FLAVONOID GLYCOSIDES IN NINE *POLYGONUM* L. TAXONS

HELENA D. SMOLARZ

Department of Pharmaceutical Botany, Medical University
Staszica 4, 20-081 Lublin, Poland
e-mail: smolarz@panaceum.am.lublin.pl

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ABSTRACT

Eighteen flavonoid glycosides were identified in 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.). The content of fourteen flavonoid glycosides was determined. Reversed Phase High Performance Liquid Chromatography was applied for separation, identification and quantitative determination of these compounds.

KEY WORDS: *Polygonum*, Polygonaceae, flavonoid glycosides, RP HPLC method.

INTRODUCTION

In the *Polygonum* L. genus (Polygonaceae) flavonoids, tannins, anthocyanins, antraquinones, and stilbenes are characteristic chemical constituents (Kawasaki et al. 1986; Yoshitama et al. 1984; Yeh et al. 1988; Kimura et al. 1983). Flavonoids are plant secondary metabolites which play a significant role in different physiological processes. Flavonoid glycosides and free aglycones are involved in pathogenetic and symbiotic interactions with microorganisms (Dixon et al. 1994; Spaink et al. 1995). Plant-derived flavonoids possess multidirectional pharmacological activity. The variety of in vitro and in vivo investigations have shown that selected flavonoids possess antiviral (Kaul et al. 1985), antibacterial (Kuroynagi et al. 1999), antiallergic (Cheong et al. 1998), anti-inflammatory (Manthley 2000) and antioxidant (Rice-Evans 2001) activities. Flavonoids exhibit anticancer and chemopreventive effects (Siess et al. 2000). Certain flavonoids possess a potency of inhibitory effect on several enzyme systems, initially connected to cel activation processes as protein tyrosine kinase, protein kinase C, phospholipase and others (Manthley 2000 and 2001). These properties evidence that flavonoids may be health-promoting, disease preventing compounds, and the plants containing them may be taken into account as potential medicinal raw materials.

Analyses of flavonoid compounds in *Polygonum* have been carried out several times and twenty six species growing all over the world have been studied. These reports state that many flavonoid glycosides were isolated or chromatographically detected in taxons studied in these works. The glycosides are as follows: rutin, hyperin, isoquercitrin, quercetin-3-O-(2"-galloyl)-glucoside, quercetin-3-O-glucuronide and eight sulphate derivatives of flavonoids from *P. hydropiper* (Kawasaki et al. 1986; Valentin and Wagner 1953; Bandjukova 1973; Haraguhi et al. 1996); rutin from *P. bistorta* (Bandjukova 1973); hyperin, quercitrin, avicularin, myricetin-3-O-rhamnoglucoside and 5,7,3',4' tetrahydroxy (3-O-rhamnoside) flavanon from *P. aviculare* (Hegnauer 1969; Bandjukova 1973; Chvorost and Komisarenko 1980); hyperin, isoquercitrin, avicularin from *P. persicaria* (Bandjukova 1973); isoquercitrin, quercetin-3-O-β--(2"-galloyl)-glucopyranoside, kaempferol-3-O-β-(2"-galloyl)-glucopyraniside and 3-hydroxy-5-methoxy-6,7-methylenodioxy-flavanon from *P. lapathifolium* ssp. *nodosum* (Isobe et al. 1979, 1980; Kuroynagi et al. 1982); rutin, hyperin, quercitrin, isoquercitrin, quercetin-3-O-β-glucuronide, quercetin 3-O-D-β-(6"-O-galloyl)-glucopyranoside, quercetin-3-O-D-(6"-O-galloyl)-galactopyranoside from *Polygonum lapathifolium* ssp. *tomentosum* (Tiwari and Massod 1977; Smolarz (in print); hyperin, avicularin, luteolin-7-O-glucoside, quercetin-7-O-glucoside from *P. amphibium* (Machkamova et al. 1970); rutin, hyperin, from *P. convolvulus* (Bandjukova 1973; Kuroynagi et al. 1982). There have been no reports on flavonoids of *P. mite* in the available literature.

Studies on phenolic acids (Smolarz 1999, 2000), stilbenes (Smolarz and Potrzebowski 2002; Smolarz and Matysik 2001), and flavonoids from *Polygonum lapathifolium* ssp. *tomentosum* (Smolarz, in print) have been previously reported. This paper is a continuation of these studies on phenolic compounds in some species of *Polygonum* genus. The aim of the present investigations is a comparative study of flavonoid glycosides on the basis flavonoids isolated from *P. lapathifolium* ssp. *tomentosum* and other standard samples.

The separation of extracts from different taxons of *Polygonum* L. and identification of flavonoid glycosides have been achieved by means HPLC, the method commonly applied to determine phenolic compounds.

MATERIAL AND METHODS

Apparatus

The chromatographic apparatus consist of Solvent Organiser (Model K-1500, Knauer, Germany), LC – pump (Model K-1001, Knauer, Germany) and UV Detector (Model K-2001, Knauer, Germany), 20 µL sample injector (Rheodyne, Cotati, CA, USA). Some of the experiments were made using a HP-1050 Hewlett-Packard chromatograph (Palo Alto, CA, USA) with 20 µl sample injector (Rheodyne, Cotati, CA, USA) and UV detector (UV-VIS) operating at 254 nm.The chromatograms were recorded with a Hewlet-Packard Model 3396 A integrator, chart speed 1 cm/ /min.

HPLC conditions

The analytical columns were: stainless steel Zorbax SB C18 (250×4.6 mm, 5 µm) steel column (Hewlet Packard) preceeded by Zorbax C-18 quard column (4 µm) and Adsorbosphere HS C 18 (Alltech, Lancashire, England) steel column (250×4.6 mm, 5 µm). The solvents used in HPLC procedure were: A – acetonitrile, B – water + acetic acid (99:1), C – water + phosphoric acid (99.5:0.5). The mobile phases in isocratic systems were: 17% A in B (1), and 17% A in C (2). The mobile phase in isocratic and gradient system was: 0 to 27 min.: 17% A in B (isocratic); 27 to 45 min.: 17 to 45% A in B (linear gradient), 45 to 60 min.: from 45 to 60% A in B (linear gradient). Chromatography was performed at room temperature.

Plant material

Herbs of the following taxons of *Polygonum* L. genus – *P. hydropiper* L., *P. bistorta* L., *P. persicaria* L., *P. aviculare* L., *P. mite* Schrank, *P. lapathifolium* ssp. *tomentosum* (Schrank) Dans, *P. lapathifolium* ssp. *nodosum* (Pers) Dans, *P. amphibium* L., *P. convolvulus* L. (*Bilderdykia convolvulus* L.) were investigated in this study. The plant materials were selected from different localities in Samoklęski and Motycz near Lublin (Poland) in 1999, and authenticated by Prof. Dr. hab. T. Krzaczek. A voucher specimens are deposited in the Department of Pharmaceutical Botany, Medical University Lublin, Poland.

Extraction

The air-dried and powdered herb (10 g) of each of the taxons was extracted twice with boiling 100 mL 80% aq. methanol, and 50% aq. methanol (1 h each extraction). The combined extracts were evaporated from the solvent under reduced pressure. The residue was treated with hot water (100 mL) and filtered. The filtrate was partitioned using sequential extraction with organic solvents: diethyl ether (1×50 mL, 4×20 mL), ethyl acetate (4×20 mL), and n-butanol (3×20 mL). The combined extracts were concentrated at 40°C under reduced pressure until syrup residue was obtained, next dissolved in 50% aqueous methanol (10 mL). The procedure was repeated three times for each plant material sample.

Standards and solvents

The quercetin-3-O-β-(6"-O-galloyl)-glucopyranoside, quercetin-3-O-β-(6"-O-galloyl)-galactopyranoside and quercetin-3-O-D-β-glucuronide were early isolated from *P. lapathifolium* ssp. *tomentosum*, and their structure was elucidated by spectroscopic methods, guaijaverin was kindly supplied by Prof. Dr hab. J. Budzianowski from University of Medicinal Sciences, Poznań, other standards were from Sigma (Sigma Chemical Co., USA), and Fluka (Fluka, Chemie AG. Switzerland ROTH (Labor Roth, Germany). All solvents used in HPLC experiments were of gradient grade.

RESULTS AND DISCUSION

In the starting investigations, the compounds extracted with 80% aqueous methanol were partitioned using sequential extraction with organic solvents: diethyl ether, ethyl acetate and n-butanol. The presence of the studied compounds in each extract was tested by HPLC. Obtained data showed that flavonoid glycosides were partitioned between these solvents to a different degree. Finally all fractions containing compounds of interest were combined for the investigations. Separation of the natural mixture of these compounds from samples and standard mixtures by HPLC was determined experimentally. Under the experiment, different isocratic and gradient techniques were tested. A satisfactory separation of flavonoid glycosides was achieved with acetonitrile +1% aq. acetic acid (17:83) mixture as solvent system (Fig. 1), and acetonitrile + 0.5% aq. phosphopic acid (17:83).

Eighteen flavonoid glycosides were detected in studied taxons of *Polygonum* L. For identification, the retention times of the peaks of the samples were composed with authentic reference compounds. Distribution of these compounds is presented in Table 1.

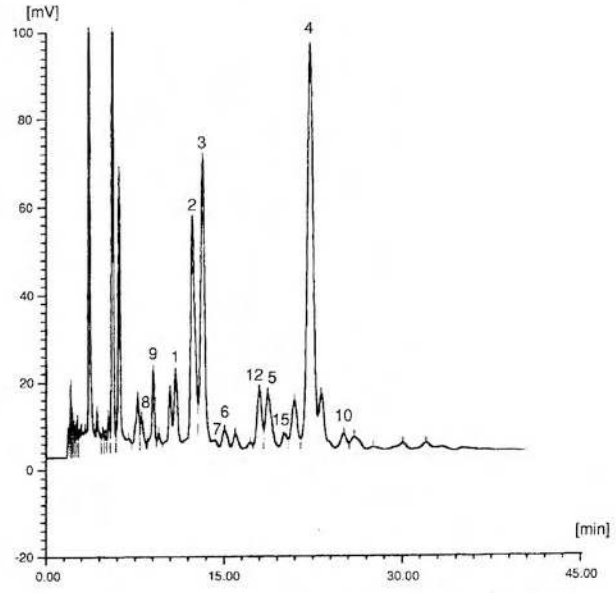

Figure 1. Separation of flavonoid glycosides from *Polygonum mite* by HPLC method on Zorbax SB C-18 column 250×4.6 mm I.D., 5 µm with quard column 4×4 mm I.D., 4 µm; 17% acetonitrile in 1% aq. solution of acetic acid; a flow rate 1 ml × min^{-1}. Numbered peaks are identified in Table 1.

TABLE 1. The distribution of flavonoid glycosides in different taxons of *Polygonum* L.

	Flavonoid glycosides	Trivial name	Ph	Pb	Pr	Pv	Pp	Pe	Pn	Pt	Pa	Pc
1	Q-3-O-rutinoside	Rutin	+	+	+	+*	+*	+*	+*	+I	+*	
2	Q-3-O-β-galactoside	Hyperin	+	+*	+	+	+	+*	+*	+I	+	
3	Q-3-O-β-glucoside	Isoquercitrin	+	+*	+	+*	+	+*	+	+I	+*	+*
4	Q-3-O-α-rhamnoside	Quercitrin	+*	+		+	+*	+*	+*	+I	+*	+
5	Q-3-O-β-arabinofuranoside	Avicularin	+			+	+	+*			+	+*
6	Q-3-O-α-arabinopyranoside	Guaijaverin						+*				+*
7	Q-3-O-β-glucuronide	Miquelianin	+	+*		+*	+*	+*	+*	+I		
8	Q-3-O-β-(6"galloyl) glucoside		+*			+*	+*	+*	+*	+I		
9	Q-3-O-β-(6"galloyl) galactoside		+*			+*	+*		+*	+I	+*	
10	Q-8-O-β-galactoside							+*			+*	
11	Q-4'-O-β-glucoside	Spiraeoside		+*		+*	+*	+*			+*	+*
12	K-3-O-rutinoside					+*	+	+*				+*
13	K-3-O-β-glucoside	Astragalin	+*	+*		+*		+*	+*	+I	+*	+*
14	I-3-O-rutinoside					+*					+*	
15	I-3-O-β-glucoside		+*			+*		+*			+*	
16	L-7-O-glucoside		+*		+							+*
17	L-8-C-glucoside	Orientin	+*		+	+*	+*				+*	+*
18	A-6-C-glucoside	Isovitexin			+*	+*						+*

+ – present, * – new for the taxons, I – isolated previously from, Q – quercetin, K – kaemperol, I – isorhamnetin, L – luteolin, A – apigenin, Ph – *P. hydropiper*, Pb – *P. bistorta* (herb), Pr – *P. bistorta* (rhizoma), Pv – *P. aviculare*, Pp – *P. persicaria*, Pe – *P. mite*, Pn – *P. lapathifolium* ssp. *nodosum*, Pt – *P. lapathifolium* ssp. *tomentosum*, Pa – *P. amphibium*, Pc – *P. convolvulus*.

The amount of fourteen glycosides was determined on the basis of calibration line, which was obtained for the concentrations of standard solutions of each glycoside for 80, 100, 120 per cent of the anticipated content of this compound in extracts. Hyperin, isoquercitrin, quercitrin, rutin, avicularin and astragalin are commonly present in studied samples and their concentrations in each taxon are shown in Fig. 2. Quercetin-3-O-arabinoside was present in *Polygonum* genus as quercetin-3-O-β-arabinofuranoside (avicularin) and quercetin-3-O-α-L-arabinopyranoside (guaijaverin). The first form has been detected in six species, the second form was detected in two taxons of *Polygonum* L. The concentration of guijaverin was small and did not go over 0.1 mg/g. Content of quercetin-3-O-β-glucuronide was: 0.07 mg/g in *P. hydropiper*; 0.17 mg/g in *P. bistorta* (herb); 0.09 mg/g in *P. aviculare*; 0.1 mg/g in *P. persicaria*; 0.09 mg/g in *P. lapathifolium* ssp. *nodosum* and 0.18 mg/g in *P. lapathifolium* ssp. *tomentosum*. Concentration of kaempferol-3-O-rutoside was: 0.1 mg/g (*P. aviculare*), 0.52 mg/g (*P. persicaria*), 0.14 mg/g (*P. mite*) and 0.05 mg/g (*P. convolvulus*), whereas the concentration of isorhamnetin-3-O-rutoside was 0.1 mg/g and 0.06 mg/g in *P. aviculare and P. perscaria* respectively. A small amount orientin was detected in *P. aviculare*, and *P. hydropiper* (0.002 mg/g), *P. bistorta*-herb (0.012 mg/g), *P. bistorta*-rhizoma (0.1 mg/g), *P. amphibium* (0.005 mg/g), *P. convolvulus* (0.002 mg/g), and trace amount in *P. persicaria* (>0.001 mg/g). Other C-glycoside isovitexin was detected in *P. bistorta*- rhizoma (0.22 mg/g), *P. convolvulus* (0.025 mg/g), and *P. aviculare* (0.001 mg/g). Studied taxons (Table 1) contain acetylated flavonoid glycosides: quercetin-3-O-β-(6"-galloyl)-glucopyranoside and quercetin-3-O-β-(6"-galloyl)-galactopyranoside. The subspecies of *P. lapathifolium* are characterised by bigger than in other taxons amount of these compounds. *Polygonum lapathifolium* ssp. *nodosum* contain 0.36 mg/g and *Polygonum lapathifolium* ssp. *tomentosum* 0.19 mg/g quercetin-3-O-β-(6"-galloyl)-glucoside. *P. mite* (0.002 mg/g), *P. aviculare* (0.01 mg/g), and *P. hydropiper* (0.001 mg/g) contain smaller amount of this compound. Content of quercetin-3-O-β-(6"-galloyl)-galactoside was: 0.14 mg/g in *P. lapathifolium* ssp. *nodosum*, 0.09 mg/g in *P. lapathifolium* ssp. *tomentosum*, 0.07 mg/g in *P. persicaria*, 0.015 mg/g in *P. aviculare* and 0.01 mg/g in *P. hydropiper*.

Acetylated flavonoids are rare in Polygonaceae, however, quercetin-3-O-(2"-galloyl)-glucoside has been isolated from *P. nodosum* and described as having a moluscidal activity, quercetin-3-O-β-(2"-galloyl)-rhamnoside was isolated from *P. filiforme* (Kawasaki et al. 1986); quercetin-3-O-β-(2"-galloyl)-glucopyranoside, quercetin-3-O-β-(2"-galloyl)-rhamnopyranoside, quercetin-3-O-β-(6"-feruloyl)-galactoside were isolated as the inhibitors of superoxide by activity-guided fractionation from *Persicaria lapathifolia* (Kim et al. 2000).

The results of presented here chromatographical investigations corrspond with data described previously (Kawasaki et al. 1986; Bandjukova 1973). Species of *Polygonum* are richest in quercetin derivatives, among them, quercetin 3-glycosides.

It is worth emphasising that the herb of *Polygonum lapathifolium* ssp. *nodosum* and *Polygonum hydropiper* occured several times more the studied compounds than in other taxons. The total amount of quantitatively determined flavonoid glycosides in *P. lapathifolium* ssp. *nodosum* was 8.3 mg/g and in *P. hydropiper* was 7.0 mg/g, whereas in the remaining taxons it ranged between 1.1 mg/g and 3.2 mg/g.

In the two taxons *P. lapathifolium* ssp. *nodosum* and *P. lapathifolium* ssp. *tomentosum* the same set of flavonoid glycosides was detected, the differences concern only the content of these compounds. This similarity suggests that it is only right that these taxons should be treated as two subspecies of *Polygonum lapathifolium* and so they are treated by most botanists.

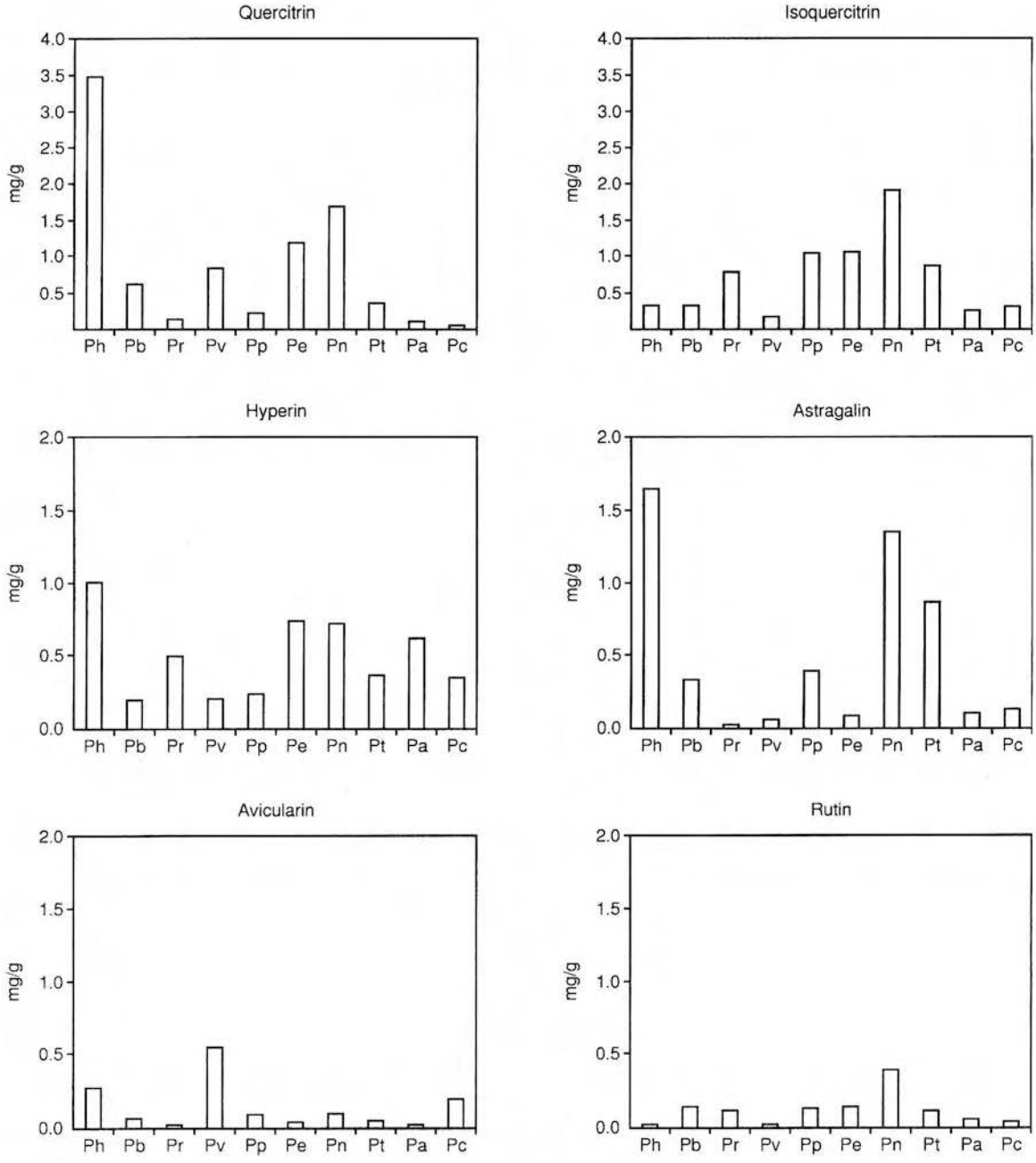

Fig. 2. Graphical presentation of selected flavonoid glycosides in the various taxons of *Polygonum* genus.
Ph – *P. hydropiper*, Pb – *P. bistorta* herb, Pr – *P. bistorta* rhizome, Pv – *P. aviculare*, Pp – *P. persicaria*, Pe – *P. mite*, Pn – *P. lapathifolium* ssp.*nodosum*, Pt – *P. lapathifolium* ssp.*tomentosum*, Pa – *P. amphibium*, Pc – *P. convolvulus* (*Bilderdykia convolvulus*).

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GLIKOZYDY FLAWONOIDOWE W DZIEWIĘCIU TAXONACH *POLYGONUM* L.

STRESZCZENIE

W następujacych taksonach *Polygonum* L.: *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, and *P. convolvulus* L. (*Bilderdykia convolvulus* L.) wykazano obecność dziewiętnastu glikozydów flawonoidowych. Określono zawartość czternastu głównych glikozydów. Badania wykonano przy użyciu wysokosprawnej chromatografii cieczowej (HPLC).

SŁOWA KLUCZOWE: *Polygonum*, Polygonaceae, glikozydy flawonoidowe, HPLC.