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EXPERIMENTAL PAPER

Accumulation of biomass and phenolic compounds in Polish and Mongolian great burnet (*Sanguisorba officinalis* L.) populations

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S u m m a r y

Eight Polish and six Mongolian great burnet populations were evaluated *ex situ* in respect of the mass of underground organs and accumulation of biologically active compounds. In the raw materials collected in autumn of the second year of plant vegetation, total content of tannins and phenolic acids as well as the content of phenolic compounds identified by HPLC were determined. Distinct differences between Polish and Mongolian populations and their high intraspecific variability, concerning analysed parameters, was observed. The mass of underground organs of Polish populations was higher than in Mongolian ones (595.0 and 523.5 g × plant^{-1}, respectively, for fresh mass). Polish populations were characterised by significantly higher total content of tannins and phenolic acids (6.02 and 1.60%, respectively) in comparison with Mongolian ones (2.89 and 0.97%, respectively). In the investigated raw materials eight phenolic compounds were identified, namely: /-/-epigallocatechin, /+/-catechin, /-/-epicatechin, /-/-epicatechin gallate, /-/-epigallocatechin gallate, astragalin, ellagic and gallic acids. In all populations, the dominating compound, was /-/-epigallocatechin. The contents of /-/-epigallocatechin, /-/-epicatechin gallate and gallic acid were distinctly higher in Polish populations.

Key words: *great burnet, populations, phenolic compounds, HPLC*

INTRODUCTION

Great burnet (*Sanguisorba officinalis* L., *Rosaceae*), is a perennial growing wild on wet grasslands, hillside meadows and woodland in Europe, Asia and northern regions of North America [1]. In Poland it occurs mostly in southern regions, whereas in Mongolia it is spread through the whole country. The raw material collected from this plant are underground organs (rhizomes and roots) known for its healing properties [1-4]. They are used in the treatment of fever, bleeding, diarrhoea, haemorrhoids and burns. In traditional Chinese medicine it is said to cool the blood, clear heat and heal wounds [3-6]. The active compounds responsible for the biological activity of great burnet roots are polyphenol compounds, in particular flavan-3-ols and phenolic acids as well as sterols and saponin compounds [1-3, 7]. The herb of great burnet is sometimes used as a medicinal raw material too. Fresh leaves of young plants are added as a seasoning to salads and meat dishes in some western countries as well [1]. So far, the raw materials of great burnet for medicinal purposes have been collected almost exclusively from wild growing plants, which are genetically and chemically differentiated [5, 8].

The aim of undertaken study was to investigate, in *ex situ* conditions, the chemical diversity of 14 great burnet populations originating both from Poland and from Mongolia.

MATERIALS AND METHODS

Plant material

Eight populations of great burnet originating from southern part of Poland (PL) and six from northern Mongolia (M) were the object of the study (tab. 1). At each wild growing population, both in Poland and Mongolia, the seeds were collected in the autumn, 2009. The species identification was made according to Broda and Mowszowicz [9] as well as Boldsaikhan [10] by Węglarz and Bączek (Department of Vegetable and Medicinal Plants, WULS-SGGW). The voucher specimens of the populations' seeds are kept in the National Centre for Plant Genetic Resources, Polish GeneBank. Seed samples, from 20–30 randomly chosen plants per population, were sown in the greenhouse in March, 2011. The seedlings were planted out at the experimental field of Department of Vegetable and Medicinal Plants in May. From each population, 40 seedlings were planted in spacing 50 × 50 cm. The underground organs were collected in the second year of plant vegetation (2012) at the end of October. They were dried at 40°C. The weight of roots per plant was determined and the raw materials were subjected to chemical analysis.

Table 1.

Geographical coordinates of great burnet natural sites

Population No.	Region of origin	Geographical coordinates	
	Polish populations		
1PL	Siemiatycze (Podlasie)	N 52°02.71´	E 022°53.12´
2PL	Pustelnik (Sudety)	N 50°51.02´	E 016°02.61´
3PL	Kamienna Góra (Sudety)	N 50°46.28´	E 016°09.44´
4PL	Marciszów (Sudety)	N 50°53.33´	E 015°30.49´
5PL	Goraj (Lubelszczyzna)	N 50°42.85´	E 022°40.54´
6PL	Łada (Lubelszczyzna)	N 50°43.81´	E 022°39.16´
7PL	Gródki (Lubelszczyzna)	N 50°46.91´	E 022°41.40´
8PL	Czarnystok (Lubelszczyzna)	N 50°38.22´	E 022°49.93´
	Mongolian populations		
8M	Archangai-Aimag area	N 48°45.56´	E 101°46.86´
13M	Khuvsgul lake area	N 50°29.77´	E 100°09.93´
20M	Khuvsgul lake area	N 50°37.00´	E 100°09.69´
22M	Khuvsgul lake area	N 50°38.18´	E 100°49.58´
27M	Kharakhorin District area	N 48°08.62´	E 101°24.57´
29M	Kharakhorin District area	N 47°27.59´	E 101°55.81´

Phytochemical analysis

Determination of the total contents of tannins and phenolic acids

The total content of tannins (expressed as pyrogallol equivalents, %) and total content of phenolic acids (expressed as caffeic acid equivalents, %) were determined spectrophotomertically, according to Polish Pharmacopoeia VIII [2]. The presented results are mean values from 3 replications.

HPLC analysis

Standards

For quantitation of investigated compounds the five-point calibration curve method was used in CLASS VP 7.3 chromatography software. Methanol standard stock solutions were prepared according to the ChromaDex's Tech Tip 0003: Reference Standard Recovery and Dilution. The solutions (0.5, 1.0, 2.0, 5.0 and 10 μl) were applied on a column in triplicate using SIL-20A. The peak table and spectra library (190-450 nm) of individual compounds were created.

Sample preparation

One gram of homogenized, air-dry raw material was extracted with 100 ml of methanol in Büchi Extraction System B-811. Soxhlet hot extraction with twenty-five extraction cycles, flushing and drying was used. After the evaporation of solvent, the residue was dissolved in 10 ml of methanol. The obtained extracts were filtered with Supelco Iso-Disc™ Syringe Tip Filter Unit, PTFE membrane, diameter 25 mm, pore size 0.20 μm and subjected to HPLC.

Parameters of separations

The analyses were performed using a Shimadzu chromatograph equipped with auto sampler SIL-20A, photodiode array detector SPD-M10A VP PDA and CLASS VP 7.3 chromatography software. A modern C-18 reversed-phase column with core-shell technology (Phenomenex Kinetex® 2.6 μm, C18, 100 A, 100 × 4.60 mm i.d.) was used as solid phase. Binary gradient of mobile phase A (deionised water adjusted to pH 3 with phosphoric acid) and B (ACN adjusted to pH 3 with phosphoric acid) was used as follows: 0 min – 10% B; 2.5 min – 20% B; 4.5 min – 20% B; 5.5 min – 70% B; 5.53 min – 70% B; 5.60 min – 10% B. The following conditions were applied: flow rate 1.0 ml×min^{-1}, oven temperature 30°C, total time of analysis 10 min, injection volume: 1 μl.

Parameters of integration

UV-spectra were recorded between 190 and 450 nm. Peak identification was confirmed by comparison of retention time and spectral data with adequate parameters of standards purchased from ChromaDex. Detection waves were applied as follows: 206 nm ((-)-epigallocatechin, (+)-catechin, (-)-epicatechin, (-)-epigallocatechin gallate, (-)-epicatechin gallate), 254 nm (ellagic acid), 264 nm (astragalin) and 280 nm (gallic acid). The content of the determined compounds was calculated in mg × 100 g^{-1} dry matter.

Statistical analysis

The results of field studies and chemical analysis were subjected to the one-way analysis of variance and Tukey's test at $\alpha=0.01$ and $\alpha=0.05$. The results comprise coefficient of variation (CV; in %) as well. They show mean values from 3 replications.

RESULTS AND DISCUSSION

Polish and Mongolian great burnet populations, investigated *ex situ*, differed significantly in respect of the mass of underground organs. The mass of those organs for Polish populations was distinctly higher than for Mongolian ones (595.0 and 523.5 g × plant^{-1}, respectively for fresh mass). However, the coefficient of variation for Mongolian populations was higher than for Polish ones (33.99 and 15.00%, respectively for fresh weight) (tab. 2). There is a little information on the accumulation of biomass in cultivated great burnet. The studies on organic cultivation of this plant undertaken in the Department of Vegetable and Medicinal Plants – SGGW showed that the air dry mass of underground organs increase from 10.06 kg × 100 m^{-2} at the blooming phase of one-year-old plants to 65.74 kg × 100 m^{-2} at the end of vegetation, in the second year of plant cultivation [11].

Table 2.

Mass of underground organs (g × plant^{-1})

Population	Fresh mass	Air dry mass
Polish populations		
1PL	656.3 C	278.4 b
2PL	482.7 E	188.4 d
3PL	559.2 D	211.6 cd
4PL	543.5 D	229.4 cd
5PL	551.7 D	245.7 c
6PL	736.6 B	324.3 a
7PL	694.8 BC	302.8 ab
8PL	535.3 D	215.1 cd
Mean $_{PL}$	**595.01***	**249.46***
CV $_{PL}$	**15.00**	**19.19**
Mongolian populations		
8M	526.9 D	220.2 cd
13M	380.7 F	158.6 e
20M	538.2 D	195.1 d
22M	315.9 F	134.0 e
27M	830.0 A	318.2 a
29M	549.3 D	224.9 cd
Mean $_{M}$	**523.50**	**208.50**
CV $_{M}$	**33.99**	**30.83**

Values in columns followed by the same letter do not differ significantly at $\alpha=0.05$.
*$p<0.05$ – concerning mean values for Polish and Mongolian population (in columns)

Obtained results revealed distinct differences in the total content of tannins between the populations originating from Poland and Mongolia as well high intraspecific variability of populations from each country. The total content of tannins in

Polish populations varied from 4.22 to 7.54% (CV=19.51%) and in Mongolian ones – from 1.90 to 3.64% (CV=25.34%) (tab. 3). According to Polish Pharmacopoeia [2], the content of these compounds should not be lower than 5.0%. All the Mongolian populations and two, out of eight, Polish populations did not fulfil the requirements. Azovtsev [12] pointed out that the total content of tannins in great burnet roots depends on the stage of plant development as well as on some ecological factors (solar radiation, elevation above sea level, air humidity) and may reach even 40%. According to these results the highest content of tannins was detected at the initial stage of budding. Similar tendency was observed by Węglarz and Bączek [11]. High diversity concerning the total content of these compounds in wild growing populations (1.1–9.7%) was also found by Pelc et al. [8].

Table 3.

Total content of tannins and phenolic acids in underground organs (%)

Population	Tannins	Phenolic acids
1PL	6.06 BC	1.63 ab
2PL	5.89 C	1.76 a
3PL	4.61 D	1.38 b
4PL	6.32 B	1.49 b
5PL	7.41 A	1.87 a
6PL	6.54 B	1.70 a
7PL	7.54 A	1.81 a
8PL	4.22 CD	1.19 bc
Mean $_{PL}$	**6.02****	**1.60****
CV $_{PL}$	**19.51**	**14.57**
8M	3.55 E	1.06 c
13M	3.14 EF	1.23 bc
20M	2.99 F	0.95 c
22M	3.64 E	1.22 bc
27M	2.10 G	0.67 d
29M	1.90 G	0.70 d
Mean $_{M}$	**2.89**	**0.97**
CV $_{M}$	**25.34**	**25.27**

Values marked with the same letter in columns do not differ at $\alpha=0.05$

$^{**}p<0.01$ – concerning mean values of Polish and Mongolian population (in columns)

There were high differences in the total content of phenolic compounds between investigated populations. The content of these compounds in underground organs of Polish populations varied from 1.19 to 1.87% (CV=14.57%) and Mongolian ones – from 0.67 to 1.23% (CV=25.27%). According to Węglarz and Bączek [9] the highest content of these compounds in great burnet underground organs was detected at the end of plant vegetation in the first year of cultivation (2.87%).

In the investigated raw materials, eight phenolic compounds were identified (fig. 1-9), including five flavan-3-ols (/-/-epigallocatechin, /+/-catechin, /-/-epicatechin, /-/-epicatechin gallate, /-/-epigallocatechin gallate), astragalin (kaempferol

3-O-glucoside) and two phenolic acids (ellagic and gallic acid). Irrespective of the population, the dominating compound was /-/-epigallocatechin. The mean content of this compound in Polish populations was 1420.28 mg × 100 g^{-1} d.m. and in Mongolian ones – 1161.70 mg × 100 g^{-1} d.m. (fig. 2). Similarly to /-/-epigallocatechin, the content of /-/-epicatechin gallate and gallic acid was distinctly higher in Polish populations (510.80 and 461.98 mg × 100 g^{-1} d.m., respectively) in comparison with Mongolian ones (106.50 and 393.55 mg × 100 g^{-1} d.m., respectively) (fig. 6, 9). The accumulation of the other identified compounds was similar in plants from Poland and Mongolia (fig. 2-4, 6-7). /-/-Epigallocatechin, /+/-catechin, /-/-epicatechin, /-/-epigallocatechin gallate, ellagic and gallic acids have been previously identified as components present both in the herb and roots of great burnet. Among them, flavan-3-ols were dominant compounds [8].

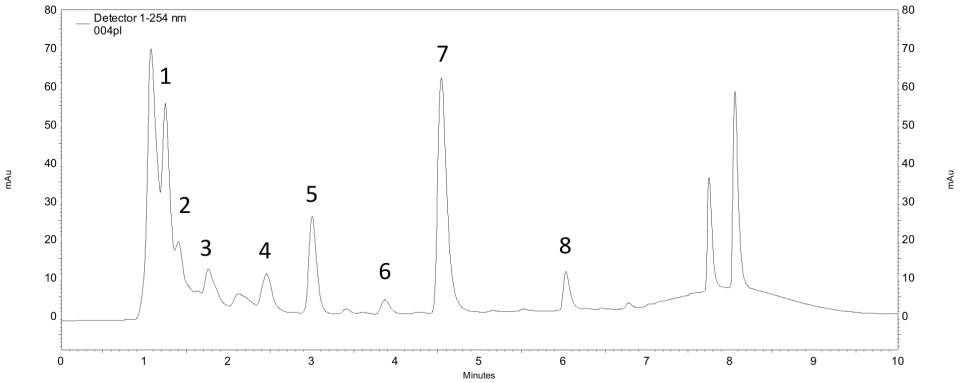

Figure 1.
HPLC chromatogram of underground organs extract: 1 – gallic acid, 2 – (-)-epigallocatechin, 3 – (+)-catechin, 4 – (-)-epicatechin, 5 – (-)-epigallocatechin gallate, 6 – (-)-epicatechin gallate, 7 – ellagic acid, 8 – astragalin

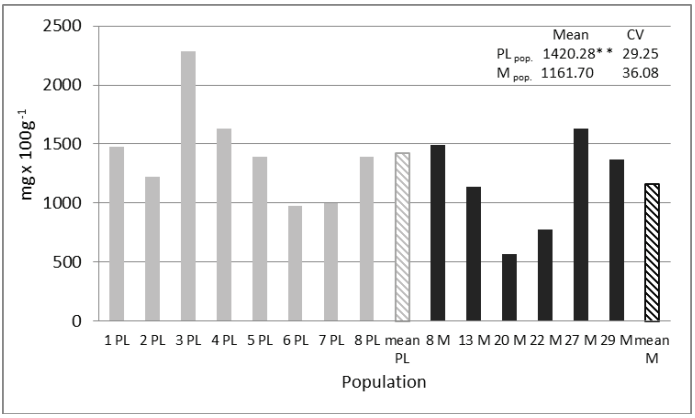

Figure 2.
Content of (-)-epigallocatechin (mg × 100 g^{-1} d.m.)

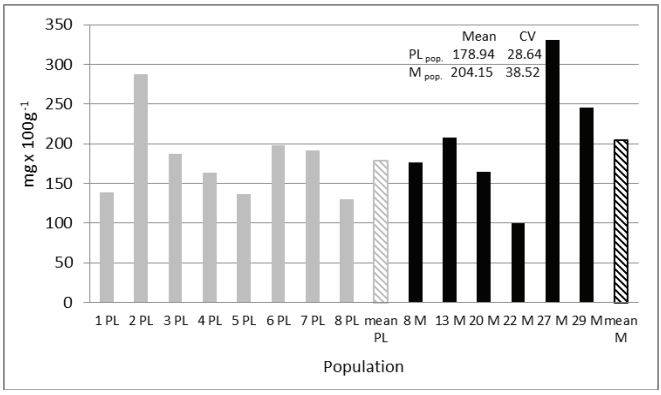

Figure 3.

Content of (+)-catechin (mg × 100 g $^{-1}$ d.m.)

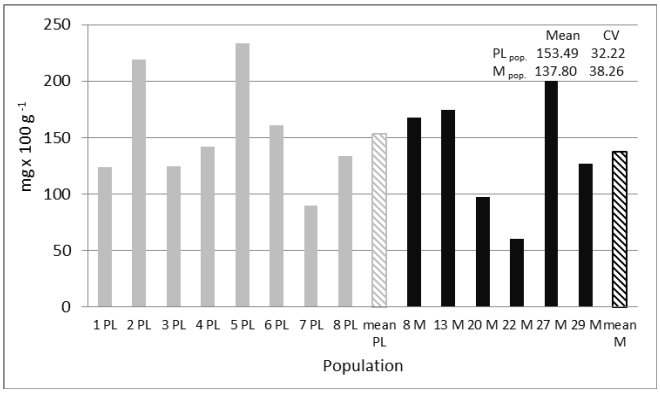

Figure 4.

Content of (-)-epicatechin (mg × 100 g $^{-1}$ d.m.)

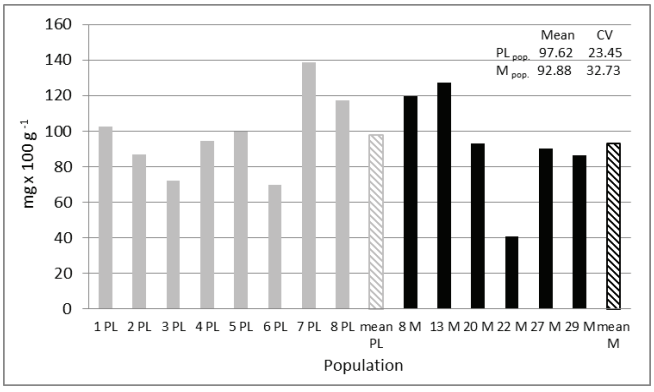

Figure 5.

Content of (-)-epigallocatechin gallate (mg × 100 g^{-1} d.m.)

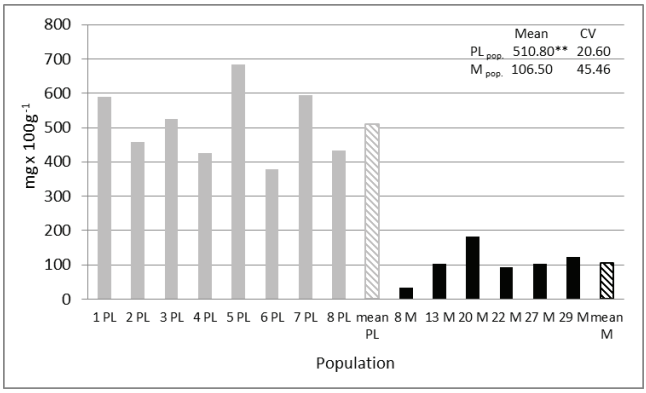

Figure 6.

Content of (-)-epicatechin gallate (mg × 100 g^{-1} d.m.)

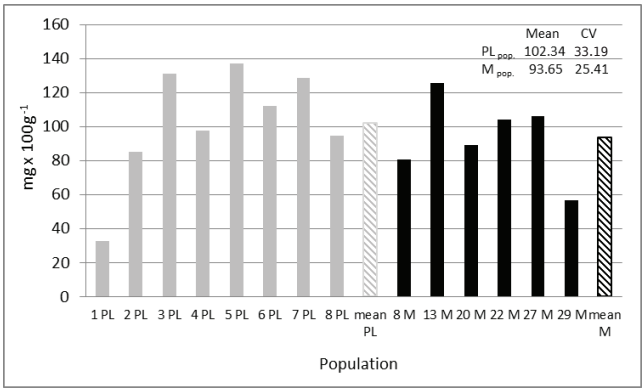

Figure 7.

Content of astragalin (mg × 100g^{-1} d.m.)

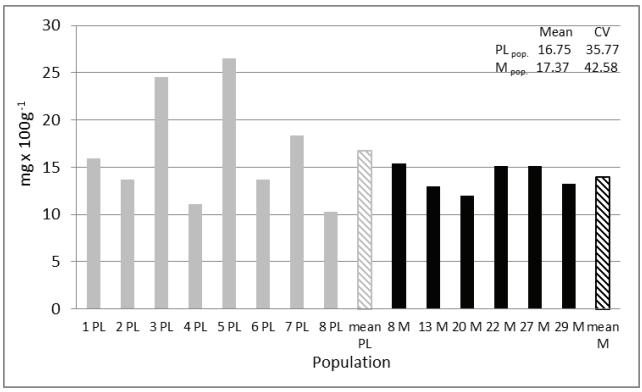

Figure 8.

Content of ellagic acid (mg × 100 g^{-1} d.m.)

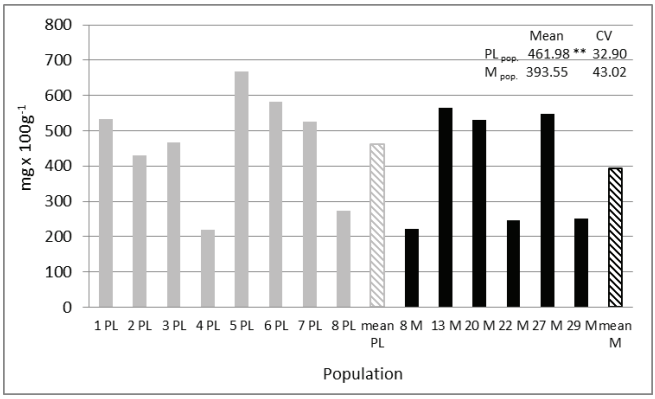

Figure 9.

Content of gallic acid (mg × 100 g^{-1} d.m.)

$^{**}p<0.01$

In recent years, the biological activity of great burnet extracts gain the interest of many scientific groups searching for new sources of medicines. Due to high content of phenolic compounds, especially flavan-3-ols and phenolic acids, this plant has become very interesting as a source of antimicrobial substances. So far, it was shown that /-/-epigallocatechin gallate, present in great burnet extracts, in combination with curcumin (isolated from *Curcuma longa* rhizomes) is very effective against multidrug-resistant *Acinetobacter baumannii* [13]. It was confirmed that extracts form this plant have strong effect against avian *E. coli* [14]. The extracts are also considered as a new, natural anti-HIV-1 drug [4]. There was also confirmed that flavan-3-ols reduce the risk of cardiovascular disease and stroke [15].

CONCLUSIONS

- The mass of great burnet underground organs (rhizomes and roots) of Polish populations was significantly higher in comparison with Mongolian ones.
- Polish populations were characterised by distinctly higher total contents of tannins and phenolic acids.
- The contents of three main phenolic compounds i.e. /-/-epigallocatechin, /-/-epicatechin gallate and gallic acid in underground organs were also distinctly higher in Polish populations of great burnet.
- Obtained results may indicate that the native populations of great burnet are better adapted to Polish environmental conditions. They are also less diversified, in respect of analysed traits, in comparison with Mongolian ones.

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GROMADZENIE BIOMASY I ZWIĄZKÓW FENOLOWYCH W POLSKICH I MONGOLSKICH POPULACJACH KRWIŚCIĄGU LEKARSKIEGO (*SANGUISORBA OFFICINALIS* L.)

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Streszczenie

Osiem polskich i sześć mongolskich populacji krwiściągu lekarskiego oceniono w warunkach uprawy, pod względem masy organów podziemnych oraz gromadzenia się w nich związków biologicznie aktywnych. W surowcach zebranych jesienią w drugim roku wegetacji oznaczono ogólną zawartość garbników i kwasów fenolowych oraz zawartość związków fenolowych zidentyfikowanych przy użyciu HPLC. Stwierdzono wyraźne różnice pomiędzy populacjami polskimi i mongolskimi oraz wysokie zróżnicowanie, pod względem badanych parametrów, w obrębie tych populacji. Masa organów podziemnych była istotnie wyższa u populacji polskich w porównaniu z mongolskimi (odpowiednio, 595,0 i 523,5 g $\times$ roślina^{-1}). Populacje polskie charakteryzowały się wyższą ogólną zawartością garbników i kwasów fenolowych (odpowiednio 6,02 i 1,60%) niż populacje mongolskie (odpowiednio 2,89 i 0,97%). W badanych surowcach zidentyfikowano osiem związków fenolowych tj. /-/-epigalokatechinę, /+/-katechinę, /-/-epikatechinę, galusan /-/-epikatechiny, galusan /-/-epigalokatechiny, astragalinę oraz kwas elagowy i galusowy. Związkiem dominującym we wszystkich populacjach była /-/-epigalokatechina. Zawartość /-/-epigalokatechiny, galusanu /-/-epikatechiny i kwasu galusowego była istotnie wyższa w organach podziemnych populacji polskich.

Słowa kluczowe: krwiściąg lekarski, populacje, związki fenolowe, HPLC