FLAVONOID GLYCOSIDES
IN NINE Polygonum L. TAXONS

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ABSTRACT

Eighteen flavonoid glycosides were identified in the following taxa of the Polygonum L. genus: P. hydropiper L., P. bistorta L., P. aviculare L., P. persicaria L., P. lapathifolium ssp. tomentosum (Schrank) DAns., P. lapa-
thifolium ssp. nodosum (Pers.) DAns., P. amphibiun L., P. mite Schrank, P. convolvulus L. (Bildendyka 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; Spanik 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 antitumour and chemopreventive effects (Stiess et al. 2000). Certain flavonoids possess a potency of inhibitory effect on several enzyme systems, initially connected to cell 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 taxaons studied in these works. The glycosides are as follows: rutin, hyperin, isoquercetin, quercetin-3-O-(2‘-galloyl)-glucoside, quercetin-3-O-glu-
curonide and eight sulphate derivatives of flavonoids from P. hydropiper (Kawasaki et al. 1986; Valentin and Wagner 1953; Bandjukova 1973; Haraguchi et al. 1996); rutin from P. bistorta (Bandjukova 1973); hyperin, quercetin, avicularin, myricetin-3-O-rhamnoglucoside and 5,7,3’,4’ tetrahydroxy (3-O-rhamnoside) flavanone from P. aviculare (Hegnauer 1969; Bandjukova 1973; Chvorost and Konisa-
renko 1980); hyperin, isoquercetin, avicularin from P. per-
sicaria (Bandjukova 1973); isoquercetin, quercetin-3-O-β-
(2’-galloyl)-glucopyranoside, kaempferol-3-O-β-(2’-gal-
loyl)-glucopyranoside and 3-hydroxy-5-methoxy-6,7-
methylenedioxy-flavanon from P. lapathifolium ssp. nodosum (Isobe et al. 1979; Kuroynagi et al. 1982); rutin, hy-
perin, quercetin, isoquercetin, quercetin-3-O-β-glucuronidi-
de, 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, luthe-
olin-7-O-glucoside, quercetin-7-O-glucoside from P. amphi-
bium (Machkanova 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 Matysi-
sk 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 Hewlett-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 guard column (4 mm) 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. nire Schrank, P. lapathifolium* ssp. *tomentosum* (Schrank) Dans, *P. lapathifolium* ssp. *nodosum* (Pers) Dans, *P. amphibium* L., *P. convolvulus* L. (*Bidentadya 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, guaiavirin 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 DISCUSSION**

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. phosphoric 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 compared with authentic reference compounds. Distribution of these compounds is presented in Table 1.
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 guaijaverin 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-rutinoside 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-rutinoside was 0.1 mg/g and 0.06 mg/g in P. aviculare and P. persicaria 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 molusidal activity, quercetin-3-O-β-(2'-galloyl)-rhamnoside was isolated from P. filifolium (Kawasaki et al. 1986); quercetin-3-O-β-(2'-galloyl)-glycopyranoside, 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 chromatographic investigations correspond 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 occurred 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 sub-species of Polygonum lapathifolium and so they are treated by most botanists.
LITERATURE CITED


Fig. 2. Graphical presentation of selected flavonoid glycosides in the various taxons of Polygonum genus.

GLIKOZODY FLAWONOIDOWE W DZIEWIĘCIU TAXONACH Polygonum L.

STRESZCZENIE


SŁOWA KLUCZOWE: Polygonum, Polygonaceae, glikozydy flawonoidalne, HPLC.