Quantitative Analysis of Flavonoids in the Flowers and Leaves of *Ficaria verna* Huds.

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The quantitative determination of flavonoid compounds in flowers and leaves of *Ficaria verna* Huds. (Ranunculaceae) was carried out in different growing seasons of the plant, using Christ-Müller's method (Polish Pharmacopoeia, 1999) and high performance liquid chromatography (HPLC) analysis after acid hydrolysis. The flavonoid content was much higher in flowers than in leaves.

Key words: Ficaria verna Huds., Flavonoids, Quantitative Determination

Introduction

Ficaria verna Huds. (Ranunculaceae), known as pilewort is a small perennial plant widely distributed throughout Europe (Szafer *et al.*, 1988; Tutin *et al.*, 1964; Jasiewicz, 1985). It is used in folk medicine as an anti-inflammatory, astringent, antibiotic and anti-hemorrhagic treatment. In particular, extracts of *F. verna* are applied to haemorrhoids by topical application as ointment or suppository (Delacroix, 1969; Docheva-Popova and Popov, 1955; Palliez *et al.*, 1968; Boulet, 1996). Previous chemical investigations of *F. verna* tubers proved the presence of triterpenoid saponins (Brisse-Le Menn *et al.*, 1990; Texier *et al.*, 1984). In the fresh plant, ranunculin and products of its enzymatic decomposition have been observed (Bonora *et al.*, 1988). Recently, in our previous papers, we have reported the identification of phenolic acids and the isolation and structure elucidation of flavonoids: kaempferol, kaempferol 3-O-glucoside, quercetin 3-O-glucoside, kaempferol 3-O-rutinoside, quercetin 3-O-rutinoside, kaempferol 3-O-rutinoside-7-O-glucoside as well as quercetin 3-O-rutinoside-7-O-glucoside and C-glycosidic derivatives of flavones: vitexin, orientin and vitexin 2″-O-glucoside from the flowers and leaves of *F. verna* (Gudej and Tomczyk, 1999; Tomczyk *et al.*, 2002; Tomczyk and Gudej, 2002a). In addition, triterpenes and sterols from flowers of pilewort were identified (Tomczyk and Gudej, 2002b). In continuation of our studies, we have now determined flavonoids in the flowers and leaves during different growing seasons of the plant by using spectrophotometric (Christ-Müller's method; Polish Pharmacopoeia, 1999) and HPLC analysis after acid hydrolysis.

Material and Methods

Plant material

The flowers (F) and leaves (L) of *F. verna* were collected in different growing seasons of the plant in the Białystok area in April/May 1999. Voucher specimens (No. FV 97004 F1-F3, L1-L4) have been deposited in the Herbarium of the Department of Pharmacognosy, Medical University of Białystok, Poland. The samples and time of harvest are given in Table I.

Quantitative analysis of flavonoids

a/ Quantitative determination using Christ-Müller's method (Polish Pharmacopoeia, 1999; Strzelecka *et al.*, 1987).

Equipment, reagents, solvents

The absorbances were measured on a SPECORD 40 UV-VIS (Jena Analytik AG, Germany). All solvents of analytical grade were purchased from POCH (Gliwice, Poland).

Content of the flavonoids in all sources was determined for quercetin and hyperoside. The results are presented in Table I.

b/ Quantitative determination using HPLC method

Instrumentation for HPLC analysis

HPLC analyses were performed using a Thermal Separation Products – TSP (USA) liquid chromatograph with UV-VIS Spectra-Focus detector, P4000 pump, injector-autosampler AS4000. Compounds were separated on a 250 mm × 4.5 mm *i.d.*, 5 μm particle, RP-C_{18} Vydac (Columbia, USA) column maintained at 36 °C.

Standards and solvents

Kaempferol (K), vitexin (V) and orientin (O) isolated from the flowers and leaves of *F. verna* were used as standards (Gudej and Tomczyk, 1999; Tomczyk *et al.*, 2002). Quercetin (Q), isovitexin (IV) and isoorientin (IO) were purchased from Roth (Karlsruhe, Germany). All HPLC-grade solvents were purchased from Merck (Darmstadt, Germany). The calibration curves were constructed for each flavonoid in the range of sample quantity 0.02–0.5 μg.

HPLC procedure

The mobile phase consisted of solvent A (0.05% trifluoroacetic acid) and solvent B (0.038% trifluoroacetic acid in 83% acetonitrile v/v) with the following gradient: 0–15 min, 15% B in A; 5–10 min, 15–70% B in A; 10–15 min, 70% B in A. The flow rate was 1 ml/min, injection volume 10 μl. The UV detection was most effective at 342 nm.

Sample preparation

1 g amount of dried and pulverized plant material (F1–F3, L1–L4, see Table I), after extraction with $CHCl_3$ in a Soxhlet apparatus, was refluxed with MeOH (50 ml, 3 ×) for 2 h and then with 50% MeOH (50 ml, 1 ×) for 2 h. The combined extracts were concentrated *in vacuo*. The residue was refluxed with 5 ml of 10% hydrochloric acid. The hydrolysate was extracted with EtOAc (50 ml, 6 ×). The EtOAc extracts was evaporated and diluted to 10 ml with MeOH.

Results and Discussion

In the final stage of investigation we evaluated the content of flavonoids in flowers and leaves of *F. verna* collected in different vegetation periods over using in parallel Christ-Müller's and after acid hydrolysis HPLC methods. The content (in mg) of flavonoids according to the Christ-Müller's method was calculated as quercetin-flavonol type compound and monoside-hyperoside. Results given as hyperoside varied from 3.1 mg/g to 5.0 mg/g in flowers and from 1.5 mg/g to 2.3 mg/g in leaves. Flavonoid capacity, in Christ-Müller's method, was the highest for flower buds and the lowest for fresh leaves and leaves picked up before plant blossoming (see Table I). At the same time the amount of flavonoid compounds was determined in the investigated samples using HPLC after acid hydrolysis, frequently applied to standardize flavonoid materials (Sticher, 1993; Hasler *et al.*, 1990). Kaempferol, quercetin, vitexin, orientin, isovitexin and isoorientin were used as standards. Addition of the latter two standards was necessary as isomerisation of 8-C-glycosides to their 6-C-glycoside derivatives took place during acid hydrolysis. Measurements were made by wavelength 342 nm. Standard curves were prepared for individual standards taking into consideration a relationship between peak area field and standard concentration. The obtained results for flavonoid standards indicate good precision of the method used.

Kaempferol and quercetin were found to dominate in flower buds of *F. verna* (see Table II). The content of C-glycoside derivatives of apigenin and

Table I. Samples, vegetation period and results of the quantitative determination of flavonoids in *F. verna* by using Christ-Müller's method (Polish Pharmacopoeia, 1999).

Sample	Vegetation period	Time of harvest	Content[a] [mg/g]	
			Hyperoside	Quercetin
F1	Flower buds	12.04.1999	5.0 (± 2.4)	3.5 (± 1.7)
F2	Flowers in full blossom	19.04.1999	3.2 (± 1.6)	2.2 (± 1.1)
F3	Flowers in late blossom	30.04.1999	3.1 (± 1.3)	2.1 (± 0.9)
L1	Fresh leaves	01.04.1999	1.6 (± 0.6)	1.1 (± 0.4)
L2	Leaves before blossoming	16.04.1999	1.5 (± 0.3)	1.0 (± 0.2)
L3	Leaves of blossoming plants	19.04.1999	2.3 (± 1.0)	1.6 (± 0.7)
L4	Leaves after blossoming	06.05.1999	1.5 (± 0.7)	1.1 (± 0.5)

[a] Dry weight.
Values in parentheses are relative standard deviations RSD (%) (n = 3).

Table II. Results of the quantitative determination of flavonoids in *F. verna* by using the HPLC method after acid hydrolysis.

Sample	Content (samples F1–L4) [mg/g][a]						
	Isoorientin	Orientin	Vitexin	Isovitexin	Quercetin	Kaempferol	Sum of flavonoids
F1	trace	0.03 (± 0.5)	trace	0.21 (± 1.5)	4.27 (± 1.3)	1.45 (± 1.2)	5.96
F2	trace	0.02 (± 1.1)	trace	0.09 (± 0.9)	3.40 (± 3.8)	2.20 (± 0.8)	5.71
F3	trace	0.40 (± 2.6)	0.83 (± 3.2)	0.49 (± 1.1)	1.82 (± 2.1)	3.20 (± 0.5)	6.74
L1	0.05 (± 0.7)	0.08 (± 0.9)	0.85 (± 2.4)	0.49 (± 2.3)	0.82 (± 0.9)	1.10 (± 2.4)	3.39
L2	0.03 (± 1.2)	0.08 (± 0.8)	0.83 (± 1.8)	0.47 (± 3.1)	0.16 (± 1.6)	0.10 (± 3.8)	1.67
L3	0.18 (± 2.1)	0.19 (± 0.6)	1.41 (± 1.2)	0.76 (± 1.6)	1.20 (± 2.3)	0.19 (± 0.9)	3.93
L4	0.14 (± 1.7)	0.27 (± 1.4)	2.05 (± 1.7)	1.29 (± 4.1)	0.96 (± 1.2)	trace	4.71

[a] Dry weight.
Values in parentheses are relative standard deviations RSD (%) (n = 6).

luteolin in all flower samples was in a much lower quantity than flavonol type compounds. *F. verna* leaves contain less flavonoids than flowers. On the contrary, in leaves C-glycoside derivatives of apigenin and luteolin were dominant. The highest content of those compounds was noted for leaves picked up from flowering and fading plants. The values obtained for flavonoids sum by Christ-Müller's method were higher than using HPLC method after acid hydrolysis, which results from a possible reaction between $AlCl_3$ and non-flavonoid compounds present in the investigated samples.

Bonora A., Botta B., Menziani-Andreoli E., and Bruni A. (1988), Organ-specific distribution and accumulation of protoanemonin in *Ranunculus ficaria* L. Biochem. Physiol. Pflanzen **183**, 451–455.

Boulet J. (1996), Se soigner par l'homéopathie. Du ROCHER, Paris, pp. 89–92 (in French).

Brisse-Le Menn F., Duclos M. P., Larpent C., Mahe C., and Patin H. (1990), HPLC analysis of hederagenin and oleanolic acid in *Ficaria ranunculoides* tubers. Analusis **18**, 250–254 (in French).

Delacroix P. (1969), Our experience with the use of an antihemorrhoidal ointment of *Ficaria* glycosides. Rev. Fr. Gynecol. Obstet. **64**, 487–488.

Docheva-Popova R. and Popov K. (1955), Investigation of the relative antibiotic actions of ranunculaceous plants. Godishnik Sofiiskiya Univ. **48**, 41–58 (in Bulgarian).

Gudej J. and Tomczyk M. (1999), Polyphenolic compounds from flowers of *Ficaria verna* Huds. Acta Pol. Pharm. – Drug Res. **56**, 475–476.

Hasler A., Sticher O., and Meier B. (1990), High-performance liquid chromatographic determination of five widespread flavonoid aglycones. J. Chromatogr. **508**, 236–240.

Jasiewicz A. (1985), Flora Polska, Rośliny naczyniowe, tom IV. PWN, Warszawa, pp. 55–56 (in Polish).

Palliez R., Delecour M., and Roussel M. (1968), Use of a new antihemorrhoid ointment of *Ficaria* glucosides in the sequel of childbirth. Lille Med. **13**, 1044.

Polish Pharmacopoeia, V. Ed. (1999). Polskie Towarzystwo Farmaceutyczne, Warszawa, pp. 56–57 (in Polish).

Sticher O. (1993), Quality of *Ginkgo* preparations. Planta Med. **59**, 2–11.

Strzelecka H., Kamińska J., Kowalski J., Malinowski J., and Walewska E. (1987), Chemiczne metody badań roślinnych surowców leczniczych. PZWL, Warszawa, pp. 82–83 (in Polish).

Szafer W., Kulczyński S., and Pawłowski B. (1988), Rośliny polskie, tom I. PWN, Warszawa, pp. 184–185 (in Polish).

Texier O., Ahond A., Regerat F., and Pourrat H. (1984), A triterpenoid saponin from *Ficaria ranunculoides* tubers. Phytochemistry **23**, 2903–2905.

Tomczyk M., Gudej J., and Sochacki M. (2002), Flavonoids from *Ficaria verna* Huds. Z. Naturforsch. **57c**, 440–444.

Tomczyk M. and Gudej J. (2002a), Quercetin and kaempferol glycosides from *Ficaria verna* flowers and their structure studied by 2D NMR spectroscopy. Polish J. Chem. **76**, 1601–1605.

Tomczyk M. and Gudej J. (2002b), Composition of lipophylic extracts from *Ficaria verna* Huds. flowers. Rocz. Akad. Med. Białymst. **47**, 213–217.

Tutin T. G., Heywood V. H., Burges N. A., Valentine D. H., Walters S. M., and Webb D. A. (1964), Flora Europaea, vol. I. University Press, Cambridge, pp. 233–234.