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ANALYSIS OF BIOACTIVE PHYTOCHEMICAL COMPOUNDS OF TWO MEDICINAL PLANTS, *Equisetum arvense* AND *Alchemila valgaris* SEEDS USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY AND FOURIER-TRANSFORM INFRARED SPECTROSCOPY

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ABSTRACT

Twenty five bioactive phytochemical compounds were identified in the methanolic extract of *Equisetum arvense* and *Alchemila valgaris*. The identification of phytochemical compounds is based on the peak area, retention time molecular weight and molecular formula. GC/MS analysis of *Equisetum arvense* revealed the existence of the Cyclohexene, 4-isopropenyl-1-methoxymethyl, α-D-Glucopyranoside, O-α-D-Glucopyranosyl, Paromomycin, 3,6,9,12-Tetraoxatetradecan-1-ol, Bicyclo[3.2.1] oct-6-ene-6,8-dimethanol, 1,7-dimethyl-4isopropyl, 2-Undecanone, 3-N,N-Dimethyllaurylammonio) propanesulfonate, d-Mannose, 3-O-Methyl-d-glucose, 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, Benzaldehyde, 2-chloro-4-hydroxy-3, Cyclopropa[3,4]cyclopenta [1,2-a]naphthalene, Furo[2,3-b]quinoline,4,6,7-trimethoxy, 2(1H)-Phenanthrenone. GC/MS analysis of *Alchemila valgaris* revealed the existence of the Levoglucosenone, Spirost-8-en-11-one, 3-hydroxy, Ethanol, 2-(9-Octadecenyloxy), 2,7-Diphenyl-1,6dioxopyridazino[4,5:2,3]pyrrolo, Estra-1,3,5(10)-trien-17β-ol, Octadecanal,2-bromo, Ethyl 9,12,15-octadecatrienote, 3-Pyridinecarboxylic acid,2,7,10-tris(acetyloxy)-1, 5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, Stigmastan-3,5-diene and Tocopherol. It contains chemical constituents which may be useful for various herbal remedy as anti-inflammatory, analgesic, antipyretic, cardiac tonic and antiasthamatic. The FT-IR analysis of both *Equisetum arvense* and *Althaea rosea* seeds proved the presence of Alkenes, Aliphatic fluoro compounds, Alcohols, Ethers, Carboxlic acids, Esters, Nitro Compounds and Alkanes.

Key words: *Alchemila valgaris*, *Equisetum arvense*, FT-IR, Gas chromatography-mass spectrometry, Phytochemicals

INTRODUCTION

Phytochemicals are chemical compounds formed during plants normal metabolic processes. *Equisetum arvense* L. (Equisetaceae, subgenus Equisetum) is a well-known and its sterile stems are used as medicines in various countries (Dos *et al.,* 2005). *Equisetum arvense* L. (horsetail) is traditionally used in the treatment of skin and for oral infections among humans throughout Iraq. It is as a bathing remedy for rheumatic diseases, gout, and in the treatment protocol of tumescence, and fractured bones in Europe (Dew *et al.,* 2007; Habauzit and Horcajada, 2008; Imad *et al.,* 2015a; Ameera *et al.,* 2015). Horsetail is rich in sterols, ascorbic acid, phenolic acids, flavonoids (D'Agostino *et al.,* 1984; Wichtl, 1994) and styrylpyrones (Veit *et al.,* 1995; Nagai *et al.,* 2004; Odabasoglu *et al.,* 1995). Several studies showed a anti-inflammatory, antimicrobial and anti-nociceptive (Broudiscou *et al.,* 2000; Mekhfi *et al.,* 2004; Dos *et al.,* 2005; Guilherme *et al.,* 2005; Aramwit and Sangcakul *et al.,* 2007).

E. arvense has a high concentration of silica (Holzhiter *et al.,* 2003) and it has been suggested that this pays a significant contribution to its medicinal properties, particularly on bone disorders (Duke *et al.,* 2002; Van and Wink, 2004; Wichtl, 2004) and diuresis (Graefe and Veit, 1999) and are antioxidant (Trouillas *et al.,* 2003), vasorelaxant (Sakurai *et al.,* 2003) antinociceptive, anti-inflammatory (Martins *et al.,* 2004) and possess germination inhibitory activity (Hiraga *et al.,* 1997). *Alchemila vulgaris* L. (Syn. *A. xanthochlora*

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Rothm.) is the most commonly used species. It is used as a folk medicine especially in north-east region of Turkey (Kaya *et al.,* 2012) and used against mild or non-specific diarrhea is approved by Commission E (Gruenwald *et al.,* 2004). Another use of *Alchemilla* species is for the adaptation to the hormonal levels of the body in case of menopause (Falchero *et al.,* 2009). It is used to heal inflammations in mouth, gynecological diseases, bleeding of the nose and furuncules. This plant is also considered to regulate the glandular activity of uterine and reduce bleeding. Uses of the infusion prepared with this plant as astringent, antidiarrhetic, antiinflammatory and antisecptic are recorded (Ivancheva *et al.,* 2006; Kiselova *et al.,* 2006).

A. vulgaris which is traditionally used of as an antiinflammatory, folk remedy in Montenegro, to treat mild and nonspecific diarrhea, menopausal complaints and dysmenorrhea as well as ulcers, eczema and skin rashes externally (Lans *et al.,* 2007; Menkovic *et al.,* 2011). It is also used as antihemorrhagic, antidiarrheal and astringent in France (Trouillas *et al.,* 2003; Kiselova *et al.,* 2006; Condrat *et al.,* 2009; Kiselova *et al.,* 2009; Oktyabrskay *et al.,* 2009; Pawlaczyk *et al.,* 2009; Condrat *et al.,* 2009). The study of Ondrejovic *et al*. (2009) showed the significantly higher antioxidant activity of methanolic extract of *A. vulgaris* in comparison with the extracts prepared using n-hexane, chloroform, ethylacetate and water as solvents (Djipa *et al.,* 2000).

In vivo studies have shown the wound healing activity of *A. vulgaris* and this activity is reported to be associated with promitotic activity in epithelial cells and myofibroblasts (Shrivastava and John, 2006; Shirivastava *et al.,* 2006; Slanc *et al.,* 2006; Imad *et al.,* 2015b). *A. vulgaris* is also reported to show inhibitory activity of pancreatic lipase in the study of Slanc *et al.* (2006). Hence, the present study was conducted to investigate the bioactive phytochemical compounds in the methanolic extract of horsetail (*Equisetum arvense*) and *Alchemila vulgaris* seeds using gas chromatography-mass spectrometry and fourier-transform infrared spectroscopy.

MATERIALS AND METHODS

Collection and preparation of plant materials

Both *E. arvense* and *A. vulgaris* were purchased from local market in Jbala, Iraq. After cleaning and removal foreign materials the seeds were stored in an air-tight container and then stored at room temperature until further use. Eighteen grams of each methanolic extract of plants powdered were soaked in fourty ml methanol for ten hours in a rotatory shaker (Huda *et al.,* 2015a). Whatman No.1 filter paper was used to separate the extract of plant and again filtered through sodium sulphate in order to remove the moisture.

Gas chromatography – mass spectrum (GC/MS) analysis

The GC-MS analysis of the plants extract was made in a (QP 2010 Plus SHIMADZU) 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 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) (Mohammed and Imad, 2013; Muhanned *et al.,* 2015; Imad *et al.,* 2014a). While the instrument was run, the computer generated a graph from the signal called a Chromatogram. Each peak in the chromatogram represents 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 (Imad *et al.,* 2014b).

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 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.,* 2015c).

Fourier transform infrared spectrophotometer (FTIR)

The samples was treated for Fourier transform infrared spectroscopy (Shimadzu, IR Affinity 1, Japan) then run at infrared region between 400 nm and 4000 nm (Huda *et al.,* 2015b).

RESULTS AND DISCUSSION

Chromatogram GC-MS analysis of the methanol extract of *Equisetum arvense* showed the presence of fourteen major peaks (Table 1 and Figure 1) and the components corresponding to the peaks were determined as follows. The First set up peak were determined to be Cyclohexene, 4-isopropenyl-1-methoxymethyl, (Supplemental Figure 2). The second peak indicated to be α-D-Glucopyranoside, O-α-D-Glucopyranosyl (Supplemental Figure 3). The next peaks were considered to be Cyclohexene, 4-isopropenyl-1-methoxymethyl, α-D-Glucopyranoside, O-α-D-Glucopyranosyl, Paromomycin, 3,6,9,12-Tetraoxatetradecan-1-ol, Bicyclo[3.2.1] oct-6-ene-6,8-dimethanol,1,7-dimethyl-4isopropyl, 2-Undecanone, 3-N,N-Dimethyllaurylammonio) propanesulfonate, d-Mannose, 3-O-Methyl-d-glucose, 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, Benzaldehyde, 2-chloro-4-hydroxy-3, Cyclopropa[3,4]cyclopenta[1,2-a]naphthalene, Furo[2,3-b]quinoline,4,6,7-trimethoxy, 2(1H)-Phenanthrenone (Supplemental Figure 4-15). Fourier-transform infrared analysis of dry methanolic extract of *Equisetum arvense* proved the presence of Alkenes, Aliphatic fluoro compounds, Alcohols, Ethers, Carboxlic acids, Esters, Alkanes, Hydrogen bonded Alcohols and Phenols which shows major peaks at 921.97, 1029.99, 1317.38, 2848.86, 3242.34 and 3242.34 (Table 2; Supplemental Figure 16). Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic extract of *Alchemila valgaris*, shown in (Table 3). The GC-MS chromatogram of the seventeen peaks of the compounds detected was shown in (Supplemental Figure 17). Chromatogram GC-MS analysis of the methanol extract of *Alchemila valgaris* showed the presence of twenty major peaks and the components corresponding to the peaks were determined as follows.

The First set up peak was determined to be Levoglucosenone (Supplemental Figure 18). The second peak indicated to be Spirost-8-en-11-one, 3-hydroxy (Supplemental Figure 19). The next peaks considered to be Ethanol, 2-(9-Octadecenyloxy), 2,7-Diphenyl-1,6dioxopyridazino[4,5:2,3]pyrrolo, Estra-1,3,5(10)-trien-17β-ol, Octadecanal,2-bromo, Ethyl 9,12,15-octadecatrienote, 3-Pyridinecarboxylic acid,2,7,10-tris(acetyloxy)-1, 5H-Cyclopropa [3,4]benz[1,2-e]azulen-5-One, Stigmastan-3,5-diene and Tocopherol (Supplemental Figure 20-28). Fourier-transform infrared analysis of dry methanolic extract of *Alchemila valgaris* proved the presence of Alkenes, Aliphatic fluoro compounds, Alcohols, Ethers, Carboxlic acids, Esters, Nitro Compounds and Alkanes which shows major peaks at 923.90, 1028.06, 1234.44, 1317.38, 2848.86 and 3064.89 (Table 4; Figure 29).

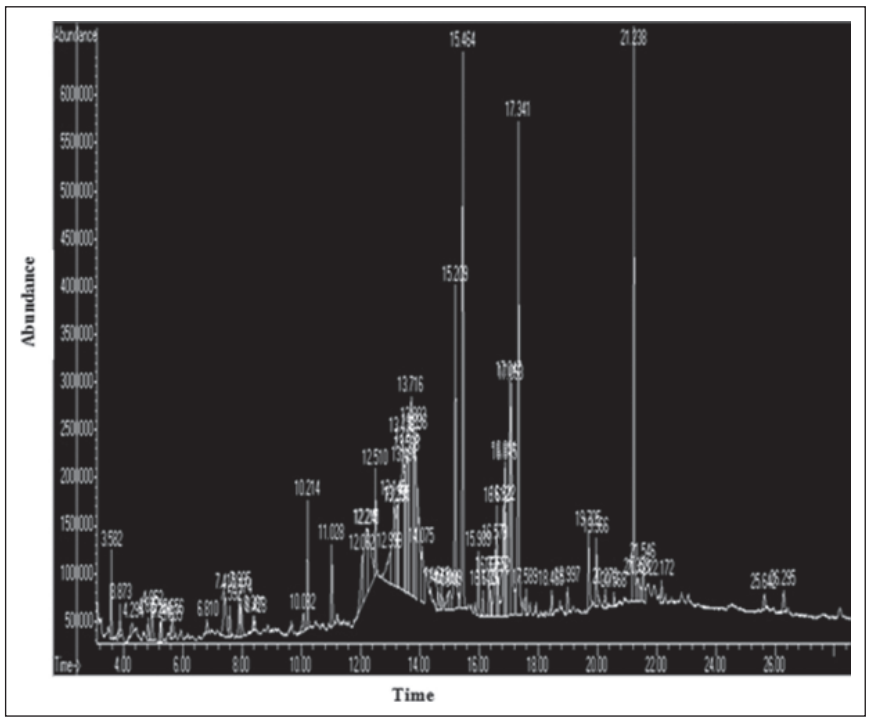


Fig. 1. GC-MS chromatogram of methanolic extract of *Equisetum arvense*.

Table 1. Major phytochemical compounds identified in methanolic extract of *Equisetum arvense*

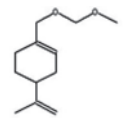
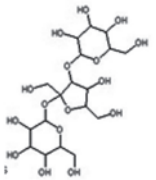
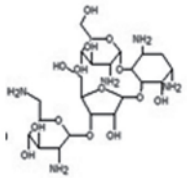
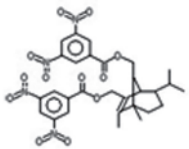
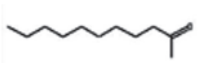
Serial No.	Phytochemical Compound	RT (min)	Formula	Molecular Weight	Exact Mass	Chemical Structure	MS Fragment- ions	Pharmacological actions
1.	Cyclohexene,4-isopropenyl-1-methoxymethyl	4.288	$C_{12}H_{20}O_2$	196	196.14633		53,79,91,119,196	anti-inflammatory effect
2.	α-D-Glucopyranoside, O-α-D-Glucopyranosyl	4.832	$C_{18}H_{32}O_{16}$	504	504.169035		60,73,85,97,113,126, 145,163,180,199	cardioprotective, neuroprotective, antidiabetic and anti-osteoporotic
3.	Paromomycin	5.250	$C_{23}H_{45}N_5O_{14}$	615	615.296303		57,67,80,94,109,124, 162,191,214,231, 248,265,287	Anti-Bacterial Agents
4.	3,6,9,12-Tetraoxatetradecan-1-ol	5.639	$C_{24}H_{42}O_6$	426	426.29814		57,89,113,135,149,161, 175,207,223,249,267, 281,295,311,325,355	Corrosion inhibitors and anti-scaling agents
5.	Bicyclo[3.2.1] oct-6-ene-6,8-dimethanol, 1,7-dimethyl-4isopropyl	6.806	$C_{29}H_{30}N_4O_{12}$	626	626.186024		55,75,120,133,159,212	New chemical compound
6.	2-Undecanone	7.407	$C_{11}H_{22}O$	170	170.167066		58,71,85	larvicidal, anti-inflammatory, analgesic, antinociceptive, antioxidant and antibiotic
7.	3-N,N-Dimethyllaurylammonio) propanesulfonate	10.126	$SC_{24}H_{32}NO_8$	335	335.249414		58,69,84,97,111, 128,152,169,194,213	Anti-Cancer

Table 1 continued...

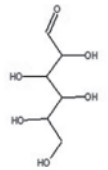
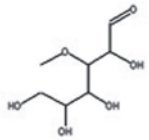
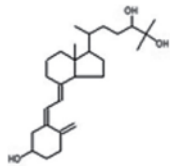
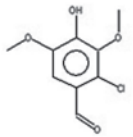
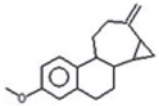
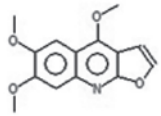
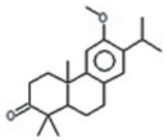
Serial No.	Phytochemical Compound	RT (min)	Formula	Molecular Weight	Exact Mass	Chemical Structure	MS Fragment- ions	Pharmacological actions
8.	d-Mannose	12.179	$C_6H_{12}O_6$	180	180.063388		60,73,85,103,149	Anti-Infective agent
9.	3-O-Methyl-d-glucose	13.718	$C_7H_{14}O_6$	194	194.079039		73,87,103,116, 145,163,177	antioxidant, anti-inflammatory, antimicrobial, pesticide and cancer preventive
10.	9,10-Secocholesta-5,7, 10(19)-triene-3,24,25-triol	16.007	$C_{27}H_{44}O_3$	416	416.329044		55,69,91,118,136, 158,176,189,207, 221,253,383,416	Antiviral, anti-Parkinsonism
11.	Benzaldehyde, 2-chloro-4-hydroxy-3	16.362	$C_9H_{94}ClO_3$	216	216.018936		77,99,127,145, 173,201,216	Antifungal and antitumor
12.	Cyclopropa[3,4]cyclopenta [1,2-a]naphthalene	17.346	$C_{18}H_{22}O$	254	254.167066		53,65,77,91,103, 115,128,159,173, 211,225,239,254	anti-inflammatory, antibacterial and antifungal
13.	Furo[2,3-b]quinoline, 4,6,7-trimethoxy	19.721	$C_{14}H_{13}NO_4$	259	259.084457		53,75,130,158, 186,216,244,259	Antitumor and anti-parasitic activity
14.	2(1H)-Phenanthrenone	21.248	$C_{21}H_{30}O_2$	314	314.22458		55,128,213,229, 257,299,314	anti-oxidant and anti-inflammatory activities

Table 2. Major phytochemical compounds identified in methanolic extract of *Alchemila valgaris*

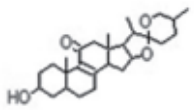
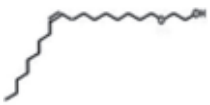
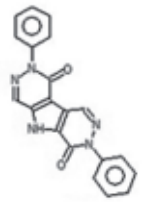
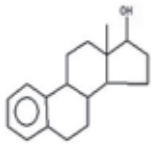
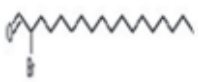
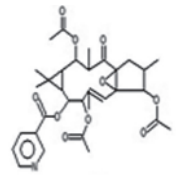
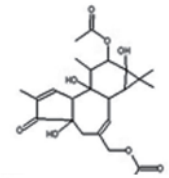
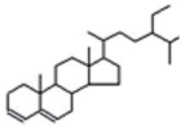
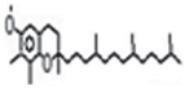
Serial No.	Phytochemical Compound	RT (min)	Formula	Molecular Weight	Exact Mass	Chemical Structure	MS Fragment- ions	Pharmacological actions
1.	Levoglucosenone	5.518	$C_6H_6O_3$	126	126.031694		53,81,98,126	anti-viral, anti-cancer and other bio-activity screening
2.	Spirost-8-en-11-one, 3-hydroxy	11.309	$C_{27}H_{40}O_4$	428	428.29266		57,69,95,135,207, 229,281,299,314, 356,395,428	antipyretic and anti-inflammatory
3.	Ethanol, 2-(9-Octadecenyloxy)	13.947	$C_{20}H_{40}O_2$	312	312.30283		55,69,82,96, 250,312	anticancer, antigonorrheal and antireverse transcriptase
4.	2,7-Diphenyl-1,6 dioxopyridazino[4,5:2,3] pyrrolo	14.404	$C_{20}H_{13}N_5O_2$	355	355.106924		51,77,93,149,165, 187,224,267, 327,355	anti-angiogenic effects and anti-tumor efficacy
5.	Estra-1,3,5(10)-trien-17β-ol	15.257	$C_{18}H_{24}O$	256	256.182714		57,73,85,97,185, 213,256	anti-arrhythmic activities
6.	Octadecanal,2-bromo	16.568	$C_{18}H_{24}BrO$	346	346.187128		57,83,95,124,224, 267,296,346	anti-inflammatory and anti-apoptotic effects
7.	Ethyl 9,12,15-octadecatrienote	17.111	$C_{20}H_{34}O_2$	306	306.25588		55,67,79,95,108, 121,135,173,213, 261,306	antioxidant, anti-inflammatory, antimicrobial and pesticide

Table 2 continued...

Serial No.	Phytochemical Compound	RT (min)	Formula	Molecular Weight	Exact Mass	Chemical Structure	MS Fragment- ions	Pharmacological actions
8.	3-Pyridinecarboxylic acid, 2,7,10-tris(acetyloxy)-1	19.011	$C_{32}H_{39}NO_{10}$	597	597.257397		55,73,105,123,151, 170,213,256,279, 294,430,475	anti-inflammatory
9.	5H-Cyclopropa[3,4]benz [1,2-e]azulen-5-one	19.040	$C_{24}H_{32}O_8$	448	448.209719		69,83,109,159,179, 213,282,310,370, 388,430	inhibits IgE synthesis (even in the presence of anti-IFN monoclonal antibody)
10.	Stigmastan-3,5-diene	25.957	$C_{29}H_{48}$	396	396.3756		55,67,81,105,147, 213,255,288, 381,396	anti-staphylococcal, antihypertensive and antiulcer activity
11.	Tocopherol	26.444	$C_{29}H_{50}O_2$	430	430.38108		57,91,137,165,205, 260,302,344,386,430	Anti-ulcer effects and antioxidants

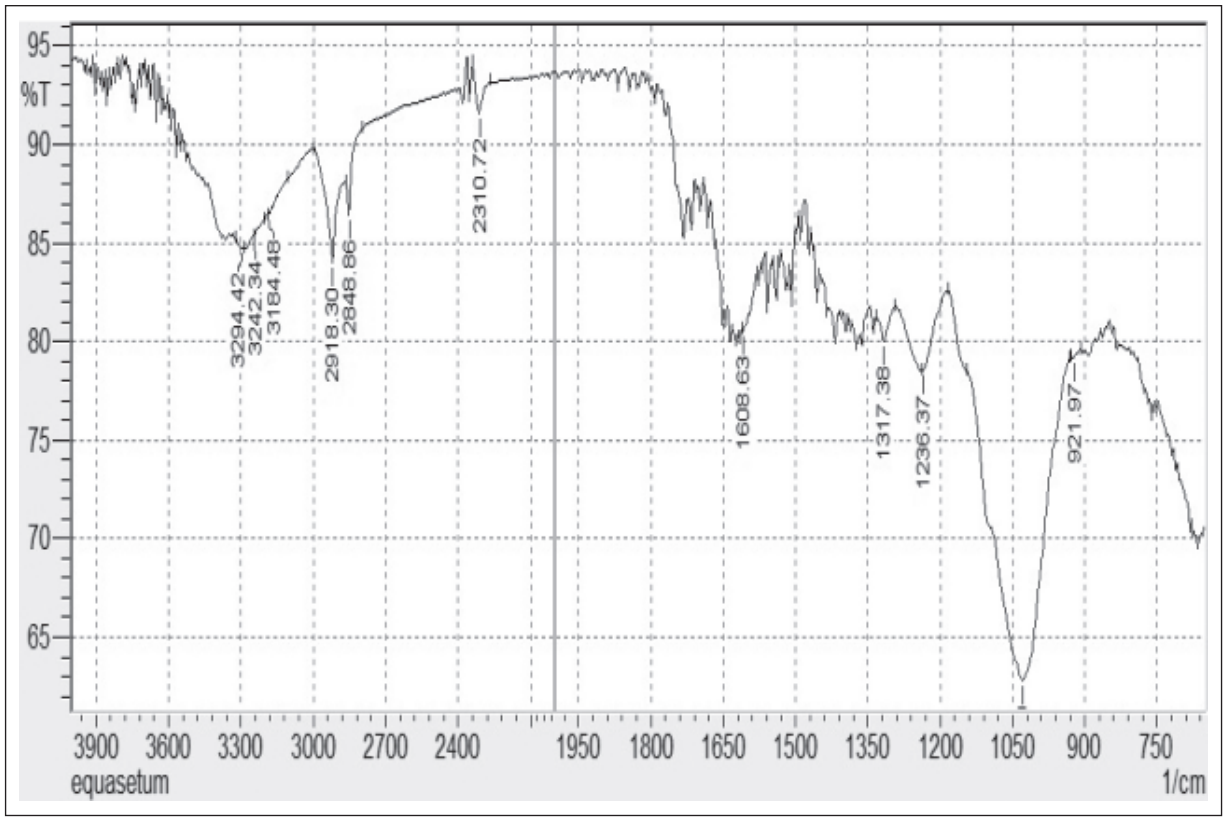


Fig. 16. FT-IR peak values of *Equisetum arvense.*

Table 3. FT-IR peak values of *Equisetum arvense*

No.	Peak (Wave number cm-^{1})	Intensity	Bond	Functional group assignment	Group frequency
1.	921.97	79.184	C-H	Alkenes	675–995
2.	1029.99	62.802	C-F stretch	Aliphatic fluoro compounds	1000–10150
3.	1236.37	78.502	C-O	Alcohols, Ethers, Carboxlic acids, Esters	1050–1300
4.	1317.38	80.070	NO2	Nitro Compounds	1300–1370
5.	1608.63	80.527	–	Unknown	–
6.	2310.72	91.627	–	Unknown	–
7.	2848.86	86.431	C-H	Alkanes	2850–2970
8.	2918.30	84.008	C-H	Alkanes	2850–2970
9.	3184.48	86.466	H-O	H-bonded H-X group	2500–3500
10.	3242.34	85.351	O-H	Hydrogen bonded Alcohols, Phenols	3200–3600
11.	3294.42	84.707	O-H	Hydrogen bonded Alcohols, Phenols	3200–3600

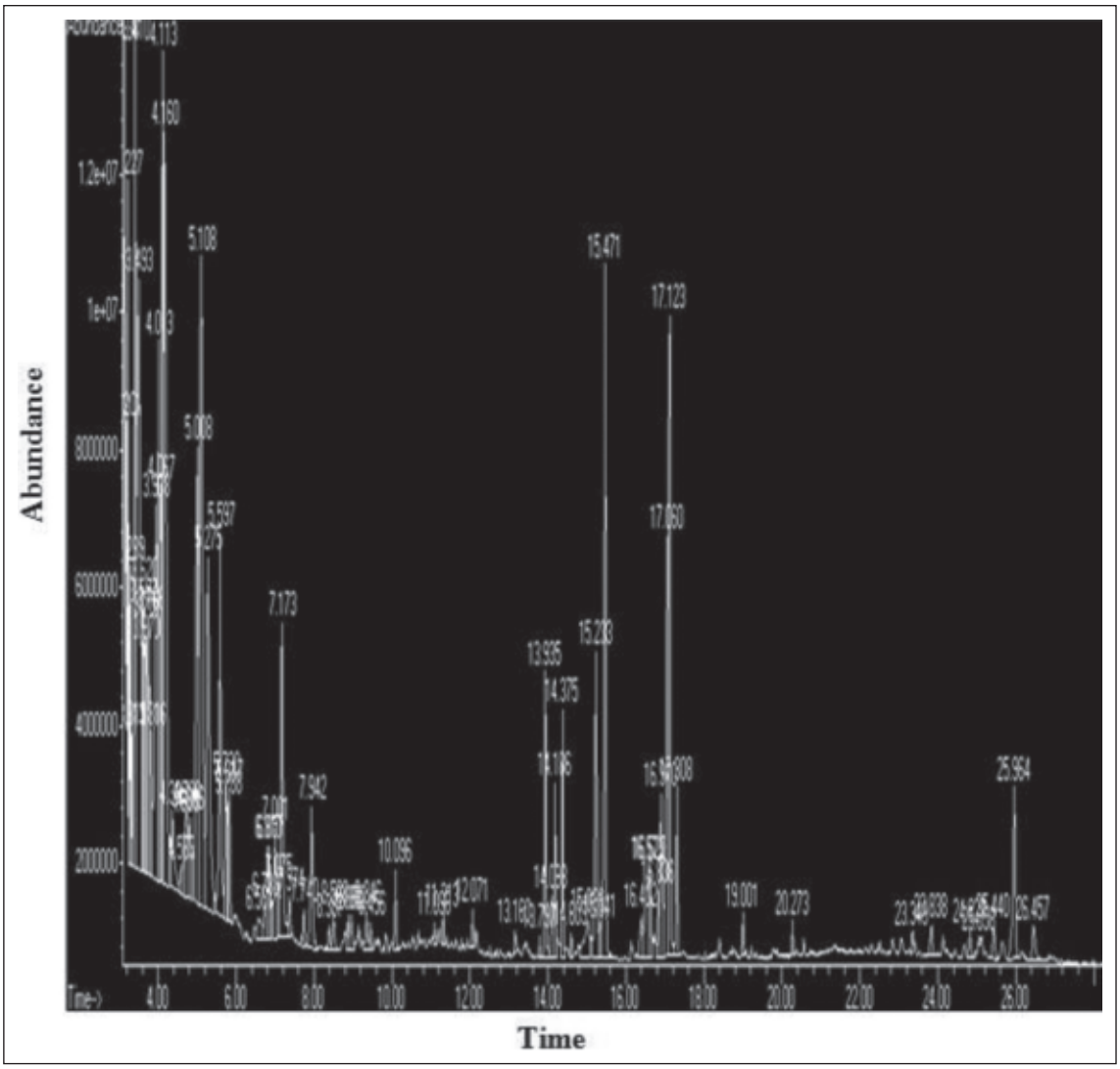


Fig. 17. GC-MS chromatogram of methanolic extract of *Alchemila valgaris*.

Table 4. FT-IR peak values of *Alchemila valgaris*

No.	Peak (Wave number cm-^{1})	Intensity	Bond	Functional group assignment	Group frequency
1.	665.44	67.975	–	Unknown	–
2.	923.90	77.642	C-H	Alkenes	675-995
3.	1028.06	62.478	C-F stretch	Aliphatic fluoro compounds	1000-10150
4.	1045.42	62.874	C-F stretch	Aliphatic fluoro compounds	1000-10150
5.	1234.44	80.863	C-O	Alcohols, Ethers, Carboxlic acids, Esters	1050-1300
6.	1317.38	80.571	NO2	Nitro Compounds	1300-1370
7.	1606.70	79.042	–	Unknown	–
8.	2308.79	92.088	–	Unknown	–
9.	2848.86	86.057	C-H	Alkanes	2850-2970
10.	2918.30	83.438	C-H	Alkanes	2850-2970
11.	3064.89	88.221	H-O	H-bonded H-X group	2500-3500
12.	3184.48	85.812	H-O	H-bonded H-X group	2500-3500

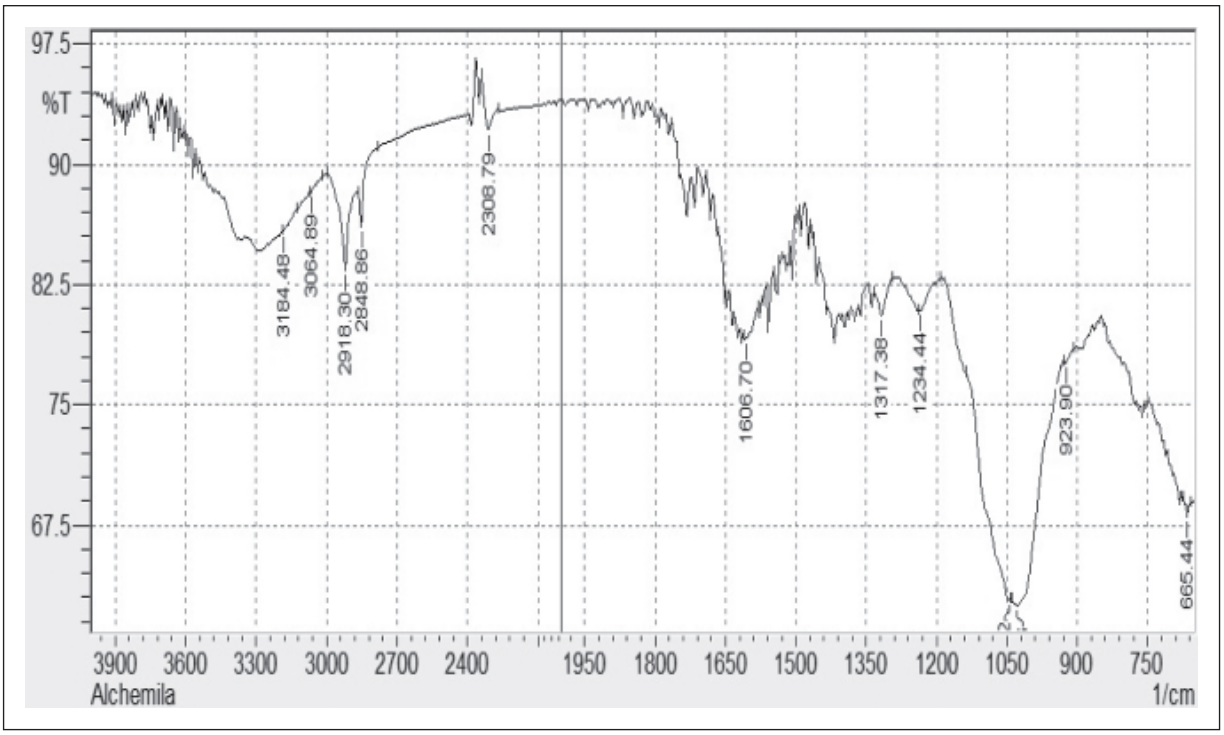


Fig. 29. Fourier-transform infrared spectroscopy peak values of *Alchemila valgaris*.

CONCLUSION

Equisetum arvense and *Alchemila valgaris* are native plants of Iraq. Thus the GC-MS analysis of methanolic extract of both plants showed a highly complex profile containing approximately twenty five components. It contain phytochemical which may be useful for various herbal formulation as antipyretic, analgesic, cardiac tonic, antiasthamatic and anti-inflammatory.

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