Morphological and chemical variability within the population of common avens *(Geum urbanum* L.*)*

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S u m m a r y

The aim of the study was an *ex situ* evaluation of morphological and chemical variability within the population of common avens. Plant height, number of shoots, stage of generative development (number of flower buds, flowers, and fruits), as well as weight of herb and underground organs were determined. In the raw materials total content of tannins and content of particular phenolic compounds identified by HPLC was determined. In underground organs also content of eugenol – the dominant constituent of essential oil was assessed. High diversity of morphological traits (especially weight of herb and underground organs) and the content of determined compounds (especially in herb) was found. Above- and underground organs differed in the composition and content of phenolic compounds. Underground organs were characterised by higher content of flavan-3-ols, but they did not contain chlorogenic acid, which was the dominant phenolic compound in herb.

Key words: *herb, underground organs, phenolic compounds, tannins, phenolic acids, eugenol*

INTRODUCTION

Common avens (*Geum urbanum* L., *Rosaceae*) is the most commonly occurring species of the *Geum* genus, both in Europe and in temperate zones of Asia and North America. It is a perennial growing in meso- and eutrophic deciduous forests,

as well as at forest clearings, roadsides and other ruderal habitats. It prefers moderately moist, sunny or semi-shady stands, and rich, slightly acidic soils [1-3]. Common avens is an ethnopharmaceutically interesting species. Both its herb and underground organs have been used in folk medicine. The main biologically active compounds in these raw materials are tannins and phenolic acids. Underground organs contain essential oil, with eugenol as a dominant constituent [1, 3-6]. The raw materials reveal antibacterial and astringent activity, they are used in digestive and respiratory tracts disorders as well as in skin diseases [2, 7].

The aim of this study was an *ex situ* evaluation of the variability within the population of common avens in respect to morphological traits of plants and content of biologically active compounds in their aboveground and underground organs.

MATERIALS AND METHODS

The object of the study was the population of common avens (*Geum urbanum* L.) originating from a natural site near Siemiatycze (Podlasie region) and grown at the experimental field of the Department of Vegetable and Medicinal Plants (WULS-SGGW) in Warsaw. In the second year of plant vegetation 10 individuals were randomly selected (from 200 individuals) for morphological and chemical evaluation. At the stage of full blooming (July 2008) the following morphological traits of plants were investigated: height, number of shoots, number of flower buds, fully developed flowers, and fruits. Herb for chemical analysis was collected at the stage of full blooming, whereas underground organs – in autumn (November 2008). Air-dry raw materials were subjected to chemical evaluation. All analyses were done in three replications.

The total content of tannins in the raw materials was determined according to Polish Pharmacopoeia [8]. For the qualitative and quantitative determination of phenolic compounds 1.0 g of air-dry, grounded raw material was extracted exhaustively with 100 ml of methanol in B-811 Extraction System (Büchi). After evaporation of solvent, the residue was dissolved in 10 ml of methanol, filtered through a Supelco IsoDisc PTFE 25 mm $\times$ 0.45 μm filter, and subjected to HPLC. The analysis was carried out using the Shimadzu chromatograph with SPD-M10A VP DAD detector equipped with Luna 5 μm C18 (2) 250 mm $\times$ 4.6 mm column (Phenomenex). The gradient of 10% acetonitrile (ACN) in water (A) and 55% ACN in water (B) was used, at the flow rate of 1.0 ml/min. The analysis lasted 40 min., recorded wave ranges were: 190-900 nm, detection wave length: 206 nm (/+/-catechin, /-/-epicatechin, /-/-epicatechin gallate, /-/-epigallocatechin, /-/-epigallocatechin gallate), 254 nm (ellagic acid), 280 nm (gallic acid), and 330 nm (chlorogenic acid). 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/100 g dry matter.

In order to determine eugenol content in the underground organs of the investigated plants the volatile compounds were extracted by SPME (Solid Phase Microextraction). 0.5 g of raw material was placed in a glass vial and poured with 5 ml of 5% NaCl solution. Carboxen-polydimethylsiloxane-divinylbenzene (CAR-PDMS-DVB) coating fibre was used for the adsorption of volatile compounds. Extraction was carried out at 60ºC for 30 minutes.

Gas chromatography of these compounds was performed on the HP 6890 chromatograph with the capillary column Carbowax 20M (length 25 m, diameter 0.32 mm, film thickness 0.3 μm). The following conditions of analysis were applied: detector temperature 250ºC, injector temperature 220ºC, carrier gas – helium with the flow rate of 2.3 ml/min. Column temperature was programmed as follows: 100ºC (2 min), temperature rise 4ºC/min to 220ºC (5 min.). The content of eugenol was calculated in mg/100 g dry matter.

The presented results comprise minimum, maximum, and mean value, as well as coefficient of variation (CV) for each of the investigated morphological traits and determined chemical compounds.

RESULTS AND DISCUSSION

The available literature on common avens concerns mainly ecology and physiology of this species [9-11]. There are few reports on chemical composition of herb and underground organs of this plant. None of these studies concerned *ex situ* evaluation of intraspecific or individual variability of this species. Taking into consideration that common avens is an allogamous, insect-pollinated plant, and that seeds of this species are dispersed by animals [9, 12], such variability may be expected. The results obtained in present study proved high diversity of the investigated population of common avens in respect to morphological and chemical traits of selected individuals. Plant height ranged from 73 to 115 cm, number of shoots – from 10 to 40 (tab. 1). Flowering of common avens and thus fruit setting is not simultaneous. At the same time on each of the investigated plants generative organs at different stages of development were found, i.e. flower buds, flowers, and fruits. The individuals differed in the number of particular generative organs. The highest coefficient of variation was found for the number of flowers (tab. 1).

The investigated plants differed markedly also in the weight of herb (44.5–191.9 g) and underground organs (22.2–62.3 g) (tab. 1). The range of variability (indicated by coefficient of variation) in respect of these parameters was similar. Both of these raw materials are considered to be rich in tannins. The content of tannins in herb of common avens collected at the stage of full blooming may amount to 20% [2, 5], and in underground organs – to 30% [1]. However, in the cited papers there is no information either on the method of determination of tannin content or chemical character of determined compounds (hydrolysable or condensed tannins). In present study the total content of tannins determined

according to Polish Pharmacopoeia [8] appeared to be relatively low: 1.16–7.44 % in herb (tab. 2), and 3.30-5.16% in underground organs (tab. 3). With the exception of one of the investigated plants, the underground organs were characterised by higher content of tannins in comparison with herb. There was no correlation between the content of tannins in above- and underground plant parts.

Table 1.

Variability within the investigated population in respect of morphological traits

	plant height [cm]	number of shoots per plant	number of generative organs per plant (at the stage of full blooming)			weight of air-dry raw material [g/plant]	
			flower buds	flowers	fruits	herb	underground organs
minimum	73	10	34	10	38	44.5	22.2
maximum	115	40	73	32	68	191.9	62.3
mean	93	25	53	19	55	111.1	32.6
CV (%)	14	35	22	37	19	46	41

CV – coefficient of variation

Table 2.

Variability within the investigated population in respect of the content of biologically active compounds in herb

	tannins (%)	flavan-3-ols [mg/100 g]		phenolic acids [mg/100 g]		
		(+)-C	(-)-ECG	chlorogenic acid	ellagic acid	gallic acid
minimum	1.16	14.02	5.83	15.63	12.77	9.87
maximum	7.44	47.02	73.65	289.51	125.84	89.18
mean	2.50	25.93	24.53	78.20	40.46	30.10
CV (%)	72	38	87	101	82	74

C – catechin, ECG – epicatechin gallate; CV – coefficient of variation

Table 3.

Variability within the investigated population in respect of the content of biologically active compounds in underground organs

	tannins (%)	flavan-3-ols [mg/100 g]					phenolic acids [mg/100 g]		eugenol [mg/100 g]
		(+)-C	(-)-EC	(-)-ECG	(-)-EGC	(-)-EGCG	ellagic acid	gallic acid	
minimum	3.30	106.56	29.36	4.35	33.69	50.20	8.33	39.12	5.61
maximum	5.16	225.97	55.99	33.58	129.93	111.08	80.84	121.83	14.54
mean	4.19	151.13	42.56	14.97	75.79	82.66	54.95	60.32	11.35
CV (%)	15	25	20	55	46	27	40	44	29

C – catechin, EC – epicatechin, ECG – epicatechin gallate, EGC – epigallocatechin, EGCG – epigallocatechin gallate;
CV – coefficient of variation

As a result of HPLC analysis three phenolic acids (gallic, ellagic, and chlorogenic acid) and two flavan-3-ols (/+/-catechin and /-/-epicatechin gallate) were identified in herb of the studied plants (tab. 2), whereas in underground organs two phenolic acids (gallic and ellagic one) and five flavan-3-ols (/+/-catechin, /-/-epicatechin, /-/-epicatechin gallate, /-/-epigallocatechin, and /-/-epigallocatechin gallate) were found (tab. 3). The presence of catechin, gallic acid and ellagic acid in underground organs of common avens has been previously reported [6].

In the majority of investigated plants the dominant phenolic compound of herb was chlorogenic acid. The individuals differed significantly in respect of its content (15.63–289.51 mg/100 g, CV=101%). In underground organs (+)-catechin was present in the highest amount (106.56–225.97 mg/100 g). The investigated individuals were much more diverse in respect of the accumulation of all determined phenolic compounds in herb than in underground organs.

Underground organs of common avens contain essential oil with eugenol as a dominant constituent (65-75%) [5]. The content of eugenol in underground organs of the investigated plants ranged from 5.61 to 14.54 mg/100 g (tab. 3).

CONCLUSIONS

The obtained results confirm the expected variability of the investigated population of common avens in respect both to morphological traits of plants (height, number of shoots, number of generative organs, and the most important from a practical point of view, weight of herb and underground organs that are used as medicinal raw materials) and accumulation of biologically active compounds in the above- and underground organs. Interindividual chemical variability concerned mainly the content of phenolic compounds in herb. However, underground organs appeared to be richer in these compounds (with exception of chlorogenic acid) in comparison with herb, thus representing greater value for phytopharmaceutical industry.

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ZRÓŻNICOWANIE MORFOLOGICZNE I CHEMICZNE W OBRĘBIE POPULACJI KUKLIKA POSPOLITEGO (*GEUM URBANUM* L.)

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S t r e s z c z e n i e

Celem pracy była ocena zróżnicowania morfologicznego i chemicznego w populacji kuklika pospolitego w warunkach jego uprawy. Określono wysokość roślin, liczbę pędów kwiatowych, stopień rozwoju generatywnego (liczbę pąków kwiatowych, kwiatów i owoców) oraz masę ziela i organów podziemnych. W zebranych surowcach oznaczono ogólną zawartość garbników oraz zawartość poszczególnych związków fenolowych zidentyfikowanych przy użyciu HPLC. W organach podziemnych określono również zawartość eugenolu – głównego składnika olejku eterycznego. Stwierdzono wyraźne zróżnicowanie badanych osobników pod względem cech morfologicznych, a zwłaszcza masy ziela i organów podziemnych, a także zawartości oznaczanych związków, szczególnie w zielu. Organy nadziemne i podziemne różniły się pod względem składu i zawartości związków fenolowych. Organy podziemne charakteryzowały się wyższą zawartością flawan-3-oli. Nie stwierdzono w nich natomiast obecności kwasu chlorogenowego, który był dominującym związkiem fenolowym w zielu.

Słowa kluczowe: *ziele, organy podziemne, związki fenolowe, garbniki, fenolokwasy, eugenol*