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FLAVONOIDS FROM *ASTERISCUS PYGMAEUS* (DC.) Coss & DR GROWN IN EGYPT

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تم فى هذا البحث فصل والتعرف على ثمانية مركبات فلافونيدية وهى ابجينين ، ٧-مثيل لوتيولين ، لوتيولين ، ٣-مثيل كوارستين ، كوارستين ، ابجينين ٧-أ-جلوكوزيد ، لوتيولين ٧-أ-جلوكوزيد ، لوتيولين ٤،٧ ثنائى الجلوكوزيد.

وقد تم التعرف على التركيب الكيميائى لهذه المركبات بإستخدام الطرق الطبيعية والكيميائية والكروماتوجرافية وكذا بإستخدام الأشعة فوق البنفسجية والرنين النووى الهيدروجينى ومقارنة هذه المركبات بعينات أصلية.

From the chloroformic fraction of the ethanolic extract of the aerial parts of Asteriscus pygmaeus (DC.) Coss & DR (Syn. Odontospermum Pygmaeum) five flavonoids were isolated and identified as apigenin, luteolin-7-O-methylether, luteolin, quercetin-3-O-methylether and quercetin. Apigenin 7-O-glucoside, luteolin 7-O-glucoside and luteolin 7,4'-di-O-glucoside were isolated from the ethyl acetate and butanol fractions. Their structures were established by physical, chemical and spectral methods.

INTRODUCTION

The genus *Asteriscus* (Family *Compositae*, tribe *Inuleae*, subtribe *Inulinae*) is a small genus common in the Mediterranean area. It is represented in Egypt with only three species[1]. The genus is placed in the Inula group close to the genera *pallenis*, *Bubonium* and *Rhanterium*[2]. The plants of the genus *Asteriscus* are known to produce compounds with potential antitumor, antispasmodic, anti-inflammatory activity and for treatment of infected wounds[3,4]

Reviewing the current literature, sesquiterpenes and flavonoids have been isolated from the genus *Asteriscus*[5]. *A. aquaticus* L. gave unsaturated sesquiterpene lactones; asteriscunolides A, B, C, D and asteriscanolide[5,6] while *A. sericeus* afforded humulenes and nerolidol derivatives[7]. Farnesol and thymol derivatives were isolated from *A. pygmaeus*[8]. Humulene derivatives[9] ,flavonoids[10] , volatile oils[11] , acetylenics[12] and bisabolone hydroperoxides[3] were isolated from *A. graveolens*. Since nothing have been reported on the flavonoidal content of *A. pygmaeus*, therefore it was thought to be interesting to study the flavonoidal content of this Egyptian plant.

Plant material

The plant material was collected in April 1991 near El-Arish, Sinai. It was identified and authenticated by Prof. Dr. A. Fayed, Professor of Plant Taxonomy, Dept. of Botany, Faculty of Science, Assiut University. The aerial parts were air-dried, powdered and kept in well closed dark container till used.

General experimental procedure

MPS were uncorrected and determined with Koffler hot stage microscope. UV spectra were recorded using Unicam 1750 spectrophotometer with Pye-Unicam AR 55 linear recorder. ^{1}H-NMR spectra were run at 400 MHz using Bruker AM 400 spectrometer in DMSO using TMS as internal standard. For column chromatography silica gel G (E. Merck) was used. Silica gel 60 F_{254} (E. Merck) was used for TLC. PC was performed using Whatmann paper No. 3 and No. 1. The chromatograms were air-

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dried and examined under UV light before and after spraying with 5% methanolic $AlCl_3$ in case of PC and 10% H_2SO_4 in case of TLC.

Extraction and isolation

The air dried powdered aerial parts (2 kg) were extracted with 75% ethanol by maceration and percolation (5Lx3). The ethanolic extract was concentrated under reduced pressure to syrupy consistency, mixed with water and successively extracted with pet. ether (2Lx4, fraction A, 24 g), chloroform (2Lx4, fraction B, 19 g), ethyl acetate (1.5Lx4, fraction C, 14 g) and n-butanol (1.5Lx4, fraction D, 32 g). Each fraction was subjected to chromatographic examination for its flavonoidal contents. Fractions C and D showed the same spots on TLC and PC, so they were collected together and given the symbol E. 10 g of fraction B were subjected to silica gel column chromatography (400 g). Elution was performed with hexane increasing the polarity with ethyl acetate gradiently, fractions 100 ml each were collected, similar fractions on TLC were pooled together and subjected to crystallization and/or preparative TLC (PTLC) for isolation of its constituents.

Fractions 28-36 eluted with hexane-ethyl acetate (1:1), by preparative PC (PPC, system III) it afforded two compounds symboled B-1 (R_f=0.47, TLC, system Ia) and B-2 (R_f=0.50, TLC, system Ia).

Fractions 44-52 eluted with hexane-ethyl acetate (2:3). By preparative TLC (PTLC), (system Ib) afforded B-3 (R_f=0.32, TLC, system Ia) and B-1.

Fractions 55-59 eluted with hexane- ethyl acetate (3:7) it gave yellow powder on repeated purification from methanol afforded compound B-4 (R_f=0.38, TLC, system Ib).

Fractions 64-77 eluted with hexane-ethyl acetate (1:4 & 1:9) purified by repeated crystallization from methanol to gave compound B-5 (R_f=0.32, TLC, system Ib).

8 g of fraction E were chromatographed over silica gel column chromatography (350 g). Elution was performed with chloroform increasing the polarity with methanol gradiently, fractions 100 ml each were collected and subjected to TLC and PC; similar fractions were mixed together and subjected to PTLC and/or PPC where three compounds were isolated and symboled E-1 ~ E-3.

Fractions 28-33 eluted with $CHCl_3$-MeOH (17:3), by PTLC, it afforded compound E-1 (R_f= 0.36, TLC, system II).

Fractions 41-47 eluted with $CHCl_3$-MeOH (8:2) showed the presence of one major and one minor spots on TLC (system II). By PTLC, it afforded compound E-2 (R_f= 0.32, TLC, system II).

Fractions 66-74 eluted with $CHCl_3$-MeOH (7:3) showed the presence of one major flavonoid spot. By PPC (system IV), it afforded compound E-3 (R_f= 0.18, TLC, system II).

Compound B-1: Obtained as yellow needles (46 mg), m.p. 345-348°C (methanol), undepressed when melted with authentic apigenin. It is soluble in ether, chloroform and methanol but insoluble in water. It has dark brown colour under UV (366 nm) changed to yellow fluorescence after spraying with $AlCl_3$. It gave positive tests for flavonoids.

Compound B-2: Obtained as yellow needles (28 mg), m.p. 310-311°C (methanol). It has the same characters as B-1.

Compound B-3: Obtained as yellow needles (54 mg), m.p. 330-332°C (methanol), undepressed when melted with authentic luteolin. It has the same characters as B-1.

Compound B-4: Obtained as yellow amorphous powder (34 mg), m.p. 213°C (methanol). It has the same characters as B-1 under UV.

Compound B-5: Obtained as yellow needles (62 mg), m.p. 313-315°C (methanol), undepressed when melted with authentic quercetin. It has yellow fluorescence under UV intensified after spraying with $AlCl_3$.

Compound E-1: Obtained as faint yellow needles (48 mg), m.p. 228-230 C (methanol). It is soluble in butanol, pyridine, ethanol and methanol but insoluble in chloroform and ether. It has dark brown spot under UV light (366 nm) changed to yellow fluorescence after spraying with $AlCl_3$.

Compound E-2: Obtained as yellow crystals (38 mg), m.p. 262-265°C (methanol). It give dark brown spot under UV light changes to yellow fluorescence after spraying with $AlCl_3$. It has the same solubility as compound E-1.

Compound E-3: Obtained as yellow amorphous powder (52 mg), m.p. 193-195°C (dec.), (methanol). It has the same characters as compounds E-1 and E-2.

The following solvent systems were used:

I) Chloroform-methanol	a) 9:1 b) 85:15
II) Chloroform-methanol-water	70:27:3
III) n-butanol-acetic acid-water	4:1:2
IV) Acetic acid	15%

Partial acid hydrolysis

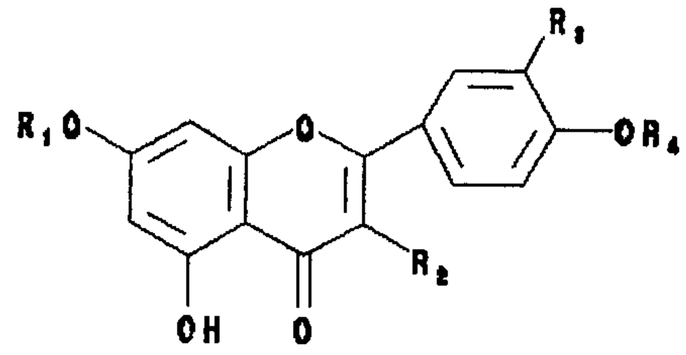
About 5 mg of each glycoside were dissolved in 5 ml methanol to which 10 ml of 2% aqueous HCl were added and the mixture was refluxed on boiling water bath for about 2 hours. A sample of hydrolysate was withdrawn with capillary glass tube every five minutes within the two hours. The samples taken were spotted on Whatmann No. 1 filter paper and the chromatogram was developed with 15% HOAc and visualised under UV light at 366 nm using ammonia vapour and 5% alcoholic $AlCl_3$ solution.

Complete acid hydrolysis

About 15 mg of each glycoside were dissolved in 10 ml methanol to which an equal volume of 10% sulphuric acid was added. The mixture was refluxed on water bath for 3 hours. The aglycone was taken with ether while the acidic mother liquor containing the sugar moiety was neutralized and used for identification of the sugar.

RESULTS AND DISCUSSION

From the aerial parts of *A. pygmaeus* (DC.) Coss & DR five flavonoid aglycones were isolated from the chloroform fraction of the alcoholic extract while three flavonoidal glycosides were isolated from the polar fraction (ethyl acetate and butanol fractions). The identification of these compounds depends on the study of physical, chemical, chromatographic as well as the spectroscopic data (UV and ^{1}H-NMR) of these compounds.



Compound	R_1	R_2	R_3	R_4
B-1	H	H	H	H
B-2	CH_3	H	OH	H
B-3	H	H	OH	H
B-4	H	OCH_3	OH	H
B-5	H	OH	OH	H
E-1	glu	H	H	H
E-2	glu	H	OH	H
E-3	glu	H	OH	glu

Fig. 1: List of the isolated compounds.

Compounds B-1, B-3, B-5 were identified as apigenin, luteolin and quercetin by the study of their physical, chemical, UV spectral data with different ionizing and complexing agents[13-16] (Table 1) and ^{1}H-NMR (Table 2) and by comparing their physical data (mp, mmp and solubility in organic solvents) and co-chromatography with authentic samples.

Compound B-2, yellow crystals, soluble in chloroform, ethyl acetate, acetone and ethanol but insoluble in water. Its UV data (Table 1) indicate that it is a flavone having ortho-dihydroxy group in ring B-, free OH at C-5 and blocked or absent -OH group at C-7[16]. ^{1}H-NMR (Table 2) showed a chelated OH at δ 13.08 for hydroxyl at C-5 and methoxyl group at δ 3.74 deduced to be at C-7 from the UV spectrum with NaOAc. Thus compound 2 was identified as luteolin 7-O-methylether.

Compound B-4, obtained as yellow powder, soluble in chloroform, ether, ethyl acetate and methanol but insoluble in water. Its UV spectral data[16] (Table 1) suggested that it is a flavone having free hydroxyl groups at C-5, C-7 and having ortho-dihydroxy groups in ring B. The ^{1}H-NMR data (Table 2) showed the presence of a methoxy group (δ 3.78). The absence of the

Table 1: UV spectral data of the Isolated compounds.

Comp. / Reagent		MeOH	+$AlCl_3$	+$AlCl_3$ +HCl	+NaOAc	+NaOAc +H_3BO_3	+NaOMe
B-1	I	338 297	384 347 301	382 342 299	376 304	340 304	394 325
	II	266	276	277	274	268	275
B-2	I	338	400	374	364	364	400
	II	256 264	272	268	256 263	256 265	272
B-3	I	339 296	425 318 300	376 335 294	365 315	420 374 301	400 329
	II	252 268	274	275 266	277	262	272
B-4	I	352	426	396 362	385	380	424
	II	257	270	265	270	260	270
B-5	I	371	438 363 303	391 360 304	396 328	392 328	412 dec.
	II	256 269	272	266	273	261	255 273
E-1	I	338 282	382 302 298	381 347 298	388 352	335	396 303
	II	265	277	278	266	267	270
E-2	I	346	428	390 366	402	372	398
	II	256 265	274	272	260	261	265
E-3	I	336	380 300	382 344 302	386 339	340	368 335
	II	255 265	274	273	266	266	258

Table 2: 400 MHz ^{1}H-NMR of the isolated compounds in DMSO-d_6

H-NO. Comp.	B-1	B-2	B-3	B-4	B-5	E-1	E-2	E-3
3	6.34	6.42	6.41	-	-	6.45	6.40	6.38
6	6.28	6.21	6.23	6.24	6.48	6.57	6.53	6.49
8	6.48	6.46	6.39	6.40	6.68	6.62	6.61	6.60
2'	7.78	7.72	7.45	7.58	7.83	7.89	7.68	7.44
3'	6.86	-	-	-	-	6.92	-	-
5'	6.86	7.04	7.12	6.81	7.18	6.92	7.14	6.88
6'	7.78	7.68	7.39	7.73	7.80	7.89	7.46	7.86
OCH_3	-	3.84	-	3.78	-	-	-	-
1"	-	-	-	-	-	5.02	5.08	4.92
1"'	-	-	-	-	-	-	-	5.12

signal around 6.3 ppm indicate the C-3 substituted flavonol; so compound B-4 was identified as quercetin 3-O-methylether.

Compounds E-1 ~ E-3: The physical, chemical and chromatographic characters of these compounds proved their flavonoidal glycosides nature[13-17].

Compound E-1: The UV analysis data (Table 1) with methanol and with different complexing and ionizing reagents[16] gave an evidence for the presence of free hydroxy group in the 5 and 4' positions indicating 7-O-glycosyl apigenin derivative. ^{1}H-NMR (DMSO) showed an apigenin pattern in addition to sugar protons. The anomeric sugar proton appear at δ 5.02 (1H,d, J= 7.6 Hz) for the ß-sugar[18]. Mild acid hydrolysis resulted in complete hydrolysis in one step. Complete acid hydrolysis afforded glucose as a sugar moiety (identified by TLC and PC using authentic sugars and systems II & III) and the aglycone was identified as apigenin (UV data and co-chromatography with authentic sample). Form all the above data, compound E-1 was identified as apigenin 7-O-ß-D-glucopyranoside.

Compound E-2: The UV data with different ionizing and complexing reagents indicate that it is a flavone having free hydroxyl groups at 5, 3' and 4' positions indicating 7-O-glycosyl luteolin. ^{1}H-NMR (DMSO) showed luteolin pattern in addition to seven protons for hexose. The anomeric sugar proton appear at δ 5.08 (1H,d, J= 7.7 Hz) for ß-sugar[18]. Mild acid hydrolysis resulted in complete hydrolysis in one step while complete acid hydrolysis afforded sugar identified as glucose (by TLC and PC using authentic sugars and systems II & III) and the aglycone was identified as luteolin (UV and co-chromatography using authentic sample). From all the above data, it is clear that compound E-2 is luteolin 7-O-ß-D-glucopyranoside.

Compound E-3: obtained as amorphous powder, soluble in methanol, ethanol, and pyridine. UV data (Table 1) exhibited that it is a flavone having free OH group at 5 and 3' positions. ^{1}H-NMR (Table 2) showed the presence of two anomeric sugar protons at δ 4.92 (J=7.4 Hz) and δ 5.12 (J= 7.5 Hz), the other sugar protons appeared between δ 3.2-4.8 (12 H, m). The signals of the aglycone were cited in Table 2. Acid hydrolysis yielded luteolin and sugar identified as glucose (PC, system III), this indicate that the sugar moiety attached to both positions 7 and 4'- is glucose. Controlled acid hydrolysis followed by fractionating the product of the hydrolysate by preparative PC; luteolin 7-O-glucoside and glucose were identified, this mean that the sugar part present in both position 7- and 4'- is glucose. The appearance of C-2' proton more downfield than C-6' proton indicate that the sugar molecule attached to C-4' not to C-3'[10]. Therefore this compound is a diglucoside and its structure is 5, 3' dihydroxy 7, 4'-diglucoside flavone (luteolin 7,4'-di-O-glucoside).

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