Artemisia annua: Biochemical products analysis of methanolic aerial parts extract and anti-microbial capacity


Department of Biology, Babylon University, Hilla City, Iraq.

ABSTRACT

Medicinal plants are potential sources of natural compounds with biological activities and therefore attract the attention of researchers worldwide. The objective of this research was to determine the chemical composition of methanolic flowers extract. The phytochemical compound screened by GC-MS method. Forty nine bioactive phytochemical compounds were identified in the methanolic extract of Artemisia annua. 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 Artemisia annua revealed the existence of the 1,2-15,16-Diepoxyhexadecane, 1-Methylcycloheptanol, 3,5-Hexadien-2-ol2-methylene, Cholestan-3-ol,2-methylene, (3ß,5α), 2,5-Octadecadiynoic acid, methyl ester, Cyclohexene,1-methyl-5-(1-methylethenyl), Cyclohexene,4-isopropenyl-1-methoxymethylmethyloxiranyl, Exo-2,7,7-trimethylbicyclo[2.2.1]heptan-2-ol, 2(3H)-Furanone,5-ethenylidihydro-5-methyl, 2H-Benzof[1]oxireno[2,3-E]benzofuran-8(9H)-one,9-[(2-dimethylamin), 2-Furmanethanol,5-ethenyltetrahydro-α,α,5-trimethyl-cis, 5,8-Decadien-2-one,5,9-dimethyl-,(E), Methyl 6-oxoheptanoate, Dodecanic acid,3-hydroxy-, Isochorone,2H-Benzof[1]oxireno[2,3-E]benzofuran-8(9H)-one,9-[(2-dimethylamin), 2(3H)-Benzo[b]furan, hexahydro-7a-methyl-, 10-Undecen-1-ol,2-methyl-, 1,4-Methanoazulen-7-ol,decahydro-1,5,5,8a-tetramethyl-,(1s-10a,3a), 3,5-Heptadien,2-ethyliden-6-methyl-, 1,6-Dimethylhepta-1,3,5-triene, 1(2H)-Naphthalenone,octahydro-4-hydroxy-,trans, Trans-Z-α-Bisabolene epoxy, 7-epi-cis-sesquisabinene hydrate, Geranyl vinyl ether, Cyclohecanone,2,2-dimethyl-5-(3-methoxyxiranyl),2α(R*),3α-,-, Spiron[4.5]decane-7-one,1,8-dimethyl-8,9-epoxy-4-isopropyl-, 6-epi-shyobunol, 3,6-Diazahomoadamantan-9-one Hydrazone, Cholestan-3-ol,2-methylene, (3ß,5α), Ingol 12-acetate, Geranyl isovalerate, 1-Ethynyl-3-trans,11-dimethylcyclohexan-4-isocarbocyclic hexahexan-1-ol, 1b,4a-Epoxy-2H-cyclonaptena,3,4)cyclopropa[8,9]cyclooctene(1,2-b), l(+)-Ascorbic acid 2,6-dihexadecanoate, 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (2Z,2Z,2), 1-Heptatriacetic, Propanoic acid, 2-[(3,2-hydroxypropoxy)tetrahydrofurane-2-y],1-[(1S)-1-m, 10,13-Diazatricyclo[7.3.1.0(4,9)]tridecan-5-ol,2carboxylic acid, 9-Octadecenamide,(2), Lupeol, 9-Desoxy-α-α-oxo-3,8,12-tri-O-acytetyl, Olefin-12-en-15,16,21,22,28,-hexol,(38,15a,16a,17a,18,22a), 2,4,6-Decatrienolic acid, 1a,2,5,5a,6,9,10,10a-6,5a-dihydro, Pregn-5-en-20-one, 3,8,11,12,14-pentaehydroxy-,(38,11a,128,148), Spirost-8-en-11-one,3-hydroxy, (38,5α,148,208,228,25R,)-y-Tocopherol, O-methyl- and 1-Phenanthrene-2,5-carboxylic acid , tetrahydro-7(2)-methyl-2-oxo. The FTIR analysis of Artemisia annua flowers proved the presence of alkenes, aliphatic fluoro compounds, esters, carboxylic acids, ethers, nitro compounds, alkanes, H-bonded HX group, hydrogen bonded alcohol and phenols. Methanolic extract of bioactive compounds of Artemisia annua was assayed for in vitro antibacterial activity against Escherichia coli, Pseudomonas aerogenosa, Proteus mirabilis, Staphylococcus aureus and Klebsiella pneumonia by using the diffusion method in agar. The diameters of inhibition zones ranged from 5.01±0.200 to 0.700±0.106 mm for all treatments.

Keywords: Anti-microbial, Bioactive compounds, GC/MS, FT-IR, Artemisia annua.

*Corresponding author
INTRODUCTION

*Artemisia annua* L. ("sweet wormwood", "qinghao") is a genus of small herbs and shrubs found in northern temperate regions (Willcox et al., 2009). *Artemisia annua* L., a plant belonging to the Asteraceae family. *A. annua* has traditionally been used in China for the treatment of fever and chills (Bora et al., 2011). *Artemisia annua* L., is an annual herb native to China and it grows naturally as a part of steppe vegetation in northern parts of Chatar and Suiyan province in China at 1,000–1,500m above sea level (Lachenmeier, 2010). The plant is now naturalised in many other countries such as Australia, Argentina, Brazil, Bulgaria, France, Hungary, Italy, Spain, Romania, the United States, and the former Yugoslavia. The plant is cropped on a large scale in China, Vietnam, Turkey, Iran, Afghanistan, and Australia. In India, it is cultivated on an experimental basis in the Himalayan regions, as well as temperate and subtropical conditions (Willcox, 2009; Valles et al., 2011; Altameme et al., 2015a). The large genus *Artemisia* comprises important medicinal plants which are currently the subject of phytochemical attention because of their biological and chemical diversity, and essential oil production. Secondary metabolism generally have a broad spectrum of bioactivity, owing to the presence of several active ingredients or secondary metabolites, which work through various modes of action. Essential oils in a plant plays a role in its survival by producing attractants for pollinators, but it also acts as a chemical defence against predators and disease. The presence of volatile oil is also reported in fruits and roots. Sesquiterpenes are the most abundant chemicals in particular, caryophyllene oxide (9.0%), caryophyllene (6.9%), (E)-farnesene (8.2%), and germacrene D (4.0%) are identified. However, only 52% of the total components were identified (Li et al., 2007; Al-Marzoqi et al., 2015; Altameme et al., 2015b). The aims of this study were analysis of nature component of *Artemisia annua* and evaluation of antibacterial activity.

MATERIALS AND METHODS

Collection and preparation of plant material

The flowers were dried at room temperature for fifteen days and when properly dried then powdered using clean pestle and mortar, and the powdered flowers were size reduced with a sieve (Altameme et al., 2015c; Hameed et al., 2015a; Idan et al., 2015). The fine powder was then packed in airtight container to avoid the effect of humidity and then stored at room temperature.

Preparation of sample

About eleven grams of the plant sample powdered were soaked in 100 ml methanol individually. It was left for 84 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 (QP 2010 Plus SHIMADZU) instrument under computer control at 70 eV (Hameed et al., 2015d). About 1μL 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 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 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. 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 constituents were separated on 30 m x 0.25 mm i.d., 0.25 μm film thickness DB-1701P column from J & W Scientific. The injector temperature was set at 250 °C and all injections were made in split mode (split 30:1). The column was initially maintained at 50 °C for 5 min with subsequent increases to 210 °C at a rate of 5 °C/min and finally held for 5 min. FID Detector temperature was set at 270 °C (Mohammed and Imad, 2013; Hameed et al., 2015b). Data acquisition and data processing using Chromulan programme. The column employed here for the separation of components was Elite 1(100% dimethyl poly siloxane) (Hameed et al., 2014). 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 (Jasim et al., 2015; Muhanned et al., 2015).

Fourier transform infrared spectrophotometer (FTIR)

The powdered sample of the Artemisia annua was treated for FTIR spectroscopy (Shimadzu, IR Affinity 1, Japan). The sample was run at infrared region between 400 nm and 4000 nm (Hameed et al., 2015c; Hamza et al., 2015).

Determination of antibacterial activity of crude bioactive compounds of Artemisia annua.

Pseudomonas aeruginosa, Klebsiella pneumoniae, E. coli, and Staphylococcus aureus were swabbed in Muller Hinton agar plates. Fifty μl of plant extract was loaded on the bored wells. The wells were bored in 0.5cm in diameter. 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 flowers extract of Artemisia annua, shown in Table 1. The GC-MS chromatogram of the 49 peaks of the compounds detected was shown in Figure 1. Chromatogram GC-MS analysis of the methanol extract of Artemisia annua showed the presence of forty nine major peaks and the components corresponding to the pounds were bated at 37°C for 24 hrs and examined. After the incubation the diameter of inhibition zones around the discs was measured.

Table 2; Figure 51
antibacterial activity namely, \textit{(staphylococcus aerueus, klebsiella pneumoniae, pseudomonas aeruginosa, E.coli. and Proteus mirabilis)}. Maximum zone formation against \textit{Klebsiella pneumoniae}, Table 3. Viuda-Martos et al. (2010) investigated the chemical composition of this species, \textit{A. annua}, cultivated in Egypt, and twenty nine components were identified, representing 93.7\% of the total oil. Padalia et al. (2011) analyzed and compared by capillary GC and GC/MS the essential oil yield and composition of the aerial parts of \textit{A. annua} growing in Uttarakhand, India, at different stages of development. Different methods were used to evaluate the antibacterial and antifungal properties and included agar disk diffusion method (Cavar et al., 2012; Li et al., 2011; Massiha et al., 2013; Gupta et al., 2009), minimal inhibition concentration (MIC) (Duarte et al., 2007; Verdian-Rizi et al., 2008; Marcos-Arias et al., 2011; Radulović et al., 2013) minimal bacterial concentration (MBC), and minimal fungicidal concentration (MFC) (Radulović et al., 2013). The main gram-positive bacteria tested with methanol, chloroform, ethanol, hexane, and petroleum ether extracts of \textit{A. annua} were \textit{Staphylococcus aureus} (Gupta et al., 2009), \textit{Enterococcus foecalis} (Massiha et al., 2013), \textit{Micrococcus luteus}, \textit{Bacillus cereus}, \textit{Bacillus subtilis}, \textit{Bacillus pumilus}, and \textit{Bacillus sp}. The gram-negative \textit{Escherichia coli}, \textit{Salmonella typhi} (Gupta et al., 2009), and \textit{Pseudomonas aeruginosa} (Massiha et al., 2013) were tested. The antifungal activity of the essential oil was also evaluated against \textit{Sclerotinia sclerotiorum}, \textit{Botrytis cinerea}, \textit{Phytophthora infestans}, and \textit{Verticillium dahliae}.

Figure 1. GC-MS chromatogram of methanolic extract of \textit{Artemisia annua}. 
Table 1. Major phytochemical compounds identified in methanolic extract of *Artemisia annua*.

<table>
<thead>
<tr>
<th>S.N.o.</th>
<th>Phytochemical compound</th>
<th>RT (min)</th>
<th>Formula</th>
<th>Molecular Weight</th>
<th>Exact Mass</th>
<th>Chemical structure</th>
<th>MS Fragmentions</th>
<th>Pharmacological actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1,2,15,16-Diepoxyhexadecane</td>
<td>3.173</td>
<td>C_{16}H_{30}O_{2}</td>
<td>254</td>
<td>254.22458</td>
<td><img src="image1" alt="Chemical structure" /></td>
<td>55,71,81,95,178,211,254</td>
<td>Antitumor and anti-inflammatory agents</td>
</tr>
<tr>
<td>2.</td>
<td>1-Methylcycloheptanol</td>
<td>3.402</td>
<td>C_{8}H_{16}O</td>
<td>128</td>
<td>128.120115</td>
<td><img src="image2" alt="Chemical structure" /></td>
<td>58,71,85,95</td>
<td>Unknown</td>
</tr>
<tr>
<td>3.</td>
<td>3,5-Hexadien-2-ol2-methyl-</td>
<td>3.585</td>
<td>C_{7}H_{12}O</td>
<td>112</td>
<td>112.088815</td>
<td><img src="image3" alt="Chemical structure" /></td>
<td>53,69,97,112</td>
<td>Anti-oxidant, anti-microbial, anti-cancer and anti-HIV</td>
</tr>
<tr>
<td>4.</td>
<td>Cholestan -3-ol ,2-methylene, (38,5α)-</td>
<td>3.739</td>
<td>C_{28}H_{46}O</td>
<td>400</td>
<td>400.370516</td>
<td><img src="image4" alt="Chemical structure" /></td>
<td>69,81,95,105,12,113,161,203,27,245</td>
<td>Anti-inflammatory and cytotoxic activities</td>
</tr>
<tr>
<td>5.</td>
<td>2,5-Octadecadiynoic acid , methyl ester</td>
<td>3.871</td>
<td>C_{19}H_{30}O_{2}</td>
<td>290</td>
<td>290.22458</td>
<td><img src="image5" alt="Chemical structure" /></td>
<td>55,67,79,91,105,117,131,145,159</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td>No.</td>
<td>Compound Description</td>
<td>Molecular Formula</td>
<td>MW</td>
<td>CAS Number</td>
<td>Molar Mass</td>
<td>Uses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>---------------------------------------------------------------------------------------</td>
<td>-------------------</td>
<td>----</td>
<td>------------</td>
<td>------------</td>
<td>----------------------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Cyclohexene, 1-methyl-5-(1-methylethenyl)</td>
<td>C_{10}H_{16}</td>
<td>136</td>
<td>136.1252</td>
<td></td>
<td>Anti-microbial agents</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Cyclohexene, 4-isopropenyl-1-methoxymethoxymethyl-</td>
<td>C_{12}H_{20}O_{2}</td>
<td>196</td>
<td>196.14633</td>
<td></td>
<td>Anti-inflammatory and anti-allergy agents</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Exo-2,7,7-trimethylbicyclo[2.2.1]heptan-2-ol</td>
<td>C_{10}H_{18}O</td>
<td>154</td>
<td>154.135765</td>
<td></td>
<td>Antioxidant and anti-inflammatory activities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>2(3H)-Furanone, 5-ethenylidihydro-5-methyl</td>
<td>C_{10}H_{18}O</td>
<td>126</td>
<td>126.0680795</td>
<td></td>
<td>Anti-malarial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>Chemical Structure</td>
<td>Molecular Formula</td>
<td>Molecular Weight</td>
<td>Spectrum Numbers</td>
<td>Description</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>-------------------</td>
<td>-------------------</td>
<td>------------------</td>
<td>-----------------</td>
<td>------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td><img src="image1" alt="Chemical Structure" /></td>
<td>C_{19}H_{32}N_{2}O_{3}</td>
<td>366</td>
<td>58,81,109,149,204,233,278,336</td>
<td>Pharmacological activities like anti-inflammatory, analgesic activities.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td><img src="image2" alt="Chemical Structure" /></td>
<td>C_{10}H_{18}O_{2}</td>
<td>170</td>
<td>59,68,81,94,111,137</td>
<td>Antiviral properties and anti-oxidative properties.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td><img src="image3" alt="Chemical Structure" /></td>
<td>C_{12}H_{20}O</td>
<td>180</td>
<td>55,69,81,91,107,122,137,165</td>
<td>Unknown.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>Compound Description</td>
<td>Molecular Formula</td>
<td>Molar Mass</td>
<td>Eyring Energy</td>
<td>Activity(s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>-------------------------------------------------------------------------------------</td>
<td>-------------------</td>
<td>------------</td>
<td>---------------</td>
<td>----------------------------------------------------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Methyl 6-oxoheptanoate</td>
<td>C₈H₁₄O₃</td>
<td>5.376</td>
<td>158</td>
<td>158.094295 Anti-cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Dodecanoic acid, 3-hydroxy-</td>
<td>C₁₂H₂₄O₃</td>
<td>5.244</td>
<td>216</td>
<td>216.1725445 Antifungal activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Isophorone</td>
<td>C₃H₁₄</td>
<td>5.450</td>
<td>138</td>
<td>138.1044655 Strong anti-platelet effect</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>2(3H)-Benzofuranone, hexahydro-7a-methyl-</td>
<td>C₉H₁₄O₂</td>
<td>5.994</td>
<td>154</td>
<td>154.09938 Anti-inflammatory, analgesic, antihypertensive, diuretic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>10-Undecen-1-αl,2-methyl-</td>
<td>C₁₂H₂₃O</td>
<td>6.411</td>
<td>182</td>
<td>182.167066 Antimicrobial and Anti-inflammatory</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

March – April 2016 RJPBCS 7(2) Page No. 1850
20. 1,4-Methanoazulen-7-ol, decahydro-1,5,5,8a-tetramethyl-[1s-(1α,3a)] 7.527 \( C_{15}H_{26}O \) 222 222.198365 55,67,79,95,107,121,136,148,165,179,189,222 Anti-Candida

21. 3,5-Heptadienal, 2-ethylidene-6-methyl 7.939 \( C_{10}H_{14}O \) 150 150.104465 53,65,91,107,121,135,150 Anti-inflammatory, anti-tumour, anti-viral activities

22. 1,6-Dimethylhept-1,3,5-triene 8.094 \( C_9H_{14}O \) 122 122.1095505 53,65,74,79,91,107,121,135,150 Antimicrobial effects

23. 1(2H)-Naphthalenone, octahydro-4-hydroxy-, trans 8.563 \( C_{10}H_{16}O_2 \) 168 168.115029 55,67,81,95,109,124,135,150,168 Anti-Candida, anti-inflammatory
24. Trans-Z-α-Bisabolene epoxide 9.026 C_{15}H_{20}O 220 220.182715

25. 7-epi-cis-sesquisabinene hydrate 9.312 C_{15}H_{20}O 222 222.198365

26. Geranyl vinyl ether 9.621 C_{12}H_{20}O 180 180.151415

27. Cyclohexanone,2,2-dimethyl-5-(3-methyloxiranyl)-,[2α(R^*),3α]-(+--)

55,67,109,121,1
59,177,220
Anti-inflammatory effects

55,69,82,93,119,161,175,204,222
Anti-inflammatory and anti-diarrheal

53,69,81,93,109,121,136,152,164,178
Antimicrobial and anti-fungal

55,69,81,95,123,153,182
New chemical compound
<table>
<thead>
<tr>
<th>No.</th>
<th>Compound Description</th>
<th>Molecular Formula</th>
<th>Molecular Weight</th>
<th>Pharmacological Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>28.</td>
<td>Spiro[4,5]decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl</td>
<td>C_{15}H_{24}O_{2}</td>
<td>236</td>
<td>Anti-inflammatory activity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29.</td>
<td>6-epi-shyobunol</td>
<td>C_{15}H_{26}O</td>
<td>222</td>
<td>Pharmacological effects, such as anti-inflammatory, antioxidant</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30.</td>
<td>3,6-Diazahomoadamantan-9-one Hydrazone</td>
<td>C_{9}H_{16}N_{4}</td>
<td>180</td>
<td>New chemical compound</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31.</td>
<td>Cholestan-3-ol, 2-methylene-(38,5a)-</td>
<td>C_{28}H_{48}O</td>
<td>400</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
32. Ingol 12-acetate  
   12.007  
   $\text{C}_{22}\text{H}_{32}\text{O}_7$  
   408  
   408.214804  
   55,122,137,151,165,192,221,245,273,301,330  
   Unknown

33. Geranyl isovalerate  
   12.162  
   $\text{C}_{15}\text{H}_{30}\text{O}_2$  
   238  
   238.19328  
   57,69,85,121,136,168,198,238  
   Anti-inflammatory, antioxidant and anti-viral activities

34. 1-Ethynyl-3,trans(1,1-dimethylethyl)-4,cis-methoxycyclohexan-1-ol  
   12.602  
   $\text{C}_{13}\text{H}_{22}\text{O}_2$  
   210  
   210.16198  
   57,70,91,104,121,151,192,210  
   Pharmacological and biological activities including anti-Candida, anti-inflammatory

35. 1b,4a-Epoxy-2H-cyclopenta[3,4]cyclopropane[8,9]cycloundec[1,2-b]o  
   12.345  
   $\text{C}_{22}\text{H}_{23}\text{O}_8$  
   424  
   424.209719  
   97,109,123,137,165,181,261,295,346,377,407  
   Unknown
<table>
<thead>
<tr>
<th>No.</th>
<th>Compound Description</th>
<th>Molecular Formula</th>
<th>Molecular Weight</th>
<th>Anti-pigmentation effect</th>
<th>Antiviral and anti-obesity properties</th>
<th>Biological activity mainly as anticancer, antineoplastic and anti-HIV</th>
<th>Anti-diabetic effect</th>
<th>Antifertility and anticancer activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>36.</td>
<td>1-(+)-Ascorbic acid 2,6-dihexadecanoate</td>
<td>C_{32}H_{60}O_{8}</td>
<td>652</td>
<td>57,73,85,98,115,129,143,157,185,199,213,256,29,7,327,353,396,414</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37.</td>
<td>9,12,15-Octadecatricinoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)</td>
<td>C_{21}H_{36}O_{4}</td>
<td>352</td>
<td>57,67,79,95,109,135,155,173,232,261,291,321,352</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>38.</td>
<td>1-Heptatriacotanol</td>
<td>C_{33}H_{56}O</td>
<td>536</td>
<td>55,81,95,147,16,1,190,257</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>39.</td>
<td>Propanoic acid, 2-[5-(2-hydroxypropyl)tetrahydrofuran-2-yl]-1-[5-(1-m)]</td>
<td>C_{21}H_{36}O_{7}</td>
<td>400</td>
<td>69,85,97,111,12,5,143,157,199,2,17,253,272,313,327,369,401</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40.</td>
<td>10,13-Dioxatricyclo[7.3.1.0(4,9)]tridecan-5-ol-2carboxylic acid, 4-me</td>
<td>C_{12}H_{26}O_{5}</td>
<td>310</td>
<td>55,69,81,93,139,152,179,211,250,278,310</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
41. 9-Octadecanamide, (Z)- 18.851  C_{18}H_{35}NO  281  281.271864  59, 72, 83, 114, 154, 184, 212, 264, 281. Anti-inflammatory and anti-cancer properties

42. Lupeol  20.327  C_{30}H_{50}O  426  426.386166  55, 68, 81, 95, 207, 315, 426. Anticancer, antiprotozoal, chemopreventive and anti-inflammatory properties

43. 9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol  20.791  C_{28}H_{40}O_{10}  536  536.262146  55, 69, 122, 207, 36, 297, 357, 417, 477. Anti-inflammatory effects

44. Olean-12-ene3,15,16,21,22,28,hexol, (3ß,15α,16α,21ß,2α)-  21.363  C_{30}H_{50}O_{6}  506  506.360739  135, 190, 207, 231, 249, 280, 298, 33, 4, 352, 439, 488. Anti-tumourogenic properties
45. 2,4,6-Decatrienoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydro  

46. Pregn-5-en-20-one,3,8,11,12,14-pentahydroxy-(38,11a,12b,14b)-  

47. Spirost-8-en-11-one,3-hydroxy-(38,5a,14b,20b,22b,25R)-  

48. (+)-γ-Tocopherol, O-methyl  

49. 1-Phenanthrenecarboxylic acid, tetradecahydro-7-(2-methoxy-2-oxoe  

55,79,91,122,14  
9,284,312,330,3  
47,380,412,478  
55,97,138,153,1  
71,209,224,242,  
274,311,344,362,  
,380  
57,69,95,135,20  
7,229,281,314,3  
56,428  
57,91,137,165,2  
05,260,302,344,  
386,430  
55,67,79,91,109,  
159,177,213,267,  
,284,316,344,37,  
6  
Antimalarial and anti-HIV  
Cardio-protective, analgesic, antimycotic, and immunomodulatory effects  
Antimicrobial, antioxidant and anti-inflammatory activities  
Anti-inflammatory and antioxidative effects  
Unknown
Figure 2. Structure of 1,2,15,16-Diepoxyhexadecane with 3.173 (RT) present in Artemisia annua.

Figure 3. Structure of 1-Methylcycloheptanol 3.402 with (RT) present in Artemisia annua.

Figure 4. Structure of 3,5-Hexadien-2-ol2-methyl with 3.585 (RT) present in Artemisia annua.

Figure 5. Structure of Cholestan -3-ol,2-methylene, (35,5s) 3.739 with (RT) present in Artemisia annua.

Figure 6. Structure of 2,5-Octadecadiynoic acid, methyl ester with 3.871 (RT) present in Artemisia annua.

Figure 7. Structure of Cyclohexene,1-methyl-5-(1-methylethenyl) with 4.054 (RT) present in Artemisia annua.
Figure 8. Structure of Cyclohexene, 4-isopropenyl-1-methoxy methoxymethyl with 4.180 (RT) present in Artemisia annua.

Figure 9. Structure of Exo-2,7,7-trimethylbicyclo[2.2.1]heptan-2-ol with 4.311 (RT) present in Artemisia annua.

Figure 10. Structure of 2(3H)-Furanone, 5-ethyldihydro-5-methyl with 4.443 (RT) present in Artemisia annua.

Figure 11. Structure of 2H-Benzof]oxireno[2,3-E]benzofuran-8(9H)-one,9[(2-(dimethylamin) with 4.672 (RT) present in Artemisia annua.

Figure 12. Structure of 2-Furanmethanol, 5-ethyldihydro-α,5-trimethyl, cis with 4.803 (RT) present in Artemisia annua.

Figure 13. Structure of 5,8-Decadien-2-one, 5,9-dimethyl, (E) with 5.164 (RT) present in Artemisia annua.
Figure 14. Structure of Methyl 6-oxoheptanoate with 5.376 (RT) present in *Artemisia annua*.

Figure 15. Structure of Dodecanoic acid,3-hydroxy with 5.244 (RT) present in *Artemisia annua*.

Figure 16. Structure of Isophorone with 5.450 (RT) present in *Artemisia annua*.

Figure 17. Structure of 2H-Benz[f]oxireno[2,3-E]benzofuran-8(9H)-one,9-[[2-(dimethylamin)] with 5.708 (RT) present in *Artemisia annua*.

Figure 18. Structure of 2(3H)-Benzofuranone, hexahydro-7a-methyl with 5.994 (RT) present in *Artemisia annua*.

Figure 19. Structure of 10-Undecen-1-al,2-methyl with 6.411 (RT) present in *Artemisia annua*. 
Figure 20. Structure of 1,4-Methanoazulen-7-ol, decahydro-1,5,5,8a-tetramethyl-1s-(1α,3a) with 7.527 (RT) present in Artemisia annua.

Figure 21. Structure of 3,5-Heptadienal, 2-ethylidene-6-methyl with 7.939 (RT) present in Artemisia annua.

Figure 22. Structure of 1,6-Dimethylhepta-1,3,5-triene with 8.094 (RT) present in Artemisia annua.

Figure 23. Structure of 1(2H)-Naphthalene, octahydro-4-hydroxy-trans with 8.563 (RT) present in Artemisia annua.

Figure 24. Structure of Trans-2α-Bisabolene epoxide with 9.026 (RT) present in Artemisia annua.

Figure 25. Structure of 7-epi-cis-sesquisabinene hydrate with 9.312 (RT) present in Artemisia annua.
Figure 26. Structure of Geranyl vinyl ether with 9.621 (RT) present in Artemisia annua.

Figure 27. Structure of Cyclohexanone,2,2-dimethyl-5-(3-methyloxiranyl)-[2α(R*),3α] with 9.804 (RT) present in Artemisia annua.

Figure 28. Structure of Spiro[4.5]decan-7-one,1,8-dimethyl-8,9-epoxy-4-isopropyl with 10.297 (RT) present in Artemisia annua.

Figure 29. Structure of 6-epi-shyobunol with 10.583 (RT) present in Artemisia annua.

Figure 30. Structure of 3,6-Diazahomoadamantan-9-one Hydrazone with 11.046 (RT) present in Artemisia annua.

Figure 31. Structure of Cholestan-3-ol,2-methylene-(3ß,5a)- with 11.670 (RT) present in Artemisia annua.
Figure 32. Structure of Ingol 12-acetate with 12.007 (RT) present in Artemisia annua.

Figure 33. Structure of Geranyl isovalerate with 12.162 (RT) present in Artemisia annua.

Figure 34. Structure of 1-Ethynyl-3,trans(1,1-dimethylethyl)-4,cis-methoxy cyclohexan-1-ol with 12.602 (RT) present in Artemisia annua.

Figure 35. Structure of 1b,4a-Epoxy-2H-cyclopenta[3,4]cyclopropa[8,9]cycloundec[1,2-b]o with 12.345 (RT) present in Artemisia annua.

Figure 36. Structure of 1(+)-Ascorbic acid 2,6-dihexadecanoate with 12.275 (RT) present in Artemisia annua.

Figure 37. Structure of 9,12,15-Octadecatetraenoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z) with 16.648 (RT) present in Artemisia annua.
Figure 38. Structure of 1-Heptatriacotanol with 17.266 (RT) present in *Artemisia annua*.

Figure 39. Structure of Propanoic acid, 2-[5-[2-hydroxypropyl][tetrahydrofuran-2-yl],-1-[5-(1-m) with 17.970 (RT) present in *Artemisia annua*.

Figure 40. Structure of 10,13-Dioxatricyclo[7.3.1.0(4,9)]tridecan-5-ol-2-carboxylic acid,4-me With 18.462 (RT) present in *Artemisia annua*.

Figure 41. Structure of 9-Octadecenamide,(Z) with 18.851 (RT) present in *Artemisia annua*.

Figure 42. Structure of Lupeol with 20.327 (RT) present in *Artemisia annua*.

Figure 43. Structure of 9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol with 20.791 (RT) present in *Artemisia annua*.
Figure 44. Structure of Olean-12-ene3,15,16,21,22,28,-hexol,(38,15α,16α,21β,22α) with 21.363 (RT) present in Artemisia annua.

Figure 45. Structure of 9-Desoxo-9-x-acetoxy -3,8,12-tri-O-acetylingol with 21.649 (RT) present in Artemisia annua.

Figure 46. Structure of 2,4,6-Decatrienoic acid , 1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydro with 21.597 (RT) present in Artemisia annua.

Figure 47. Structure of Pregn-5-en-20-one,3,8,11,12,14-pentahydroxy-(38,11α,12β,14β) with 23.697 (RT) present in Artemisia annua.

Figure 48. Structure of Spirost-8-en-11-one,3-hydroxy-(38,5α,14β,206,228,25R) with 26.289 (RT) present in Artemisia annua.

Figure 49. Structure of (+)-y-Tocopherol,Omethyl with 26.495 (RT) present in Artemisia annua.
Figure 50. Structure of 1-Phenanthrene carboxylic acid, tetradecahydro-7-(2-methoxy-2-oxe with 27.611 (RT) present in Artemisia annua.

Figure 51. FT-IR peak values of Artemisia annua.

Table 2. FT-IR peak values of Artemisia annua.

<table>
<thead>
<tr>
<th>No.</th>
<th>Peak (Wave number cm⁻¹)</th>
<th>Intensity</th>
<th>Bond</th>
<th>Functional group assignment</th>
<th>Group frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>665.44</td>
<td>61.059</td>
<td>-</td>
<td>Unknown</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>777.31</td>
<td>64.617</td>
<td>C-H</td>
<td>Alkenes</td>
<td>675-995</td>
</tr>
<tr>
<td>3.</td>
<td>894.97</td>
<td>74.403</td>
<td>C-H</td>
<td>Alkenes</td>
<td>675-995</td>
</tr>
<tr>
<td>4.</td>
<td>1028.06</td>
<td>57.221</td>
<td>C-F stretch</td>
<td>Aliphatic fluoro compounds</td>
<td>1000-10150</td>
</tr>
<tr>
<td>5.</td>
<td>1155.36</td>
<td>72.616</td>
<td>C-O</td>
<td>Alcohols, Ethers, Carboxlic acids, Esters</td>
<td>1050-1300</td>
</tr>
<tr>
<td>6.</td>
<td>1242.16</td>
<td>72.142</td>
<td>C-O</td>
<td>Alcohols, Ethers, Carboxlic acids, Esters</td>
<td>1050-1300</td>
</tr>
<tr>
<td>7.</td>
<td>1315.45</td>
<td>56.648</td>
<td>NO2</td>
<td>Nitro Compounds</td>
<td>1300-1370</td>
</tr>
<tr>
<td>8.</td>
<td>1417.68</td>
<td>74.421</td>
<td>C-H</td>
<td>Alkanes</td>
<td>1340-1470</td>
</tr>
<tr>
<td>9.</td>
<td>1616.35</td>
<td>62.969</td>
<td>-</td>
<td>Unknown</td>
<td>-</td>
</tr>
<tr>
<td>10.</td>
<td>1732.08</td>
<td>79.650</td>
<td>-</td>
<td>Unknown</td>
<td>-</td>
</tr>
<tr>
<td>11.</td>
<td>2306.86</td>
<td>92.190</td>
<td>-</td>
<td>Unknown</td>
<td>-</td>
</tr>
<tr>
<td>12.</td>
<td>2848.86</td>
<td>82.386</td>
<td>H-O</td>
<td>H-bonded H-X group</td>
<td>2500-3500</td>
</tr>
<tr>
<td>13.</td>
<td>2918.30</td>
<td>77.950</td>
<td>C-H</td>
<td>Alkanes</td>
<td>2850-2970</td>
</tr>
<tr>
<td>14.</td>
<td>3064.89</td>
<td>85.717</td>
<td>H-O</td>
<td>H-bonded H-X group</td>
<td>2500-3500</td>
</tr>
<tr>
<td>15.</td>
<td>3273.20</td>
<td>79.265</td>
<td>O-H</td>
<td>Hydrogen bonded Alcohols, Phenols</td>
<td>3200-3600</td>
</tr>
<tr>
<td>16.</td>
<td>3361.93</td>
<td>79.184</td>
<td>O-H</td>
<td>Hydrogen bonded Alcohols, Phenols</td>
<td>3200-3600</td>
</tr>
</tbody>
</table>
Table 3. Zone of inhibition (mm) of test bacterial strains to *Artemisia annua* bioactive compounds and standard antibiotics.

<table>
<thead>
<tr>
<th>/ Antibiotics</th>
<th>Artemisia annua</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>Klebsiella pneumonia</em></td>
</tr>
<tr>
<td><em>Artemisia annua</em></td>
<td></td>
<td>5.01±0.200</td>
</tr>
<tr>
<td>Cefotoxime</td>
<td>1.33±0.240</td>
<td>2.00±0.371</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>2.42±0.561</td>
<td>0.70±0.106</td>
</tr>
<tr>
<td>Rifambin</td>
<td>0.96±0.100</td>
<td>1.09±0.320</td>
</tr>
</tbody>
</table>

**CONCLUSION**

From the results obtained in this study, it could be concluded that *Artemisia annua* possesses remarkable antibacterial activity, which is mainly due to 3,5-Heptadienal, Naphthalenone and Lupeol. According to these findings, it could be said that the methanolic extract act as antibacterial agents.

**ACKNOWLEDGEMENT**

I wish to express my deepest gratitude to Prof. Dr. Adul-Kareem for his valuable contributions and support throughout this study. I would also like to express my gratitude to Dr. Huda for his valuable suggestions and comments.

**REFERENCES**


