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Full Length Research Paper

Biochemical analysis of *Origanum vulgare* seeds by fourier-transform infrared (FT-IR) spectroscopy and gas chromatography-mass spectrometry (GC-MS)

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Medicinal plants are potential sources of natural compounds with biological activities, and therefore attract the attention of researchers worldwide. The objective of this research is to determine the chemical composition of methanolic seed extract. The phytochemical compound screened by spectroscopy and gas chromatography-mass spectrometry (GC-MS) method. Sixteen bioactive phytochemical compounds were identified in the methanolic extract of *Origanum vulgare*. 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 *O. vulgare* revealed the existence of the 1,7-Dioxaspiro[5,5]undec-2-ene, 2,4-Dihydroxy-2,5-dimethyl-39(2H)-furan-3-one, 2,4-Difurobenzene, 1-benzyloxy, α-D-Glucopyranoside, O-α- Glucopyranosyl, 4-Hexenal, 6-hydroxy-4-methyl,dimethyl acetal, acetate, 4H-pyran-4-one,2,3,-dihydro-3,5-dihydroxy-6-methyl, Benzofuran, 4-Amino-1,5-pentandioic acid, 2-Methoxy-4-vinylphenol, d-Mannose, 7-Isopropyl-10-methyl-1-oxo-1,5-dithia-spiro[5,5]undecane-2-carboxy, Phytol, Cis-Vaccenic acid, N-Methyl-Nbenzyltetradecanamine, 3,8,8-Trimethoxy-3-piperidyl-2,2-binaphthalene-1,1,4,4-tetrone, and 17-(1,5-Dimethylhexyl)10,13-dimethyl. The FTIR analysis of *O. vulgare* seeds proved the presence of alkenes, aliphatic fluoro compounds, alcohols, ethers, carboxlic acids, esters and hydrogen bonded alcohols, phenols.

Key words: GC/MS, bioactive compounds, FT-IR, *O. vulgare*.

INTRODUCTION

Origanum vulgare L. is a perennial aromatic herb belonging to the family *Lamiaceae* (Skoula and Harborne, 2002) used for thousands of years as spices and as local medicines in traditional medicine (Altameme et al., 2015). *O. vulgare* L. is the most wide spread among all the species within the genus. It is distributed all over Europe, West and Central Asia up to Taiwan (Ietswaart, 1980; Hameed et al., 2015).

Aerial flowering parts of *O. vulgare* subsp. *viride* are used in Iranian traditional medicine as diuretic, stomachic, antineuralgic, antitussive and expectorant (Afsharypuor et al. 1997). *O. vulgare* plays a primary role

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among culinary herbs in world trade (Oliver, 1997). Taxonomic studies on the basis of morphological characters have led to the discrimination of several subspecies. Ietswaart (1980), distinguished six subspecies of *O. vulgare*, that is, *hirtum*, *vulgare*, *virens*, *viride*, *gracile* and *glandulosum*. Only *O. vulgare* L. subspecies *hirtum* has the leaf anatomy which corresponds to that of commercially marketed European oregano (Skoula and Harborne, 2002).

Oregano is used worldwide both as spice and crude drug, which is mainly provided by species of *Origanum* genus (Franz and Novak, 2002). Oregano was found to be strong antimicrobial agent and had a significant spasmolytic effect on smooth muscle (Hameed et al., 2015). The fumigant toxicity and insecticidal effect of oregano essential oils for storeroom insects has also been proved (Marn et al., 1999; Imad et al., 2015). Many herbs are commonly used in home-type cure therapies, complementary medicine and modern medicine because of their perceived antioxidant, antimicrobial, anticancer, etc. properties. Origanum species are counted among these herbs, since they show high activities according to their assessment for the above biological properties (Mechergui et al., 2010; Hussein et al., 2015). A remarkable phytochemical polymorphism with several chemotypes is also reported by several studies on this species that shows marked spatial segregation in nature (D'antuono et al., 2000; Radušiene et al., 2005; Hameed et al., 2015c).

Furthermore, *O. vulgare* has an antioxidant property and is applied in human health. Cervato et al. (2000), prove that the antioxidant activities of extracts of oregano's leaves (both aqueous and methanolic extracts) can inhibit all phases of lipid peroxidative process (Sahin et al., 2004; Singh, 2007; Hussein et al., 2015). The biological activity of essential oils and herb extracts cause a high pharmaceutical and industrial interest in *O. vulgare*, since antimicrobial, antifungal, insecticidal and antioxidative effects have been reported (Kulisic et al. 2004; Bakkali et al. 2008; et al., 2015). *Origanum* tea is a treatment for indigestion, coughs, and to stimulate menstruation. The oil of *Origanum* is used for toothache, and in some cosmetics. Its leaves and flowering stems are natural antiseptics because of high thymol content (Bhat et al., 2002; Singh, 2003; Dalpe, 2004; Bilalis et al., 2011).

The study, aim to study the analysis of chemical compounds of *Origanum vulgare* seeds by fourier-transform infrared (FT-IR) spectroscopy and gas chromatography-mass spectrometry (GC-MS).

MATERIALS AND METHODS

Collection and preparation of plant material

The seeds were dried at room temperature for two weeks, and when properly dried then powdered using clean pestle and mortar, and the powdered plant was size reduced with a sieve. The fine powder was then packed in airtight container to avoid the effect of humidity and then stored at room temperature. About thirteen grams of the plant sample powdered were soaked in 100 ml methanol individually. It was left for 96 h 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 (Jasim et al., 2015).

Gas chromatography – mass spectrum analysis

The GC-MS analysis of the plant extract was made in a (Agilent 7890 A) instrument under computer control at 70 eV. About 1µL of the methanol extract was injected into the GC-MS using a micro syringe and the scanning was done for 45 min (Imad et al., 2014a). 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 (Mohammed and Imad, 2013; Imad et al 2014b; Kareem et al., 2015).

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 1 ml per min. The electron gun of mass detector liberated electrons having energy of about 70eV. The column employed here for the separation of components was Elite 1(100% dimethyl poly siloxane). The identity of the components in the extracts was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. Compounds were identified by comparing their spectra to those of the Wiley and NIST/EPA/NIH mass spectral libraries (Imad et al., 2014c; Muhanned et al., 2015).

RESULTS AND DISCUSSION

Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic seeds extract of *O. vulgare*, shown in Table 1. The GC-MS chromatogram of the 16 peaks of the compounds detected was shown in Figure 1. Chromatogram GC-MS analysis of the methanol extract of *O. vulgare* showed the presence of sixteen major peaks and the components corresponding to the peaks were determined as follows. The First set up peak were determined to be 1,7-Dioxaspiro[5,5]undec-2-ene Figure 2. The second peak

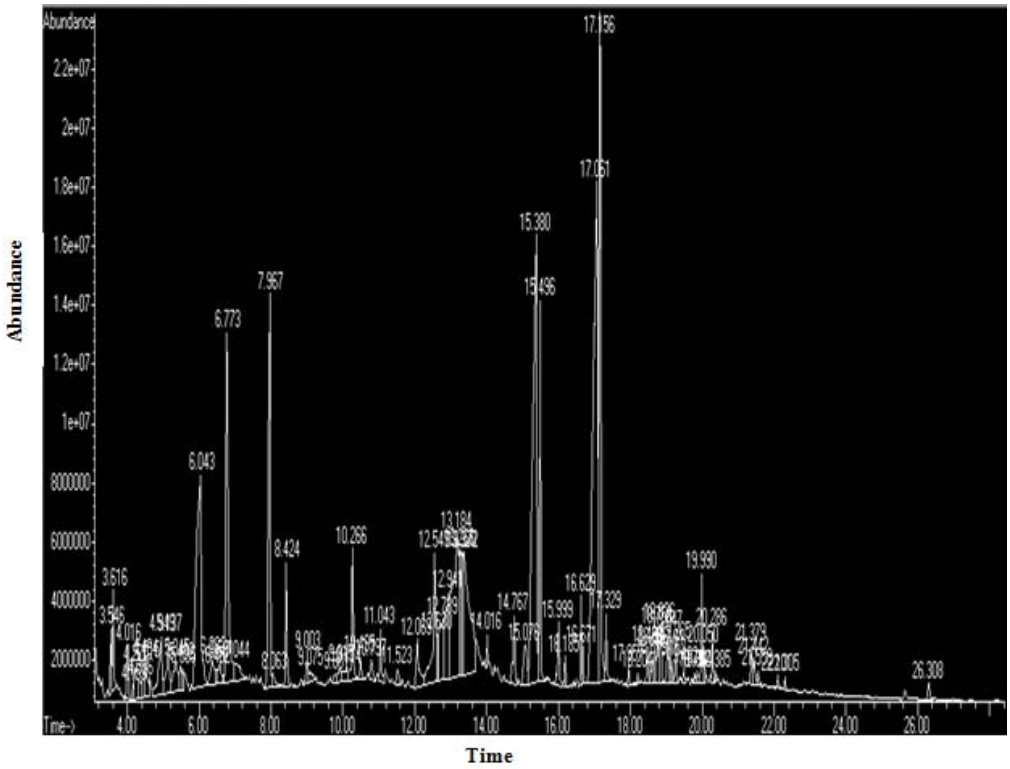


Figure 1. GC-MS chromatogram of methanolic extract of *O. vulgare*.

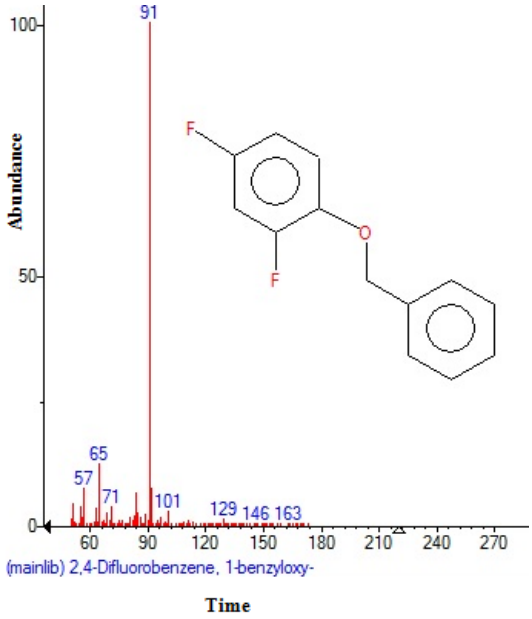


Figure 2. Mass spectrum of 2,4-Difurobenzene, 1-benzyloxy with Retention Time (RT)= 4.460.

indicated to be 2,4-Dihydroxy-2,5-dimethyl-39(2H)-furan-3-one Figure 3. The next peaks considered to be 2,4-Difurobenzene, 1-benzyloxy, α-D-Glucopyranoside, O-α-Glucopyranosyl, 4-Hexenal, 6-hydroxy-4-methyl,dimethyl acetal, acetate, 4H-pyran-4-one,2,3,-dihydro-3,5-dihydroxy-6-methyl, Benzofuran, 4-Amino1, 5-pentandiotic

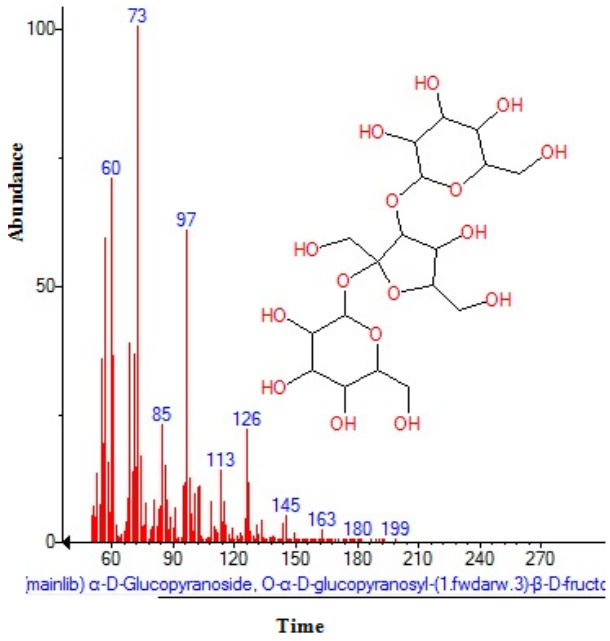


Figure 3. Mass spectrum of α-D-Glucopyranoside, O-α-Glucopyranosyl with Retention Time (RT)= 4.975.

Figure 5. Mass spectrum of 2,4-Dihydroxy-2,5-dimethyl-39(2H)-furan-3-one with Retention Time (RT)= 4.020.

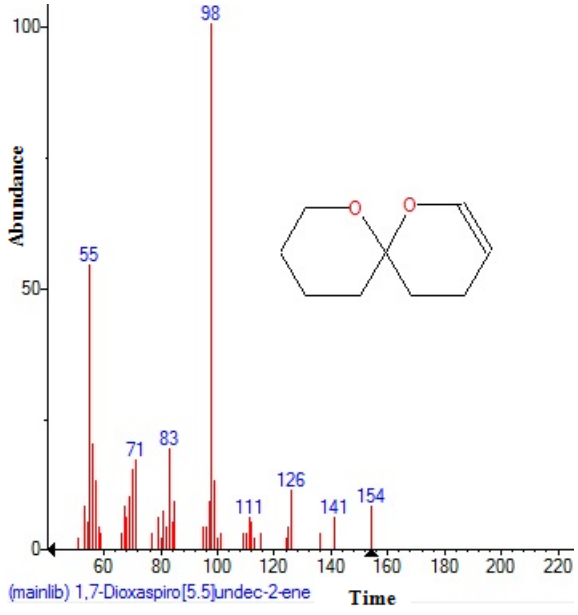


Figure 4. Mass spectrum of 1,7-Dioxaspiro[5,5]undec-2-ene with Retention Time (RT)= 3.585.

pentandioic acid, 2-Methoxy-4-vinylphenol, d-Mannose, 7-Isopropyl-10-methyl-1-oxo-1,5-dithia spiro[5,5]undecane-2-carboxy, Phytol, Cis-Vaccenic acid, N-Methyl-Nbenzyltetradecanamine, 3,8,8-Trimethoxy-3-piperidyl-2,2-binaphthalene-1,1,4,4-tetrone, and 17-(1,5-Dimethylhexyl)10, 13-dimethyl (Figure 4 to 17). The FTIR

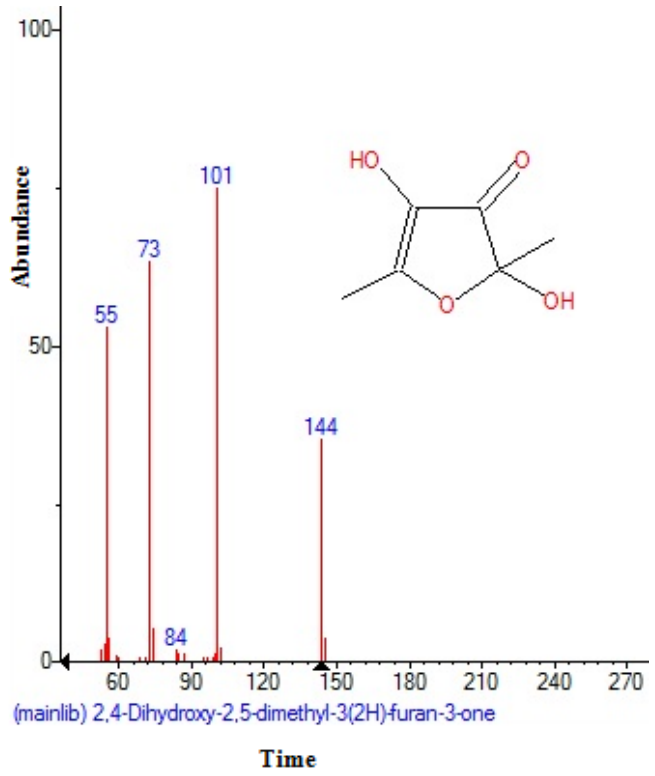


Figure 5. Mass spectrum of 2,4-Dihydroxy-2,5-dimethyl-39(2H)-furan-3-one with Retention Time (RT)= 4.020.

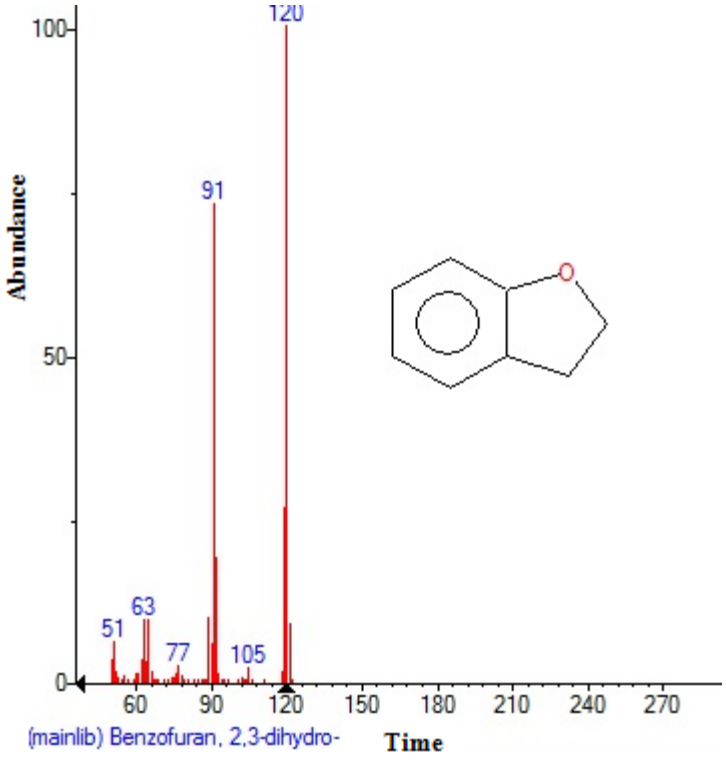


Figure 6. Mass spectrum of Benzofuran with Retention Time (RT)= 6.760.

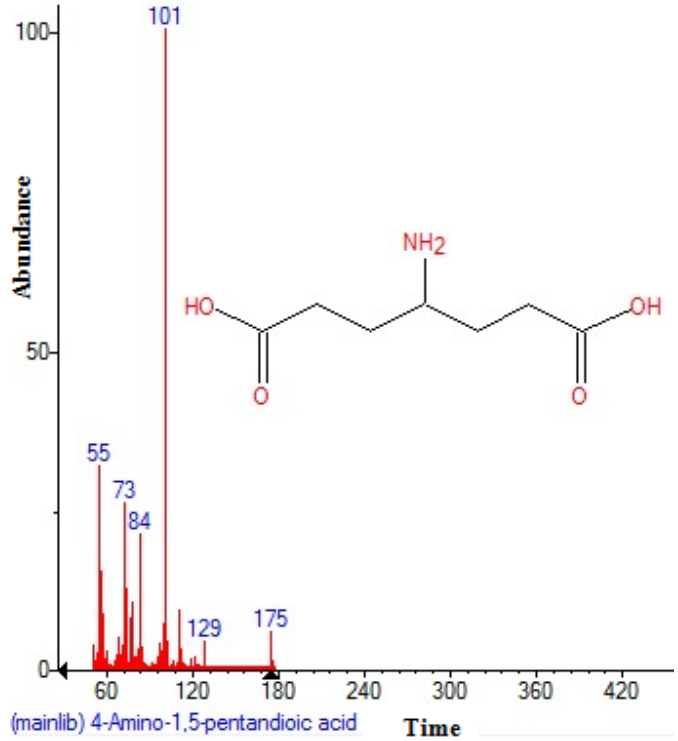


Figure 7. Mass spectrum of 4-Amino-1,5-pentandioic acid with Retention Time (RT)= 6.320.

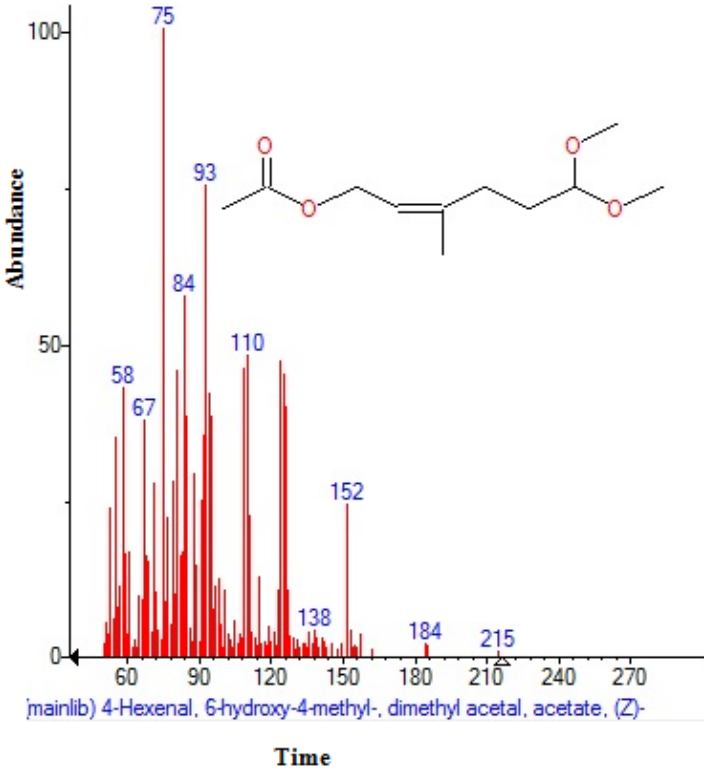


Figure 8. Mass spectrum of 4-Hexenal, 6-hydroxy-4-methyl,dimethyl acetal, acetate with Retention Time (RT)= 5.158.

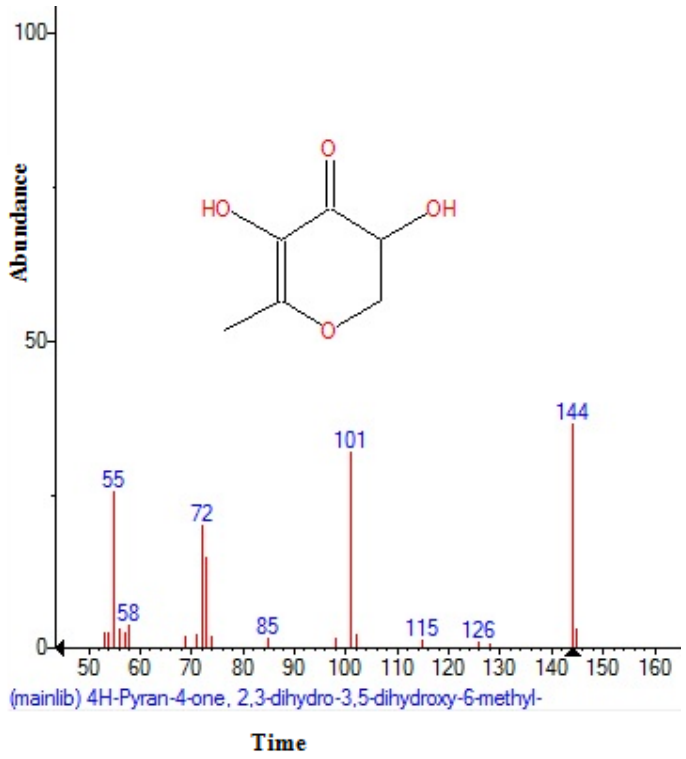


Figure 9. Mass spectrum of 4H-pyran-4-one,2,3,-dihydro-3,5-dihydroxy-6-methyl with Retention Time (RT)= 6.011.

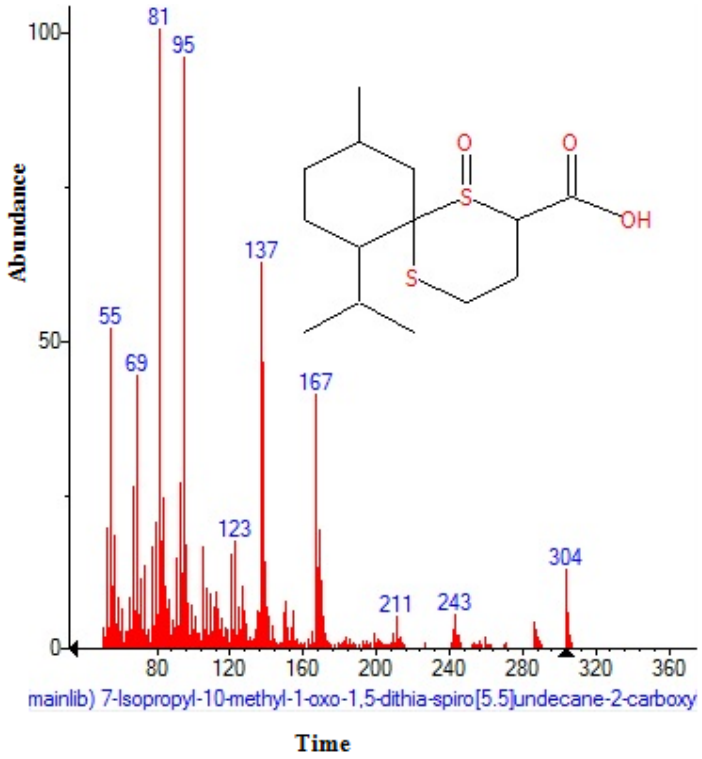


Figure 10. Mass spectrum of 7-Isopropyl-10-methyl-1-oxo-1,5-dithia-spiro[5,5]undecane-2-carboxy with Retention Time (RT)= 14.743.

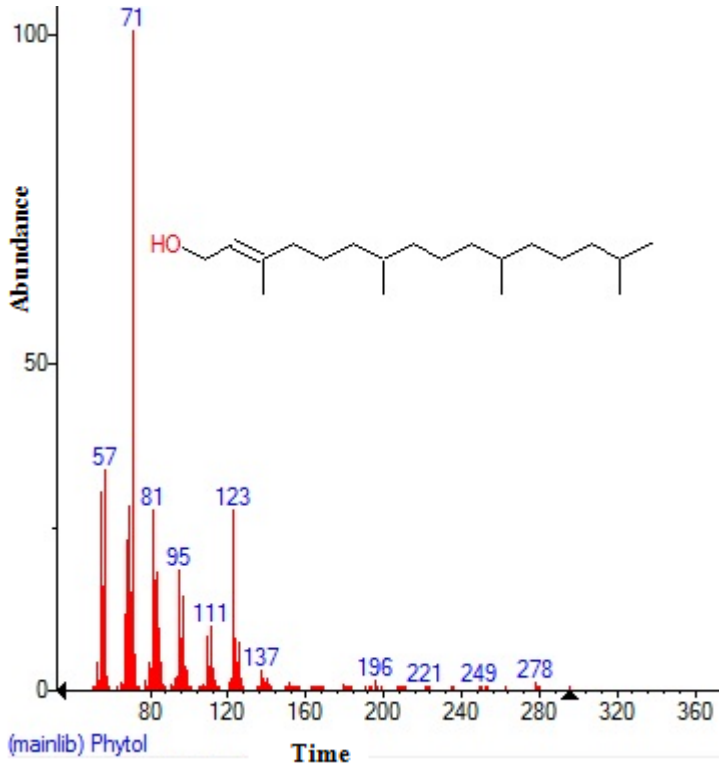


Figure 11. Mass spectrum of Phytol with Retention Time (RT)= 16.625.

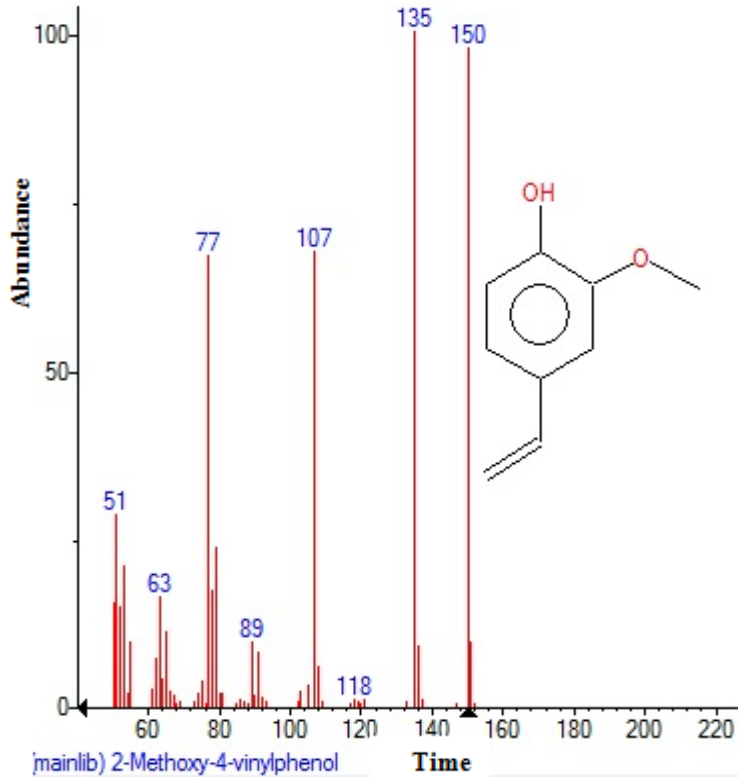


Figure 12. Mass spectrum of 2-Methoxy-4-vinylphenol with Retention Time (RT)= 7.974

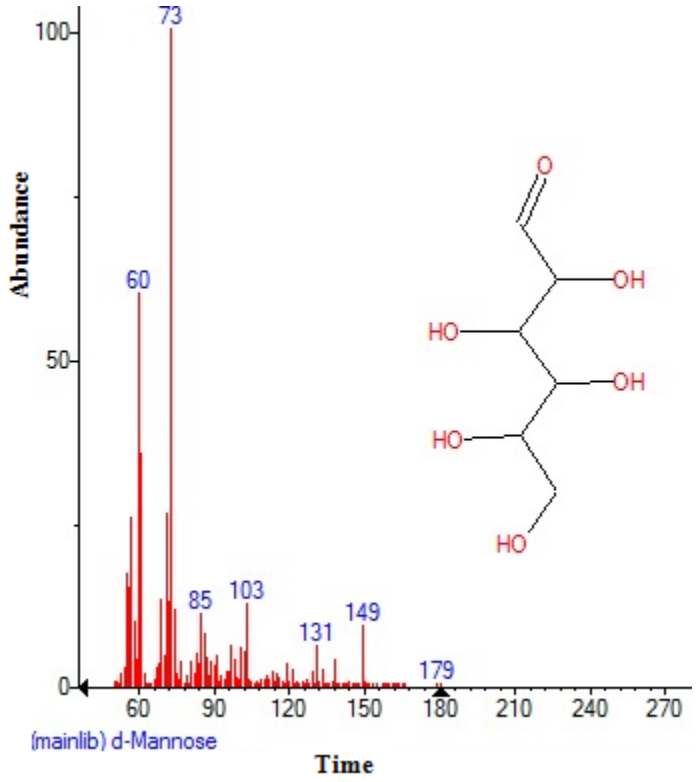


Figure 13. Mass spectrum of d-Mannose with retention time (RT)= 13.215.

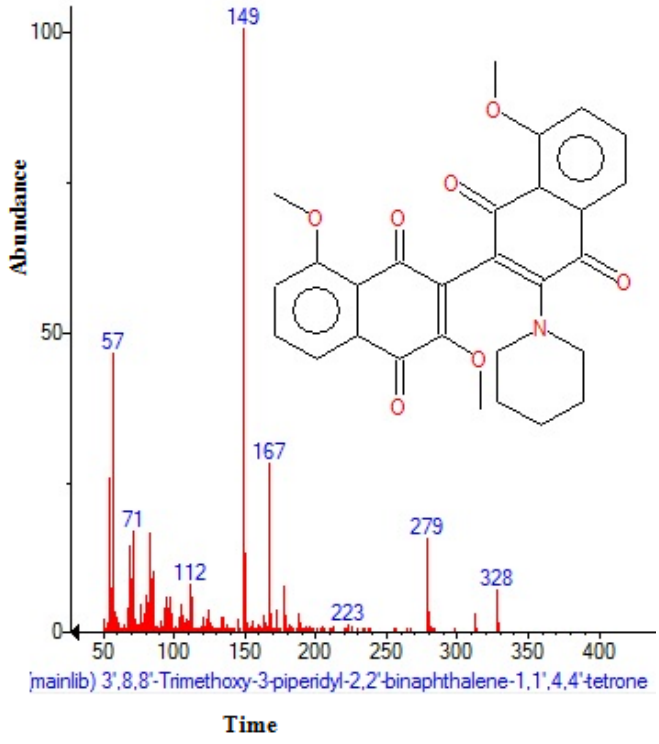


Figure 14. Mass spectrum of 3,8,8-Trimethoxy-3-piperidyl-2,2-binaphthalene-1,1,4,4-tetrone with retention time (RT)= 20.293.

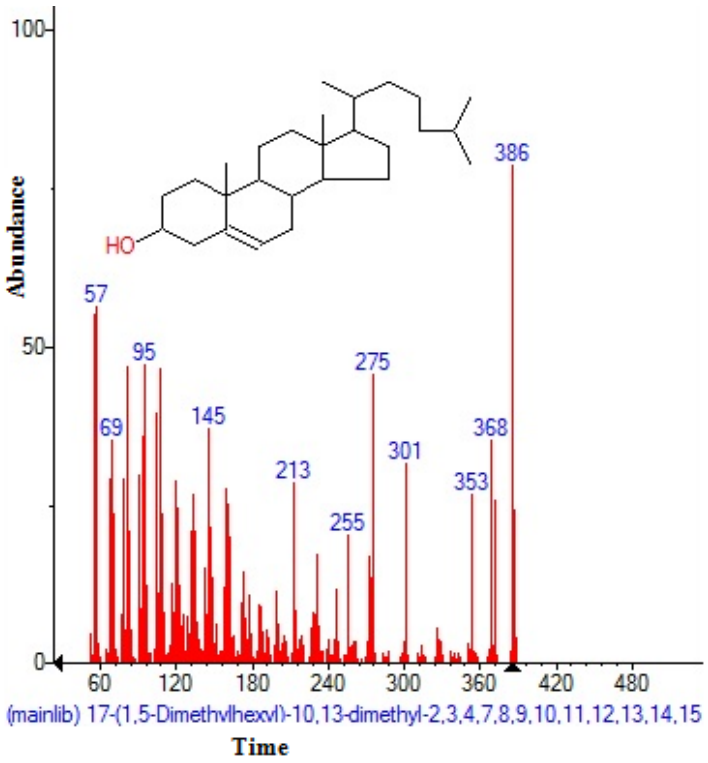


Figure 15. Mass spectrum of 17-(1,5-Dimethylhexyl)10,13-dimethyl with Retention Time (RT)= 26.284.

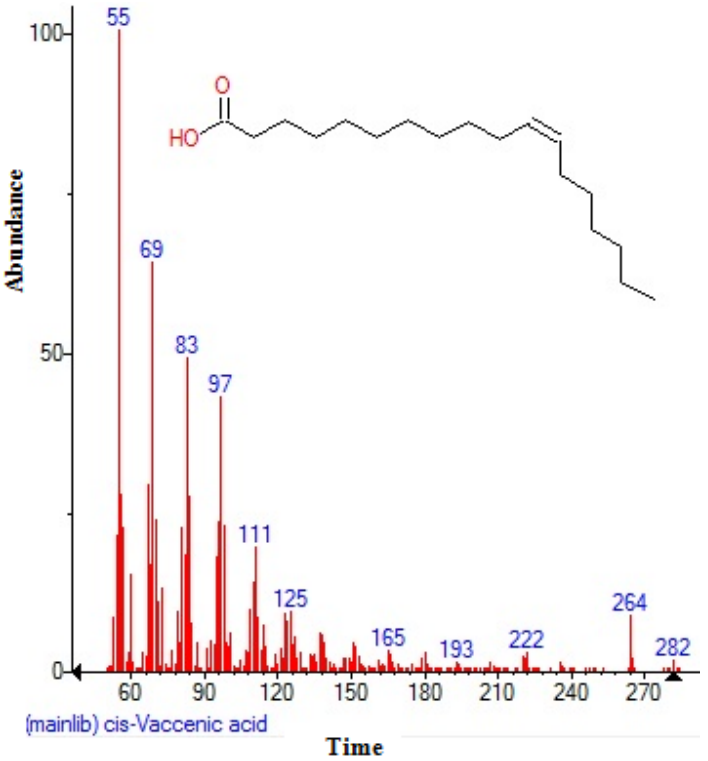


Figure 16. Mass spectrum of Cis-Vaccenic acid with retention time (RT)= 17.014

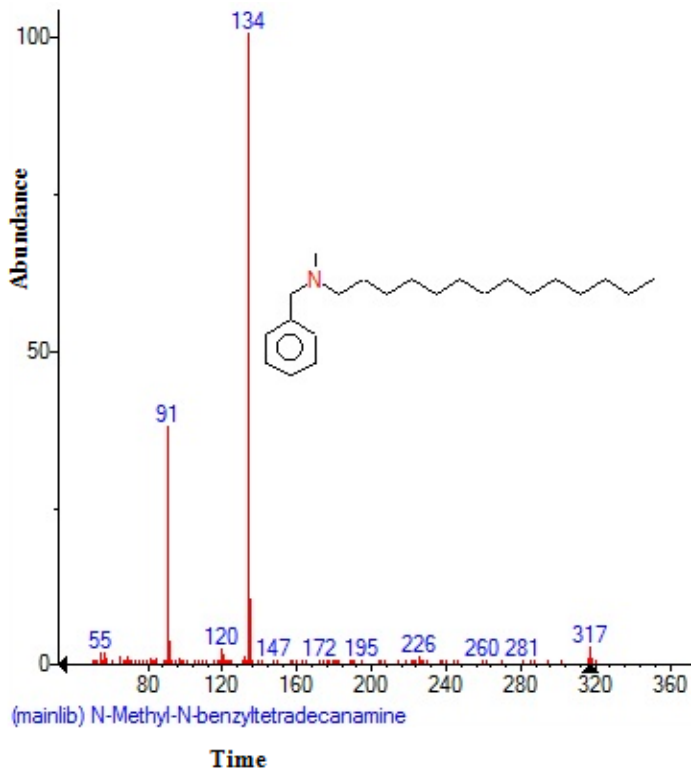


Figure 17. Mass spectrum of N-Methyl-Nbenzyltetradecanamine with Retention Time (RT)= 18.485.

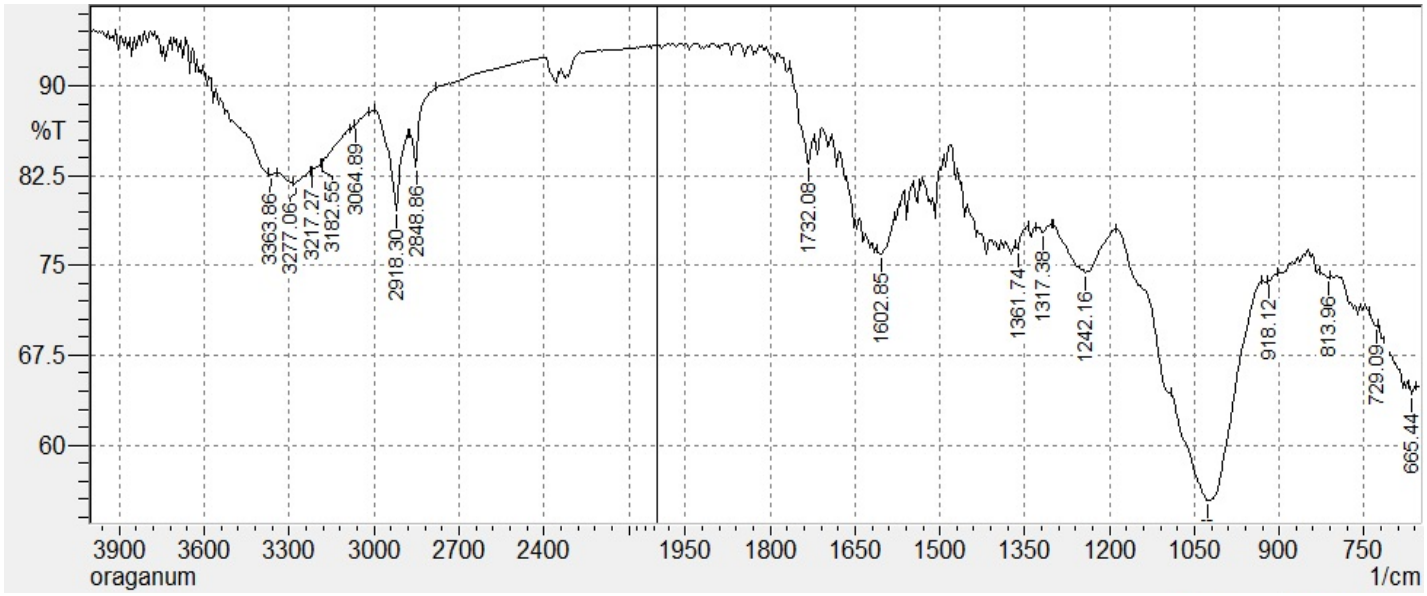


Figure 18. FT-IR peak values of *O. vulgare*

analysis of *O. vulgare* seeds proved the presence of alkenes, aliphatic fluoro compounds, alcohols, ethers, carboxlic acids, esters and hydrogen bonded alcohols, phenols which shows major peaks at 729.09, 1026.13, 1242.16, 1317.38, 2918.30, 3064.89 and 3363.86 (Table 2 and Figure 18).

Table 1. Major phytochemical compounds identified in methanolic extract of *O. vulgare*.

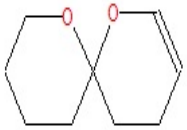
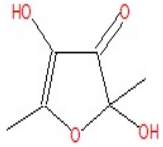
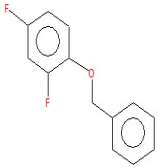
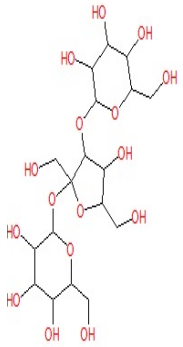
S/N	Phytochemical compound	RT(min)	Formula	Molecular weight	Exact mass	Chemical structure	MS Fragmentations	Pharmacological actions
1.	1,7-Dioxaspiro[5,5]undec-2-ene	3.585	$C_9H_{14}O_2$	154	154.09938		55,71,83,98,111, 126,141,154	Anti-inflammatory activity and anti-oxidative effects
2.	2,4-Dihydroxy-2,5-dimethyl-39(2H)-furan-3-one	4.020	$C_6H_8O_4$	144	144.042258		55,73,84,101,144	New chemical compound
3.	2,4-Difurobenzene, 1-benzyloxy	4.460	$C_{13}H_{10}F_2O$	220	220.069971		57,65,71,91,101, 129,146,163	Antihistamic and antibacterial
4.	α-D-Glucopyranoside, O-α- Glucopyranosyl	4.975	$C_{18}H_{32}O_{16}$	504	504.169035		60,73,85,97,113, 126,145,163,180 ,199	Antidiabetic and anti-osteoporotic

Table 1. Cont'd.

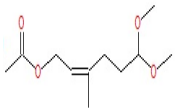
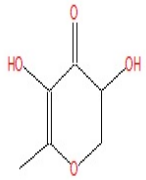
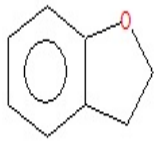
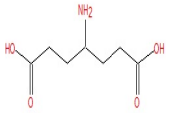
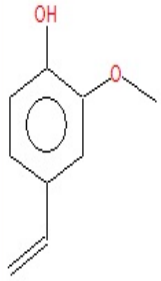
5.	4-Hexenal, 6-hydroxy-4-methyl,dimethyl acetal, acetate	5.158	$C_{11}H_{20}O_4$	216	216.136159		58,67,75,84,93,110,138,152,184,215	Anagestic, anticoagulant and antipyretic
6.	4H-pyran-4-one,2,3,-dihydro-3,5-dihydroxy-6-methyl	6.011	$C_6H_8O_4$	144	144.042258		55,58,72,85,101,115,126,144	Anti-tussive, antibacterial activities and antioxidant
7.	Benzofuran	6.760	$C_8H_8O_4$	120	120.0575147		51,63,77,91,105,120	Antimicrobial and anti-inflammatory activities.
8.	4-Amino-1,5-pentandioic acid	6.320	$C_7H_{13}NO_4$	175	175.084458		55,73,84,101,129,175	Anti-cancer agents
9.	2-Methoxy-4-vinylphenol	7.974	$C_9H_{10}O_2$		150.06808		51,63,77,89,107,118,135,150	Anti-inflammatory effect

Table 1. Cont'd.

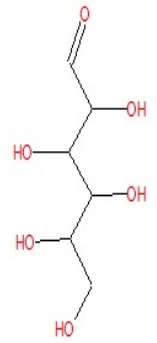
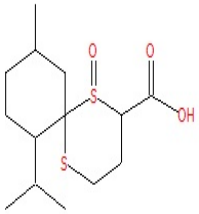
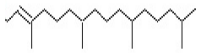
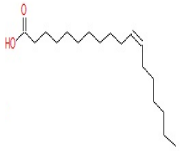
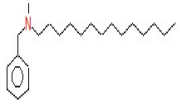
10	d-Mannose	13.215	$C_6H_{12}O_6$	180	180.063388		60,73,85,103,131,149,179	Anti-allergic and anti-bacterial
11.	7-Isopropyl-10-methyl-1-oxo-1,5-dithia-spiro[5,5]undecane-2-carboxy	14.743	$4H_{24}O_3S_2$	304	304.116686		55,69,81,95,123,137,167,211,243,304	New chemical compound
12.	Phytol	16.625	$C_{20}H_{40}O$	296	296.307917		57,71,81,95,111,123,137,196,221,249,278	Anti-inflammatory and antinociceptive activities
13.	Cis-Vaccenic acid,	17.014	$C_{18}H_{24}O_2$	282	282.25588		55,69,83,97,111,125,165,193,222,264,282	Anti-carcinogenic effect
14.	N-Methyl-Nbenzyltetradecanamine	18.485	$C_{22}H_{39}N$	317	317.30825		55,91,120,134,147,172,195,226,260,281,317	Anti ulcer agents

Table 1. Cont'd.

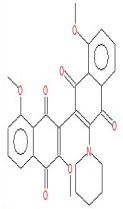
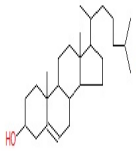
15.	3,8,8-Trimethoxy-3-piperidyl-2,2-binaphthalene-1,1,4,4-tetrone,	20.293	$C_{23}H_{28}NO_7$	487	487.163101		57,71,112,149167,223,279,328	Anticancer, antiarthritic and anti-inflammatory
16.	17-(1,5-Dimethylhexyl)10,13-dimethyl	26.284	$H_{69}NO_6Si_3$	386	386.354866		57,69,95,145,213,255,275,301,	Anti-HIV Agents

Table 2. FT-IR peak values of methanolic extract of *O. vulgare*

No.	Peak (Wave number cm-I)	Intensity	Bond	Functional group assignment	Group frequency
1.	665.44	64.274	-	Unknown	-
2.	729.09	69.940	C-H	Alkenes	675-995
3.	813.96	74.033	C-H	Alkenes	675-995
4.	918.12	73.759	C-H	Alkenes	675-995
5.	1026.13	55.406	C-F stretch	Aliphatic fluoro compounds	1000-10150
6.	1242.16	74.424	C-O	Alcohols, Ethers, Carboxlic acids, Esters	1050-1300
7.	1317.38	77.763	NO2	Nitro Compounds	1300-1370
8.	1361.74	76.351	NO2	Nitro Compounds	1300-1370
9.	1602.85	75.990	-	Unknown	-
10.	1732.08	83.527	-	Unknown	-
11.	2848.86	83.173	-	Unknown	-
12.	2918.30	79.612	C-H	Alkanes	2850-2970

Table 2. Cont'd.

13.	3064.89	86.700	H-O	H-bonded H-X group	2500-3500
14.	3182.55	83.546	H-O	H-bonded H-X group	2500-3500
15.	3217.27	82.889	O-H	Hydrogen bonded Alcohols, Phenols	3200-3600
16.	3277.06	81.843	O-H	Hydrogen bonded Alcohols, Phenols	3200-3600
17.	3363.86	82.552	O-H	Hydrogen bonded Alcohols, Phenols	3200-3600

CONCLUSION

O. vulgare is native plant of Iraq. It contain chemical constitutions which may be useful for various herbal formulation as anti-inflammatory, analgesic, antipyretic, cardiac tonic and antiasthamatic.

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Conflict of interest

Authors have none to declare

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