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PHARMACOGNOSTICAL STUDY OF GYNANDROPSIS PENTAPYHLLA (HURHUR) GROWING IN EGYPT

Part III: Lipids and Flavonoids of the Leaves.

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ABSTRACT

From the air-dried powdered leaves of Gynandropsis pentaphylla (Hurhur), we isolated and identified centaureidin (5,7,3 trihydroxy, 3,6,4 trimethoxy flavone), Kaempferol, Kaempferol-3-O-diglucoside, quercitrin, myricitrin, ∝- and B-amyrin, taraxasterol and B-sitosterol.

The fatty acids capric, lauric, myrestic, palmitic, palmitoleic, stearic, oleic and linoleic were detected and estimated.

INTRODUCTION

Gynandropsis pentaphylla (Hurhur) (Capparidaceae) is an annual herb which grows as a weed in Egypt[1,2] and tropical countries[3]. The plant has a wide reputation as a valuable remedy for various diseases[4-6].

Reviewing the current literature, nothing could be traced dealing with the study of Gynandropsis pentaphylla (Hurhur) constituents other than the isothiocynate-producing glycosides and the seed fat[7]. Therefore it was considered of interest to undertake a detailed chemical investigation of this plant. Previously, the authors described the macro-and micromorphology of the different organs of the herb[8,9].

EXPERIMENTAL

General Experimental Procedures:

Melting points were determined on a koffler hot plate and were uncorrected. All UV-spectra were in methanol using Bausch and Lomb spectronic 2000 and IR spectra were in KBr using Perkin-Elmer 467 spectrophotometer. GLC were conducted on a Perkin-Elmer FII gas chromatograph with flame ionization detector. Mass spectral measurements were at 70 ev using 3V micromass zab IF, ^{1}H-and ^{13}C-NMR using jeol EX90Q. Column chromatography was performed on silica gel (Merck) and polyamide. Silica gel G.F.254, (Merck) was used for TLC and Whatmann paper for PC. Acetylation was done by acetic anhydride/pyridine method[10].

Plant Material:

The leaves of Gynandropsis pentaphylla (Hurhur) were collected in October 1982, from plants growing in the Experimental Station of Medicinal Plants, Faculty of Pharmacy, Assiut University. Identification of the plant was confirmed by late Prof. Dr. Foad Y. Amin. Prof. of Floriculture, Faculty of Agriculture, Assiut University.

Extraction and Isolation:

The air-dried powdered leaves (2 kg) were extracted by percolation with ethyl alcohol 80%. The extract was concentrated under reduced pressure to syrupy consistency and successively shaken with pet.ether (60-80°C), followed by chloroform. Each extract was subjected to chromatographic examination and studied separately.

Pharmacognostical Study of Gynandropsis Pentapyhlla (Hurhur) Growing in Egypt Part III: Lipids and Flavonoids of the Leaves.

Chromatographic Systems:

Systems for TLC:

Adsorbent: silica gel G;solvent system I: pet. ether-ethyl acetate (9:1)

Systems for PC:

Whatmann 3MM; Solvent system II:t.BuOH-HOAc-H_2O (3:1:1); system III:n.BuOH-HOAc-H_2O(4:1:5).

A- Petroleum-Ether Extract (Fraction I):

The pet. ether extract (30 g.) was saponified[11] to allow the separation of saponifiable and unsaponifiable fractions. The fatty acids, remaining in the mother liquor after extraction of the unsapoifiable matter were esterified to their methyl esters[12] and analysed by gas-liquid chromatography.

The following operating contitions were adopted column,5 feet lomg,4 mm i.d., packed with 10% polypropylene glycol adepate on 80-100 mesh chromosorb W; column temperature, 200°C; carrier gas, nitrogen at a flow rate of 60 ml/min; hydrogen flow rate, 40 ml/min; air flow rate 500 ml/min; sample size, 1µl of 15% solution of the methyl ester in ether.

The unsaponifiable fraction (5 g.) was fractionated over silica gel column (180 g., 120 x 5 cm) using solvents in order of increasing polarities starting with pet.ether, then pet.ether-ethyl acetate. Fractions (500 ml each) were collected and subjected to TLC study using system I (TLC). Four compounds were isolated and designated 1-4.

B- Chloroform Extract (Fraction II):

The residue from the chloroform fraction (9 g.) was examined on PC using Whatmann 3MM with system III. Two flavonoidal components were detected and isolated by preparative PC adopting the same solvent system. The isolated compounds were designated 5 and 6 (Table 1).

Compound 5:

MS showed M^+ at m/z 360 (100) and characteristic peaks at 345 (60) $(M-CH_3)^+$, 182(15), 151(15), 148(10). ^{1}H-NMR (as TMS ether in CCL_4, TMS) showed δ:7.89 (1H,dd,J=2.5, 9Hz, H-6`), 7.61 (1H, d, J=2.5 Hz, H-2`), 7.04 (1H,d, J=9Hz, H-5`), 6.51 (1H, S, H-8), 3.81, 3.89, 3.93 (3 S, 3OMe).

C- [illegible]queous Layer (Fraction III):

The aqueous layer (remained after fractionation of the concentrated alcoholic extract), was evaporated under reduced pressure (5 g.). The residue was chromatographed over polyamide column (400 g. 140 x 6 cm) using methanol-water (1:1). Fractions (200 ml) each were collected and examined by TLC and P.C. three pure flavonoidal compounds were isolated and labelled 7-9.

Acid Hydrolysis:

Each of the isolated glycosides was separatly dissolved in N/2 H_2SO_4 mixed with an equal volume of ethanol and refluxed for 2 hours. The agylcones were extracted with ether, purified and subjected to TLC and spectral studies. gars were identified from their gas-liquid chromatograms after trimethylsilation[13]. GLC analysis was performed, using 3% OV-1 on silanized chromosorb column (column temperature; 160°C; injection port and detector temperature 260-300°C; helium flow rate 30 ml/min). Identification of the sugars was performed using standard sugars (Sigma Chem. CO.) analysed under the same conditions.

Mild Acid Hydrolysis:

Ten mg. of each glycoside were separately dissolved in N/10 H_2SO_4 (10 ml), mixed with an equal volume of ethanol and refluxed for 2 hours. A sample of the hydrolysate was withdrawn every 5 minutes during the first 20 minutes, then every 10 minutes during the remaining period and spotted on PC (3MM) using 15% glacial acetic acid as solvent system.

Compound 7:

^1H-NMR (as TMS ether in $CHCl_3$, TMS) δ : 8.03(2H,d,H-2', H-6', J= 9Hz), (2H,d, H-3', H-5', J=9Hz), 6.51 (1H,d, H-8, J=2Hz), 6.31(1H,d, H-6, J=2Hz), 5.98 (1H, d, H-1'', J= 8Hz), 5.11 (1H,d, H-1''',J= 7Hz), 3.85 (12H,m,diglucosyl.

^{13}C-NMR (DMSO-d_6) δ: 156.3 (C-2), 132.4 (C-3), 177.1 (C-4), 161.[illegible] (C-5), 99.45 (C-6), 163.9(C-7), 94.31(C-8), 156,3(C-9), 103.92(C-10), 121.1(C-1'), 130.91(C-2'), 115.1(C-3'), 159.5(C-4'), 115.1(C-5'), 130.79(C-6'), 99.9[illegible]-1''), 81.7(C-2''), 75.12(C-3''), 69.93(C-4''), 75.68(C-5''), 61.54(C-6''), 103.1 C-1'''), 74.2(C-2'''), 75.37(C-3'''), 70.18(C-4'''), 76.1(C-5'''), 61.98(C-6''').

Pharmacognostical Study of Gynandropsis Pentapyhlla (Hurhur) Growing in Egypt Part III: Lipids and Flavonoids of the leaves.

RESULTS AND DISCUSSION

The air dried powdered leaves of Gynandropsis pentaphylla (Hurhur) were extracted with ethanol and the concentrated extract was fractionated using petroleum ether (Fraction I), chloroform (Fraction II) and the left aqueous residue (Fraction III). Each of the three fractions was subjected to chromatographic studies

By chromatographing the unsaponifiable matter of (Fraction I) over silica column, four compounds were isolated.

On the basis of co-chromatography, mixed m.p., physical properties, chemical tests, acetate formation and comparison of IR spectra, compounds 1-4 were found to be B-amyrin, α-amyrin, taraxasterol and B-sitosterol respectively.

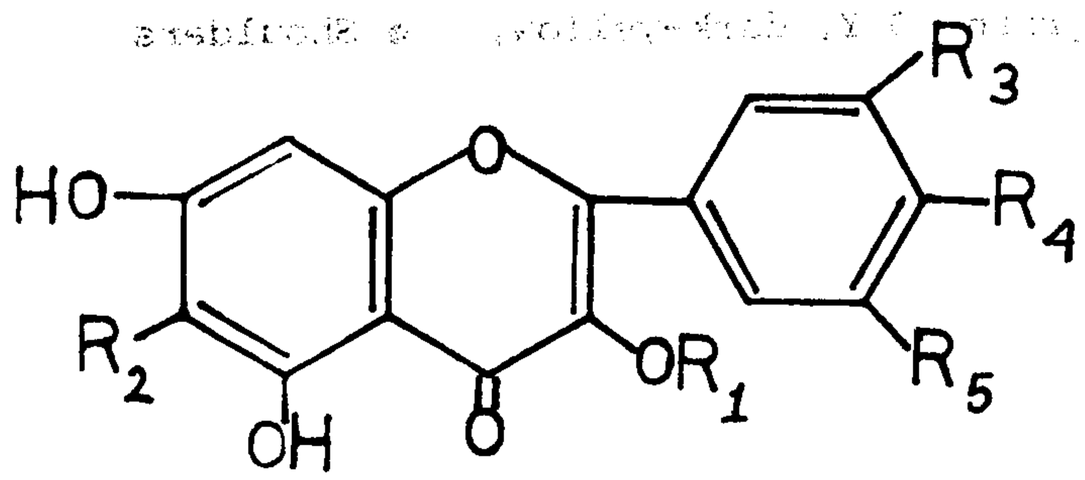
The fatty acids methyl esters were analyzed by gas-liquid chromatography. Their identification was carried out by direct comparison of the retention times of standard methyl esters (Sigma Chem) with those of unkown esters under identical conditions. The quantitative estimation was based on the internal normalization method using the peak area measurements. The following fatty acids were detected and estimated: capric (2.86%), lauric (6.46%), myrestic (8.96%), palmitic (33.93%) Palmitoleic (14.86%), stearic (2.44%) oleic (15.87%) and linoleic (12.48%).

Chromatographic examination of the chloroform extract (Fraction II), revealed the presence of two flavonoidal aglycones[14] (designated 5&6) and they were isolated and purified by preparative PC. Their physical and chromatographic characters in addition to spectral data (Table 1), compare favourably with those published for centaureidin[15] and kaempferol[16] respectively.

From the aqueous residue left after fractionation of the alcoholic extract (Fraction III), three compounds (7-9) were isolated after chromatography over polyamide column.

Compounds 7-9 gave positive tests of flavonoids[14]. By mild acid hydrolysis, compound 7 only, hydrolyzed on two steps, indicating its biside nature which was confirmed by the appearance of a signal at δ 3.85 in the ^{1}H-NMR spectrum, for 2 glucose molecules attached to one another and also gave evidence that the glycoside is attached in the C-3 position[16]. The ^{13}C-NMR spectrum of compound 7 confirmed that the sugars are attached to one another in the C-2'' position[17].

Acid hydrolysis of the isolated compounds (7-9), gave aglycones which were proved by m.p., co-chromatography and UV data to be kaempferol, quercetin and myricetin respectively. The sugar moieties were identified by GLC to be, glucose for compound 7, and rhamnose for compounds 8 and 9 respectively. Comparing the UV-spectral data (Table 2) with different complexing and ionizing agents for both the intact glycosides and their corresponding aglycones, it was proven that the sugars are attached to C-3 in the three compounds. Therefore these compounds were proved to be kaempferol-3-O-diglucoside, quercitrin and myricitrin respectively.



1- R_1=Me, R_2=R_4=OMe, R_3=OH, R_5=H (Centaureidin).

2- R_1=glucoglucosyl (1-2), R_2=R_3=R_5=H, R_4=OH (Kaempferol-3-O-diglucoside)

3- R_1=rhamnose, R_2=R_5=H, R_3=R_4=OH (Quercitrin).

4- R_1=rhamnose, R_2=H, R_3=R_4=R_5=OH (Myricitrin).

Pharmacognostical Study of Gynandropsis Pentaphlla (Hurhur) Growing in Egypt Part III: Lipids and Flavonoids of the Leaves.

Table 1: Chromatographic Properties and UV data for the Flavonoids 5 and 6 Isolated from the Chloroform Extract (Fraction II):

Compound	Amounts isolated (mg)	h_{R_f} in systems* colour				M.P.°C.	UV λ_{max} in (nm)					
		I	II	UV	Alcl UV3		MeOH	NaOMe	$AlCl_3$	$AlCl_3$/Hcl	NaOAC	NaOAc /H_3BO_3
5	25	78	87	P.	D.Y.	204-206	253, 269, 269, 347	269, 303•, 392	304• 378	265, 305•, 366	269, 372,	255, 266• 354
6	70	53	67	Y.G	Y.G	278-281, 320•, 367 (decomp)	252 266, 316	278, 268, 416	260 350, 420	258• 270, 348, 425	274, 300, 387,	267, 300• 320• 370

* h_{R_f} on paper chromatogragpy. syst I: t.BuOH-HOAc-H_2O (3:1:1) Syst II n. BuOH-HOAc-H_2O (4:1:5). Y.G.: yellowish-green, D.Y. dark-yellow, • Shoulders.

P. : pink.

Table 2: Physico-Chemical Characters as well as Spectral Data of Compounds 7-9(Fraction III)

Characters	Compounds 7	Compound 8	Compound 9
M.P.	147-149°C	178-180°C	197-199°C
h_{R_f}*	51	42	39
Mild acid hydrolysis	Two steps	One step	One step
Acid hydrolysis	Kaempterol+glucose	Quercetin + rhamnose	Myricetin+rhamnose
UV-data, nm.			
MeOH	269,337	256,265 sh, 305 sh,350	250, 270 sh,300 sh, 350.
NaOMe	279,323,399	272,322,393(decomp)	258sh,280sh,322, 423(decomp)
$AlCl_3$	273,297sh,348,415.	275,300sh,330,425	265,310sh,430
$AlCl_3$/HCl	275,338,392	270,300sh,350,400	266,270sh,300sh,415
NaOAc	276,326,374	270,320sh,370	265,325 (decomp)
NaOAc/H_3BO_3	268,322,356	260,300sh,362	253,300sh,385
Identification	Kaempferol-3-O-diglucoside	Quercetin-3-O-rhamnoside	Myricetin-3-O-rhamnoside

* h_{R_f}, P.C., Whatmann No 3, system n-BuOH-HOAc-H_2O(4:1:5)

Pharmacognostical Study of Gynandropsis Pentapyhlla (Hurhur) Growing in Egypt
Part III: Lipids and Flavonoids of the Leaves.

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دراسة عقاقيرية لنبات الجيناندروبس بنتافيلا (هرهر) المنزرع فى مصر
الجزء الثالث : الدهون والفلافونويدات فى الاوراق

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جورجيا ـ الولايات المتحدة الامريكية

لقد قام الباحثون باستخلاص أوراق النبات بالكحول الايثيلى ٨٠٪ وبعد تركيز هذة الخلاصة ثم تجزئتها بالمذيبات التالية : البترول الايثيرى والكلوروفورم وتم تركيز الجزء المائى المتبقى بعد التجزئه لدراسته .

لقد أمكن فصل أربعة مركبات من الجزء الغير متصبن لخلاصة البترول الاثيرى وتم التعرف عليها بالصفات الطبيعية والطيفية وهى كالتالى: الفا أميرين ـ بيتا أميرين وتركساستيرول وبيتاسيتوستيرول ... وتم أيضا التعرف على وجود ثمانية أحماض دهنية باستخدام كروماتوجرافيا الغاز وهى كابريك ـ لاوريك ـ ميرستيك-بالمانيك بالميتوليك ـ ستياريك ـ أوليك ولينوليك .

وبدراسة خلاصة الكلوروفورم أمكن فصل والتعرف على فلافونويدات حرة وهى السنتوردين والكامبيفيرول وذلك باستعمال الطرق الطيفية المختلفة (فوق البنفسجية ومطياف الكتلة والرنين النووى المغناطيسى) .

ومن المركز المائى (المتبقى بعد التجزئة بالمذيبات المختلفة) تم فصل ودراسة ثلاثة جلوكوزيدات فلافونية بالطرق الطبيعية والطيفية المختلفة ،ووجد أنها كامبيفيرول ـ ٣ ـ أ ـ ثنائى الجلوكوز وكورسترين وميرسترين .

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