

## Full Length Research Paper

# Phytochemical screening of methanolic dried galls extract of *Quercus infectoria* using gas chromatography-mass spectrometry (GC-MS) and Fourier transform-infrared (FT-IR)

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The main objective of this study was to determine the phytochemical composition from the dried galls of *Quercus infectoria*, using methanolic extraction and report the main functional components by using infrared (IR) technique. The phytochemical compound screened by gas chromatography-mass spectrometry (GC-MS) method. Twelve bioactive phytochemical compounds were identified in the methanolic extract of *Q. infectoria*. The identification of phytochemical compounds is based on the peak area, retention time molecular weight, and molecular formula. GC-MS analysis of *Q. infectoria* revealed the existence of the Cis-p-mentha -1(7),8-dien-2-ol, 3-Nonynoic acid, Urea, N,N'-bis(2-hydroxyethyl)-, 3-Trifluoroacetoxypentadecane, Pterin -6-carboxylic acid, 2,2-Difluoroheptacosanoic acid,  $\gamma$ -Sitosterol, Spirost-8-en-11-one, 3-hydroxy-, (3 $\beta$ ,5 $\alpha$ ,14 $\beta$ ,20 $\beta$ ,22 $\beta$ ,25R)-, Curan,16,17-didehydro-, (20xi)-, 17.alfa.21 $\beta$ -28,30-Bisnorhopane, Ethyl iso-allocholate, Milbemycin B,6,28-anhydro-15-chloro-25-isopropyl-13-dehydro-5-. The Fourier transform-infrared (FTIR) analysis of *Q. infectoria* proved the presence of alkenes, aliphatic fluoro compounds, nitro compounds, alkanes, hydrogen bonded alcohols, and phenols.

**Key words:** *Quercus infectoria*, Fourier transform-infrared (FT-IR), gas chromatography-mass spectrometry (GC-MS) analysis, phytochemicals.

## INTRODUCTION

*Quercus infectoria* is an oak tree of the family Fagaceae in the Mediterranean area, especially in Greece, Syria,

Iran, and Asia Minor (Samuelsson, 1999). The galls arise on young branches of this tree as a result of attack by

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female gasp-wasp *Adleria gallae-tinctoria* and *Cynips gallae tinctoria* by deposition of the eggs (Greenish, 1999). *Q. infectoria* is a small tree widely distributed in Greece, Asia Minor, and Iran. It has been evaluated in terms of its pharmacological effects and it was found that it had antiparkinsonian, antitremorine, antiinflammatory, antidiabetic, and antioxidant effects (Aivazi and Vijayan, 2009; Altameme et al., 2015a). Traditionally, galls are used in postpartum practice (Soon et al., 2007) and in the treatment of diarrhea, hemorrhage, and skin disease (Greenish, 1999; Hameed et al., 2015a). The galls of *Q. infectoria* were documented to possess antibacterial (Basri et al., 2005; Darogha, 2009), anti-MRSA (Chusri and Voravuthikunchai, 2009), antiviral (Hussein et al., 2000), antifungus (Yamunarani et al., 2005; Yoshikawa et al., 2007; Hameed et al., 2015b), and anti-inflammatory activities. Previous investigation revealed that the ethanol extract of the nutgalls consists of tannins, flavonoids, and steroidal compounds (Rukayadi et al., 2006; Chusri and Voravuthikunchai, 2009; Mekseepalard et al., 2010). The constituents of the galls of *Q. infectoria* comprise a large amount of tannins, gallic acid, syringic acid, ellagic acid, beta sitosterol, amentoflavone hexamethyl ether, isocryptomerin, methyl betulate, methyl olenate, and hexagalloyl glucose (Lodhi et al., 2012; Hameed et al., 2015c). Larvacidal activity of the gall extracts of *Q. infectoria* was initially reported against *Anopheles stephensi* (Aivazi and Vijayan, 2009). The main constituents of the galls are tannin (50 to 70%) with small amount of free gallic acid and starch. The present study aimed to analyze the methanol extract of *Q. infectoria* galls.

## MATERIALS AND METHODS

### Collection and preparation of plant

The dried galls were purchased from local market in Hilla city, middle of Iraq. After thorough cleaning and removal of foreign materials, the dried galls were stored in airtight container to avoid the effect of humidity and then stored at room temperature until further use (Altameme et al., 2015b; Hameed et al., 2015d).

### Preparation of sample

About 15 g of the plant sample powdered were soaked in 75 ml methanol for 14 h in a rotatory shaker. Whatman No.1 filter paper was used to separate the extract of the plant. The filtrates were used for further phytochemical analysis (Hussein et al., 2015; Jasim et al., 2015; Hamza et al., 2015). It was again filtered through sodium sulphate in order to remove the traces of moisture.

### Gas chromatography-mass spectrum (GC-MS) analysis

GC-MS technique was used in this study to identify the components present in the extract which was carried out at Indian Institute of Science, Bangalore. The GC-MS analysis of the plant extract was made in a Agilent 7890 A instrument under computer control at 70 eV (Kareem et al., 2015; Imad et al., 2014a). 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. 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 is, the bigger 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) (Imad et al., 2014b). 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 Mass/Charge (M/Z) ratio obtained was calibrated from the graph obtained, which was called 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/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) (Mohammed and Imad, 2013; Imad et al., 2014c). The identity of the components in the extracts was assigned by comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures (Yang et al., 2010).

## RESULTS AND DISCUSSION

GC-MS analysis of compounds was carried out in methanolic dried galls extract of *Q. infectoria* and is shown in Table 1. The GC-MS chromatogram of the twenty peaks of the compounds detected as shown in Figure 1. Chromatogram GC-MS analysis of the methanol extract of *Q. infectoria* showed the presence of twenty major peaks and the components corresponding to the peaks were determined as follows. The first set up peak were determined to be Cis-p-mentha -1(7),8-dien-2-ol (Figure 2). The next peaks were considered to be 3-Nonynoic acid, Urea, N,N'-bis(2-hydroxyethyl)-, 3-Trifluoroacetoxypentadecane, Pterin -6-carboxylic acid, 2,2-Difluoroheptacosanoic acid, γ-Sitosterol, Spirost-8-en-11-one, 3-hydroxy-, (3β,5α,14β,20β,22β,25R)-, Curan,16,17-didehydro-, (20xi)-, 17.alfa.21β-28,30-Bisnorhopane, Ethyl iso-allocholate, Milbemycin B,6,28-anhydro-15-chloro-25-isopropyl-13-dehydro-5 (Figures 3 to 13). Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects. Further continued exploration of plant derived antimicrobials is needed today. The FTIR analysis of *Q. infectoria* proved the presence of alkenes, aliphatic fluoro compounds, nitro compounds Table 2, alkanes, hydrogen bonded alcohols and phenols which show major peaks at 744.52, 806.25, 920.05, 1026.13,

**Table 1.** Major phytochemical compounds identified in methanolic extract of *Quercus infectoria*.

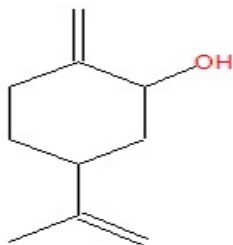
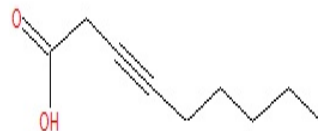
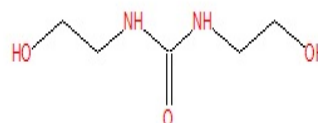
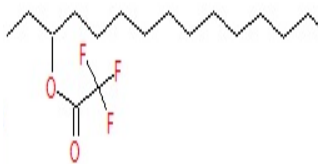
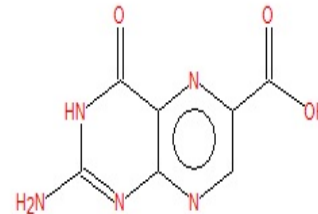
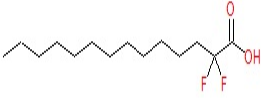
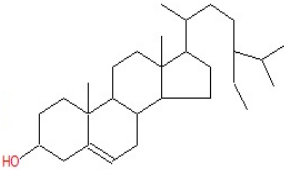
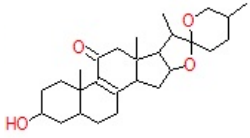
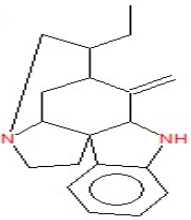
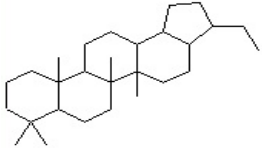
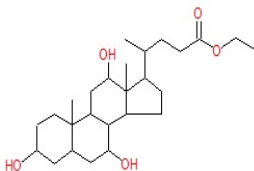
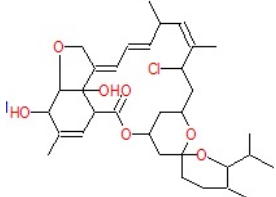
S/N	Phytochemical compound	RT (min)	Formula	Molecular weight	Exact mass	Chemical structure	MS Fragment-ions	Pharmacological actions
1	Cis-p-mentha -1(7),8-dien-2-ol	4.523	C <sub>10</sub> H <sub>16</sub> O	152	152.120115		55, 67, 79, 91, 109, 119, 134, 152	New chemical compound
2	3-Nonynoic acid	4.140	C <sub>9</sub> H <sub>14</sub> O <sub>2</sub>	154	154.09938		55, 70, 79, 94, 97, 108, 125, 139, 149	Anti- bacterial activity
3	Urea , N,N'-bis(2-hydroxyethyl)-	5.559	C <sub>5</sub> H <sub>12</sub> N <sub>2</sub> O <sub>3</sub>	148	148.084792		61, 81, 132, 146	Anti-bacterial and anti-tumor activities
4	3-Trifluoroacetoxypentadecane	6.205	C <sub>17</sub> H <sub>31</sub> F <sub>3</sub> O <sub>2</sub>	324	324.227615		55, 69, 77, 83, 91, 97, 111, 125, 138, 153, 163	Antimicrobial, anti-inflammatory
5	Pterin -6-carboxylic acid	7.127	C <sub>7</sub> H <sub>5</sub> N <sub>5</sub> O <sub>3</sub>	207	207.039239		57, 69, 105, 122, 149, 163, 177, 207	Anti-psychotic, mood-stabilizer and anti-parasite

Table 1. Cont'd

6	2,2-Difluoroheptacosanoic acid	12.688	$C_{14}H_{26}F_2O_2$	264	264.190086		57, 71, 85, 101, 129, 151, 165, 185, 207, 237, 264	Antimicrobial activity
7	$\gamma$ -Sitosterol	14.388	$C_{29}H_{50}O$	414	414.386166		55, 69, 81, 145, 161, 213, 255, 303, 329, 354, 381, 396, 414	Anti-inflammatory activity
8	Spirost-8-en-11-one, 3-hydroxy-, (3 $\beta$ ,5 $\alpha$ ,14 $\beta$ ,20 $\beta$ ,22 $\beta$ ,25R)-	17.295	$C_{27}H_{40}O_4$	428	428.29266		57, 69, 77, 95, 109, 135, 159, 173, 207, 229, 267, 281, 299, 314, 327, 356, 405	Estrogenic, progestogenic and anti-inflammatory effects
9	Curan,16,17-didehydro-, (20xi.)-	17.649	$C_{19}H_{24}N_2$	280	280.193949		55, 69, 83, 110, 130, 144, 182, 225, 243, 280	New chemical compound
10	17.alpha.21 $\beta$ -28,30-Bisnorhopane	18.559	$C_{28}H_{48}$	384	384.3756		81, 95, 109, 149, 163, 177, 191, 217, 246, 299, 328, 369, 384	New chemical compound
11	Ethyl iso-allocholate	22.479	$C_{26}H_{44}O_5$	436	436.318874		55, 69, 81, 95, 213, 253, 400, 418	Anti-inflammatory activity and anti-infective

**Table 1.** Cont'd

12	Milbemycin B,6,28-anhydro-15-chloro-25-isopropyl-13-dehydro-5-	27.743	$C_{33}H_{47}ClO_7$	590	590.301033		55, 78, 95, 151, 209, 282, 348, 390, 426, 462, 518	Anti-infective effect
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**Table 2.** FT-IR peak values of methanolic seeds extract of *Quercus infectoria*.

No.	Peak (Wave number $cm^{-1}$ )	Intensity	Bond	Functional group assignment	Group frequency
1	744.52	48.444	C-H	Alkenes	675-995
2	761.88	47.875	C-H	Alkenes	675-995
3	806.25	65.347	C-H	Alkenes	675-995
4	821.68	65.697	C-H	Alkenes	675-995
5	869.90	57.620	C-H	Alkenes	675-995
6	920.05	63.145	C-H	Alkenes	675-995
7	1002.98	35.856	C-F stretch	Aliphatic fluoro compounds	1000-10150
8	1026.13	32.232	C-F stretch	Aliphatic fluoro compounds	1000-10150
9	1070.49	49.397	C-F stretch	Aliphatic fluoro compounds	1000-10150
10	1195.87	36.381	C-O	Alcohols, ethers, carboxylic acids, esters	1050-1300
11	1313.52	45.329	NO <sub>2</sub>	Nitro compounds	1300-1370
12	1442.75	64.028	C-H	Alkanes	1340-1470
13	1533.41	73.386	-	Unknown	-
14	1608.63	60.035	-	Unknown	-
15	1693.50	64.149	-	Unknown	-
16	2430.31	90.556	-	Unknown	-
17	2578.83	88.049	-	Unknown	-
18	2704.20	86.084	-	Unknown	-
19	2850.79	83.910	C-H	Alkanes	2850-2970
20	2922.16	81.091	C-H	Alkanes	2850-2970
21	2954.95	80.623	C-H	Alkanes	2850-2970
22	3232.70	71.274	O-H	Hydrogen bonded alcohols, phenols	3200-3600
23	3277.06	70.546	O-H	Hydrogen bonded alcohols, phenols	3200-3600
24	3296.35	70.436	O-H	Hydrogen bonded alcohols, phenols	3200-3600
25	3313.71	70.372	O-H	Hydrogen bonded alcohols, phenols	3200-3600

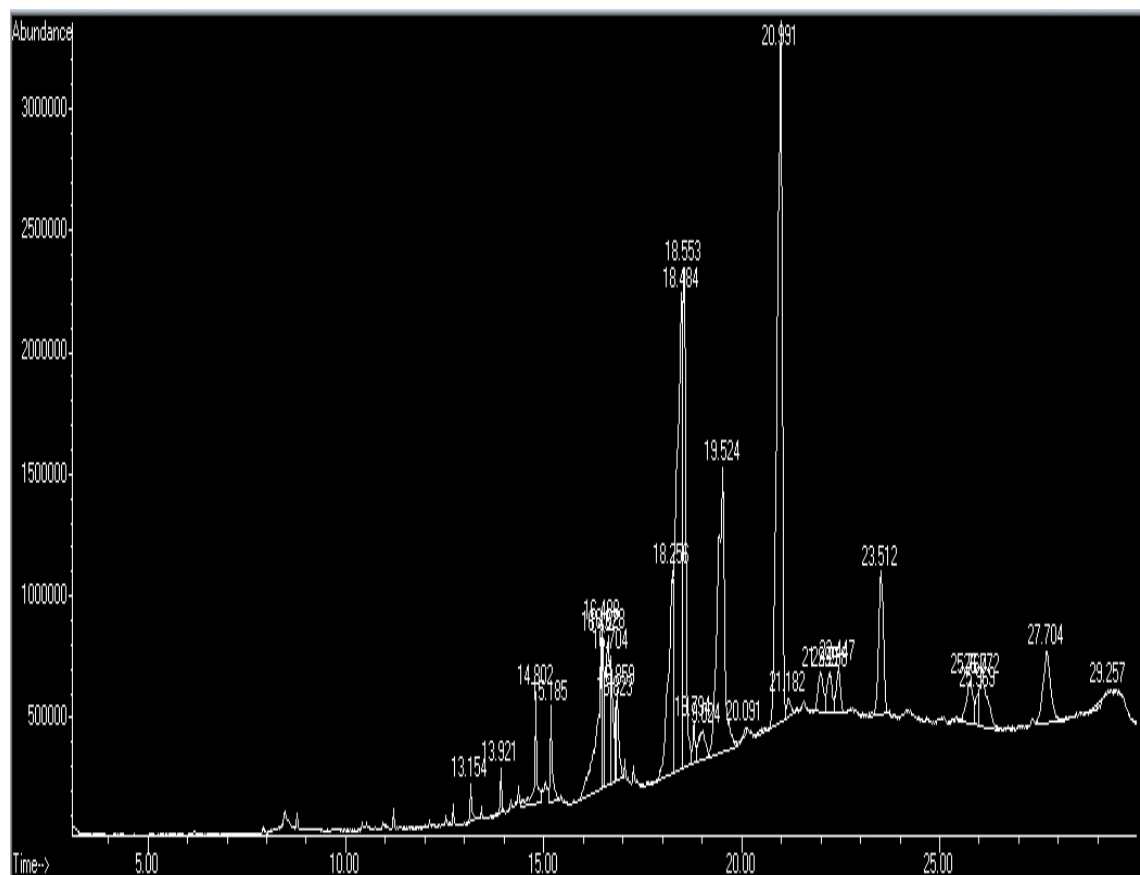


Figure 1. GC-MS chromatogram of methanolic extract of *Quercus infectoria*.

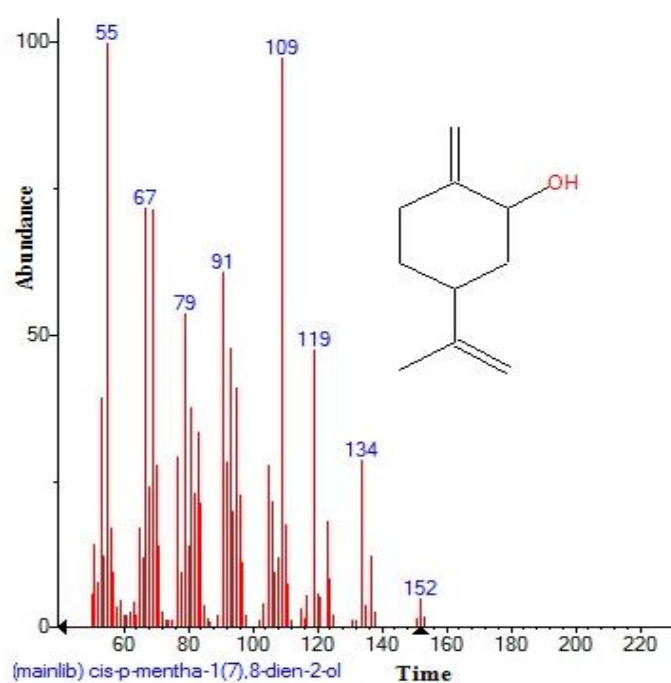


Figure 2. Structure of Cis-p-mentha-1(7),8-dien-2-ol present in *Quercus infectoria* using GC-MS analysis.

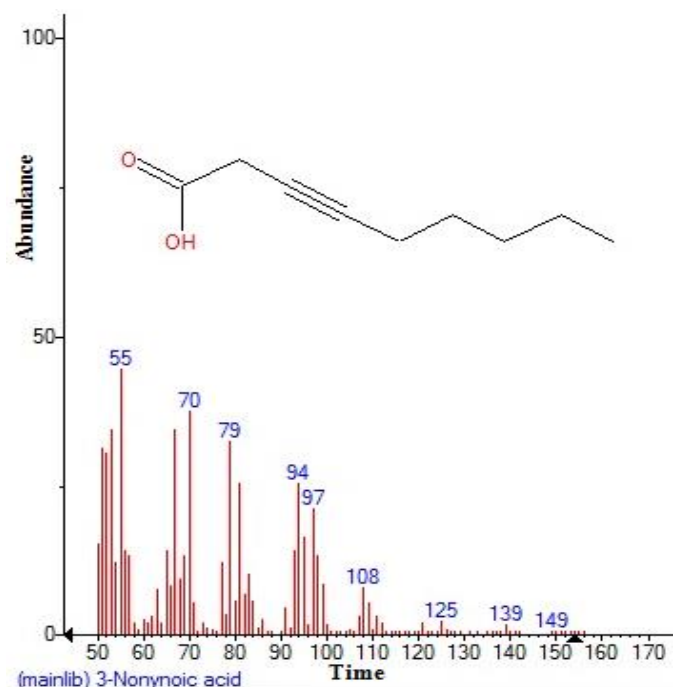
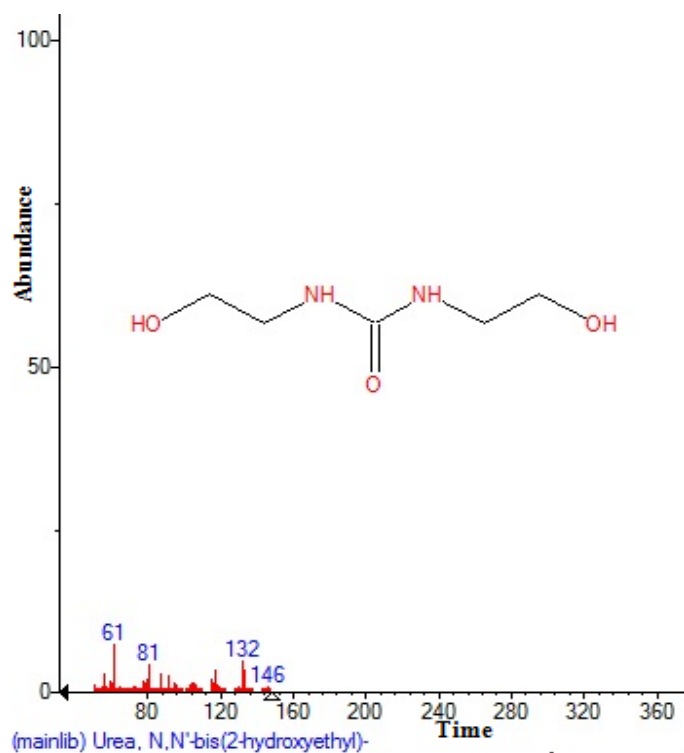
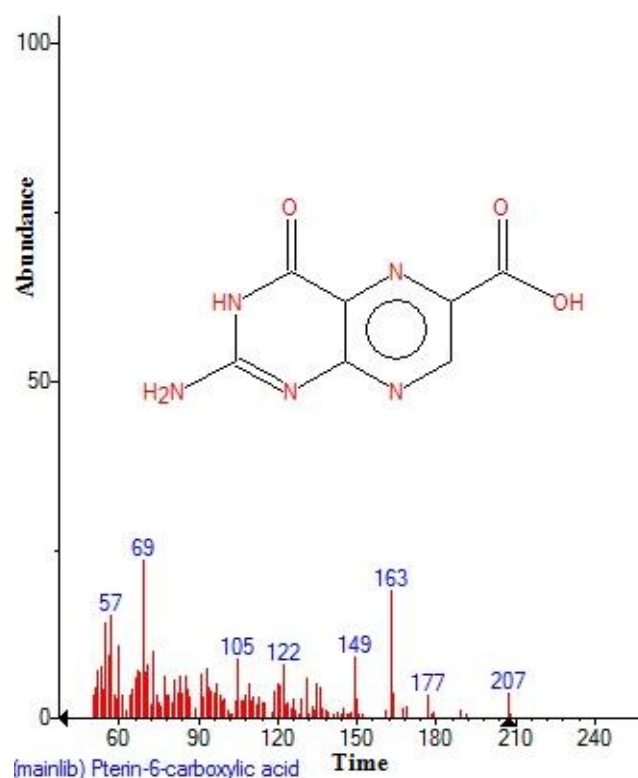


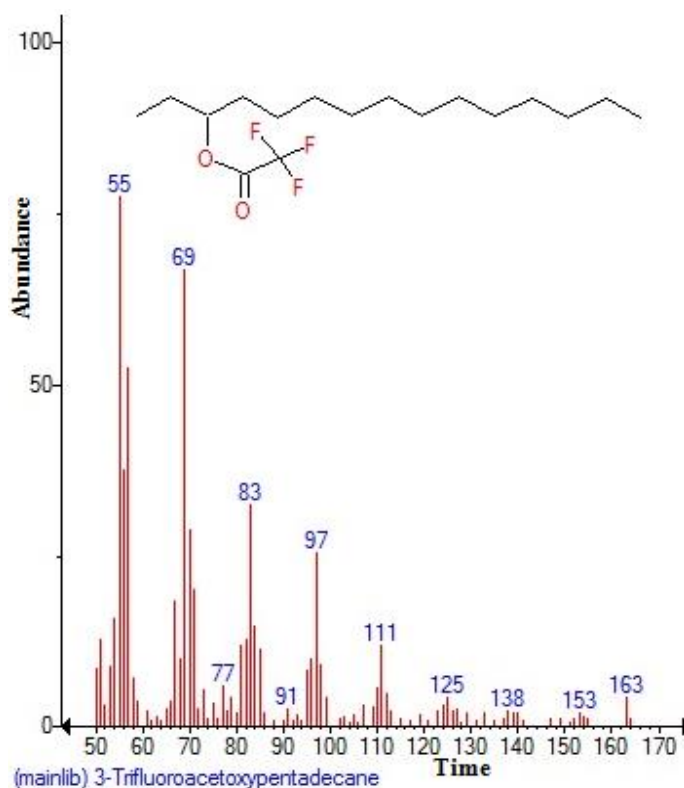
Figure 3. Structure of 3-Nonynoic acid present in *Quercus infectoria* using GC-MS analysis.



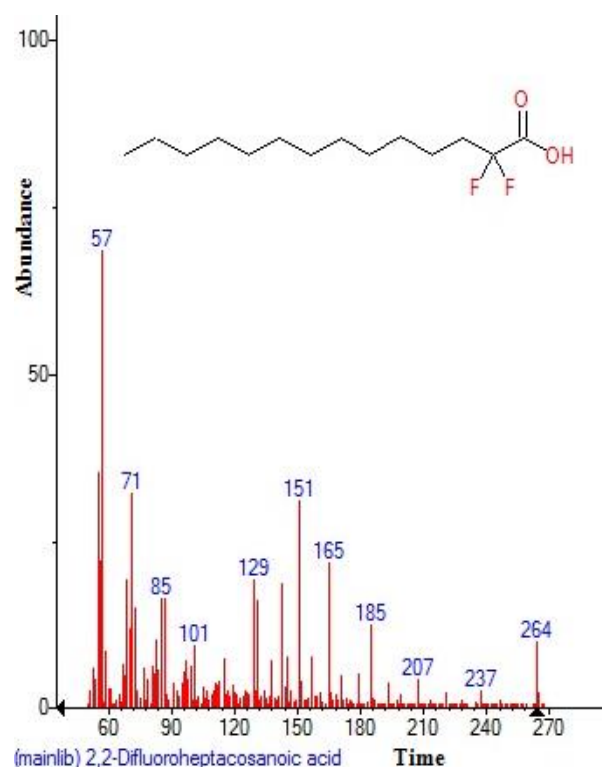
**Figure 4.** Structure of Urea, N,N'-bis(2-hydroxyethyl) present in *Quercus infectoria* using GC-MS analysis.



**Figure 6.** Structure of Pterin-6-carboxylic acid present in *Quercus infectoria* using GC-MS analysis.

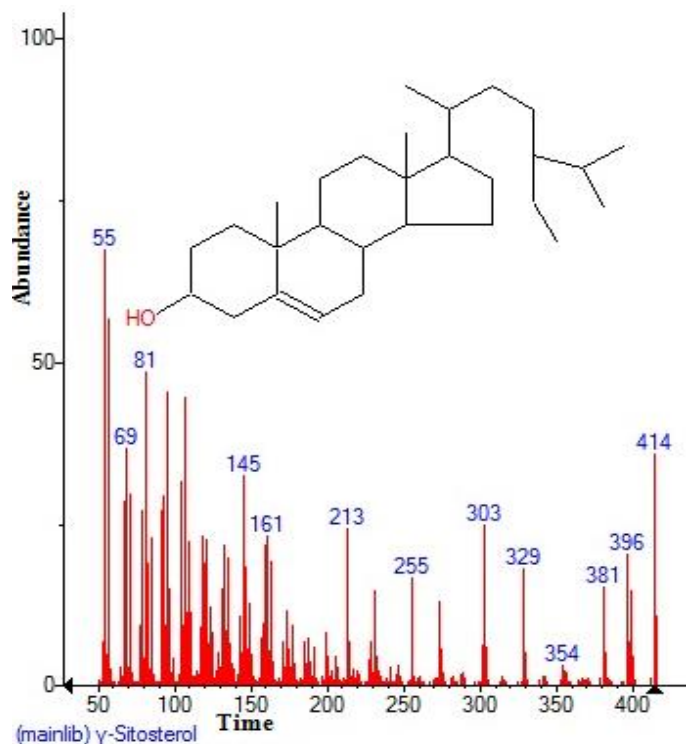


**Figure 5.** Structure of 3-Trifluoroacetoxy-pentadecane present in *Quercus infectoria* using GC-MS analysis.

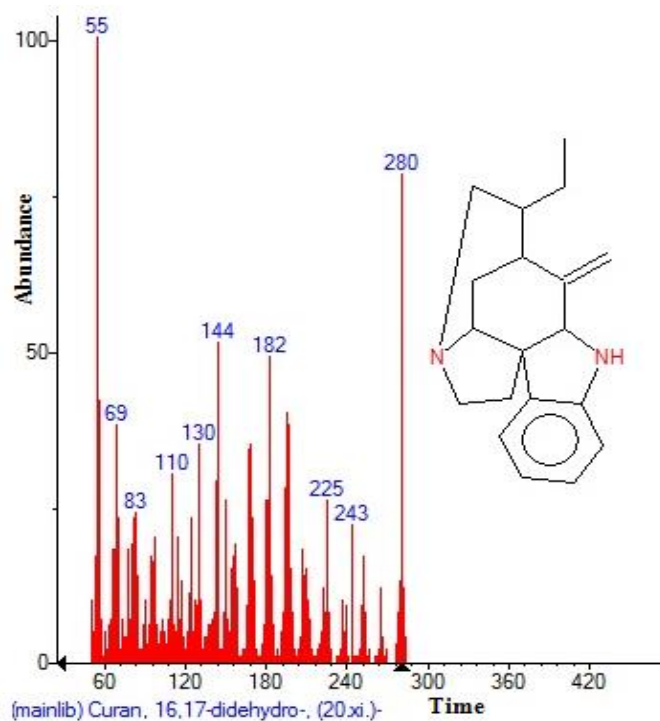


**Figure 7.** Structure of 2,2-Difluoroheptacosanoic acid present in *Quercus infectoria* using GC-MS analysis.

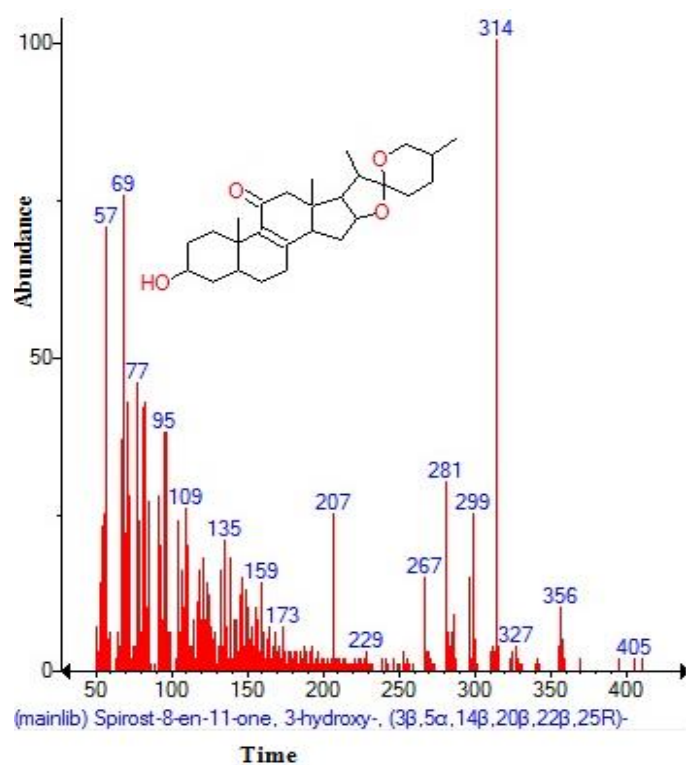




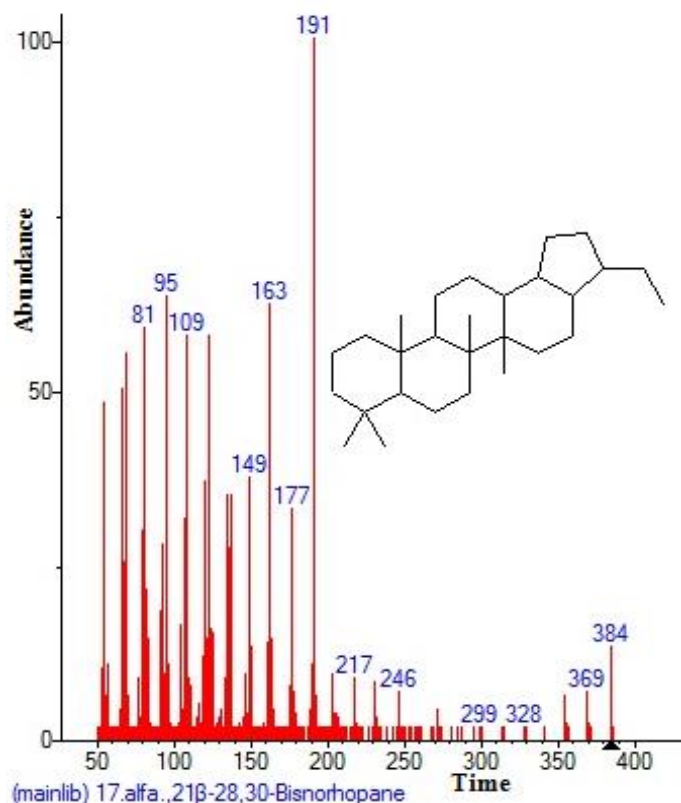
**Figure 8.** Structure of  $\gamma$ -Sitosterol present in *Quercus infectoria* using GC-MS analysis.



**Figure 10.** Structure of Curan, 16,17-didehydro-, (20xi)- present in *Quercus infectoria* using GC-MS analysis.

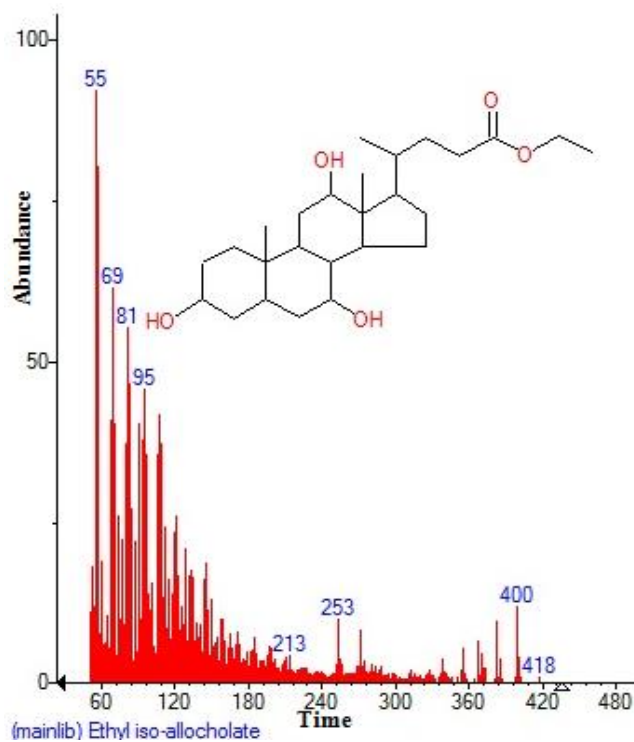


**Figure 9.** Structure of Spirost-8-en-11-one, 3-hydroxy-, (3 $\beta$ ,5 $\alpha$ ,14 $\beta$ ,20 $\beta$ ,22 $\beta$ ,25R) present in *Quercus infectoria* using GC-MS analysis.

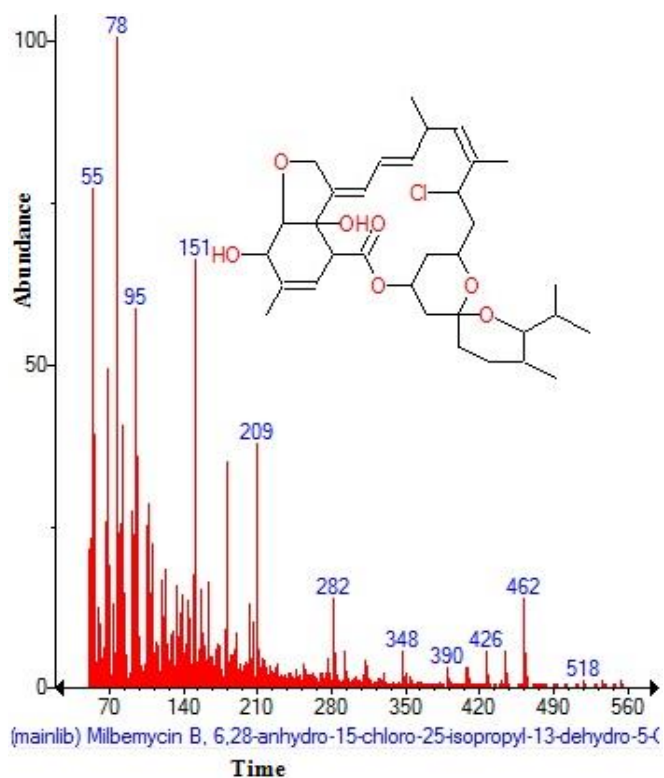


**Figure 11.** Structure of 17.α.,21 $\beta$ -28,30-Bisnorhopane present in *Quercus infectoria* using GC-MS analysis.





**Figure 12.** Structure of Ethyl iso-allocholate present in *Quercus infectoria* using GC-MS analysis.



**Figure 13.** Structure of Milbemycin B, 6,28-anhydro-15-chloro-25-isopropyl-13-dehydro-5- present in *Quercus infectoria* using GC-MS analysis.

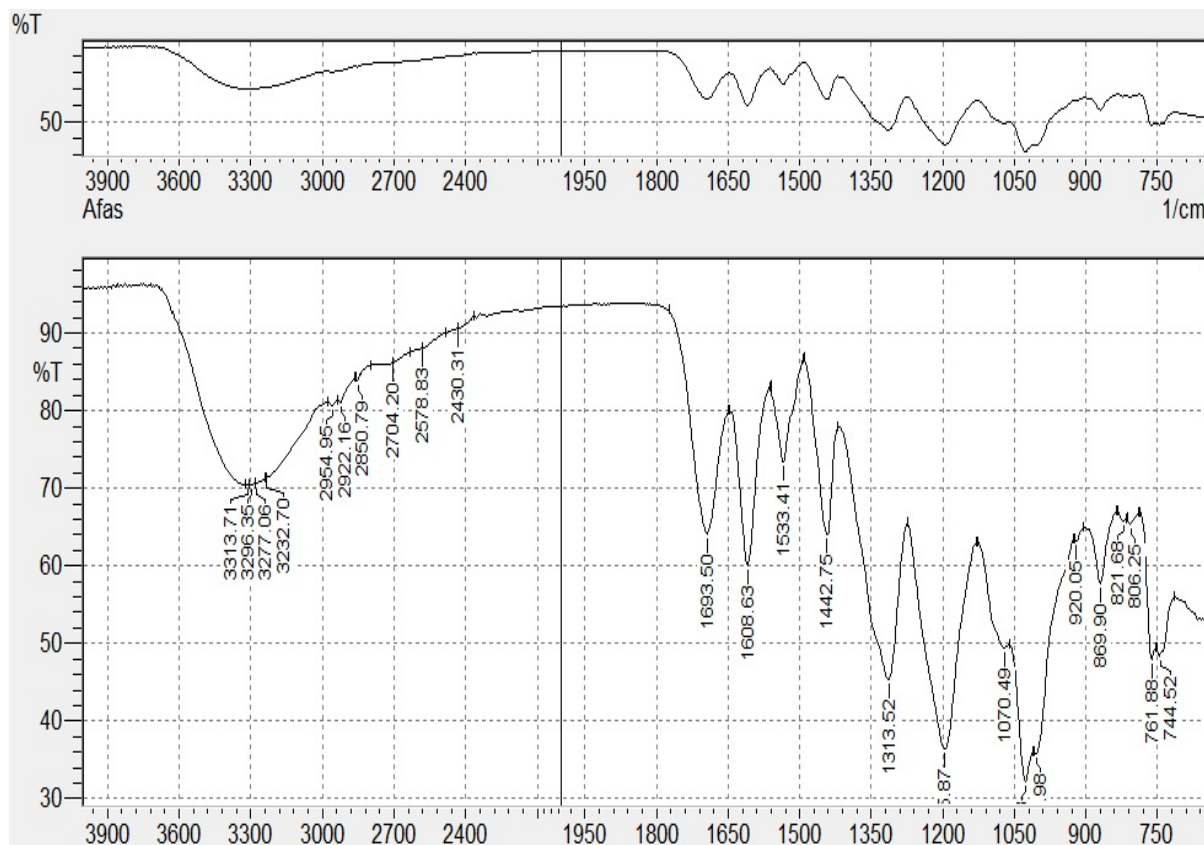


Figure 14. FT-IR profile of *Quercus infectoria*.

1195.87, 1313.52, 1442.75, 2850.79, 2954.95, 3232.70, and 3313.71 (Figure 14).

## Conclusion

*Q. infectoria* is a native plant of Iraq. Thus, the GC-MS analysis of methanolic extract of *Q. infectoria* showed a highly complex profile containing approximately twelve components. This study may be useful to further explore the pharmacological and biosynthetic activity of the plants.

## ACKNOWLEDGEMENT

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