CHROMATOGRAPHICAL ANALYSIS OF PHENOLIC ACIDS IN SOME SPECIES OF *POLYGONUM* L. GENUS. PART 2 QUANTITATIVE DETERMINATION OF THE MAJOR COMPONENTS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

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ABSTRACT

Nine major phenolic acids (protocatechuic, gentisic, p-hydroxybenzoic, vanillic, caffeic, syringic, p-coumaric, ferulic, synapic) were investigated in 9 species of the genus *Polygonum* L. The total amount of these compounds equated 65.8 µg/g of dry herb *in Polygonum persicaria* L., 61.2 µg in *P. convolvulus* L., 59.1 µg in *P. lapathifolium ssp. nodosum* (Pers.) Dans, 53.3 µg in *P. bistorta* L., 42.1 µg in *P. mite* Schrank, 38.2 µg in *P. lapathifolium ssp. tomentosum* (Schrank) Dans, 37 µg in *P. amphibium* L., 33.2 µg in *P. hydropiper* L., 31.1 µg in *P. aviculare* L., and 14,1 µg in rhizome *P. bistorta* L.

Among the analysed phenolic acids, synapic acid (28.6 µg/g) in *P. persicaria* L., protocatechuic acid (34 µg/g) in *P. lapathifolium ssp. nodosum* (Pers) Dans, and ferulic acid (21 µg/g) in *P. bistorta* L., were dominating.

KEY WORDS: *Polygonum*, phenolic acids, HPLC.

INTRODUCTION

Phenolic acids occuring in plants exibit pharmacological activity. The therapeutic effects, are shown by the following acids: gallic, caffeic, vanillic, p-coumaric acid – antibacterial and anti – inflamatory activity (Masquelier 1965; Kroes 1992); ferulic – antibacterial, antiphlogistic and antypyretic activites (Kohlmünzer 1993; Negver 1978); caffeic, chlorogenic acid – immunostimulatory activity (Sawicka 1994), ellagic acid – anticartinogenic properties (Laacra-Pina 1996).

Biological activity of plant preparations depends on the composition of active ingredients and on their concentration. Therefore the aim of the study is to estimate the content of major phenolic acids in 10 raw materials of the genus *Polygonum* L., using HPLC as the best method for plant material examinations (Dzido 1994).

MATERIAL AND METHODS

Plant Material

Herbs of nine taxons of *Polygonum* L. – *P. persicaria* L., *P. mite* Schrank, *P. convolvulus* L., *P. lapathifolium ssp. nodosum* (Pers) Dans, *P. lapathifolium ssp. tomentosum* Schrank. *P. amphibium* L., *P. aviculare* L., *P. bistora* L., *P. hydropiper* L., and rhizome *P. bistorta* L. were used in my study. The plant materials were collected from different places in Samoklęski near Lublin in 1996. 50 g samples of dry herbs and rhizome were analysed. A procedure for the isolation of fraction containing free phenolic acids (A) has been earlier described (Smolarz 1999).

Sample clean-up

Samples with free phenolic acids were purified from fatty components and chlorophyl by SPE (solid phase extraction). The fractions of phenolic acids were dissolved in 50% methanol and applied to microcolumns (Octadecyl, 500 mg, 3 ml, J.T. Backer, Germany). Previously the microcolumns were washed with 10 ml of metanol and conditioned with water (10 ml). The samples were passed through the column of the sorbent under reduced pressure using chamber SPE-12G (J.T. Backer, Germany).

HPLC – conditions

The experiment was carried out using Hewlett – Packard (Palo, Alto, CA, USA) Model 1050 liquid chromatograph with a 20mn sample injector (Rheodyne, Cotati, CA, USA) and a UV detector (UV-VIS) operated at 254 nm.

The stainless-steel column 200 × 4.6 mm J.D, was filled with 5 mm Hypersil ODS (Shandon, Cheshire, UK). The isocratic mobile phase consisted of methanol – water (25:75) with 5% v/v acetic acid. The flow rate was 1 ml/min. The chromatograms were recorded with a Hewlett – Pacard Model 3396A reporting integrator, chart speed 0.5 cm/min. Chromatography was preformed at room temperature.

RESULTS AND DISCUSSION

The content of nine phenolic acids was detemined by RP – HPLC method. The retention times of the peaks of the samples were compared with authentic reference compounds (Table 1). The amount of each phenolic acid was determined on the basis of comparison of peak heights.

TABLE 1. Retention times of phenolic acids.
Column 200 × 4.6 mm, I.D. 5 µm Hypersil ODS. Eluent: MeOH – H_2O (25+75) with 5% v/v acetic acid.

No Standard	t.R (min)
1. protocatechuic acid	4.8
2. gentisic acid	5.8
3. p – hydroxybenzoic acid	8.1
4. vanillic acid	10.1
5. caffeic acid (trans)	9.4
5'. caffeic acid (cis)	11.0
6. syringic acid	11.9
7. p – coumaric acid (trans)	16.2
7'. p – coumaric acid (cis)	20.9
8. ferulic acid (trans)	22.6
8'. ferulic acid (cis)	26.3
9. synapic acid	30.9

I show that phenolic acids concentration is higher in herbs and lower in rhizome (Fig. 2). The amount of individual phenolic acids in *Polygonum bistorta* L. does not exceed 2.9 µg/g in dry rhizome tissue. The concentration of most phenolic acids in the herb of *P. bistorta* is bigger than in rhizome, except caffeic acid, which in the herb occurs in small amount. The protocatechuic acid is present in big amount in the following herbs: *Polygonum bistorta* L. (13.5 µg/g), *Polygonum convolvus* L. (12.7 µg/g) and *Polygonum amphibium* L. (11.4 µg/g) in *Polygonum lapathifolium ssp. nodosum* (Pers.) Dans it is predominant among the analysed compounds (34 µg/g). The herb *Polygonum bistorta* L. stands out against other drugs in terms of content of ferulic acid (22 µg/g).

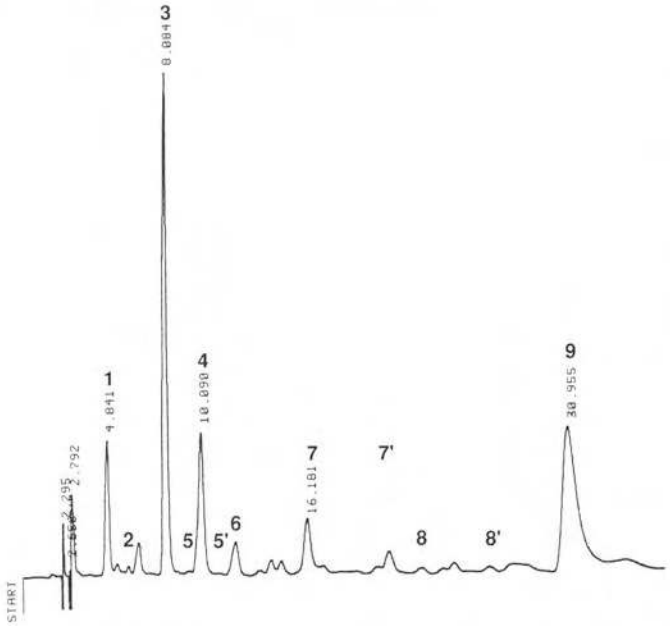

Fig. 1. HPLC chromatogram of phenolic acids from herb of *Polygonum persicaria* L. Numbered peaks are identified in Table 1.

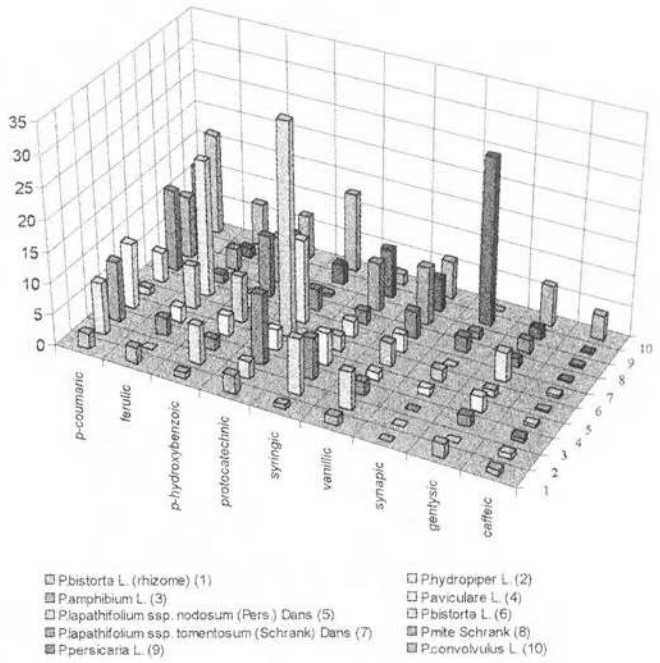

Fig. 2. Content and distribution of phenolic acids in the investigated plants (in µg/g).

The p – coumaric acid is the major component *in Polygonum aviculare* L. (10.5 µg/g), *P. mite* Schrank (10.1 µg/g), *P. convolvulus* L. (16.5 µg/g), *P. lapathifolium ssp. tomentosum* (Schrank) Dans (13.1 µg/g), *P. amphibium* L. (9.7 µg), and *P. hydropiper* L. (8.5 µg/g). Caffeic acid is present in the investigated raw materials in small amount (0.3-3.9 µg/g). The gentisic acid is usually present in plant in the bounded state. Therefore in the free state its concentration was low and it ranged from 1 µg to 6.5 µg/g; in *P. hydropiper* L. it was not found. The synapic acid was found in six out of nine of the analysed raw materials. The *P. persicaria* L. is a species which includes 26.8 µg/g of this acid in one gram of dry harb, which is about 39% of all phenolic acids investigated by HPLC method. The chromatogram of phenolic acids from herb *P. persicaria* L. (Fig. 1) shows predominant peak (No 9) of synapic acid. In other plants the content of synapic acid ranges from 1 µg to 2.5 µg/g. The content of vanillic acid varies between 1.3 µg (*P. aviculare*) and 3.9 µg (*P. mite*). The content of syringic acid ranges between 0.8 µg (rhizome bistorta) and 8.9 µg/g (*P. hydropiper*).

Figure 3 shows us the total amount of phenolic acids. Large concentration of nine phenolic acids was detected *in P. persicaria* L. (65.8 µg/g), *P. convolvulus* L. (61.2 µg/g), *P. bistorta* L. (53.3 µg/g) and *P. lapathifolium ssp. nodosum* (Pers.) Dans (59.1 µg/g). Lower amnount of phenolic acids was detected in *P. lapathifolium ssp. tomentosum* (Schrank) Dans (38.2 µg/g) *P. hydropiper* L. (33.2 µg/g), *P. amphibium* L. (37 µg/g), *P. mite* Schrank (42.1 µg/g), *P. aviculare* L. (31.1 µg/g). Of all the analysed drugs rhizome bistorta showed the smallest content of these acids.

On the basis of the obtained results one can expect that, due to considerable amount of phenolic acids, *Polygonum persicaria* L., *Polygonum convolvulus* L. and *Polygonum lapathifolium ssp. nodosum* (Pers.) Dans, may have synergistic influence on biological activity of those drugs.

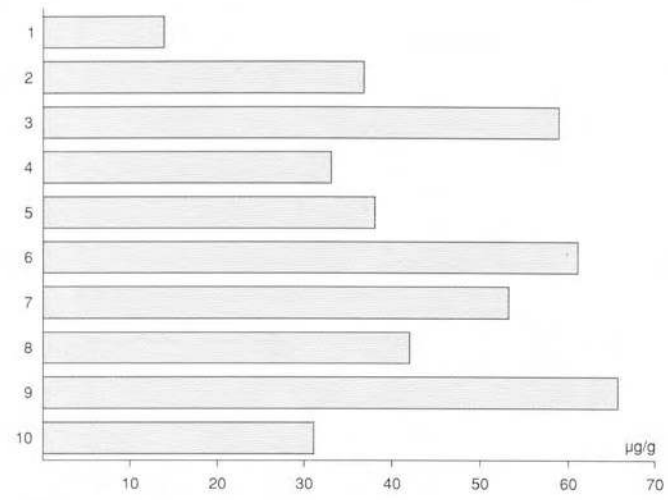

Fig. 3. The total amount of detected phenolic acids (in µg/g).
1 – *P. bistorta* L. (rhizome), 2 – *P. amphibium* L., 3 – *P. lapathifolium ssp. nodosum* (Pers.) Dans, 4 – *P. hydropiper* L., 5 – *P. lapathifolium ssp. tomentosum* (Schrank) Dans, 6 – *P. convolvulus* L., 7 – *P. bistorta* L., 8 – *P. mite* Schrank, 9 – *P. persicaria* L., 10 – *P. aviculare* L.

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CHROMATOGRAFICZNA ANALIZA KWASÓW FENOLOWYCH W KILKU GATUNKACH Z RODZAJU *POLYGONUM* L. CZ. 2 ILOŚCIOWE OZNACZENIE GŁÓWNYCH SKŁADNIKÓW METODĄ WYSOKOSPRAWNEJ CHROMATOGRAFII CIECZOWEJ

STRESZCZENIE

Przy użyciu RP-HPLC określono zawartość kwasu protokatechowego, gentyzowego, p-hydroksybenzoesowego, wanilinowego, kawowego, syryngowego, p-kumarowego, ferulowego i synapowego w 9 taksonach z rodzaju *Polygonum* L. Sumaryczna zawartość tych składników wynosiła od 65,8 µg/g suchego ziela w *P. persicaria* L., 61,2 µg w *P. convolnlus* L., 59,1 µg w *P. lapathifolium ssp. nodosum* (Pers.) Dans, 53,3 µg w *P. bistorta* L., 42,1 µg w *P. mite* Schrank., 38,2 µg w *P.lapathifolium ssp. tomentosum* (Schrank) Dans, 37 µg w *P. amphibium* L., 33,2 µg w *P. hydropiper* L., 31,1 µg w *P. aviculare* L. i 14,1 µg w kłączu *P. bistorta* L. Spośród analizowanych kwasów w *P. persicaria* L. dominował kwas synapowy (28,6 µg/g), w *P. lapathifolium ssp nodosum* (Pers.) Dans, – protokatechowy (34 µg/g) a w *P. bistorta* L. w największym stężeniu występował kwas ferulowy (21 µg/g).

SŁOWA KLUCZOWE: Polygonum, kwasy fenolowe, HPLC.