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Phytochemical Components Analysis by Using Gas Chromatography-mass and Fourier transform-Infrared Techniques of *Cressa cretica* L. Flowers Extracts

Aseel Mohammad Omran[1], Nidaa Adnan Abu-Seraj[2]

Ibtihal Muiz Al Husaini[3]

[1] College of pharmacy, Department of Pharmacognosy,. University of Babylon , Hilla, Iraq, Biomaster2007@yahoo.com
[2] College of science, Department of Biology, University of Babylon, Hilla, Iraq , seragdcnidaa@yobabylon.com
[3] College of science, Department of Biology, University of Babylon, Hilla, Iraq, ebtihalmuiz@yahoo.com
*Corresponding author email: Biomaster2007@yahoo.com

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Abstract:

This study aimed to investigate the phytochemical components of *Cressa cretica* L.(flowers) using Gas chromatography- Mass spectrum (GC-MS) and Fourier transform infrared spectrophotometer (FTIR) techniques. The secondary metabolites analysis of *C.cretica* extracts revealed the presence of phenols, alkaloids, terpenes, flavonoids and glycosides. The results of GC-MS reported thirty chemical compounds in flower's extract. The results of the FTIR confirmed the presence of large numbers of functional groups in flowers of the plant and these functional groups which varied according to the solvent type. More of these functional groups were alcohols, phenols, alkanes, alkyl halides, aldehydes, carboxylic acids, aromatics, nitro compounds and amines.

Key words:

Cressa cretica, flowers, phytochemical compounds, GC-Mass, FTIR..

Citation:

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Introduction

Cressa cretica is a small erect shrub, usually cultivates in sandy or unclear saline environments [1]. It is a salt tolerant plant, common in coastal area, along the landward edge of marshes [2]. All parts of this plant used as a glue and decoction to treated infection with fungi, asthma, blood purifier and eczema [3] .

The floating parts of *C. cretica* have five flavonoids which they are querctin, quercetin-3-B-O-D-glucoside, kaempferol-(1-6)-B-D-glucoside, and quercetin-3-O-a-L rhamno-(1-6)-B-D-glucoside (rutin) [4]. Hussain *et al*. [5] found seven from the phytochemicals by using colom chromatography including triacontanoic acid, hydroxy-4-octacosanone, 23,nor-12-uresene, B-amyrin, stigmasterol, ursolic acid, and stigmasterol-3-o-B-D-glucoside. Raje et al [6] recorded four types of heavy metals lead, zinc, copper and nickel by Atomic absorption spectroscopy (AAS). Weber [2] showed that the seeds of *C. cretica* used as source of eatable oil, The oil quantity ranges from 22% - 25%.

Pirzada *et al*. [7] found many of basic elements including Al, Ca, Cu, Fe, Mg, Mn, P, S and Zn in the herbal plant *C. cretica*, by using atomic absorption and U V spectrophotometry.

Materials and methods

Preparation of extracts:-

Aqueous extract:-

10 g from dried powdered flowers was taken and put in a conical flask (500 ml), added distilled water (250 ml), and boiled on slow heat for 2h, then filtered through 8 layers of muslin cloth and centrifuged at 5000 RPM for 10 min. The supernatant was collected [8]. Concentrated the extract in an oven at 45^0C until dryness. Dried extracts were stored at 4^0C for further use [9].

Organic solvent extract:-

Three types of organic solvents were used with different polarity: methanol, ethyl acetate, hexane [10]. 10 gm of the powdered plant part was soaked in the conical flask (250 ml) containing 200 ml of solvent, plugged with cotton, and then put in a horizontal shaker at 140-220 RPM for 24 h. After that it was filtered through 8 layers of muslin cloth and centrifuged at 5000 RPM for 10min, the supernatant was collected

[8].Concentrated the extracts in an oven at 45oc until dryness. Dried extracts was stored at 4oc for further use [9J].

Qualitative phytochemical tests:

1- Determination pH for plant extracts

Before the qualitative phytochemical testes and for the purpose of as certaining whether there are differences in the pH level of plant extracts used, there were dissolving 10 gm of plant powder extracted in 50 ml of distilled water and left at room temperature for 10 minutes. Done measuring pH by PH meter for the plant extracts used in the experiment [11].

2- Test for alkaloids (Dragendorff's reagent):

20 g from Bismuth Nitrate in 40 ml distilled water and 16 g from sodium Iodide in 40 ml distilled water, mixed together and added 1-2 ml from this reagent to 5 ml from the extract, a prominent orange color was indicated the test as positive [12]

3- Test for phenols:

The extract (50 mg) was dissolved in 5 mL of distilled water. To this, 3ml of 10% lead acetate were added. A bulky white precipitate indicated the presence of phenol compounds [13].

4- Test for saponins:

Mixed one ml of extract with one ml of distilled water, then shaked vigorously, a stable foam indicate the presence of saponin [14].

5- Test for tannins:

A- Ferric chloride

A portion of extract was dissolved in water, then filtrate it, Ferric chloride (10%) was added. The appearance of bluish black color indicates the presence of tannins [14].

6- Test for flavonoids:

Melted 10 gm of plant powder in 50 ml of ethanol (95%) and then nominated by A. 10 ml of ethanol (50%), added to 10 ml of potassium hydroxide (50%) and nominated by B. Mix equal amount of solution A and B, the appearance color yellow as evidence of a flavonoids [15].

7- Test for glycosides:

Using Fehling's test to determination the presence of glycosides by mixed equal volume of Fehling's A (Copper sulphate in distilled water) and Fehling's B (Potassium tartarate and sodium hydroxide in distilled water) , few drops of this reagent was added to 5 ml of the extract and boiled, red precipitate was formed, if sugars are present [16].

8- Test for terpenes:

Dissolve 1 ml of plant extract in 2 ml chloroform, added to the solution a drop of anhydrous acetic acid and a drop of concentrated sulfuric acid, the appearance of brown color recorded as positive indicator to the presence of terpenes [17].

analysis phytochemicals by Gas chromatography-Mass spectrum (GC-MS) :-

The GC-MS analysis of the plant extract was made in an instrument (QP 2010 Plus SHIMADZU) under computer control at 70 eV [18; 19]. Injected about 1 µl of the methanol extract into the GC-MS using a micro syringe, after that the scanning was done for 45 min. When the compounds were separated, they eluted from the column and entered adetector which had the ability to create an electronic signal whenever a compound was detected. Then the signal obtained had been processed by the computer. Retention time (RT) was calculated as the time from when the injection was made (Initial time) to when elution occurred is referred.

Helium gas was used as a carrier and an eluent. The flow rate of helium gas was set to one ml per minute. The electron gun of mass detector liberated electrons having energy was about 70eV. The column employed here for the separation is siloxane. By the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures [18 ,19 ,20]

Analysis the functional groups in the plant by Fourier Transform Infrared spectrophotometer (FTIR)

The powdered sample of each plant parts was extracted with aqueous, methanol, ethyl acetate, hexane to be treated for reading by FTIR spectroscopy (Shimadzu, IR Affinity 1, Japan). Each sample was run at infrared region between 400 and 4000 nm [21, 22].

Results and discussion

Qualitative Secondary metabolites Tests

Table (1) represent the secondary metabolites constituents that present in the flowers of *Cressa cretica* L. by using different solvents (water, methanol, ethyl acetate, hexane). The qualitative analysis of all extracts appear the presence of phenols, alkaloids, tannins, terpenes, and glycosides by using all solvents that used in this study , flavonoids only appear in the methanol extract but not found when using other solvents in all parts, that may be because of the high polarity of flavonoids so it was needed to a high polarity solvent like methanol, Ghasemzadeh *et al.* [23] found that the concentration of flavonoids increase with the increasing of the polarity of solvents when using three types of solvent (methanol, acetone and chloroform), he found that methanol give a high concentration from flavonoids then acetone and chloroform . The results show that the saponin was not present with all solvents by using foam test. Also the results show that the PH of all extracts appears from neutral to acetic status.

These results agree with the results of Khare *et al.* [24] which found the presence of alkaloids, tannins, glycosides in *C. cretica* by using four types of solvents (water, ethanol, petroleum ether, chloroform) while flavonoids found only in the ethanol extract by using ammonia test, and there were no appearance to saponin in *C. cretica* by using foam test in all solvents that used, that's appear that *C. cretica* poor in saponin but rich with other types of phytochemicals like alkaloids, phenols, tannins, terpenes, and glycosides.

Table (1). Secondary metabolites tests for various extracts

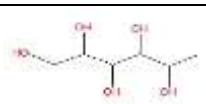
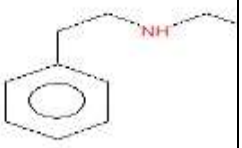
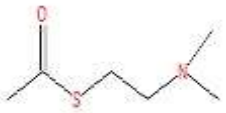
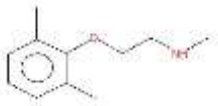
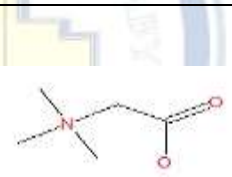
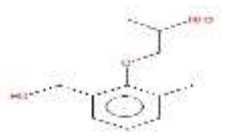
Solvent type / chemicals	Aqueous	Methanol	Ethyl acetate	Hexane
PH	6.1	5.8	5.7	5.6
Alkaloids	+	+	+	+
Phenols	+	+	+	+
Tannin	-	-	-	-
Glycosides	+	+	+	+
Saponin	-	-	-	-
Flavonoids	-	+	-	-
Terpenes	+	+	+	+

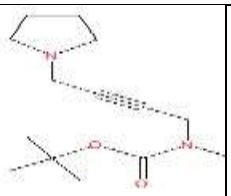
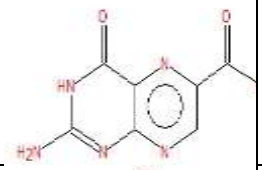
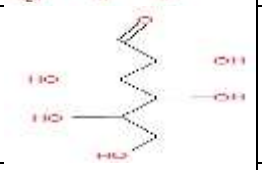
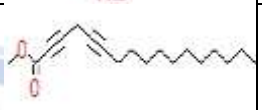
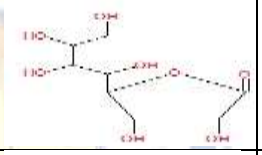
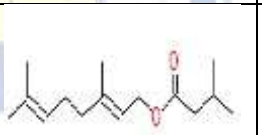
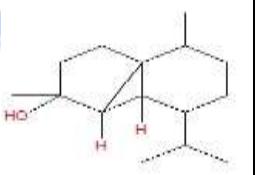
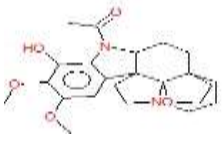
GC-MS analysis of *C. cretica* L. Flowers methanolic extract

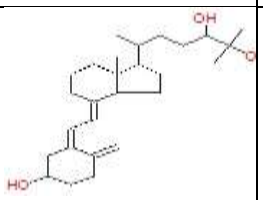
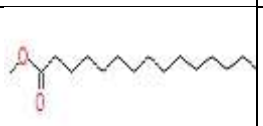
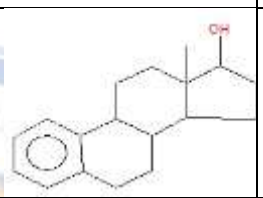
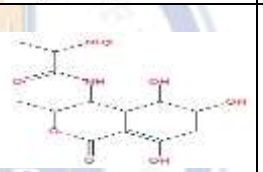
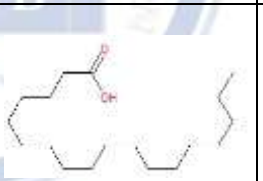
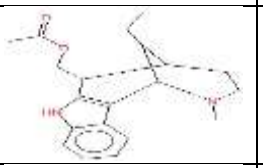
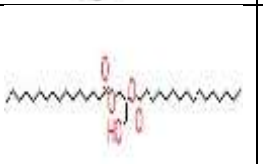
Chromatogram GC-MS analysis of methanol extract of flowers of *C. cretica* showed the presence of 30 compounds. The chemical compound, structural formula, molecular weight and exact mass were as shown in (table 4-2). The spectrum profile of GC-MS showed the presence of thirty major peaks and these compounds were variable compound and had different chemical nature such as alkaloids (N-Ethyl-2-phenethylamine; Ethanamine, 2-(2,6-dimethylphenoxy)-N-methyl- ; Aspidospermidin-17-ol, 1-acetyl-19,21-epoxy-15,16-dimethoxy) , also flowers contain several types of acids like fatty acids (5,7- Dodecadiyn-1,12-diol), fatty acid ester (2,5-Octadecadiynoic acid , methyl ester; Hexadecanoic acid, methyl ester; Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester), stearic acid (Octadecanoic acid), carboxylic acid (Propanoic acid ,2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17) , and flowers contain alcohol compounds (4-epi-cubedol; Estra-1,3,5(10)trien-17β-ol), hydrocarbons (Actinobolin; d-Mannose) , also flowers contain sterols (Campesterol; Stigmasterol; γ-Sitosterol).

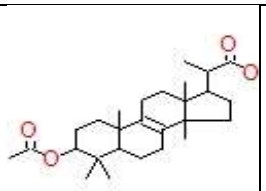
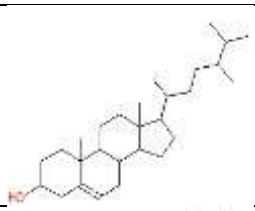
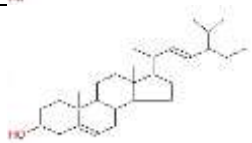
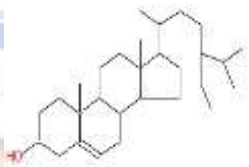
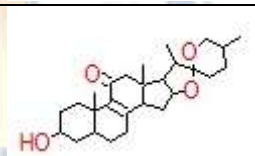
Most of phytochemicals that mentioned in table (4-2) had pharmacological action like Octadecanoic acid which act as Cancer preventive [25], Pterin -6-carboxylic acid act as anti-psychotic and anti-parasite [22]. γ-Sitosterol act as anti-inflammatory activity [22]. Geranyl isovalerate act as antifungal activity [26]. d-Mannose act as anti-allergic and anti-bacterial [21]. Desulphosinigrin act as anticancer [27]. Tertbutyloxyformamide , N-methyl-N-[4-(1-pyrrolidinyl)-2-butynyl had anti histaminc properties [28].

Table (2). Major phytochemical compounds identified in *C. cretica* flowers

Serial No.	Phytochemical compound	RT (min)	Molecular Weight	Exact Mass	Chemical structure	MS Fragment- ions	Nature of compound
1.	1-Deoxy-d-mannitol	3.258	166	166.084124		61,73,87, 103,131,166	carbohydrates
2.	N-Ethyl-2-phenethylamine	4.077	149	149.120449		58,65,77, 91,132,149	Monoamine alkalods
3.	Ethanethioic acid, S-[2-(dimethylamino)ethyl ester	4.546	147	147.071785		58,71,81, 103,147	Organic sulfur compound
4.	Ethanamine , 2-(2,6-dimethylphenoxy)-N-methyl-	4.821	179	179.131014		51,58,65, 77,91,105 ,121,136, 160,179	alkaloids
5.	Methanaminium , 1-carboxy-N,N,N-trimethyl- ,hydroxide , inner	5.587	117	117.0789785		58,73,77, 85,91,100 ,116	Nitrogen compound
6.	Desulphosinigrin	7.287	279	279.077658		57,60,64, 69,73,76, 85,91,103 ,117,127, 145,160	Glycoside
7.	Benzenemethanol , 2-(2-aminopropoxy)-3-methyl-	7.865	195	195.125929		58,65,77, 91,121,145,178,195	Aromatic compound
8.	4-(2,5-Dihydro-3-methoxyphenyl)butylamine	9.232	181	181.146665		55,65,77, 91,107,121,134,150	Volatile oil

9.	Tertbutyloxyformamide , N-methyl-N-[4-(1-pyrrolidinyl)-2-butynyl	9.616	252	252.183778		57,70,84,95,108,122,151,161,179,195,221,252	Alcohol
10.	Pterin-6-carboxylic acid	10.279	207	207.039239		57,69,105,122,149,163,177,207	CH2O2 derivates
11.	d-Mannose	11.401	180	180.063388		60,73,103,149	Carbohydrates
12	2,5-Octadecadiynoic acid , methyl ester	12.116	290	290.22458		55,67,79,91,105,117,131,145	Fatty acid ester
13	l-Gala-l-ido-octonic lactone	11.893	238	238.068868		61,73,84,112,127,142,159,189,220	Carbonyl compound
14	Geranyl isovalerate	12.078	238	238.19328		57,69,85,93,103,121,129,136,154,168,183,198,219	terpenes
15	4-epi-cubedol	12.551	222	222.198365		55,81,91,105,119,133,147,161,189,207,222	Sesequiterpines
16	D-Fructose , diethyl mercaptal , pentaacetate	12.911	496	496.14369		60,69,97,113,129,154,185,213,245,273,316	Carbohydrates
17	Aspidospermidin-17-ol, 1-acetyl-19,21-epoxy-15,16-dimethoxy	13.238	414	414.215471		69,83,97,111,138,160,171,202	alkaloids

18	5,7-Dodecadiyn-1,12-diol	13.867	194	194.13068		55,79,91,105,115,148,179	Fatty acid
19	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol,(3β,5Z,7E)-	14.199	416	416.329044		55,69,91,118,136,158,176,207,221,253,383,398	sterols
20	Hexadecanoic acid , methyl ester	14.554	270	270.25588		55,74,87,97,115,129,143,185,213,227,239,270	Fatty acid ester
21	Estra-1,3,5(10)trien-17β-ol	14.908	256	256.182714		57,73,85,97,129,157,185,213,241,256	alcohol
22	Actinobolin	15.732	300	300.132137		57,65,89,111,137,165,192,213,239,282	Hydrocarbon derivativ
23	Octadecanoic acid	16.802	284	284.27153		60,73,83,97,115,129,143,157,171,185,199,227,241,255,284	Stearic acid
24	Dascarpidan-1-methanol , acetate (ester)	18.530	326	326.199429		69,83,97,124,180,222,256,326	Ester
25	Hexadecanoic acid , 1-(hydroxymethyl)-1,2-ethanediyl ester	19.738	568	568.506676		57,73,83,98,129,213,239,256,299,313,331,423	Fatty acid ester

26	Propanoic acid ,2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17	20.121	430	430.30831		55,69,121,187,213,233,281,337,355,415	Carboxylic acid
27	Campesterol	27.359	400	400.370516		55,81,145,161,213,255,289,315,382,400	sterols
28	Stigmasterol	28.017	412	412.370516		55,83,133,213,255,300,351,369,412	Sterols
29	γ-Sitosterol	29.168	414	414.386166		55,69,81,145,161,213,255,273,303,329,396,414	sterols
30	Spirost-8-en-11-one, 3-hydroxy-,(3β,5α,14β,20β,22β,25R)-	29.499	428	428.29266		69,95,135,207,229,281,314,356,428	Hydrocarbon

Functional groups analysis by FTIR of different extracts of *C. cretica* L. Flowers

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The results of FTIR peak values and functional groups were represented in table (3). Each sample was run at infrared region between 400 and 4000 nm. The presence of various functional groups of different compounds was found.

The absorption spectra of original sample flowers are show in figure 1 which give 10 peaks . The dominant peaks in case of flowers were observed at 667.37and 1014.56 cm^{-1} represents alkyl halides compound. The band at 694.37and 900.76 cm^{-1} represents alkenes. The peaks at 1022.27, 1095.57 and 1236.37cm^{-1} represents aliphatic amines. The peak at 1317.38 cm^{-1} represent nitro compounds.

The absorption spectra of the aqueous extracts of flowers sample were shown in figure 2, which appear the highest number of peaks (20). The peaks at 665.44, 875.68 and 896.90 cm^{-1} represents alkenes. The peaks at 1028.06, 1099.43, 1149.65 and 1242.16 cm^{-1} represents aliphatic amines. The peaks at 1317.38 cm^{-1} represent nitro compounds. The peak at1616.35 cm^{-1} represent amines. The peaks at 2848.86 and 2918.03 cm^{-1} represent alkanes. The peaks at 3143.97, 3211.48, 3253.91 and 3282.84 cm^{-1} represents carboxylic acids. The peaks at 3390.86 and 3419.79 cm^{1} represents amines, amides. The peaks at 3523.95 and 3566.38 cm^{-1} represents Alcohol. The peak at 1541.12 cm^{-1} was unknown.

The absorption spectra of the methanol extracts of flowers sample were shown in figure 3, which appear lowest number of peaks (7). The peaks at 756.10 and 875.68 cm^{-1} represents alkenes. The peaks at 1022.27, 1149.57 and 1238.30 cm^{-1} represents aliphatic amines. The peak at 1317.31cm^{-1} represent nitro compounds. The peak at 2918.30 cm^{-1} represents alkanes.

The absorption spectra of the ethyl acetate extracts of flowers sample are shown in figure 4. which give 10 peaks. The peaks at 667.37 and 875.68 cm^{-1} represents Alkenes. The peaks at 1022.27, 1095.57, 1145.57 and 1244.09cm^{-1} represents aliphatic amines. The peak at 1317.38 cm^{-1} represents nitro compounds. The peak at 1616.35 cm^{-1} represents amines. The peak at 2850.79 and 667.37 cm^{-1} alkanes.

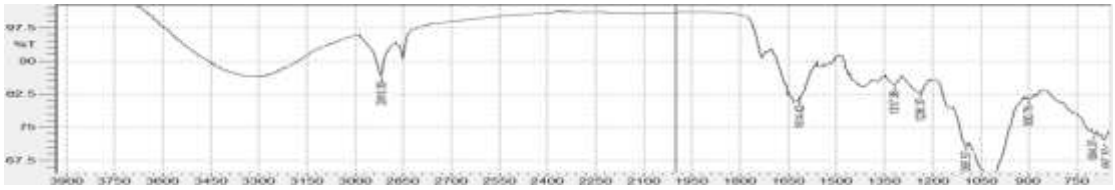
The absorption spectra of the hexane extracts of flowers sample are shown in figure 5. which give 16 peaks. The peaks at 665.44, 875.68 and 896.90 cm^{-1} represents alkenes. The peaks at 1026.13, 1095.57 and 1240.23 cm^{-1} represents aliphatic amines. The peak at 1317.38 cm^{-1} represents nitro compounds. The peak at 1616.35 cm^{-1} represents amines. The peak at 2850.79 and 2918.30 cm^{-1} represents alkanes. The peaks at 3145.90 , 3199.91, 3244.27 and 3280.92 cm^{-1} represents carboxylic acids. The peak at 3346.50 cm^{-1} represents amines, amides.

The differentiation in the functional groups with differentiation solvent type may be due to the variable in ability of these solvents to extract these functional groups. This study agree with other studies like Sahaya *et al* [29] found a differentiation in the

functional groups according to the peaks appearance with the differentiation of the solvent (Ethanol, Acetone, Petroleum ether, Chloroform) in the plant *Vitex altissima*. Carboxilic acids used as antioxidants, radio, and cytoprotector [30].

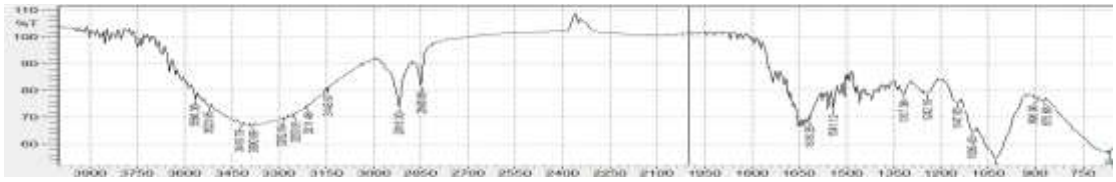
Table (3): FTIR peak values and functional groups of different extracts of flowers

original		Aqueous		Methanol		Ethyl acetate		Hexane	
Peak values	**Functional groups**	**Peak values**	**Functional groups**	**Peak values**	**Functional groups**	**Peak values**	**Functional groups**	**Peak values**	**Functional groups**
667.37	alkyl halides	665.44	Alkenes	756.10	Alkenes	667.37	Alkenes	665.44	Alkenes
694.37	Alkenes	875.68	Alkenes	875.68	Alkenes	875.68	Alkenes	875.68	Alkenes
900.76	Alkenes	896.90	Alkenes	1022.27	aliphatic amines	1022.27	aliphatic amines	896.90	Alkenes
1014.56	alkyl halides	1028.06	aliphatic amines	1149.57	aliphatic amines	1095.57	aliphatic amines	1026.13	aliphatic amines
1022.27	aliphatic amines	1099.43	aliphatic amines	1238.30	aliphatic amines	1145.57	aliphatic amines	1095.57	aliphatic amines
1095.57	aliphatic amines	1149.65	aliphatic amines	1317.31	nitro compounds	1244.09	aliphatic amines	1240.23	aliphatic amines
1236.37	aliphatic amines	1242.16	aliphatic amines	2918.30	alkanes	1317.38	nitro compound	1259.52	**Unknown**
1317.38	nitro compounds	1317.38	nitro compound			1616.35	amines	1317.38	nitro compounds
1614.42	amines	1541.12	**unknown**			2850.79	alkanes	1616.35	Amines
2918.30	alkanes	1616.35	amines			667.37	alkanes	2850.79	Alkanes
		2848.86	alkanes					2918.30	Alkanes
		2918.03	alkanes					3145.90	carboxylic acids
		3143.97	carboxylic acids					3199.91	carboxylic acids
		3211.48	carboxylic acids					3244.27	carboxylic acids
		3253.91	carboxylic acids					3280.92	carboxylic acids
		3282.84	carboxylic acids					3346.50	amines, amides
		3390.86	amines, amides						
		3419.79	amines, amides						
		3523.95	alcohol						
		3566.38	alcohol						



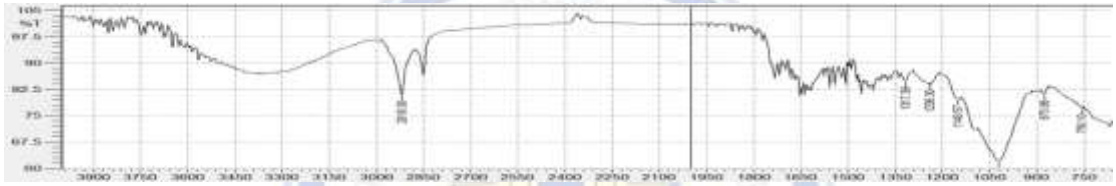
Wavenumber (cm-^{1})

Figure (1). FT-IR profile solid analysis of *Cressa cretica* (Flowers original)



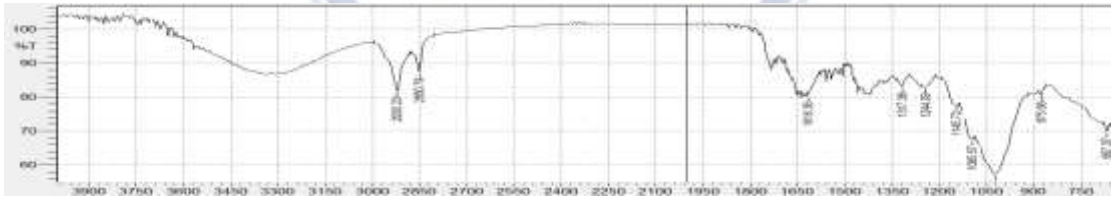
Wavenumber (cm-^{1})

Figure (2). FT-IR profile solid analysis of water extract of *Cressa cretica* (Flowers)



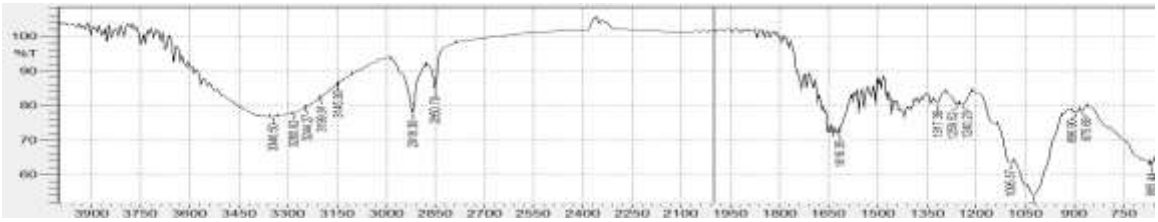
Wavenumber (cm-^{1})

Figure (3). FT-IR profile solid analysis of methanolic extract of *Cressa cretica* (Flowers)



Wavenumber (cm-^{1})

Figure (4). FT-IR profile solid analysis of ethyl acetate extract of *Cress cretica* (Flowers)



Wavenumber (cm-^{1})

Figure (5). FT-IR profile solid analysis of hexane extract of *Cressa cretica* (Flowers)

Conclusion

Cressa cretica L. is a natural plant in Iraq. The GC-MS analysis of methanolic of flowers extract showed a highly complex profile containing about thirty four constituents. The FT-IR analysis appear 20 functional groups in aqueous solution, 16 functional groups in hexane solution, 10 functional groups in ethyl acetate and original solution, and 7 functional groups in methanol solution, this plant contain phytochemical compounds may be suitable for treatment many diseases as herbal drug such as anti-inflamatory, anti-bacterial, anti-fungal and others.

Conflict of interests.

There are non-conflicts of interest.

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الخلاصة

تهدف هذه الدراسة الى الكشف عن المركبات الفعالة في ازهار نبات الشويل باستخدام جهاز الكروماتوغرافيا الغازية وجهار المطياف الضوئي للاشعة تحت الحمراء باستخدام مذيبات مختلفة وهي الماء المقطر الاميثانول والهكسان والاثيل اسيتيت. اظهرت النتائج وجود العديد من المركبات الفعالة مثل الفينولات, القلويدات, التربينات, الفلافونويدات والكلايكوسيدات. اظهرت نتائج الكروماتوغرافيا الغازية وجود 30 نوع من المركبات الكيميائية الفعالة في ازهار نبات الشويل, واظهرت نتائج التحليل الكيائي بجهاز المطياف الضوئي للاشعة تحت الحمراء وجود عدد كبير من المجاميع الفعالة وتختلف هذه المجاميع باختلاف نوع المذيب المستخدم , معظم هذه المجاميع هي عبارة عن فينول, كحول, الكان, الكين, الديهايد, حامض الكربوكسلك وغيرها.

الكلمات الدالة: ازهار، مركبات كيميائية نباتية، كروماتوغرافيا الغاز – مطياف الكتلة، جهاز "فوريه" لتحويل طيف الأشعة تحت الحمراء