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

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From the chloroformic fraction of the ethanolic extract of the aerial parts of Asteriscus pygmaeus (DC.) Coss & DR (Syn. Odontospernum Pygmaeum) five flavonoids were isolated and identified as apigenin, luteolin-7-O-methyl ether, luteolin, quercetin-3-O-methyl ether 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 species¹. The genus is placed in the Inula group close to the genera pallenis, Bubonium and Rantherium². The plants of the genus Asteriscus are known to produce compounds with potential antitumor, antispasmodic, anti-inflammatory activity and for treatment of infected wounds³.⁴

Reviewing the current literature, sesquiterpenes and flavonoids have been isolated from the genus Asteriscus⁵. A. aquaticus L. gave unsaturated sesquiterpene lactones; asteriscunolides A, B, C, D and asteriscanolide⁵,⁶ while A. sericeus afforded humulenes and nerolidol derivatives⁷. Farnesol and thymol derivatives were isolated from A. pygmaeus⁸. Humulene derivatives⁹, flavonoids⁵, volatile oils¹⁰, acetylenics¹¹ and bisabolone hydroperoxides¹² 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. ¹H-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 F₅₂₄ (E. Merck) was used for TLC. PC was performed using Whatmann paper No. 3 and No. 1. The chromatograms were air-
dried and examined under UV light before and after spraying with 5% methanolic AlCl₃ in case of PC and 10% H₂SO₄ 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 gradually, 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 (Rₛ = 0.47, TLC, system Ia) and B-2 (Rₛ = 0.50, TLC, system Ia).

Fractions 44-52 eluted with hexane-ethyl acetate (2:3). By preparative TLC (PTLC), (system Ib) afforded B-3 (Rₛ = 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 (Rₛ = 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 (Rₛ = 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 CHCl₃-MeOH (17:3), by PTLC, it afforded compound E-1 (Rₛ = 0.36, TLC, system II).

Fractions 41-47 eluted with CHCl₃-MeOH (8:2) showed the presence of one major and one minor spots on TLC (system II). By PTLC, it afforded compound E-2 (Rₛ = 0.32, TLC, system II).

Fractions 66-74 eluted with CHCl₃-MeOH (7:3) showed the presence of one major flavonoid spot. By PPC (system IV), it afforded compound E-3 (Rₛ = 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 AlCl₃. 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 AlCl₃.

**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 AlCl₃.
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 AlCl₃. 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 AlCl₃,
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 ¹H-
NMR) of these compounds.

![Fig. 1: List of the isolated compounds.](image)

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 agents¹³-¹⁶
(Table 1) and ¹H-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-7¹⁶. ¹H-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
data¹⁸ (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
¹H-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.

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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 anumeric sugar proton appear at $\delta$ 5.02 (1H,d, J = 7.6 Hz) for the $\beta$-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-$\beta$-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 anumeric sugar protons at $\delta$ 4.92 (J = 7.4 Hz) and $\delta$ 5.12 (J = 7.5 Hz), the other sugar protons appeared between $\delta$ 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).
REFERENCES


