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COMPARATIVE STUDY ON THE FREE FLAVONOID AGLYCONES IN HERBS OF DIFFERENT SPECIES OF *POLYGONUM* L.

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Abstract: The flavonoid aglycones were studied in the herbs of the following taxons of the *Polygonum* L. genus: *P. hydropiper* L., *P. bistorta* L., *P. aviculare* L., *P. persicaria* L., *P. lapathifolium* ssp. *tomentosum* (Schrank) Dans, *P. lapathifolium* ssp. *nodosum* (Pers.) Dans, *P. amphibium* L., *P. mite* Schrank, *P. convolvulus* L. (*Bilderdykia convolvulus* L.) by means of the RP–HPLC method.The content of taxifolin, quercetin, quercetin–3–methyl ether, kaempferol, myritcetin, luteolin, isorhamnetin and rhamnetin were determined.

Keywords: *Polygonum, Polygonaceae,* flavonoid aglycones, RP HPLC method.

The genus *Polygonum* L. (Polygonaceae) contains a large number of species widely distributed in the world. Three species growing in Poland: *Polygonum hydropiper* L., *Polygonum aviculare* L. and *Polygonum bistorta* L. are sources of pharmacopoeial drugs. *Polygonum persicaria* L. has been used in folk medicine. These plants show antiseptic, diuretic, cholagogic and antihaemorrhagic activity. The active components of these species are phenolic compounds, mainly flavonoids and tannins (1). *Herba Polygoni avicularis* is a typical flavonoid drug according to FP V.

This paper aims at detecting flavonoid aglycones in some taxons of *Polygonum* L. genus growing in Poland. These compounds and their glycosides possess a wide range of pharmaceutical properties (2;3) and their content in plant influences the activity of the plant material.

Flavonoid aglycones have successfully been used in several plants in taxonomic aspect (4) and can be helpful in classification of the investigated species.

Ealier reports on the aglycones in the studied taxons describe quercetin, kaempferol, luteolin, myricetin and isorhamnetin in *P. aviculare* (5), quercetin, kaempferol, myricetin and isorhamnetin in *P. hydropiper* (6), quercetin and kaempherol in *P. persicaria* (7;8), quercetin and rhamnetin in *P. lapathifolium* ssp. *tomentosum* (9), quercetin, kaempferol, taxifolin and myricetin in *P. lapathifolium* ssp. *nodosum* (10;11), quercetin and kaempferol in *P. convolvulus* and quercetin, kaempferol in *P. amphibium* (6). There have been no reports on flavonoid aglycones of *Polygonum bistorta* L. and *Polygonum mite* L. in the available literature.

EXPERIMENTAL

General experimental procedures

The RP–HPLC method was applied to qualitative and quantitative determination of flavonoid aglycones in the plant extracts. Analyses were performed on an HP–1050 Hewlett–Packard chromatograph (Palo Alto, CA, USA) with a 20 µl sample injector (Rheodyne, Cotati, CA, USA) and a UV detector (UV–VIS) operating at 254 nm. The chromatograms were recorded with a Hewlett–Packard Model 3396 A integrator, chart speed 0.5

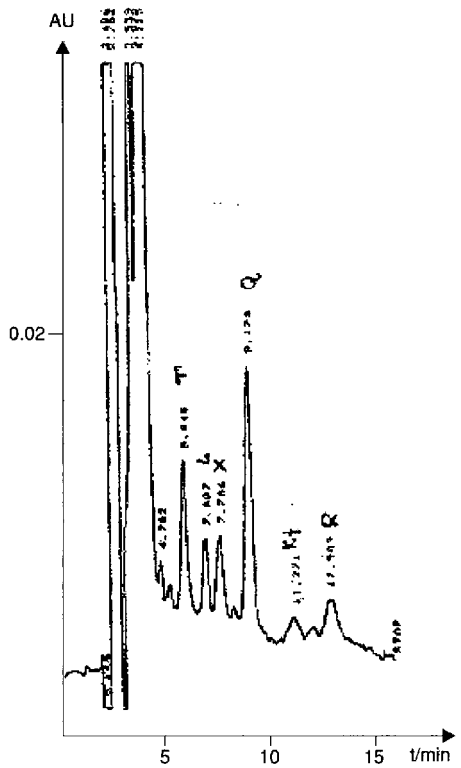

Figure 1. HPLC chromatogram of flavonoid aglycones from the diethyl ether extract of *Polygonum hydropiper* L. T– taxifolin, L– luteolin, X– quercetin–3–methyl ether, Q– quercetin. Kf– kaempferol, R– rhamnetin.

cm/min. The column (250 × 4.6 mm) was packed with 5 μm Adsorbosphere HS C 18 (Alltech, England). Chromatography was performed at room temperature.

Mobile phase system was determined experimentally. Three modifiers (methanol, acetonitrile and tetrahydrofurane) were studied for the separation of a standard mixture solution and for the investigation of natural mixtures in the binary water – organic reversed phase systems. In this case, a satisfactory separation of compounds in plant extract was achieved with THF–H_2O–HOAc (42.5 : 56.5 : 1) mixture as the solvent system (Figure 1). The retention times of the peaks of the samples were compared with authentic compounds. Quantitative determination of the content of each flavonoid aglycone was made on the basis of linear dependence between peak heights and concentration.

Plant material

Herbs of nine taxons of *Polygonum* L. genus – *P. amphibium* L., *P. hydropiper* L., *P. bistorta* L., *P. persicaria* L., *P. mite* Schrank, *P. aviculare* L., *P. lapathifolium* ssp. *tomentosum* (Schrank) Dans, *P. lapathifolium* ssp. *nodosum* (Pers) Dans, and *P. convolvulus* L. (*Bilderdykia convolvulus* L.) were investigated in this work. The plant materials were collected in Samoklęski and in Motycz near Lublin (Poland) in 2000. Voucher specimens are deposited in the Department of Pharmaceutical Botany, Medical University of Lublin, Poland.

Extraction

The air–dried and powdered herbs (10 g) of each of the taxons were extracted with 100 ml of 80% aq. methanol three times (1h each extraction). The combined extract was evaporated to dryness and the residue was dissolved in water (100 ml) and extracted with diethyl ether (1 × 50 ml, 4 × 20 ml). The Et_2O extract was evaporated and the residue was dissolved in ethanol (10 ml).

Standards and solvents

The quercetin, kaempferol, myricetin were from Fluka (Chemie AG. Switzerland), taxifolin and luteolin were from Sigma (Chemical Co., USA), quercetin–3–methyl ether was isolated from *Polygonum amphibium* L. and its structure was elucidated by spectroscopic methods (12), isorhamnetin and rhamnetin were from ROTH (Labor Roth, Germany). All solvents used in HPLC experiments were of gradient grade.

RESULTS AND DISCUSSION

As a result of the investigations carried out on *Polygonum* L. species, the following eight aglycones: taxifolin, quercetin–3–methyl ether, quercetin, kaempferol, luteolin, myricetin, isorhamnetin and rhamnetin were detected. Quercetin and kaempferol were present in all analysed samples and the amount of quercetin in all the cases was significantly higher than the amount of kaempferol. The biggest amount of quercetin occurred in the herbs *Polygonum bistor-*

Table 1. The content of the flavonoid aglycones in different taxons of *Polygonum* L. (in mg/g)

No.	Taxons	T R_T=5.9 min	L R_T=7.0 min	M R_T=7.3 min	Qme R_T=7.7 min	Q R_T=9.2 min	I R_T=10.7 min	K R_T=11.2 min	R R_T=12.9 min
1.	*P. hydropiper*	0.63	0.19	–	0.19	0.39	0.12	0.11	–
2.	*P. bistorta*	0.27	0.08	–	0.20	1.0	0.15	0.03	0.07
3.	*P. aviculare*	0.2	0.15	0.07	0.02	0.1	0.04	0.25	–
4.	*P. persicaria*	0.13	0.23	–	0.24	0.05	–	0.03	0.08
5.	*P. lapathifolium* ssp. *tomentosum*	0.28	–	–	0.11	0.65	0.2	0.2	0.1
6.	*P. lapathifolium* ssp. *nodosum*	0.19	–	–	0.15	0.95	0.11	0.15	–
7.	*P. amphibium*	0.1	–	–	0.48	0.17	0.05	0.07	–
8.	*P. mite*	0.16	0.07	–	0.25	0.26	–	0.03	0.02
9.	*P. convolvulus*	–	0.72	0.08	–	0.08	0.12	0.08	0.19

T – taxifolin, L – luteolin, M – myricetin, Qme – quercetin – 3 – methyl ether, Q – quercetin, I – isorhamnetin, K – kaempferol, R – rhamnetin.

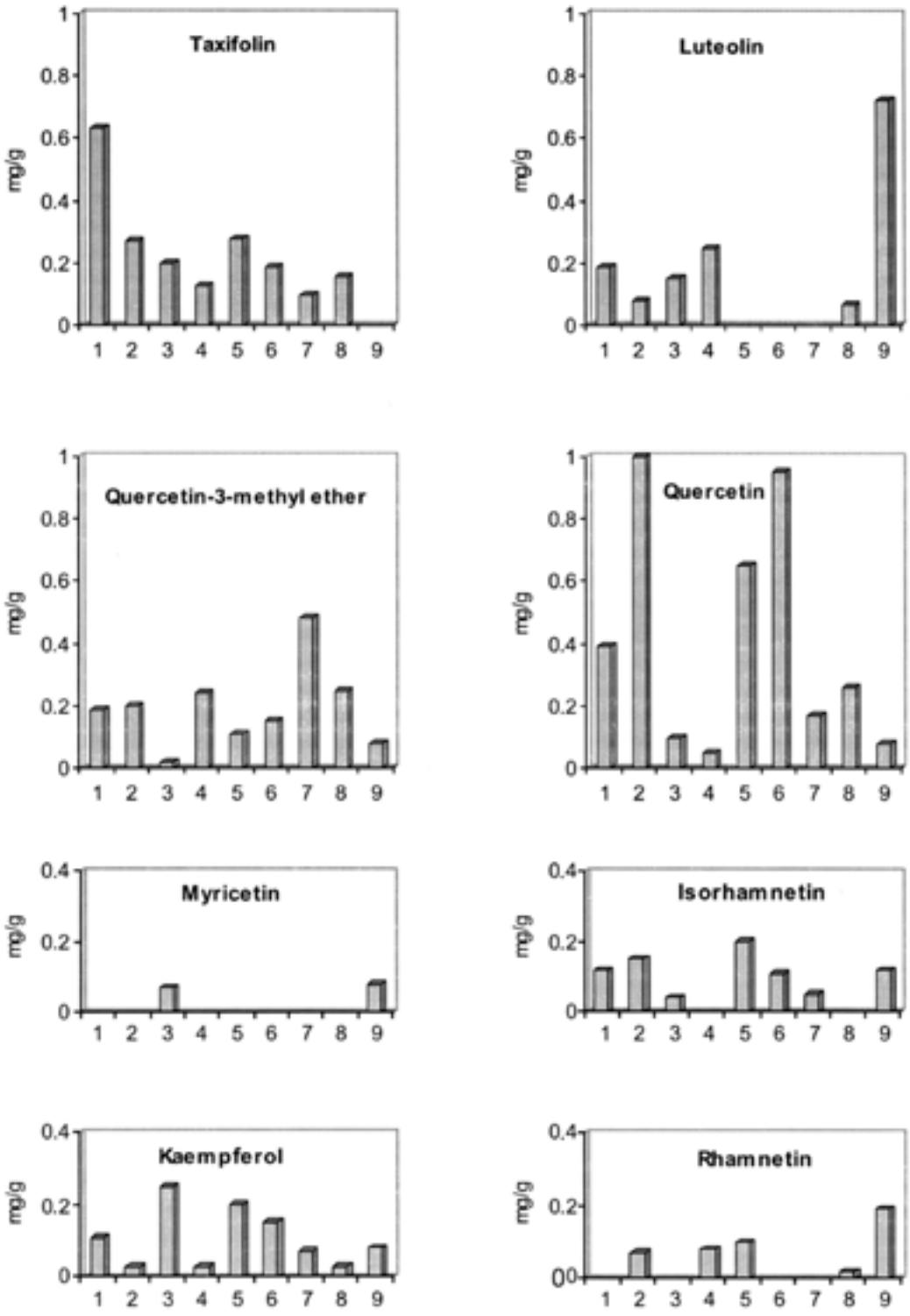

Figure 2. Graphical presentation of the flavonoid aglycones content in various taxons of *Polygonum* L.: *P. hydropiper* – 1; *P. bistorta* – 2; *P. aviculare* – 3; *P. persicaria* – 4; *P. lapathifolium* ssp. *tomentosum* – 5; *P. lapathifolium* ssp. *nodosum* – 6; *P. amphibium* – 7; *P. mite* – 8; *P. convolvulus* – 9.

ta L. (1 mg/g), *P. lapathifolium* ssp. *nodosum* L. (0.95 mg/g) and *P. lapathifolium* ssp. *tomentosum* L. (0.65 mg/g). Taxifolin and quercetin–3–methyl ether were detected in eight out of nine taxons of *Polygonum* L.. Taxifolin was the major flavonoid aglycone in the herb of *P. hydropiper* L. (0.63 mg/g) and quercetin–3–methyl ether was the major component in the herb of *P. amphibium* L. (0.48 mg/g). In the herb of *P. convolvulus* L. both, taxifolin and quercetin–3–methyl ether were not detected and the luteolin was dominating aglycone (0.72 mg/g). Isorhamnetin was present in all the investigated plants except for *P. persicaria* L. and *P. mite* L. Rhamnetin was present in a small amount in five taxons of *Polygonum* L. and myricetin was detected only in *P. aviculare* L. and *P. convolvulus* L. (Table 1).

Comparison of the free aglycones content in the various taxons of *Polygonum* L. genus determined by the RP HPLC method is presented in Figure 2.

The total amount of these determined aglycones in the studied plant was: *P. hydropiper* – 1.63 mg/g, *P. bistorta* – 1.8 mg/g, *P. aviculare* – 0.83 mg/g, *P. persicaria* – 0.78 mg/g, *P. lapathifolium* ssp. *tomentosum* – 1.54 mg/g, *P. lapathifolium* ssp. *nodosum* – 1.55 mg/g, *P. amphibium* – 0.87 mg/g, *P. mite* – 0.79 mg/g, *P. convolvulus* – 1.27 mg/g.

Among the investigated aglycones, quercetin–3–methyl ether was detected in all the studied taxons except for *Polygonum convolvulus* L., and taxifolin was confirmed in *P. lapathifolium* ssp. *nodosum* L. and shown in seven other taxons. Furthremore, rhamnetin was confirmed in *P. lapathifolium* ssp. *tomentosum* L. and detected in four other taxons. Luteolin was determined neither in *P. lapathifolium* L. subspecies nor in *P. amphibium* L., and isorhamnetin was confirmed in *P. hydropiper* L. and *P. aviculare* L. and detected in five other taxons.

Pawłowski, in „Flora Polska" (13) described *Polygonum convolvulus* L. in the genus *Polygonum* L. In the second edition of „Flora Polski" (14), the classification of *Polygonum convolvulus* L. was changed. The species was classified as *Bilderdykia convolvulus* (L.) Dumort syn. *Polygonum convolvulus* L. and described in genus *Bilderdykia* L.. Taxifolin and quercetin–3–methyl ether less commonly occurring in plant material, are present in all the investigated species except for *Polygonum convolvulus* L.. The major aglycone in *P. convolvulus* L. is luteolin, whereas in other species the most frequently dominating aglycone is quercetin or its derivatives. The above presented results confirm a certain dissimilarity of this species from other studied species.

The aglycones identified in the studied samples show multidirectional pharmacological activity. Antioxidant properties are shown by quercetin, kaempferol, taxifolin, luteolin and myricetin (15). Quercetin and luteolin possess antialergic properties (16;17). Taxifolin inactivated cytotoxic substances (18). Quercetin, myricetin, and taxifolin were shown to be highly effective in inhibiting the reverse transcriptase activity (19). These and other properties of aglycones, e.g. anti–influenza, antivirus and antitumour effect (16), may influence the pharmacological activity of the studied plant material.

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