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Antibacterial Effect of Ginger (*Zingiber officinale*) Roscoe and Bioactive Chemical Analysis using Gas Chromatography Mass Spectrum

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ABSTRACT

The objectives of this research was to determine the chemical composition of roscoe extract from methanol and evaluation of antibacterial activity. The phytochemical compound screened by GC-MS method. Forty eight bioactive phytochemical compounds were identified in the methanolic extract of *Zingiber officinale*. The identification of phytochemical compounds is based on the peak area, retention time molecular weight, molecular formula, MS Fragment-ions and pharmacological actions. GC-MS analysis of *Zingiber officinale* revealed the existence of the Octanal, 2-Naphthale namine,1,2,4a,5,6,7,8,8a-octahydro-4a-methyl, 1-(Cyclopropyl-nitro-methyl)-cyclopentanol, Endo-Borneol, Decanal, 1,2-15,16-Diepoxyhexadecane, Propanal,2-methyl-3-phenyl, Benzeneacetic acid ,4-(1H-1,2,3,4-tetrazol-1-yl), Ascaridole epoxide, 2-Methoxy-4-vinylphenol, 6-epi-shyobunol, Phenol,2-methoxy-5-(1-propenyl)-,E, Alfa.-Copaene, 8-Isopropenyl-1,5-dimethyl-cyclodeca-1,5-diene, Bicyclo[3.1.0]hexane-6-methanol,2-hydroxy-1,4,4-trimethyl, 7-epi-cis-sesquisabinene hydrate, Alloaromadendrene, Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl, 1,3-Cyclohexadiene ,5-(1,5-dimethyl-4-hexenyl)-2methyl-,[S-(R*,S*)], Aromadendrene oxide, 1,6,10-Dodecatrien-3-ol,3,7,11-trimethyl-,(E), 4-((1H)-3-Hydroxy-1-propenyl)-2-methoxyphenol, Butan-2-one,4-(3-hydroxy-2-methoxyphenyl), Longipinocarveol,trans, Cholestan-3-ol,2-methylene-,(3ß,5á)-, Bicyclo[4.4.0] dec-2-ene-4-ol,2-methyl-9-(prop-1-en-3-ol-2-yl)-, Corymbolone, Estra-1,3,5(10)-trien-17ß-ol, 1-Heptatriacotanol, Fenretinide, Folic acid, Spiro[4.5]decan-7-one,1,8-dimethyl-8,9-epoxy-4-isopropyl-, 7H-6,9a-Methano-4H-cyclopenta[9,10] cyclopropa[5,6]cyclodeca[1, Gingerol, 1b,4a-Epoxy-2H-cyclopenta[3,4]cyclopropa [8,9]cycloundec[1,2-b]o, Cyclopropa[5,6]-A-nor-5á-androstane-3,7-dione,3´,6ß-dihydro-17ß-h, Olean-12-ene-3,15,16,21,22,28-hexol,(3ß,15á,16á,21ß,22á)-, Benz[e]azulen-3(3aH)-one,4,6a,7,8,9,10,10a,10b-octahydro-3a,8,1, Naphthalene, decahydro-1-pentadecyl-, 13-Docosenamide,(Z)-, 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3ß,5Z,7E)-, n-(2,4-Dinitrophenyl)-N´-13-(2,6,6-trimethyl-cyclohex-1-enyl)propylider, n-(2,4-Dinitrophenyl)-N´-13-(2,6,6-trimethyl-cyclohex-1-enyl)propylider, Ingol 12-acetate, 2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetrae, Piperine, 2-Methylcortisol, 9-Desoxo-9-x-acetoxy-3,8,12,-tri-O-acetylingol and Propanoic acid ,2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl. Methanolic extract of bioactive compounds of *Zingiber officinale* was assayed for in vitro antibacterial activity against

Proteus mirabilis,Escherichia coli, *Pseudomonas aerogenosa, Proteus mirabilis, Staphylococcus aureus* and *Klebsiella pneumonia* by using the diffusion method in agar. The zone of inhibition were compared with different standard antibiotics. The diameters of inhibition zones ranged from 4.93±0.290 to 0.89±0.210 mm for all treatments.

Keywords: Antibacterial, GC/MS, Bioactive compounds, *Zingiber officinale*

INTRODUCTION

Ginger (*Zingiber officinale* Roscoe, fam. Zingiberaceae) is a perennial herb, slender perennial plant that reaches the height of two feet and has greenish yellow flowers resembling orchids. The rhizome is horizontal, branched, fleshy, aromatic, white or yellowish to brown. Leaves are narrowly or linear-lanceolate, up to 20 cm long and 1.5-2 cm wide. The dried rhizome of ginger contains approximately 1-4% of volatile oils which are the medicinally active constituents and are also responsible for the characteristic odour and taste. Flowers are produced in a dense spike, yellow green with purple endings. This plant is widely distributed in South-Eastern Asia[1]. 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[2]. Other pharmacological actions of ginger and compounds isolated from it include anti-inflammatory, antioxidant[3] ; hypoglycemic[4] ; analgesic, antiplatelet[5], antiemetic[6,7], antithrombotic, anti-tumorigenic, radio protective, antimicrobial, antifungal actions[8,9]. The major pungent compounds in ginger include potentially active gingerols, which can be converted to shogaols, zingerone, and paradol. 6-gingerol appears to be responsible for characteristic taste of ginger and together with 6-shogaol have been shown to have antipyretic, analgesic, anti-inflammatory, anti-tussive and hypotenssive effects[10,11]. Patients with chronic and painful diseases often seek alternative therapy, and currently ginger is one of the most popular herbal medications for inflammatory diseases[12]. In food industry, both pathogenic and food spoilage bacteria can attach and form a biofilm on food contact surfaces and food product, on the other hand *Z. officinale* is widely used as spice, so the aim of this study was ginger effectiveness in preventing this problem through the evaluation of antibacterial activity of methanolic extract of *Z. officinale*.

MATERIALS AND METHODS

Collection and preparation of plant material

The roscoe were dried at room temperature for ten days and when properly dried then powdered using clean pestle and mortar, and the powdered plant was size reduced with a sieve. The fine powder was then packed in airtight container to avoid the effect of humidity and then stored at room temperature[13,14].

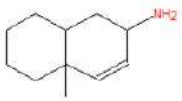
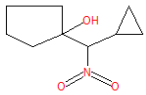
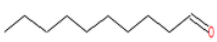
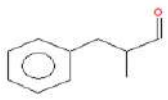
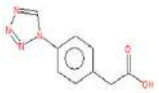
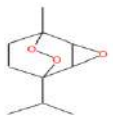
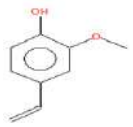
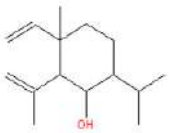
Preparation of sample

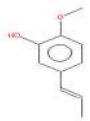
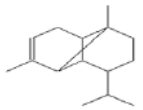
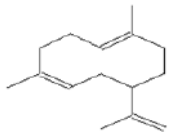
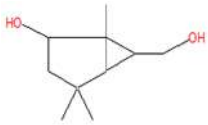
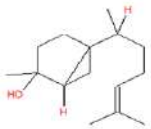
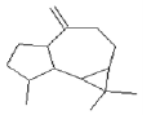
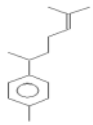
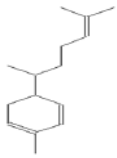
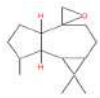
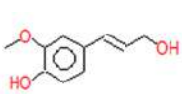
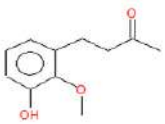
About seven grams of the plant sample powdered were soaked in 80 ml methanol individually. It was left for 72 hours so that alkaloids, flavonoids and other constituents if present will get dissolved. The methanol extract was filtered using Whatman No.1 filter paper and the residue was removed[15,16].

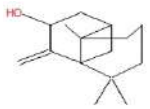
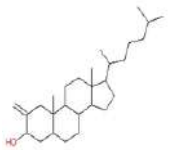
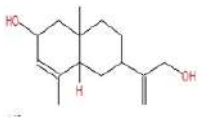
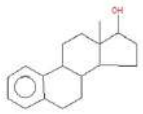
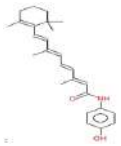
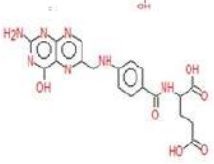
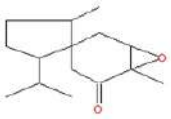
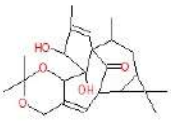
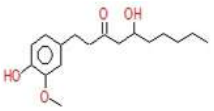
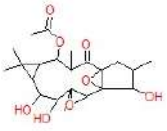
Gas chromatography – Mass Spectrum analysis

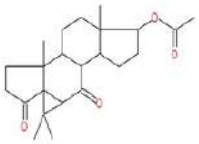
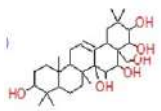
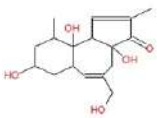
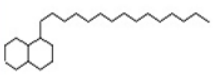
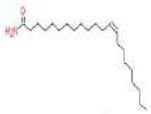
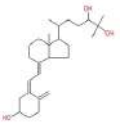
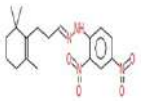
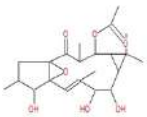
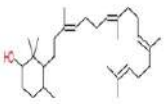
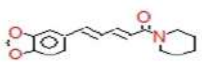
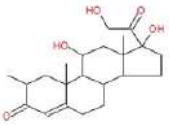
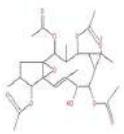
The GC-MS analysis of the plant extract was made in a (789 Agilent) instrument under computer control at 70 eV. About 1ìL of the methanol extract was injected into the GC-MS using a micro syringe and the scanning was done for 45 minutes[17,18]. As the compounds were separated, they eluted from the column and entered a detector which was capable of creating an electronic signal whenever a compound was detected. The greater the concentration in the sample, bigger was the signal obtained which was then processed by a computer. The time from when the injection was made (Initial time) to when elution occurred is referred to as the retention time (RT).While the instrument was run, the computer generated a graph from the signal called Chromatogram. Each of the peaks in the chromatogram represented the signal created when a compound eluted from the Gas chromatography column into the detector[19,20]. The x-axis showed the RT and the y-axis measured the intensity of the signal to quantify the component

Table 1: Bioactive Chemical Compounds Identified In Methanolic Extract of *Zingiber Officinale*

S. No.	Phytochemical compound	RT (min)	Formula	Molec. Weight	Exact Mass	Chemical structure	MS Fragment-ions	Pharmacological actions
1	Octanal	3.888	$C_{85}H_{16}O$	128	128.12		56,69, 84,100,128	Antioxidant activity and anti -inflammatory activities
2	2-Naphthalenamine, 1,2,4a,5,6,7,8,8a-octahydro-4a-methyl	4.987		165	165.152		55,67,81, 96,109,121, 135,150,165	Anti-inflammatory, analgestic and antimicrobial properties
3	1-(Cyclopropyl-nitro -methyl)-cyclopentanol	5.702		185	185.105		55,69,85, 95,121,139	Anti-muscarinic properties.
4	Endo-Borneol	5.982	$C_{10}H_{18}O$	154	154.136		55,67,79,95, 110,121, 139,152	Antinociceptive and anti-inflammatory activities
5	Decanal	6.417	$C_{10}H_{20}O$	156	156.151		57,70,82, 95,112,128, 138,155	Anti-Salmonella agents and antioxidant activity
6	1,2-15,16-Diepoxyhexadecane	6.697	$C_{16}H_{30}O2$	254	254.225		55,71,81, 95,178, 211,254	Antitumor and anti-inflammatory agents
7	Propanal,2-methyl-3-phenyl	6.932	$C_{10}H_{12}O$	148	148.089		51,63,77, 91,105,119, 133,148	Various biological activities such as anti-inflammatory
8	Benzeneacetic acid, 4-(1H-1,2,3,4-tetrazol-1-yl)	7.338		204	204.065		51,77,89, 104,131, 149,204	Antimicrobial
9	Ascaridole epoxide	7.75	$C_{10}H_{16}O_3$	184	184.11		55,69,79, 91,97,107, 117,135, 150,168	Anti-carcinogenic effects
10	2-Methoxy-4-vinylphenol	7.928	$C_9H_{10}O_2$	150	150.068		51,77,89, 107,121,135	Antioxidant, anti microbial and anti inflammatory.
11	6-epi-shyobunol	8.208	$C_{15}H_{26}O$	222	222.198		55,67,81,93, 109,121,136, 153,161, 189,207, 222	Anti-inflammatory, antinociceptive and antipyretic effects

12	Phenol,2-methoxy-5-(1-propenyl)-,E	8.414	$C_{10}H_{12}O_2$	164	164.084		55,65,77, 91,121, 131,149,164	Anti-plasmodial activity, cytotoxic effect, antipyretic, induce apoptosis
13	Alfa.-Copaene	8.717	$C_{15}H_{24}$	204	204.188		55,69,77,91, 105,119,133, 147,161, 175,189,204	Anti-Bacterial Agents
14	8-Isopropenyl-1, 5-dimethyl- cyclodeca-1, 5-diene	8.9	$C_{15}H_{24}$	204	204.188		53,68,81,	Anticancer, anti-inflammatory, gastro protective effects
15	Bicyclo[3.1.0] hexane-6-methanol, 2-hydroxy-1,4, 4-trimethyl	9.055	$C_{10}H_{18}O_2$	170	170.131		55,67,81, 95,139, 152,170	Anti-Candida and anti-inflammatory
16	7-epi-cis-sesquisabinene hydrate	9.335	$C_{15}H_{26}O$	222	222.198		55,69,82,93, 105,119,133, 147,161,175, 189,204,222	Anti-cancer
17	Alloaromadendrene	9.845	$C_{15}H_{24}$	204	204.188		55,69,79,91, 105,119,133, 147,161, 175,189,204	Anti-Inflammatory and antinociceptive effects
18	Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl	10.037	$C_{15}H_{22}$	202	202.172		55,65,69,77, 83,91,95,105, 119,132,145, 159,187,202	Antimicrobial and anti-inflammatory.
19	1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2methyl -,[S-(R*,S*)]	10.228	$C_{15}H_{24}$	204	204.188		56,69,77, 93,105,119, 133,161,204	Antioxidant, *anti*-inflammatory and antinoci-ceptive activities
20	Aromadendrene oxide	10.869	$C_{15}H_{24}O$	220	220.183		55,81,91,133, 147,177,189, 205,220	Anti HIV5,6, antifungal and antimicrobial
21	1,6,10-Dodecatrien-3-ol,3,7, 11-trimethyl-,(E)	10.972	$C_{15}H_{26}O$	222	222.198		55,69,81,93, 107,123,136, 148,161,189, 204	Antidiabetic, hepatoprotective and anti-inflammatory activities
22	4-((1H)-3-Hydroxy-1-propenyl)-2 -methoxyphenol	11.224	$C_{10}H_{12}O_3$	180	180.079		51,65,77,91, 103,124,131, 137,147, 163,180	Antioxidant, anti microbial and anti inflammatory.
23	Butan-2-one, 4-(3-hydroxy-2-methoxyphenyl)-	12.248	$C_{11}H_{14}O_3$	194	194.094		51,65,77,91, 107,119,137, 151,161, 179,194	Anti-inflammatory, antidiabetic, antilipolytic, antidiarrhoeic

24	Longipinocarveol, trans	12.631	$C_{15}H_{24}O$	220	220.183		55,69,81,95, 109,118,133, 159,177,187, 202,220	Antidepressant, anti-malarial, anticonvulsant and antioxidant
25	Cholestan-3-ol, 2-methylene-, (3ß,5±)-	12.837	$C_{28}H_{48}O$	400	400.371		69,81,95, 121,203, 245,315,400	Activities such as anti-inflammatory and cytotoxic activities
26	Bicyclo[4.4.0] dec-2-ene-4-ol, 2-methyl-9-(prop-1-en-3-ol-2-yl)-	13.427	$C_{15}H_{24}O_2$	236	236.178		55,69,91, 119,143,157, 185,203,236	Local anesthetic and anti-inflammatory
27	Corymbolone	14.268	$C_{15}H_{24}O_2$	236	236.178		55,69,93,109, 135,203,218	Anti-fungal agent
28	Estra-1,3,5(10) -trien-17ß-ol	15.24	$C_{18}H_{24}O$	256	256.183		57,73,85,97, 129,157,185, 213,241,256	Antioxidant, anti-inflammatory , antifungal and antibacterial
29	1-Heptatriacotanol	15.166	$C_{37}H_{76}O$	536	536.59		55,81,95,107, 121,133,147, 161,190,201, 229,244,257	Antioxidant, anticancer, anti inflammatory and to sexhormone activity
30	Fenretinide	16.059	$C_{26}H_{33}NO_2$	391	391.251		58,69,81,95, 109,135,161, 202,255,391	Anti-tumor
31	Folic acid	15.675		441	441.14		65,84,93,120, 137,177,202, 263,278,310, 364	Increased content of glutathione and glutathione peroxidase activity
32	Spiro[4.5]decan-7-one,1,8-dimethyl -8,9-epoxy-4-isopropyl	15.034	$C_{15}H_{24}O_2$	236	236.178		55,69,81,95, 109,123,137, 151,193,208	Anti-inflammatory activity
33	7H-6,9a-Methano -4H-cyclopenta [9,10]cyclopropa [5,6]cyclodeca[1,	17.495	$C_{23}H_{32}O_5$	388	388.225		55,77,91,121, 136,162,190, 237,266,284, 330,370	Unknown
34	Gingerol	18.799	$C_{17}H_{26}O_4$	294	294.183		55,77,91,119, 137,150, 194,205,294	Biological activities including anti-inflammatory and anti-oxidative
35	1b,4a-Epoxy-2H-cyclopenta[3,4] cyclopropa[8,9] cycloundec[1,2-b]o	18.897	$C_{22}H_{32}O_8$	424	424.21		69,97,109,123, 137,152,165, 181,237,261, 295,317,346	Unknown

36	Cyclopropa[5,6] -A-nor-5±- androstane-3,7 -dione,3´,6ß-dihydro -17ß-h	19.795	$C_{23}H_{32}O_4$	424	424.21		55,97,135, 177,216,276, 316,344,372	Unknown
37	Olean-12-ene-3, 15,16,21,22,28 -hexol,(3ß,15±, 16±,21ß,22±)-	20.533	$C_{30}H_{50}O_6$	506	506.361		#######	Anti-tumourogenic properties
38	Benz[e]azulen -3(3aH)-one,4,6a,7, 8,9,10,10a,10b -octahydro-3a,8,1	20.768	$C_{17}H_{24}O_5$	308	308.162		53,79,109, 159,181,209, 244,272, 290,308	Unknown
39	Naphthalene, decahydro-1 -pentadecyl-	22.095	$C_{25}H_{48}$	348	348.376		55,67,81,109, 137,171,194, 219,243,266, 289,317,348	Antioxidant activity and Anti bacterial Activity
40	13-Docosenamide, (Z)	22.175	$C_{22}H_{43}NO$	337	337.334		59,72,83,112, 126,184,240, 294,320,337	Antinociceptive and anti-inflammatory activities
41	9,10-Secocholesta -5,7,10(19)-triene-3, 24,25-triol, (3ß,5Z,7E)-	22.811	$C_{27}H_{44}O_3$	416	416.329		55,69,118, 136,158,207, 253,383,416	Unknown
42	n-(2,4-Dinitrophenyl)- N´-13-(2,6,6-trimethyl -cyclohex-1-enyl) propylider	22.862		360	360.18		69,95,121,137, 163,177,224, 296,343,360	Unknown
43	Ingol 12-acetate	22.914	$C_{22}H_{32}O_7$	408	408.215		69,95,121, 137,163,177, 224,296, 343,360	Anti-inflammatory activity
44	2,2,4-Trimethyl- 3-(3,8,12,16- tetramethyl- heptadeca-3, 7,11,15-tetrae	23.554	$C_{30}H_{52}O$	428	428.402		55,69,81,95, 175,271,341, 428	Anti-inflammatory activity
45	Piperine	23.629	$C_{17}H_{19}NO_3$	285	285.136		63,84,115,143, 173,201,256, 285	Apoptotic, anti-pyretic and analgesic activities
46	2-Methylcortisol	24.195	$C_{22}H_{32}O_5$	376	376.225		55,91,135,161, 177,241,274, 299,317, 358,376	Anti-Inflammatory
47	9-Desoxo- 9-x-acetoxy-3, 8,12,-tri- O-acetylingol	25.242	$C_{28}H_{40}O_{10}$	536	536.262		55,69,122, 207,236,297, 357,417, 477	Anti-inflammatory effects

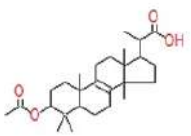
48	Propanoic acid ,2-(3-acetoxy -4,4,14-trimethy-landrost-8-en-17-yl	25.986	$C_{27}H_{42}O_4$	430	430.308		55,69,121,159, 233,281,337, 355,415	Antihyperglycem, hypolipidemic and anti-microbial.

Table 2: Zone Of Inhibition (Mm) Of Test Bacterial Strains To *Zingiber Officinale* Bioactive Compounds And Standard Antibiotics

Bacteria	Plant (*Zingiber officinale*) / *Zingiber officinale*	Antibiotics Streptomycin	Rifambin	Cefotoxime
Pseudomonas eurogenosa	4.01±0.188	0.92±0.210	1.04±0.300	1.183±0.100
Escherichia coli	2.99±0.311	1.500±0.141	0.99±0.240	2.20±0.170
Klebsiella pneumonia	4.93±0.290	2.02±0.361	1.05±0.161	0.93±0.150
Staphylococcus aureus	3.75±0.910	1.00±0.102	2.00±0.140	1.00±0.301
Proteus mirabilis	1.99±0.200	2.00±0.180	2.06±0.300	0.89±0.210

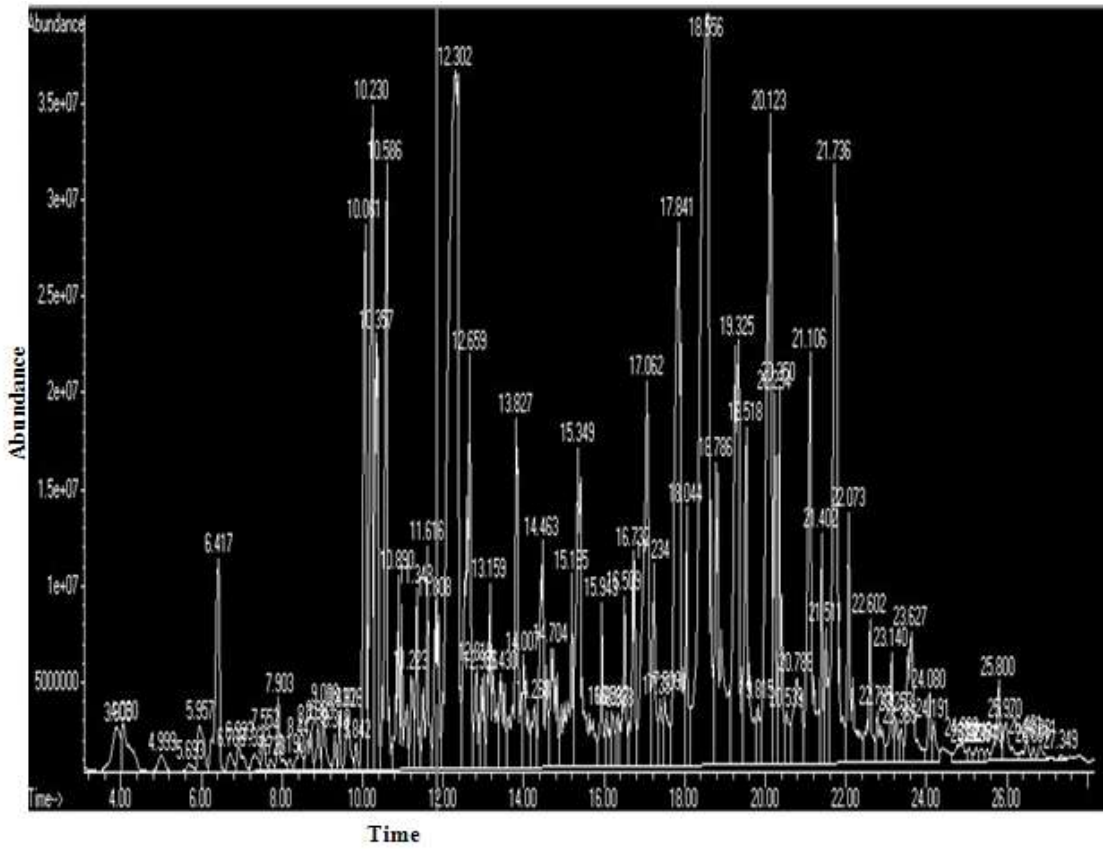


Fig. 1: GC-MS chromatogram of methanolic extract of *Zingiber officinale*

in the sample injected. As individual compounds eluted from the gas chromatographic column, they entered the electron ionization (mass spectroscopy) detector, where they were bombarded with a stream of electrons causing them to break apart into fragments. The fragments obtained were actually charged ions with a certain mass[21]. The M/Z (mass / charge) ratio obtained was calibrated from the graph obtained, which was called as the Mass spectrum graph which is the fingerprint of a molecule. Before analyzing the extract using gas Chromatography and Mass Spectroscopy, the temperature of the oven, the

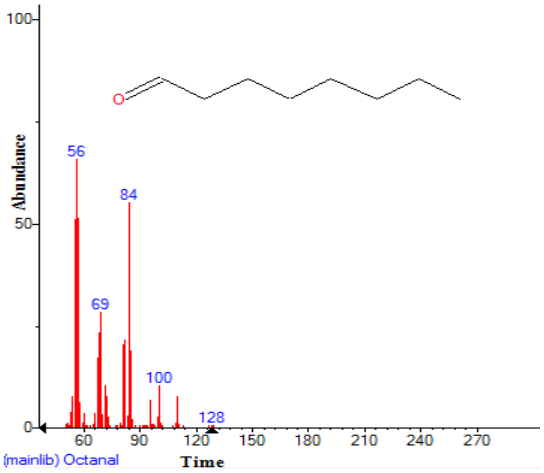


Fig. 2: Structure of Octanal present in *Zingiber officinale* with retention time=3.888 using GC-MS analysis

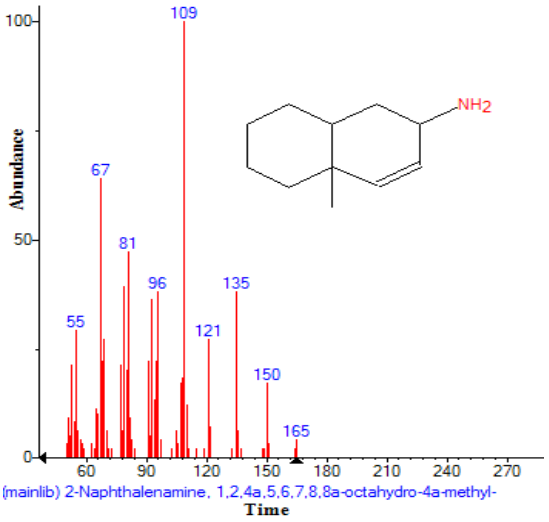


Fig. 3: Structure of 2-Naphthalenamine, 1,2,4a,5,6,7,8,8a-octahydro-4a-methyl present in *Zingiber officinale* with retention time= 4.987 using GC-MS analysis

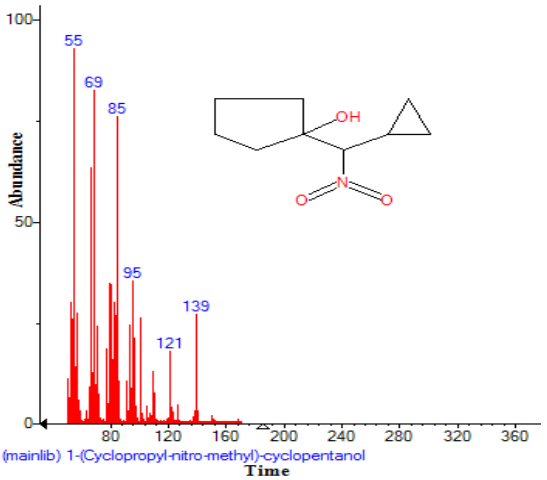


Fig. 4: Structure of 1-(Cyclopropyl-nitro-methyl)-cyclopentanol present in *Zingiber officinale* with retention time= 5.702 using GC-MS analysis

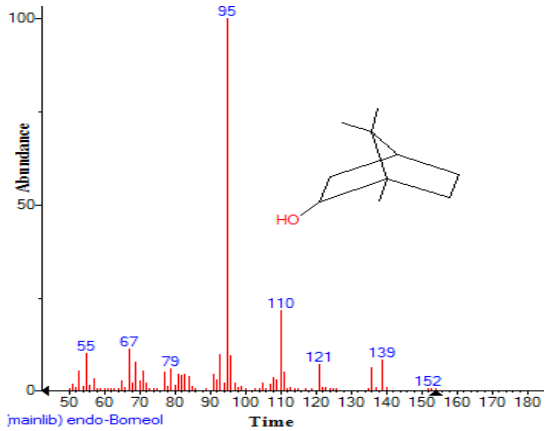


Fig. 5: Structure of Endo-Borneol present in *Zingiber officinale* with retention time= 5,982 using GC-MS analysis

flow rate of the gas used and the electron gun were programmed initially. The temperature of the oven was maintained at 100°C. Helium gas was used as a carrier as well as an eluent. The flow rate of helium was set to 1ml per minute. The electron gun of mass detector liberated electrons having energy of about 70eV.The column employed here for the separation of components was Elite 1(100% dimethyl poly siloxane). The identity of the components in the extracts was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library

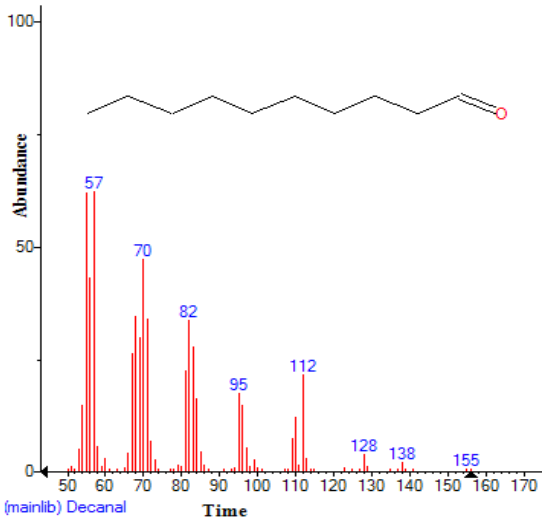


Fig. 6: Structure of Decanal present in *Zingiber officinale* with retention time= 6.417 using GC-MS analysis

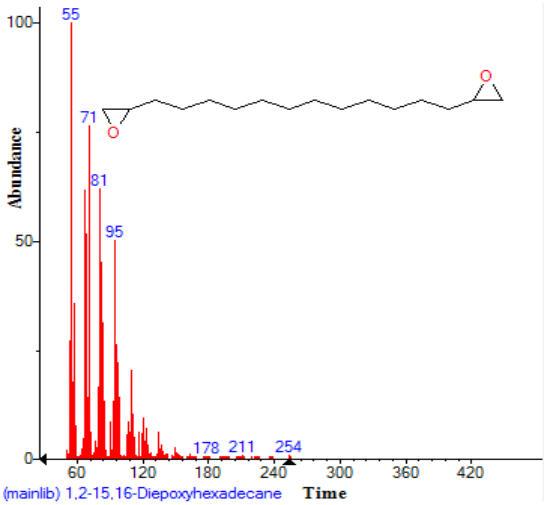


Fig. 7: Structure of 1,2-15,16-Diepoxyhexadecane present in *Zingiber officinale* with retention time= 6.697 using GC-MS analysis

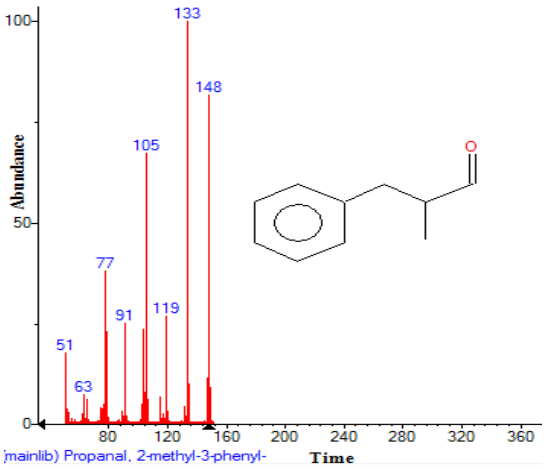


Fig. 8: Structure of Propanal,2-methyl-3-phenyl present in *Zingiber officinale* with retention time= 6.932 using GC-MS analysis

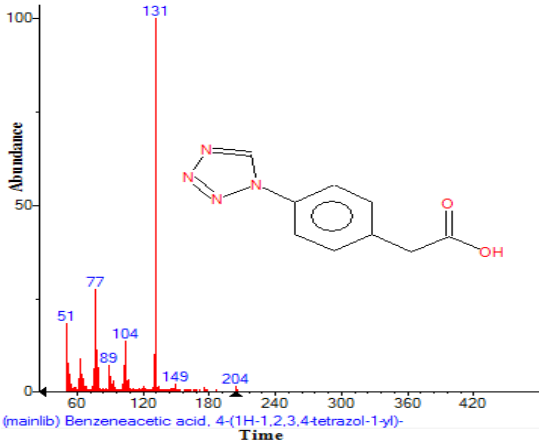


Fig. 9: Structure of Benzeneacetic acid ,4-(1H-1,2,3,4-tetrazol-1-yl) present in *Zingiber officinale* with retention time= 7.338 using GC-MS analysis

and also with published literatures. Compounds were identified by comparing their spectra to those of the Wiley and NIST/EPA/NIH mass spectral libraries[22].

Determination of antibacterial activity of crude bioactive compounds of *Zingiber officinale*

The test pathogens (*Klebsiella pneumoniae, Pseudomonas aeruginosa, Klebsiella pneumoniae, E. coli,* and *Staphylococcus aureus)* were swabbed in Muller-Hinton agar plates. 60ìl of plant extract was loaded on the bored wells. The wells were bored in 0.5cm in diameter[24]. The plates were incubated at 37C° for 24 hrs and examined. After the incubation the diameter of inhibition zones around the discs was measured.

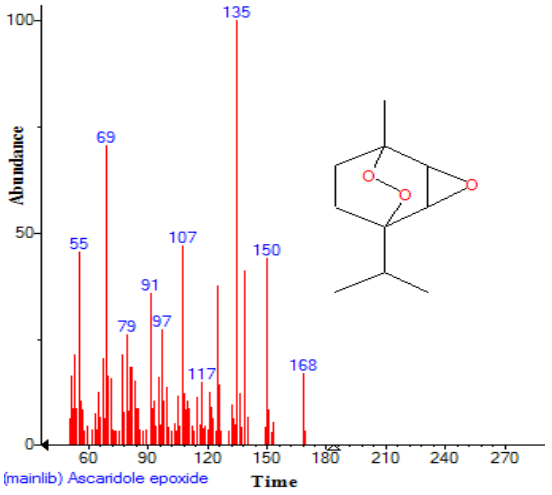


Fig. 10: Structure of Ascaridole epoxide present in *Zingiber officinale* with retention time= 7.750 using GC-MS analysis

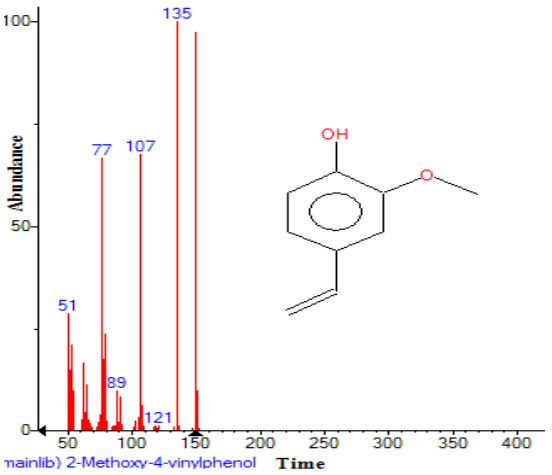


Fig. 11: Structure of 2-Methoxy-4-vinylphenol present in *Zingiber officinale* with retention time= 7.928 using GC-MS analysis

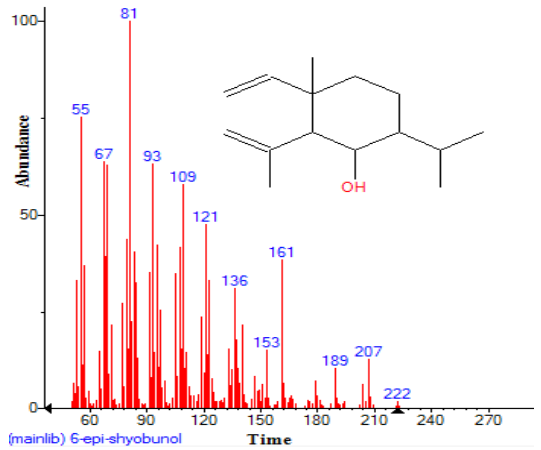


Fig. 12: Structure of 6-epi-shyobunol present in *Zingiber officinale* with retention time= 8.208 using GC-MS analysis

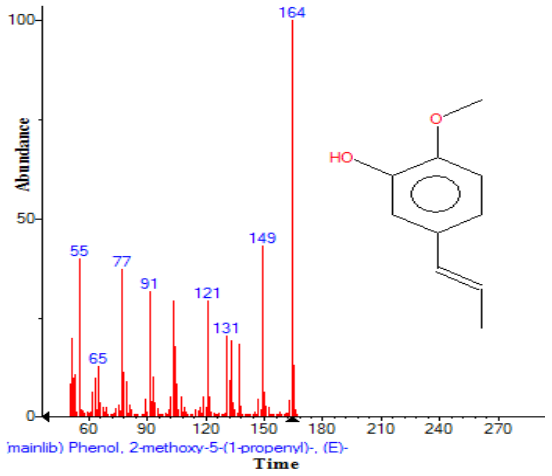


Fig. 13: Structure of Phenol,2-methoxy-5-(1-propenyl)-,E present in *Zingiber officinale* with retention time= 8.414 using GC-MS analysis

RESULTS AND DISCUSSION

Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic roscoe extract of *Zingiber officinale*, shown in Table 1. The GC-MS chromatogram of the 48 peaks of the compounds detected was shown in Figure 1. Chromatogram GC-MS analysis of the methanol extract of *Zingiber officinale* showed the presence of forty eight major peaks and the

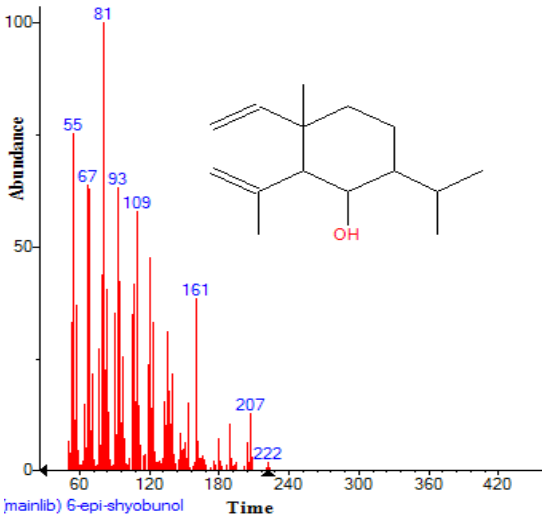


Fig. 14: Structure of 6-epi-shyobunol present in *Zingiber officinale* with retention time= 8.614 using GC-MS analysis

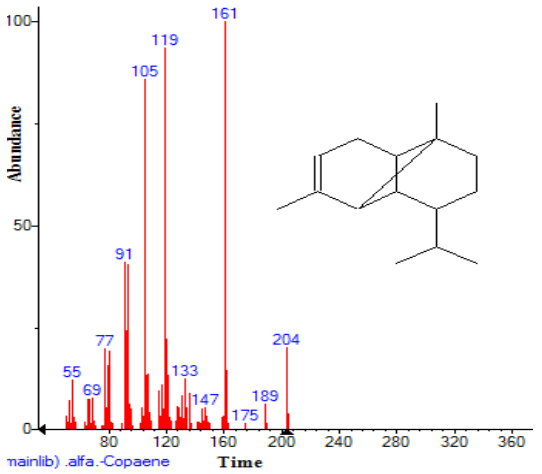


Fig. 15: Structure of Alfa.-Copaene present in *Zingiber officinale* with retention time= 8.717 using GC-MS analysis

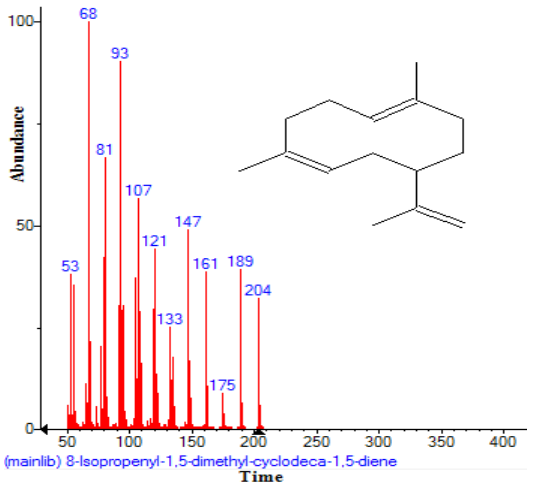


Fig. 16: Structure of 8-Isopropenyl-1,5-dimethyl-cyclodeca-1,5-diene present in *Zingiber officinale* with retention time= 8.900 using GC-MS analysis

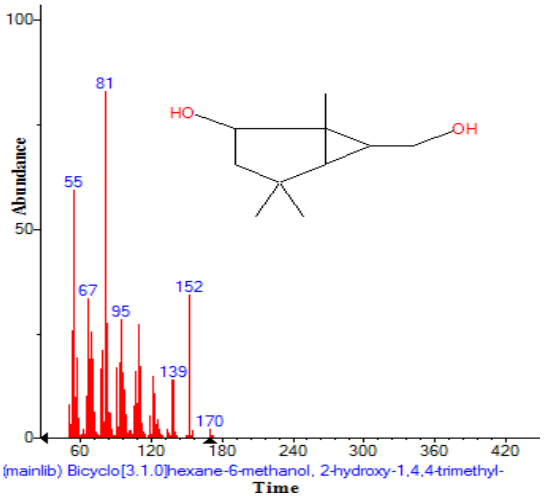


Fig. 17: Structure of Bicyclo[3.1.0]hexane-6-methanol,2-hydroxy-1,4,4-trimethyl present in *Zingiber officinale* with retention time= 9.055 using GC-MS analysis

components corresponding to the peaks were determined as follows. The First set up peak were determined to be Octanal Figure 2. The second peak indicated to be, 2-Naphthalenamine,1,2,4 a,5,6,7,8,8a-octahydro-4a-methyl, Figure 3. The next peaks considered to be 1-(Cyclopropyl-nitro-methyl)-cyclopentanol, Endo-Borneol, Decanal, 1,2-15,16-Diepoxyhexadecane, Propanal,2-methyl-3-phenyl, Benzeneacetic acid ,4-(1H-1,2,3,4-tetrazol-1-yl), Ascaridole epoxide, 2-Methoxy-4-vinylphenol, 6-epi-shyobunol, Phenol,2-methoxy-5-(1-propenyl)-,E, Alfa.-Copaene, 8-Isopropenyl-1,5-dimethyl-

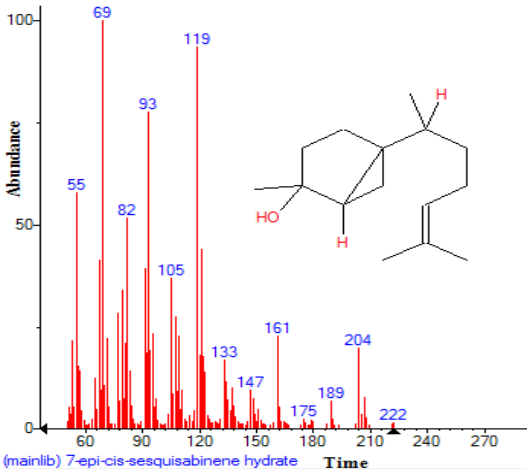


Fig. 18: Structure of 7-epi-cis-sesquisabinene hydrate present in *Zingiber officinale* with retention time= 9.335 using GC-MS analysis

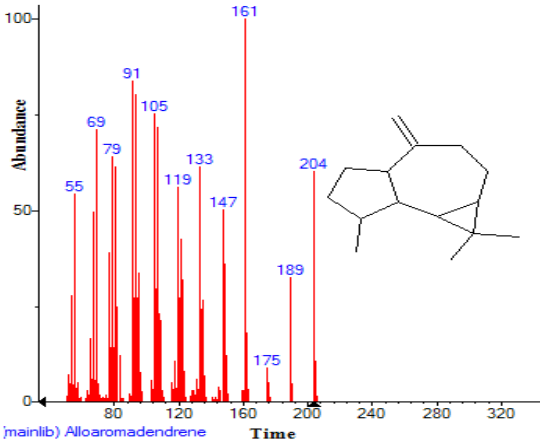


Fig. 19: Structure of Alloaromadendrene present in *Zingiber officinale* with retention time= 9.845 using GC-MS analysis

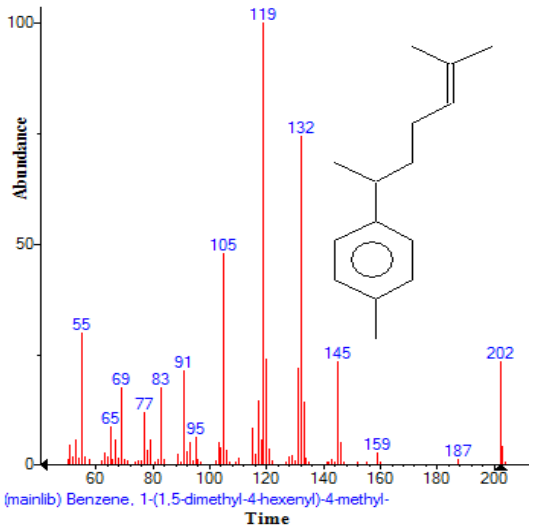


Fig. 20: Structure of Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl present in *Zingiber officinale* with retention time= 10.037 using GC-MS analysis

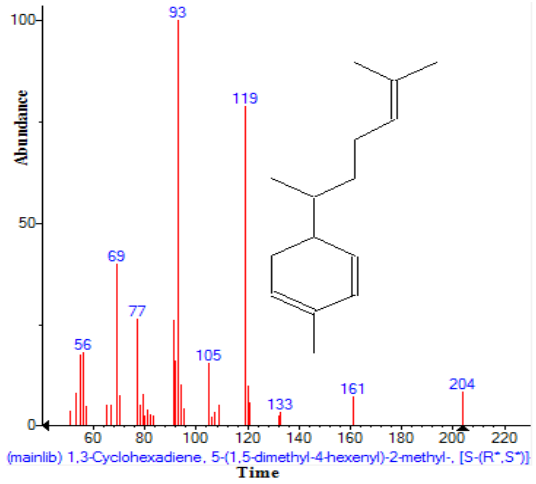


Fig. 21: Structure of 1,3-Cyclohexadiene ,5-(1,5-dimethyl-4-hexenyl)-2methyl-,[S-(R*,S*)] present in *Zingiber officinale* with retention time= 10.228 using GC-MS analysis

cyclodeca-1,5-diene, Bicyclo[3.1.0]hexane-6-methanol,2-hydroxy-1,4,4-trimethyl, 7-epi-cis-sesquisabinene hydrate, Alloaromadendrene, Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl, 1,3-Cyclohexadiene ,5-(1,5-dimethyl-4-hexenyl)-2methyl-,[S-(R*,S*)], Aromadendrene oxide, 1,6,10-Dodecatrien-3-ol,3,7,11-trimethyl-,(E), 4-((1H)-3-Hydroxy-1-propenyl)-2-methoxyphenol, Butan-2-one,4-(3-hydroxy-2-methoxyphenyl), Longipinocarveol,trans, Cholestan-3-ol,2-methylene-

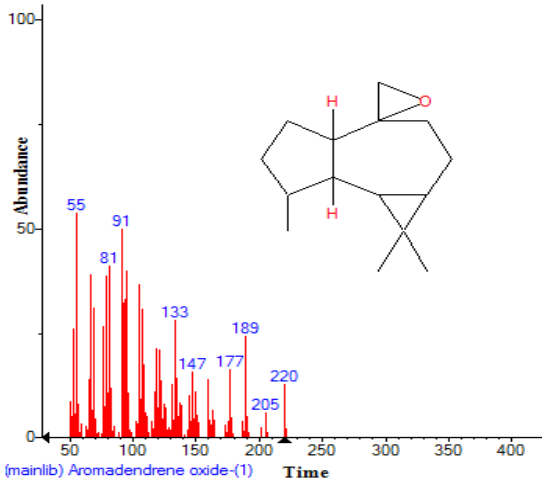


Fig. 22: Structure of Aromadendrene oxide present in *Zingiber officinale* with retention time= 10.869 using GC-MS analysis

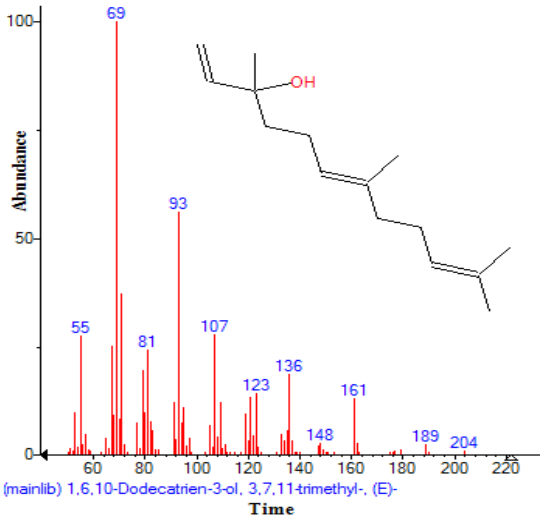


Fig. 23: Structure of 1,6,10-Dodecatrien-3-ol,3,7,11-trimethyl-,(E) present in *Zingiber officinale* with retention time= 10.972 using GC-MS analysis

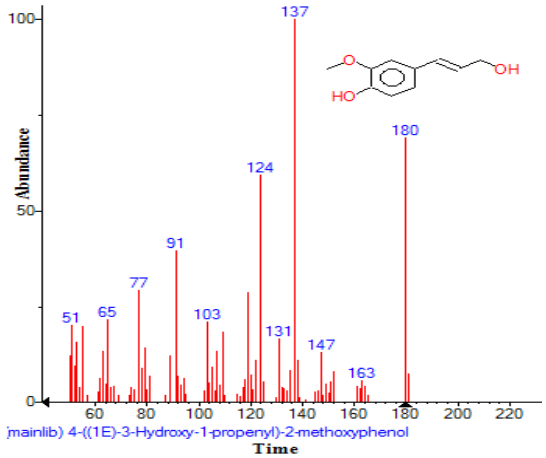


Fig. 24: Structure of 4-((1H)-3-Hydroxy-1-propenyl)-2-methoxyphenol present in *Zingiber officinale* with retention time= 11.224 using GC-MS analysis

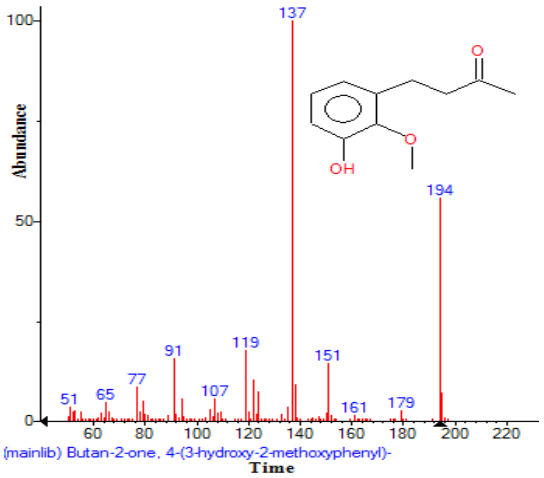


Fig. 25: Structure of Butan-2-one,4-(3-hydroxy-2-methoxyphenyl) present in *Zingiber officinale* with retention time= 12.248 using GC-MS analysis

,(3ß,5á)-, Bicyclo[4.4.0]dec-2-ene-4-ol,2-methyl-9-(prop-1-en-3-ol-2-yl)-, Corymbolone, Estra-1,3,5(10)-trien-17ß-ol, 1-Heptatriacotanol, Fenretinide, Folic acid, Spiro[4.5]decan-7-one,1,8-dimethyl-8,9-epoxy-4-isopropyl-, 7H-6,9a-Methano-4H-cyclopenta[9,10] cyclopropa[5,6]cyclodeca[1, Gingerol, 1b,4a-Epoxy-2H-cyclopenta[3,4]cyclopropa [8,9]cycloundec[1,2-b] o, Cyclopropa[5,6]-A-nor-5á-androstane-3,7-

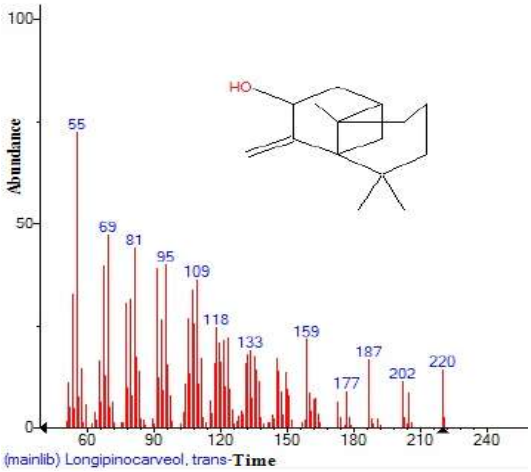


Fig. 26: Structure of Longipinocarveol,trans present in *Zingiber officinale* with retention time= 12.631 using GC-MS analysis

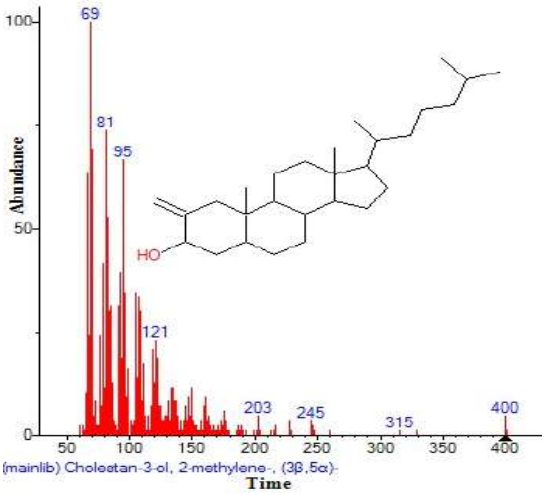


Fig. 27: Structure of Cholestan-3-ol,2-methylene-,(3ß,5α) present in *Zingiber officinale* with retention time= 12.837 using GC-MS analysis

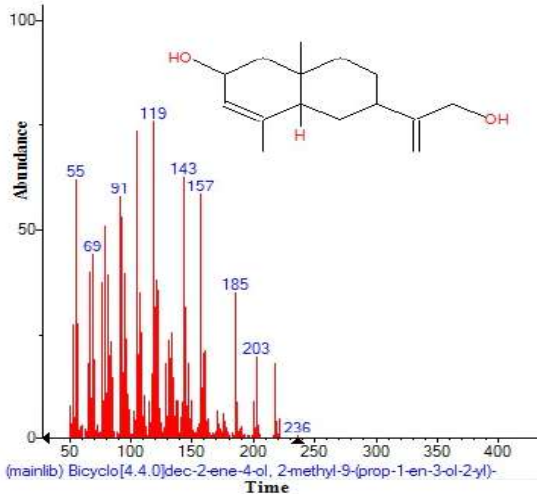


Fig. 28: Structure of Bicyclo[4.4.0]dec-2-ene-4-ol,2-methyl-9-(prop-1-en-3-ol-2-yl) present in *Zingiber officinale* with retention time= 13.427 using GC-MS analysis

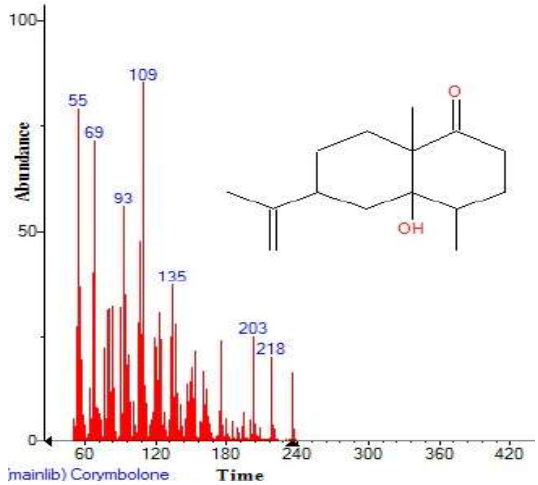


Fig. 29: Structure of Corymbolone present in *Zingiber officinale* with retention time= 14.268 using GC-MS analysis

dione,3´,6ß-dihydro-17ß-h, Olean-12-ene-3,15,16,21,22,28-hexol,(3ß,15á,16á,21ß,22á)-, Benz[e]azulen-3(3aH)-one,4,6a,7,8,9,10,10a,10b-octahydro-3a,8,1, Naphthalene, decahydro-1-pentadecyl-, 13-Docosenamide,(Z)-, 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3ß,5Z,7E)-, n-(2,4-Dinitrophenyl)-N´-13-(2,6,6-trimethyl-cyclohex-1-enyl)propylider, n-(2,4-Dinitrophenyl)-N´-13-(2,6,6-trimethyl-cyclohex-1-enyl)propylider, Ingol 12-acetate, 2,2,4-Trimethyl-3-

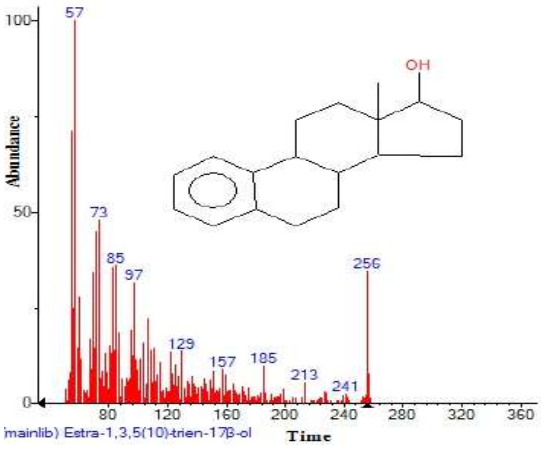


Fig. 30: Structure of Estra-1,3,5(10)-trien-17ß-ol present in *Zingiber officinale* with retention time= 15.240 using GC-MS analysis

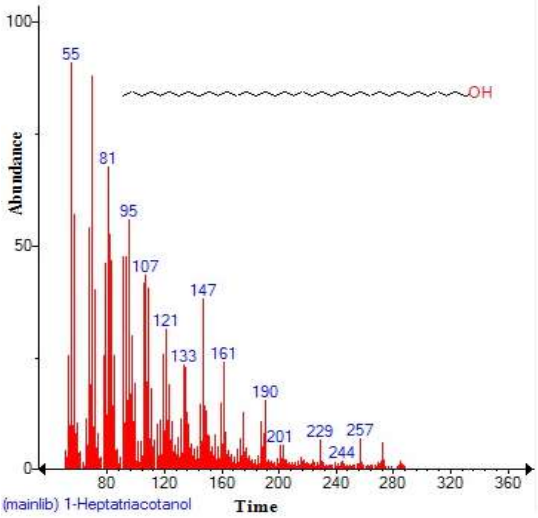


Fig. 31: Structure of 1-Heptatriacotanol present in *Zingiber officinale* with retention time= 15.166 using GC-MS analysis

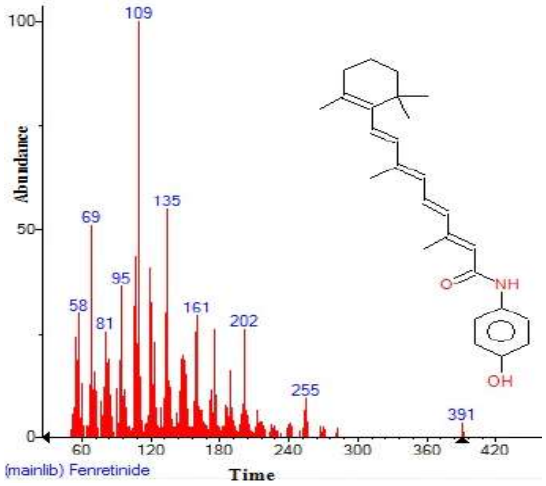


Fig. 32: Structure of Fenretinide present in *Zingiber officinale* with retention time= 16.059 using GC-MS analysis

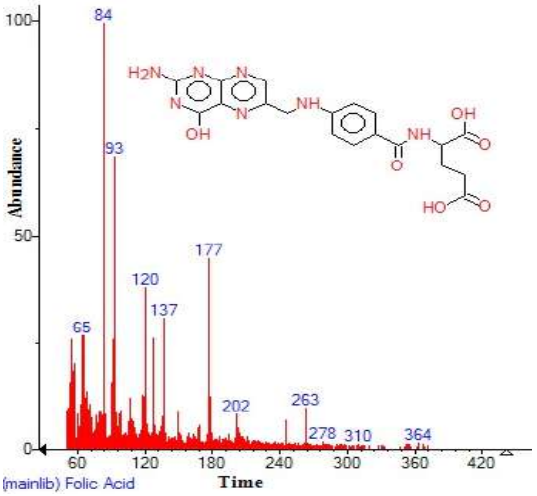


Fig. 33: Structure of Folic acid present in *Zingiber officinale* with retention time= 15.675 using GC-MS analysis

(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetrae, Piperine, 2-Methylcortisol, 9-Desoxo-9-x-acetoxy-3,8,12,-tri-O-acetylingol and Propanoic acid ,2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl. (Figure 4-50). In this study five clinical pathogens selected for antibacterial activity namely, (*staphylococcus aeureus, klebsiella pneumoniae, pseudomonas aeroginosa, E.coli.* and *Proteus mirabilis*. Maximum zone formation against *Klebsiella pneumoniae,* Table 2.

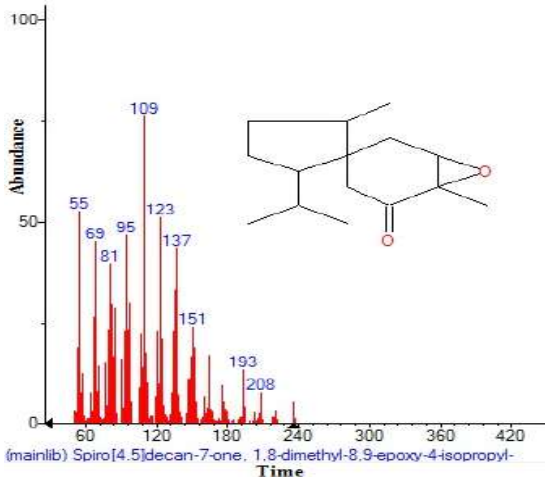


Fig. 34: Structure of Spiro[4.5]decan-7-one,1,8-dimethyl-8,9-epoxy-4-isopropyl present in *Zingiber officinale* with retention time= 15.034 using GC-MS analysis

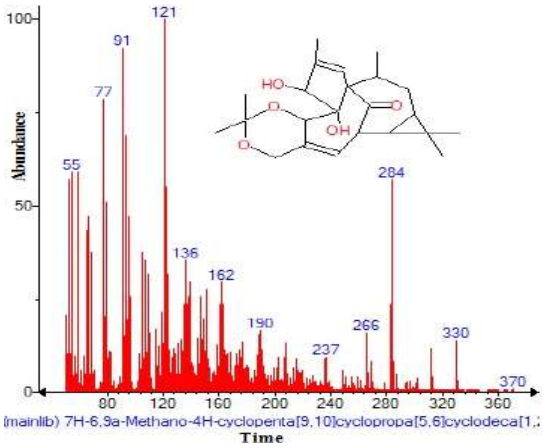


Fig. 35: Structure of 7H-6,9a-Methano-4H-cyclopenta[9,10]cyclopropa[5,6]cyclodeca[1, present in *Zingiber officinale* with retention time= 17.495 using GC-MS analysis

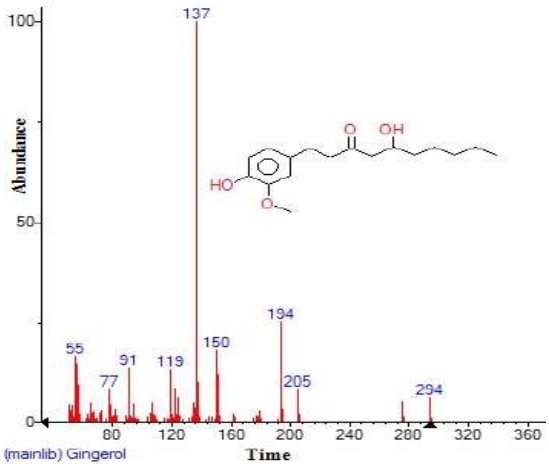


Fig. 36: Structure of Gingerol present in *Zingiber officinale* with retention time= 18.799 using GC-MS analysis

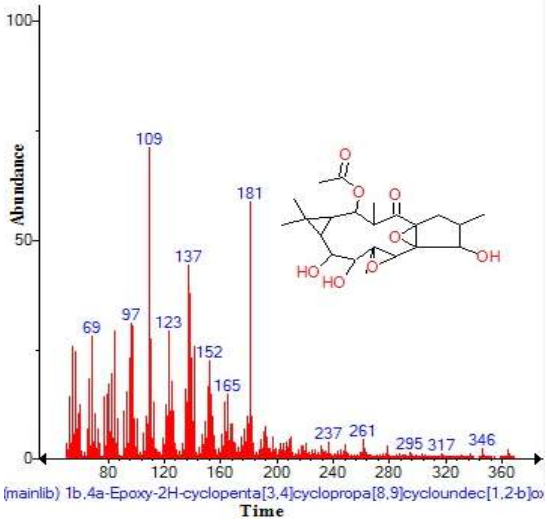


Fig. 37: Structure of 1b,4a-Epoxy-2H-cyclopenta[3,4]cyclopropa[8,9] cycloundec[1,2-b]o present in *Zingiber officinale* with retention time= 18.897 using GC-MS analysis

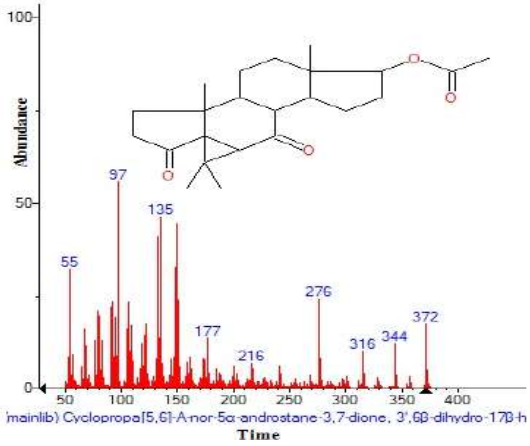


Fig. 38: Structure of Cyclopropa[5,6]-A-nor-5á-androstane-3,7-dione,3´,6ß-dihydro-17ß-h present in *Zingiber officinale* with retention time= 19.795 using GC-MS analysis

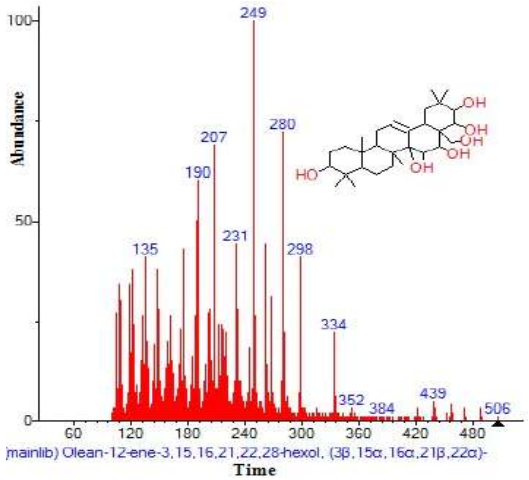


Fig. 39: Structure of Olean-12-ene-3,15,16,21,22,28-hexol,(3ß,15á,16á,21ß,22á) present in *Zingiber officinale* with retention time= 20.533 using GC-MS analysis

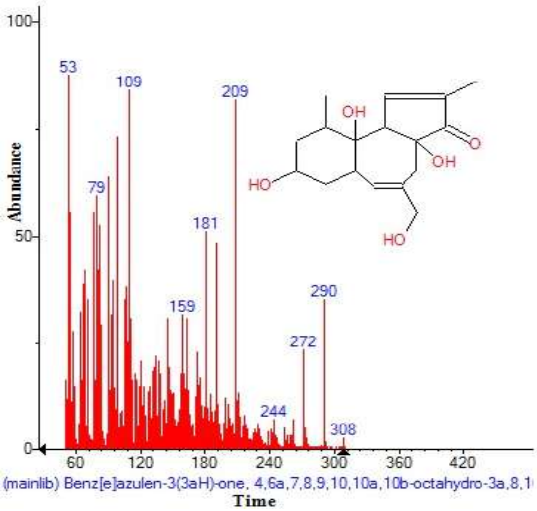


Fig. 40: Structure of Benz[e]azulen-3(3aH)-one,4,6a,7,8,9,10,10a,10b-octahydro-3a,8,1 present in *Zingiber officinale* with retention time= 20.768 using GC-MS analysis

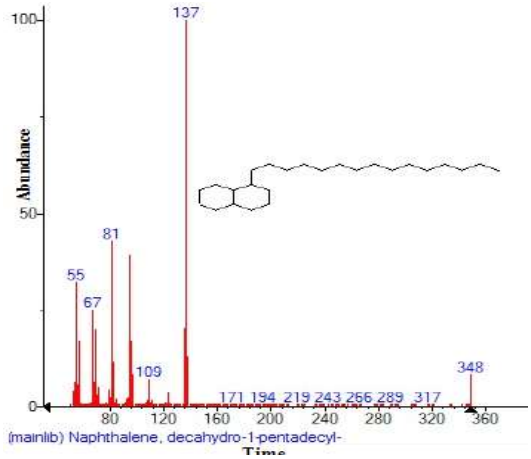


Fig. 41: Structure of Naphthalene,decahydro-1-pentadecyl present in *Zingiber officinale* with retention time= 22.095 using GC-MS analysis

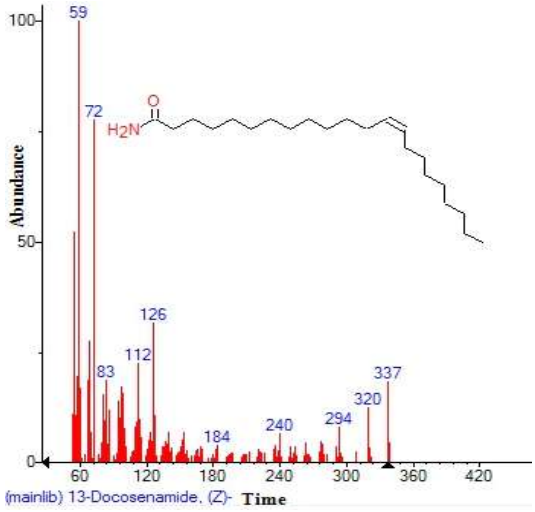


Fig. 42: Structure of 13-Docosenamide,(Z) present in *Zingiber officinale* with retention time= 22.175 using GC-MS analysis

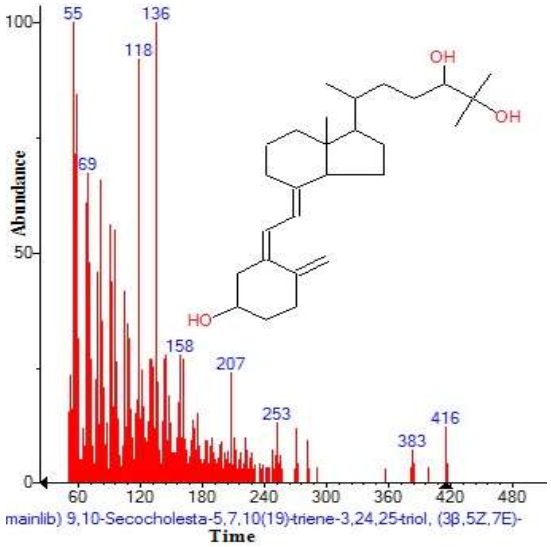


Fig. 43: Structure of 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3ß,5Z,7E) present in *Zingiber officinale* with retention time= 22.811 using GC-MS analysis

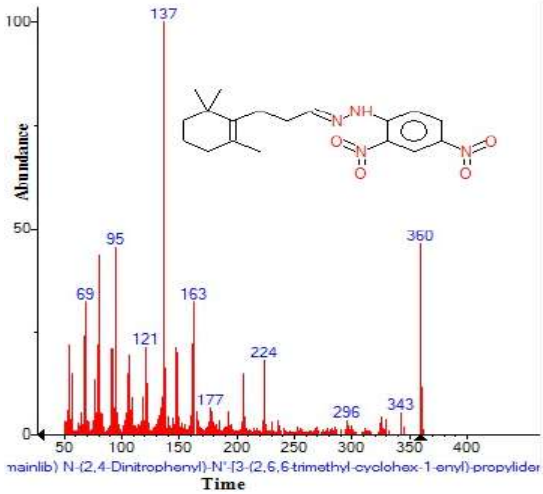


Fig. 44: Structure of n-(2,4-Dinitrophenyl)-N´-13-(2,6,6-trimethyl-cyclohex-1-enyl)propylider present in *Zingiber officinale* with retention time= 22.862 using GC-MS analysis

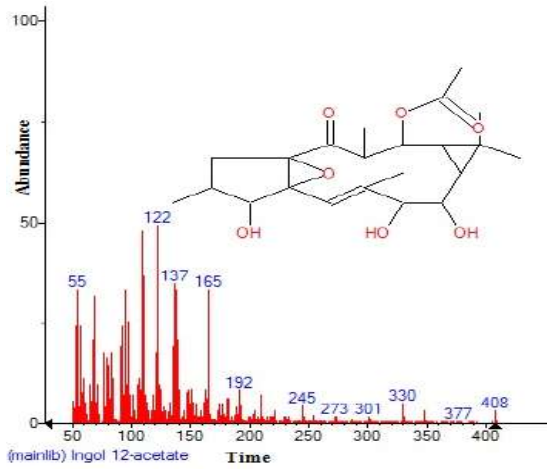


Fig. 45: Structure of Ingol 12-acetate present in *Zingiber officinale* with retention time= 22.914 using GC-MS analysis

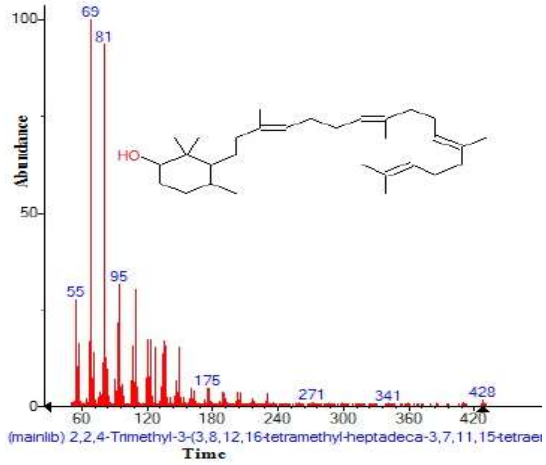


Fig. 46: Structure of 2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetrae present in *Zingiber officinale* with retention time= 23.554 using GC-MS analysis

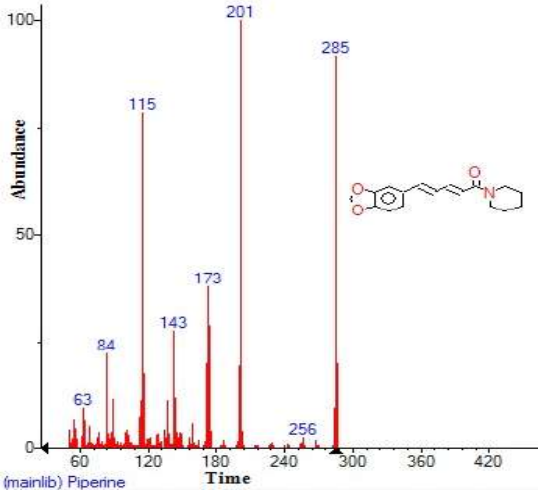


Fig. 47: Structure of Piperine present in *Zingiber officinale* with retention time= 23.629 using GC-MS analysis

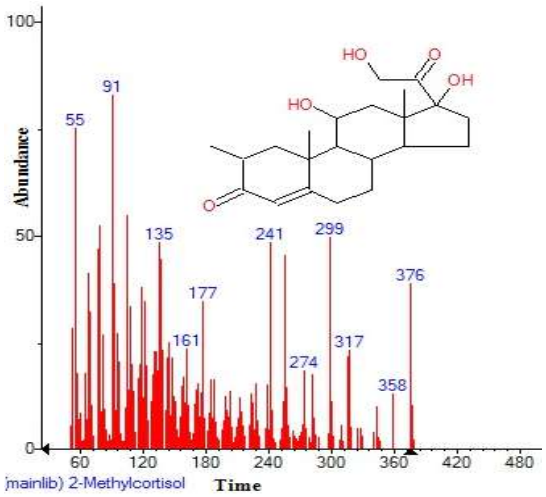


Fig. 48: Structure of 2-Methylcortisol present in *Zingiber officinale* with retention time= 24.195 using GC-MS analysis

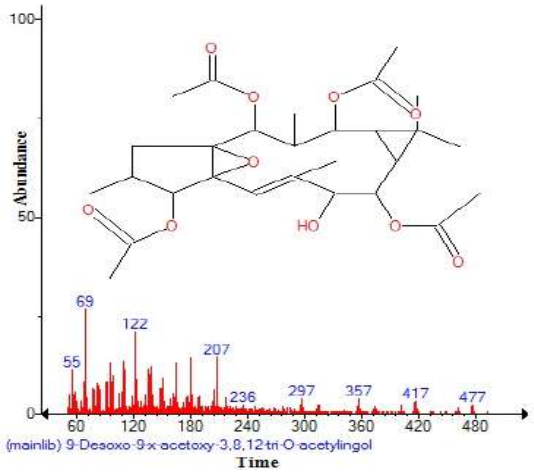


Fig. 49: Structure of 9-Desoxo-9-x-acetoxy-3,8,12,-tri-O-acetylingol present in *Zingiber officinale* with retention time= 25.242 using GC-MS analysis

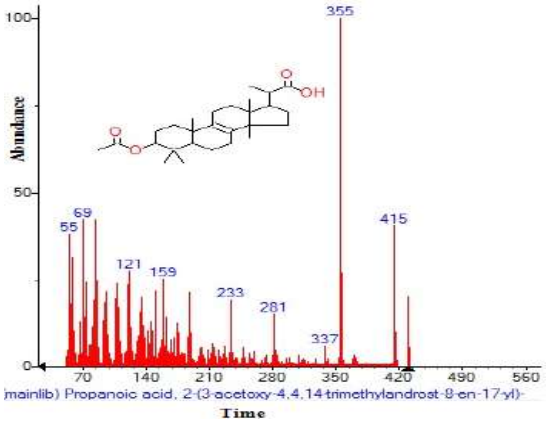


Fig. 50: Structure of Propanoic acid ,2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl present in *Zingiber officinale* with retention time= 25.986 using GC-MS analysis

CONCLUSION

From the results obtained in this study, it could be concluded that *Zingiber officinale* possesses remarkable antimicrobial activity, which is mainly due to naphthalenamine, decanal, and alfa.-copaene. According to these findings, it could be said that the methanolic extract act as antibacterial agents.

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