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Research Article

ISOLATION OF ASTRAGALIN FROM IRAQI *CHENOPODIUM ALBUM*

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ABSTRACT

Objective: *Chenopodium album* L is the species of the genus chenopodium. The Greek name *Chenopodium* means goosefoot. The plant is native to Asia and Europe. The analysis of the constituents of the Iraqi plant was performed using gas chromatography and high-performance liquid chromatography techniques.

Methods: Thin-layer chromatography (TLC) and high-performance TLC chromatographic techniques were used for the detection and isolation of the active constituent found in the plant. Spectral analysis such as nuclear magnetic resonance (NMR) and UV was used to confirm the chemical structure of the compound isolated.

Results: Astragalin was isolated and identified by comparison with standard kaempferol 3-O-β-glucoside (astragalin) which was detected as the major glycoside in the polar fraction of the plant. Further, identification of the compound was performed by ^{1}H-NMR spectroscopy.

Conclusion: Astragalin is the major flavonoid glycoside found in the plant.

Keywords: *Chenopodium*, Astragalin, Thin-layer chromatography, High-performance liquid chromatography, Nuclear magnetic resonance.

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INTRODUCTION

Medicinal plants are used from a very long interval of time for the treatment of several diseases [1]. *Chenopodium album* Linn. is a fast-growing shrub, growing almost everywhere in nitrogen-rich soil, especially on wasteland. The herb is a common weed during summer and winter in the field of wheat, barley, mustard, and gram and reduces their yield [2]. The plant used as culinary vegetables which is useful as anticancer agent. The leaves and young shoots may be eaten, either steamed or cooked, but should be eaten in moderation due to high levels of oxalic acid (Fig. 1) [3].

The widespread uses of *C. album* in traditional medicine have resulted in considerable chemical analysis of the plant and its active principles. From the phytochemical point of view, the plant was reported to contain flavonoids, glycosides, alkaloids, terpenes, sterols, and saponins [4,5].

The flavonoid glycoside (astragalin)

Kaempferol 3-O-β-glucoside (astragalin) is one of the major flavonoid glycosides found in a variety of plants [6]. Astragalin is receiving increasing attention due to its various health benefits and biological activities including antioxidant, anthelmintic, anti-inflammatory, anti-HIV, and antiallergic effects [7-11].

The aim of this study is to investigate the presence of astragalin in the Iraqi *C. album* and to isolate it as a pure compound.

MATERIALS AND METHODS

Plant material

The aerial parts (stems and leaves) of *C. album* were collected from area Al-Yousifiya in Baghdad and identified by the national herbarium in Abu-Ghraib, Baghdad.

The plant material was collected during March and dried at room temperature in the shade, then grinded as powder and weighed.

Extraction and detection of flavonoids and flavonoid glycosides

The powdered aerial parts of *C. album* (200 g) were defatted with hexane (1400 mL). The defatted plant material was further extracted with ethanol (1500 mL) using Soxhlet extractor. The ethanolic extract was concentrated by evaporation under reduced pressure using rotary evaporator. Then, distilled water (35 mL) was added to the ethanolic extract, and the extract partitioned with ethyl acetate (50 mL) and allowed to settle overnight.

The lower aqueous layer (400 mL) was collected and labeled as fraction A, while the upper ethyl acetate layer was collected and labeled as fraction B.

Isolation of flavonoid glycoside

Column chromatography

A part of the ethyl acetate phase (fraction B) was concentrated using rotary evaporator and subjected to column chromatography (1 M × 4 cm) over silica gel (60 mesh) and eluted with (ethyl acetate:methanol:water, 15:1.25:1). The fractions (20) were collected by monitoring on thin-layer chromatography (TLC) using (ethyl acetate:methanol:water, 15:1.25:1) as mobile phase and the spot was detected by UV. The collected fractions (fractions 8–15) were combined and concentrated to dryness using rotary evaporator. Further, purification carried out using preparative TLC.

Preparative TLC

The combined fraction was further purified by preparative TLC performed on silica gel GF254 precoated plates (0.5 mm and 1 mm thickness) and developed with (ethyl acetate:methanol:water, 15:1.25:1), the separated bands were visualized under UV light (254 nm), bands at R_f = 0.5 were scrapped off and eluted with acetone and methanol. Solvent was evaporated by rotary evaporator.

Preparative high-performance liquid chromatography (preparative HPLC)

Ethyl acetate phase (fraction B) was subjected to preparative HPLC using C_{18} column with a mobile phase (acetonitrile:water) as isocratic mixture (30:70) and a flow rate of 10 ml/min, detection at 347 nm, run time 10 min. Kaempferol 3-O-glucoside peak was collected off the column according to comparison with reported retention time [12].

Fig. 1: Iraqi *Chenopodium album*

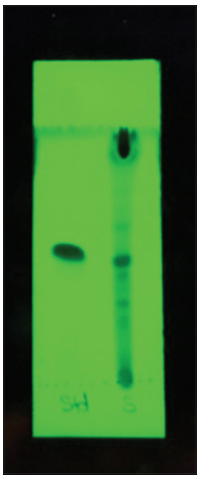


Fig. 2: Analytical thin-layer chromatography of fraction (B) compared with standard kaempferol 3-O-β-glucoside

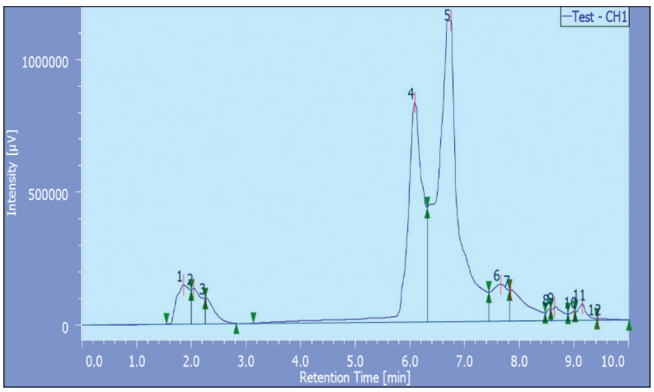


Fig. 3: Preparative high-performance liquid chromatography for ethyl acetate extract of *Chenopodium album*

The R_f value of the isolated compound that is supposed to be kaempferol 3-O-glucoside was determined by TLC using solvent system (ethyl acetate:methanol:water, 15:1.25:1) in comparison with standard kaempferol 3-O-glucoside.

High-performance TLC (HPTLC)

HPTLC analysis was performed to confirm that the compound isolated by preparative HPLC is kaempferol 3-O-β-glucoside.

Table 1: R_f values of standard kaempferol 3-O-β-glucoside and *Chenopodium album* extract

Solvent system	R_f of astragalin standard	R_f of extract
Ethyl acetate:methanol: H_2O (15:1.25:1)[131]	0.52	0.51

HPTLC analysis was performed using silica gel 60 F 254 plates (10 cm × 20 cm). The standard kaempferol 3-O-β-glucoside (3 µl), the compound isolated by preparative HPLC (2 µl), and ethyl acetate phase (fraction B) (2 µl) were applied automatically on the plate by CAMAG Linomat 5. The plate after the application of the samples was developed in developing chamber using (ethyl acetate:methanol:water, 15:1.25:1), as the solvent system. The plates were air dried after development and scanned under UV (348 nm) using CAMAG TLC scanner 4.

Ultraviolet spectroscopy

The UV (MeOH) spectrum of the isolated compound was recorded (200–300 nm) to detect the lambda max of the isolated compound compared with standard material.

Fourier transform-infrared spectroscopy (FTIR)

The isolated compound was analyzed using FTIR spectrophotometer.

Nuclear magnetic resonance (NMR)

The isolated compound was resuspended in deuterated methanol for ^{1}H-NMR analysis that was performed on BRUKER 400 MHz using tetramethylsilane as internal standard.

RESULTS AND DISCUSSION

Isolation of kaempferol 3-O-β-glucoside (astragalin)

Analytical TLC of ethyl acetate phase (fraction B) confirmed the presence of kaempferol 3-O-β-glucoside (astragalin) in the plant extract in comparison with standard kaempferol 3-O-β-glucoside as represented in Table 1 and Fig. 2.

Preparative HPLC of ethyl acetate phase (fraction B) indicated the presence of kaempferol 3-O-β-glucoside (astragalin) as the major glycoside and isolated it by comparison with reported retention time in literature [13]. Fig. 3 shows preparative HPLC chromatogram of the plant extract with the peak numbered 5 corresponding to retention time of 6.750 min represented kaempferol 3-O-β-glucoside as compared with reported retention time of kaempferol 3-O-β-glucoside and accounted for 50.75% of total extract as illustrated in Table 2.

Kaempferol 3-O-β-glucoside (astragalin) was isolated by column chromatography and further purified by preparative TLC and combined with isolated astragalin from preparative HPLC. The isolated compound was identified by analytical TLC, HPTLC, UV, FTIR, and ^{1}H-NMR. A standard kaempferol 3-O-β-glucoside (astragalin) was bought from Sigma-Aldrich to confirm that the isolated flavonoid is astragalin, not similar compound.

Isolated compound from the Iraqi plant was subjected to HPTLC along with the standard kaempferol 3-O-β-glucoside (astragalin) and ethyl acetate phase (fraction B). HPTLC analysis showed that standard kaempferol 3-O-β-glucoside has maximum R_f value of 0.34 as shown in Fig. 4 and Table 3.

HPTLC chromatogram of the extract Fig. 5 showed 17 peaks at different R_f values with well-defined peak at maximum R_f = 0.29 that represents 33% of the total extract composition as shown in Table 4. By comparison with R_f value of the standard kaempferol 3-O-β-glucoside, the observed peak identified as kaempferol 3-O-β-glucoside.

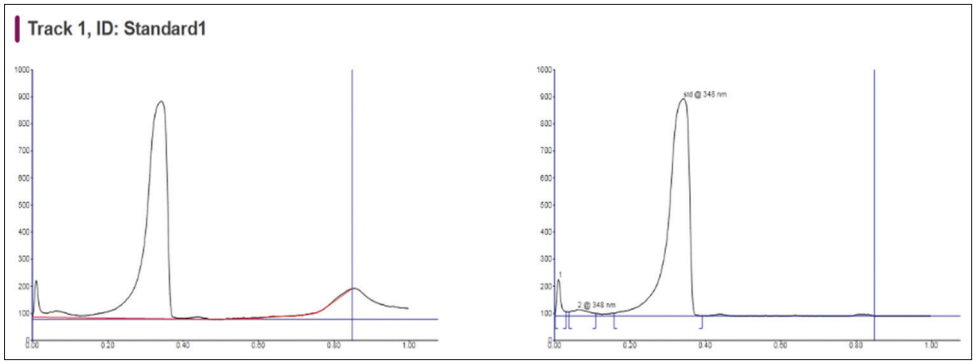


Fig. 4: High-performance thin-layer chromatography chromatogram of standard kaempferol 3-O-β-glucoside

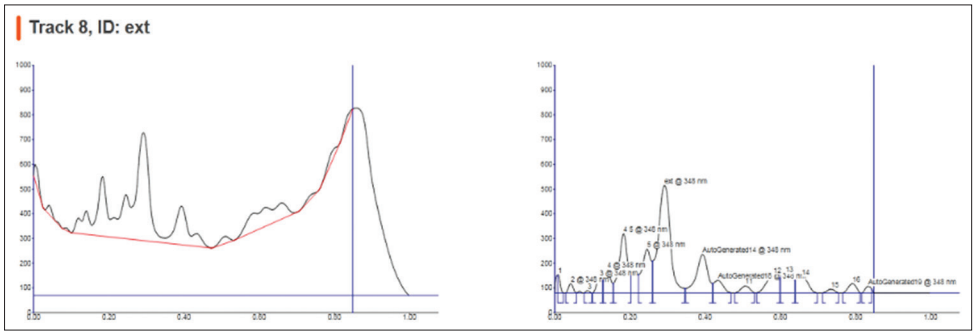


Fig. 5: High-performance thin-layer chromatography chromatogram of Iraqi *Chenopodium album* extract

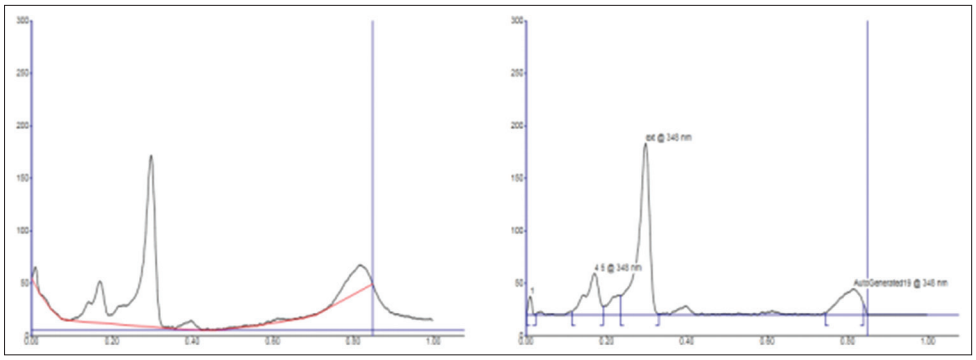


Fig. 6: High-performance thin-layer chromatography chromatogram of the isolated compound from Iraqi *Chenopodium album* extract

Table 2: Retention time and corresponding percent area and height of preparative HPLC of *Chenopodium album* extract

Peak name	CH	tR (min)	Area (µV sec)	Height (µV)	Area %	Height%
S	1	1.858	2378737	146280	4.055	5.244
Unknown	1	2.042	1755755	133538	2.993	4.788
Unknown	1	2.275	1125377	97884	1.918	3.509
Unknown	1	6.083	16137499	825086	27.509	29.581
Unknown	1	6.750	29773755	1130130	50.754	40.517
Unknown	1	7.650	2887320	138962	4.922	4.982
Unknown	1	7.833	2583489	115946	4.404	4.157
Unknown	1	8.550	251572	45372	0.429	1.627
Unknown	1	8.642	710660	51735	1.211	1.855
Unknown	1	8.983	238664	34732	0.407	1.245
Unknown	1	9.150	703735	62688	1.200	2.247

HPTLC chromatogram of the isolated compound from Iraqi plant Fig. 6 showed a well-defined peak at maximum R_f = 0.30 as shown in Table 5. By comparison with R_f value of the standard kaempferol 3-O-β-glucoside (astragalin), the observed peak identified as kaempferol 3-O-β-glucoside. Fig. 7 shows the 3D spectra of all tracks scanned at 348 nm.

Structure elucidation of kaempferol 3-O-β-glucoside (astragalin)

UV λ_{max} (MeOH): 267, 351 nm for standard astragalin and λ_{max} =267, 362 nm for the isolated compound as shown in Fig. 8-10.

FTIR (KBr): Broad 3416 cm^{-1} (OH), 1744 cm^{-1} (C=O), 1656 cm^{-1} (C=C, aromatic) Fig. 11 [12].

Table 3: R_f values and corresponding peak areas of standard kaempferol 3-O-β-glucoside

Peak	Start R_f	Start height	Max R_f	Max height	Max%	End R_f	End height	Area	Area%	Assigned substance
1	0.00	5.3	0.01	135.7	14.09	0.03	17.0	1133.0	3.21	Unknown*
2	0.04	15.9	0.06	24.3	2.52	0.11	10.4	860.3	2.44	2
3	0.16	11.0	0.34	802.8	83.39	0.39	1.8	33338.2	94.36	Std

Table 4: R_f values and corresponding peak areas of Iraqi *Chenopodium album* extract

Peak	Start	Start height	Max R_f	Max height	Max%	End R_f	End height	Area	Area%	Assigned substance
1	0.01	73.3	0.01	75.0	4.50	0.02	0.2	399.6	1.25	Unknown*
2	0.03	0.3	0.04	37.5	2.25	0.06	1.0	348.3	1.09	2
3	0.08	0.7	0.09	10.4	0.62	0.10	0.3	77.9	0.24	Unknown*
4	0.10	0.1	0.12	62.1	3.73	0.13	51.1	689.1	2.15	3
5	0.13	51.7	0.14	94.5	5.67	0.16	38.8	1214.3	3.79	4
6	0.16	40.0	0.18	239.4	14.35	0.20	70.7	4053.1	12.66	45
7	0.22	75.6	0.25	178.5	10.70	0.26	129.6	3251.9	10.16	5
8	0.26	130.5	0.29	435.8	26.13	0.35	19.5	10641.5	33.24	Ext
9	0.35	19.2	0.39	155.6	9.33	0.42	38.9	3599.3	11.24	Autogenerated 14
10	0.42	39.5	0.44	51.3	3.08	0.47	0.2	967.5	3.02	Autogenerated 18
11	0.48	0.2	0.51	28.8	1.73	0.53	0.7	519.3	1.62	Unknown*
12	0.54	1.0	0.58	74.3	4.45	0.60	63.9	1826.9	5.71	Unknown*
13	0.60	64.3	0.62	76.7	4.60	0.64	52.8	1732.9	5.41	Unknown*
14	0.64	52.9	0.66	65.4	3.92	0.70	0.2	1506.2	4.70	Unknown*
15	0.71	0.4	0.74	16.0	0.96	0.76	0.3	243.4	0.76	Unknown*
16	0.77	0.5	0.79	38.5	2.31	0.81	1.0	625.3	1.95	Unknown*
17	0.82	0.4	0.84	27.9	1.67	0.84	17.0	319.3	1.00	Autogenerated 19

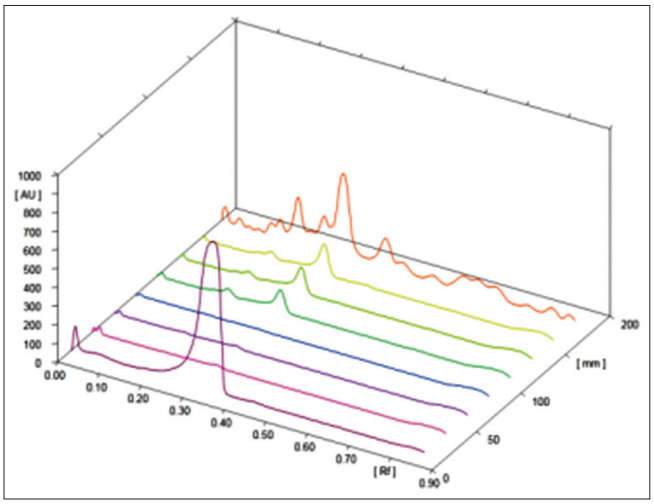


Fig. 7: The three-dimensional spectra of all tracks scanned at 348 nm

Fig. 8: High-performance thin-layer chromatography plate of the isolated compound and the plant extract compared with standard kaempferol 3-O-β-glucoside under short UV (254 nm)

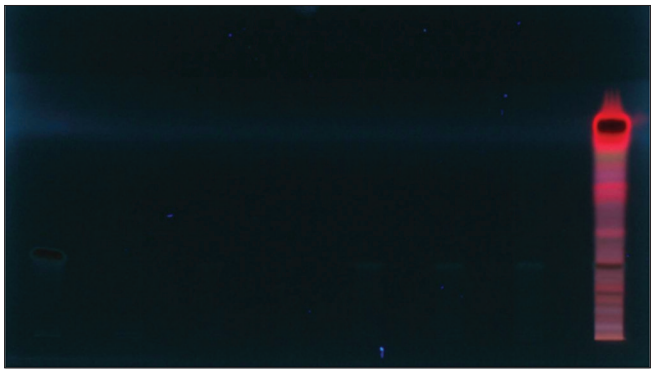


Fig. 9: High-performance thin-layer chromatography plate of the isolated compound and the plant extract compared with standard kaempferol 3-O-β-glucoside under long UV (366 nm)

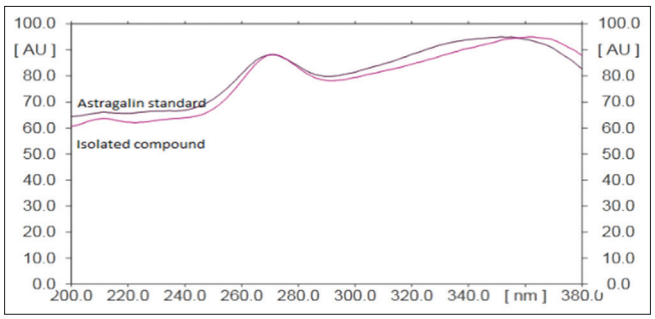


Fig. 10: UV spectra of standard kaempferol 3-O-β-glucoside and isolated compound

As shown in Fig. 12 and Table 6, the ^{1}H-NMR spectrum of the isolated compound indicated that B ring protons, H_2' and H_6' signals were gave doublet at 7.61–7.59 ppm. H_3' and H_5' proton signals were observed at 6.93–6.91 ppm as a doublet. H_8 and H_6 protons were at 6.31 ppm and 6.14 ppm, respectively, as singlet. Based on these findings and by comparison of NMR data, the aglycone identified as kaempferol. The anomeric proton (H-1") of the hexose appeared as a doublet at 5.28 ppm. H_2'' and CH_2 appeared as multiplet at 3.96 ppm and 3.72 ppm,

Table 5: R_f values and corresponding peak areas of the isolated compound from Iraqi *Chenopodium album* extract

Peak	Start R_f	Start height	Max R_f	Max height	Max%	End R_f	End height	Area	Area%	Assigned substance
1	0.00	2.6	0.01	17.9	7.30	0.02	0.8	118.3	2.11	Unknown*
2	0.11	3.0	0.17	39.5	16.09	0.19	8.0	970.5	17.31	45
3	0.24	18.2	0.30	163.6	66.57	0.33	0.9	3547.8	63.29	Ext
4	0.75	1.5	0.82	24.7	10.04	0.84	10.4	968.9	17.28	Autogenerated19

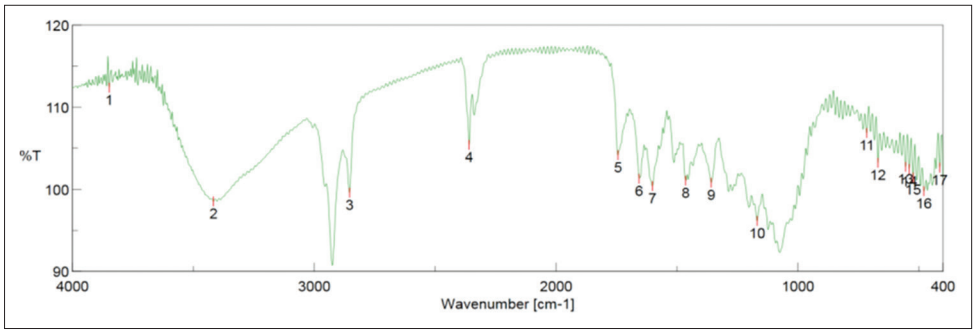


Fig. 11: Infrared spectrum of the compound isolated from Iraqi *Chenopodium album*

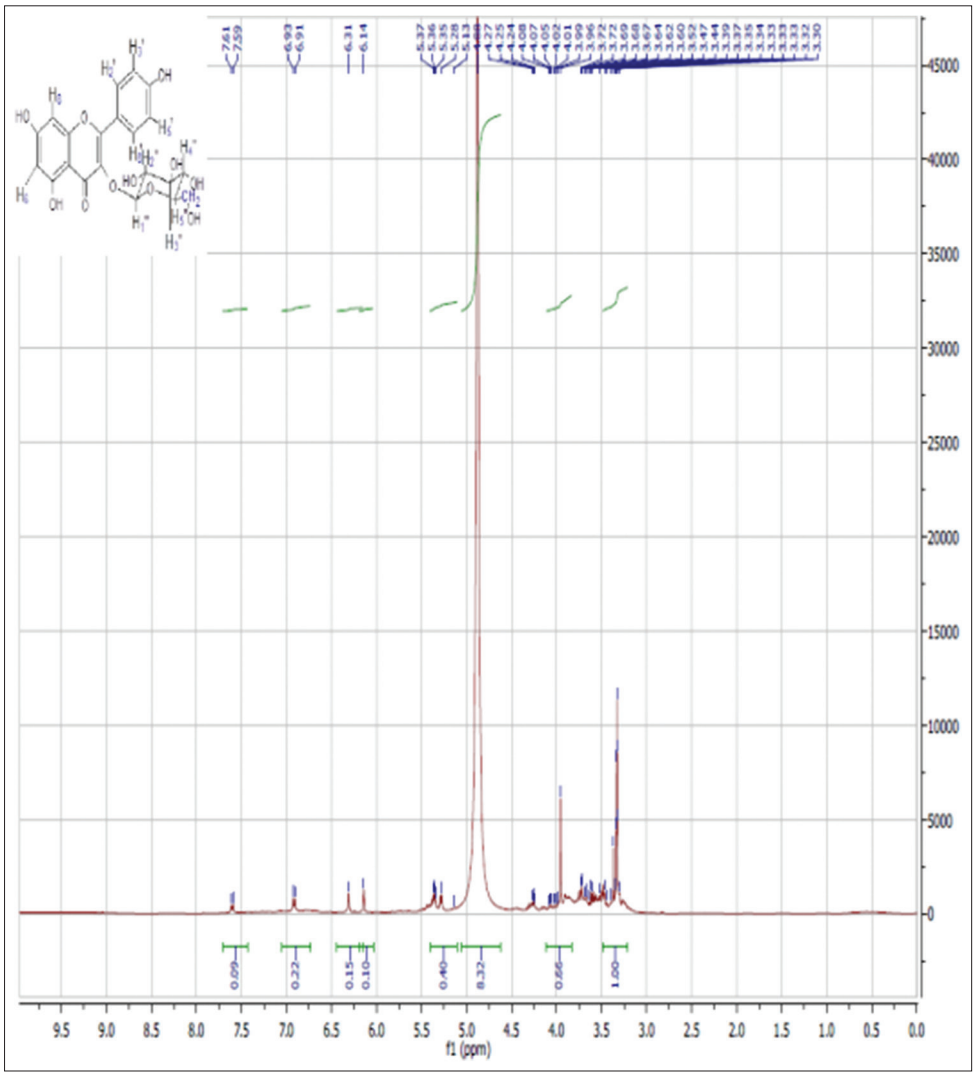
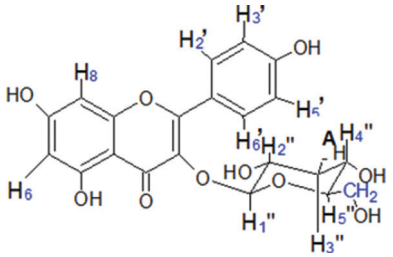


Fig. 12: ^{1}H-nuclear magnetic resonance of kaempferol 3-O-β-glucoside in deuterated methanol

Table 6: ^{1}H-NMR data and their interpretation of kaempferol 3-O-β-glucoside



Group	Chemical shift ppm	Number of H	Interpretation
H_5"	3.47	1	Multiplet, for proton of the sugar moiety
H_3", H_4"	3.52-3.62	2	Multiplet, for proton of the sugar moiety
CH_2	3.72	2	Multiplet, for proton CH_2 group
H_2"	3.96	1	Multiplet, for proton of the sugar moiety
H_1"	5.28	1	Doublet, for proton of the sugar moiety
H_6	6.14	1	Singlet, for proton of the A ring
H_8	6.31	1	Singlet, for proton of the A ring
H_3', H_5'	6.91–6.93	2	Doublet, for proton of the B ring
H_2', H_6'	7.59–7.61	2	Doublet, for proton of the B ring

respectively, H_3", H_4" gave signal at 3.52–3.62 ppm as multiplet and H_5" appeared as multiplet at 3.47 ppm [13]. Therefore, the isolated compound was assumed to be kaempferol 3-O-β-glucoside (astragalin).

CONCLUSION AND RECOMMENDATION

Kaempferol 3-O-β-glucoside (astragalin) is present in the Iraqi plant as a major glycoside. Quantitative phytochemical analysis of Iraqi *C. album* is necessary to determine the quantity of its important chemical constituents including astragalin.

Clinical study of the plant extract must be performed to assist the use of this plant as herbal infusion for health benefit [14].

Astragalin is a useful compound; it was first isolated from plant supplement in 1997 and marketed to be used for its antioxidant activity [15].

AUTHORS' CONTRIBUTION

Dr. Widad was the supervisor of the research. Anwar Mehdi was MSc student; she performs the work on this plant. Dr. Ayad is a chemist; he contributes through explaining NMR and IR data.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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