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SHORT COMMUNICATION

FLAVONOID AGLYCONES AND PHYTOSTEROLS FROM THE *ERIGERON ACRIS* L. HERB

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Abstract: Four flavonoid aglycones (apigenin, kaempferol, luteolin, quercetin) were isolated from methanolic extract from the herb of *Erigeron acris* L. (Asteraceae). In this extract five phytosterols (campesterol, chondrillasterol, stigmast-7-en-3-ol(5α,3α), stigmasterol and spinasterone) were also identified.

Keywords: *Erigeron acris* (Asteraceae), herb, flavonoid aglycones, phytosterols

Erigeron acris L. (syn. *E. acer*) – blue fleabane is a member of the sunflower family, subfamily *Asteroideae,* tribe *Astereae*. It is North American and has become a weed in Europe. The plant is biennial or perennial, possesses well-branched erect stems ended with the flower-heads. Florets of two kinds: outer with short erect rays, pinkinsh-purple; the inner yellow disc florets, slender tubular. Leaves, basal, oblong lancet-like to spatulate, hairy. The flowering time is between May and September. Blue fleabane occurs on eskers, in dry grassland, sandy pastures and on walls (1,2). In some countries this plant is applicable in folk medicine, e.g. in Italy roots are used to relieve tooth-aches and arthritic pains (3), in Spain herb is used as a digestive (4). There is only a few phytochemical studies on *E. acris*. In the essential oil of Lachnophyllum an ester was found (5). The contemporary studies (GC and GC-MS) have shown that the major constituents of the essential oil from herb are monoterpenoid and sesquiterpenoid hydrocarbons (6). From leaves pyromeconic acid β-D-glucoside was isolated (7). In neutral lipid fraction of herb linoleic, linolenic, oleic, palmitic, stearic and palmitoleic acids were identified (8). Flavonoid composition of the plant has not been too well investigated; by preparative paper chromatography luteolin 7-O-glucoside was isolated (9), the content of total flavonoids and scutellarin was determined (10). In this work identification of phytosterols and isolation and identification of flavonoids is described.

EXPERIMENTAL

General techniques

The spectral analysis in UV was taken according to the method by Mabry et al. (11), on a Specord 40 UV-VIS Spectrophotometer (Jena Analytic AG). The ^1H NMR spectra were run in CD_3OD on a Bruker 200F spectrometer (200 MHz).

Chromatographic analysis

Phytosterols

Column chromatography (CC), Silica gel 60 (Merck); TLC, Silica gel 60 F_{254} (Merck): S1 hexane, S2 hexane-benzene step gradient, S3 benzene, S4 benzene-$CHCl_3$ step gradient, S5 $CHCl_3$;

TLC, Silica gel 60 F_{254}: S6 benzene-$CHCl_3$-MeOH (10:15:2, v/v/v), chromatogram analyzed after spraying with Liebermann-Burchard reagent;

GC and GC-MS: analysis by GC was carried out using a CARLO-ERBA INSTRUMENTS chromatograph HRGC 5300 Mega series, equipped with FID detector and HP-5 column, 30 m × 0.32 mm, film thickness of stationary phase 0.25 μm; GC-MS analysis on FISONS INSTRUMENTS GC 8000 apparatus connected with mass detector MD 800 using HP-5 column, 30 m × 0.32 mm, film thickness 0.32 μm. Experimental conditions: oven temperature programmed from 200°C to 320°C at 6°C/min (for both GC and GC/MS); injector and detector temperature 330°C; helium as the carrier gas at a flow rate of 1.5 mL/min; the mass spectrometer was operated at 70 eV. The identification of the com-

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pounds was based on a comparison of retention times and mass spectra with those of authentic samples and with NIST MS Library.

Flavonoids

CC, Sephadex LH-20, S7 50% MeOH, S8 MeOH;

TLC, cellulose powder (Merck): S9 n-BuOH-CH_3COOH-H_2O (4:1:5, v/v/v) organic phase, S10 CH_3COOH-hydrochloric acid-H_2O (30:3:10, v/v/v); chromatograms were analyzed in $UV_{366\ nm}$ before and after spraying with 0.1% Naturstoffreagenz A reagent (Roth).

CC, Polyamide (Roth): S10 EtOAc and EtOAc-MeOH step gradient.

Plant material

E. acris L. flowering herb was collected in July and August 2003 near Bielsk Podlaski. A voucher specimen is deposited in the Herbarium of the Department of Pharmacognosy, Medical University of Białystok (Poland).

Extraction and isolation

The air-dried grinded herb (1000 g) was extracted with hot MeOH under reflux (213 g brown-green extract). MeOH-extract was dissolved in $CHCl_3$ and filtered. The $CHCl_3$ solution was concentrated, applied on Silica gel column and eluted with solvent systems S1-S5. The separation was monitored with the same solvent systems by TLC on Sigel plates. From fractions 364-409 eluted with hexane-benzene (6:4 and 5:5, v/v) after crystallization and re-crystallization in hexane-benzene (1:1, v/v) 68 mg of white substance was obtained. Chromatographic analysis (TLC, S6), after spraying with Liebermann-Burchard reagent, showed sterol character confirmed by GC and GC-MS.

The residue after filtration of $CHCl_3$ solution (110 g), was dissolved in 50% MeOH and separated on Sephadex LH-20 column using S7 and S8 as eluents (monitoring by TLC, solvent systems S9 and S10, respectively). Altogether 14 fractions were obtained; 1-10 from 50% MeOH and 11-14 from MeOH. Chromatographic analysis of fraction 11 (TLC, S10) showed the presence of about five flavonoid aglycones. This fraction was further separated on Polyamide with S11 as an eluent. The separation was controlled by TLC using S10. From fractions 57-84 eluted with EtOAc compound **I** (13 mg), from 127-142 eluted with EtOAc and EtOAc-MeOH (9:1, v/v) compound **II** (10 mg), from 164-223 (EtOAc-MeOH 9:1, v/v) compound **III** (125 mg) and from fractions 238-250 (EtOAc-MeOH 8:2, v/v) compound **IV** (505 mg) were obtained.

Identification

Gas chromatography and GC-MS revealed the presence of five phytosterols: campesterol, chon-

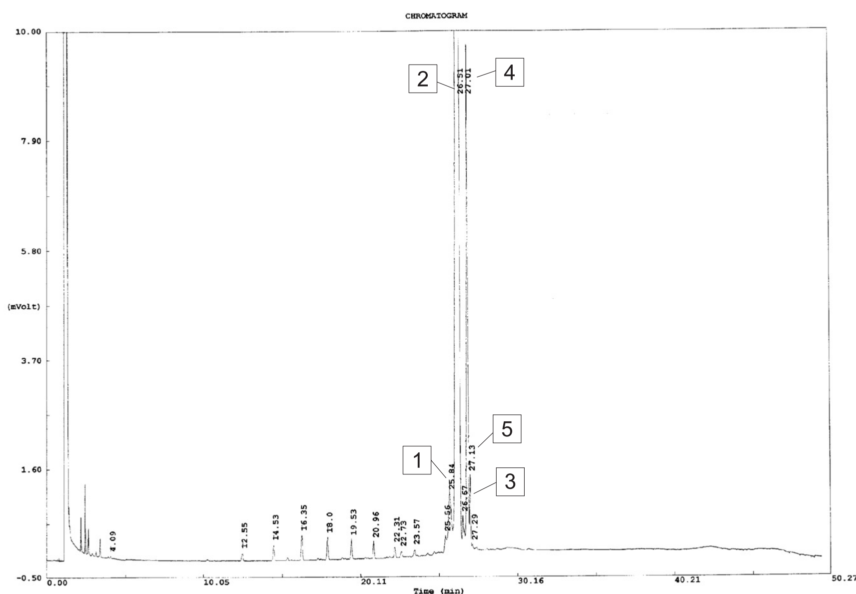

Figure 1. GC chromatogram of the phytosterols mixture from the herb of *E. acris* L.

Table 1. Phytosterols identified in *E. acris* L. herb.

Peak	t_R (min)	M^+ (m/z)	Identified compounds	Area %
1	25.84	400	campesterol	2.5
2	26.51	412	chondrillasterol	78.13
3	26.67	412	stigmasterol	1.5
4	27.01	414	stigmast-7-en-3-ol	13.95
5	27.13	410	spinasterone	0.51

drillasterol, stigmasterol, stigmast-7-en-3-ol(3α,5α) and spinasteron. (Table 1 and Figure 1).

Flavonoid compounds obtained in crystalline form from 80% MeOH were identified by co-TLC in S10 and by comparison of their UV and ^{1}H NMR data with those in the literature (11, 12).

Apigenin [**I**] ^{1}H NMR (CD_3OD): δ (ppm) 6.2 (1H, d, *J*=2.1 Hz, H-6), 6.45 (1H, d, *J*=2.1 Hz, H-8), 6.59 (1H, s, H-3), 6.93 (2H, d, *J*=8.9 Hz, H-3' and H-5'), 7.85 (2H, d, *J*=8.9 Hz, H-2' and H-6')

Kaempferol [**II**] ^{1}H NMR (CD_3OD): δ (ppm) 6.15 (1H, d, *J*=2.1 Hz, H-6), 6.36 (1H, d, *J*=2.1 Hz, H-8), 6.89 (2H, d, *J*=9.0 Hz, H-3' and H-5'), 8.06 (2H, d, *J*=9.0 Hz, H-2' and H-6'),

Luteolin [**III**] ^{1}H NMR (CD_3OD): δ (ppm) 6.18 (1H, d, *J*=2.1 Hz, H-6), 6.43 (1H, d, *J*=2.1 Hz, H-8), 6.66 (1H, s, H-3), 6.88 (1H, d, *J*=8.5 Hz, H-5'), 7.39 (1H, d, *J*=2.1 Hz, H-2'), 7.41 (1H, dd, *J*=2.1 and 8.5 Hz, H-6')

Quercetin [**IV**] ^{1}H NMR (CD_3OD): δ (ppm) 6.1 (1H, d, *J*=2.1 Hz, H-6), 6.37 (1H, d, *J*=2.1 Hz, H-8), 6.87 (1H, d, *J*=8.5 Hz, H-5'), 7.6 (1H,dd, *J*=2.1 and 8.5 Hz, H-6'), 7.74 (1H, d, *J*=2.1 Hz, H-2')

RESULTS

The herb of *Erigeron acris* L. was investigated. From $CHCl_3$ soluble fraction of methanolic extract after column chromatography on silica gel a mixture of five phytosterols was obtained, recognized by GC and GC-MS analysis as campesterol, chondrillasterol, stigmasterol, stigmast-7-en-3-ol(3α,5α) and spinasteron. The residue after filtration of $CHCl_3$ solution was chromatographed on Sephadex LH-20 column. Fraction 11 contained flavonoid aglycones mixture which was separated on Polyamide column and four pure compounds were obtained. There were apigenin (**I**), kaempferol (**II**), luteolin (**III**), and quercetin (**IV**). This is the first mention about phytosterols in *E. acris* and flavonoid aglycones were isolated for the first time.

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