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# STUDY OF THE LIPID AND FLAVONOID CONTENTS OF ASTRAGALUS CREMOPHILOS BOISS. GROWING IN EGYPT

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## ABSTRACT

*From.pet.ether extract of Astragalus cremophilos Boiss. B-sitosterol and ceryl alcohol were isolated from the unsaponifiable fraction as well as fatty acids were studied by GLC. In addition, seven flavonoidal compounds were isolated: Kaempferol, quercetin, luteolin, luteolin-7-O-glucoside, kaempferol-3-O-glucoside (astragalin), apigenin-7-O-glucoside, quercetin-3-O-rhamnoside, (quercetrin) and rutin from the methanolic extract of the plant.*

## INTRODUCTION

Live stock poisoning due to the ingestion of various Astragalus species (Leguminpsae), has been known for many years[1].

Stermitz and others stated that the toxicity of Astragalus is due to the poisonous nitroaliphatic compounds that exist naturally as glucosides[2,3].

MacCracken et al[4] studied 12 Astragalus species for their cytot-toxicity. They found a relationship between

the cytotoxicity of the plants and their anti-tumor activity on mouse and human tumor. This is to a great extent in accordance with the early reported uses of different Astragalus plants in Chinese folkmedicine[5,6].

Revewing the current literature nothing was traced dealing with the study of Astragalus cremophilos Boiss. growing in Egypt, therefore,this part deals with the study of lipid and flavonoid content of the plant.

**Plant materials:**

The plant was collected in March 1981 from flowering plants growing wild, 50 km. East to Quena. Identity of the plant was kindly confirmed by Dr. A. Fayed , Assistant Prof. of Taxonomy, Faculty of Science , Assiut University. Samples collected were air-dried then reduced to fine powdered.

## EXPERIMENTAL

One kg. of the powdered herb was successively extracted with pet.ether (b.r.60-80°C) and methanol. Each extract was separately concentrated under reduced pressure and studied as follows:

**Study of Lipid content:**

10g. of the pet.ether extract were saponified[7] with 0.5 N ethanolic KOH. The unsaponifiable matter was extracted with ether and obtained after evaporation of the solvent. It was investigated by TLC using Benezene-ethyl acetate 4:1 v/v system and 50% $H_2SO_4$ as spraying reagent (after heating at 110°C for 5 minutes). Six spots were located.

The unsaponifiable fraction was chromatographed on aluminium oxide column, using(Benzene-ethyl acetate

gradient) as solvent system. Each fraction was separately concentrated under reduced pressure to small volume and subjected to TLC screening.

Two crystalline substances A & B were obtained.

**Substance A:**

The crystals melted at 136-137 C and showed no depression when mixed with authentic B-sitosterol. It gave a green colour with Liebermann-Burchard test.

**Substance B:**

Colourless crystals m.p. 80-81 $^{o}$C, dark-brown spot with antimony trichloride and sulphuric acid spray reagents. Having $hR_f$ 20 and 82 in benzene-pet.ether (3:1) v/v) and benzene-ethyl alcohol (9:1 v/v) respectively. Its acetate melted at 66-67 $^{o}$C,co-chromatography with authentic samples of ceryl alcohol was superimposable.

The substance B found to be ceryl alcohol through colour test, chromatographic behaviour and mixed m.p.

**Fatty acids:**

The mother liquor left after extraction of unsapcnifiable matter was acidified with dil.$H_2SO_4$ and extracted with ether to give the free fatty acids.

The methyl esters were prepared by using methanol and $H_2SO_4$ method[8]. The resulting methyl esters were analysed by GLC, adopting the following operating conditions.

Column temperature: 200$^{o}$C kept isothermal; Carrier gas: Nitrogen with flow rate of 40 ml/min.; Hydrogen flow rate: 40 ml/min.; Air flow rate 600-700 ml/min.; Attenuation: $20x10^{-2}$; Chart speed: 600-700 mm/hour; sample size:1 ul. of 2% of methyl ester in acetone; flame ionization detector.

Indentification of the fatty acids under investigation

was accomplished by comparing in each case the relative retention time of their methyl esters with those of pure available authentics.

The quantitative estimation of each fatty acids was carried out according to peak area measurement. The results obtained are compiled in Table 1.

**Study of the falvonoid content:**

The methanolic extract residue was triturated with water, the aqueous solution was exhausted with ether and ethyl acetate.

Chromatographic screening of the ether extract by TLC on silica gel G using chloroform-methanol (9:1v/v) as system revealed the presence of 5 flavonoidal spots.

Chromatographing the ether extract over silica gel column, eluted by chloroform and chloroform methanol in increasing polarities led to the isolation of quercetin, kaempferol and -luteolin as aglycones. Their structures were illustrated by physical, chemical, spectral, colour reactions in UV and co-chromatography using authentic samples.

The ethyl acetate soluble fraction also chromatographed using cellulose column, elution was started with chloroform then chloroform methanol gradient. Subsequent preparative PC was carried out using whatman No. 3 MM and 15% HOAc as solvent system. Five flavonoidal glycodides (As 1 to As 5) were isolated.

## RESULTS AND DISCUSSION

From the Table 1 it was found that linoleic, oleic, arachidic, palmetic, myrestic and lauric acids are present in plant under investigation.

**As 1: Luteolin-7-O-glucoside:**

Yellowish-green crystals, mp. 257°C, $hR_f$ 36 in n-butanol-acetic acid-water (4:1:5 v/v) system 1 and 29 in 15% HOAc sys-

tem 2. UV (MeOH) 260, 355 nm; + NaOMe 263, 300, 394 nm; + NaOAc 260, 365, 400 nm; + NaOAc/$H_3BO_3$ 260, 372 nm; + $AlCl_3$ 274,328,432 nm; + $AlCl_3$/HCl 273, 358, 387 nm.

**Acid Hydrolysis:**

10 mg. of the compound As 1 was refluxed with 50 ml. of 10% HCl for 3 hours. The aglycone was extracted with ether, while the sugar moiety in the hydrolysate was examined by PC using n-butanol-pyridine-water(6:3:4 v/v) as a system and sprayed with aniline hydrogen phthalate.

The compound was hydrolysed to luteolin and glucose as a sugar.

The above data confirmed that "As 1" is luteolin-7-O-glucoside[9].

**As 2: Kaempferol-3-O-glucoside (Astragalin):**

Yellow substance, brown colour in UV and yellowish-green after exposing to ammonia, mp. 195°C,$hR_f$ 27 and 63 in systems 1 and 2. UV (MeOH) 260,365 nm; + NaOAc 265, 406 nm; + NaOAc/$H_3BO_3$ 265,352 nm; + $AlCl_3$ 260,400 nm; + $AlCl_3$/HCl 270,398 nm;+ NaOMe 270,389 nm.

Acid hydrolysis afforded kaempferol aglycone and glucose. (astragalin)[9].

**As 3: Quercetin-3-O-rhamnoside (quercetrin):**

Pale yellow crystals mp. 180°C, yellowish-green colour in UV after exposing to ammonia, $hR_f$66 and 43 in both system 1 & 2 UV (MeOH) 260,360 nm; + NaOAc 270, 428 nm; + NaOAc/$H_3BO_3$ 260 , 386 nm; + $AlCl_3$ 260, 458 nm;+$AlCl_3$/HCl 270,426 nm;+NaOMe 291, 457 nm. Acid hydrolysis afforded quercetin aglycone and sugar rhamnose.

The compound "As 3" is quercetin-3-O-rhamnoside (quercetrin).

**As 4: Apigenin-7-O-glucoside:**

Yellowish-green crystals, $hR_f$59 and 25 in both systems 1 and 2. Purple colour in UV, green after exposing to ammonia

solution.

UV (MeOH) 267,332 nm; + NaOMe 267,302,386 nm;+$AlCl_3$ 276, 299,383 nm;+$AlCl_3$/HCl 276,300,384 nm;+NaOAc 267,357,385 nm;+ NaOAc/$H_3BO_3$ 267,335 nm.

Acid hydrolysis revealed apigenin aglycone and sugar glucose.

The compound "As 4" is apigenin-7-O-glycoside.

As 5: Quercetin-3-O-rhamnoglucoside (Rutin):

Pale yellow substance, mp. 190°C has $hR_f$ 15 and 58 in systems 1 and 2 brown colour in UV and yellowish-green after exposing to ammonia.

Acid hydrolysis revealed quercetin aglycone, glucose and rhamnose. Co-chromatography with authentic rutin was superimposable.

The compound " As 5" is quercetin-3-O-rhamnoglucoside (rutin).

From the above data it is concluded that the preliminary phytochemical study of the overground portion of Astragalus cremophilos Boiss. revealed the presence of free and combined flavonoids in the ether and ethyl acetate extracts. Column chromatography of the ether extract led to the isolation and identification of quercetin, luteolin and kaempferol, while fractionation of the ethyl acetate extract yielded five flavonoidal glycosides. By extensive physical, chemical, spectral analysis as well as acid hydrolysis and co-chromatography with authentic samples confirmed the isolation and identification of luteolin-7-O-glucoside , quercetin-3-O-rhmnoside(quercetrin),apigenin-7-O-glucoside, kaempferol-3-O-glucoside(astragalin) and quercetin-3-O-rhmnoglucoside (rutin).

Kaempferol-3-O-glucoside, quercetin and quercetin glucoside are widly distributed in Astragalus species, while luteolin-7-O-glucoside is very rare in Astragalus plants.

Table 1: Results of GLC analysis of the methyl esters of the fatty acids obtained from the Astragalus cremophilos boiss.

| *Peak No.* | *Rel.to Oleic* | *% of fatty acids* | *Authentic* |
|---|---|---|---|
| 1 | 0.11 | 2.4 | lauric |
| 2 | 0.22 | 6.2 | myrestic |
| 3 | 0.33 | 2.99 | -------- |
| 4 | 0.36 | 5.63 | -------- |
| 5 | 0.55 | 11.51 | palmetic |
| 6 | 0.66 | 4.69 | -------- |
| 7 | 0.66 | 4.46 | -------- |
| 8 | 0.78 | 2.63 | -------- |
| 9 | 0.91 | 9.97 | stearic |
| 10 | 1.00 | 15.51 | oleic |
| 11 | 1.16 | 20.31 | linoleic |
| 12 | 1.35 | 5.63 | -------- |
| 13 | 1.52 | 13.37 | arachidic |

rel.to:relative to oleic acid

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# دراسة المحتوى الفلافونيدى والدهنى
# لنبات الأستراجالس كريموفيلوس بويس
# الذى ينمو فى مصر

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نبات الأستراجالس من النباتات السامه للماشيه ، هذا بالأضافه الى تأثيره واستخداماته فى علاج السرطان .

وقد وجد الباحثون أن هذا النوع من النبات يحتوى على مواد نيتروجينيفاتيه ويرجع اليها تأثيره السام .

فى هذا البحث تمت دراسة المواد المتصبنه من خلاصة الايثير البترولى وذلك باستخدام كروماتوجرافيا العمود مستخدما أكسيد الألومنيوم وقد تم فصل مادة البيتاسيتوستيرول والكحول السيريلى ـ كما تمت دراسة الأحماض الدهنيه وذلك باستخدام كروماتوجرافيا الغاز وقد أمكن التعرف على حمض لوريك ميريستك ، بالميتيك ، استيريك ، أوليك ، لينوليك وأرشيدك .

أما الخلاصه الكحوليه فقد تم استخلاصها تعاقبيا باستخدام الايثير وخلات الأيثيل ـ وأمكن التعرف على الفلافونيدات الحره فى الأيثير وهى كوارستين ليوتيولين وكمبهفيرول .

أما الفلافونيدات التى تذوب فى خلات الايثيل فقد أمكن فصلها باستخدام كروماتوجرافيا العمود مستخدما مادة السليولوز وأمكن فصل والتعرف على خمسة فلافونيدات فى صورة جلوكوزيدات وذلك بدراسة خواصها الفيزيائية تحت الأشعة الفوق بنفسجيه وكواشف الألوان والحلماه الحمضيه . كذلك أمكن دراسة الكواشف الأيونية وتأثيرها على هذه المركبات . واتضح من هذه الدراسه أن هذه المركبات هى : ٧ـ جلوكوزيد ابجنين ـ٧ـأ جلوكوزيد ، كوارستين ـ٣ـأ ـ رامنوزيد ( كوارستيرين ) .

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