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Mirosław Angielczyk, Katarzyna Bączek, Anna Geszprych, Jarosław L. Przybył , Zenon Węglarz

Department of Vegetable and Medicinal Plants, Warsaw University of Life Sciences – SGGW, Nowoursynowska 159, 02-776 Warsaw, Poland

CHEMICAL DIVERSITY OF SILVERWEED (*POTENTILLA ANSERINA* L.) GROWING AT THE EDGES OF ARABLE FIELDS

ABSTRACT

The medicinal usage of silverweed herb (Anserinae herba) is primarily related to the presence of phenolic compounds. Three populations of silverweed (Potentilla anserina L.) occurring at the edges of arable fields in Podlasie area (north-eastern Poland) were compared in respect of the content and composition of phenolics. Herb collected from the investigated populations differed in the content of determined compounds. The tannin content was very low (0.81-0.91%). The total flavonoid content ranged from 0.48 to 0.60%. Phenolic acid content was much higher (1.38-2.26%). Three flavan-3-ols: (+)-catechin, (-)-epicatechin, and (-)-epigallocatechin, four flavonol glycosides: rutoside, quercetin-3-O-glucoside, isorhamnetin-3-O-glucoside, and kaempferol-3-O-glucoside, as well as one phenolic acid (ellagic acid) were identified as a result of HPLC analysis of methanolic extracts from the investigated raw materials. The most considerable differences between populations concerned the content of (+)-catechin (87.0-199.3 mg × 100 g⁻¹), (-)-epigallocatechin (141.9-290.6 mg × 100 g⁻¹), and rutoside (192.3-386.0 mg × 100 g⁻¹) in herb.

Key words: catechins, flavonoids, phenolic acids, populations, secondary metabolites, silverweed herb, tannins

INTRODUCTION

Silverweed (*Potentilla anserina* L., *Rosaceae*) is a perennial stoloniferous plant occurring mainly in moist meadows and pastures, often on river banks, and in ruderal plant communities. This species is rarely a weed of economic concern in arable fields, but can be troublesome in field margins, especially in low-input system (Gabriel and Tscharntke 2007, Hyvönen and Salonen 2002). Silverweed propagates both by seeds and vegetatively, according to environmental conditions (Eriksson 1985). Silverweed herb is used as a medicinal raw material mainly because of its anti-diarrheic and spasmolytic activity. Antimicrobial and antiviral properties of silverweed extracts have been also proved (Tomczyk and Latte 2009, Tomczyk *et al.* 2008, Zhao *et al.* 2008). Medicinal usage of silver-

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weed stems from the presence of phenolic compounds, especially tannins and flavonoids (Wichtl 2004).

As yet, intraspecific chemical diversity of this species has not been taken into consideration when collecting raw material from wild growing plants. The aim of this preliminary study was to compare populations of silverweed growing at the edges of arable fields in three locations in Podlasie area (north-eastern Poland) in respect of the content of phenolic compounds.

MATERIAL AND METHODS

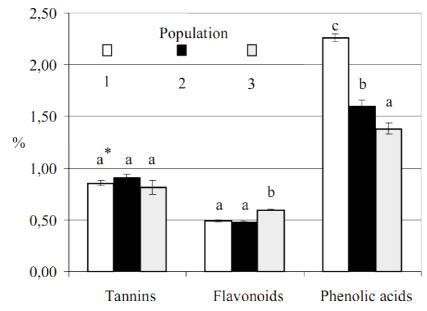
Plant samples were obtained from three populations of silverweed occurring on the edges of arable fields: population 1 – near Brańsk (N 52° 44.296' E 22° 49.397', 120 m above sea level), population 2 - in Spieszyn (N 52° 39.683' E 22° 48.959', 125 m), and population 3 – in Koryciny-Borki (N 52° 39.412' E 22° 45.987', 122 m). Plants were harvested at the beginning of blooming stage (first ten days of June 2008) and dried at 40°C. The content of three groups of phenolic compounds (flavonoids, phenolic acids, and tannins) in the samples was determined using spectrophotometric methods (Farmakopea Polska VI, 2002). Contents of flavonoids were expressed as quercetin equivalents, phenolic acids - as caffeic acid, and tannins - as pyrogallol. For the separation and identification of individual phenolic compounds, 1 g of dry raw material was extracted with methanol in Büchi B-811 Extraction System. After evaporation of the solvent, the residue was dissolved in 5 ml of methanol, filtered (Supelco IsoDisc PTFE 25 mm \times 0.45 μ m), and subjected to HPLC. The analysis was carried out using a Shimadzu chromatograph with SPD-M10A VP DAD detector and a Supelco LC RP 18 column (5 μm, 250 mm × 4.6 mm). a gradient of 10% acetonitrile in water and 55% acetonitrile in water at pH 3 was applied. Other analysis conditions used were: flow rate 1 ml × min⁻¹, oven temperature 28°C, time of analysis 60 min, recorded wave range: 190-450 nm, detection wave length: 206 nm (flavan-3-ols), 254 nm (rutoside, ellagic acid, quercetin-3-O-glucoside, isorhamnetin-3-O-glucoside), and 264 nm (kaempferol-3-O-glucoside). Peaks were identified by comparison of retention time and spectral data with adequate parameters of standards. Quantification was based on the peak area. The content of the determined compounds was calculated in $mg \times 100 g^{-1}$ dry matter.

The results were analysed statistically for differences between sampled populations with ANOVA (Tukey's HSD test at the 0.05 significance level) in Statgraphics Plus for Windows v. 4.1.

RESULTS AND DISCUSSION

The medicinal usage of silverweed herb (Anserinae herba) is primarily related to the presence of phenolic compounds. Silverweed herb is considered to be a tannin-rich raw material with the content of these compounds reaching 10%

(Strzelecka and Kowalski 2000, Wichtl 2004). According to DAC (German Drug Codex Supplement to the Pharmacopoeia), the tannin content in silverweed herb should not be less than 2% (Wichtl 2004). In our study the content of tannins in the silverweed herb appeared to be very low (0.81-0.91%) (Fig. 1). These compounds reveal antidiarrheic, anti-inflammatory, antibacterial, antioxidant, and antimutagenic activity (Borkowski and Miłkowska 1997, Schimmer and Lindenbaum 1995). Flavonoids constitute another group of active compounds in silverweed herb. They are considered to be responsible for the spasmolytic and choleretic activity of the raw material (Youngken et al. 1949). The total content of flavonoids in the investigated plant material was rather low (0.48-0.60%). Phenolic acids were present in higher amount, but their content in the raw materials obtained from the studied populations differed considerably (1.38-2.26%) (Fig. 1).



* For particular groups of compounds values marked with the same letter do not significantly differ at =0.05 (Tuckey's test)

As a result of HPLC analysis of methanolic extracts from the investigated raw materials eight phenolic compounds were identified (Table 1). Three of them were recognised as flavan-3-ols: (+)-catechin, (-)-epicatechin, and (-)-epigallocatechin (Table 1). The presence of (+)-catechin in silverweed herb has been previously reported by Kombal and Glasl (1995). The raw material obtained from the population 1 was distinguished by highest content of this compound. The population 3 was characterised by significantly lower content of all three catechins in comparison with the populations 1 and 2.

 $\label{eq:table 1} Table~1~$ Content of identified phenolic compounds in silverweed herb (mg $\times~100~g^{-1})$

	Population		
Phenolic compound	1	2	3
(-)-Epigallocatechin	290.6 b*	278.3 b	141.9 a
(+)-Catechin	199.3 с	169.4 b	87.0 a
(-)-Epicatechin	302.3 b	312.8 b	198.1 a
Rutoside	386.0 с	234.7 b	192.3 a
Quercetin-3-O-glucoside	420.2 b	535.9 с	314.1 a
Isorhamnetin-3-O-glucoside	136.9 b	133.5 b	103.3 a
Kaempferol-3-O-glucoside	78.4 b	68.0 ab	58.3 a
Ellagic acid	405.8 b	426.0 c	363.0 a

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Four flavonol glycosides (rutoside, quercetin-3-O-glucoside, isorhamnetin-3-O-glucoside, and kaempferol-3-O-glucoside), and one phenolic acid (ellagic acid) were also identified in the evaluated raw materials. Kombal and Glasl (1995) have found eleven flavonol glycosides in the aerial parts of silverweed grown in Germany, including quercetin-3-O-glucoside and kaempferol-3-O-glucoside. Ellagic acid has been previously reported as a constituent of silverweed herb by Krzaczek (1984). The herb collected from the plants of the population 3 was characterised by significantly lower content of all determined flavonol glycosides and ellagic acid in comparison with the raw materials obtained from the populations 1 and 2. The population 1 was characterised by higher content of rutoside and lower content of quercetin-3-O-glucoside and ellagic acid than the population 2. Distinctly lower content of all determined phenolic compounds in the herb obtained from the population 3 seems to be a result of light conditions. Plants of this population were shaded by broadleaf trees – silver birch (*Betula pendula* Roth) and black poplar (*Populus nigra* L.).

CONCLUSIONS

Herb collected from the investigated populations of silverweed differed in the content of determined phenolic compounds.

Population 3 was distinguished by significantly lower content of all identified compounds in comparison with two others. The most considerable differences concerned the content of (+)-catechin (87.0-199.3 mg \times 100 g⁻¹), (-)-epigallocatechin (141.9-290.6 mg \times 100 g⁻¹), and rutoside (192.3-386.0 mg \times 100 g⁻¹).

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