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Isolation of Astragalin from *Cressa cretica* cultivated in Iraq

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Abstract

Cressa cretica L. Fam: Convolvulaceae (morning glory). Is a halophytic dwarf shrub grows in sandy, saline land. The aims of the study were isolation, quantification, and identification of kaempferol-3-O glucoside (astragalin) of *Cressa cretica* aerial parts .the presence of Kaempferol 3- O- β- glucoside (astragalin) was detected as the major glycoside in the polar fraction by TLC, HPTLC and HPLC comparing with astragalin standard. Astragalin was isolated from Iraqi plant as yellow powder by preparative TLC and then it was determined by HPTLC. The identification and the structural elucidation of isolated astragalin were performed by[1] H-nuclear magnetic resonance, [13]C-nuclear magnetic resonance), infrared, and ultraviolet.Results showed that the quantity of Kaempferol3-O- glucoside (astragalin) is 0.3% which is extracted under optimal conditions (80% aqueous ethanol and 80°c for 3 hrs.).

Keywords: Astragalin, *Cressa cretica*, Halophytic, Isolation.

INTRODUCTION

According to the World Health Organization (WHO), more than 70% of the population uses medicinal plants in their primary health care, mainly of herbal sources, This is especially the case in third world countries where the cost of consulting a doctor and the price of medication cannot be afforded by many People.[1,2] despite herbal medicine has been used since the beginning of life, however, one of the greatest challenges is the fact that our knowledge of how plants actually affect human physiology is poor. Therefore many researchers are going on in this field and plants are claiming various medicinal uses is increased.[3] *Cressa cretica* is a halophytic dwarf shrub grows in sandy, saline land. It is a small plant with a woody base and many stems. The leaves of the plant are small and compressed. The flowers are small and their color is white.[4] From the phytochemical point of view, the plant was reported to contain: Coumarins, sterols, alkaloids, tannins, glycosides (cardiac glycoside, anthraquinone glycoside), protein, carbohydrate, flavonoids, unidentified sugars, and high salt content.[5–9] Kaempferol-3-*O*-glucoside (Astragalin) is one of the major flavonoid found in a variety of plants.[10,11]
astragalin is receiving increasing attention due to its varies health benefiting and biological activities including anti-oxidative, anti-inflammatory anti-HIV, anti-allergic effects, besides this astragalin is responsible for the color of different beans and has the potential to extract it and market as a nutritionally important food supplement.[12] The aerial parts of *Cressa cretica L*. yielded five flavonoids that were identified as quercetin (**1**), *quercetin-3-O-glucoside*(**2**), kaempferol-3-*O*-glucoside (**3**), kaempferol-3-*O*rhamnoglucoside (**4**), and rutin (**5**).[13] Chemically flavonoids are based upon a fifteen-carbon skeleton consisting of two benzene rings (A and B) linked via a heterocyclic pyrane ring (C).[14]
The widespread uses of *Cressa cretica* in traditional medicine have resulted in considerable chemical analysis of the plant and its active principles. Available information indicates that hydroalcoholic extract of *Cressa cretica* was assayed against different carcinoma cell lines.[15]
The aim of this study is to investigate the presence of astragalin in the Iraqi Cressa cretica L.
To the best of our knowledge, this study is the first work studied the qualitative-quantitative analysis and isolation with the structural elucidation of astragalin of *Cressa cretica* L. cultivated in Iraq

METHODS

General procedures

Ultraviolet (UV) spectra were recorded in MeOH using a CAMAG system, IR spectra in KBR disk on Fourier-transform IR (FTIR) (Jasco-6100), H-NMR spectrum was measured on BRUKER AVANCE III 500 MHz apparatus high-performance thin-layer chromatography (HPTLC) analysis was carried out using CAMAG system (Switzerland), pre-coated silica gel GF254 (aluminum TLC) from Merck co., and the standard astragalin from Sigma-Aldrich chemicals Co. All the solvents were from Sigma-Aldrich chemicals Co and Merk chemical company.

Plant material

The aerial parts (stems and leaves) of *Cressa cretica* were collected from area Al-Msiab and identified by the national herbarium in Abu-Ghraib, Baghdad.
The plant material was collected during October and dried at room temperature in the shade, then grinded as powder and weighed.

Preparation of aqueous methanolic extract

The powdered aerial parts of *Cressa cretica* (180 g) were defatted with hexane (1200mL), using soxhlet extractor 250 x 6 units. The defatted plant material was further extracted with ethanol 80% (1500 mL) using soxhlet extractor at extraction temperature in the range (60-80°C) for 3 hours. The ethanolic extract was concentrated to (250 mL) by evaporation under reduced pressure using a rotary evaporator at (50°C). Then distilled water (100mL) was added to the ethanolic extract, and the extract partitioned with ethyl acetate (350mL) and allowed to settle overnight.
The lower aqueous layer (300mL) was collected and labeled as fraction A, while the upper ethyl acetate layer was collected and labeled as fraction B.

Chromatographic analysis for the detection of astragalin

TLC
TLC analysis of extract in comparison with astragalin standard was done by development with (ethyl acetate: methanol: water, 15:1.25:1) as a mobile phase.[16]
The HPTLC analysis was conducted to detect the presence of kaempferol 3-O-β- glucoside in the extract prepared with the same concentration
The HPTLC analysis was conducted to detect the presence of astragalin. The HPTLC analysis was performed by using HPTLC silica gel 60 GF 254s (10x20 cm), the layer thickness was 0.5 mm. The standard astragalin 2 µL and the sample (2µL) from extract were applied automatically on the plate by CAMAG Linomat 5. The plate was automatically submerged into an automatic developing chamber (ADC2 CAMAG) using solvent system (ethyl acetate: methanol: water, 15:1.25:1, v/v/v), with migration distance about 7.5 cm. The plates were air-dried after development and scanned under UV (366 and 245 nm) using CAMAG TLC scanner 4. The data were processed using win CATS software.
HPLC
HPLC analysis was performed for detection and estimation of Astragalin in the ethyl acetate extract fraction (B). The extract was analyzed by (HLPC) method with UV detection. The HPLC analysis was carried out by prominence HPLC system (SYKAM)

and the separation was performed in a reversed phase (RP) ODS-C18 column (25 cm x4.6 mm x5µ m). The mixture was passed through a 0.45 µm PVDF membrane and then 20 µL of each sample was injected into the HPLC system. The separation was done by elution with isocratic mixtures, 80% methanol as solvent A and 20%(water with 0.1% acetic acid) as solvent (B). A flow rate was set as 0.8ml/min for 10 minutes, detected by UV at 360. The astragalin was detected according to the retention time of the standard astragalin.[17]

Isolation and purification of flavonoid glycoside

The ethyl acetate layer (fraction B) purified by preparative TLC. The purification technique performed on (20x20 cm) glass plates pre-coated with silica gel GF 254; prepared manually with 0.5 mm thickness. After that the sample (fraction B) applied as a concentrated solution in a raw spots by glass pasture pipette four times on each plate, one should wait after each application until all the solvent is evaporated, the elution system was (ethyl acetate: methanol: water, 15:1.25:1), the separated bands were visualized under UV light (254nm) as shown in the figure (3), bands at R_f= 0.5 were scrapped off with comparison with standard, and then eluted with acetone and methanol. The solvent was evaporated by rotary evaporator and weighed it. Then the band is further purified on another glass plates with 0.25 mm thickness with another solvent system(chloroform: methanol, 9:1)[18]

Identification of the isolated compound

Spectrometric analysis

Chemical structure elucidation was obtained by IR, UV, ^{1}H NMR, and ^{13}C-NMR.

Result And Discussions

Chromatographic analysis for detection of astragalin.

The extract of aerial parts was analyzed by HPLC and HPTLC.

TLC

The result showed the best separation of compounds in the ethyl acetate extract and a fluorescent blue spot of astragalin was showed under 254 nm of UV light with Rf value 0.50 in comparison with standard astragalin as shown in (fig:1) and table (1).

Table (1): R_f values of Astragalin in *Cressa cretica* extract and standard

Solvent system	Value of R_f of astragalin standard	R_f value of extract
Ethyl acetate: methanol, water, (15:1.25:1)	0.52	0.50

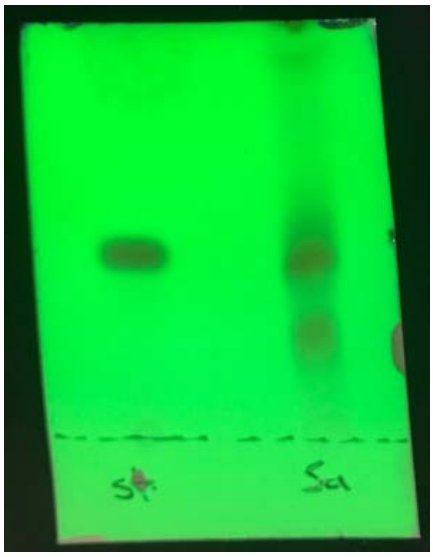
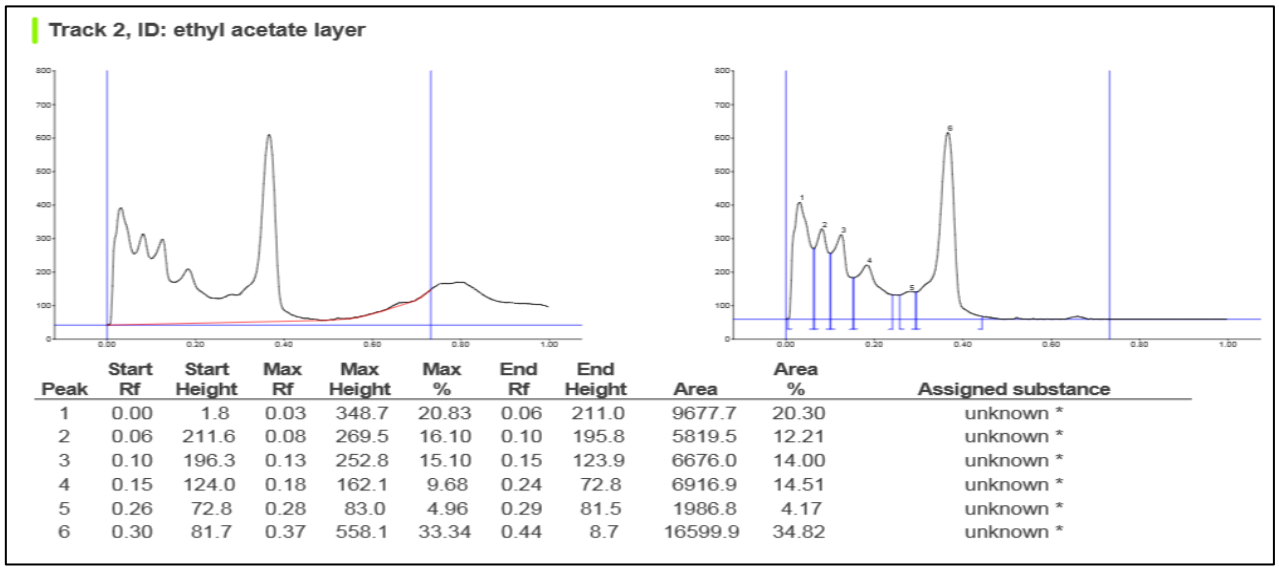


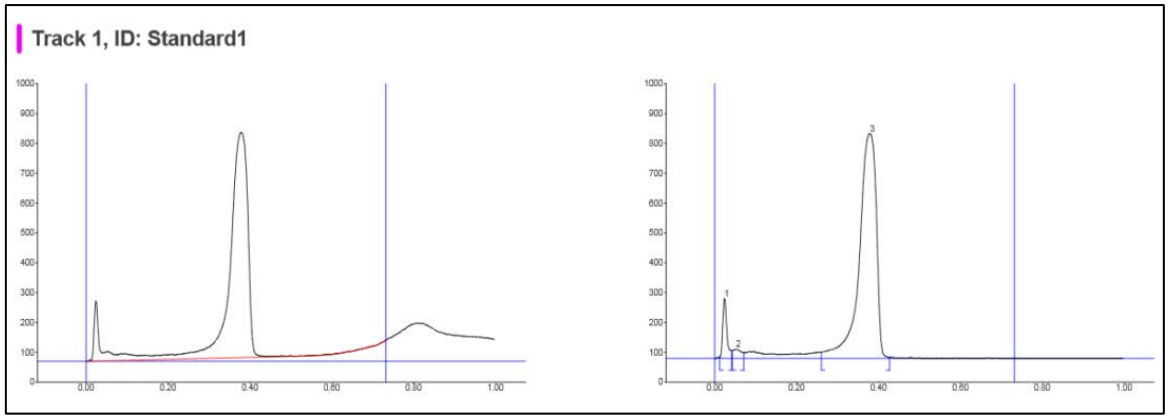
Figure (1): Analytical TLC of astragalin standard and *Cressa cretica* extract.

HPTLC chromatogram of the ethyl acetate layer shows 6 peaks at different R_f values with well-defined peak number 6 at maximum R_f of 0.37 that represents percent area about 34.82% of the extract compositions as shown in (Fig.2). With reference to R_f value of standard astragalin as shown in (fig.3), the observed peak identified as astragalin.



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.00	1.8	0.03	348.7	20.83	0.06	211.0	9677.7	20.30	unknown *
2	0.06	211.6	0.08	269.5	16.10	0.10	195.8	5819.5	12.21	unknown *
3	0.10	196.3	0.13	252.8	15.10	0.15	123.9	6676.0	14.00	unknown *
4	0.15	124.0	0.18	162.1	9.68	0.24	72.8	6916.9	14.51	unknown *
5	0.26	72.8	0.28	83.0	4.96	0.29	81.5	1986.8	4.17	unknown *
6	0.30	81.7	0.37	558.1	33.34	0.44	8.7	16599.9	34.82	unknown *

Fig (2): High-performance thin-layer chromatography chromatogram of extract from aerial parts cultivated in Iraq

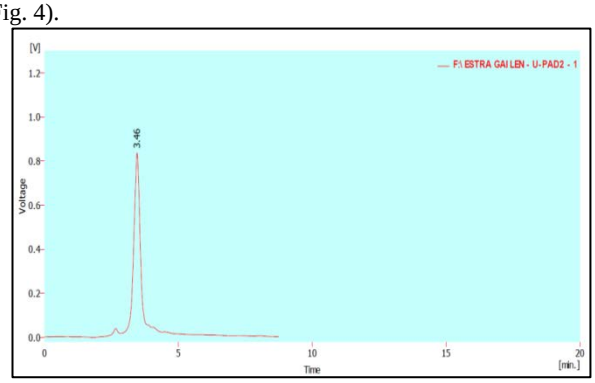


Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	4.5	0.02	201.1	20.39	0.04	26.9	1629.8	6.11	unknown *
2	0.04	27.0	0.05	30.9	3.13	0.07	20.0	523.7	1.96	unknown *
3	0.26	21.2	0.38	754.2	76.48	0.43	4.0	24529.1	91.93	unknown *

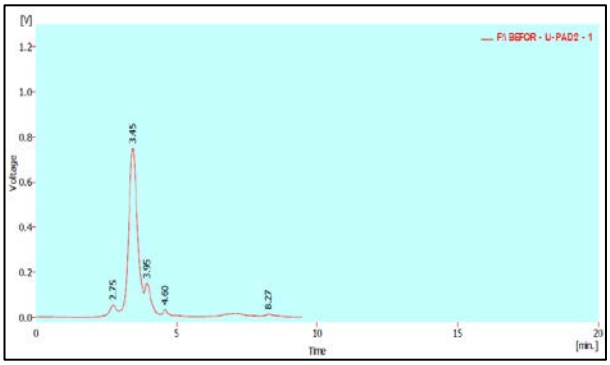
Fig (3): HPTLC chromatogram of standard Astragalin.

HPLC

HPLC analysis showed astragalin peak at R_t 3.45 min representing 80.2% of the total compositions of extract compared with a retention time of astragalin standard (3.46 min) as shown in (Fig. 4).



A



B

Fig. 4: The high-performance liquid chromatography analysis of ethyl acetate extract of *Crassa cretica*, (a) peak of standard astragalin was detected at 3.46 min (b) astragalin in the extract was detected in 3.45 min.

Total astragalin content

The amount of astragalin was isolated and quantified about 0.3% w/v in aerial parts extract, (Fig. 5) shows the separation bands of astragalin extract by preparative TLC.

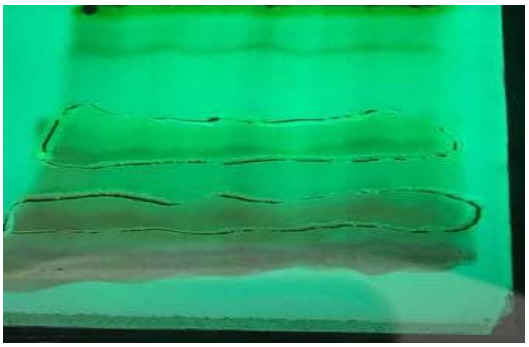


Fig 5: Preparative thin-layer chromatography for isolation of astragalin

Spectral identification of isolated compound

The isolated compound obtained as a yellow powder with R_f=0.5, chemical investigation of this compound indicated the structure of astragalin (Fig. 6) and 13C-NMR together with IR, 1H-NMR, and spectral data confirmed the molecular formula to be (C21H20O11). The maximum UV absorption of astragalin acid was 351 nm in comparison with standard 352 nm (figure 7).

Structure elucidation by FT-IR for isolated compound showed the presence of hydroxyl groups of aromatic rings as broadband centered at 3364 cm-1, and presence bands at 1666 cm-1 of carbonyl group, 1607, 1560, 1452(C=C, Ar), 1286 (=C-O-C), 1180 (C-OH) as shown in figure (8). The spectral data are agreement with previously reported literature for the same compound.[19]

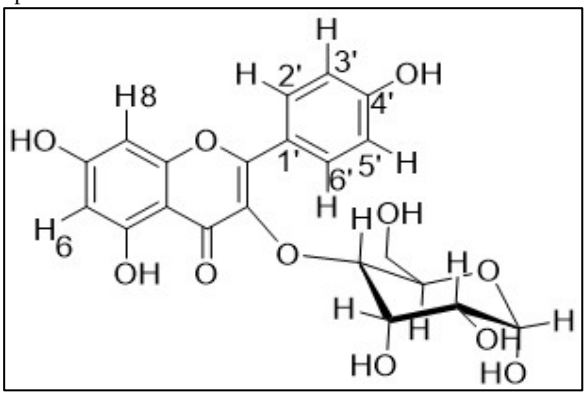


Fig 6: Chemical structure of astragalin

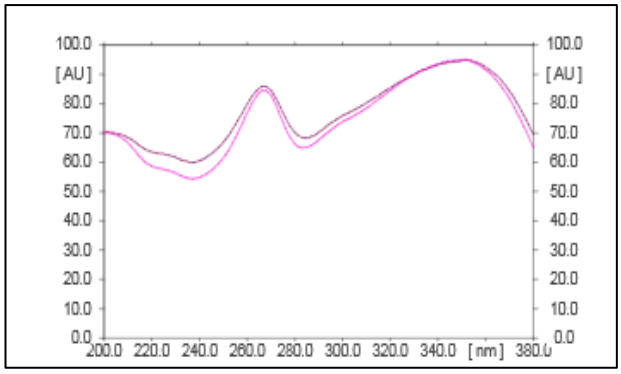


Fig (7): UV-spectra of standard kaempferol 3- O-β-glucoside and isolated compound.

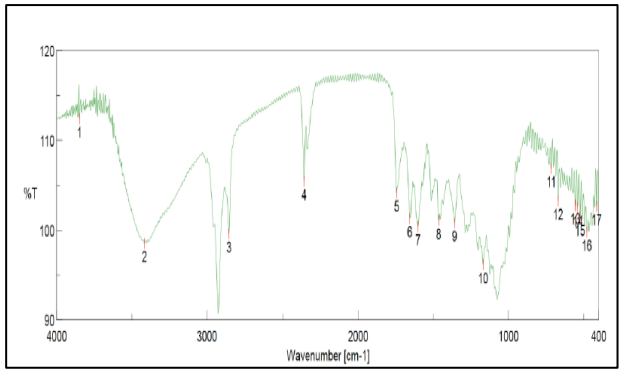


Fig (8): IR spectrum of the compound isolated from Iraqi *Cressa cretica.*

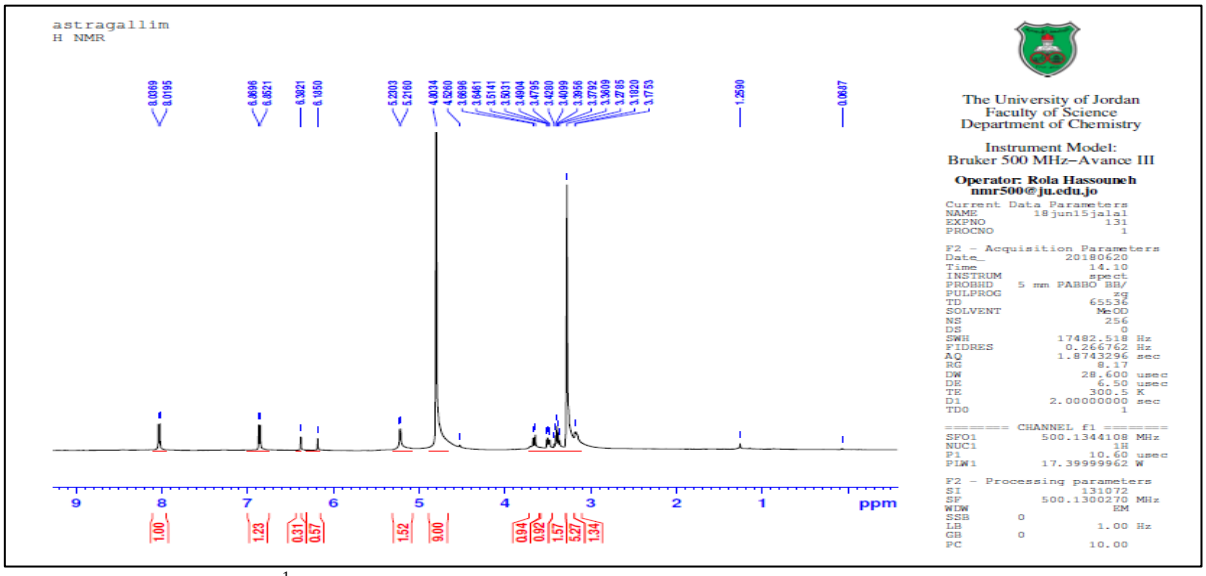
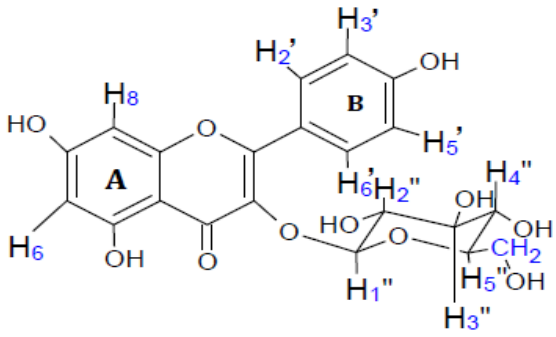


Fig (9):[1]HNMR spectrum of kaempferol 3- O-β-glucoside in deuterated methanol.

Table (2): [1]H-NMR Data and their Interpretation of kaempferol 3-O-β-glucoside.



Group	Chemical shift ppm	No. of H	Interpretation
H5''	3.17	1	multiplet, for a proton of the sugar moiety
H_3'',H_4''	3.409-3.379	2	multiplet, for a proton of the sugar moiety
CH_2	3.514	2	doublet, for proton CH_2 group
H_2''	3.66	1	doublet, for a proton of the sugar moiety
H_1"	5.23-5.21	1	doublet, for a proton of the sugar moiety
H_6	6.185	1	singlet, for a proton of the A ring
H_8	6.382	1	singlet, for a proton of the A ring
H_3', H_5'	6.86-6.85	2	doublet, for a proton of the B ring
H_2', H'$_6$	8.01-8.03	2	doublet, for a proton of the B ring

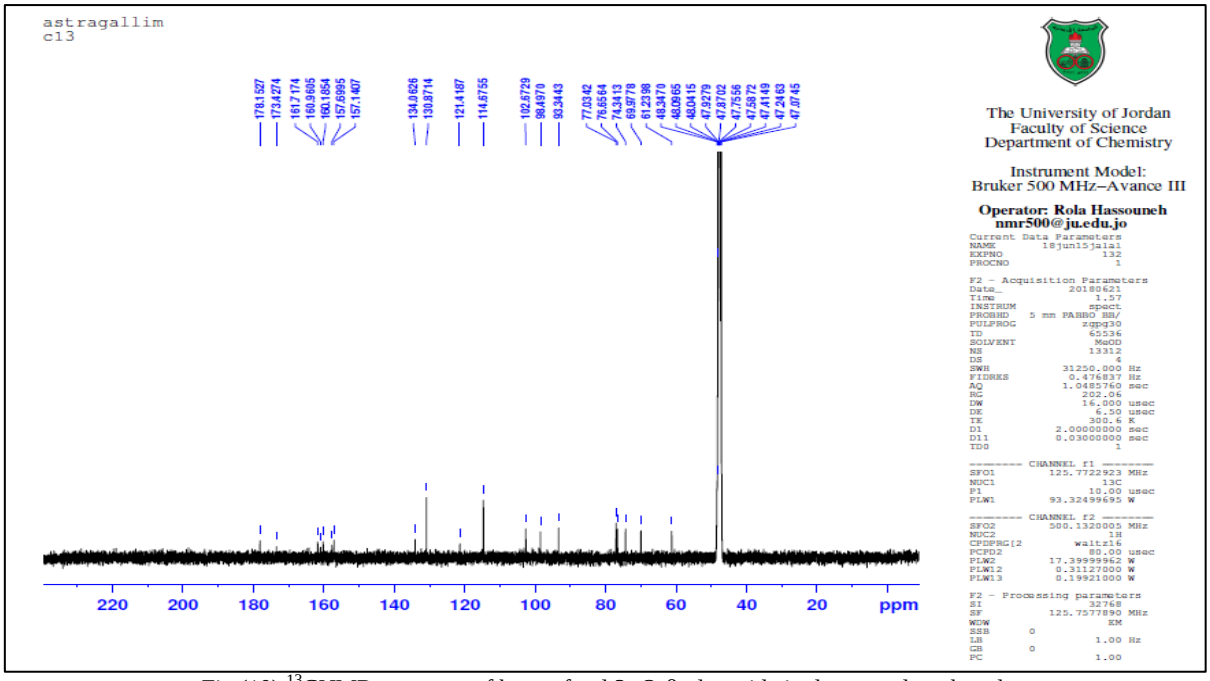


Fig (10):[13]CNMR spectrum of kaempferol 3- O-β-glucoside in deuterated methanol.

The [1]H-NMR spectrum of the isolated compound indicated that B ring protons, H_2' and H_6' signals were given doublet at 8.01- 8.03 ppm. H_3' and H_5' proton signals were observed at 6.86-6.85 ppm as a doublet.H_8 and H_6 protons were at 6.382 and 6.185 ppm, respectively as a singlet. Based on these findings and by comparison of NMR data, the aglycone identified as kaempferol. The aromatic proton (H-$_1$") of the hexose appeared as a doublet at 523ppm. H_2" and CH_2 appeared as a doublet at 3.669 ppm and 3.514 ppm respectively, H_3", H_4"gave signal at 3.409-3.379 ppm as multiplet and H_5" appeared as a multiplet at 3.175 ppm.[20] Therefore the isolated compound was assumed to be kaempferol 3-O-β- glucoside (Astragalin). as shown in (fig:9) & (table 2)
[13]C-NMR shows characteristic peaks in which broadband decoupling present 19 different signals, with highest chemical shift for (C=O) at 178.15 and C=C attached to (OH) groups at range 173.42-160.9, other C=C signals at range 157.69-102.67, while those carbons attach to (OH) group at range 76.65-61.23ppm. The peaks assigned in [13]C NMR corresponded to the Ref.[21] as shown in (fig.10).

Conclusion

The quantity of Kaempferol 3- O- β- glucoside (astragalin) is (0.3% W/W) present in the Iraqi plant as a major glycoside. Astragalin was extracted from *Cressa cretica* under optimal conditions (80% aqueous ethanol and 80°c for 3 hrs.). We suggest that these isolated compounds are a suitable candidate for further clinical and pharmacological study

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