Metabolites Profiling of ginger (*Zingiber officinale* Roscoe) rhizome using Gas Chromatography-Mass Spectrometry (GC-MS) and its Antibacterial potential on *Staphylococcus aureus* and *Escherichia coli*.

Methodology: The GC-MS and phytochemical screening of the aqueous ginger (*Zingiber officinale* Roscoe) rhizome extract were determined using standard procedures. Antibacterial activities were determined by agar well diffusion methods. The minimum inhibitory concentrations (MIC) were determined using standard procedure.

Results: The result of the GC-MS analysis shows that thirty six compounds were identified in the ginger (*Zingiber officinale* Roscoe) rhizome using GC-MS analysis with tridecane with molecular formula of $C_{13}H_{28}$ being the most abundant with peak area of 16.94% and retention time of 12.849. The phytochemical screening shows that the plant contains saponins, alkaloids, glycoside, simple phenolics, tannins, flavonoids carbohydrates and reducing sugar. The study shows that at 250 mg/ml, the aqueous ginger extract exhibited little or no response with zone of inhibition of 9.85 ± 0.39 and 8.19 ± 1.33 against *Escherichia coli* and *Staphylococcus aureus* respectively. The extract exhibited weak response antibacterial activity against *E. coli* and moderate response against *S. aureus* with zone of inhibition of 13.62 ± 2.03 and 16.73 ± 1.83 at 500 mg/dl respectively. Augmentin showed moderate and strong response with zone of inhibition of 17.23 ± 1.67 and 21.13 ± 1.34 against *E. coli* and *S. aureus* at concentration of 7.50 mg/ml respectively. At 15 mg/ml, augmentin showed strong response with zone of inhibition of 23.00 ± 2.88 against *E. coli* and potent response with zone of inhibition of 30.50 ± 2.64 against *S. aureus*. The Minimum inhibitory concentration (MIC) values for the aqueous ginger are 125 and 250 mg/ml for *S. aureus* and *E. coli* and 7.81 and 15.63 for augmentin solution for the same organisms respectively.

Conclusions: The aqueous ginger (*Zingiber officinale* Roscoe) rhizome contains secondary metabolites and possesses poor antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* and may prevent pathogenic diseases caused by these organisms.

Keywords: Antimicrobial activity; *Escherichia coli*; GC-MS; ginger; *Staphylococcus aureus*.

1. Introduction

Ginger (*Zingiber officinale* Roscoe), rhizome belongs to the Zingiberaceae family and the plant has been consumed as a spice and an herbal medicine used for the treatment of various diseases for a very long period of time [1]. Studies have shown the different bioactive compounds in ginger and the main compounds are terpene and phenolic compounds. The phenolic compounds are mainly gingerols, shogaols, and paradols, which account for the various bioactivities of ginger [2]. The pungency of fresh ginger is mainly due to the gingerols, whereas the pungency of dried ginger is primarily due to the presence of shogaols, mainly 6-shogaol, which are dehydrated forms of gingerols [3]. 6-gingerol and 6-shogaol are the two active components found in ginger which produce a depressor response on blood pressure at lower doses in cardiovascular system [4], because of these health-promoting properties of the plant, ginger can be considered as an active ingredient that can be added to food substances for reducing the risk of cardiovascular disease[5, 6].

Secondary metabolites are substances manufactured by plants that exert a wide range of effects on the plant itself and on other living organisms. They act as antimicrobials, maintain perennial growth, and induce flowering, fruit set and abscission. Over 50,000 secondary metabolites have been discovered in the plant kingdom. Metabolite profiling requires an analytical system that can generate useful datasets and identify the compounds of interest. To date, many techniques, such as liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC-MS), and nuclear magnetic resonance (NMR) have been widely employed for metabolite profiling [7]. Among these techniques, GC-MS has the advantages of low cost compared to the other analytical methods, high reproducibility, high resolution, highly repeatable mass spectral fragmentation, and few matrix effects [8].

Metabolite profiles have been obtained from various medicinal plants, including: *Curucuma* species [9], *Carica papaya* leaf [10], *Hunteria umbellata* seed extract [11], hexane leaf, stem and root extracts of *Azadirachta indica* A. Juss [12], methanolic root, stem and leaf extracts of *Vernonia amygdalina* [13], *Carica papaya* seed oil [14], *Rehmannia glutinosa* using GC-MS combined with multivariate statistical analysis [15] and *Azadirachta indica* root [16].

Medicinal herbs rely on secondary plant metabolites for their metabolism and actions. Ginger has been found to possess various biological activities, such as anti-inflammatory [17], antioxidant [18], anticancer [19] and antimicrobial [20] activities. Furthermore, numerous studies have shown that ginger possesses the potential to prevent and ameliorate the effect of several diseases,

such as obesity [21], diabetes mellitus [22], neurodegenerative diseases [23], respiratory disorders [24] and cardiovascular diseases [25].

Escherichia coli is a gram negative, rod-shaped bacterium that is common inhabitant of the animal and human gut, and may also be found in vegetation, soil and water. It is the leading pathogen causing urinary tract infections [26-29] and is among the most common pathogens causing blood stream infections [30], otitis media, diarrhea, meningitis, wounds and other complications in humans [26, 31, 32]. *Escherichia coli* is also the most common cause of food and water-borne human diarrhea worldwide and in developing countries, causing many deaths in children under the age of five years. [33].

Staphylococcus aureus is a gram positive bacterium and they cause wide range of infections in human and animals [34]. They are found on human skin and mucous membranes. However, it can also be found in other areas of human contact including water, soil and food products [34]. They causes serious infections like bacteremia, septic arthritis, pneumonia, wound sepsis, septicemia, osteomyelitis, endocarditis, food poisoning, bone and joint infections and toxic shock syndrome [34]. The study evaluates the secondary metabolites profiling of ginger (*Zingiber officinale* Roscoe) rhizome using GC-MS and its antibacterial potential on *Staphylococcus aureus* and *Escherichia coli*

2. MATERIALS AND METHODS

2.1 Collection of ginger Plant

The ginger (*Zingiber officinale* Roscoe), rhizome was purchased from Ikorodu market and stored in a refrigerator in the Department of Chemical Sciences (Biochemistry unit), Lagos State University of Science and Technology.

2.2 Gas Chromatography-Mass Spectrometry (GC-MS) analysis of ginger

GC-MS analysis of the *Zingiber officinale* rhizome was carried out on an Agilent technology 7890 GC system equipped with a mass spectrometric detector (MSD) as described by Momoh et al. [35].

2.2.1 Detection of components

Analysis of mass spectrum GC-MS was conducted by the database of the National Institute Standard and Technique (NIST) which contained more than 62,000 patterns. The spectrum of the unidentified compound was compared with the spectrum of the identified compounds stored in the National Institute Standard and Technique library. The names, molecular weight, structure of the compounds in the test material were determined.

2.3 Preparation of aqueous garlic extract

Aqueous *Zingiber officinale* extract was prepared according to the method described by Momoh et al. [36]. The ginger was cleaned with water to remove any adhering soil on their surfaces. 100g of garlic was taken after the removal of the outer skin surfaces and cut into small pieces by sterile scalpel. The small pieces were blended with 200 ml sterile distilled water using sterile blender for 5 minutes. The homogenized mixture was filtered using white cloth, centrifuged at $2000 \times g$ for 10 minutes and the clear supernatant was used for the experiment. The filtered extract was used for the study within 4 hours of preparation.

2.4 Preliminary phytochemical analysis

The presence of glycosides, tannins, saponin, reducing sugars, alkaloids, flavonoids were determined by qualitative procedures [37, 38].

2.5 Test organisms

To study the antibacterial activity of aqueous *Zingiber officinale* extract against two bacterial strains (*Staphylococcus aureus* a gram-positive bacterium with ATCC #6538 and *Escherichia coli* a gram negative bacterium with ATCC # 25922) were used for the study. The two microorganisms were maintained at 4°C on Nutrient Agar slant in the Department of Chemical Sciences and fresh subcultures were made before use.

2.5.1 Inoculum preparation

A loopful of isolated colonies of the two organisms (*Staphylococcus aureus* and *Escherichia coli*) were inoculated separately into 4 ml of peptone water, incubated at 37°C for 4 hours. These actively growing bacterial suspensions were then adjusted with peptone water to obtain turbidity

visually comparable to that of 0.5 McFarland standards using standard procedure [34]. The 0.5 McFarland standard was prepared by mixing 0.5ml of 1.75% (w/v) barium chloride dehydrate ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$) with 99.5 ml of 1% (v/v) H_2SO_4 . This turbidity was equivalent to approximately 1×10^8 colony forming units per ml (CFU/ml) [34].

2.5.2 Determination of diameter of zone of inhibition using agar well diffusion method

Agar well-diffusion method was employed for the determination of the antibacterial activity of aqueous ginger extract. Twenty four hours of broth culture of the two microorganisms (*Staphylococcus aureus* and *Escherichia coli*) were suspended into sterile nutrient broth. It was standardized by gradually adding 9% normal saline to compare its turbidity to McFarland standard of 0.5 which is approximately 1×10^8 colony forming units per ml. Petri-dishes were prepared by loading about 25 ml of an autoclaved nutrient agar on sterile plates and left to solidify. 100 μl of a standardized culture (adjusted to 0.5 McFarland) of the two different organisms were added onto the different agar plates. Then, the surface of each plate was drilled using a sterile cork borer (6 mm) and 3 wells were punched out on each plate followed by loading of 100 μl of the aqueous ginger extract of different concentration in the wells and allowed to diffuse at room temperature for 2 hours. The plates were incubated at 37°C for 18-24 hours for bacterial pathogens. The diameters of the inhibition zone (mm) were measured. The susceptibility of the two different organisms to different concentration of aqueous ginger extracts were assayed using standard procedures [11, 34]. The experiment was repeated thrice, for each replicate, the readings were taken in three different fixed directions and the average values were recorded [11, 34]. The inhibitory responses were classified as potent response, +++++, zone diameter >30 mm; strong response, +++, zone diameter between 21-30 mm; moderate response, ++, zone diameter between 16-20 mm; weak response, +, zone diameter between 10-15 mm; and little or no response, zone diameter <10 mm [34, 39].

2.5.3 Minimum inhibitory concentration (MIC) of aqueous *Zingiber officinale* extract

Minimum inhibition concentration is the lowest concentration of ginger extract that inhibited the growth of the test organisms as indicated by the absence of visible turbidity in the tube compared with the control tubes [16, 34]. The MIC of the aqueous ginger rhizome extract was determined according to standard method [16, 34]. The MIC of the aqueous ginger extract was assayed using serial dilution method. In this method, a total of 1 ml of Mueller-Hinton broth was poured to a set of different test tubes and autoclaved. Subsequently, 1 ml of 100% aqueous ginger extract

(2g/ml) was poured to the first separate test tube to make a concentration of 50%, and two-fold serial dilutions were made by transferring 1 ml from one tube to another. Then, an overnight broth culture of the different test organisms were adjusted to McFarland turbidity standard and 100 μ l of the different cell suspensions were added to each of the separate tubes. The tubes were incubated aerobically at 37°C for 18 hours. Negative control tube was made by pouring 1ml of normal saline instead of the aqueous ginger extract. The lowest concentration of the dilution without bacterial growth was considered as the minimum inhibition concentration.

2.6 Statistical Analysis

All analyses were carried out in triplicate determination and results were expressed as mean \pm SD. Student's *t*-test was used for comparison. The data analysis was done using one way analysis of variance (ANOVA) Post Hoc Turkey Graph Pad prism computer software version 5.01. *P*-value < 0.05 was considered significant.

3. RESULTS

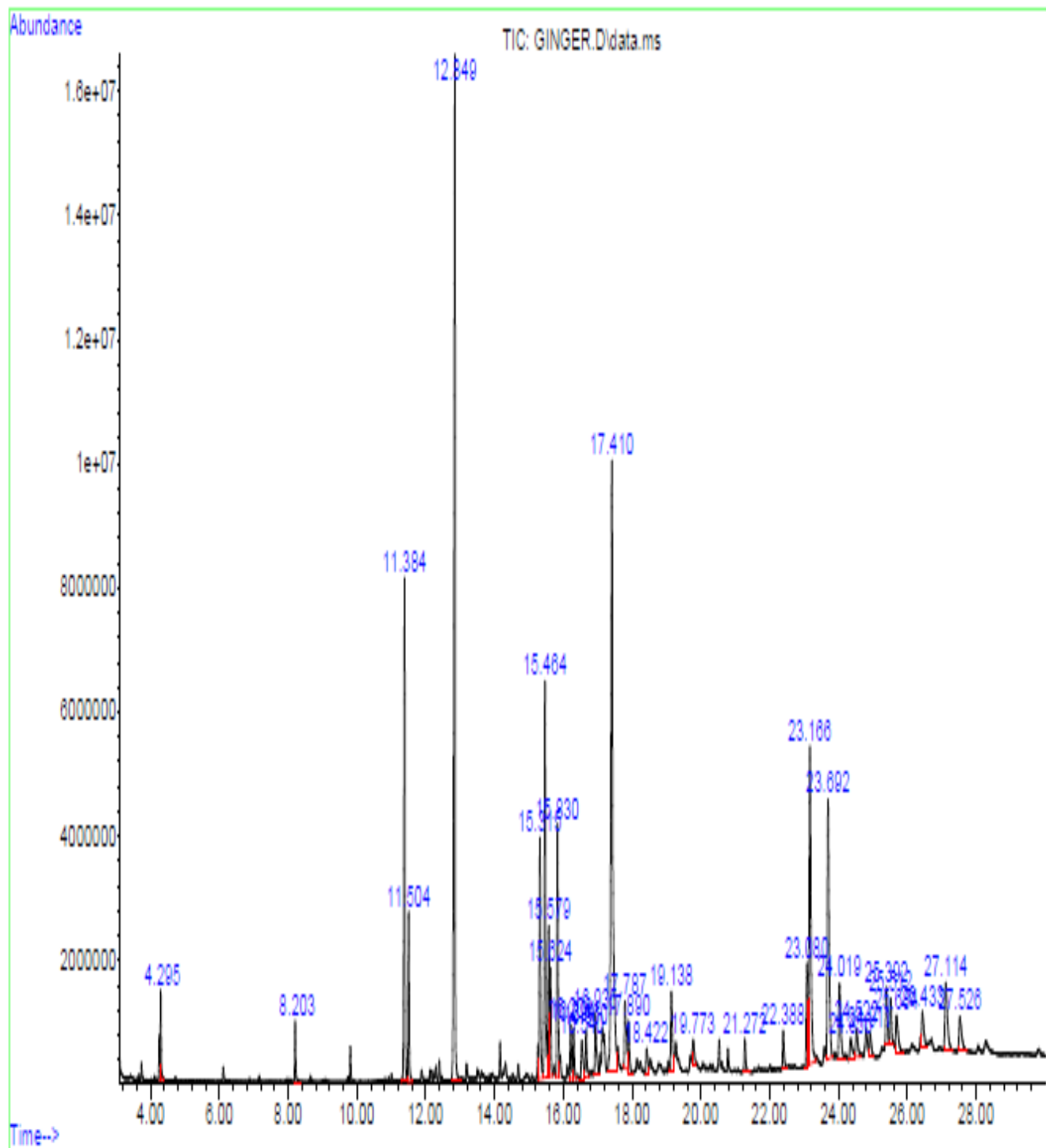


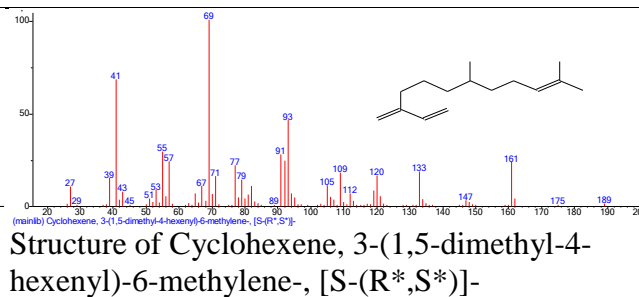
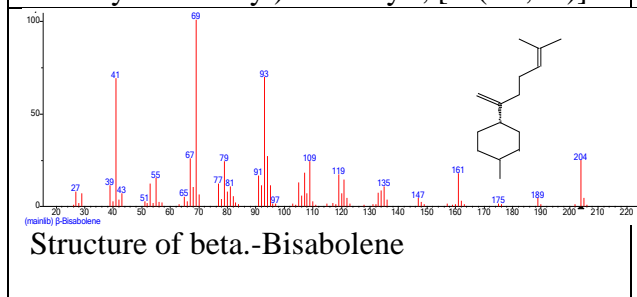
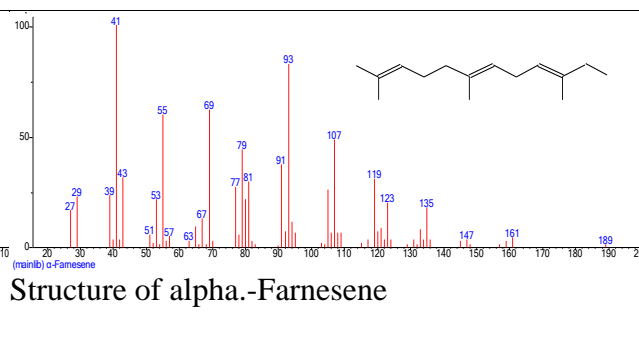
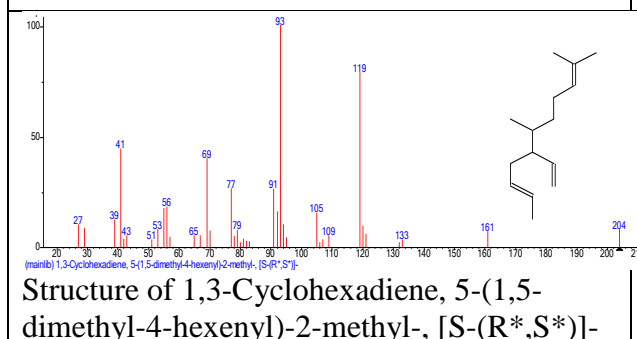
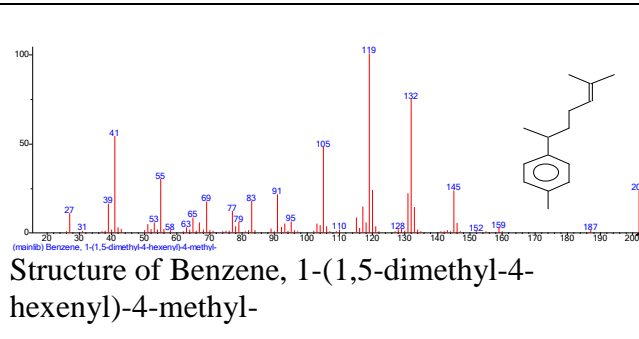
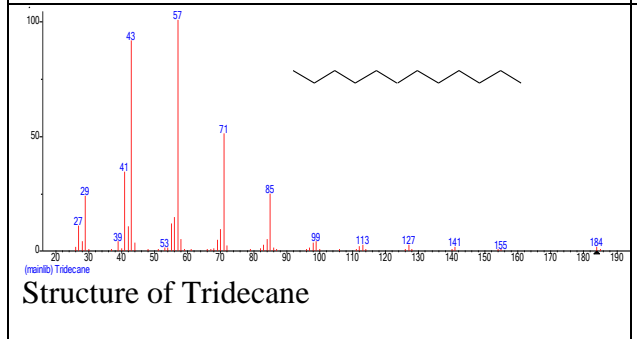
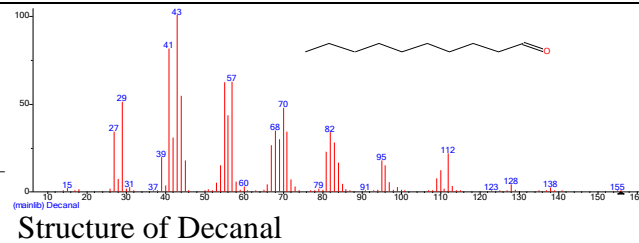
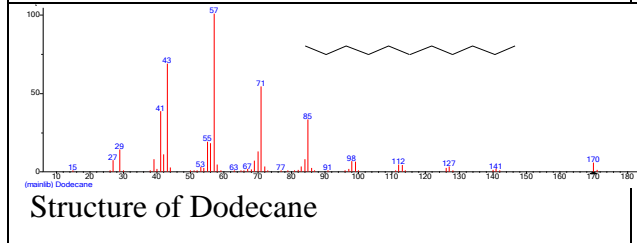
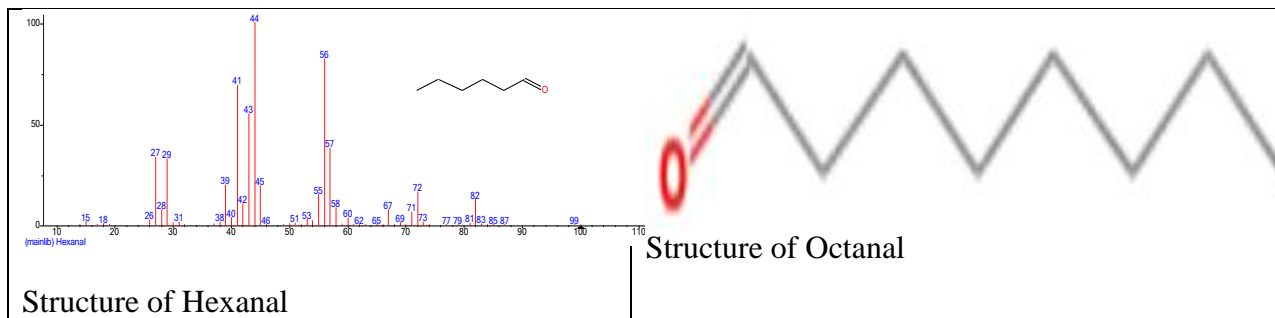
Figure 1: Gas-Chromatography–Mass Spectrometry chromatogram of ginger (*Zingiber officinale*) rhizome.

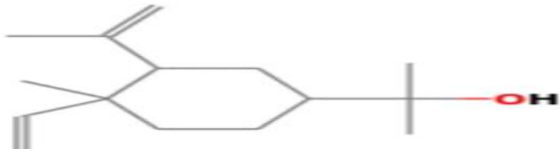
Table 1: Compounds found in the ginger (*Zingiber officinale*) rhizome analyzed using Gas-Chromatography–Mass Spectrometry.

PK#	RT	Peak Area (%)	Name of the compound	Molecular Formulae	Molecular Weight (g/mol)	Ref#	CAS#	Qual.
1	4.295	1.00	Hexanal	C ₆ H ₁₂ O	100.1589	3833	000066-25-1	90
2	8.203	0.76	Octanal	C ₈ H ₁₆ O	128.2120	12694	000124-13-0	90
3	11.384	6.58	Dodecane	C ₁₂ H ₂₆	170.3348	39972	000112-40-3	97
4	11.504	2.19	Decanal	C ₁₀ H ₂₀ O	156.2652	29133	000112-31-2	98
5	12.849	16.94	Tridecane	C ₁₃ H ₂₈	184.3614	51394	000629-50-5	96
6	15.315	3.58	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	C ₁₅ H ₂₂	202.3352	66866	000644-30-4	99
7	15.464	5.85	1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]-	C ₁₅ H ₂₄	204.3511	68761	000495-60-3	94
8	15.579	2.31	alpha.-Farnesene	C ₁₅ H ₂₄	204.3511	68573	000502-61-4	81
9	15.624	1.51	beta.-Bisabolene	C ₁₅ H ₂₄	204.3511	68571	000495-61-4	98
10	15.830	3.89	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	C ₁₅ H ₂₄	204.3511	68741	020307-83-9	93
11	16.208	0.91	Cyclohexanemethanol, 4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-methylethenyl)-, [1R-(1.alpha.,3.alpha.,4.beta.)]-	C ₁₅ H ₂₆ O	222.3663	85863	000639-99-6	83
12	16.294	0.82	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-	C ₁₅ H ₂₆ O	222.3663	85747	007212-44-4	91
13	16.540	1.00	4-(1-Hydroxyallyl)-2-methoxyphenol	C ₁₀ H ₁₂ O	180.2005	47432	112465-50-6	96

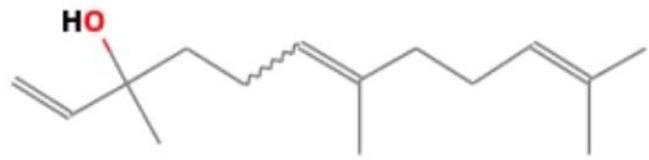
14	16.660	0.87	trans-Sesquisabinene hydrate	C ₁₅ H ₂₆ O	222.3663	85739	145512-84-1	95
15	16.935	1.33	(1S,2R,5R)-2-Methyl-5-((R)-6-methylhept-5-en-2-yl)bicyclo[3.1.0]hexan-2-ol	C ₁₅ H ₂₆ O	222.3663	85811	058319-05-4	87
16	17.410	15.95	Butan-2-one, 4-(3-hydroxy-2-methoxyphenyl)-	C ₁₁ H ₁₄ O ₃	194.23	59345	303187-89-5	98
17	17.787	1.65	3-Cyclohexene-1-methanol, .alpha., 4-dimethyl-.alpha.-(4-methyl-3-pentenyl)-, [R-(R*,R*)]-	C ₁₅ H ₂₆ O	222.3663	85832	023178-88-3	56
18	17.890	1.04	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	C ₁₅ H ₂₆ O	222.3663	85748	004602-84-0	55
19	18.422	0.63	1,2-Cyclohexanediol, cyclic sulfite, trans-	C ₆ H ₁₀ O ₃ S	162.21	33637	019456-19-0	41
20	19.138	1.72	2-Butanone, 4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-, (R)-	C ₁₃ H ₂₂ O	194.3132	59811	039721-65-8	38
21	19.773	0.53	Cyclopropane carboxamide, 2-cyclopropyl-2-methyl-N-(1-cyclopropylethyl)-	C ₁₃ H ₂₁ NO	207.31	71615	331416-19-4	51
22	21.272	0.62	1,6,10,14-Hexadecatetraen-3-ol, 3, 7,11,15-tetramethyl-, (E,E)-	C ₂₀ H ₃₄ O	290.4834	150239	001113-21-9	59
23	22.388	0.74	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-(.+/-.)-	C ₃₀ H ₅₀ O	426.7174	249597	097232-74-1	80
24	23.080	2.23	(E)-1-(4-Hydroxy-3-methoxyphenyl) dec-3-en-5-one	C ₁₇ H ₂₄ O ₃	276.3707	136509	863913-65-9	95
25	23.166	7.39	3-Decanone, 1-(4-hydroxy-3-methoxyphenyl)-	C ₁₇ H ₂₆ O ₃	278.3865	138319	027113-22-0	98
26	23.692	6.36	1-(4-Hydroxy-3-methoxyphenyl) dec-4-en-3-one	C ₁₇ H ₂₄ O ₃	276.3707	136506	000555-66-8	98
27	24.019	2.00	1-(4-Hydroxy-3-methoxyphenyl) decane-3,5-dione	C ₁₇ H ₂₄ O ₄	292.4	151730	061871-71-4	96
28	24.356	0.61	Vanillin	C ₈ H ₈ O ₃	152.15	26591	000121-33-5	45
29	24.522	0.75	5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl) decan-3-	C ₁₇ H ₂₆ O ₄	294.391	153613	039886-76-5	97

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30	24.911	0.59	1-(4-Hydroxy-3-methoxyphenyl) dodecan-3-one	C ₁₉ H ₃₀ O ₃	306.4397	165516	027113-23-1	93
31	25.392	1.25	1-(4-Hydroxy-3-methoxyphenyl) oct-4-en-3-one	C ₁₅ H ₂₀ O ₃	248.32	109653	211176-76-0	74
32	25.512	0.79	(3R,5S)-1-(4-Hydroxy-3-methoxyphenyl) decane-3,5-diyl diacetate	C ₂₁ H ₃₂ O ₆	380.4752	227162	143615-75-2	99
33	25.684	1.19	1-(3,4-Dimethoxyphenyl) decane-3,5-diyl diacetate	C ₂₂ H ₃₄ O ₆	394.5018	235343	053254-52-7	42
34	26.433	0.93	(E)-1-(4-Hydroxy-3-methoxyphenyl) tetradec-3-en-5-one	C ₂₁ H ₃₂ O ₃	332.4770	190321	1278586-98-3	95
35	27.114	2.27	1-(4-Hydroxy-3-methoxyphenyl) tetradec-4-en-3-one	C ₂₁ H ₃₂ O ₃	332.5	190316	036752-54-2	97
36	27.526	1.21	1-(4-Hydroxy-3-methoxyphenyl) tetradecane-3,5-dione	C ₂₁ H ₃₂ O ₄	348.5	204403	079067-90-6	97

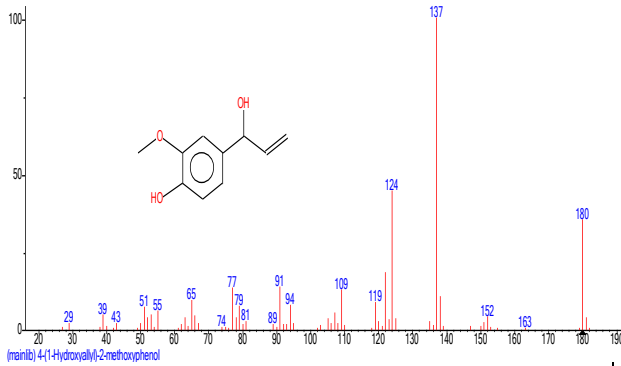




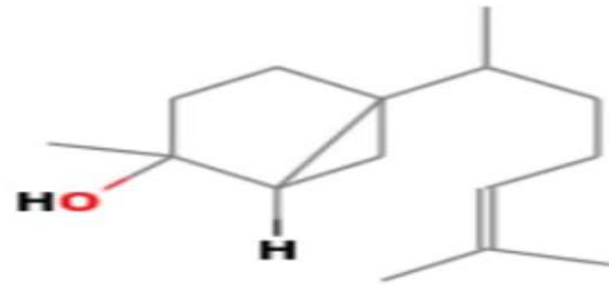
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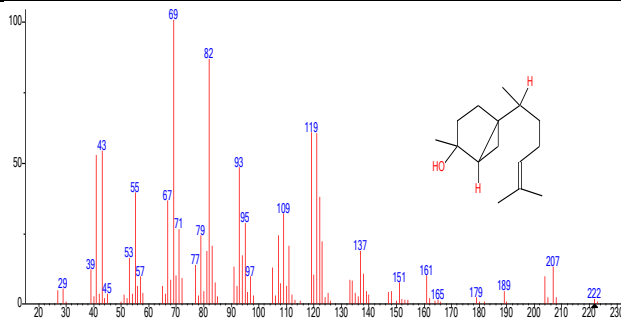
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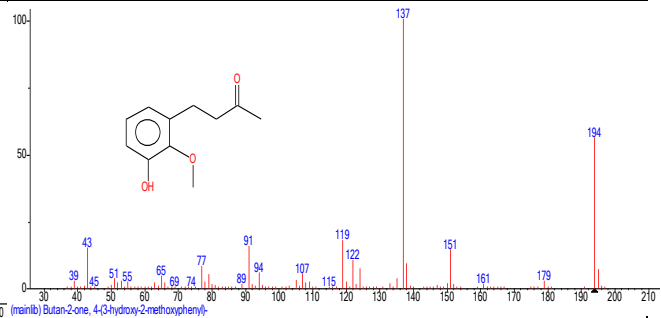
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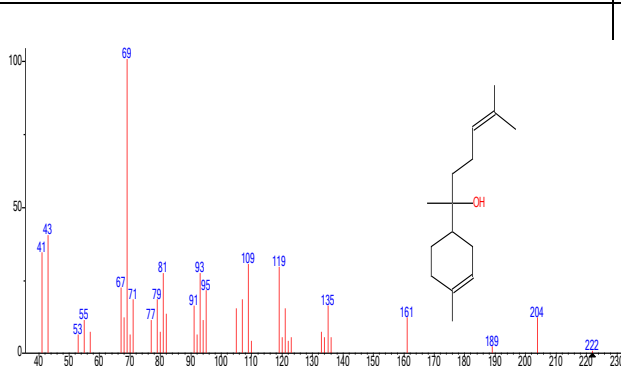
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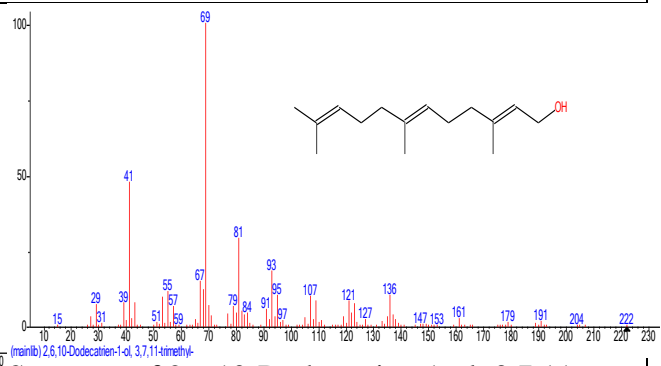
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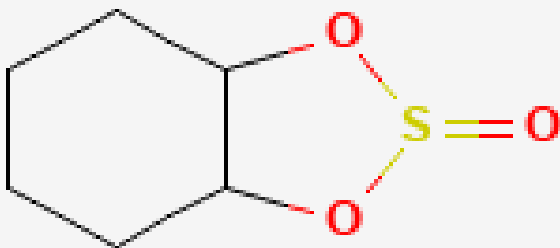
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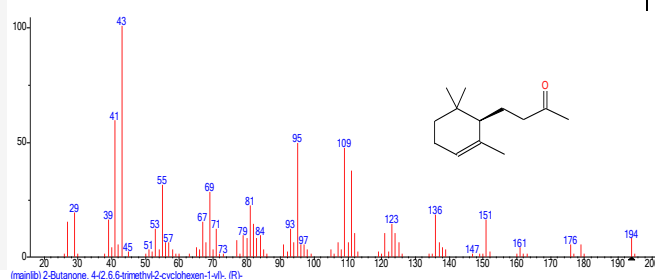
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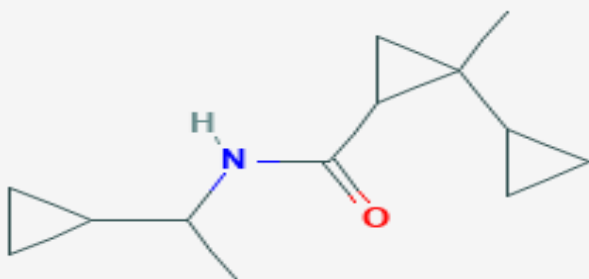
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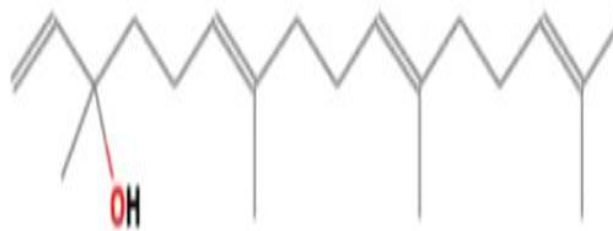
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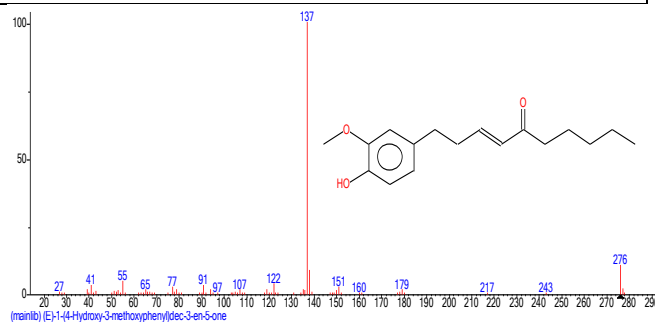
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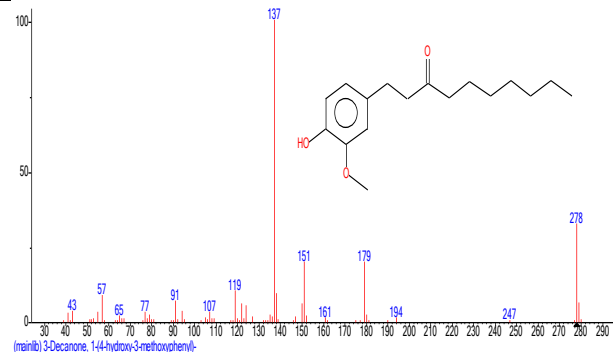
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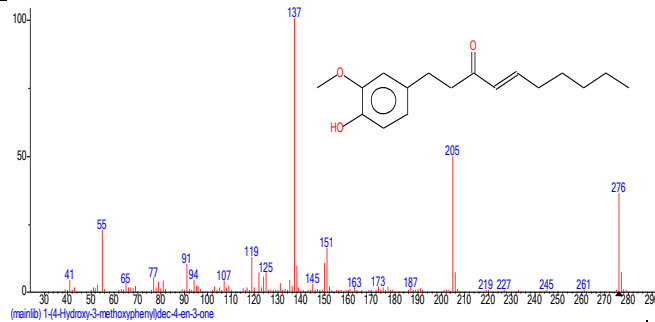
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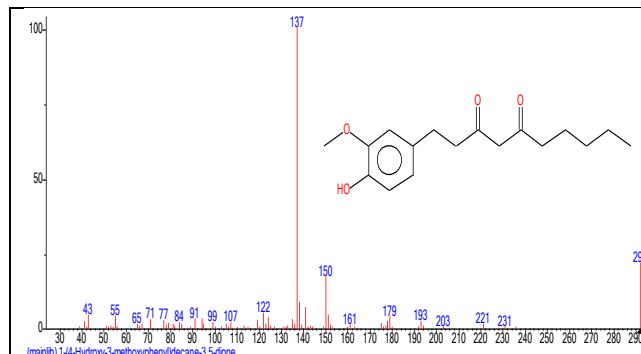
Structure of (E)-1-(4-Hydroxy-3-methoxyphenyl) dec-3-en-5-one



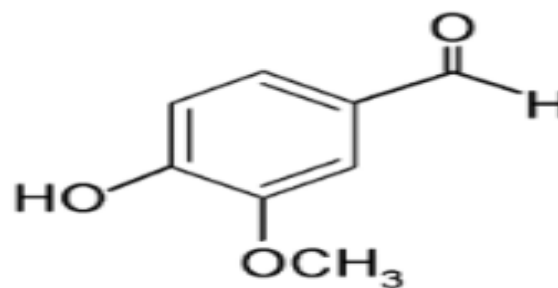
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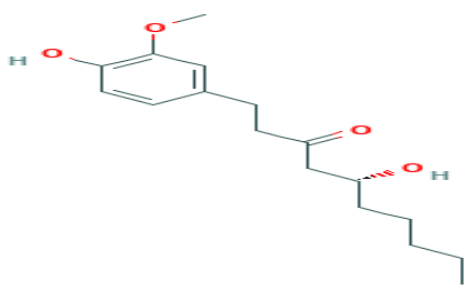
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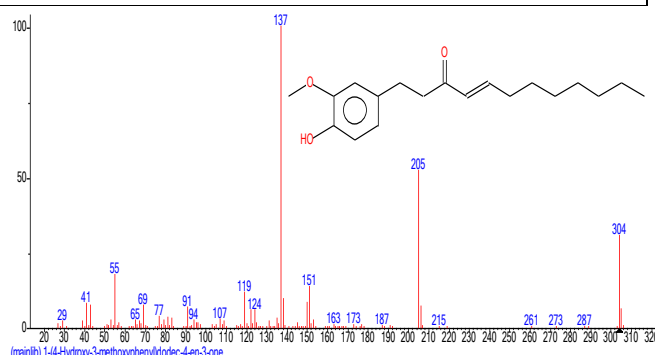
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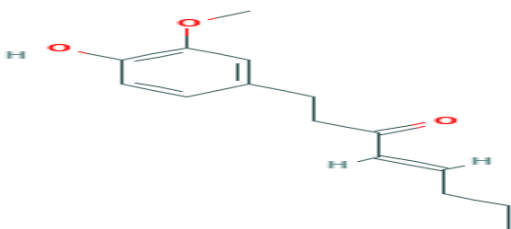
Structure of Vanillin



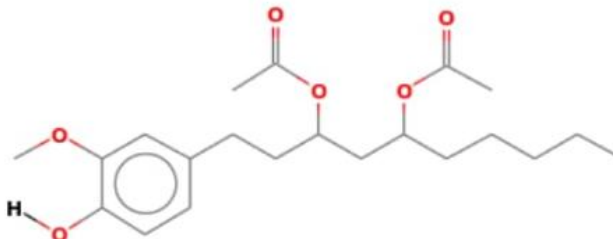
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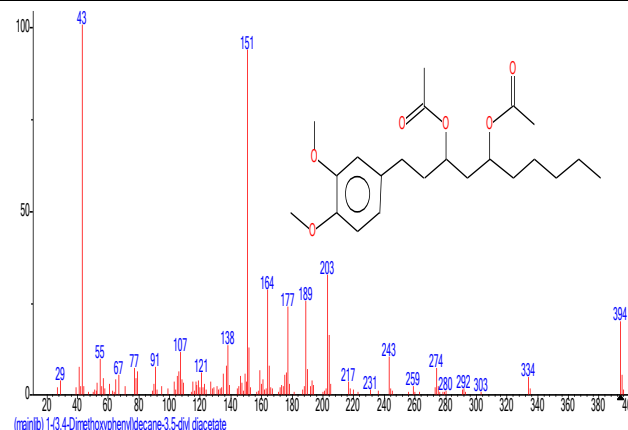
Structure of 1-(4-Hydroxy-3-methoxyphenyl)dodecan-3-one



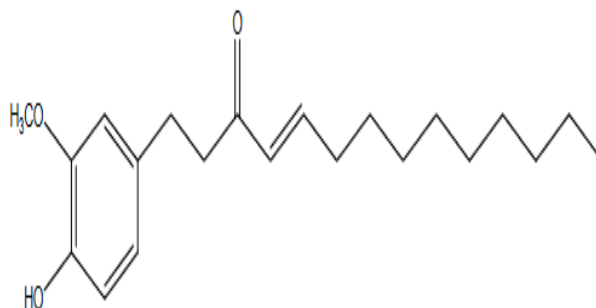
Structure of 1-(4-Hydroxy-3-methoxyphenyl)oct-4-en-3-one



Structure of (3R,5S)-1-(4-Hydroxy-3-methoxyphenyl)decane-3,5-diyl diacetate



Structure of 1-(3,4-Dimethoxyphenyl)decane-3,5-diyl diacetate



Structure of (E)-1-(4-Hydroxy-3-methoxyphenyl)tetradec-4-en-3-one

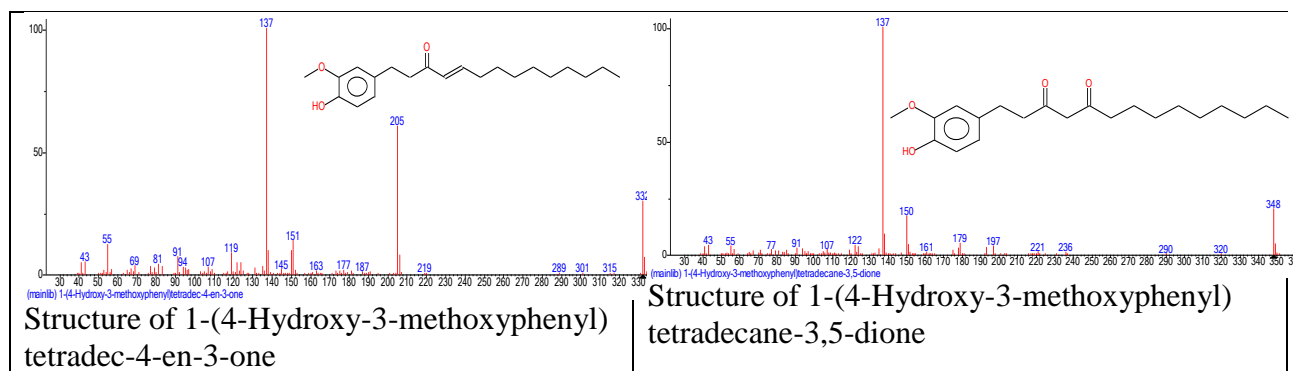


Figure 2. Mass spectrum and structure of 36 different compounds obtained during GC-MS analysis of ginger rhizome.

Table 2: The qualitative phytochemical constituents of aqueous ginger extract.

Phytochemical constituent	Inference
Saponins	Present
Tannins	Present
Simple phenolics	Present
Flavonoids	Present
Glycosides	Present
Alkaloids	Present
Carbohydrate	Present
Reducing sugar	Present

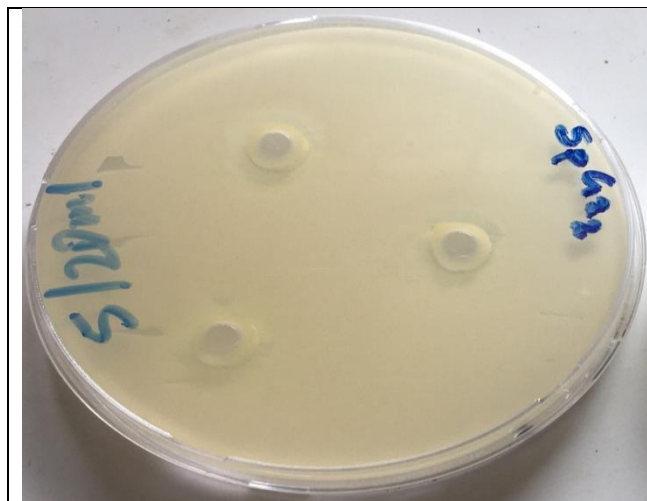


Figure 3a: Zone of inhibition at 250 mg/ml of the aqueous extract of ginger against *Staphylococcus aureus*

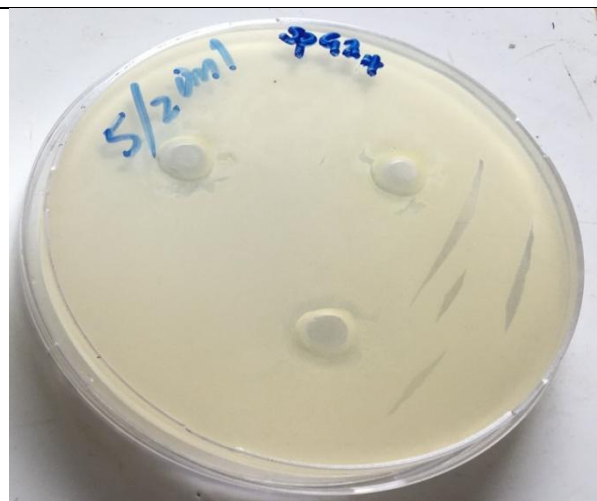


Figure 3b: Zone of inhibition at 250 mg/ml of the aqueous extract of ginger against *Staphylococcus aureus*

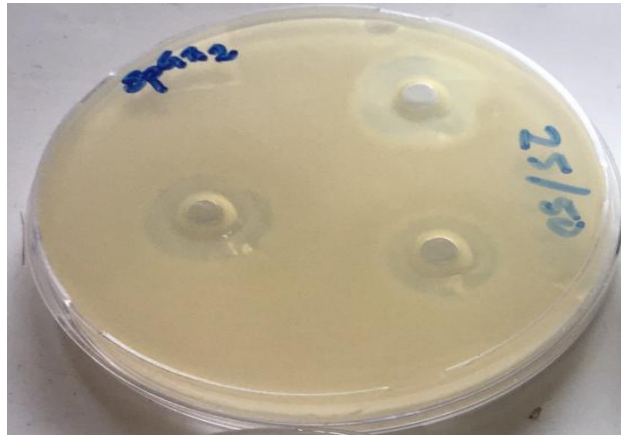


Figure 3c: Zone of inhibition at 500 mg/ml of the aqueous extract of ginger against *Staphylococcus aureus*

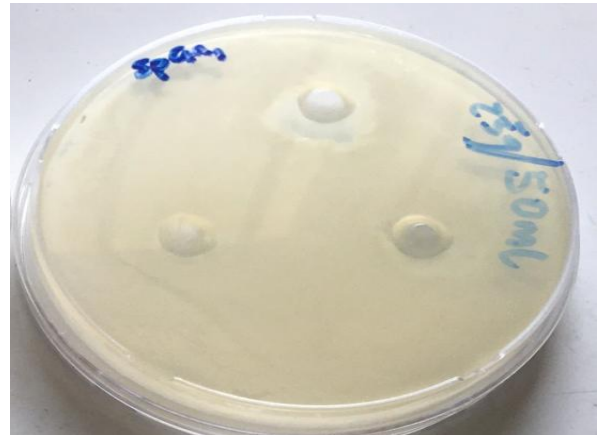


Figure 3d: Zone of inhibition at 500 mg/ml of the aqueous extract of ginger against *Staphylococcus aureus*



Figure 3e: Zone of inhibition at 250 mg/ml of the aqueous extract of ginger against *Escherichia coli*



Figure 3f: Zone of inhibition at 500 mg/ml of the aqueous extract of ginger against *Escherichia coli*

Figure 3. Zone of inhibition of, aqueous extracts of ginger (*Zingiber officinale*) rhizome against *Staphylococcus aureus* and *Escherichia coli* at 250 and 500 mg/ml.

Table 3. Zone of inhibition of aqueous extract of ginger (*Zingiber officinale*) against *Escherichia coli* and *Staphylococcus aureus*

Test organisms	Aqueous extract of ginger concentration (mg/ml)	Zone of inhibition for aqueous extract of ginger (mm)	Interpretation	Concentration of Augmentin solution used (mg/ml)	Zone of inhibition of Augmentin solution (mm)	Interpretation
<i>Escherichia coli</i>	250	8.19±1.33 ^c	little or no response	7.50	17.23±1.674 ^c	++
<i>Staphylococcus aureus</i>	250	9.85±0.39 ^c	little or no response	7.50	21.13±1.34 ^c	+++
<i>Escherichia coli</i>	500	13.62±2.03 ^b	+	15	23.00±2.88 ^b	+++
<i>Staphylococcus aureus</i>	500	16.73±1.83 ^a	++	15	30.50±2.64 ^a	++++

Values are represented as mean ± SD. A comparison across the column was done using One way ANOVA Post Hoc Turkey test. The superscript a has the highest value followed by b and c has the lowest value. A. P<0.05 was considered statistically significant. The inhibitory responses were classified as potent response, +++++, zone diameter >30 mm; strong response, +++, zone diameter between 21-30 mm; moderate response, ++, zone diameter between 16-20 mm; weak response, +, zone diameter between 10-15 mm; and little or no response, zone diameter <10 mm.

3.2. Minimum Inhibitory Concentration (MIC) for aqueous ginger and Augmentin antibiotic against *Staphylococcus aureus* and *Escherichia coli*

The values for the MICs of aqueous ginger (*Zingiber officinale*) and Augmentin antibiotic against *Staphylococcus aureus* and *Escherichia coli* are shown in Table 4 below:

Table 4: Minimum inhibitory concentration (MIC) of aqueous extract of *Zingiber officinale* rhizome and Augmentin antibiotic against *Staphylococcus aureus* and *Escherichia coli*

ORGANISMS	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
MIC for aqueous extract of ginger (mg/ml)	125.00	250.00
MIC for Augmentin solution (mg/ml)	7.81	15.63

4. DISCUSSION

Ginger rhizomes and its products have been used widely as a food spice as well as in herbal medicine for the protection and treatment of diseases. Figure 1 shows the Gas-Chromatography–Mass Spectrometry chromatogram of ginger rhizome. A total of 36 compounds were identified consisting of two prominent compounds and 34 minor compounds (Table 1). The two major compounds and their percentage abundance are: Tridecane with molecular formula of $C_{13}H_{28}$ (RT=12.849 and peak area=16.94%) and Butan-2-one, 4-(3-hydroxy-2-methoxyphenyl)- with molecular formula of $C_{11}H_{14}O_3$ (RT=17.410 and peak area=15.95%).

In one of our study, it was observed that the GC-MS analysis of aqueous ginger extract contains 11 different compounds: Propane, 1-chloro-2-nitro-, Cyclopropene, 1-methyl-3-(2-methyl cyclopropyl)-, 1,3-Cyclohexadiene, 5-(1,5-dimethy 4-hexenyl)-2-methyl-, [S-(R*,S*)]-, Preg-4-en-3-one, 17.alpha.-hydroxy-17.beta.-cyano, (E)-.beta.-Famesene, 3-Bromo-N (3,5dichlorophenyl) -benzamide, TMS derivative, Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis-[(trimethylsilyl)oxy]-, 2,5-Dihydroxybenzoic acid, 3TMS derivative, Cyclodecasiloxane, eicosamethyl-, 3-Isopropoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)-trisiloxane and 1,1,3,3,5,5,7,7,9,9,11,11,13,13- tetradecamethylheptasiloxane.

1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]- was the most abundant compound in the aqueous ginger extract with peak area of 33.98 % and retention time of 14.554 [40]. Two highly alkylated gingerols, 10-gingerol and 12-gingerol, seem to be effective in inhibiting the growth of oral pathogens at a MIC range of 6–30 µg/mL and killing these oral pathogens at a minimum bactericidal concentration range of 4–20 µg/mL [41]. In another study, it was observed that Four ginger components namely, 6-dehydrogingerdione, 10-gingerol, 6-shogaol, and 6-gingerol have shown antibacterial effects against extensively drug-resistant *Acinetobacter baumannii* [42]. Keith and Singletary [43] study enumerates the biological importance of vanillin to include the following: pain relief, antidepressant, antisickling, antianxiety, protect against nerve damage and neurodegeneration, correct blood glucose and lipid dysregulation. 1-(4-Hydroxy-3-methoxyphenyl) decane-3,5-dione is also called gingerdione and possess vital medicinal uses. 5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl) decan-3-one found in the rhizome of ginger is also called 6-gingerol. 6-Gingerol is one of the primary bioactive phenylpropanoid of the rhizome of ginger and has been reported to have a pharmacological activities including; antioxidant effect, anti-cancer, anti-inflammation, anti-oxidation and possess cytotoxic activity and Inhibit of angiogenesis, [44]. 1-(4-Hydroxy-3-methoxyphenyl) oct-4-en-3-one is also called 4-Shogaol and possesses medicinal uses. 1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]- is also called alpha-Zingiberene. 1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]- was the most abundant compound found in the aqueous ginger extract according to the GC-MS analysis carried out by Momoh et al. [40] Zingiberene is the primary terpenoid in ginger. (E)-1-(4-Hydroxy-3-methoxyphenyl) tetradec-4-en-3-one compound found in the plant that is used in this study is also called 10-Shogaol. A study shows that 10-Shogaol possesses antioxidant activity, promoted human normal epidermal keratinocytes and dermal fibroblasts cell growths. It enhances growth factor production in transforming growth factor-β (TGF-β), platelet derived growth factor-αβ (PDGF-αβ) and vascular endothelial growth factors (VEGF) of both cells. In the in vitro wound healing assay for 12 or 24 h, with 10-shogaol, the fibroblasts and keratinocytes migrated more rapidly than the vehicle control group [45].

The preliminary qualitative analysis of the different secondary metabolites present in the aqueous extracts of ginger was investigated. The aqueous ginger showed that they contain simple phenolics, alkaloids, glycosides, saponins, flavonoids, tannins, carbohydrate and reducing sugar.

Tannins which are phenolic compounds tend to dissolve in water and tend to be polar. Terpenoids are fat soluble. One of the terpenoids which has the potential as an antimicrobial is triterpenoid. Flavonoids are generally more soluble in water or polar solvents because they bond with hydroxyl groups. Glycosides are compounds that contain non-sugar and sugar components. Saponins are generally in the form of glycosides so they tend to be polar. Saponins are surface active compounds that produce foam if shaken in water. This happens because saponins have polar and non-polar groups that will form micelles. When the micelle is formed the polar group will face out while the non-polar groups face inside so it looks like foams. The antimicrobial activity of plants is due to saponins, essential oils, tannins, phenolic compounds and flavonoids [46]. It is interesting to note that even crude extracts of these plants showed good activity against multidrug resistant strains where modern antibiotic drug has limited effect.

Escherichia coli and *Staphylococcus aureus* were selected for the study and tested against aqueous ginger and Augmentin. In our study, the aqueous ginger extract exhibited moderate response antimicrobial activity against *Staphylococcus aureus* and weak response against *Escherichia coli* with zone of inhibition of 16.73 ± 1.83 and 13.62 ± 2.03 at 500 mg/dl respectively. The study shows that at 250 mg/ml, the aqueous ginger extracts exhibited little or no response with zone of inhibition of 9.85 ± 0.39 and 8.19 ± 1.33 against *S. aureus* and *E. coli* respectively. The poor activity of the aqueous ginger used in this study may be due to the low concentration of the active ingredients (5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl) decan-3-one also called 6-gingerol has 0.75%, 1-(4-Hydroxy-3-methoxyphenyl) dec-4-en-3-one (6-Shogaol) has 6.36%, (E)-1-(4-Hydroxy-3-methoxyphenyl) tetradec-3-en-5-one (10-Isoshogaol) has 0.93%, 1-(4-Hydroxy-3-methoxyphenyl) decane-3,5-dione (Gingerdione) has 2.00%, 1-(4-Hydroxy-3-methoxyphenyl) tetradecane-3,5-dione (10-Gingerdione) has 1.21%, 1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]- (Zingiberene) has 5.85% etc) found in the ginger as obtained during GC-MS analysis (Table 1).

Several natural spices and herbs have been developed into natural effective antimicrobial agents against many pathogenic microorganisms [47]. *Zingiber officinale* has been reported to have antifungal, antibacterial and antiviral activities [48, 49]. Different research works have shown the antimicrobial activities of ginger. A study has shown the antifungal activity of *Zingiber officinale* essential oil on *Fusarium verticillioides* and it reduces the biosynthesis of ergosterol; affecting membrane integrity; decreasing the production of fumonisin B1 and fumonisin B2. [50]. The

formation of biofilm is a factor that can cause infection and antimicrobial resistance. A study found that *Zingiber officinale* inhibited the growth of a multidrug-resistant strain of *Pseudomonas aeruginosa* by affecting membrane integrity and inhibiting biofilm formation [51]. Furthermore, treatment with ginger extract blocked biofilm formation via a reduction in the level of bis-(3'-3')-cyclic dimeric guanosine monophosphate (c-di-GMP) in *Pseudomonas aeruginosa* PA14 [52]. Methanolic fraction and crude extract of ginger inhibited biofilm formation, glucan synthesis, and the adherence of *Streptococcus mutans* by downregulating virulence genes. Consistent with the in-vitro study, a reduction in caries development caused by *Streptococcus mutans* was found in a treated group of rats [53]. Furthermore, an in vitro study revealed that gingerone-A and 6-shogaol found in ginger exhibited an inhibitory effect on *Staphylococcus aureus* by inhibiting the activity of 6-hydroxymethyl-7, 8-dihydropterin pyrophosphokinase (HPPK) in pathogen [54]. 6-Hydroxymethyl-7,8-dihydropterin pyrophosphokinase (HPPK) is an enzyme of the folic acid biosynthetic pathway that catalyzes the magnesium-dependent pyrophosphorylation of 6-hydroxymethyl-7,8-dihydropterin, utilising ATP to form 6-hydroxymethyl-7,8-dihydropterin pyrophosphate. The product formed can be used for design of inhibitors with a potential therapeutic value. *Zingiber officinale* essential oil exhibited inhibitory activity against *Fusarium verticillioides* with an MIC of 2500 µg/mL [50]. Ginger has been shown to be effective against the growth of both gram-negative and gram-positive bacteria like: *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus* [55].

In a study carried out by Samuel-Penu and Baridakara [56], it was observed that the aqueous extract of ginger (*Zingiber officinale*) did not inhibit any growth of bacteria both at 100 and 50% concentrations while the ethanolic extract of ginger inhibited and a zone diameter of 11 mm was recorded both for *Staphylococcus epidermidis* and *Bacillus sp.* at the 100% concentration. In another study, it was observed that the zone diameter for ethanolic extract of ginger rhizomes against *E.coli* varies from 8.50±0.12 to 15.50±0.30 at concentration between 25 to 200 µg/ml and 9.30±0.32 to 13.55±0.20 for *S. aureus* at the same concentration [57]. In a research work carried out by Njobdi1 et al. [58], it was observed that at a concentrations of 10, 20, 30 , and 40 mg/ml, the zones of inhibition of dried *Z. officinale* extracts on *S. aureus* were 11.00 ±1.41 mm, 13.5 ± 0.71 mm, 14.00±2.66 mm and 17.5 ± 0.87 mm respectively and on *E. coli* were 6.00 ± 2.83 mm, 7.5 ± 2.12 mm, 8.00 ± 2.83 mm and 14.5± 6.08 mm respectively. Fresh ginger showed 15.00±1.40 mm and 12.00±2.83 mm at 100% and 50% concentrations respectively on *S. aureus*

and 15.00 ± 3.54 mm and 13.00 ± 2.66 mm on *E. coli* respectively but has no effect at 25% and 12.5% concentration on both organisms. Gull et al. [59] study shows that the aqueous extract of ginger **inhibits the growth of** different pathogenic bacteria like: *E.coli*, *S. aureus*, *S.typhi*, *S. epidermidis*, *K. pneumonia*, *Shigella*, *B. subtilis*, and *P. aeruginos* with zone of inhibition ranging from 11 ± 0 to 13 ± 0.47 mm with concentration ranging from 25 to 200.

Amoxicillin/clavulanic acid, also known as co-amoxiclav or amox-clav, sold under the brand name augmentin, is an antibiotic medication used for the treatment of a number of bacterial infections. It is a combination consisting of amoxicillin, a β -lactam antibiotic, and potassium clavulanate, a β -lactamase inhibitor. It is specifically used for otitis media, urinary tract infections, streptococcal pharyngitis, pneumonia, cellulitis, and animal bites. Augmentin showed moderate response with zone of inhibition of 17.23 ± 1.67 against *Escherichia coli* and strong response with zone of inhibition of 21.13 ± 1.34 against *Staphylococcus aureus* at concentration of 7.50 mg/ml respectively. At 15 mg/ml, the drug (augmentin) showed strong response with zone of inhibition of 23.00 ± 2.88 against *E. coli* and potent response with zone of inhibition of 30.50 ± 2.64 against *S. aureus*. In a study consisting of 973 bacteria isolates, 823 were *E. coli* and 150 were *Klebsiella spp*. More of the organisms were found to be susceptible to amoxicillin-clavulanic acid than Ampicillin-sulbactam, regardless of the susceptibility testing methodology used in their study [60]. Njobdi1 et al. [58] study shows that Augmentin has a zone of inhibition of 21.00 ± 1.41 mm against *E. coli*. Gram negative bacteria (*Escherichia coli*) was more resistant than gram positive bacteria (*Staphylococcus aureus*), since they have lower zone of inhibition for both the aqueous ginger and augmentin solution as shown in Table 3. These variations in inhibition may be because of differences in the composition and structure surface between gram positive and gram negative bacteria. In addition to the cell wall and cell membrane, gram negative bacteria have an outer membrane composed of a phospholipid bilayer, which may be protective barrier against the ginger extract and augmentin solution used. Furthermore, the cell walls of gram positive bacteria have a large amount of peptidoglycan and a small amount of lipid, while in the case of gram negative bacteria, due to the presence of an outer membrane, a large amount of lipid and a small amount of peptidoglycan is found in the cell wall. In addition to that, gram negative bacteria have an additional outer membrane on their cell wall, the entry of secondary metabolites from ginger and the augmentin solution may be interrupted and its effects are lesser of the cell serious. However, gram positive bacteria lack the outer membrane

and therefore they are more susceptible to, easily entering of these compounds. The study shows that the Minimum inhibitory Concentration (MIC) values for the aqueous ginger for *Staphylococcus aureus* and *Escherichia coli* are 125 and 250 mg/ml and 7.81 and 15.63 mg/ml for augmentin solution for the same organisms respectively.

MIC's of ginger for *E. coli* (175 mg/ml), *Staphylococcus aureus* (125 mg/ml) and *Salmonella* (150 mg/ml) was observed in a study carried out by Virenda et al. [55]. Ponmurugan and Shyamkumar 2012 [57] study shows that ethanolic extract of ginger rhizomes has MICs of 75.60 and 68.45 against *E. coli* and *S. aureus* respectively. Gull et al. [59], study indicate that aqueous extract of ginger has an MICs of 0.1 and 0.6 mg/ml for *E.coli* and *S. aureus* respectively. MIC of dried and fresh *Z. officinale* extracts on *E. coli* and *S. aureus* isolates are both 2.5 mg/ml respectively [58].

5. CONCLUSION

This study shows that thirty six compounds were identified in the ginger (*Zingiber officinale* Roscoe) rhizome using GC-MS analysis with tridecane with molecular formula of $C_{13}H_{28}$ being the most abundant with peak area of 16.94% and retention time of 12.849. The preliminary phytochemical analysis of the extract of *Zingiber officinale* shows the presence of secondary metabolites like saponins, alkaloids, glycoside, Simple phenolics, tannins, flavonoids, carbohydrates and reducing sugar. The aqueous ginger extract has poor antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* due to the low concentration of the active compounds obtained during GC-MS analysis. This is an indication that the ginger extract has antibacterial potential.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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