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 pharmaco logical actions. GC-MS analysis of Zingiber officinale revealed the existence of the Octanal, 2-Naphthalalamine,1,2,4a,5,6,7,8,8a-octahydro-4a-methyl, 1-(Cyclopropyl-nitro-methyl)-cyclopentanol, Endo-Borneol, Decanal, 1,2,15,16-Diepoxyhexadecane, Propanol,2-methyl-3-phenyl, Benzeneacetic acid, 1H-1,2,3,4-tetrazol-1-yl), Ascaridole epoxide, 2-Methoxy-4-vinyphenol, 6-epi-shpybunol, 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,5a)-, Bicyclo(4.4.0) dec-2-ene-4-ol,2-methyl-9-(prop-1-en-3-ol-2-yl)-, Corymbolone, Estr-1-3,5(10)-trien-17β-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]cycloocta[1, Gingerol, 1b,4a-Epoxycyclopenta[3,4]cyclopropa[8,9]cyclobuta[1,2,3]cyclopenta[5,6]-A-nor-5a-androstan-3,7-dione,3´,69-dihydro-17β-, Olean-12-ene-3,15,16,21,22,28-hexol,(38,15a,16a,21ß,22αa)-, Benz(e)azulen-3(3aH)-one,4,6a,7,8,9,10,10a,10b-octahydro-3a,8,1, Naphthalene, decahydro-1-pentadecyl-, 13-Docosanamide,(Z),3-10-Seccocholesta-5,7,10(19)-triene-3,24,25-triol, (38,52,Z,E)-, n-(2,4-Dinitrophenyl)-N-13-(2,6,6-trimethyl-cyclohex-1-ethyl)propyldier, n-(2,4-Dinitrophenyl)-N- 13-(2,6,6-trimethyl-cyclohex-1-ethyl)propyldier, 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. 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. Other pharmacological actions of ginger and compounds isolated from it include anti-inflammatory, antioxidant; hypoglycemic; analgesic, antiplatelet; antiemic; anti-thrombotic, anti-tumorigenic, radio protective, antimicrobial, antifungal actions. 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 hypotensive effects. Patients with chronic and painful diseases often seek alternative therapy, and currently ginger is one of the most popular herbal medications for inflammatory diseases. 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 air tight container to avoid the effect of humidity and then stored at room temperature.

**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.

**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. 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 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. The x-axis showed the RT and the y-axis measured the intensity of the signal to quantify the component.
<table>
<thead>
<tr>
<th>S. No.</th>
<th>Phytochemical</th>
<th>RT (min)</th>
<th>Formula</th>
<th>Molec. Weight</th>
<th>Exact Mass</th>
<th>Chemical structure</th>
<th>MS Fragment ions</th>
<th>Pharmacological actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Octanal</td>
<td>3.888</td>
<td>C₈H₁₆O</td>
<td>128</td>
<td>128.12</td>
<td><img src="image" alt="Octanal structure" /></td>
<td>56,69, 84,100,128</td>
<td>Antioxidant activity and anti-inflammatory activities</td>
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<tr>
<td>2</td>
<td>2-Naphthalenamine, 1,2,4a,5,6,7,8,8a-octahydro-4a-methyl</td>
<td>4.987</td>
<td>C₁₆H₁₆O</td>
<td>165</td>
<td>165.152</td>
<td><img src="image" alt="2-Naphthalenamine structure" /></td>
<td>55,67,81, 96,109,121,135,150,165</td>
<td>Anti-inflammatory, analgesic and antimicrobial properties</td>
</tr>
<tr>
<td>3</td>
<td>1-(Cyclopropyl-nitro-methyl)-cyclopentanol</td>
<td>5.702</td>
<td>C₂₀H₂₁O</td>
<td>185</td>
<td>185.105</td>
<td><img src="image" alt="1-(Cyclopropyl-nitro-methyl)-cyclopentanol structure" /></td>
<td>55,69,85, 95,121,139</td>
<td>Anti-muscarinic properties</td>
</tr>
<tr>
<td>4</td>
<td>Endo-Borneol</td>
<td>5.982</td>
<td>C₁₀H₁₆O</td>
<td>154</td>
<td>154.136</td>
<td><img src="image" alt="Endo-Borneol structure" /></td>
<td>55,67,79,95, 110,121, 139,152</td>
<td>Antinociceptive and anti-inflammatory activities</td>
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<td>5</td>
<td>Decanal</td>
<td>6.417</td>
<td>C₁₀H₂₀O</td>
<td>156</td>
<td>156.151</td>
<td><img src="image" alt="Decanal structure" /></td>
<td>57,70,82, 95,112,128, 138,155</td>
<td>Anti-Salmonella agents and antioxidant activity</td>
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<td>6</td>
<td>1,2-15,16-Diepoxyhexadecane</td>
<td>6.697</td>
<td>C₁₉H₃₆O₂</td>
<td>254</td>
<td>254.225</td>
<td><img src="image" alt="1,2-15,16-Diepoxyhexadecane structure" /></td>
<td>55,71,81, 95,178, 211,254</td>
<td>Antitumor and anti-inflammatory agents</td>
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<tr>
<td>7</td>
<td>Propanal,2-methyl-3-phenyl</td>
<td>6.932</td>
<td>C₁₀H₁₂O</td>
<td>148</td>
<td>148.089</td>
<td><img src="image" alt="Propanal,2-methyl-3-phenyl structure" /></td>
<td>51,63,77, 91,105,119, 133,148</td>
<td>Various biological activities such as anti-inflammatory</td>
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<td>8</td>
<td>Benzeneacetic acid, 4-(1H-1,2,3,4-tetrazol-1-yl)</td>
<td>7.338</td>
<td>C₁₂H₁₄O₄</td>
<td>204</td>
<td>204.065</td>
<td><img src="image" alt="Benzeneacetic acid, 4-(1H-1,2,3,4-tetrazol-1-yl) structure" /></td>
<td>51,77,89, 104,131, 149,204</td>
<td>Antimicrobial</td>
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<td>9</td>
<td>Ascaridole epoxide</td>
<td>7.75</td>
<td>C₁₅H₂₂O₃</td>
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<td>184.11</td>
<td><img src="image" alt="Ascaridole epoxide structure" /></td>
<td>55,69,79, 91,97,107, 117,135, 150,168</td>
<td>Anti-carcinogenic effects</td>
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<td>10</td>
<td>2-Methoxy-4-vinylphenol</td>
<td>7.928</td>
<td>C₁₀H₁₀O₂</td>
<td>150</td>
<td>150.068</td>
<td><img src="image" alt="2-Methoxy-4-vinylphenol structure" /></td>
<td>51,77,89, 107,121,135</td>
<td>Antioxidant, anti microbial and anti inflammatory.</td>
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<td>11</td>
<td>6-epi-shyobunol</td>
<td>8.208</td>
<td>C₁₆H₂₆O</td>
<td>222</td>
<td>222.198</td>
<td><img src="image" alt="6-epi-shyobunol structure" /></td>
<td>55,67,81,93, 109,121,136, 153,161, 189,207, 222</td>
<td>Anti-inflammatory, antinociceptive and antipyretic effects</td>
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<tr>
<td>No.</td>
<td>Compound Name</td>
<td>Molecular Formula</td>
<td>Molecular Weight</td>
<td>MRD</td>
<td>MWD</td>
<td>Activity</td>
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<td>12</td>
<td>Phenol,2-methoxy-5-(1-propenyl)-E</td>
<td>C_{12}H_{15}O_{3}</td>
<td>204</td>
<td>91</td>
<td>131</td>
<td>Anti-plasmodial activity, cytotoxic effect, antipyretic, induce apoptosis</td>
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<td>13</td>
<td>Alfa-Copaene</td>
<td>C_{15}H_{24}</td>
<td>204</td>
<td>55</td>
<td>82</td>
<td>Anti-Bacterial Agents</td>
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<td>14</td>
<td>8-Isopropenyl-1,5-dimethyl-cyclodeca-1,5-diene</td>
<td>C_{16}H_{24}</td>
<td>204</td>
<td>53</td>
<td>81</td>
<td>Anticancer, anti-inflammatory, gastro protective effects</td>
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<td>15</td>
<td>Bicyclo[3.1.0]hexane-6-methanol, 2-hydroxy-1,4,4-trimethyl</td>
<td>C_{16}H_{24}O_{2}</td>
<td>222</td>
<td>55</td>
<td>82</td>
<td>Anti-Candida and anti-inflammatory</td>
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<tr>
<td>16</td>
<td>7-epi-cis-sesquisabinene hydrate</td>
<td>C_{19}H_{20}O</td>
<td>222</td>
<td>55</td>
<td>82</td>
<td>Anti-cancer</td>
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<td></td>
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<td>17</td>
<td>Alloaromadendrene</td>
<td>C_{18}H_{24}</td>
<td>204</td>
<td>55</td>
<td>82</td>
<td>Anti-Inflammatory and anti-inflammatory</td>
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<td>18</td>
<td>Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl</td>
<td>C_{16}H_{24}O_{2}</td>
<td>202</td>
<td>55</td>
<td>82</td>
<td>Anti-HIV5,6, antifungal and antimicrobial</td>
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<td>19</td>
<td>1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2methyl-[S-(R*,S*)]</td>
<td>C_{16}H_{24}O_{2}</td>
<td>204</td>
<td>56</td>
<td>82</td>
<td>Antioxidant, anti-inflammatory and antinociceptive activities</td>
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<tr>
<td>20</td>
<td>Aromadendrene oxide</td>
<td>C_{17}H_{26}O_{3}</td>
<td>220</td>
<td>55</td>
<td>82</td>
<td>Anti HIV5,6, antifungal and antimicrobial</td>
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<td>21</td>
<td>1,6,10-Dodecatrien-3-ol,3,7,11-trimethyl-(E)</td>
<td>C_{16}H_{26}O_{3}</td>
<td>222</td>
<td>55</td>
<td>82</td>
<td>Anti-diabetic, hepatoprotective and anti-inflammatory activities</td>
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<td>22</td>
<td>4-((1H)-3-hydroxy-1-propenyl)-2-methoxyphenol</td>
<td>C_{15}H_{23}O_{2}</td>
<td>180</td>
<td>51</td>
<td>82</td>
<td>Antioxidant, anti microbial and anti inflammatory</td>
<td></td>
<td></td>
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<tr>
<td>23</td>
<td>Butan-2-one, 4-(3-hydroxy-2-methoxyphenyl)-</td>
<td>C_{16}H_{26}O_{3}</td>
<td>194</td>
<td>51</td>
<td>82</td>
<td>Anti-inflammatory, anti-diabetic, antilipolytic, antidiarrhoeic</td>
<td></td>
<td></td>
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</tbody>
</table>
24 Longipinocarveol, trans 12.631 $\text{C}_{15}\text{H}_{24}\text{O}$ 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 $\text{C}_{26}\text{H}_{40}\text{O}$ 400 400.371 69,81,95,121,203,245,315,400 Activities such as anti-inflammatory and cytotoxic activities


27 Corymbolone 14.268 $\text{C}_{15}\text{H}_{24}\text{O}_2$ 236 236.178 55,69,93,109,135,203,218 Anti-fungal agent

28 Estra-1,3,5(10)-trien-17ß-ol 15.24 $\text{C}_{18}\text{H}_{24}\text{O}$ 256 256.183 57,73,85,97,129,157,185,203,244 Antioxidant, anti-inflammatory, antifungal and antibacterial

29 1-Heptatriacotanol 15.166 $\text{C}_{16}\text{H}_{32}$ 536 536.59 55,81,95,107,121,133,147,161,203,229,244,257 Antioxidant, anticancer, anti inflammatory and to sexhormone activity

30 Fenretinide 16.059 $\text{C}_{26}\text{H}_{33}\text{NO}_2$ 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 $\text{C}_{15}\text{H}_{24}\text{O}_2$ 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]cyclopropane[5,6]cyclo[1,2-b] [388.225] 17.495 $\text{C}_{23}\text{H}_{32}$ 388 Increased content of glutathione and glutathione peroxidase activity

34 Gingerol 18.799 $\text{C}_{17}\text{H}_{26}\text{O}_4$ 294 294.183 55,77,91,119,137,150,194,205,294 Biological activities including anti-inflammatory and anti-oxidative

36. Cyclopropa[5,6]-A-nor-5±-androstane-3,7-dione,3´,6ß-dihydro-17ß-hexol,(3ß,15±,16±,21ß,22±)-

37. Olean-12-ene-3,15,16,21,22,28-hexol,(3ß,15±,16±,21ß,22±)-octahydro-3a,8,1

38. Benz[e]azulen-3(3aH)-one,4,6a,7,8,9,10,10a,10b-octahydro-3a,8,1

39. Naphthalene, decaphydro-1-pentadecyl-

40. 13-Docosenamide, (Z)-

41. 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3ß,5Z,7E)-

42. n-(2,4-Dinitrophenyl)-N´-13-(2,6,6-trimethyl-cyclohex-1-enyl)propylider

43. Ingol 12-acetate

44. 2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetrae

45. Piperine

46. 2-Methylcortisol

47. 9-Desoxo-9-x-acetoxyl-3,8,12,tri-O-acetylingol

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

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Plant (<em>Zingiber officinale</em>)</th>
<th>Antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Zingiber officinale</em></td>
<td>Streptomycin</td>
</tr>
<tr>
<td><em>Pseudomonas euorgenosa</em></td>
<td>4.01±0.188</td>
<td>0.92±0.210</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>2.99±0.311</td>
<td>1.04±0.300</td>
</tr>
<tr>
<td><em>Klebsiella pneumonia</em></td>
<td>4.93±0.290</td>
<td>1.05±0.161</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>3.75±0.910</td>
<td>2.00±0.140</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>1.99±0.200</td>
<td>2.06±0.300</td>
</tr>
</tbody>
</table>

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 spectrometry) 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\(^2\). 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
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.
and also with published literatures. Compounds were identified by comparing their spectra to those of the Wiley and NIST/EPA/NIH mass spectral libraries\textsuperscript{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. 60\textmu l of plant extract was loaded on the bored wells. The wells were bored in 0.5cm in diameter\textsuperscript{24}. The plates were incubated at 37°C for 24 hrs and examined. After the incubation the diameter of inhibition zones around the discs was measured.


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-nitromethyl)-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-...
cyclohepta-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)-2-methyl-[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
(3β,5α)-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-17β-ol, 1-Heptatriacotanol, Fenretinide, Folic acid, Spiro[4.5]decan-7-one, 1,8,8-dimethyl-8,9-epoxy-4-isopropyl-, 7H-6,9a-Methano-4H-cyclopenta[9,10]cyclopropane[5,6]cycloeca[1], Gingerol, 1b,4a-Epoxy-2H-cyclopenta[3,4]cyclopropane[8,9]cyclodec[1,2-b] o, Cyclopropa[5,6]-A-nor-5α-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-(3β,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, $3\prime,6\beta$-dihydro-17β-h, Olean-12-ene-3,15,16,21,22,28-hexol, (3β,15α,16α,21β,22α)-
Benz[e]azulen-3(3aH)-one, 4,6a,7,8,9,10,10a,10b-octahydro-3a,8,1, Naphthalene, decahydro-
Pentadecyl-, 13-Docosanamide, (Z)-, 9,10-
Secocholesta-5,7,10(19)-triene-3,24,25-triol, 
(3β,5Z,7E)-, n-(2,4-Dinitrophenyl)-N´-13-(2,6,6-
trimethyl-cyclohex-1-enyl)propylyder, n-(2,4-
Dinitrophenyl)-N´-13-(2,6,6-trimethyl-cyclohex-1-
enyl)propylyder, Ingol 12-acetate, 2,2,4-Trimethyl-3-

Fig. 30: Structure of Estra-1,3,5(10)-trien-17β-
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-Desoxy-9-x-acetoxy-3,8,12,-tri-O-acetyiligol 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 aureus, klebsiella pneumoniae, pseudomonas aeroginosa, E.coli. and Proteus mirabilis). Maximum zone formation against Klebsiella pneumoniae, Table 2.
Fig. 38: Structure of Cyclopropa[5,6]-A-nor-5α-androstane-3,7-dione, 3',6β-dihydro-17β-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, (3β,15α,16α,21β,22α) present in *Zingiber officinale* with retention time = 20.533 using GC-MS analysis.

Fig. 40: Structure of Benz[e]azulen-3(3aH)-one, 4,6α,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-Docosanamide, (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)propylidene 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
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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REFERENCES