

## Detection of terpenes of Iraqi *Artemisia abrotanum* L. by GC/MS in hexane extract

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### Abstract:

*Artemisia abrotanum* L. is recently introduced plant in Iraq, in this study we conducted GS/MS for detection of terpene that are group of constituents abundant in this species qualitatively and quantitatively. We used hexane extract for the detection depending on the like dissolve like proce-

dure of extraction since they are mostly nonpolar compounds, they dissolve in nonpolar solvent like hexane thus the extraction procedure is conducted with this solvent. New compound was detected with hexane extract that wasn't present in previous studies.

**Key words:** terpenes, essential oil

الكشف عن التربينات لنبته الكولا العراقية (*Artemisia abrotanum* L.) بواسطة

كروماتوغرافيا الغاز ومطياف الكتلة لمستخلص الهسكزان

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### الخلاصة:

نبات الكولا من النباتات المستزرعة في العراق حاليا حيث اجرينا في هذه الدراسة تحاليل كمييه ونوعيه لمستخلص النبات باستخدام مذب الهكسان وبتقنيه كروماتوغرافيا الغاز ومطياف الكتلة لتشخيص التربينات التي هيه جزء من مكونات العطره لهذا الجنس من النبات. واطهرت النتائج عن وجود مركبات جديده اخرى لم يتم تشخيصها في دراسات سابقه .

الكلمات المفتاحية: تربينات ، زيوت العطره

### Introduction

*Artemisia abrotanum* L. are some fragrant varieties with significant part of the time, they are used for remedial reason and additionally tried for the nearness of the auxiliary metabolites like: coumarins, flavonoids, terpene subsidiaries and phenolic acids. A large number of which are of pharmacological impact. For instance, spasmolytic flavonoids have been

distinguished from the methanol concentrate of Southernwood (*Artemisia abrotanum* L.) as the principals fundamentally in charge of watched smooth muscle loosening up movement of this plant. southernwood has a place with the *Artemisia* class. It is utilized as a sweet-smelling plant and in customary drug for

the treatment of an assortment of clutters, for example, contaminations (or fiery sicknesses) of the upper respiratory tract <sup>[1]</sup>. Concentrates on the confinement and portrayal of pharmacologically dynamic constituents have concentrated on the essential oil, which contains a few bioactive particles that give the plant its mitigating, expectorant, spasmolytic, germ-free and antimicrobial exercises Volatile oils are separated into two classes dependent on their biosynthesis <sup>[2]</sup> Acetate-mevalonic acid

pathway that is called terpenes and Shikimic acid-phenylpropanoid pathway that are aromatic. Terpenes are dynamic mixes in plant with in excess of 20,000 known structures, terpenoid structures might be separated into isoprene units which produce by acetic acid derivation mevalonate pathway. Unpredictable oils are utilized for its enhancing, fragrance, restorative action, beginning material for different mixes <sup>[3]</sup>.



**Figure (1):** picture of *Artemisia abrotanum*

Terpenoids are volatile substances which give plants and flowers their fragrance. They occur widely in the leaves and fruits of higher plants, conifers, citrus and eucalyptus. Most natural terpenoids hydrocarbon have the general formula  $(C_5H_8)_n$  where the value of n or number of carbon atoms present in the structure.

As it can be Monoterpenoids ( $C_{10}H_{16}$ ), Sesquiterpenoids ( $C_{15}H_{24}$ ), Diterpenoids ( $C_{20}H_{32}$ ), Sesterpenoids ( $C_{25}H_{40}$ ), Troterpenoids ( $C_{30}H_{48}$ ), Tetraterpenoids ( $C_{40}H_{64}$ ) <sup>[4-6]</sup>.

Each class of terpenes can be further subdivided into subclasses according to the number of rings present in the structure as Acyclic terpenoids: They contain open structure, Monocyclic terpenoids: They contain one ring in the

structure, Bicyclic terpenoids: They contain two rings in the structure, Tricyclic terpenoids: They contain three rings in the structure and Tetracyclic terpenoids: They contain four rings in the structure <sup>[7]</sup>.

Terpene have wide range of biological activities though new constituent's we identified like lupeol exhibit anticancer against variety of cell line like MC7 cell that shows activity against it by more than  $50\mu M$ , antiprotozoal, chemo preventive and anti-inflammatory <sup>[8]</sup>. eucalyptol shows promising pharmacological activity as gastro protective, bronchodilator, anti-inflammatory effect, vasodilator effect and exhibit ant nociceptive properties thereby, indicating a potential calmative and depressant action on the central

nervous system [9]. camphor shows antitussive, nasal decongestant, expectorant, analgesic properties, antipruritic and modulate the activities of hepatic enzymes involved in phase I and phase II drug metabolism [10]. Borneol is used for analgesia and anesthesia in traditional Chinese and Japanese medicine [11].

## Material and methods

### Collection of the plant

*Artemisia abrotanum* L. is collected from algriaate area in November 2018. The Aerial part of the plant was cut, washed with water and dried under shield at room temperature for three days then grinded by mechanical grinder to fine powder

### Extraction procedure

Powder plant of the arial part of *Artemisia abrotanum* L. was weighted to 50g and added to two thimbles of two 250 ml soxhlet apparatus then added hexane 500ml divided for the two soxhlet. The extraction process continues till clearances then we collect the hexane extract filter and evaporate by rotary evaporator till dryness. The extracted was weight 1.561g and sent to GC/MS at the ministry of science and technology and tested for the presence of terpenes.

### Terpenes test

five ml of plant hexane extract were mixed with 2 ml of chloroform followed by adding 3 ml of concentrated (H<sub>2</sub>SO<sub>4</sub>). A layer of the reddish-brown coloration was formed at the interface indicates positive result [12].

## Result and discussion

### Terpene detection

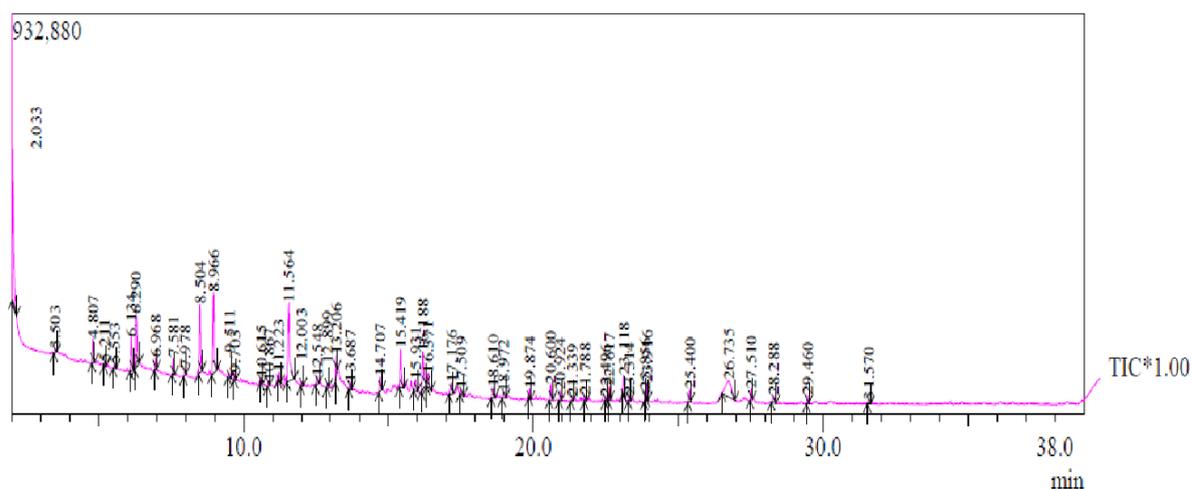
Terpenes are large group of volatile, unsaturated hydrocarbons commonly found in the essential oils of plants, especially cannabis, conifers, and citrus trees. These compounds are responsible for a plant's aromatic fragrance that is found in plant resin glands, or trichomes [13]. Terpenes give reddish- brown ring up on reaction with H<sub>2</sub>SO<sub>4</sub> that is indicate of positive result of as seen in figure (2).



Figure (2): terpene test

### GC/MS analysis

Analysis of hexane extract of aerial parts of *Artemisia abrotanum* L. was performed by GC/MS. The result revealed the presence of 3-carene, thujene, o-cymene, eucalyptol, sabinene, isopregol, camphor, borneol, myrtenol, germacrene D, caryophyllene, cedrene, caryophyllene oxide, Pseudosarsasapogenin-5,20-dien and lupeol that was confirmed by GC-MS and the chromatogram as in figure (3) and table (1).



**Figure (3): GC/MS chromatogram of *Artemisia abrotanum* L.**

Chromatogram showed peaks for each compound summarized in table (1) with retention times that is identified by

comparing their mass spectra with a database library of National Institute of Standard and Technology (NIST08).

**Table (1): Essential oil constituent of hexane extract of *Artemisia abrotanum* L.**

Name	Peak	Retention time	Area%	Mass peak (m/z)	Mass spectrum shown in figure:
Eucalyptol	7	6.293	7.19	154	5
Camphor	11	8.508	6.31	152	8
Borneol	12	8.967	8.96	139	9
Lupeol	45	26.733	7.00	218	17
3-carene	3	4.808	2.00	136	4
Isopergol	9	7.583	1.14	154	7
Cedrene	24	14.708	1.38	204	14
Caryophyllene oxide	28	16.375	1.99	220	15
Sabinene	8	6.967	0.77	154	6
Myrtenol	13	9.508	0.47	152	10
Germacrene D	21	12.900	0.27	204	12
Caryophyllene	23	13.683	0.54	204	13
Citronellol	41	23.317	0.38	157	16
trans-Piperitol	14	9.700	0.38	154	11

The mass spectrum is shown in the following figures (4-17):

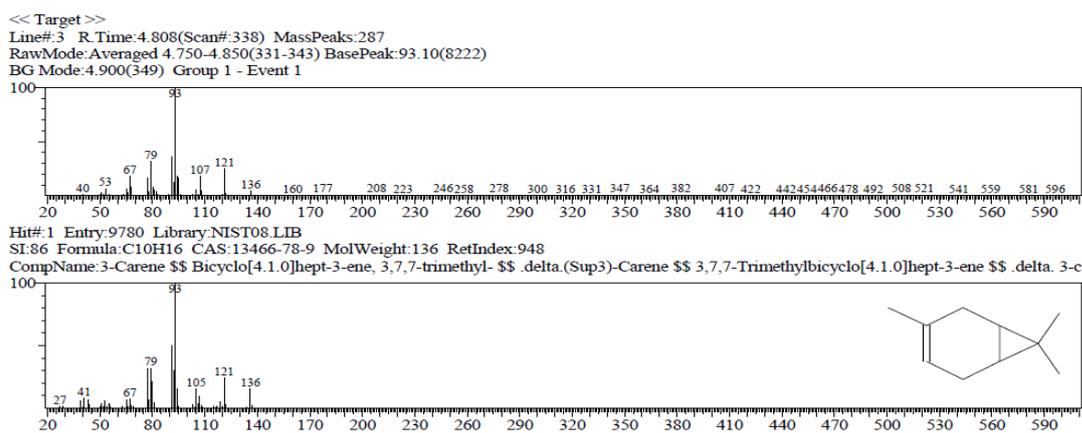


figure (4): mass spectrum of carene

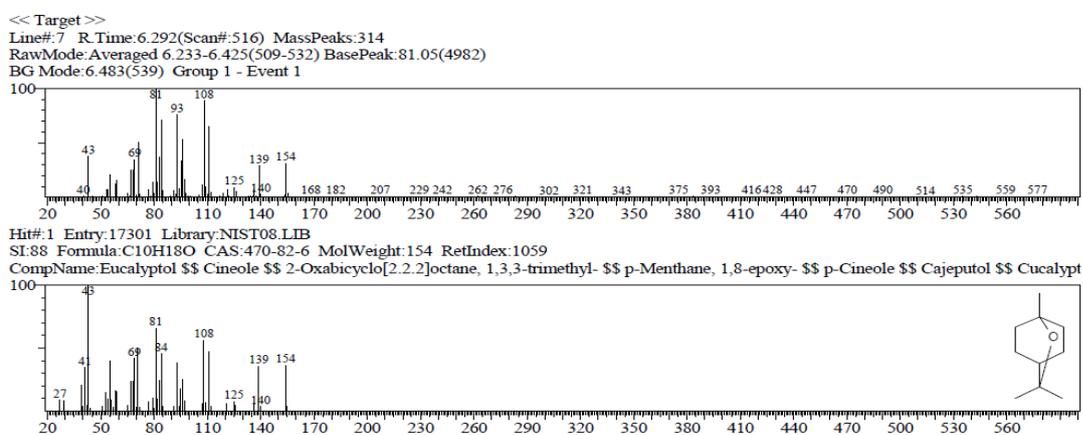


figure (5): mass spectrum of Eucalyptol

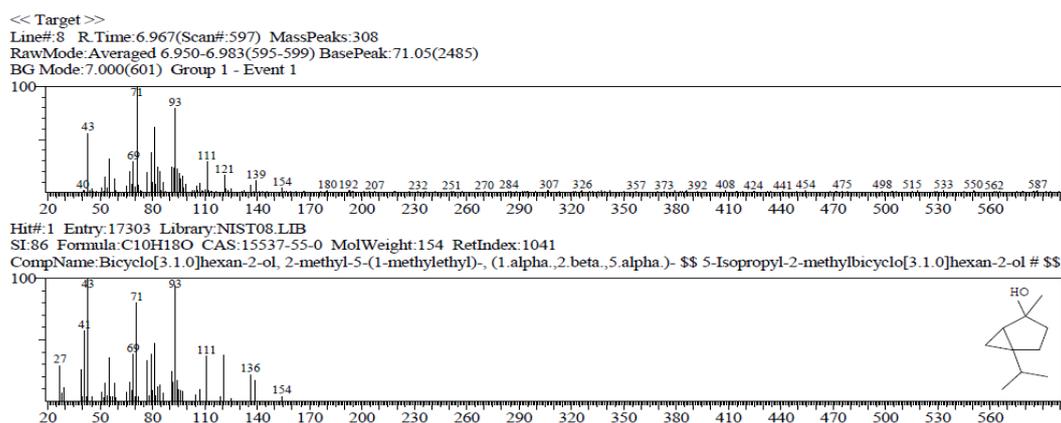


figure (6): mass spectrum of Sabinene

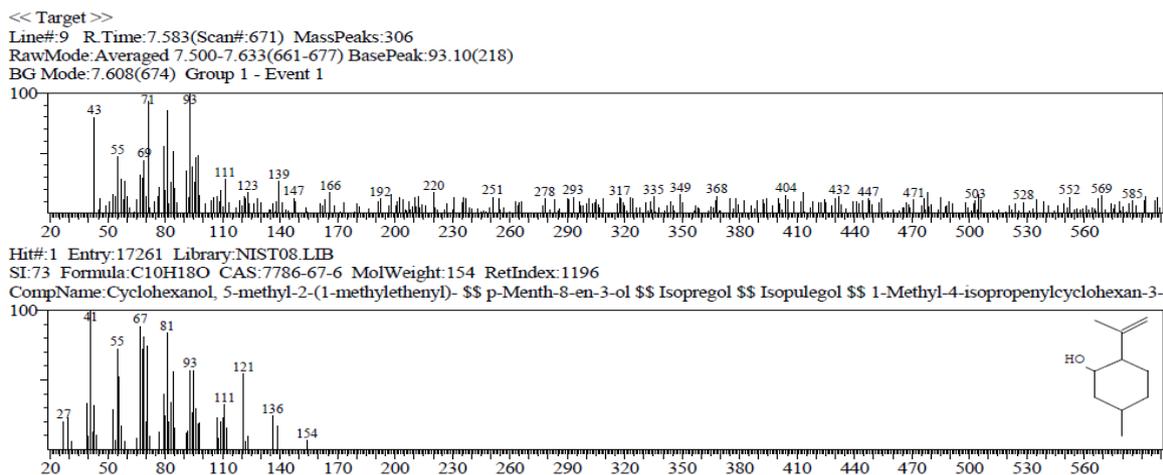


figure (7): mass spectrum of Isopregol

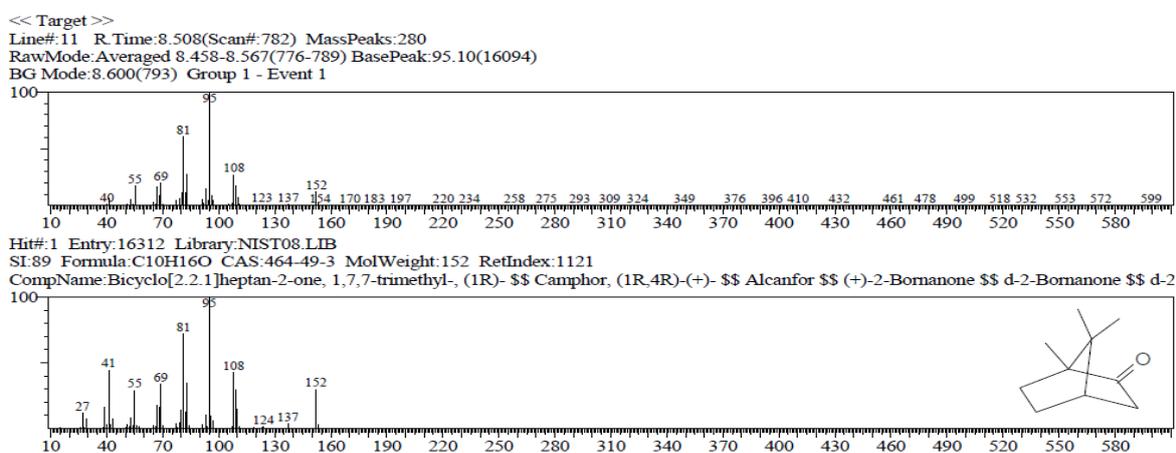


figure (8): mass spectrum of Camphor

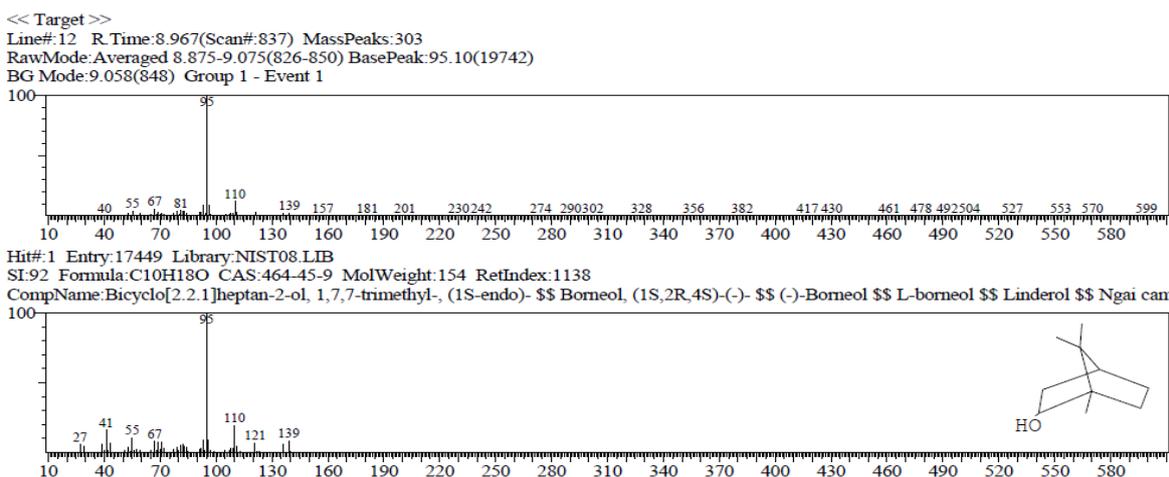
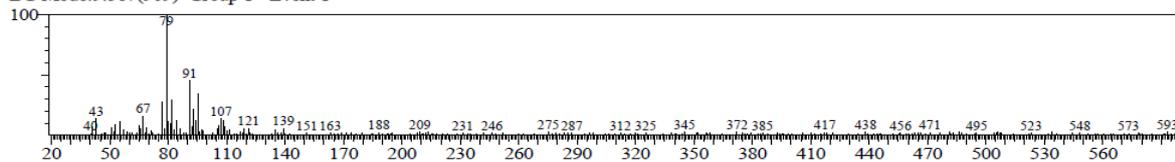


figure (9): mass spectrum of Borneol

&lt;&lt; Target &gt;&gt;

Line#:13 R.Time:9.508(Scan#:902) MassPeaks:312  
 RawMode:Averaged 9.458-9.550(896-907) BasePeak:79.05(1436)  
 BG Mode:9.567(909) Group 1 - Event 1



Hit#:1 Entry:16233 Library:NIST08.LIB  
 SI:83 Formula:C10H16O CAS:19894-97-4 MolWeight:152 RefIndex:1191  
 CompName:(-)-Myrtenol \$\$ Bicyclo[3.1.1]hept-2-ene-2-methanol, 6,6-dimethyl-, (1R)- \$\$

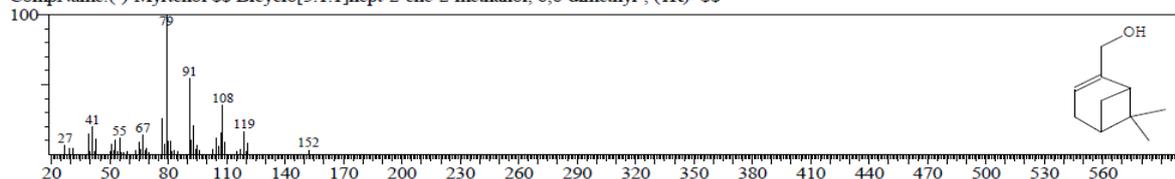
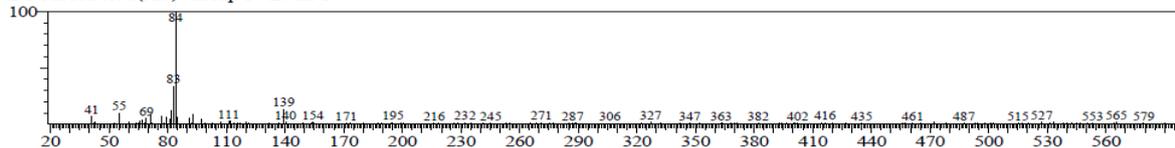


figure (10): mass spectrum of Myrtenol

&lt;&lt; Target &gt;&gt;

Line#:14 R.Time:9.700(Scan#:925) MassPeaks:301  
 RawMode:Averaged 9.658-9.742(920-930) BasePeak:84.05(2295)  
 BG Mode:9.658(920) Group 1 - Event 1



Hit#:1 Entry:17415 Library:NIST08.LIB  
 SI:78 Formula:C10H18O CAS:16721-39-4 MolWeight:154 RefIndex:1175  
 CompName:2-Cyclohexen-1-ol, 3-methyl-6-(1-methylethyl)-, trans- \$\$ p-Menth-1-en-3-ol, trans- \$\$ trans-p-Menth-1-en-3-ol \$\$ trans-Piperitol \$\$ 6-I

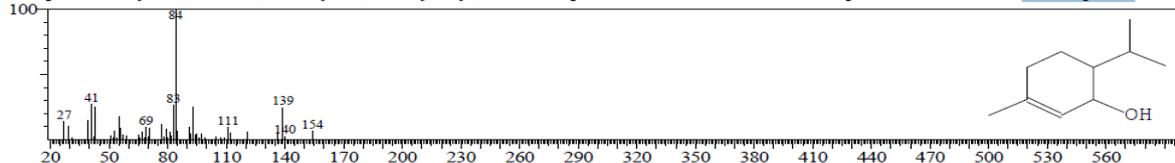
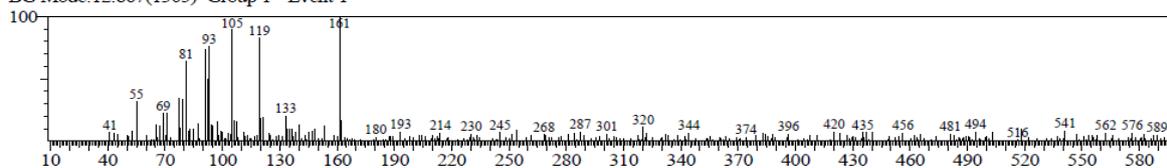


figure (11): mass spectrum of trans-Piperitol

&lt;&lt; Target &gt;&gt;

Line#:21 R.Time:12.900(Scan#:1309) MassPeaks:301  
 RawMode:Averaged 12.875-12.942(1306-1314) BasePeak:161.30(492)  
 BG Mode:12.867(1305) Group 1 - Event 1



Hit#:1 Entry:45510 Library:NIST08.LIB  
 SI:76 Formula:C15H24 CAS:23986-74-5 MolWeight:204 RefIndex:1515  
 CompName:1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]- \$\$ Germacene D \$\$ 8-Isopropyl-1-methyl-5-methylene-1,6-cy

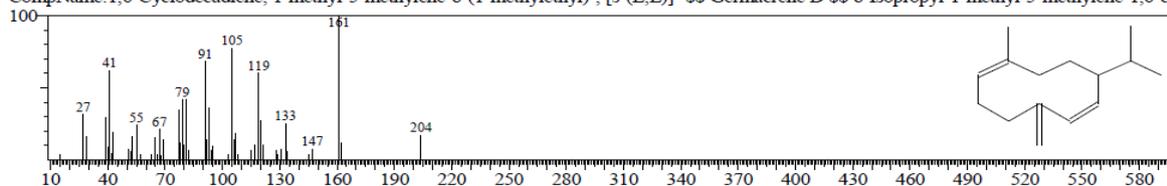
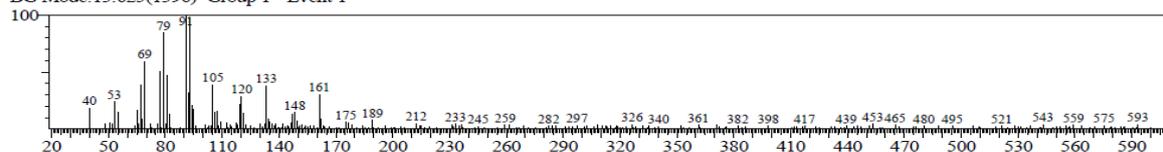


figure (12): mass spectrum of Germacene D

&lt;&lt; Target &gt;&gt;

Line#:23 R.Time:13.683(Scan#:1403) MassPeaks:291  
 RawMode:Averaged 13.667-13.733(1401-1409) BasePeak:91.05(1032)  
 BG Mode:13.625(1396) Group 1 - Event 1



Hit#:1 Entry:45431 Library:NIST08.LIB

SI:83 Formula:C15H24 CAS:87-44-5 MolWeight:204 RetIndex:1494

CompName:Caryophyllene \$\$ Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-, [1R-(1R\*,4E,9S\*)]- \$\$

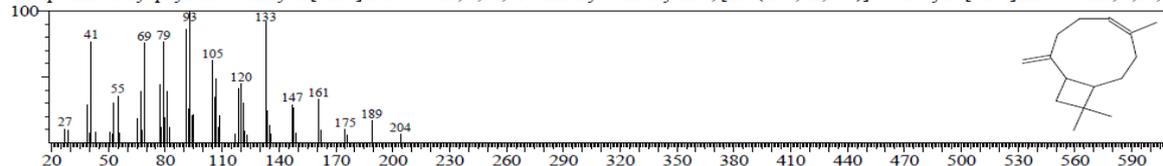
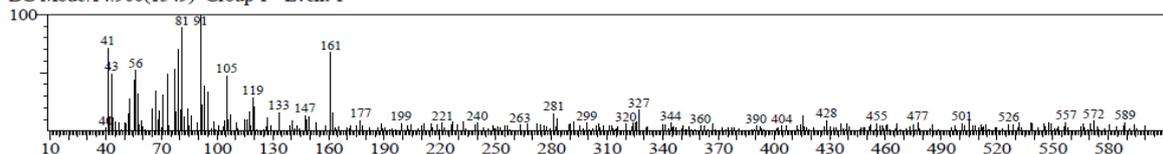


figure (13): mass spectrum of Caryophyllene

&lt;&lt; Target &gt;&gt;

Line#:24 R.Time:14.708(Scan#:1526) MassPeaks:319  
 RawMode:Averaged 14.525-14.858(1504-1544) BasePeak:91.05(455)  
 BG Mode:14.900(1549) Group 1 - Event 1



Hit#:1 Entry:45384 Library:NIST08.LIB

SI:74 Formula:C15H24 CAS:11028-42-5 MolWeight:204 RetIndex:1398

CompName:Cedrene \$\$ Cedr-8(15)-ene # \$\$

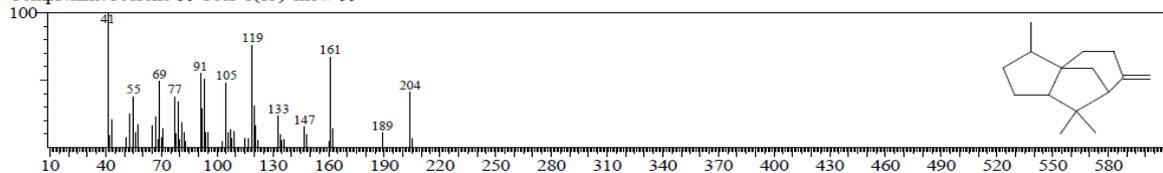
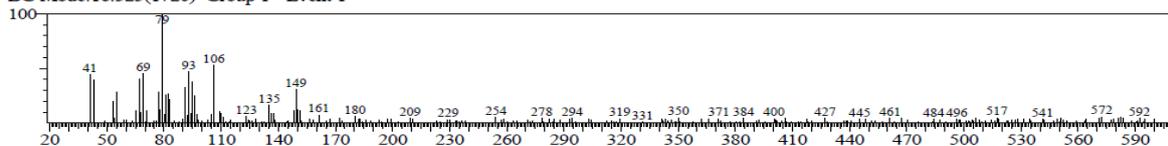


figure (14): mass spectrum of Cedrene

&lt;&lt; Target &gt;&gt;

Line#:28 R.Time:16.375(Scan#:1726) MassPeaks:285  
 RawMode:Averaged 16.308-16.475(1718-1738) BasePeak:79.05(678)  
 BG Mode:16.325(1720) Group 1 - Event 1



Hit#:1 Entry:55919 Library:NIST08.LIB

SI:78 Formula:C15H24O CAS:1139-30-6 MolWeight:220 RetIndex:1507

CompName:Caryophyllene oxide \$\$ 5-Oxatricyclo[8.2.0.0(4,6)]dodecane, 4,12,12-trimethyl-9-methylene-, [1R-(1R\*,4R\*,6R\*,10S\*)]- \$\$

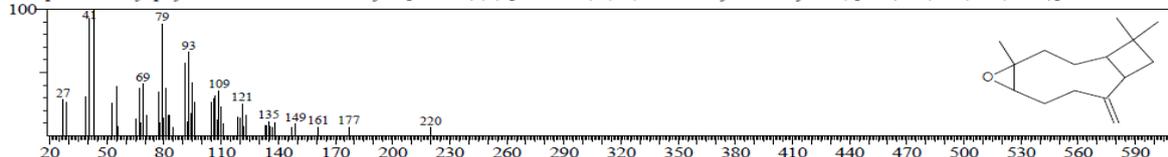


figure (15): mass spectrum of Caryophyllene oxide

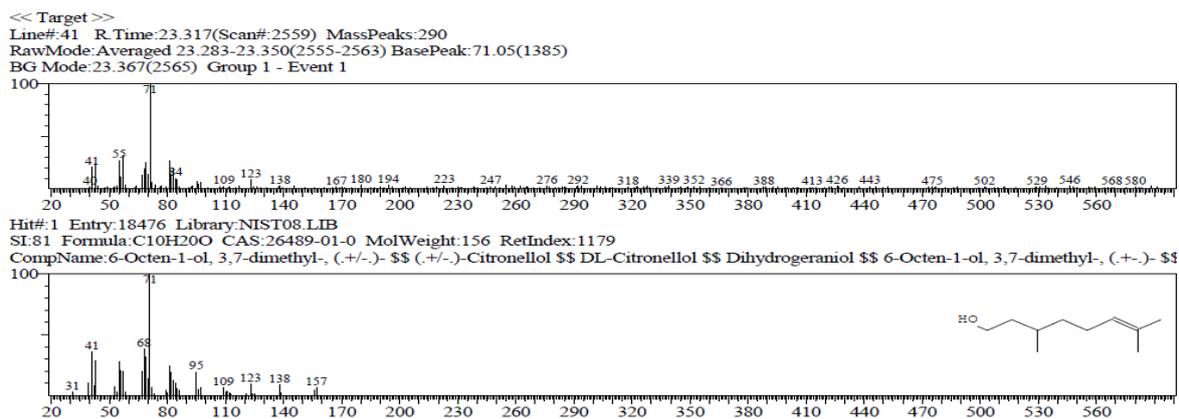


figure (16): mass spectrum of Citronellol

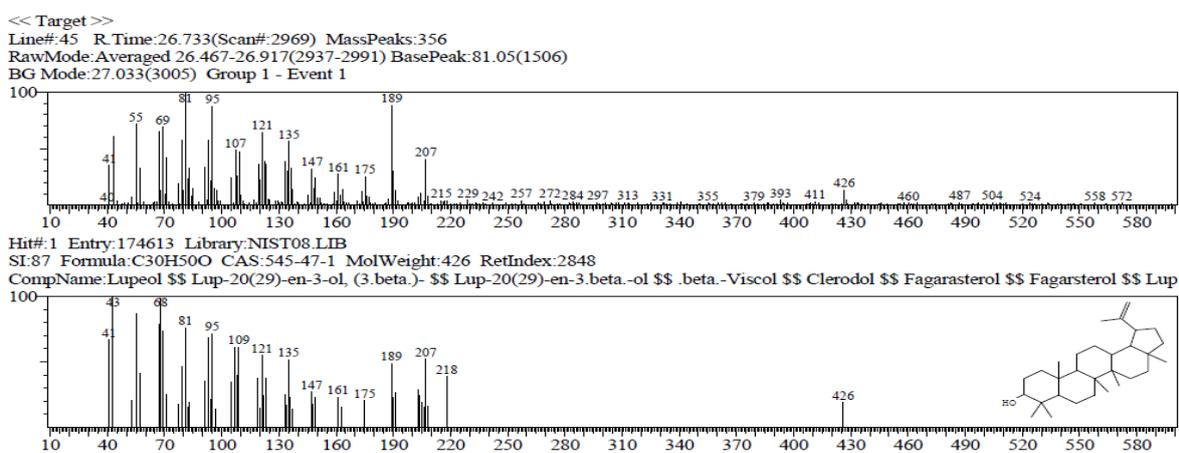


figure (17): mass spectrum of Lupeol

The analysis shows the presence of major constituents like Eucalyptol (7.19%), camphor (6.31%), Borneol (8.96%), Lupeol (7.00%) and to less extent 3-Carene (2.00%), o-Cymene (2.93%), Isopergol (1.14%), Cedrene (1.38%), Caryophyllene oxide (1.99%) with minor constituents as Sabinene (0.77%), Myrtenol (0.47%), GermacreneD (0.27%), Caryophyllene (0.54%), Citronellol (0.38%), Pseudosarsapogenin (0.49%) and trans-Piperitol(0.38%) in the hexane extract of *Artemisia abrotanum*. The result shows matching of four compounds although there is different percentage of some the matching essential oil constituents that was done by Sarah and co-workers with the presence of cymene (3.60%), camphor (0.09%), borneol (8.38%) and Eucalyptol (1.12%) [14,15].

By comparing the constituent of essential oil with the one from Serbia the result shows cross matching with constituents like caryophyllene oxide (1.6%), caryophyllene (0.6%), trans-Piperitol (0.6%), camphor (3.5%) with major constituent 1, 8-cineole (10.5 %), bisabolol oxide A acetate (8.7%), germacrene D (6.5 %) and Borneol (6.0 %) [16].

## Conclusion

This study has concluded that Iraqi cultivated *Artemisia abrotanum* L. contain terpenes in which its proven by chemical test and via gas chromatography mass spectroscopy (GC/MS) of its hexane extract. hexane use used as solvent for extraction and detection of terpene quantitatively and qualitatively by GC/MS

by like dissolve like procedure of extraction by which this method is proven to be accurate and powerful.

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