Cytotoxic Activity of Iraqi *Cressa Cretica*

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Abstract:

Cressa cretica Fam: Convolvulaceae (morning glory). These plants are native to the tropical and subtropical areas of the world. Nutritional analysis provide it to be a potential source of minerals, energy, carbohydrate, alpha-tocopherol, edible oil. From phytochemical point of view, the plant was reported to contain: coumarins, sterols, alkaloids, tannins, glycosides, flavonoids, unidentified sugars and high salt content. Ethyl acetate layer and ethanolic layer were examined for cytotoxic activity.Kampferol-3-O-glucoside (Astragalin) was isolated as amorphous powder from the aerial parts of Cressa cretica. The flavonoid in the Iraqi plant was detected by TLC. Its presence was confirmed by HPLC.

Key words: Cressa cretica, Astragalin, HPLC analysis

التاثير السمي لنبات الشويل العراقي

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الخلاصة:

الشويل هو نبات ينتمي لعائله (بهاء الصباح)، النبات يتواجد طبيعيا في الاماكن الاستوائيه وشبه الاستوائيه من العالم . التحليل الغذائي للنبات اثبت امكانيه اعتباره مصدرا للمعادن، الطاقه، الكاربوهيدرات، الفا توكوفيرول وزيوت نباتيه. من وجهه النظر الكيمونباتيه النبات يحتوي: كومارين، ستيرولات، قلويدات، تانين، كلايكوسيدات، فلافينويدات، سكريات وكميات عاليه من الاملاح.مستخلص النبات الجاف من الايثل اسيتيت ومستخلص النبات الجاف من الايثانول تم دراسه تأثيرهم على الخلايا السرطانيه. تم فصل الكامفيرول كلايكوسايد (الاستراكالين) كبادر من الاجزاء الهوائيه للنبات، الفلافونويد في النبات العراقي تم الكشف عنه باستخدام تقنيه استشراب الطبقه الرقيقه (TLC)، وثم تأكيد وجوده باستخدام تقنيه (HPLC) مع الاستراكالين القياسي .

الكلمات المفتاحية: الشويل، الاستراكالين، التحليل بواسطة HPLC

Introduction:

Cressa cretica figure (1), is a perennial subshrub or herb, usually much-branched up to 40 cm height the smallest adult samples are about 5-6 cm high, while the highest ones are approximately 20cm. most of the samples are about 10 cm high. [1]

Stems figure (2), are at first erect and then become decumbent, apparently short-lived, gray appressed pilose to sericeous. [2]

Seeds figure (3), are 3–4 mm long, glabrous and smooth, and shining to reticulate, dark brown.

Leaves figure (4), on main branches are often larger than those on branchlets, the blade 1–12 mm long, lanceolate, ovate or elliptic- to scale-like, sessile, or shortly petiolate [3].

Figure (1)

Figure (2)

Figure (3)

Figure (4)

Nutritional analysis proved it to be a potential source of energy, carbohydrate, alpha-tocopherol and minerals (sodium, potassium, magnesium, zinc).[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]

Ethyl acetate extract of *Cressa cretica* at both doses 100mg/kg and 200mg/kg caused significant decrease of mitotic index in both bone marrow cells and spleen cells when compared to negative control (DMSO) ($P<0.05$).[6]

Kampferol-3-O- Glucoside (Astragalin)

Astragalin **1** is one of the major flavonoid found in variety of plants, astragalin is receiving increasing attention due to its varies health benefiting and biological activities including anti-oxidative, anti-inflammatory anti-HIV, anti-allergic effects, anti-tumor beside this astragalin is responsible for the color of different beans and has the potential to extract it and market as nutritionally important food supplement.[7] (Astragalin) was isolated from the aerial parts of *Cressa cretica*.[8] preliminary study of this article is to investigate the presence of this compound as a major component in Iraqi *Cressa cretica* and its cytotoxic effect on two cells cancer (breast and ovarian cancer) since it is an important compound in therapy.

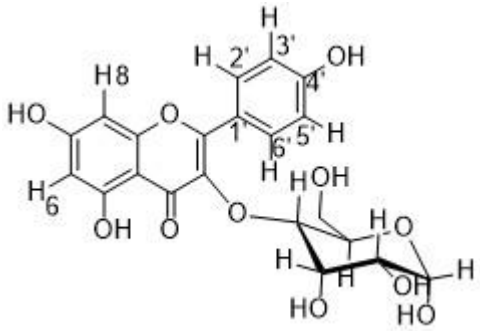

Figure (5): Chemical structure of Astragalin

Material and Methods

Plant Material

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

Extraction

Powder plant (30g) was extracted by soxhlet apparatus with ethanol (80%, 150 mL). The extract concentrated to (80mL) and divided in to two fractions; the first (40mL) was evaporated by rotary evaporator to dryness and taken as the ethanolic extract. The other fraction (40mL) was taken and distilled water (25mL) was added, and then partitioned by ethyl acetate (30mLx3), the ethyl acetate layer was dried by anhydrous sodium sulphate and evaporated to dryness and collected as ethyl acetate layer.[9]

Preparation of Cell Cultures

MCF-7 cell line Michigan Cancer Foundation-7 and SKOV-3 [1] were obtained from the Iraq biotech Cell Bank Unit and maintained in RPMI-1640 supplemented with 10% Fetal bovine, 100 units/mL penicillin, and 100 µg/mL streptomycin. Cells were passaged using Trypsin-EDTA reseeded at 50% confluence twice a week, and incubated at 37 °C.[10]

Cytotoxicity of *Cressa Creica* Extracts

Different concentrations of the both extracts were prepared (3.125, 6.25, 12.5, 25, 50, 100µg/mL) to determine the cytotoxic effect, the MTT cell viability assay was conducted on 96-well plates. Cell lines were seeded at 1 × 104 cells/well. A confluent monolayer was achieved after 24 hrs. Cells were treated with the tested compounds. Cell viability was measured after 72 hrs. of treatment by removing the medium, adding 28 µL of 2 mg/mL solution of MTT (and incubating the cells for 1.5 h at 37 °C. After removing the MTT solution, the crystals remaining in the wells was solubilized by the addition of 130 µL of DMSO (Dimethyl Sulphoxide) followed by 37 °C incubation for 15 min with shaking. The absorbency was determined on a microplate reader at 492 nm (test wavelength); the assay was performed in triplicate.[11] The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated as the following equation:-

Inhibition rate =

$$\frac{A - B}{A} * 100$$

Where A and B are the optical density of control & the optical density of test.

High Performance Liquid Chromato-Graphy (HPLC)

HPLC analysis was performed for detection and estimation of major components of ethyl acetate extract. 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 reversed phase (RP) ODS-C18 column (25 cm x4.6 mm x5µ m). The mixture was passed through 0.45 µm PVDF membrane and then 20 µL of each sample was injected in to 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 retention time of the standard astragalin.

Result

Ethyl acetate and ethanoic extracts both showed an efficient cytotoxic activity.

Ethyl Acetate Extract

The result of cytotoxic activity of ethyl acetate extract showed highly significant cytotoxic effect on both MCF-7 and SKOV-3 cell lines in dose dependent manner. The result of cytotoxic effect on human ovarian carcinoma cells was presented in table (1) and figure (6), while the result on breast carcinoma cells was presented in table (2) and figure (7).

Upon measuring the inhibition concentration (IC50) value for the wells incubated with ethyl acetate extract, the average of the reading was 14.25µg/mL,17.3µg/mL on both human ovarian carcinoma cells (SKOV-3) and breast carcinoma cells (MCF-7) respectively.

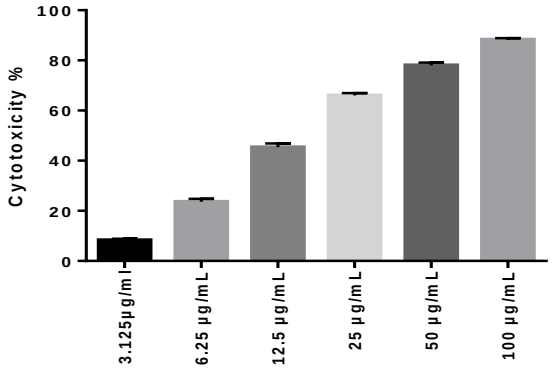

Figure (6): Cytotoxic Effect on SKOV-3Cell Line

Table 1: Mean Value of Cytotoxic Effect on SKOV-3 Cell Line

Con.	3.125 µg/ml	6.25 µg/mL	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL
Mean	8.251	23.59	45.36	66.04	78.08	88.32
P value	0.0021**	0.0009**	0.0004**	0.0001**	0.0001**	0.0001**

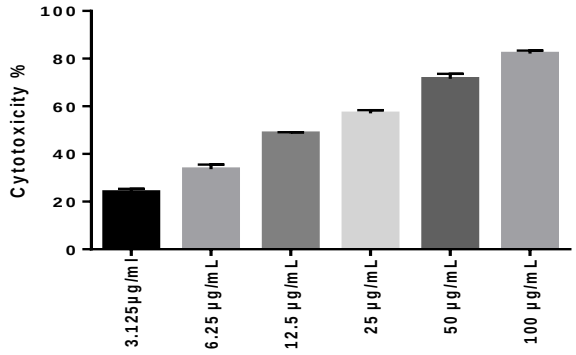

Figure (7): Cytotoxic Effect on MCF-7 Cell Line

Table 2: Mean Value of Cytotoxic Effect on MCF-7 Cell Line

Con.	3.125µg/ml	6.25µg/mL	12.5µg/mL	25µg/mL	50µg/mL	100µg/L
Mean	24.03	33.60	48.67	57.02	71.54	82.12
P value	0.0257*	0.0265*	0.0043**	0.0109*	0.0130*	0.0068**

Ethanol Extract

The results of cytotoxic effect for ethanolic extract on human ovarian carcinoma cells and breast carcinoma cells were presented in the tables (3&4) and clarified in figures (8&9) respectively, which revealed ethanolic extract have significant cytotoxic effect. Upon measuring the inhibition concentration (IC50) value for the wells incubated with ethanol extract, the average of all reading was 44.2 µg/mL, 48.52 µg/mL on both human ovarian carcinoma cells (SKOV-3) and breast carcinoma cells (MCF-7) respectively.

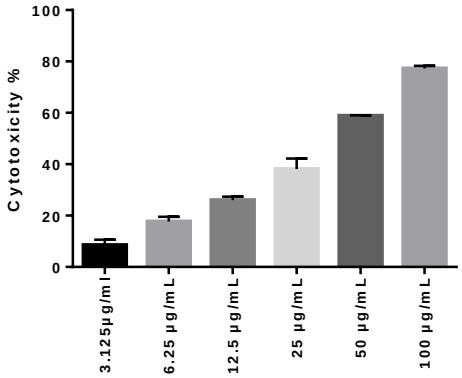

Figure (8): Cytotoxic Effect on SKOV-3 Cell line

Table 3: Mean Value of Cytotoxic Effect on SKOV-3 Cell line

Con.	3.125µg/mL	6.25µg/mL	12.5µg/mL	25µg/mL	50µg/mL	100µg/mL
Mean	8.501	17.67	26.04	38.12	58.85	77.27
P value	0.1111	0.0479*	0.0234*	0.0480*	0.0017**	0.0060**

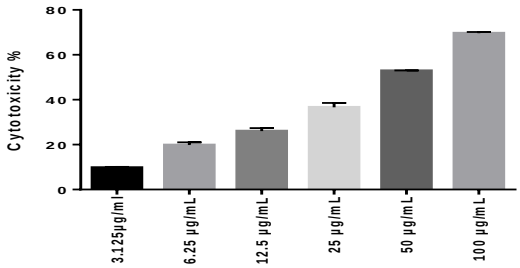

Figure (9): Cytotoxic Effect on MCF-7 Cell Line

Table 4: Mean Value of Cytotoxic Effect on MCF-7 Cell Line

Con.	3.125µg/ml	6.25µg/mL	12.5 µg/mL	25µg/mL	50µg/mL	100µg/mL
Mean	9.768	19.85	26.04	36.65	52.92	69.65
P value	0.0151*	0.0274*	0.0234*	0.0234*	0.0010**	0.0032**

HPLC Analysis of the Ethyl Acetate Layer

HPLC analysis of the ethyl acetate layer with isocratic elution resulted in the separation of astragalin as the major compound together with several minor components. Astragalin was determined by comparison of the actual retention time (Rt) to retention time of astragalin standard (3.46) as seen in figure (10).

Figure (11) showed the presence of different chemical constituents with major peak at Rt 3.45minutes representing percent area of 80.2%of the total compositions as illustrated in Table (5). As compared to retention time of standard astragalin, this compound is confirmed to be astragalin.

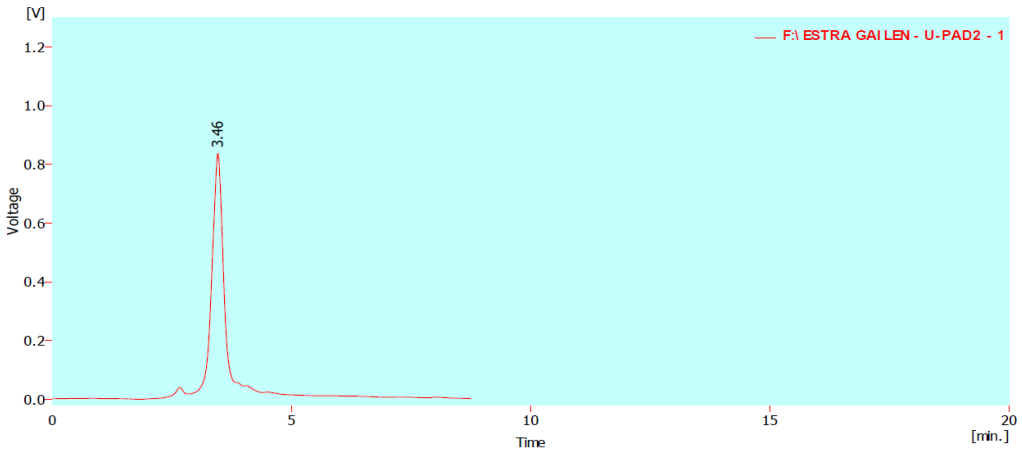

Figure (10): HPLC Chromatogram of Standard Astragalin

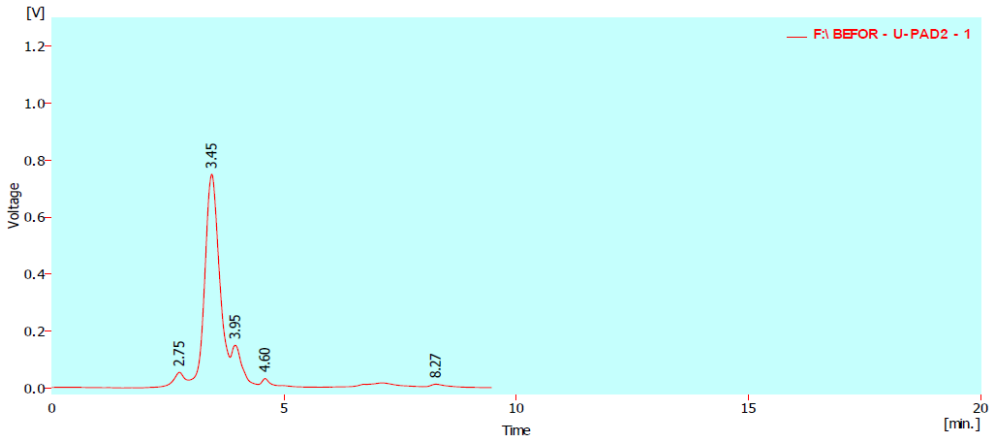

Figure (11): HPLC Chromatogram of Ethyl Acetate Extract of Aerial Part of *Cressa Cretica*

Table (5): Retention Time and Peak Areas of HPLC Analysis for Extract of *Cressa Critica* Aerial Parts.

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.752	190.041	23.088	3.4	5.1	0.14
2	3.448	4526.133	345.167	80.2	76.4	0.20
3	3.952	654.314	58.878	11.6	13.0	0.16
4	4.596	121.740	16.246	2.2	3.6	0.12
5	8.272	150.308	8.153	2.7	1.8	0.27
	Total	5642.536	451.531	100.0	100.0	

Discussion

As one of the most common causes of death worldwide, cancer takes almost 7 million lives each year.

Natural compounds isolated from medicinal plants are promising resources for the discovery of novel chemotherapeutic drugs.[12] Flavonoids are phenolic substances with potential antitumor function.[13] Such as astragalin which previously reported to exert highly cytotoxic effects on HepG2 (Hepatoma G2) cells.[14] Four novel findings may account for the apoptotic effect of Astragalin treatment against NSCLC (non-small cell lung cancer) cells: (1) activation of caspases; (2) alteration of the Bax:Bcl-2 ratio; (3) reduction of LPS- or TNFα-induced nuclear translocation of NF-κB; and (4) inhibition of MAPKs and PI3K/Akt pathway.[15] Astragalin treatment to control the growth of cancer cells might be a potential therapeutic

strategy. It may circumvent adverse side effects and drug resistance that frequently occurred in current cancer therapy.[16]

Interestingly, these results in comparison to previous studies showed similar findings were ethyl acetate fraction of Cressa cretica had a cytotoxic activity against carcinoma cells. Another significant aspect was that the plant also had flavonoid glycosides like kaempferol, quercetin, and rutin, which contribute to different biological activities. In general,

Kaempferol glycosides have been found to be a major component in Iraqi Cressa cretica and responsible for the cytotoxic activity.[17] Consequently, this can explain the results found by our study the highest percentage of cell growth inhibition was observed in ethyl-acetate extract approximately were three times more than ethanol extract which has been also reported by other studies.[18]

Conclusion

Cressa cretica possess significant cytotoxic effect on both human ovarian and breast carcinoma cells. Probably due to the presence of astragalin as a major component. These results suggest that Astragalin may a promising cancer therapeutic drug that warrants further investigation into its potential clinical application.

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