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

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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-methyl, Cholestan -3-ol ,2-methylene, (3ß,5α), 2,5-Octadecadiynoic acid , methyl ester, Cyclohexene,1-methyl-5-(1-methylethenyl), Cyclohexene,4-isopropenyl -1-methoxymethoxymethyl, Exo-2,7,7-trimethylbicyclo[2.2.1]heptan-2-ol, 2(3H)-Furanone,5-ethenyldihydro-5-methyl, 2H-Benzo[f]oxireno[2,3-E]benzofuran-8(9H)-one,9[[[2-(dimethylamin), 2-Furanmethanol,5-ethenyltetrahydro-α,α,5-trimethyl-,cis-, 5,8-Decadien-2-one,5,9-dimethyl-,(E)-, Methyl 6-oxoheptanoate, Dodecanoic acid,3-hydroxy-, Isophorone, 2H-Benzo[f]oxireno[2,3-E]benzofuran-8(9H)-one,9-[[[2-(dimethylamin), 2(3H)-Benzofuranone, hexahydro-7a-methyl-, 10-Undecen-1-al,2-methyl-, 1,4-Methanoazulen-7-ol,decahydro-1,5,5,8a-tetramethyl-,[1s-(1α,3a), 3,5-Heptadienal,2-ethylidene-6-methyl-, 1,6-Dimethylhepta-1,3,5-triene, 1(2H)-Naphthalenone,octahydro-4-hydroxy-,trans, Trans-Z-α-Bisabolene epoxide, 7-epi-cis-sesquisabinene hydrate, Geranyl vinyl ether, Cyclohexanone,2,2-dimethyl-5-(3-methyloxiranyl)-,[2α(R*),3α]-(.+-.)-, Spiro[4.5]decan-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(1,1-dimethylethyl)-4,cis-methoxycyclohexan-1-ol, 1b,4a-Epoxy-2H-cyclopenta [3,4]cyclopropa[8,9]cycloundec[1,2-b]o, I-(+)-Ascorbic acid 2,6-dihexadecanoate, 9,12,15-Octadecatrinoic acid , 2,3-dihydroxypropyl ester , (Z,Z,Z)-, 1-Heptatriacotanol, Propanoic acid , 2-[5-(2-hydroxypropyl)tetrahydrofuran-2-yl]-,1-[5-(1-m), 10,13-Dioxatricyclo[7.3.1.0(4,9)]tridecan-5-ol-2carboxylic acid, 9-Octadecenamide,(Z)-, Lupeol, 9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol, Olean-12-ene3,15,16,21,22,28,-hexol,(3ß,15α,16α,21ß,22α)-, 2,4,6-Decatrienoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydro, Pregn-5-en-20-one,3,8,11,12,14,-pentahydroxy-,(3ß,11α,12ß,14ß)-, Spirost-8-en-11-one,3-hydroxy-, (3ß,5α,14ß,20ß,22ß,25R-,(+)-y-Tocopherol,O-methyl- and 1-Phenanthrenecarboxylic acid ,tetradecahydro-7-(2-methoxy-2-oxoe. The FTIR analysis of *Artemisia annua* flowers proved the presence of alkenes, aliphatic fluoro compounds, alcohols, ethers, carboxlic acids, esters, nitro compounds, alkanes, H-bonded H-X group, hydrogen bonded alcohols 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*.

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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.

MATRIALS 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 ºC and all injections were made in split mode (split 30:1). The column was initially maintained at 50 ºC for 5 min with subsequent increases to 210 ºC at a rate of 5 ºC/min and finally held for 5 min. FID Detector temperature was set at 270 ºC (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 37C° for 24 hrs and examined. After the incubation the diameter of inhibition zones around the discs was measured.

RESULTS AND DISCUSION

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 peaks were determined as follows. The first set up peak were determined to be 1,2-15,16-Diepoxyhexadecane **Figure 2**. The second peak indicated to be 1-Methylcycloheptanol Figure 3. The next peaks considered to be 3,5-Hexadien-2-ol2-methyl, Cholestan -3-ol ,2-methylene, (3ß,5α), 2,5-Octadecadiynoic acid , methyl ester, Cyclohexene,1-methyl-5-(1-methylethenyl), Cyclohexene,4-isopropenyl -1-methoxymethoxymethyl, Exo-2,7,7-trimethylbicyclo[2.2.1]heptan-2-ol, 2(3H)-Furanone,5-ethenyldihydro-5-methyl, 2H-Benzo[f]oxireno[2,3-E]benzofuran-8(9H)-one,9[[[2-(dimethylamin), 2-Furanmethanol,5-ethenyltetrahydro-α,α,5-trimethyl-,cis-, 5,8-Decadien-2-one,5,9-dimethyl-,(E)-, Methyl 6-oxoheptanoate, Dodecanoic acid,3-hydroxy-, Isophorone, 2H-Benzo[f]oxireno[2,3-E]benzofuran-8(9H)-one,9-[[[2-(dimethylamin), 2(3H)-Benzofuranone, hexahydro-7a-methyl-, 10-Undecen-1-al,2-methyl-, 1,4-Methanoazulen-7-ol,decahydro-1,5,5,8a-tetramethyl-,[1s-(1α,3a), 3,5-Heptadienal,2-ethylidene-6-methyl-, 1,6-Dimethylhepta-1,3,5-triene, 1(2H)-Naphthalenone,octahydro-4-hydroxy-,trans, Trans-Z-α-Bisabolene epoxide, 7-epi-cis-sesquisabinene hydrate, Geranyl vinyl ether, Cyclohexanone,2,2-dimethyl-5-(3-methyloxiranyl)-,[2α(R*),3α]-(.+-.)-, Spiro[4.5]decan-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(1,1-dimethylethyl)-4,cis-methoxycyclohexan-1-ol, 1b,4a-Epoxy-2H-cyclopenta[3,4]cyclopropa[8,9]cycloundec[1,2-b]o, I-(+)-Ascorbic acid 2,6-dihexadecanoate, 9,12,15-Octadecatrinoic acid , 2,3-dihydroxypropyl ester , (Z,Z,Z)-, 1-Heptatriacotanol, Propanoic acid , 2-[5-(2-hydroxypropyl)tetrahydrofuran-2-yl]-,1-[5-(1-m), 10,13-Dioxatricyclo[7.3.1.0(4,9)]tridecan-5-ol-2carboxylic acid, 9-Octadecenamide,(Z)-, Lupeol, 9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol, Olean-12-ene3,15,16,21,22,28,-hexol,(3ß,15α,16α,21ß,22α)-, 2,4,6-Decatrienoic acid , 1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydro, Pregn-5-en-20-one,3,8,11,12,14,-pentahydroxy-,(3ß,11α,12ß,14ß)-, Spirost-8-en-11-one,3-hydroxy-,(3ß,5α,14ß,20ß, 22ß,25R)-, (+)-y-Tocopherol,O-methyl- and 1-Phenanthrenecarboxylic acid ,tetradecahydro-7-(2-methoxy-2-oxoe (**Figure 3-50**). The FTIR analysis of *Artemisia annua* flowers proved the presence of Alkenes, Aliphatic fluoro compounds, Alcohols, Ethers, Carboxlic acids, Esters, Nitro Compounds, Alkanes, H-bonded H-X group, Hydrogen bonded Alcohols and Phenols which shows major peaks at 777.31, 1028.06, 1155.36, 1315.45, 1417.68, 2848.86, 2918.30, 3273.20 and 3361.93 (**Table 2; Figure 51**). 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 3**. Viuda-Martos et al. (2010) investigated the chemical composition of this species, *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 *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; Radulovi´c et al., 2013) minimal bacterial concentration (MBC), andminimal fungicidal concentration (MFC) (Radulovi´c et al., 2013). The main gram-positive bacteria tested with methanol, chloroform, ethanol, hexane, and petroleum ether extracts of *A. annua* were *Staphylococcus aureus* (Gupta et al., 2009), *Enterococcus faecalis* (Massiha et al., 2013), *Micrococcus luteus*, *Bacillus cereus*, *Bacillus subtilis*, *Bacillus pumilus*, and *Bacillus sp*. The gram-negative *Escherichia coli*, *Salmonella typhi* (Gupta et al., 2009), and *Pseudomonas aeruginosa* (Massiha et al., 2013) were tested. The antifungal activity of the essential oil was also evaluated against *Sclerotinia sclerotiorum*, *Botrytis cinerea*, *Phytophthora infestans*, and *Verticillim dahliae*.

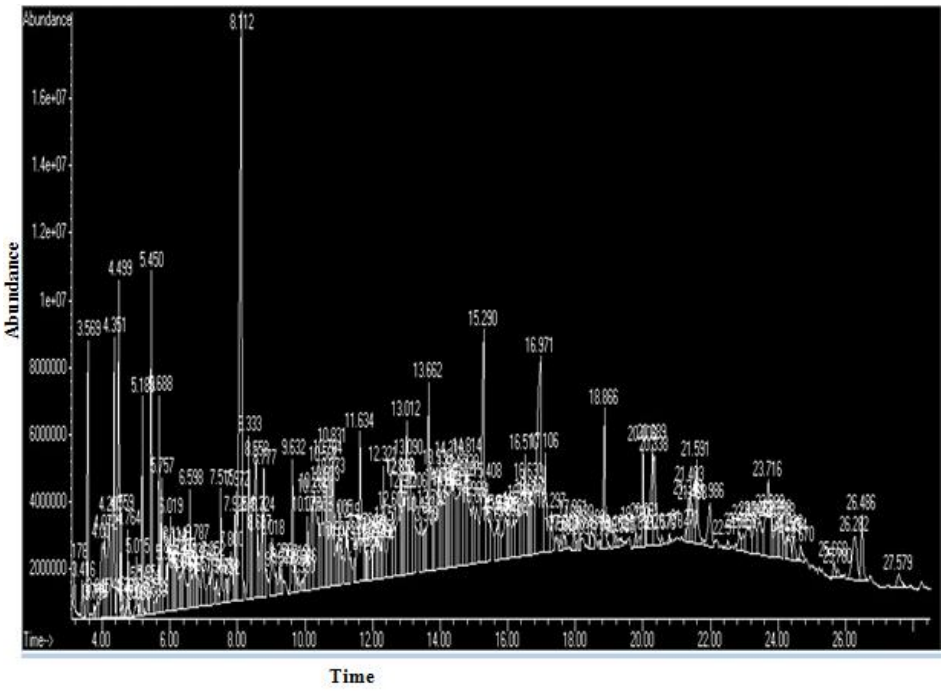

Figure 1. GC-MS chromatogram of methanolic extract of *Artemisia annua*.

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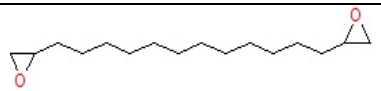
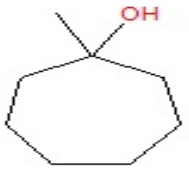
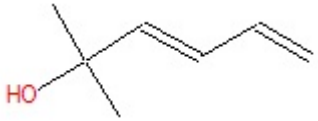
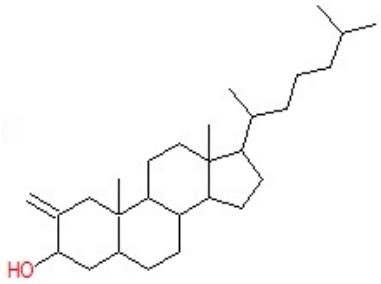
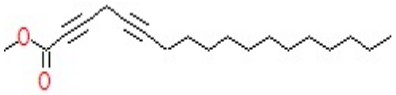
Table 1. Major phytochemical compounds identified in methanolic extract of *Artemisia annua*.

S.No.	Phytochemical compound	RT (min)	Formula	Molecular Weight	Exact Mass	Chemical structure	MS Fragment-ions	Pharmacological actions
1.	1,2-15,16-Diepoxyhexadecane	3.173	$C_{16}H_{30}O_2$	254	254.22458		55,71,81,95,178, 211,254	Antitumor and anti-inflammatory agents
2.	1-Methylcycloheptanol	3.402	$C_8H_{16}O$	128	128.120115		58,71,85,95	Unknown
3.	3,5-Hexadien-2-ol2-methyl-	3.585	$C_7H_{12}O$	112	112.088815		53,69,97,112	Anti-oxidant, anti-microbial, anti-cancer and anti-HIV
4.	Cholestan -3-ol ,2-methylene, (3ß,5α)-	3.739	$C_{28}H_{48}O$	400	400.370516		69,81,95,105,121,133,161,203,227,245	Anti-inflammatory and cytotoxic activities
5.	2,5-Octadecadiynoic acid , methyl ester	3.871	$C_{19}H_{30}O_2$	290	290.22458		55,67,79,91,105, 117,131,145,159	Anti-inflammatory

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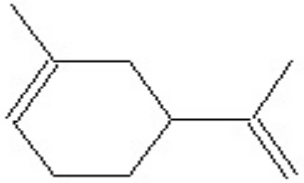
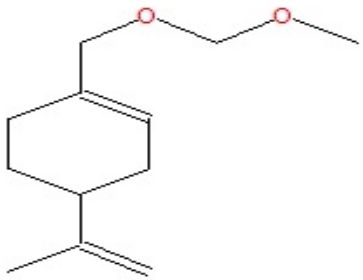
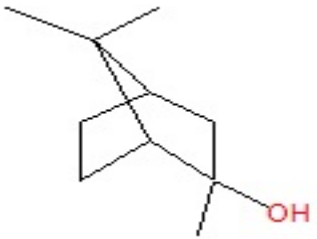
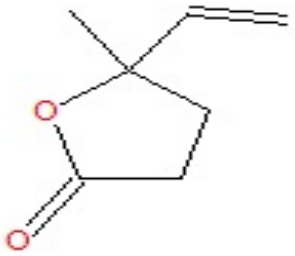
6.	Cyclohexene,1-methyl-5-(1-methylethenyl)	4.054	$C_{10}H_{16}$	136	136.1252		53,68,79,93,107,136	Anti-microbial agents
7.	Cyclohexene,4-isopropenyl -1-methoxymethoxymethyl -	4.180	$C_{12}H_{20}O_2$	196	196.14633		53,67,79,91,119,164,196	Anti-inflammatory and anti-allergy agents
8.	Exo-2,7,7-trimethylbicyclo[2.2.1]heptan-2-ol	4.311	$C_{10}H_{18}O$	154	154.135765		55,71,81,93,107,121,136,154	Antioxidant and anti-inflammatory activities
9.	2(3H)-Furanone,5-ethenyldihydro-5-methyl	4.443	$C_{10}H_{18}O$	126	126.0680795		55,67,71,83,93,99,111,126	Anti-malarial

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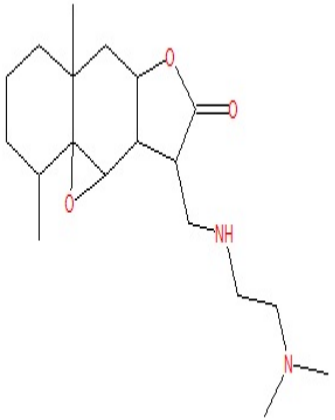
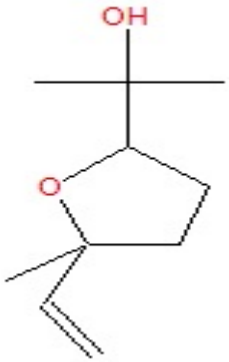
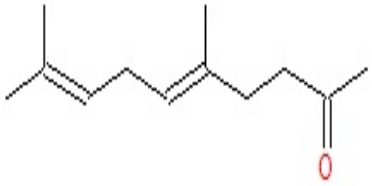
10.	2H-Benzo[f]oxireno[2,3-E]benzofuran-8(9H)-one,9[[[2-(dimethylamin)	4.672	$C_{19}H_{32}N_2O_3$	366	366.241293		58,81,109,149,204,233,278,336	Pharmacological activities like anti-inflammatory, analgesic
11.	2-Furanmethanol,5-ethenyltetrahydro-α,α,5-trimethyl-,cis-	4.803	$C_{10}H_{18}O_2$	170	170.13068		59,68,81,94,111,137	Antiviral properties and anti-oxidative properties
12.	5,8-Decadien-2-one,5,9-dimethyl-,(E)-	5.164	$C_{12}H_{20}O$	180	180.151415		55,69,81,91,107,122,137,165	Unknown

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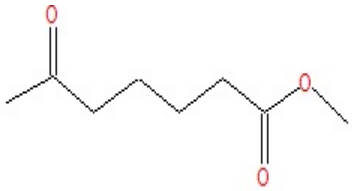
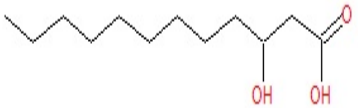
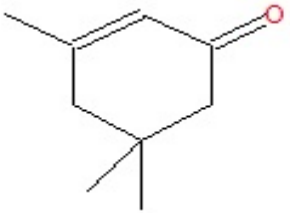
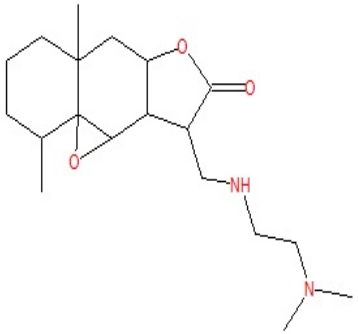
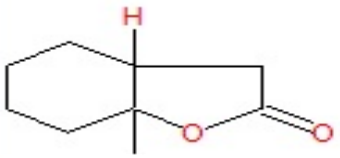
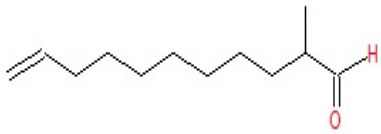
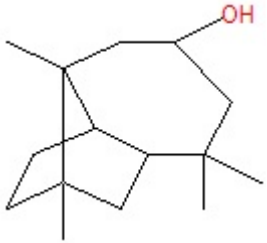
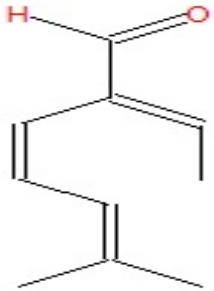
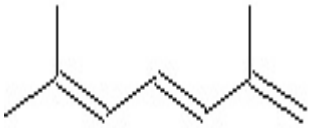
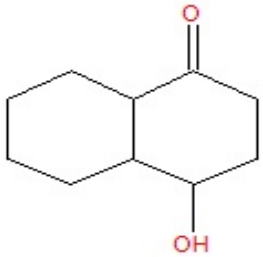
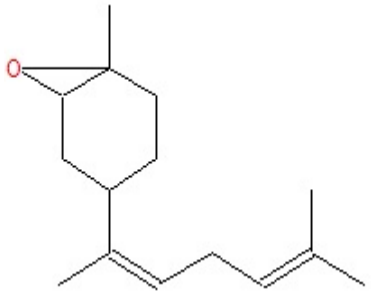
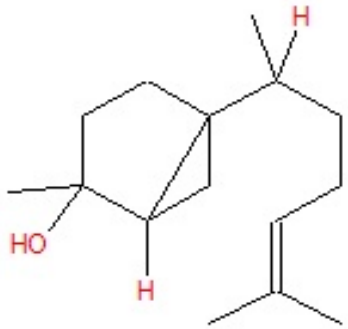
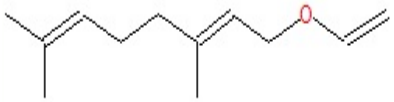
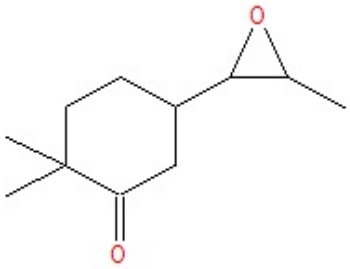
14.	Methyl 6-oxoheptanoate	5.376	$C_8H_{14}O_3$	158	158.094295		55,84,111,126,143	Anti-cancer
15.	Dodecanoic acid,3-hydroxy-	5.244	$C_{12}H_{24}O_3$	216	216.1725445		55,69,83,96,112,126,138,151,180	Antifungal activity
16.	Isophorone	5.450	C_9H_{14}	138	138.1044655		54,67,77,82,91,95,103,110,123,138	Strong anti-platelet *effect*
17.	2H-Benzo[f]oxireno[2,3-E]benzofuran-8(9H)-one,9-[[[2-(dimethylamin)	5.708	$C_{19}H_{32}N_2O_3$	336	336.241293		58,71,81,91,109,123,135,149,173,185,204,219	Antitumor, anti-inflammatory, antifungal, pesticidal and insecticidal
18.	2(3H)-Benzofuranone, hexahydro-7a-methyl-	5.994	$C_9H_{14}O_2$	154	154.09938		55,68,83,95,111,121,139,154	Anti-inflammatory, analgesic, antihypertensive, diuretic
19.	10-Undecen-1-al,2-methyl-	6.411	$C_{12}H_{22}O$	182	182.167066		58,69,81,97,112,125,151,167,182	Antimicrobial and Anti- inflammatory

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	150.1044655		53,65,91,107,121,135,150	Anti-inflammatory, anti-tumour, anti-viral activities
22.	1,6-Dimethylhepta-1,3,5-triene	8.094	$C_9H_{14}O$	122	122.1095505		53,65,74,79,91,107,122	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	$C_{15}H_{24}O$	220	220.182715		55,67,109,121,159,177,220	Anti-inflammatory effects
25.	7-epi-cis-sesquisabinene hydrate	9.312	$C_{15}H_{26}O$	222	222.198365		55,69,82,93,119,161,175,204,222	Anti-inflammatory and anti- diarrheal
26.	Geranyl vinyl ether	9.621	$C_{12}H_{20}O$	180	180.151415		53,69,81,93,109,121,136,152,164,178	Antimicrobial and anti-fungal
27.	Cyclohexanone,2,2-dimethyl-5-(3-methyloxiranyl)-,[2α(R*),3α]-(.+-.)-	9.804	$C_{11}H_{18}O_2$	182	182.13068		55,69,81,95,123,153,182	New chemical compound

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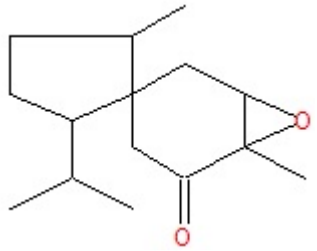
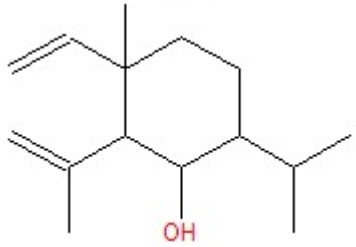
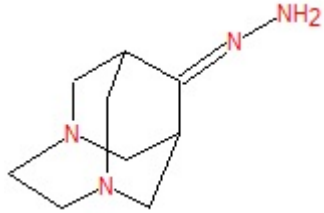
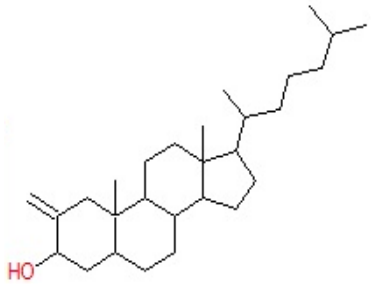
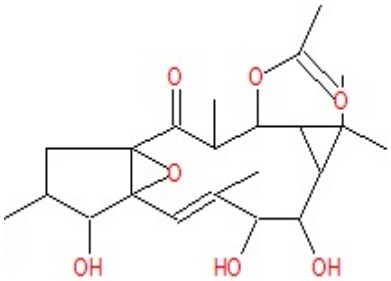
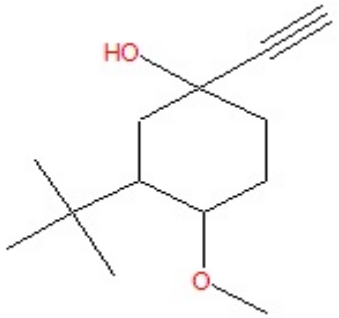
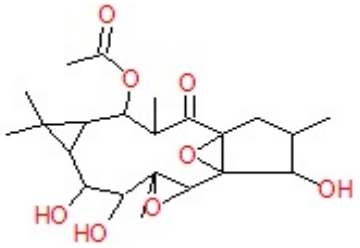
28.	Spiro[4.5]decan-7-one,1,8-dimethyl-8,9-epoxy-4-isopropyl	10.297	$C_{15}H_{24}O_2$	236	236.17763		55,69,81,95,109,123,137,151,165,175,193,208,236	Anti-inflammatory activity
29.	6-epi-shyobunol	10.583	$C_{15}H_{26}O$	222	222.298365		55,67,81,93,109,121,136,153,161,189,207,222	Pharmacological effects, such as anti-inflammatory, antioxidant
30.	3,6-Diazahomoadamantan-9-one Hydrazone	11.046	$C_9H_{16}N_4$	180	180.137497		58,72,95,121,135,164,180	New chemical compound
31.	Cholestan-3-ol,2-methylene-,(3ß,5α)-	11.670	$C_{28}H_{48}O$	400	400.370516		69,81,95,105,121,133,149,175,203,227,245,260	Unknown

32.	Ingol 12-acetate	12.007	$C_{22}H_{32}O_7$	408	408.214804		55,122,137,151, 165,192,221,245 ,273,301,330	Unknown
33.	Geranyl isovalerate	12.162	$C_{15}H_{26}O_2$	238	238.19328		57,69,85,121,13 6,168,198,238	Anti-inflammatory, antioxidant and anti-viral activities
34.	1-Ethynyl-3,trans(1,1-dimethylethyl)-4,cis-methoxycyclohexan-1-ol	12.602	$C_{13}H_{22}O_2$	210	210.16198		57,70,91,104,12 1,151,192,210	Pharmacological and biological activities including anti-Candida, anti-inflammatory
35.	1b,4a-Epoxy-2H-cyclopenta[3,4]cyclopropa[8,9]cycloundec[1,2-b]o	12.345	$C_{22}H_{23}O_8$	424	424.209719		97,109,123,137, 165,181,261,295 ,346,377,407	Unknown

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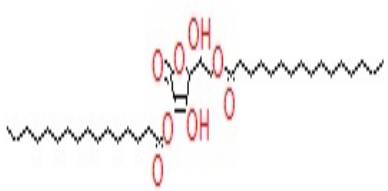
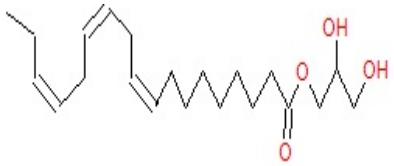
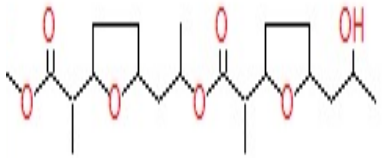
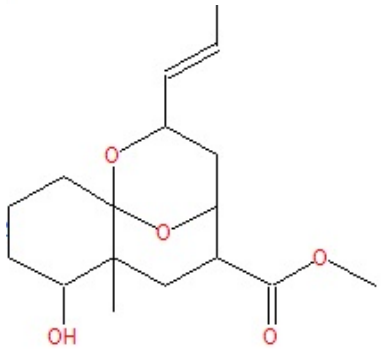
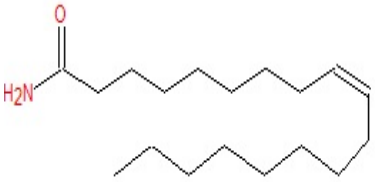
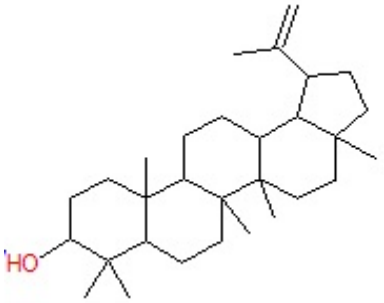
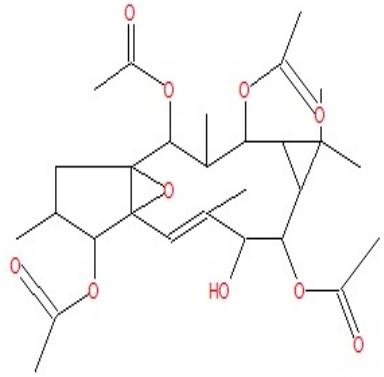
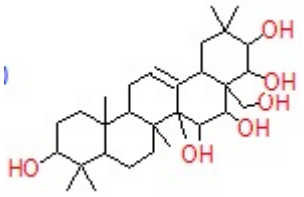
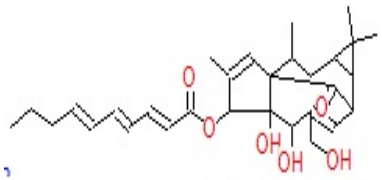
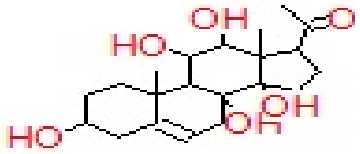
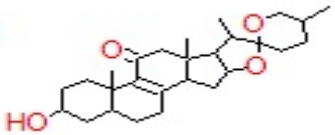
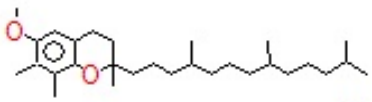
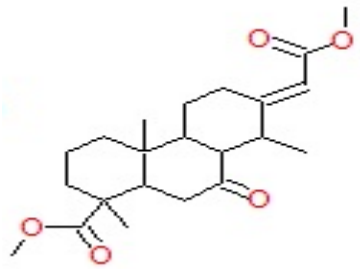
36.	I-(+)-Ascorbic acid 2,6-dihexadecanoate	15.275	$C_{38}H_{68}O_8$	652	652.49142		57,73,85,98,115,129,143,157,185,199,213,256,297,327,353,396,414	Anti-pigmentation effect
37.	9,12,15-Octadecatrinoic acid , 2,3-dihydroxypropyl ester , (Z,Z,Z)-	16.648	$C_{21}H_{36}O_4$	352	352.26136		57,67,79,95,109,135,155,173,232,261,291,321,352	Antiviral and anti-obesity properties
38.	1-Heptatriacotanol	17.266	$C_{37}H_{76}O$	536	536.58962		55,81,95,147,161,190,257	Biological activity mainly as anticancer, antineoplastic and anti-HIV
39.	Propanoic acid , 2-[5-(2-hydroxypropyl)tetrahydrofuran-2-yl]-,1-[5-(1-m)	17.970	$C_{21}H_{36}O_7$	400	400.246103		69,85,97,111,125,143,157,199,217,253,272,313,327,369,401	Anti-diabetic effect
40.	10,13-Dioxatricyclo[7.3.1.0(4,9)]tridecan-5-ol-2carboxylic acid,4-me	18.462	$C_{17}H_{26}O_5$	310	310.178024		55,69,81,93,139,152,179,211,250,278,310	Antifertility and anticancer activity

41.	9-Octadecenamide,(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		55,69,122,207,236,297,357,417,477	Anti- inflammatory effects
44.	Olean-12-ene3,15,16,21,22,28,-hexol,(3ß,15α,16α,21ß,22α)-	21.363	$C_{30}H_{50}O_6$	506	506.360739		135,190,207,231,249,280,298,334,352,439,488	Anti-tumourogenic properties

45.	2,4,6-Decatrienoic acid , 1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydro	21.597	$C_{30}H_{40}O_6$	496	496.28249		55,79,91,122,149,284,312,330,347,380,412,478	Antimalarial and anti-HIV
46.	Pregn-5-en-20-one,3,8,11,12,14,-pentahydroxy-,(3ß,11α,12ß,14ß)-	23.697	$C_{21}H_{32}O_6$	380	380.219889		55,97,138,153,171,209,224,242,274,311,344,362,380	Cardio-protective, analgesic, anti-mycotic, and immunomodulatory effects
47.	Spirost-8-en-11-one,3-hydroxy-,(3ß,5α,14ß,20ß,22ß,25R)-	26.289	$C_{27}H_{40}O_4$	428	428.29266		57,69,95,135,207,229,281,314,356,428	Antimicrobial, antioxidant and anti-inflammatory activities
48.	(+)-y-Tocopherol,O-methyl	26.495	$C_{29}H_{50}O_2$	430	430.38108		57,91,137,165,205,260,302,344,386,430	Anti-inflammatory and antioxidative effects
49.	1-Phenanthrenecarboxylic acid ,tetradecahydro-7-(2-methoxy-2-oxoe	27.611	$C_{22}H_{32}O_5$	376	376.224974		55,67,79,91,109,159,177,213,267,284,316,344,376	Unknown

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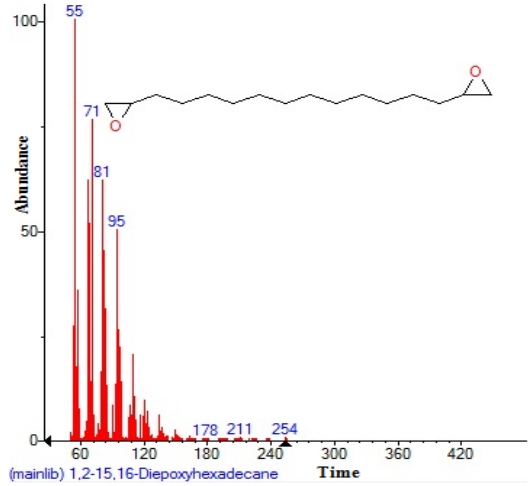

Figure 2. Structure of 1,2-15,16-Diepoxyhexadecane with 3.173 (RT) present in *Artemisia annua*.

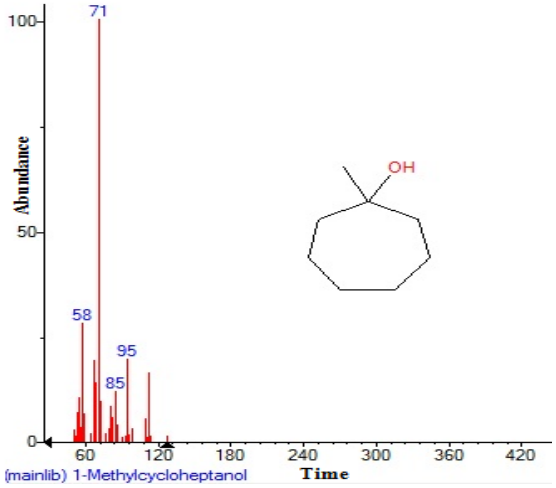

Figure 3. Structure of 1-Methylcycloheptanol 3.402 with (RT) present in *Artemisia annua*.

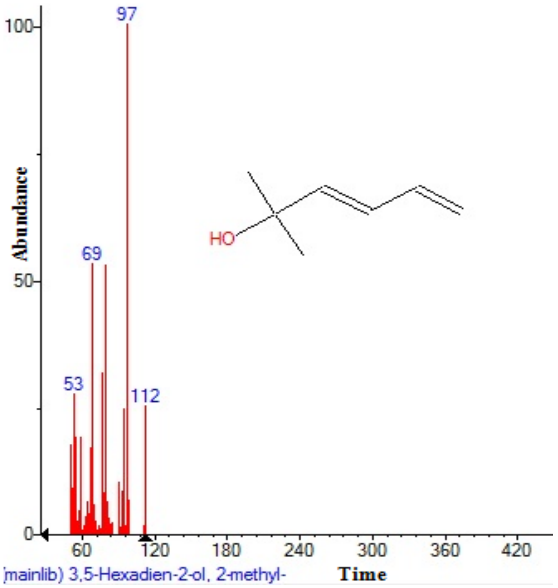

Figure 4. Structure of 3,5-Hexadien-2-ol2-methyl with 3.585 (RT) present in *Artemisia annua*.

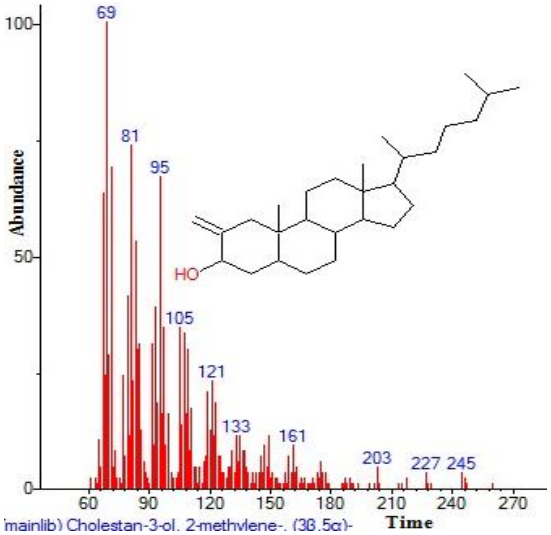

Figure 5. Structure of Cholestan -3-ol ,2-methylene, (3ß,5α) 3.739 with (RT) present in *Artemisia annua*.

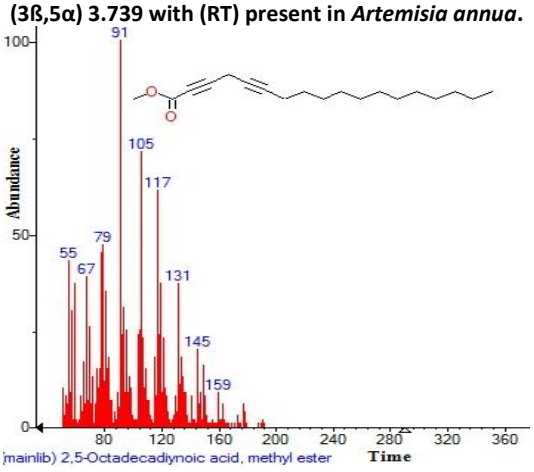

Figure 6. Structure of 2,5-Octadecadiynoic acid , methyl ester with 3.871 (RT) present in *Artemisia annua*.

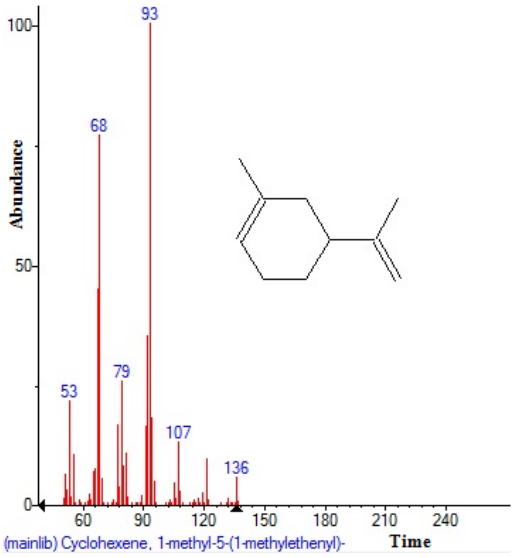

Figure 7. Structure of Cyclohexene,1-methyl-5-(1-methylethenyl) with 4.054 (RT) present in *Artemisia annua*.

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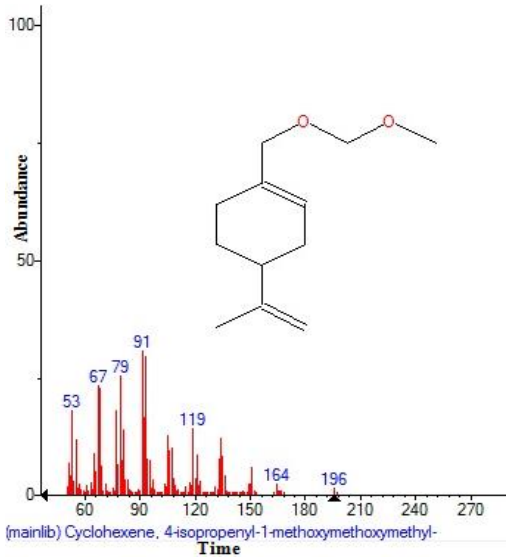

Figure 8. Structure of Cyclohexene,4-isopropenyl -1-methoxymethoxymethyl with 4.180 (RT) present in *Artemisia annua*.

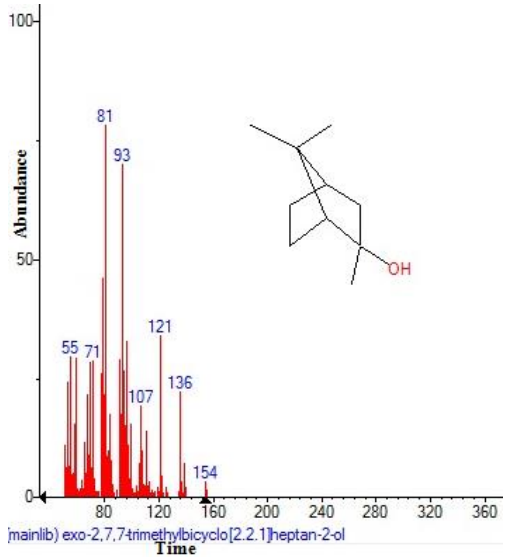

Figure 9. Structure of Exo-2,7,7-trimethylbicyclo[2.2.1]heptan-2-ol with 4.311 (RT) present in *Artemisia annua*.

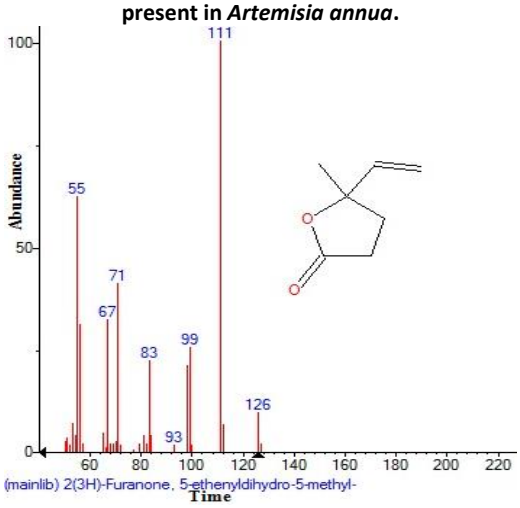

Figure 10. Structure of 2(3H)-Furanone,5-ethenyldihydro-5-methyl with 4.443 (RT) present in *Artemisia annua*.

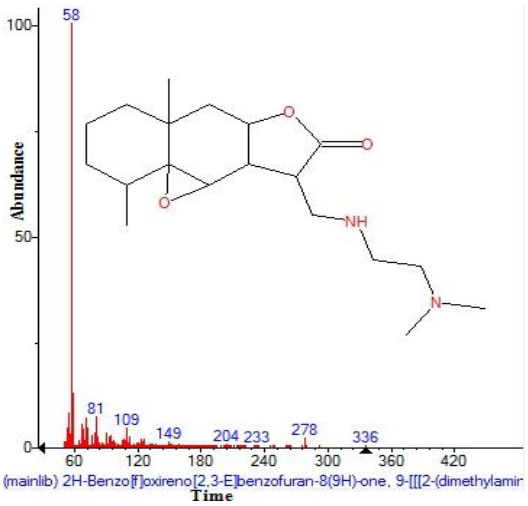

Figure 11. Structure of 2H-Benzo[f]oxireno[2,3-E]benzofuran-8(9H)-one,9[[[2-(dimethylamin) with 4.672 (RT) present in *Artemisia annua*.

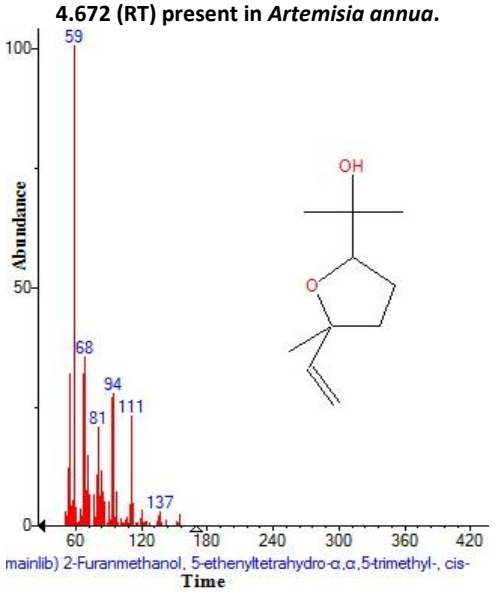

Figure 12. Structure of 2-Furanmethanol,5-ethenyltetrahydro-α,α,5-trimethyl-,cis with 4.803 (RT) present in *Artemisia annua*.

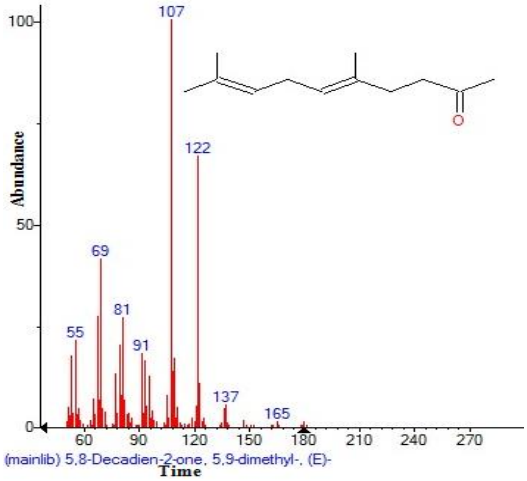

Figure 13. Structure of 5,8-Decadien-2-one,5,9-dimethyl-,(E) with 5.164 (RT) present in *Artemisia annua*.

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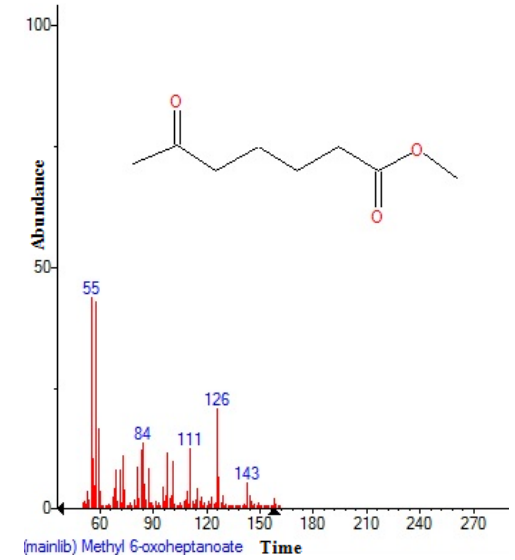

Figure 14. Structure of Methyl 6-oxoheptanoate with 5.376 (RT) present in *Artemisia annua*.

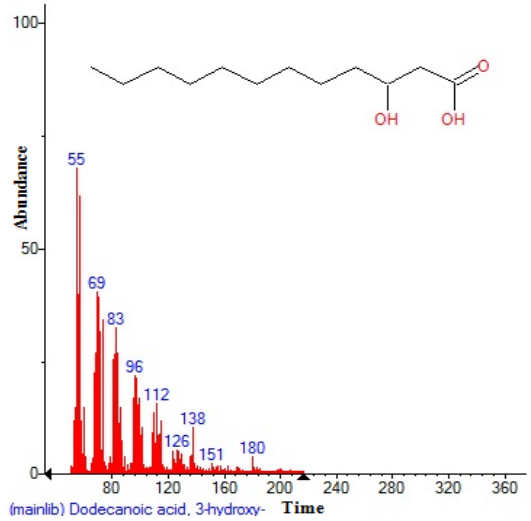

Figure 15. Structure of Dodecanoic acid,3-hydroxy with 5.244 (RT) present in *Artemisia annua*.

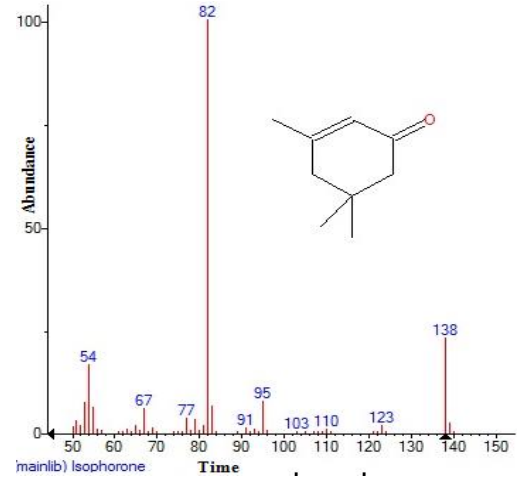

Figure 16. Structure of Isophorone with 5.450 (RT) present in *Artemisia annua*.

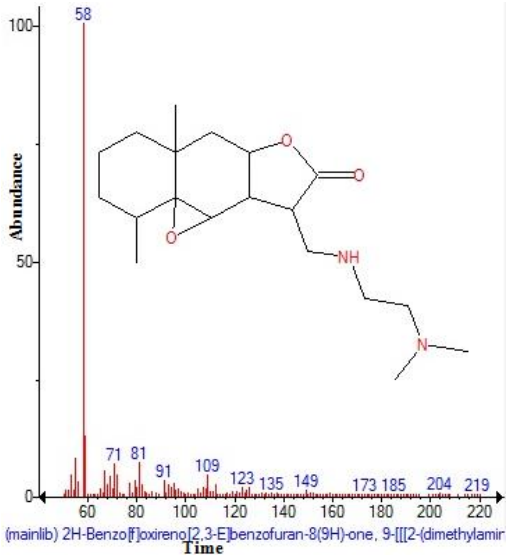

Figure 17. Structure of 2H-Benzo[f]oxireno[2,3-E]benzofuran-8(9H)-one,9-[[[2-(dimethylamin) with 5.708 (RT) present in *Artemisia annua*.

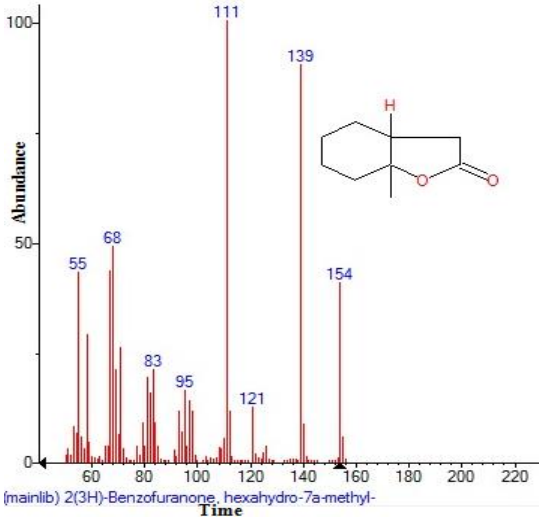

Figure 18. Structure of 2(3H)-Benzofuranone, hexahydro-7a-methyl with 5.994 (RT) present in *Artemisia annua*.

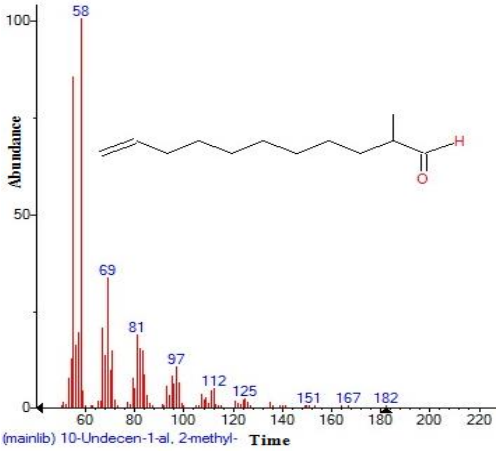

Figure 19. Structure of 10-Undecen-1-al,2-methyl with 6.411 (RT) present in *Artemisia annua*.

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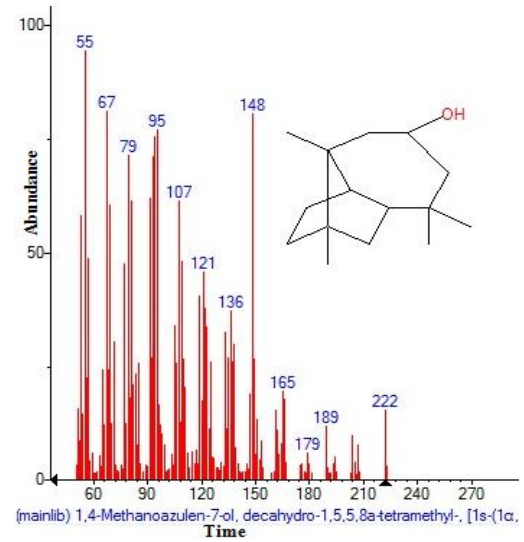

Figure 20. Structure of 1,4-Methanoazulen-7-ol,decahydro-1,5,5,8a-tetramethyl-,[1s-(1α,3a) with 7.527 (RT) present in *Artemisia annua*.

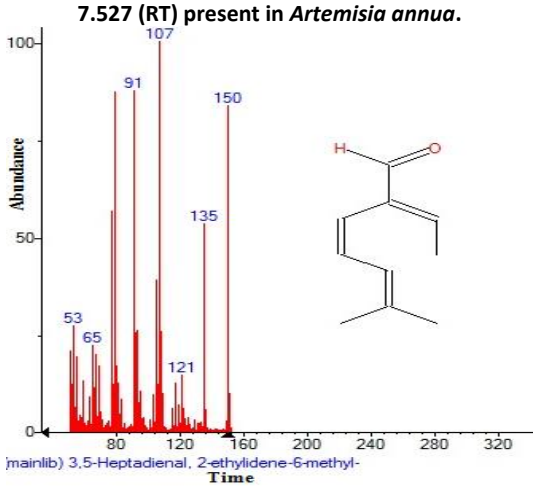

Figure 21. Structure of 3,5-Heptadienal,2-ethylidene-6-methyl with 7.939 (RT) present in *Artemisia annua*.

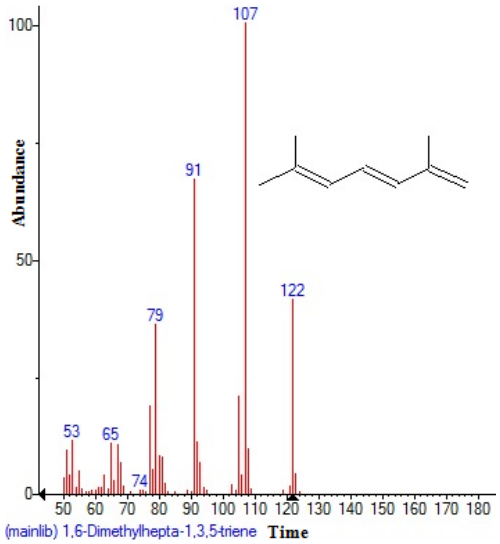

Figure 22. Structure of 1,6-Dimethylhepta-1,3,5-triene with 8.094 (RT) present in *Artemisia annua*.

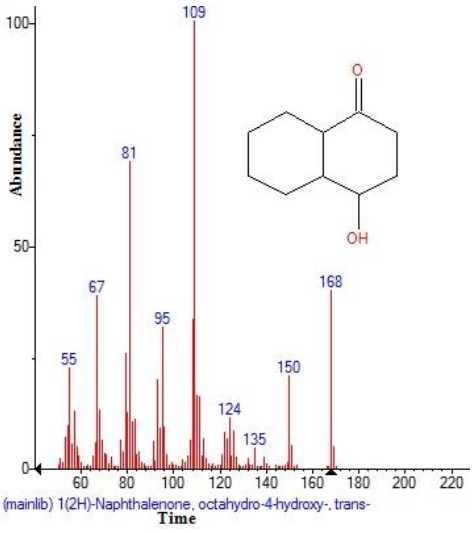

Figure 23. Structure of 1(2H)-Naphthalenone,octahydro-4-hydroxy-,trans with 8.563 (RT) present in *Artemisia annua*.

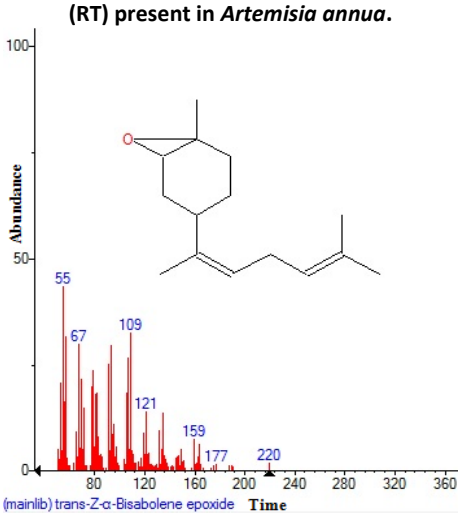

Figure 24. Structure of Trans-Z-α-Bisabolene epoxide with 9.026 (RT) present in *Artemisia annua*.

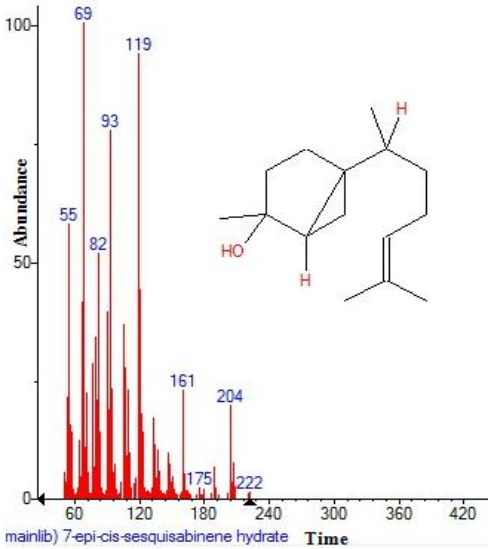

Figure 25. Structure of 7-epi-cis-sesquisabinene hydrate with 9.312 (RT) present in *Artemisia annua*.

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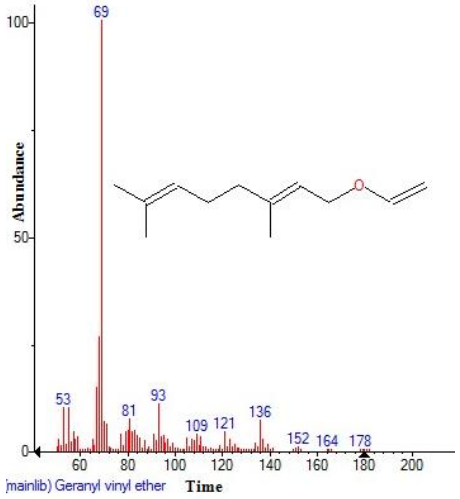

Figure 26. Structure of Geranyl vinyl ether with 9.621 (RT) present in *Artemisia annua*.

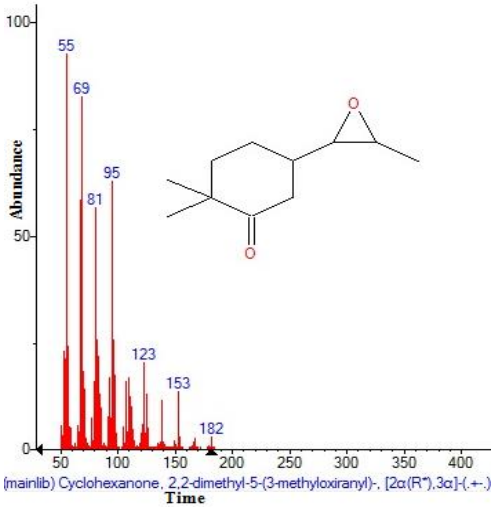

Figure 27. Structure of Cyclohexanone,2,2-dimethyl-5-(3-methyloxiranyl)-,[2α(R*),3α] with 9.804 (RT) present in *Artemisia annua*.

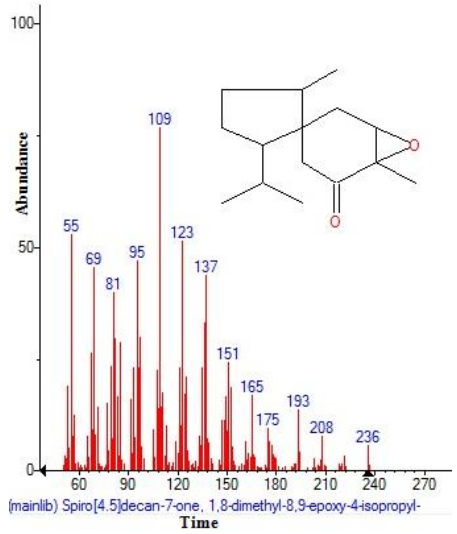

Figure 28. Structure of Spiro[4.5]decan-7-one,1,8-dimethyl-8,9-epoxy-4-isopropyl with 10.297 (RT) present in *Artemisia annua*.

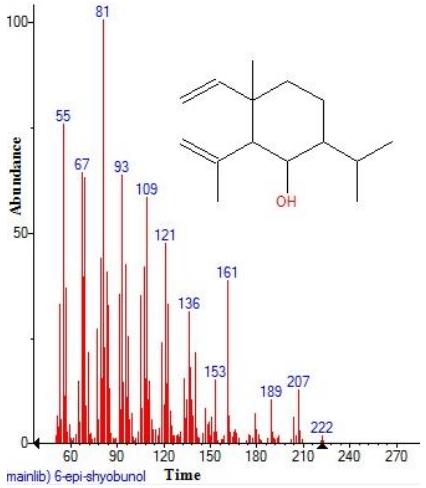

Figure 29. Structure of 6-epi-shyobunol with 10.583 (RT) present in *Artemisia annua*.

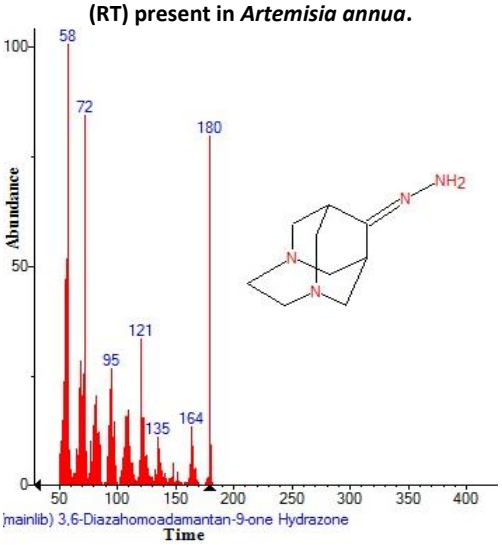

Figure 30. Structure of 3,6-Diazahomoadamantan-9-one Hydrazone with 11.046 (RT) present in *Artemisia annua*.

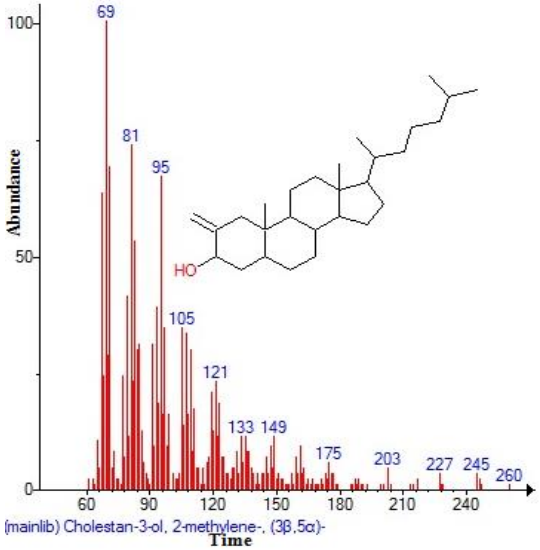

Figure 31. Structure of Cholestan-3-ol,2-methylene-,(3ß,5α) with 11.670 (RT) present in *Artemisia annua*.

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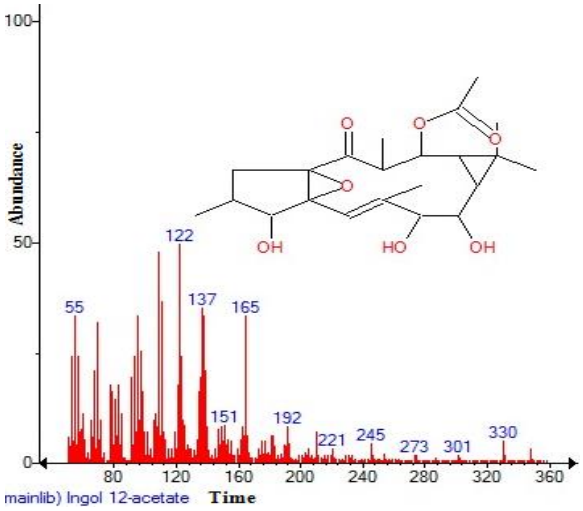

Figure 32. Structure of Ingol 12-acetate with 12.007 (RT) present in *Artemisia annua*.

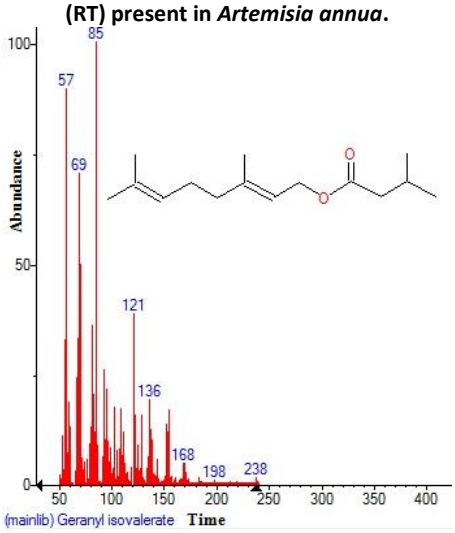

Figure 33. Structure of Geranyl isovalerate with 12.162 (RT) present in *Artemisia annua*.

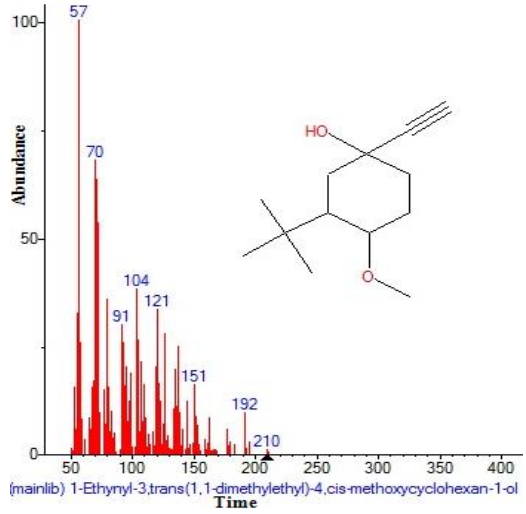

Figure 34. Structure of 1-Ethynyl-3,trans(1,1-dimethylethyl)-4,cis-methoxycyclohexan-1-ol with 12.602 (RT) present in *Artemisia annua*.

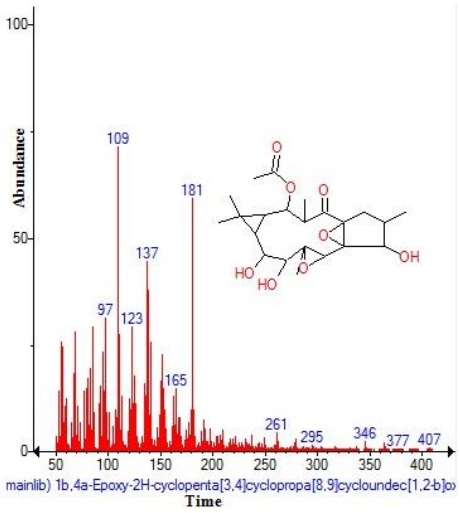

Figure 35. Structure of 1b,4a-Epoxy-2H-cyclopenta[3,4]cyclopropa[8,9]cycloundec[1,2-b]o with 12.345 (RT) present in *Artemisia annua*.

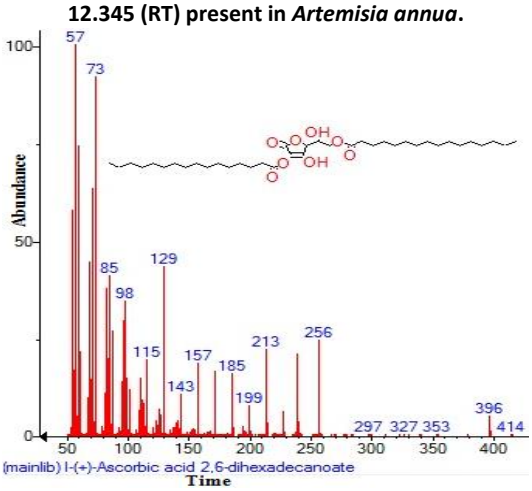

Figure 36. Structure of l-(+)-Ascorbic acid 2,6-dihexadecanoate with 12.275 (RT) present in *Artemisia annua*.

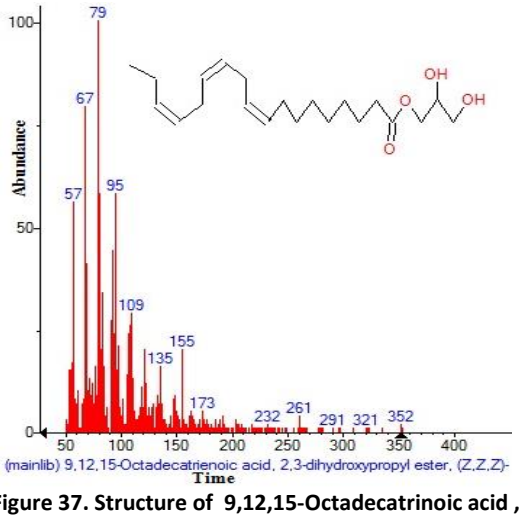

Figure 37. Structure of 9,12,15-Octadecatrinoic acid , 2,3-dihydroxypropyl ester , (Z,Z,Z) with 16.648 (RT) present in *Artemisia annua*.

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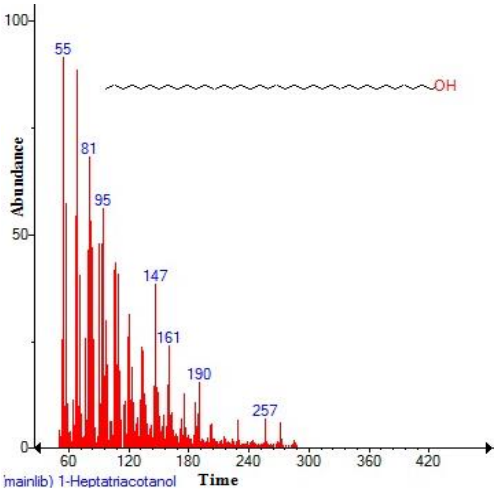

Figure 38. Structure of 1-Heptatriacotanol with 17.266 (RT) present in *Artemisia annua*.

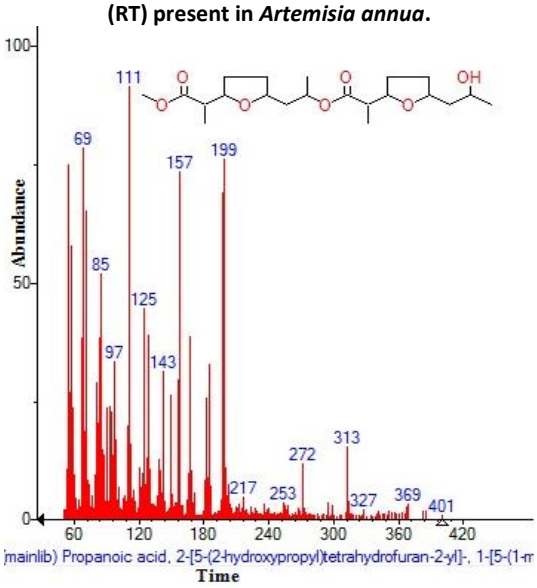

Figure 39. Structure of Propanoic acid , 2-[5-(2-hydroxypropyl)tetrahydrofuran-2-yl]-,1-[5-(1-m) with 17.970 (RT) present in *Artemisia annua*.

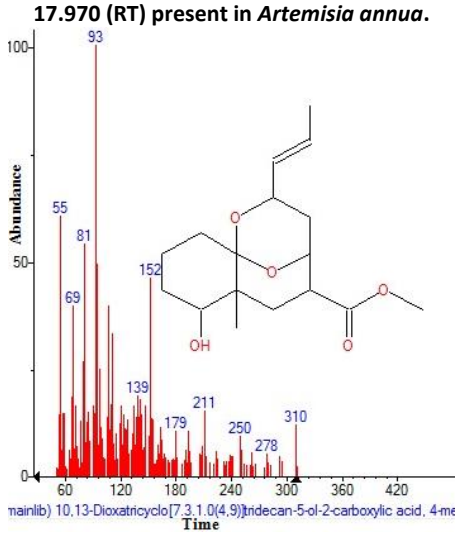

Figure 40. Structure of 10,13-Dioxatricyclo[7.3.1.0(4,9)]tridecan-5-ol-2carboxylic acid,4-me
With 18.462 (RT) present in *Artemisia annua*.

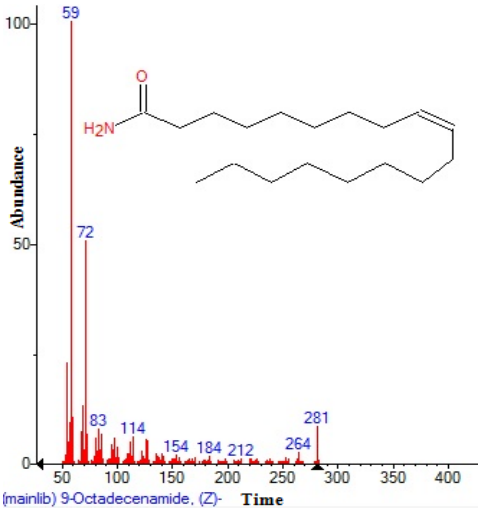

Figure 41. Structure of 9-Octadecenamide,(Z) with 18.851 (RT) present in *Artemisia annua*.

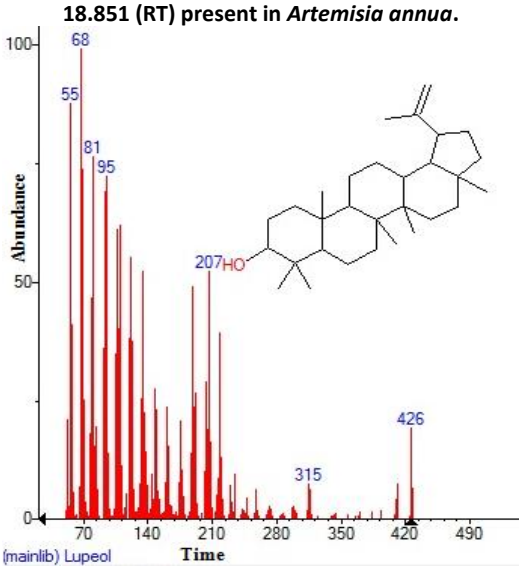

Figure 42. Structure of Lupeol with 20.327 (RT) present in *Artemisia annua*.

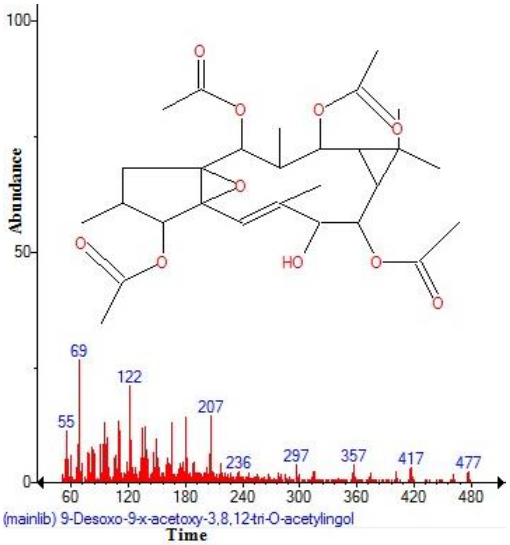

Figure 43. Structure of 9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol with 20.791 (RT) present in *Artemisia annua*.

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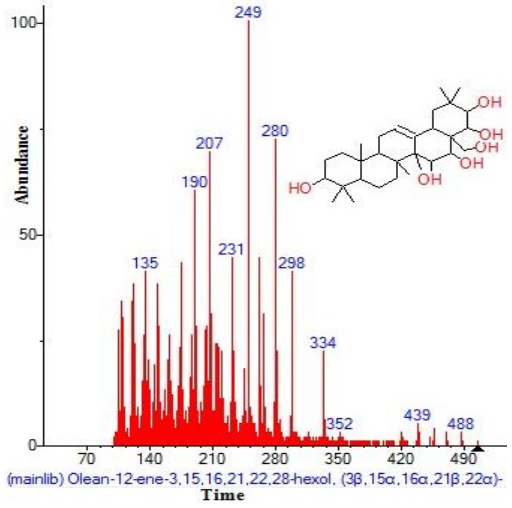

Figure 44. Structure of Olean-12-ene3,15,16,21,22,28,-hexol,(3ß,15α,16α,21ß,22α) with 21.363 (RT) present in *Artemisia annua*.

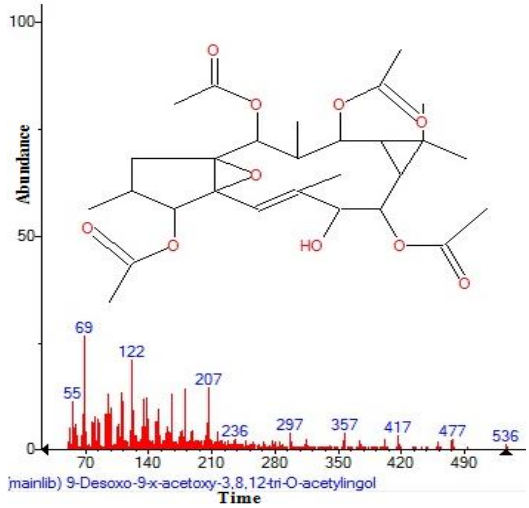

Figure 45. Structure of 9-Desoxo-9-x-acetoxy -3,8,12-tri-O-acetylingol with 21.649 (RT) present in *Artemisia annua*.

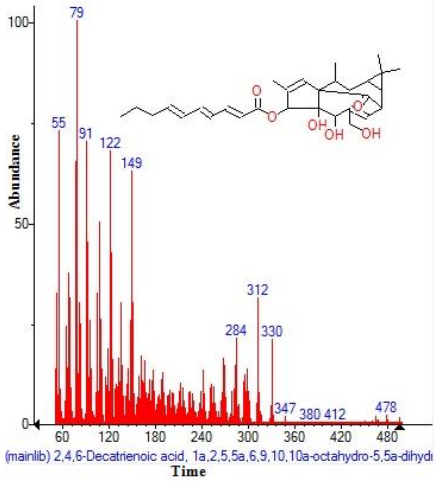

Figure 46. Structure of 2,4,6-Decatrienoic acid , 1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydro with 21.597 (RT) present in *Artemisia annua*.

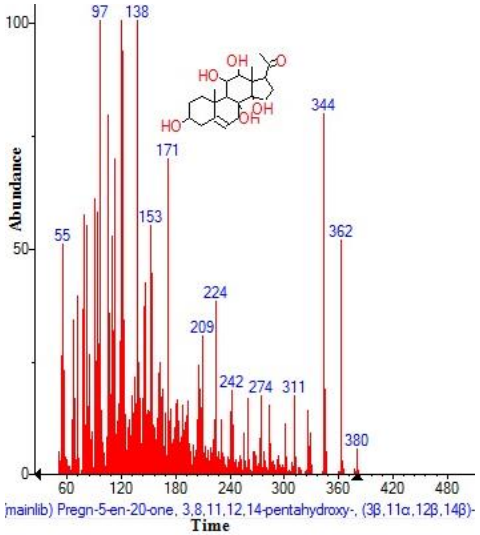

Figure 47. Structure of Pregn-5-en-20-one,3,8,11,12,14,-pentahydroxy-,(3ß,11α,12ß,14ß) with 23.697 (RT) present in *Artemisia annua*.

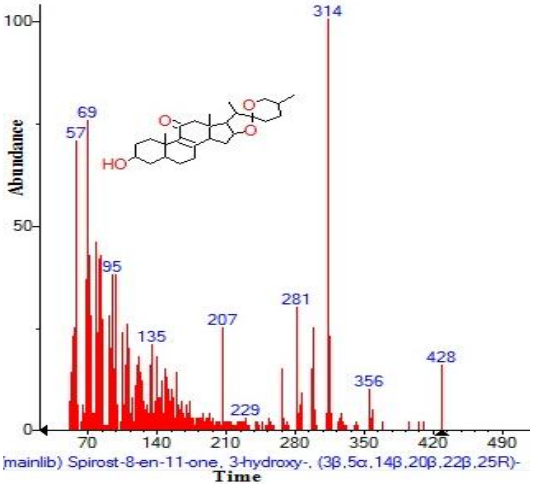

Figure 48. Structure of Spirost-8-en-11-one,3-hydroxy-,(3ß,5α,14ß,20ß,22ß,25R) with 26.289 (RT) present in *Artemisia annua*.

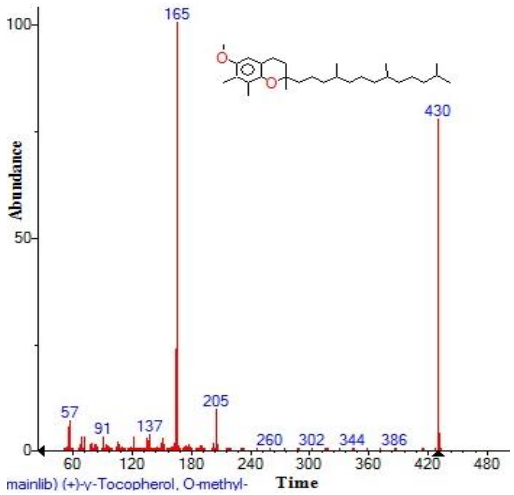

Figure 49. Structure of (+)-y-Tocopherol,O-methyl with 26.495 (RT) present in *Artemisia annua*.

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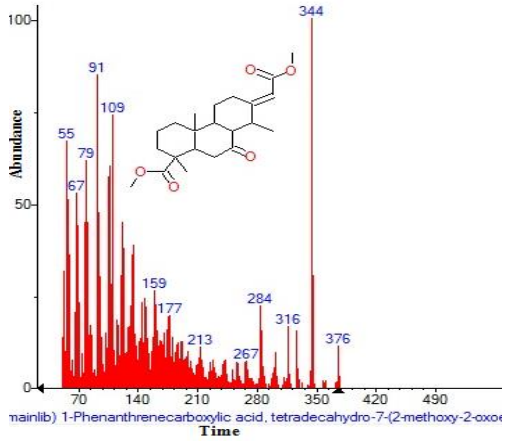

Figure 50. Structure of 1-Phenanthrenecarboxylic acid ,tetradecahydro-7-(2-methoxy-2-oxoe with 27.611 (RT) present in *Artemisia annua*.

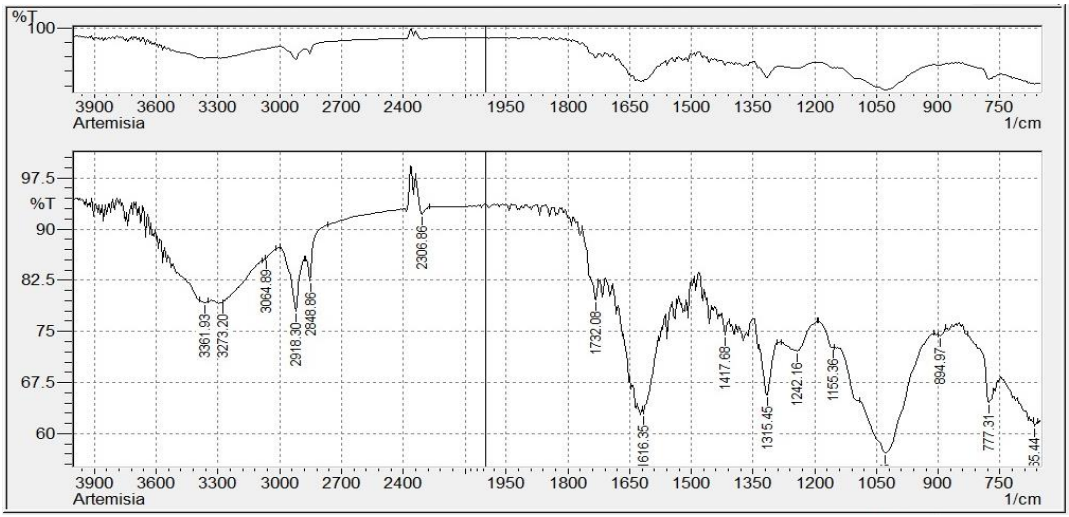

Figure 51. FT-IR peak values of *Artemisia annua*.

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

No.	Peak (Wave number cm-^{l})	Intensity	Bond	Functional group assignment	Group frequency
1.	665.44	61.059	-	Unknown	-
2.	777.31	64.617	C-H	Alkenes	675-995
3.	894.97	74.403	C-H	Alkenes	675-995
4.	1028.06	57.221	C-F stretch	Aliphatic fluoro compounds	1000-10150
5.	1155.36	72.616	C-O	Alcohols, Ethers, Carboxlic acids, Esters	1050-1300
6.	1242.16	72.142	C-O	Alcohols, Ethers, Carboxlic acids, Esters	1050-1300
7.	1315.45	56.648	NO2	Nitro Compounds	1300-1370
8.	1417.68	74.421	C-H	Alkanes	1340-1470
9.	1616.35	62.969	-	Unknown	-
10.	1732.08	79.650	-	Unknown	-
11.	2306.86	92.190	-	Unknown	-
12.	2848.86	82.386	H-O	H-bonded H-X group	2500-3500
13.	2918.30	77.950	C-H	Alkanes	2850-2970
14.	3064.89	85.717	H-O	H-bonded H-X group	2500-3500
15.	3273.20	79.265	O-H	Hydrogen bonded Alcohols, Phenols	3200-3600
16.	3361.93	79.184	O-H	Hydrogen bonded Alcohols, Phenols	3200-3600

Table 3. Zone of inhibition (mm) of test bacterial strains to *Artemisia annua* bioactive compounds and standard antibiotics.

/ Antibiotics *Artemisia annua*	Bacteria				
	Klebsiella pneumonia	*Escherichia coli*	*Pseudomonas eurogenosa*	*Staphylococcus aureus*	*Proteus mirabilis*
Artemisia annua	5.01±0.200	3.17±0.150	3.64±0.208	4.89±0.210	2.00±0.131
Cefotoxime	1.33±0.240	2.00±0.371	1.39±0.180	0.99±0.607	1.09±0.194
Streptomycin	2.42±0.561	0.700±0.106	1.00±0.427	0.91±0.122	0.99±0.210
Rifambin	0.96±0.100	1.09±0.320	0.95±0.510	1.81±0.140	1.46±0.290

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.

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