Acta Poloniae Pharmaceutica – Drug Research, Vol. 58 No. 5 pp. 401–404, 2001

ISSN 0001-6837
Polish Pharmaceutical Society

SHORT COMMUNICATIONS

QUALITATIVE AND QUANTITATIVE CHROMATOGRAPHIC INVESTIGATION OF FLAVONOIDS IN *BELLIS PERENNIS* L.

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Abstract: The qualitative and quantitative analysis of flavonoids in the flowers and leaves of naturally growing *B. perennis* and in the flowers of cultivated variations *'rosea'* and *'rubra'* were carried out. The qualitative analysis was carried out by TLC and compared with flavonoid compounds isolated from flowers of common daisy. The quantitative determinations were carried out by Christ–Müller's method and by HPLC after acid hydrolysis. Similar flavonoids were found in all the flowers, while differences were noted in the flavonoid composition of the leaves. The flavonoid contents were higher in the flowers than in the leaves.

Keywords: HPLC, TLC, flavonoids, *Bellis perennis* L.

Bellis perennis L. (Asteraceae) is a common plant used in folk medicine and homeopathy as an expectorant, diuretic, anti–inflammatory, antispasmotic, antiexudatic. The known chemical components of *B. perennis* are triterpenoid saponins, phenolic acids, polyacetylenes (1, 2, 3). From the flowers of *B. perennis* of apigenin, apigenin 7–O–β–D–glucoside, apigenin 7–O–β–D–glucuronide, apigenin 7–O–β–D–methylglucuronide, apigenin 7–O–β–D–(6″–E–caffeoyl)–glucoside, kaempferol, kaempferol 3–O–β–D–glucoside, isorhamnetin 3–O–β–D–galactoside, isorhamnetin 3–O–β–D–(6″–acetyl)–galactoside, and quercetin were isolated (4, 5, 6). In this paper we present qualitative and quantitative investigations of flavonoids in the flowers and leaves of wildly growing *B. perennis* and in the flowers of cultivated variations 'rosea' and 'rubra'.

EXPERIMENTAL

Plant material

The flowers and leaves of *B. perennis* were collected from plants wildly growing in Białystok in June 1998. The flowers from variations *'rosea'* and *'rubra'* were collected from plants cultivated in the Garden of Medical Plants in Białystok (June 1998). Voucher specimens are deposited in the herbarium of the Department of Pharmacognosy, Medical Academy, Białystok, Poland.

Qualitative analysis of flavonoids by TLC

Standards: Flavonoids isolated from the flowers of *B. perennis* and isorhamnetin from Fluka were used as standards.

Sample preparation: The dried and powdered plant material (5 g) was extracted with $CHCl_3$ in a Soxhlet apparatus. After chloroform extraction, the material was extracted with boiling MeOH (5 × 250 cm^3). The solvent was distilled off completely from the combined MeOH extracts and the residues were diluted with 50 cm^3 of water and heated for 1 h. After separation of the balast substances, successive extraction was performed with $CHCl_3$ (3 × 50 cm^3), Et_2O (10 × 50 cm^3), AcOEt (10 × 50 cm^3), n–BuOH (10 × 50 cm^3). After distilling off the solvents, the residues were diluted with 10 cm^3 of MeOH. The Et_2O, EtOAc, n–BuOH extracts were investigated by TLC on silica gel glass plates (Merck) in the solvent system EtOAc–HCOOH–H_2O (18:1:1) and the Et_2O extracts additionally on cellulose glass plates (Merck) in the solvent system CH_3COOH–conc.–HCl–H_2O (30:3:10). Spots of the flavonoids were localized in UV light after spraying with a 1% methanolic solution of $AlCl_3$.

Quantitative analysis of flavonoids

a) Quantitative determination by Christ–Müller's method (7)

Content of the flavonoids in the sources were counted over apigenin and quercetin. Apigenin and quercetin isolated from the flowers of *B. perennis* were used as standards. The calibration curves were constructed in the range of sample quantity 0.050–2.000 mg.

b) Quantitative determinations by HPLC after hydrolysis

HPLC equipment – liquid chromatograph – TSP with a UV–VIS – Spectra–Focus (200–360 nm) detector, a P4000 pump, an injector – autosampler AS 4000.

A column – Vydac RP–C_{18} (250 × 4,6 mm ID).

Solvents – all the solvents used were of analytical – reagent grade for HPLC (Merck).

Standards – apigenin, kaempferol and quercetin isolated from the flowers of *B. perennis* and isorhamnetin from Fluka were used as standards. The calibration curves were constructed for each aglycone in the range of sample quantity 0.05–1.00 μg.

Sample preparation – the dried, powdered plant material (1 g) after extraction with $CHCl_3$ in the Soxhlet apparatus, was refluxed with MeOH (3 × 50 cm^3) and with 50% MeOH (1 × 50 cm^3) for 2 h. The combined MeOH extracts were evaporated under vacuum. The residue was refluxed for 3 h with 5 cm^3 of 10% hydrochloric acid. The hydrolysate was extracted with EtOAc (5 × 50 cm^3). The EtOAc extract was evaporated under vacuum and diluted to 10 cm^3 with MeOH.

HPLC procedure

Solvents: A – 0,05% TFA

B – 0,038% TFA in 83% ACN

Linear gradient: 30–33% B in A 0–15 min.

Flow rate: 1 cm^3/min.

Detection: 254 nm

Injection volume: 1 μl for the determination of apigenin in the flowers and 10 μl for the determination of apigenin in the leaves and for remainder samples.

RESULTS AND DISCUSSION

In qualitative research of the whole set of flavonoids, using the TLC method, it was im-

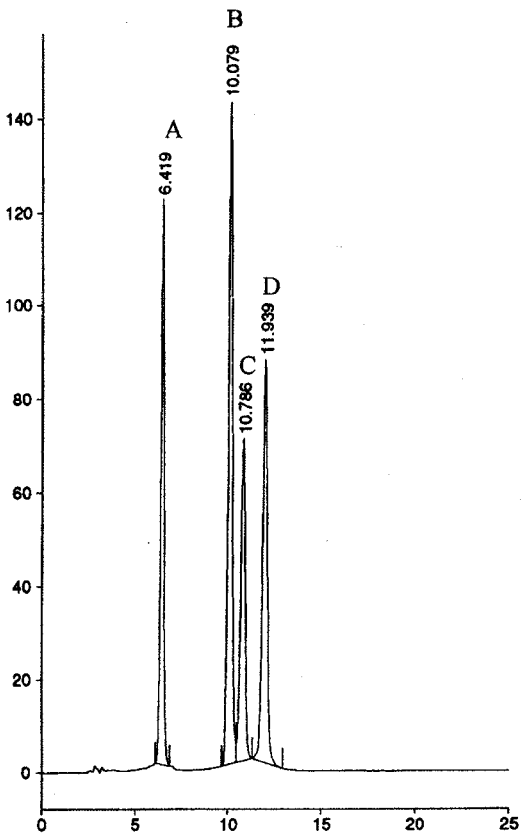

Figure 1. A chromatogram of the aglycones standards (A – quercetin, B – apigenin, C – kaempferol, D – isorhamnetin)

Table 1. Results of the quantitative determination of the flavonoids in *Bellis perennis* by using Christ–Müller's method

Source	Average content (%) by counting over	
	apigenin	quercetin
Flowers of *B.p.*	1.326	0.246
Leaves of *B.p.*	0.678	0.128
Flowers of *B.p.* var. *'rosea'*	1.348	0.253
Flowers of *B.p.* var. *'rubra'*	1.065	0.200

Table 2. Parameters of the equations of a least–squares regression and retention times of standards

Standard	Slope	Intercept	Correlation coeficient	Number of points	Range of sample quanitity (μg)	Retention time
quercetin	40.39	3.533	0.998	5	0.05–1	6.42
apigenin	18.47	1.264	0.999	5	0.05–1	10.08
kaempferol	35.07	1.654	0.999	5	0.05–1	10.79
isorhamnetin	25.30	–2.00	0.999	5	0.05–1	11.94

Table 3. Results of the quantitative determination of the flavonoids in *B. perennis* by using the HPLC method after hydrolysis

Source	Content in material (%)				
	quercetin	apigenin	kaempferol	isorhamnetin	sum of aglycones
Flowers of *B.p.*	0.013	0.10	0.007	0.019	0.229
Leaves of *B.p.*	0.008	0.008	trace	trace	0.016
Flowers of *B.p.* var. *'rosea'*	0.012	0.35	0.005	0.017	0.384
Flowers of *B.p.* var. *'rubra'*	0.012	0.36	0.009	0.025	0.406

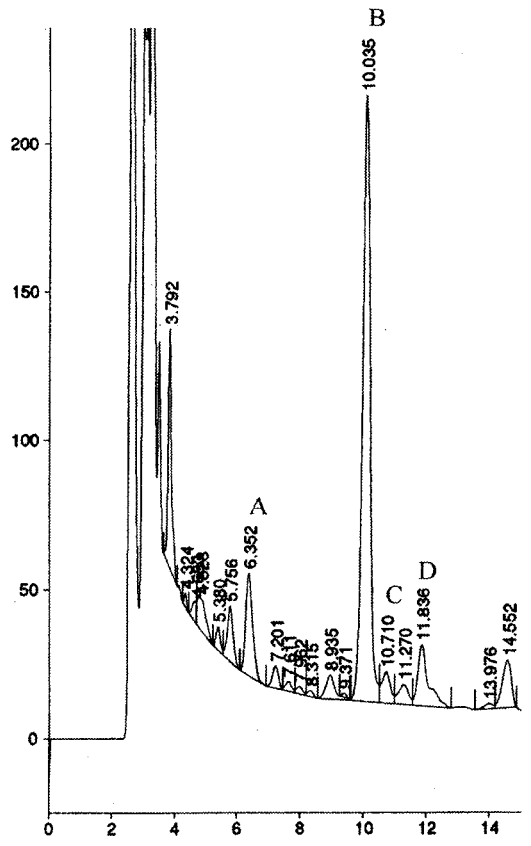

Figure 2. HPLC analysis of the hydrolysed MeOH extract from the flowers of *Bellis perennis* wildly growing (A – quercetin, B – apigenin, C – kaempferol, D – isorhamnetin)

perative to fractionate the MeOH extracts, to get a better separation. In the flowers of *B. perennis* wildly growing and also in cultivated variations *'rosea'*, *'rubra'*, the presence of apigenin, apigenin 7–O–β–D–glucoside, apigenin 7–O–β–D–glucuronide, apigenin 7–O–β–D–methylglucuronide, kaempferol, kaempferol 3–O–β–D–glucoside, isorhamnetin 3–O–β–D–galactoside, isorhamnetin 3–O–β–D–(6″–acetyl)–galactoside, and quercetin were detected. Besides, in the flowers of wildly growing daisy the apigenin 7–O–β–D–(6″–E–caffeoyl)–glucoside occurred. In the leaves of *B. perennis* apigenin, apigenin 7–O–β–D–glucoside, apigenin 7–O–β–D–glucuronide, kaempferol, kaempferol 3–O–β–D–glucoside, isorhamnetin 3–O–β–D–galactoside and quercetin occurred.

Contents of the flavonoids in the investigated sources was indicated by Christ–Müller's method by counting over apigenin and quercetin. The results of the determination are given in Table 1. Contents of the flavonoids counting over apigenin oscillated from 0.7% in the leaves of *B. perennis* wildly growing to 1.35% in the flowers of the cultivated variation *'rosea'*, and over quercetin figured from 0.13% in the leaves to 0.25% in the flowers of variation *'rosea'*.

Also quantitative composition of the flavonoids in the investigated sources were indicated using the HPLC method after acid hydrolysis. Figure 1 shows a recorded chromatogram of the separated aglycone standards. Figure 2 shows the HPLC analysis of the hydrolysed MeOH extract from the flowers of *B. perennis* wildly growing. Quantitative determination of the content of flavonoid aglycones made on the basis of linear dependence between the peak areas and their concentration. The parameters of the equations of a least–squares regression and retention times of the standards are listed in Table 2. The results of quantitative determination by the HPLC method after acid hydrolysis are shown in Table 3. The aglycones amounted from 0.016% in the leaves to 0.41% in the flowers of the cultivated variation 'rubra'. In the flowers, derivatives of the apigenin prevailed.

The appreciable content of the flavonoids in the flowers of *B. perennis* wildly growing also in the flowers of cultivated variations, may suggest, that these compounds are responsible for some

therapeutical effects of these sources. The flowers of common daisy could be numbered to valuable flavonoid sources.

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Received: 21.03.2001