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Full Length Research Paper

Identification of five newly described bioactive chemical compounds in methanolic extract of *Mentha viridis* by using gas chromatography - mass spectrometry (GC-MS)

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The bioactive compounds were screened by gas chromatography-mass spectrometry (GC-MS) method. Twenty one bioactive phytochemical compounds were identified in the methanolic extract of *Mentha viridis* using GC-MS method. The identification of phytochemical compounds is based on the peak area, retention time, molecular weight and molecular formula. GC-MS analysis of *M. viridis* revealed the existence of the 3,6-Octadecadiynoic acid, methyl ester, 2,5-Dimethyl-4-hydroxy-3(2H)-furanone, 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, benzofuran, R-Limonene, 2-methoxy-4-vinylphenol, 2-hydroxy-5-methylbenaldehyde, tetra-acetyl-d-xylonic nitrile, Ficusin, Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl, n-Hexadecanoic acid, 7-Methyl-Z-tetradecen-1-ol acetate, Ethyl 9,12,15-octadecatrienoate, Methyl 19-methyl-eicosanoate, Ethyl iso-allocholate, and Tocopherol. Five new bioactive chemical compounds 3-(N,N-Dimethyllayrylammonio), 1b,4a-eboxy-2H-cyclopenta [3,4]cyclopropal[8,9] cycloundec, 5H-Cyclopropa[3,4]benz [1,2-e]azulene-5-one, 2,2,4-Trimethyl-3-(3,8), 12,16-tetramethyl-hepta deca, and 4H-Cyclopropa[5,6]benz [1,2:7,8] azulene[5,6-b] oxiren-4-one are described and may in future be suitable sources for phytotherapy purposes. Mentha viridis contain chemical constitutions which may be useful for various herbal formulation exhibiting cardiac tonic, analgesic, antiasthamatic, anti-inflammatory and antipyretic properties.

Key words: Bioactive compounds, chromatography-mass spectrometry (GC-MS) analysis, *Mentha viridis*.

INTRODUCTION

Mentha viridis, (Lamiaceae family) commonly known as garden or green mint, is originally a native of the Mediterranean region (Grieve, 2013), and widely distributed in Euroasia, Australia and South Africa have

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been grown in damp or wet places (Gulluce et al., 2007; Mkaddem et al., 2009; Ozturk et al., 2009). Lamiaceae family consists of 200 genera and more than 4000 species (Ramesh et al., 2007). The phytochemical investigations on the Mentha species revealed that they possessed flavonoids and their glycosides, phenolic compounds, triterpenoids, steroids, and lignans (Monte et al., 1998; Areias et al., 2001; Ali et al., 2002; Zheng et al., 2007). Many species within this family are medicinal plants that apply in food, row and cooked forms and in human disease therapy (Santos et al., 2012).

Mentha spp. has been used for liver complaints due to its anti-inflammatory and treatment of bronchitis, nausea, flatulence, anorexia, and ulcerative colitis (Zhang et al., 2006). Essential oil formation in the plants is highly dependent on climatic conditions, especially day length, irradiance, temperature, and water supply (Franz and Novak, 2010). The leaves, flowers and stems of the Mentha species have been used as carminative, antispasmodic, antiemetic, stimulant, analgesic, and emmenagogue in traditional medicine all around the world. Their leaves have also been consumed as herbal tea and spice (Iscan et al., 2002).

Mentha species usually contain the monoterpene menthol in their constitutions, food products, menthol, and cosmetic (Simões et al., 2007; Oliveira et al., 2014). The chemical composition of the essential oils is influenced by factors such as leaf development and the emergence of new organs, which may lead to lower concentrations of these metabolites caused by translocation, as well as by effects such as seasonality, rain levels, and the stress to which the plant is exposed. These effects can directly influence the quantity and quality of the constituents in the essential oil (Gobbo et al., 2007; Mkaddem et al., 2009). The objective of this research was to determine the phytochemical composition of methanolic extract of *Mentha viridis*.

MATERIALS AND METHODS

Collection and preparation of plant material

Mentha viridis seeds were purchased from local market in Hilla city, middle of Iraq. After thorough cleaning and removal of foreign materials, the seeds were stored in airtight container to avoid the effect of humidity and then stored at room temperature until further use (Ameera et al., 2015; Huda et al., 2015a).

Preparation of sample

About seventeen grams of methanolic extract of *Mentha viridis* powdered were soaked in twenty five ml methanol for ten h in a rotatory shaker. Whatman No.1 filter paper was used to separate the extract of plant. The filtrates were used for further phytochemical analysis. It was again filtered through sodium sulphate in order to remove the traces of moisture (Huda et al., 2015b).

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 (Mohammed and Imad, 2013). About 1 µl of the methanol extract was injected into the GC-MS using a micro syringe and the scanning was done for 45 min. 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 1 ml per min. The electron gun of mass detector liberated electrons having energy of about 70 eV. The column employed here for the separation of components was Elite 1 (100% dimethyl poly siloxane) (Imad et al., 2014; Muhanned et al., 2015). 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.

RESULTS AND DISCUSSION

Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic seeds extract of *Mentha viridis*, shown in (Table 1). The GC-MS chromatogram of the 21 peaks of the compounds detected was shown in (Figure 1). Chromatogram GC-MS analysis of the methanolic extract of *Mentha viridis* showed the presence of twenty one major peaks and the components corresponding to the peaks were determined as follows. The First set up peak were determined to be 3,6-Octadecadiynoic acid, methyl ester (Figure 2). The second peak indicated to be 2,5-Dimethyl-4-hydroxy-3(2H)-furanone (Figure 3). The next peaks considered to be 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, Benzofuran, R-Limonene, 2-Methoxy-4-vinylphenol, 2-Hydroxy-5-methylbenaldehyde, 3-(N,N-dimethyllayrylammonio), Tetraacetyl-d-xylonic nitrile, Ficusin, Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl., n-Hexadecanoic acid, 7-Methyl-Z-tetradecen-1-ol acetate, Ethyl 9,12,15-octadecatrienoate, Methyl 19-methyl-eicosanoate, 1b,4a-eboxy-2H-cyclopenta, (3,4) cyclopropal, (8,9) cycloundec, 5H-Cyclopropa, (3,4) benz 1,2-e]azulene-5-one, 2,2,4-Trimethyl-3-(3,8, 12,16-tetramethyl-hepta deca), 4H-Cyclopropa (5,6) benz, (1,2:7,8) azuleno (5,6-b) oxiren-4-one, Ethyl iso-allocholate, and Tocopherol. (Figures 4 to 22). *M. viridis* was found to be more active at lower concentration against all the pathogenic bacterial strains and these results could be due to differences in chemical composition of the oils (Oumzil et al., 2002; Zenasni et al., 2008; Imad et al., 2015). The earlier report on the essential oil of *M. viridis* from Tunisie revealed the presence of carvone, 1,8-cineole, and limonene

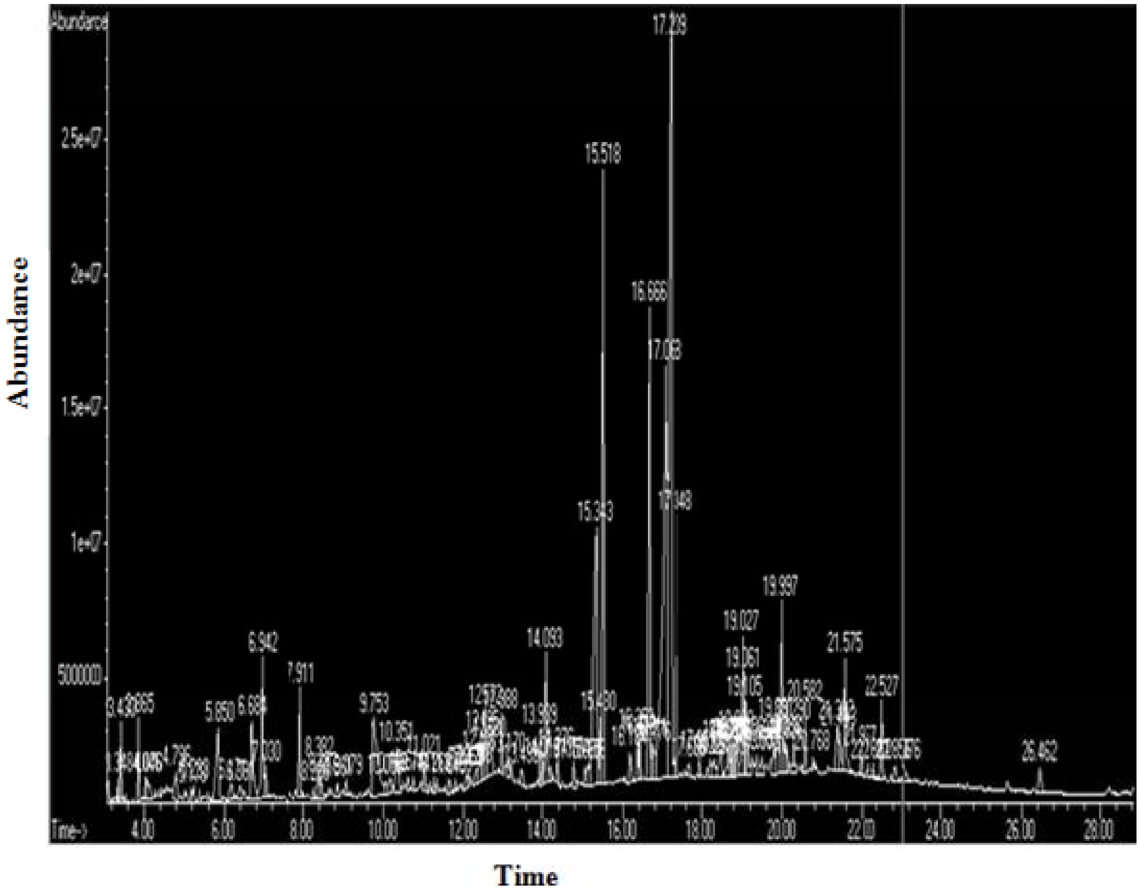


Figure 1. GC-MS chromatogram of methanolic extract of *Mentha viridis* leaves.

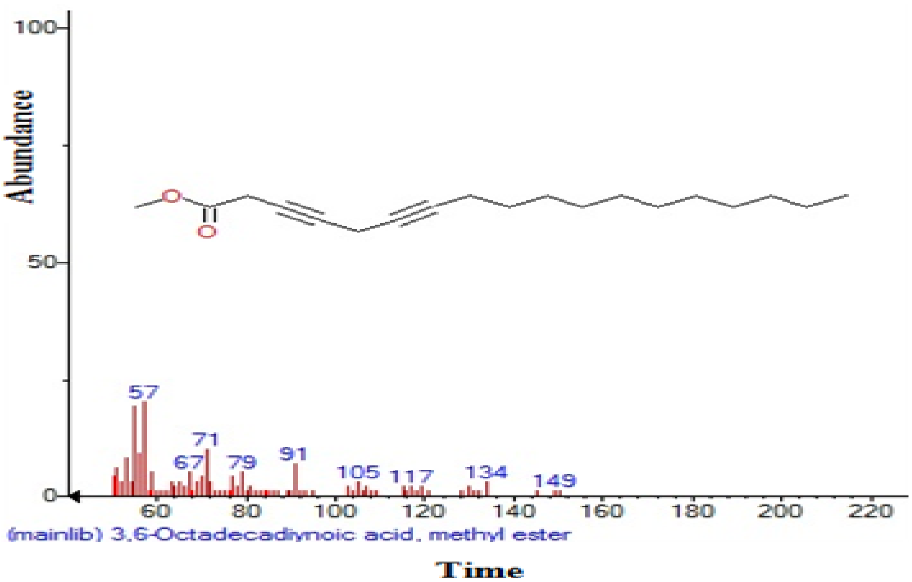


Figure 2. Mass spectrum of 3,6-Octadecadiynoic acid, methyl ester.

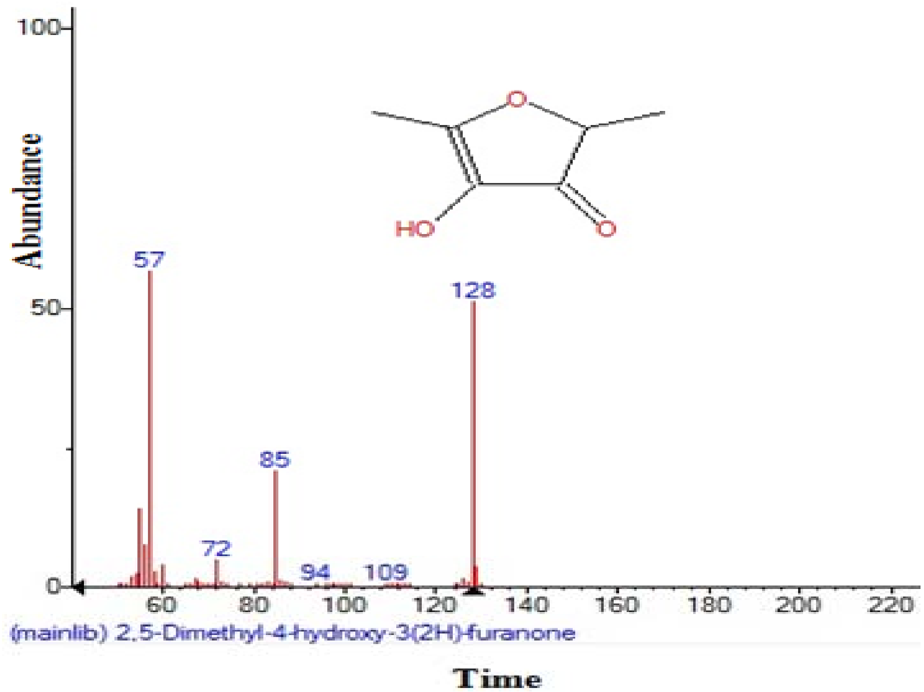


Figure 3. Mass spectrum of 2,5-Dimethyl-4-hydroxy-3(2H)-furanone.

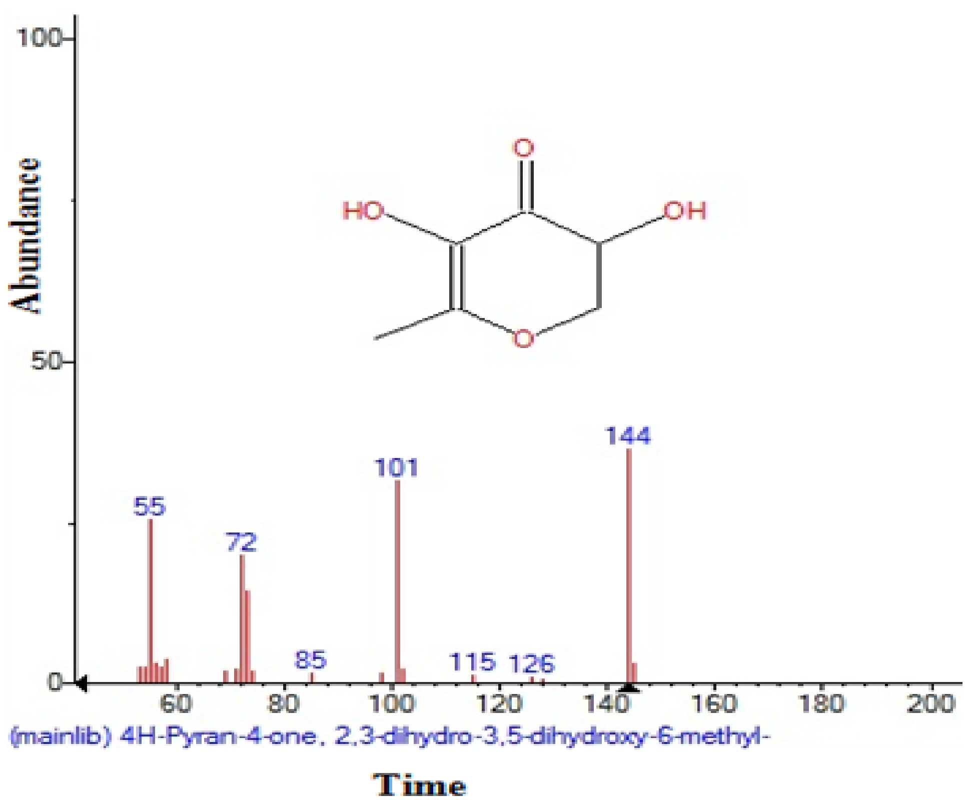


Figure 4. Mass spectrum of 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl.

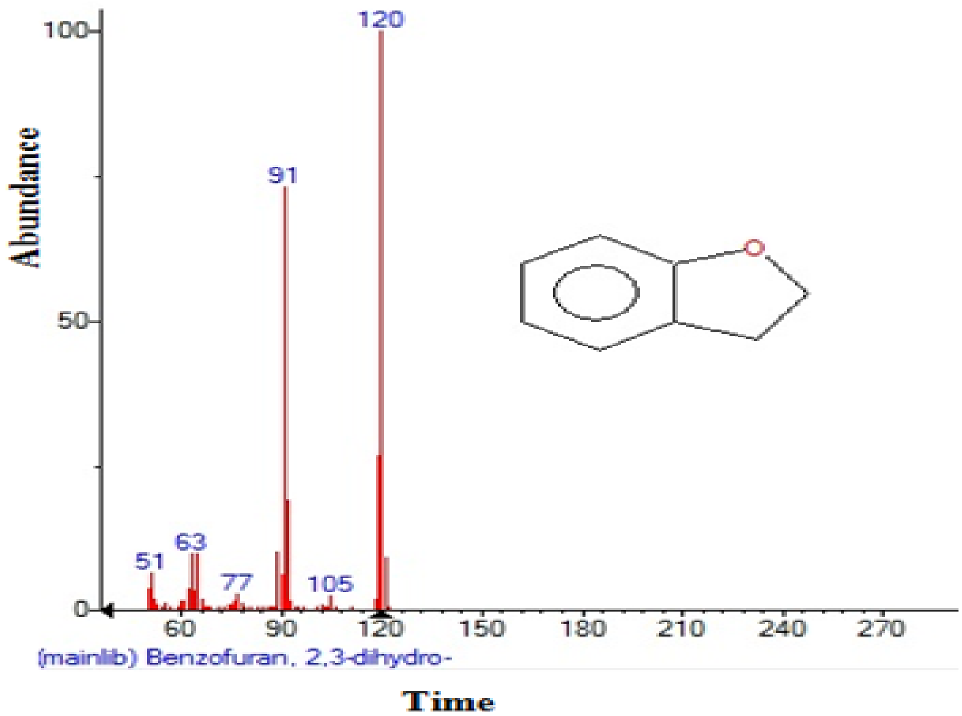


Figure 5. Mass spectrum of Benzofuran.

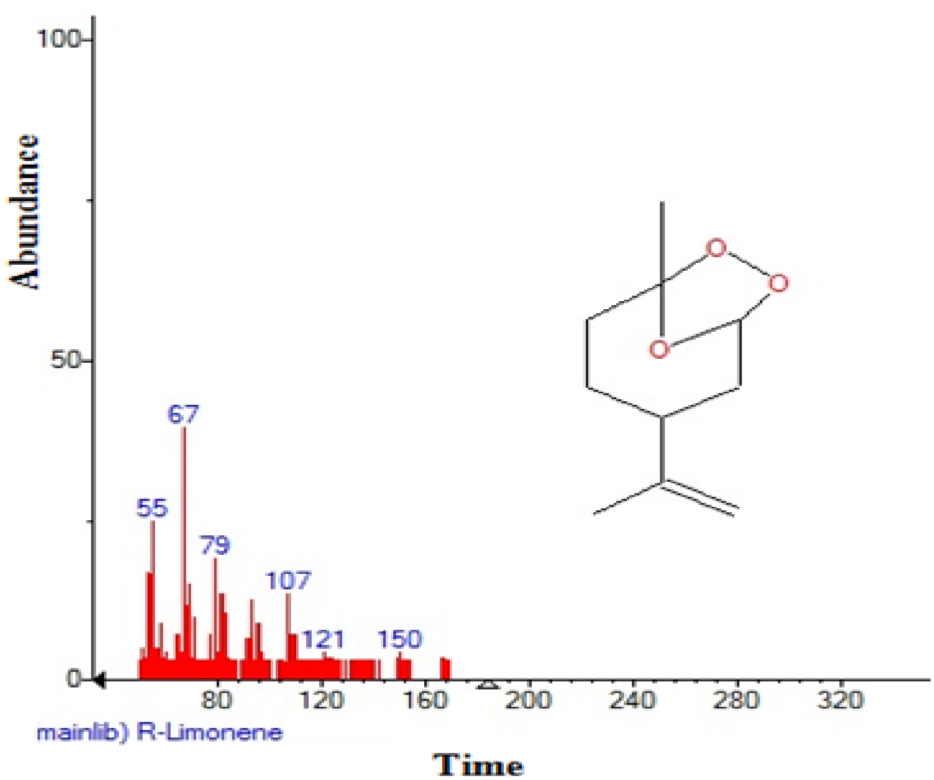


Figure 6. Mass spectrum of R-Limonene.

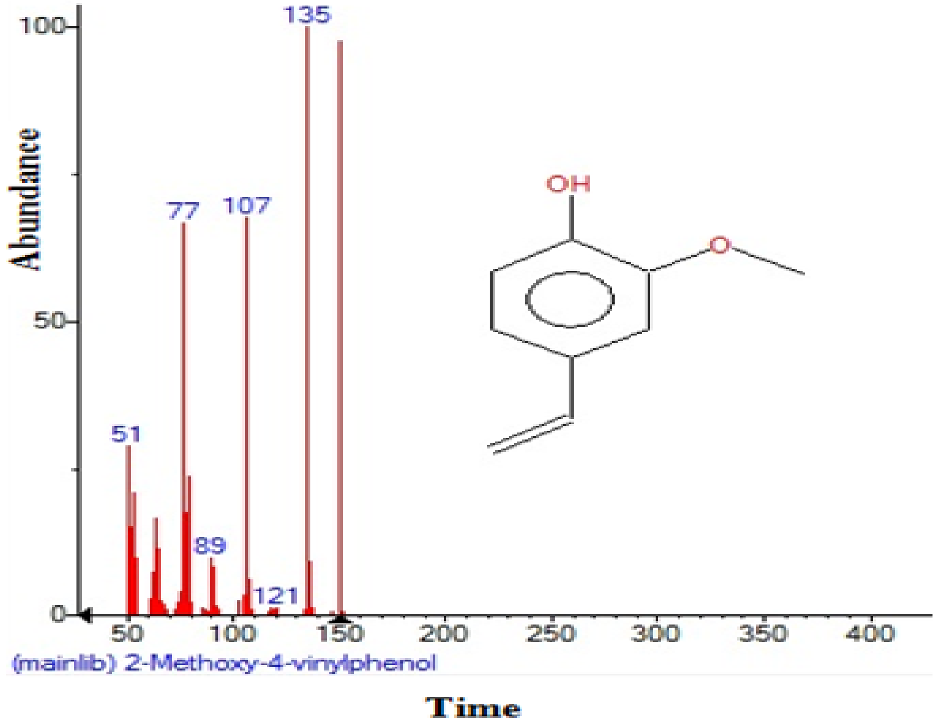


Figure 7. Mass spectrum of 2-Methoxy-4-vinylphenol.

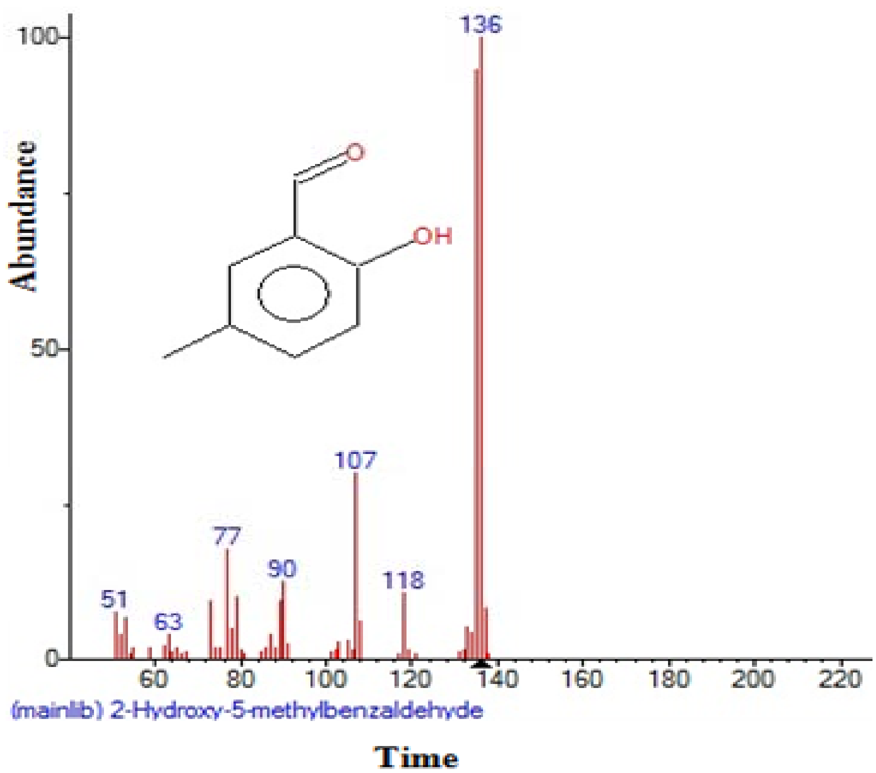


Figure 8. Mass spectrum of 2-Hydroxy-5-methylbenaldehyde.

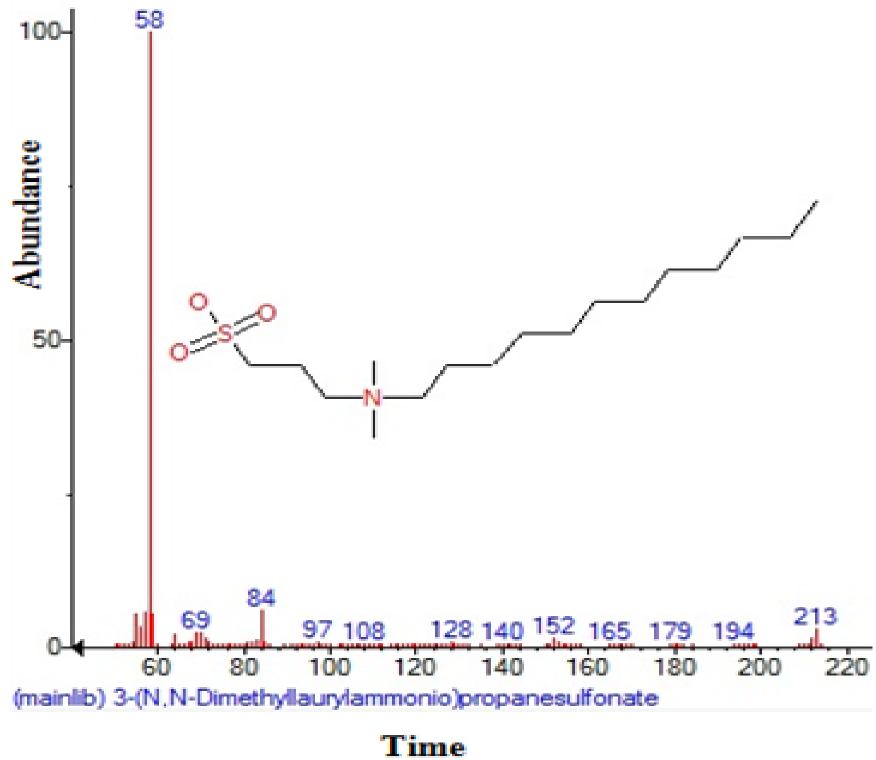


Figure 9. Mass spectrum of 3-(N,N-Dimethyllayrylammonio).

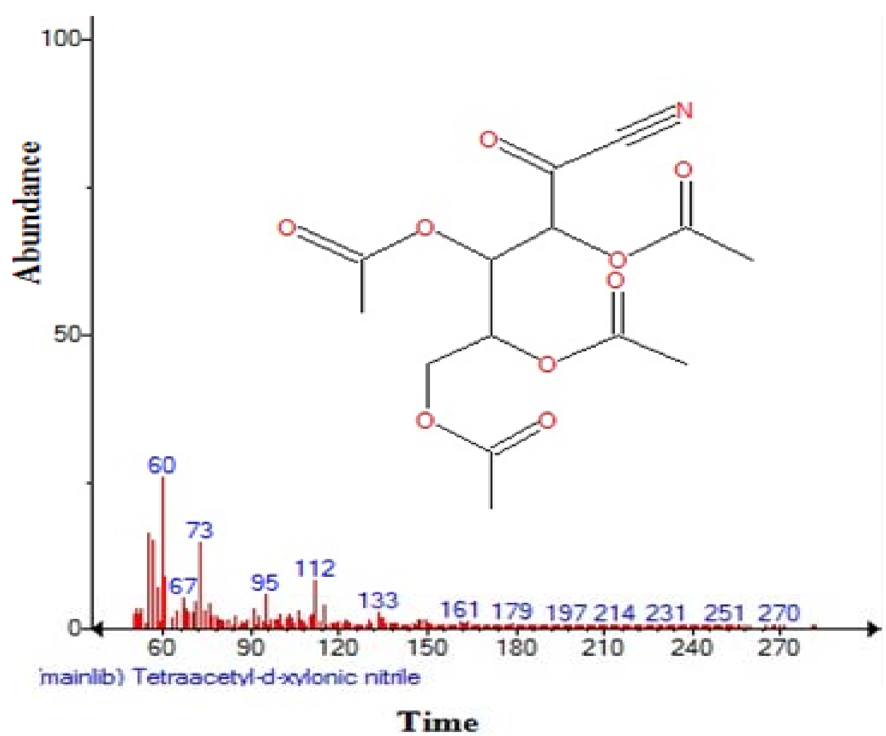


Figure 10. Mass spectrum of Tetraacetyl-d-xylonic nitrile.

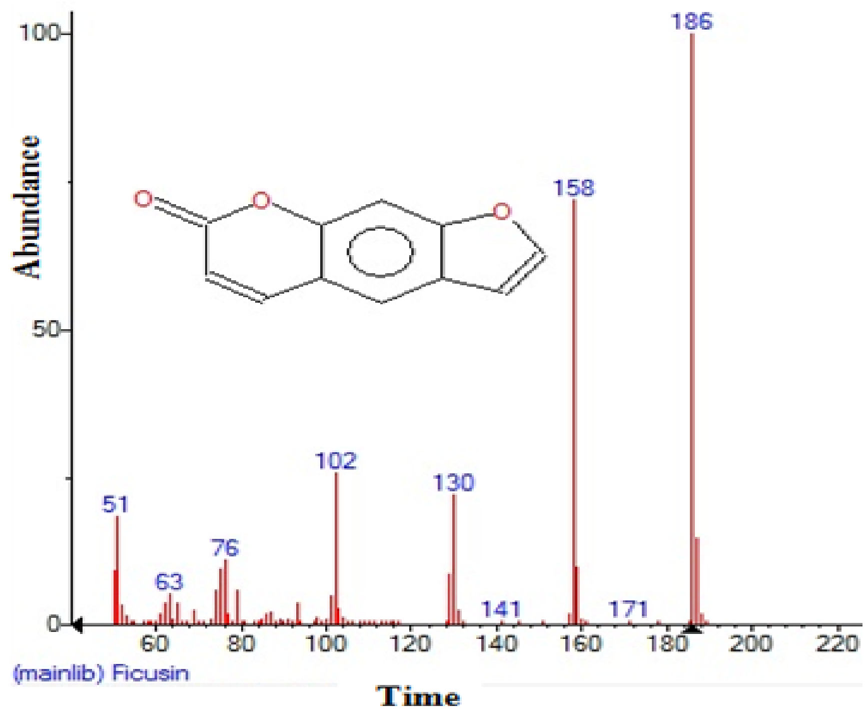


Figure 11. Mass spectrum of Ficusin.

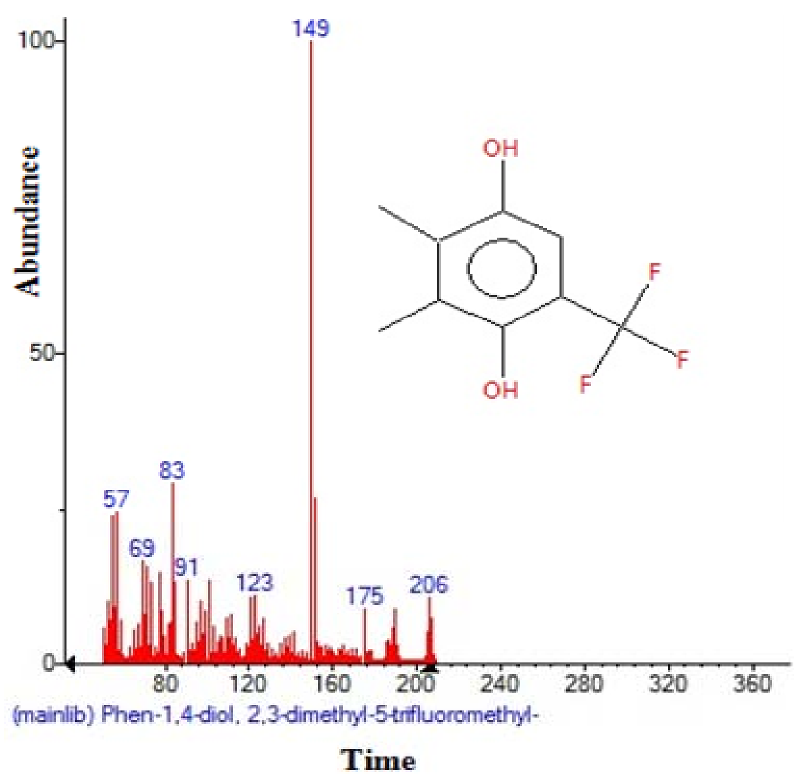


Figure 12. Mass spectrum of Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl.

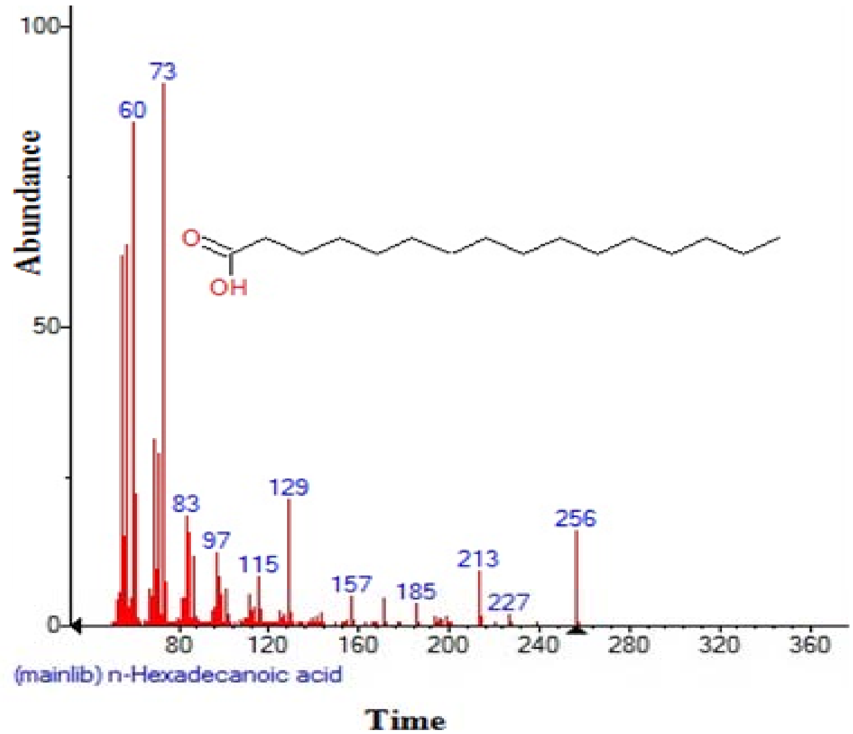


Figure 13. Mass spectrum of n-Hexadecanoic acid.

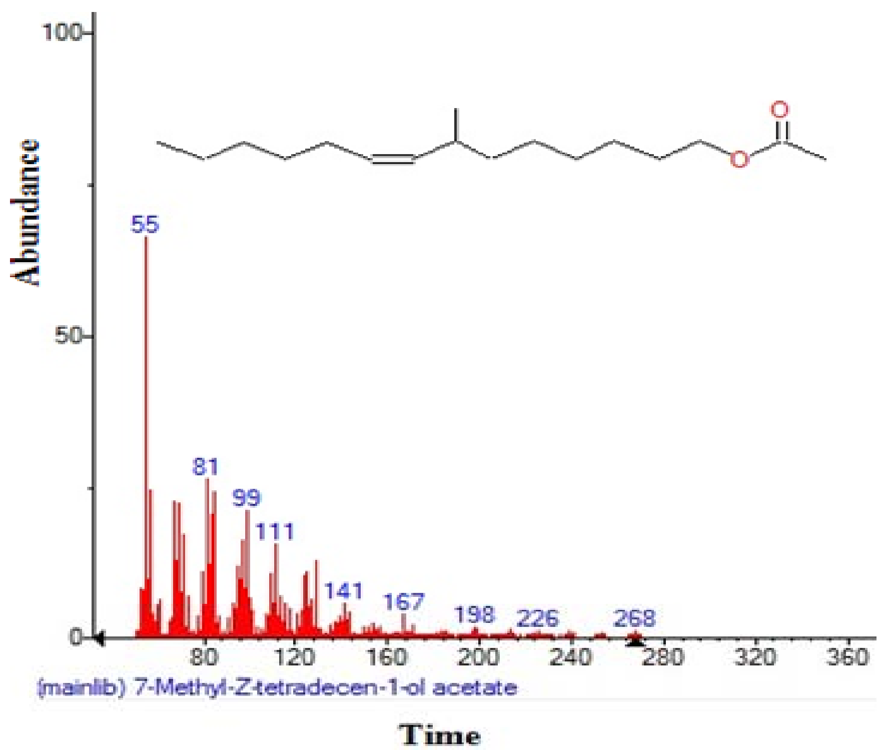


Figure 14. Mass spectrum of 7-Methyl-Z-tetradecen-1-ol acetate.

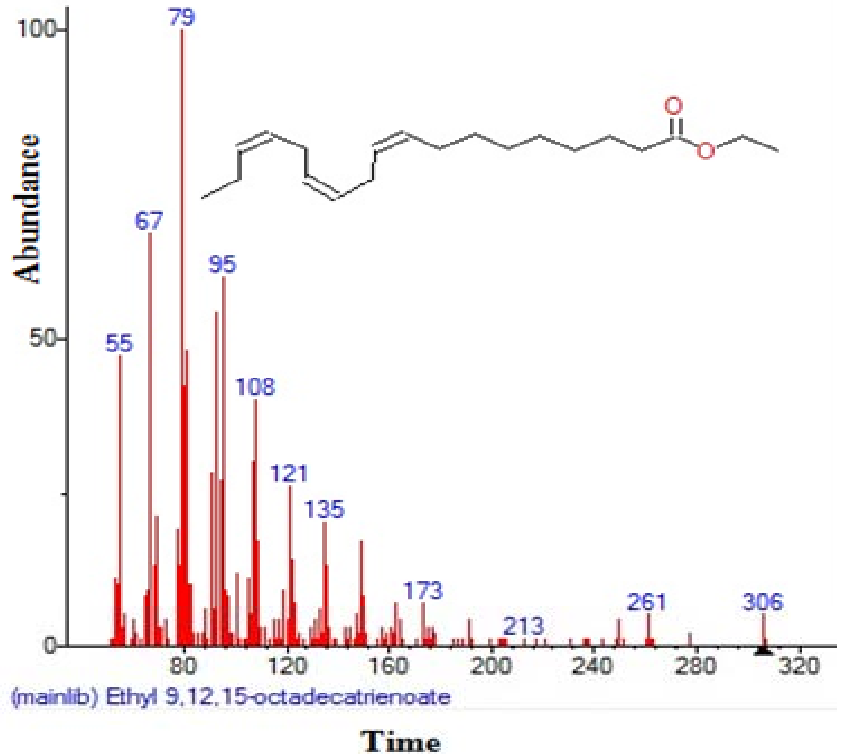


Figure 15. Mass spectrum of Ethyl 9,12,15-octadecatrienoate.

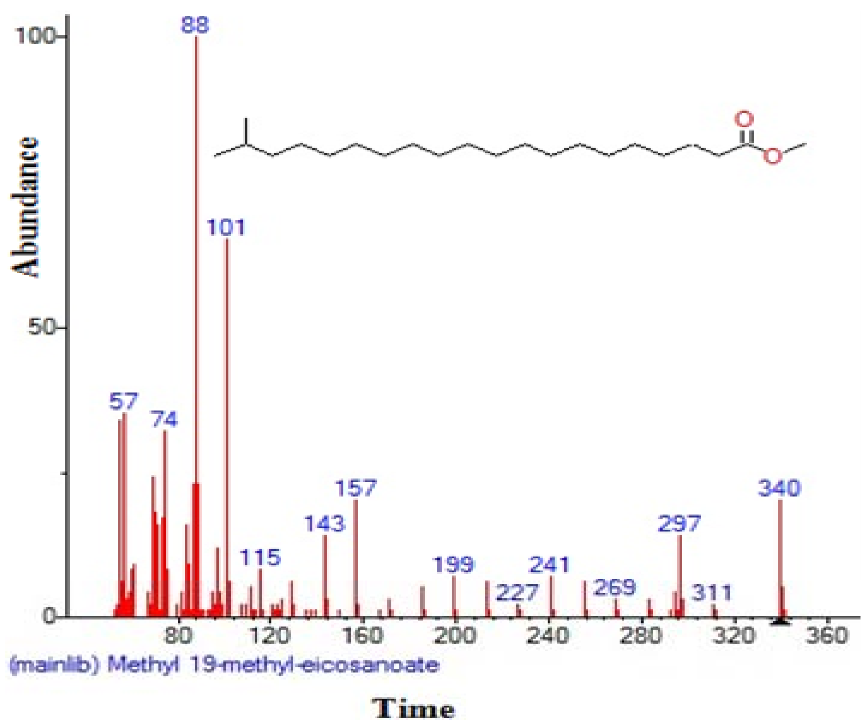


Figure 16. Mass spectrum of Methyl 19-methyl-eicosanoate.

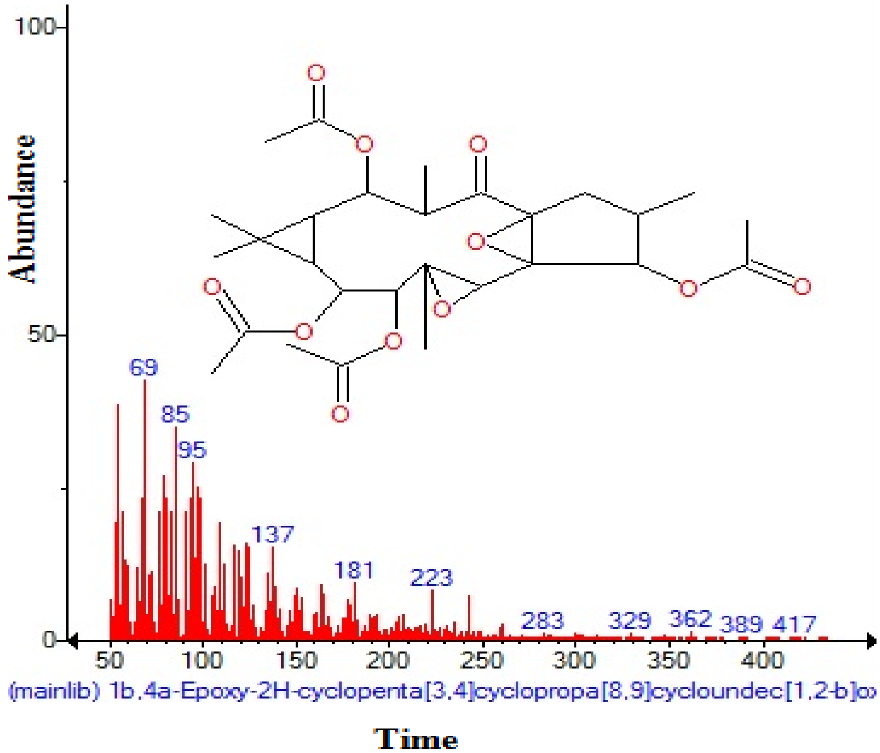


Figure 17. Mass spectrum of 1b,4a-eboxy-2H-cyclopenta[3,4]cyclopropal[8,9] cycloundec.

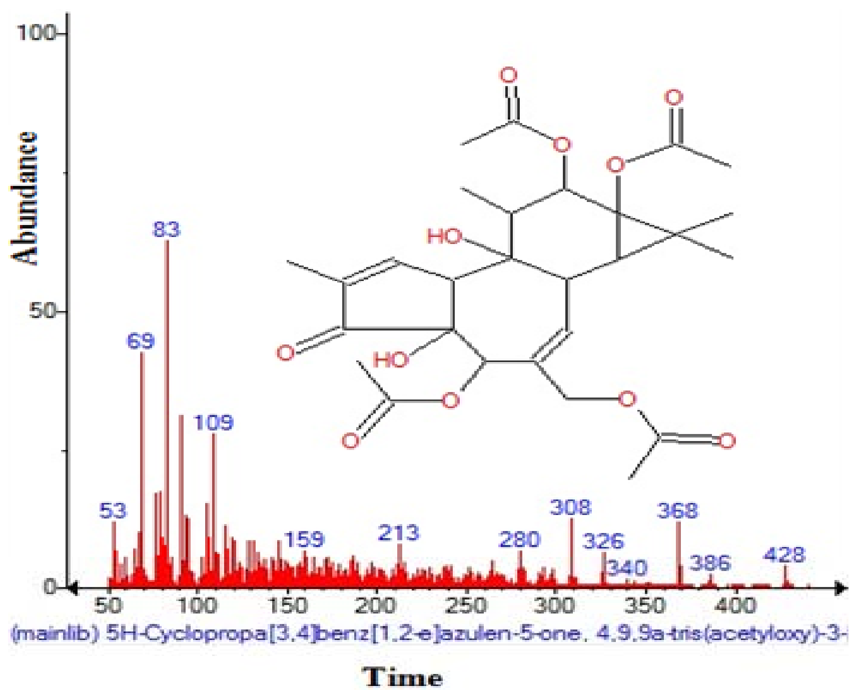


Figure 18. Mass spectrum of 5H-Cyclopropa[3,4]benz[1,2-e]azulene-5-one.

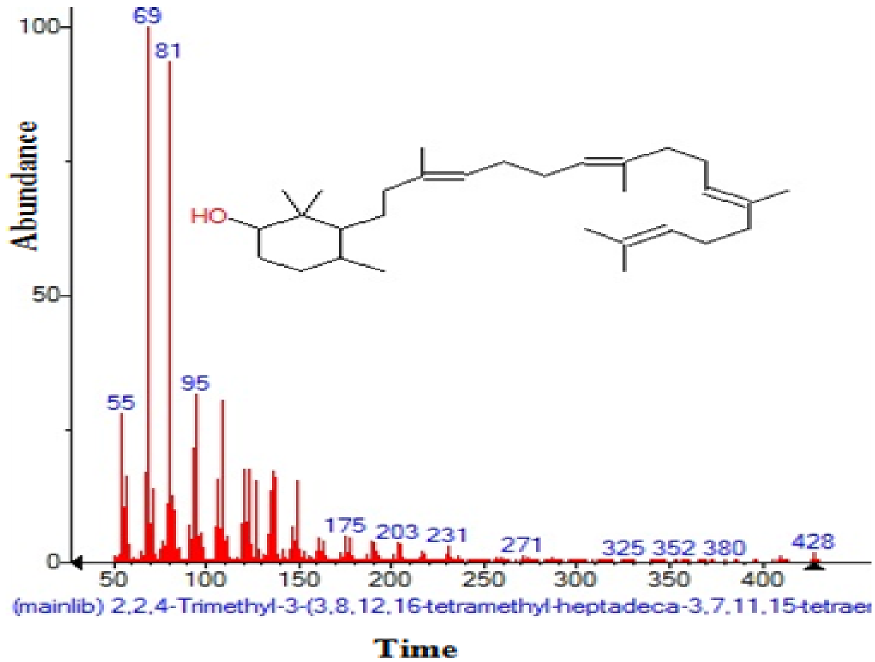


Figure 19. Mass spectrum of 2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca.

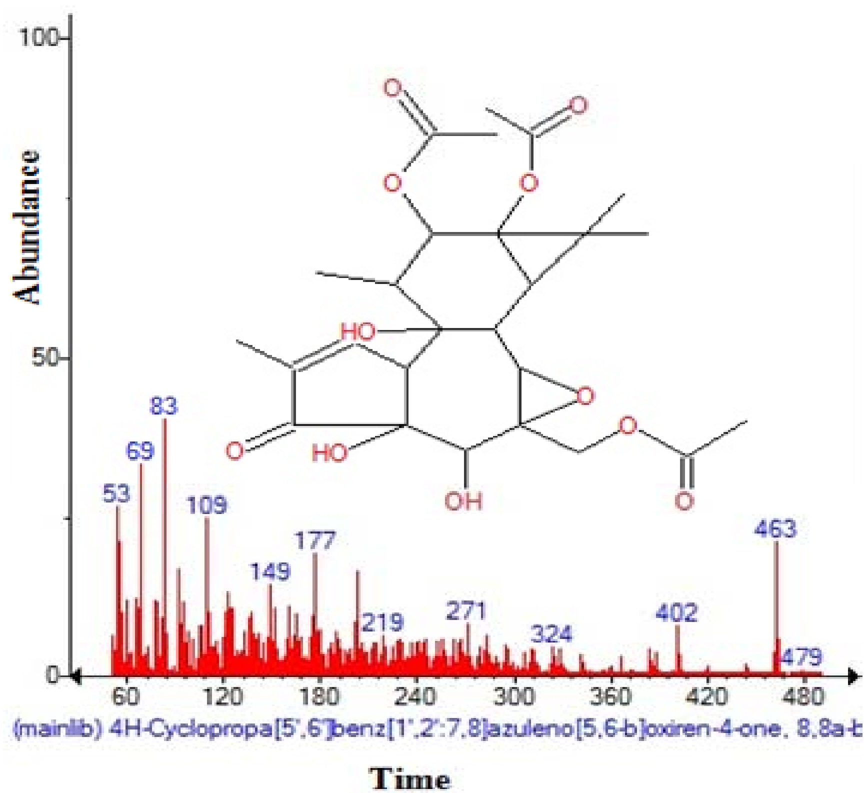


Figure 20. Mass spectrum of 4H-Cyclopropa[5,6]benz[1,2:7,8]azuleno[5,6-b]oxiren-4-one.

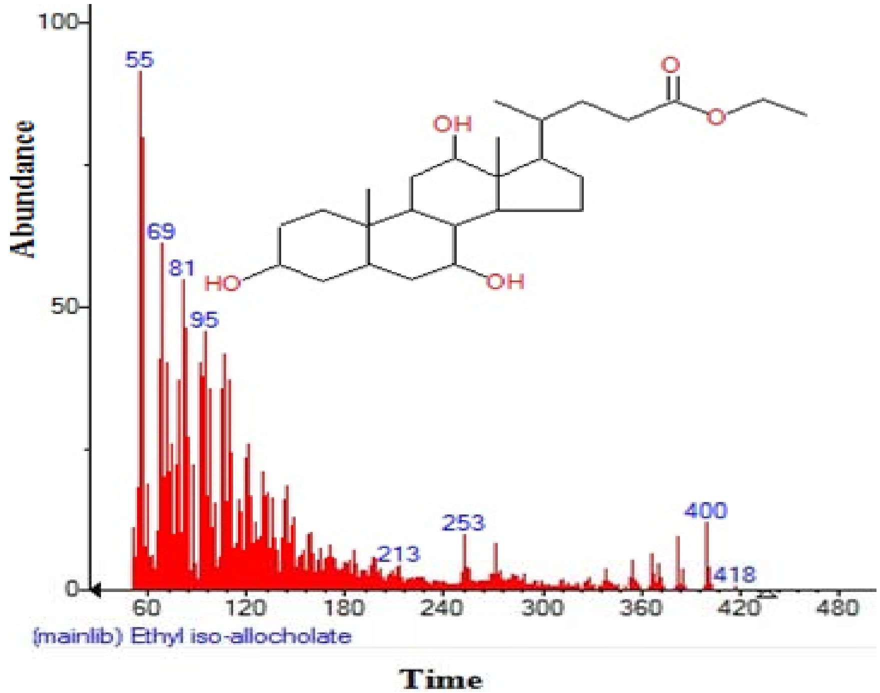


Figure 21. Mass spectrum of Ethyl iso-allocholate.

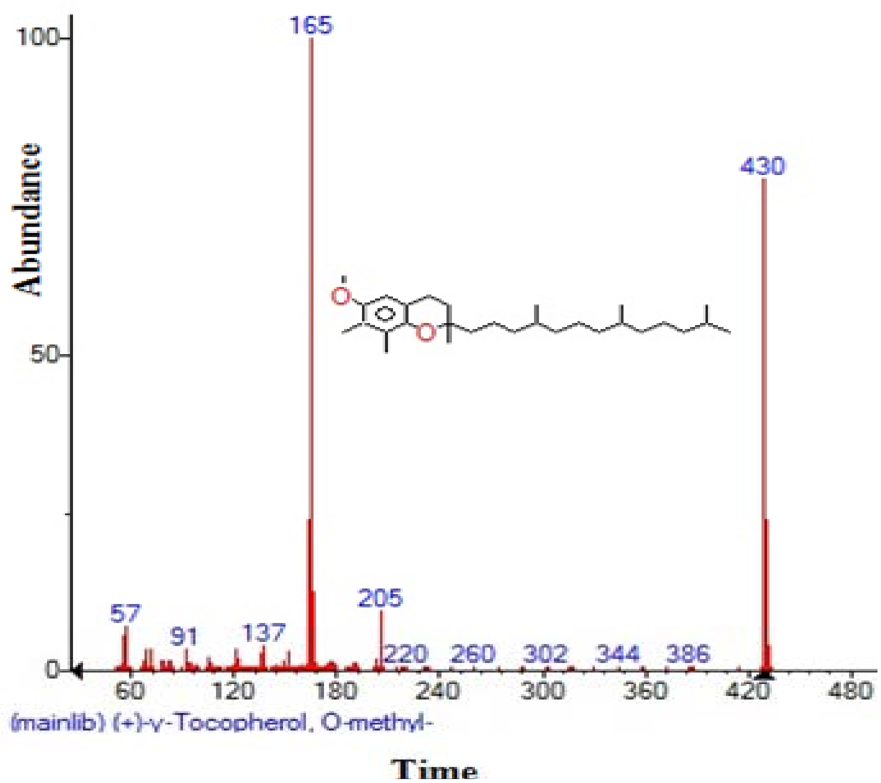


Figure 22. Mass spectrum of Tocopherol.

Table 1. Major phytochemical compounds identified in methanolic extract of *Mentha viridis* leaves.

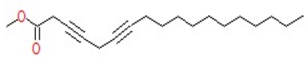
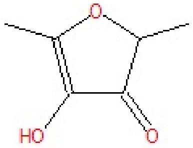
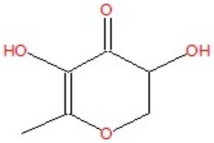
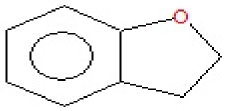
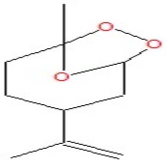
S/No.	Phytochemical compound	RT(min)	Formula	Molecular weight	Exact mass	Chemical structure	Pharmacological actions
1.	3,6-Octadecadiynoic acid, methyl ester.	4.088	$C_{19}H_{30}O_2$	290	290.22458		Anti-asthma, pesticides, Neurons protective, anti-inflammatory and hepato-protective property
2.	2,5-Dimethyl-4-hydroxy-3(2H)-furanone.	4.775	$C_6H_8O_3$	128	128.047344		Anti-cataract effects and anti-oxidative activities
3.	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl.	5.850	$C_6H_8O_4$	144	144.042258		Anti-diabetic and anti-oxidant activity
4.	Benzofuran.	6.686	C_8H_8O	120	120.0575147		Anti-inflammatory and analgesic effects
5.	R-Limonene.	7.006	$C_{10}H_{16}O_3$	184	184.109944		Anti-anxiety and anti-Inflammatory

Table 1. Cont'd.

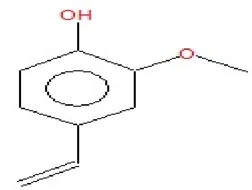
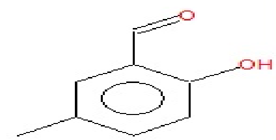
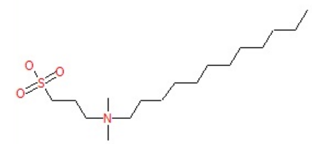
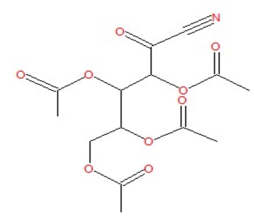
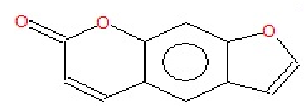
6.	2-Methoxy-4vinylphenol.	7.922	$C_9H_{10}O_2$	150	150.06808		Anti-inflammatory effect
7.	2-Hydroxy-5-methylbenaldehyde.	9.787	$C_8H_8O_2$	136	136.052424		Anti-Inflammatory and antioxidant
8.	3-(N,N-Dimethyllayrylammonio).	10.336	$C_{17}H_{37}NO_3S$	335	335.249414		New chemical compound (not found in PubChem Compound)
9.	Tetraacetyl-d-xylonic nitrile.	12.597	$C_{14}H_{17}NO_9$	343	343.090332		Anti-tumor and anti-oxidant
10.	Ficusin.	14.090	$C_{11}H_6O_3$	186	186.031694		Anti-oxidant and antimicrobial activity

Table 1. Cont'd.

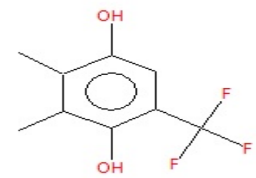
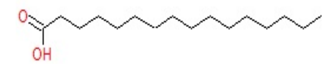
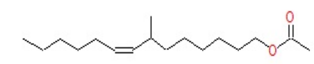
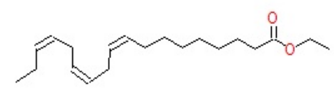
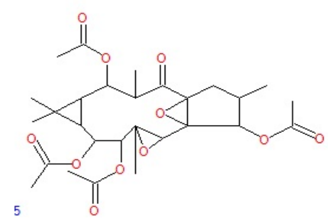
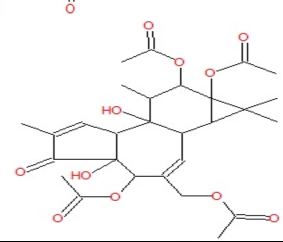
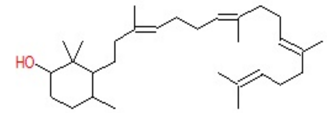
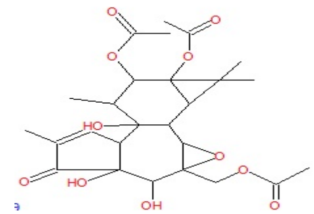
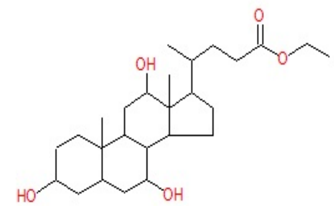
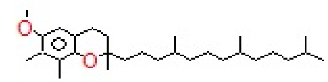
11.	Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl.	14.170	$C_9H_9F_3O_2$	206	206.055464		Antioxidant, antithrombotic and anti-tuberculosis activity
12.	n-Hexadecanoic acid.	15.349	$C_{16}H_{32}O_2$	256	256.24023		Antioxidant, anti-inflammatory, antimicrobial, pesticide and cancer preventive.
13.	7-Methyl-Z-tetradecen-1-ol acetate.	16.190	$C_{17}H_{32}O_2$	268	268.24023		Anti cancer, anti-inflammatory, hepatoprotective
14.	Ethyl 9,12,15-octadecatrienoate.	17.191	$C_{20}H_{34}O_2$	306	306.25588		Antimicrobial and pesticide
15.	Methyl 19-methyl-eicosanoate.	19.028	$C_{22}H_{44}O_2$	340	340.334131		Antifungal and antigenotoxic
16.	1b,4a-eboxy-2H-cyclopenta [3,4]cyclopropal[8,9] cycloundec.	19.263	$C_{28}H_{38}O_{11}$	550	550.241413		New chemical compound (not found in PubChem Compound)
17.	5H-Cyclopropa[3,4]benz [1,2-e]azulene-5-one.	20.602	$C_{28}H_{36}O_{11}$	548	548.22576		New chemical compound (not found in PubChem Compound)

Table 1. Cont'd.

18.	2,2,4-Trimethyl-3-(3,8, 12,16-tetramethyl-hepta deca.	22.519	$C_{30}H_{52}O$	428	428.401815		New chemical compound (not found in PubChem Compound)
19.	4H-Cyclopropa[5,6]benz [1,2:7,8]azuleno[5,6-b] oxiren-4-one.	22.833	$C_{26}H_{34}O_{11}$	522	522.210114		New chemical compound (not found in PubChem Compound)
20.	Ethyl iso-allocholate.	23.125	$C_{26}H_{44}O_5$	436	436.18874		Antimicrobial, anti-inflammatory
21.	Tocopherol	26.455	$C_{29}H_{54}O_2$	430	430.38108		Antioxidant and anti-inflammatory

(Mkaddem et al., 2009), while the study from Morocco showed that the oil contains high pulegone content (Talbaoui et al., 2012). The essential oil composition of *M. viridis* reported from Tunisie were carvone, 1,8- cineole, and limonene (Mkaddem et al., 2009), while that from Morocco contained high pulegone content (Talbaoui et al., 2012). The major compounds identified by Joshi and Sharma (2014) were cis-ocimenone (61.7%), limonene (10.5%), and trans-carveol (5%). The other minor constituents were α-selinene (1.7%), isodihydrocarveol acetate (1.5%), Z-jasmone (1.3%), 1,8-cineole (1.2%), and cis-carveol (1.0%). The essential oils of two species of mentha and showed strong antimicrobial activite (Hajlaoui et al., 2009). Studies of Bang (2007) examined biological activities of essential oil of mentha on pathogen of potato. Among the identified phytocompounds have the property of antimicrobial activities, antioxidant and inhibit several pathogenic parasites (Stainer et al., 1986; Singh et al., 1998; Prescott et al., 1999; Purohit and Vyas, 2004; Sasikumar et al., 2003; Santh, 2006; Sazada et al., 2009). However, further research is required to better understand the scientific and biotechnological basis values of applied phytotherapy.

Conclusion

Mentha viridis is native plant of Iraq. It contains chemical constitutions which may be useful for various herbal formulation as anti-inflammatory, analgesic, antipyretic, cardiac tonic and antiasthmatic.

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Conflicts of interest

Authors have none to declare.

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