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EXPERIMENTAL PAPER

Intraspecific variability of yarrow (*Achillea millefolium* L. s.l.) in respect of developmental and chemical traits

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Summary

The aim of study was to determine the variability of 20 yarrow populations introduced into *ex situ* conditions, in respect of selected developmental traits as well as content and composition of biologically active compounds (essential oil, tannins, flavonoids and phenolic acids). Field experiment was established at the Experimental Station, Department of Vegetable and Medicinal Plants. Morphological observations and harvest of raw material were carried out in the second year of plant vegetation, at the beginning of blooming. Investigated populations differed significantly in respect of developmental features as well as content and composition of identified compounds. The highest differences among the populations concerned fresh mass of herb (0.46–1.79 kg per plant), number of shoots per m^2 (64–243) and length of the longest internode (42–158 mm). Total content of essential oil ranged from 0.10 to 1.00%. Among 24 identified compounds *β*-pinene, 1,8-cineole, terpinene-4-ol, nerolidol and chamazulene were the dominants. According to content of these compounds, three chemotypes were distinguished within investigated populations,

i.e.: β-pinene, β-pinene + chamazulene and 1,8-cineole type. Content of tannins ranged from 0.38 to 0.90%. Four flavonoids were identified and apigenin 7-glucoside was present in the highest amount (from 9.87 to 475.21 mg × 100 g^{-1}), while the highest differences between populations concerned the content of luteolin-3',7-diglucoside. Within phenolic acids, three compounds (caffeic acid derivatives) were identified. Rosmarinic acid was the dominant one (75.64–660.54 mg × 100 g^{-1}), while cichoric acid differentiated investigated populations the most.

Key words: *yarrow, populations, chemotypes, essential oil, phenolic compounds*

INTROCUCTION

Yarrow (*Achillea millefolium* L. *sensu lato*) is a perennial, medicinal plant from *Asteraceae* family, widely distributed throughout the temperate and boreal zones of the Northern Hemisphere [1]. It represents a highly polymorphic group of closely related species, subspecies, microspecies and hybrids, which differ in the ploidy level, morphology and chemical composition [2-5]. These various taxa are difficult to differentiate one from another and are classified together in pharmacopeial monographs under the name *Achillea millefolium* L. [6, 7].

Yarrow herb (*Millefolii herba*) is a valuable raw material listed in the European Pharmacopoeia and described by European Medicines Agency as a traditional herbal drug [8]. It is rich mainly in essential oil (up to 1%) containing predominantly: chamazulene, 1,8-cineole, β-pinene, sabinene, borneol, camphor and nerolidol. The chemical composition of yarrow essential oil is quite variable and, according to major compounds, the species creates different chemotypes [9-11]. This raw material also contains considerable amounts of phenolic compounds, i.e. flavonoids, phenolic acids and tannins. Among flavonoids, the most numerous are derivatives of luteoline and apigenine [12-16], while phenolic acids are represented mainly by chlorogenic acid and dicaffeoylquinic acids [14, 15]. Another important group of substances present in yarrow herb are sesquiterepene lactones proazulens (absent in essential oil), namely: guaianolides, germacranolides, eudesmanolides and longipinene derivatives [7, 17].

Due to such a wide range of biologically active compounds, yarrow herb reveals various pharmacological activities, i.e. antispasmodic, choleretic, antimicrobial, anti-inflammatory, antioxidant, anticancer, hepatoprotective and gastroprotective [18, 19]. In general, antispasmodic activity is attributed primarily to flavonoids, dicaffeoylquinic acids are shown to mediate the choleretic effects, sesquiterpenes cause anti-inflammatory result, while essential oil is responsible for antibacterial and antifungal properties [20-25]. Yarrow herb is used to prepare tea infusions as well as ethanolic tinctures, applied mainly in gastrointestinal disorders (flatulence, cramps), as a bitter aromatic medicine in a temporary loss of appetite, to stimulate bile secretion, and externally in skin and mucous membranes inflammations, as well as a wound healing remedy.

In folk medicine, this raw material is also used for menstrual problems and as a diaphoretic agent [7].

Yarrow herb is one of the most important herbal raw materials collected from the wild in Poland. It occurs naturally in different habitats, within a wide range of environments, mainly on grasslands, meadows, as well as ruderal and segetal communities [26, 27]. In regard of the requirements of herbal industry concerning a high quality of raw materials, it is recommended to collect yarrow herb from ecologically clean stands, away from industrial areas, roads and highways. Although yarrow is a collective, variable species, this fact is not taken into account in practice, while harvesting raw material. Therefore, it is crucial to investigate the differences between yarrow wild growing populations in respect of traits important for utility.

In the previous study, the chemical variability of yarrow populations growing wild in *in situ* conditions was determined [28]. The aim of present work was to determine the variability of selected yarrow populations introduced into *ex situ* conditions, in terms of some developmental traits as well as content and composition of biologically active compounds (essential oil, flavonoids, phenolic acids and tannins) in herb. *Ex situ* conditions allow to eliminate a variable influence of environmental factors affecting plants growing wild on natural sites and in consequence to show differences between populations more precisely.

MATERIAL AND METHODS

Plant material

Twenty yarrow populations originating from eastern Poland, introduced to *ex situ* conditions, were the objects of the study (tab. 1). In August 2011, at each identified natural site, the vegetative cuttings were collected from 20 randomly chosen yarrow plants and used for establishing a field experiment. The field experiment was performed in the randomized block design, in tree replications, at the Experimental Station of Department of Vegetable and Medicinal Plants, WULS-SGGW. On a single plot, 30 cuttings were planted out, in 60 × 40 cm spacing. Nitrogen (50 kg N × ha^{-1}), phosphorous (50 kg P_2O_5 × ha^{-1}) and potassium (80 kg K_2O × ha^{-1}) fertilization was applied before planting. The morphological observations and harvest of raw material was carried out in the second year of plant vegetation (2013), at the beginning of blooming (June). Collected herb was dried at 35°C and subjected to chemical analysis.

The voucher specimens of the populations' seeds are kept in the National Centre for Plant Genetic Resources (Polish Gene Bank), while representative plants grow in the collection of medicinal and aromatic plants of Department of Vegetable and Medicinal Plants.

Table 1.

Geographical localization of natural sites of yarrow populations

Population No./site		Coordinates	
1	Zdory	N 53°42’	E 021°46’
2	Drohiczyn	N 52°24’	E 022°38’
3	Kózki	N 52°35’	E 022°87’
4	Drohiczyn Wąwóz	N 52°23‘	E 022°39’
5	Zajęczniki	N 52°23’	E 022°42’
6	Siemiatycze	N 52°30’	E 022°53’
7	Gródki	N 50°46’	E 022°41’
8	Czarnystok	N 50°38’	E 022°49’
9	Radecznica	N 50°44’	E 022°48’
10	Czernięcin Poduchowny	N 50°49’	E 022°50’
11	Sędziejowice	N 50°34’	E 020°40’
12	Chomentówek	N 50°31’	E 020°38’
13	Podłęże	N 50°33’	E 020°34’
14	Kozubów	N 50°26’	E 020°29’
15	Skowronno	N 50°32’	E 020°30’
16	Bóbrka	N 49°25’	E 022°26’
17	Bukowiec	N 49°19’	E 022°24’
18	Terka	N 49°18’	E 022°25’
19	Wołkowyja	N 49°20’	E 022°25’
20	Krzemienna	N 49°42’	E 022°12’

Developmental characteristics

Developmental characteristics was performed according to the List of Descriptors elaborated in the Medicinal and Aromatic Plants Working Group, European Cooperative Programme for Plant Genetic Recourses. Directly before harvest of raw material (June, 2013) the following morphological observations were carried out: plant height (cm), number of shoots per m^2, length of the longest internode (mm), number of nodes per shoot, number of leaves per shoot, length of leaf (mm), width of leaf (mm), ratio of leaf length to width, length and width of inflorescence (mm). Fresh mass of herb (kg × $plant^{-1}$) was determined, as well. At each population, the observations were conducted in five plants, in three replications.

Chemical analysis

Total contents of tannins (expressed as pyrogallol equivalents, %) and essential oil were determined according to European Pharmacopoeia 8^{th}. The analysis of

essential oil was carried out by GC/MS and GC/FID, while the composition of flavonoids and phenolic acids was determined using HPLC. All measurements were performed in triplicate.

Analysis of essential oils by GC/MS and GC/FID

The qualitative GC-MS analysis was carried out using Shimadzu GC-MS QP210S gas chromatograph equipped with Phenomenex Zebron ZBFFAP polar column (30 m × 0.25 μm × 0.25 μm film thickness). The operating conditions were as follows: oven temperature 2 min isothermal at 60°C, then rising at 4°C per min to 210°C and held isothermal for 5 min. Injector temperature: 210°C. The carrier gas (He) flow was 1.1 ml × min^{-1}. The split ratio was 1:20. Diluted samples (1/100 v/v, in n-hexane:isopropanol) of 1 μl were injected at 210°C by auto sampler. Ion source temperature -220°C, ionization voltage 70 eV. Mass spectra were scanned in the range 40-500 amu. Essential oil compounds identification was based on comparison of mass spectra from the Mass Spectral Database, as following: NIST08, NIST27, NIST147, Wiley7N2, PAL 600, and on comparison of retention indices (RI) relative to retention times of a series of n-hydrocarbons (C7-C30) with those reported in literature.

The quantitative GC-FID analysis was performed using a Hewlett Packard 6890 gas chromatograph equipped with a flame ionization detector (FID) and capillary, polar column HP 20M (25 m × 032 mm × 0.3 μm film thickness). The analysis was carried out using the following temperature programme: oven temperature isotherm at 60°C for 2 min, then it was programmed from 60°C to 220°C at a rate of 4°C per min and held isothermal at 220°C for 5 min. Injector and detector temperatures were, respectively, at 220°C and 260°C. The carrier gas (He) flow was 1.1 ml × min^{-1}. The split ratio was 1:70. Manually injection of 0.5 μl essential oil was applied. Component identification was confirmed by comparison of their retention times with those of pure authentic samples and by means of their linear retention indices (RI) relative to the series of n-hydrocarbons (C7-C30), under the same operating conditions. The percentage composition of the essential oils was computed by the normalization method from the GC peak areas, without the use of correction factors.

Analysis of phenolic acids and flavonoids by HPLC

Commercially available standards (ChromaDex®) were separately dissolved in 10 ml volumetric flask with MeOH according to the ChromaDex's Tech Tip 0003: Reference Standard Recovery and Dilution and used as a standard stock solutions. Further calibration levels were prepared by diluting these solutions with methanol in 10 ml volumetric flasks (injected volume ranges: 10, 50, 100, 200, 500 and

1000 μl). As the seventh calibration level, the mixture of all stock solutions was used.

The working solutions were injected (1 μl) on a column in six replicates (n=6) using SIL-20A to generate a seven-point calibration curve, using CLASS VP™ 7.3 chromatography software. The peak table and spectra library (190-450 nm) of individual compounds were created. Standard curve parameters were calculated with statistical service e-stat (http://www.chem.uw.edu.pl/stat/e-stat/). Limits of detection (LOD) were determined at a signal-to-noise ratio (S/N) of 3 from the chromatograms of standards at the lowest concentrations. Limits of quantification (LOQ) were determined at a signal-to-noise ratio (S/N) of 10.

Air-dry, finely powdered and homogenized raw material (1.000 g of leaves) was extracted with 100 ml of methanol in Büchi Labortechnik AG Extraction System B-811. Soxhlet hot extraction with 25 extraction cycles, flushing and drying was used. After 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.

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 Å, 100 × 4.60 mm i.d.) was used as a 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: 0.01 min – 18% B; 0.50 min – 18% B; 5.50 min – 30% B; 5.54 min – 95% B; 6.50 min – 95% B; 6.51 min – 18% B; 11.00 min – STOP. The following conditions were applied: flow rate 1.2 ml × min^{-1}, oven temperature 35°C, total time of analysis 11 min, injection volume: 1 μl.

UV-spectra were recorded between 190 and 450 nm. Peak identification was conducted by comparison of retention time and UV-spectra recorded between 190 and 800 nm of standards. Detection wave applied: 300 nm (chlorogenic acid, cichoric acid), 330 nm (rosmarinic acid), 336 nm (apigenin, apigenin-7-glucoside), 347 nm (luteolin, luteolin-3',7-diglucoside). The content of the determined compounds was calculated in mg per 100 g of dry matter.

Statistical analysis

Data were subjected to statistical analysis using Statistica® software. The mean values were compared by using the one way analysis of variance (ANOVA) followed by Tukey's multiple range test. The differences between means were deemed to be significant at $p<0.05$. The coefficient of variation (CV) was determined as well.

RESULTS AND DISSCUSION

Taking into consideration the number of wild growing medicinal plant species in Poland and their resources, most of them occur in the eastern part of Poland. This area, in comparison with other parts of our country, is economically less developed and characterised by relatively higher biodiversity. Thus, it is regarded as one of the most ecologically clean regions in Europe. In this area yarrow herb is collected for commercial purposes in high quantities [29, 30].

When regards the collection of herbal raw material from natural sites, some medicinal plants are treated as collective species. The example could be the flower of lime, obtained from two species: small-leaved lime (*Tilia cordata* Mill.) and large-leaved lime (*Tilia plathyphyllos* Scop.). In this case the individual species are not distinguished when it comes to harvest of raw materials, considered to be homogenous [31]. Yarrow also represents a collective taxon. According to Danihelka and Rotreklova [32], in central Europe the *A. millefolium* group is divided into seven species: *A. collina, A. millefolium, A. pannonica, A. pratensis, A. asplenifolia, A. setacea, A. roseo-alba*. Many authors claim that the developmental, morphological and chemical diversity within this group of species is very high [5, 26, 33-35]. Nevertheless, there is only a little information on intraspecific variability of yarrow in Poland [36-38].

In the presented study, it was observed that investigated populations were the most differentiated when fresh mass of herb (from 0.46 to 1.79 kg; CV 0.38), number of shoots per m^2 (from 64 to 243; CV 0.36) and the length of the longest internode (from 42 to 158 mm; CV 0.38) are regarded. Plant's height varied between 65.2 and 93.2 cm, while the number of nodes per shoot from 7.2 to 23.2. The length and width of inflorescence ranged from 48 to 100 mm and from 43 to 96 mm, respectively. Population No.15 was distinguished by the highest number of leaves per shoot (26.3) and ratio of leaf length to width (9.4), while population No.18 was characterized by the longest and the widest leaves (20.9 and 3.6 mm, respectively) (tab. 2). Total content of essential oil ranged from 0.10% in herb of population No. 4 to 1.00% in populations No. 3 and 14 (tab. 3). According to European Pharmacopeia 8^{th} the total content of essential oil in yarrow herb should not be lower than 0.20% [6]. Rahimmalek *et al.* [11] provided the values from 0.10 to 2.70%, while the results given by Boscovic *et al.* [33] indicated a content of 0.08%. According to Giorgi *et al.* [39], this values ranged between 0.16 and 0.22%. In present study, 24 components were identified, with a domination of β-pinene, 1,8-cineole, terpinene-4-ol, nerolidol and chamazulene. According to the major compounds in essential oil (tab. 2), among the investigated yarrow populations three chemotypes could be distinguished, i.e. β-pinene (populations No. 2, 5, 14, 19, 20), β-pinene + chamazulene (populations No. 7, 10, 16, 15,) and 1,8-cineole type (populations No. 3, 6, 13). These results are in good agreement with the researches conducted in the area of Baltic countries, i.e. Lithuania or Estonia [9, 10, 33, 34, 40]. For instance, Mockute and Judzentiene

[9] distinguished four chemotypes within 40 samples from Lithuania, i.e. chamazulene + *β*-pinene, *β*-pinene + 1,8-cineole, borneol + camphor, *trans*-nerolidol + *β*-pinene, while Gudaityte and Venskutonis [10] selected the following six chemotypes: α-pinene + *β*-pinene; *β*-caryophyllene + 1,8-cineole + α-phellandrene; *β*-pinene + α-phellandrene + chamazulene, *β*-myrcene; *β*-pinene + camphor + trans nerolidol, *β*-pinene [9, 10]. In contrary, yarrow essential oil originating from Turkish populations contained in the highest amount D-cadinene, limonene and alloaromadendrene, while sabinene, 1,8-cineole, *cis*-chrysanthenol, germacrene D, copaene and borneol were dominants in populations located in Iran [41-44]. These data as well as results obtained in our investigations indicate a remarkable chemical polymorphism within populations of *Achillella millefolium* s.l. Moreover, it seems that chemical composition of yarrow essential oil depends on the geographical localization of populations. According to Rumińska [38], chamazulene free forms occur mainly in the West and South Poland, while forms rich in this compound are located predominantly in the Central and East Poland. Taking into account the collective character of yarrow, many authors agree that sesquiterpenes, i.e. both proazulenes and chamazulene can be used as chemotaxonomic indicators. It is known that a correlation exists between the presence of proazulenes and the chromosome number in plants belonging to *Achillea millefoilum* group. As a rule, only tetraploid species (*A. collina* and *A. pratensis*) contain azulenes, while most of other yarrow karyotypes (diploid, hexaploid and octoploid) are azulene-free [2, 26, 32, 35]. In turn, octaploid forms (*A. pannonica*) are distinguished by the highest content of monoterpenes in essential oil [2]. Given the antiphlogistic activity of sesquiterpenes (i.e. chamazulene) and traditional usage of yarrow herb, it was supposed that only forms containing these compounds may be interesting from the medicinal point of view. Nevertheless, monoterpenes such as *β*-pinene and 1,8-cineole also reveal an important pharmacological activity, i.e. antibacterial and antifungal, so yarrow forms rich in these substances could be taken into consideration, as well [7, 31]. Taking into account that this raw material is collected mainly from natural sites, some authors tried to elaborate a marker method, based on plant's morphological features, that could be used for predicting chemical composition of populations. They claim that tetraploid forms (containing azulenes) are characterized by higher number of nodes and higher productivity in comparison to hexaploids [26, 45]. However, results obtained in our studies do not indicate any relationship between the presence of chamazulene in essential oil and the above mentioned features of plants belonging to investigated populations (tab. 2, 3).

According to Benedek *et al.* [14], another group of substances present in yarrow herb, important both from therapeutic and chemotaxonomic point of view, are phenolic compounds. The present results (tab. 4) indicate that the investigated yarrow populations differed significantly in the content of tannins and chemical composition of flavonoids and phenolic acids. The content of tannins ranged from 0.38% in population No.17 to 0.90% in population No. 6 (CV 0.26), which corresponded with results obtained by Špinarová and Petříková [32]. In contrary,

Table 2.

Developmental traits of investigated yarrow populations

Population No./site		Number of shoots per m^2	Number of nodes per shoot	Length of the longest internode [mm]	Plant height [cm]	Number of leaves per shoot	Lenght of leaf [cm]	Width of leaf [cm]	Ratio of leaf lenght to width	Lenght of inflores-cence [mm]	Width of inflores-cence (mm)	Fresh mass of herb (kg × $plant^{-1}$)
1	Zdory	147 c	15.4 bc	50 d	83.0 b	14.4 b	12.0 c	2.5 b	4.8 c	64 b	96 a	1.19 ab
2	Drohiczyn	195 b	14.6 bc	50 d	78.0 c	13.6 b	12.0 c	1.8 bc	6.6 b	65 b	88 ab	1.70 a
3	Kózki	137 cd	12.2 c	75 bc	80.6 b	14.0 b	11.4 c	1.8 bc	6.3 b	66 b	78 ab	0.91ab
4	Drohiczyn Wąwóz	243 a	17.0 b	82 bc	68.9 cd	20.0 a	14.4 b	2.9 ab	4.9 c	48 bc	43 c	1.42 a
5	Zajęczniki	86 ef	23.2 a	84 bc	72.1c	25.3 a	14.9 b	2.4 b	6.2 b	55 bc	56 bc	1.79 a
6	Siemiatycze	212 ab	15.6 bc	52 d	75.3 c	14.3 b	8.2 d	1.7 bc	4.7 c	66 b	58 bc	1.17 ab
7	Gródki	134 c	11.2 c	92 b	93.0 a	14.0 b	19.9 a	2.2 b	9.0 a	77 ab	63 bc	1.06 ab
8	Czarnystok	198 b	10.8 c	102 b	76.6 c	15.4 b	12.8 c	3.1 a	4.1 c	81 ab	68 bc	0.82 b
9	Radecznica	92 e	12.8 c	42 de	83.0 b	14.0 b	8.9 d	2.1 b	4.2 c	90 a	62 bc	1.02 ab
10	Czernięcin Pod.	64 f	14.0 bc	47 de	75.6 c	16.6 b	11.0 c	1.7 bc	6.4 b	59 bc	60 bc	0.55 c
11	Sędziejowice	122 cd	10.4 c	90 b	93.1 a	12.6 c	11.9 c	3.2 a	3.7 d	100 a	69 b	1.25 a
12	Chomontówek	178 bc	9.0 cd	158 a	90.8 a	13.3 bc	14.0 b	2.7 b	5.1 bc	80 ab	68 b	1.26 a
13	Podłęże	128 cd	11.6 c	68 c	93.0 a	13.6 bc	15.8 b	1.8 bc	8.5 a	96 a	75 b	1.35 a
14	Kozubów	215 ab	15.4 bc	78 bc	76.8 c	13.8 bc	7.8 d	2.6 c	3.0 d	69 b	81ab	0.91ab
15	Skowronno	92 e	23.0 a	47 de	77.2 c	26.3 a	10.4 c	1.1 bc	9.4 a	72 b	59 bc	0.79 b
16	Bóbrka	145 c	17.0 b	42 de	76.3 c	16.6 b	11.4 c	1.7 bc	6.7 b	69 b	61 bc	0.46 cd
17	Bukowiec	134c	13.0 c	104 b	65.2 d	14.6 b	14.0 b	2.2 b	6.3 b	74 ab	61 bc	0.55 c
18	Terka	133 c	10.0 c	80 bc	93.2 a	11.3 c	20.9 a	3.6 a	5.8 bc	94 a	74 b	0.62 bc
19	Wołkowyja	72 ef	7.2 d	94 b	70.8 c	11.0 c	15.4 b	3.3 a	4.6 c	87 ab	82 ab	0.75 b
20	Krzemienna	105 cd	9.4 d	60 c	74.2 c	10.0 c	10.7 c	2.1 b	5.1 c	75 ab	79 ab	0.57 c
	Mean	141.7	13.64	74.85	79.84	15.25	12.89	2.33	5.8	74.35	69.05	1.01
	SD	50.7	4.2	28.4	8.7	4.2	3.4	0.7	1.7	14.0	1.26	0.4
	CV	0.36	0.31	0.38	0.11	0.28	0.27	0.28	0.30	0.19	0.18	0.38

Values in columns marked with different letters differ at $p<0.05$, Tukey's test

Table 3.

Total content and chemical composition of essential oil from herb of investigated yarrow populations (%)

Population No.	β-Pinene type							β-Pinene + chamazulene type					1,8-Cineole type				Others							
	RI	2	5	14	19	20	mean	7	10	16	15	mean	3	6	13	mean	1	4	8	9	11	17	18	mean
Total content		0.35	0.50	1.00	0.30	0.40	0.51	0.42	0.60	0.58	0.37	0.49	1.00	0.43	0.30	0.58	0.43	0.10	0.28	0.23	0.17	0.33	0.20	0.25
α-Pinene	1028	4.45	3.55	5.91	1.87	3.27	3.81	3.27	3.10	5.38	6.77	4.63	7.08	8.29	8.95	8.11	0.83	8.87	2.22	1.23	17.52	6.27	1.26	5.46
Camphene	1088	0.24	0.58	0.44	4.16	0.13	1.11	0.16	0.19	0.08	0.02	0.11	0.33	4.03	0.68	1.68	0.24	0.22	0.23	0.66	-	-	0.29	0.33
β-Pinene	1113	23.08	22.08	42.05	19.92	28.08	27.04	27.10	25.40	28.33	13.25	23.52	11.81	9.94	2.42	8.06	2.26	-	18.40	2.36	0.52	1.49	10.07	5.85
Sabinene	1124	3.15	2.62	1.71	-	8.10	3.90	9.32	5.90	-	1.27	5.50	4.12	4.17	1.16	3.15	4.37	2.23	4.64	-	1.82	12.95	-	5.20
β-Myrcene	1166	0.23	0.37	-	0.84	0.30	0.44	0.45	0.66	0.46	-	0.52	0.23	0.24	0.18	0.22	0.20	0.38	0.11	-	-	-	0.62	0.33
α-Terpinene	1182	0.76	0.95	0.60	0.26	0.54	0.62	0.21	0.69	0.81	-	0.57	0.51	0.45	0.20	0.39	-	-	0.16	-	-	-	-	0.16
1,8-cineole	1203	4.66	10.85	5.10	5.79	5.61	6.40	6.64	4.44	5.17	4.66	5.23	15.05	15.15	15.84	15.35	7.39	11.04	3.43	0.20	5.74	8.44	2.37	5.52
Limonene	1209	1.49	0.62	0.42	1.60	0.95	1.02	0.46	1.24	1.31	1.22	1.06	1.14	0.95	0.37	0.82	0.65	1.12	0.33	0.05	0.88	1.32	1.48	0.83
γ-Terpinene	1248	0.21	0.56	0.48	0.58	0.59	0.48	1.45	0.07	0.38	0.80	0.68	0.17	0.48	0.26	0.30	0.35	0.17	0.50	0.29	0.26	1.73	1.12	0.63
p-Cymene	1273	1.86	0.14	0.33	0.34	0.47	0.63	0.22	0.31	0.21	-	0.25	0.26	0.99	0.35	0.53	0.14	-	0.09	0.08	-	-	0.35	0.17
Camphor	1509	2.03	4.12	0.92	0.39	0.42	1.58	0.29	0.38	0.24	0.24	0.29	1.93	3.33	3.82	3.03	0.80	0.53	0.60	1.73	2.49	0.65	1.64	1.21
Linalool	1540	0.32	0.63	0.38	0.84	0.28	0.49	0.45	0.43	0.36	-	0.41	1.14	0.56	0.35	0.68	0.48	-	0.57	0.41	-	-	1.05	0.63
Bornyl acetate	1576	0.28	-	0.38	0.28	0.45	0.35	0.59	0.28	4.03	-	1.63	-	0.26	-	0.26	4.65	-	0.63	4.31	2.12	-	-	2.93
Terpinen-4-ol	1584	6.32	1.60	3.12	5.26	8.82	5.02	11.58	3.29	6.47	15.87	9.30	4.52	5.09	2.01	3.87	12.99	16.91	4.71	1.67	9.56	6.35	7.34	8.50
β-Caryophylloene	1593	4.43	0.59	0.22	0.28	1.50	1.40	0.35	0.13	0.02	1.12	0.41	0.98	0.38	0.97	0.78	0.24	0.25	0.25	0.47	1.53	0.20	0.74	0.53
β-Terpineol	1622	0.40	0.83	-	0.24	0.34	0.45	0.79	0.40	0.62	-	0.60	0.55	0.21	3.07	1.28	0.18	9.45	0.71	0.73	0.78	0.22	0.74	1.83
α-Humulene	1657	1.41	0.25	0.38	0.87	1.30	0.84	1.70	1.66	1.03	2.49	1.72	-	3.04	2.01	2.53	0.56	3.12	1.29	1.63	0.44	1.26	0.27	1.22
α-Terpineol	1681	1.78	1.90	4.64	1.59	0.97	2.18	1.57	0.45	1.39	1.55	1.24	2.62	12.77	0.97	5.45	0.28	1.03	5.93	10.08	1.00	1.85	1.63	3.11
Borneol	1687	5.63	1.08	5.44	0.68	3.44	3.25	3.52	2.97	3.70	8.61	4.70	2.61	1.27	1.80	1.89	2.51	11.08	4.66	1.75	8.98	1.39	1.37	4.53
Citronellol	1756	0.53	0.37	-	0.43	0.58	0.48	0.83	0.04	0.17	-	0.35	4.15	-	0.33	2.24	-	-	0.80	0.60	1.00	-	2.94	1.34
Nerol	1795	0.81	1.16	0.49	0.67	0.10	0.65	0.30	0.68	0.16	-	0.38	-	1.03	0.88	0.96	0.35	-	0.30	0.58	1.77	-	0.56	0.71
Caryophyllene oxide	1955	-	0.58	0.29	0.67	-	0.51	3.09	-	-	-	3.09	-	-	0.75	0.75	0.24	-	2.41	4.99	1.33	0.13	-	1.82
Nerolidol	2024	3.84	1.02	0.91	1.82	2.10	1.94	2.16	1.35	0.93	1.84	1.57	4.37	2.67	1.02	2.69	5.29	15.75	16.65	2.41	5.11	2.27	1.84	7.05
Eugenol	2144	6.92	0.23	1.65	0.21	0.14	1.83	1.07	0.07	-	-	0.57	7.59	2.01	-	4.80	1.64	0.03	3.22	1.18	-	3.51	14.01	3.93
Bisabolol	2197	0.34	2.60	0.43	1.51	-	1.22	1.36	-	-	-	1.36	2.80	0.38	3.37	2.18	0.30	-	2.83	2.67	0.78	0.26	1.85	1.45
Izoeugenol	2230	1.17	0.59	0.45	1.71	0.22	0.83	1.00	0.33	0.18	-	0.50	6.32	0.58	0.24	2.38	0.37	2.36	3.03	2.81	1.56	4.20	1.72	2.29
Viridiflorol	2245	-	0.23	0.33	0.64	-	0.40	0.35	0.55	0.39	1.19	0.62	0.36	0.17	6.80	2.44	1.13	12.77	1.56	30.45	4.98	-	0.50	8.57
Chamazulene	2370	7.93	0.57	1.07	0.74	0.27	2.12	15.79	33.26	19.56	40.67	27.32	2.17	1.49	0.55	1.40	1.84	6.08	1.20	0.83	1.58	2.70	1.60	2.26

Table 4.

Total content of tannis (%) as well as content of determined flavonoids and phenolic acids (mg × 100 g^{-1})

Population No./site		Tannins	Flavonoids				Phenolic acids		
			Luteolin	Luteolin 3',7-diglucoside	Apigenin	Apigenin 7-glucoside	Rosmarinic acid	Chlorogenic acid	Cichoric acid
1	Zdory	0.52 d	83.54 c	92.54 cd	50.02 d	271.45 c	169.87 cd	25.36 cd	105.46 c
2	Drohiczyn	0.63 c	57.62 d	140.54 c	75.54 cd	245.54 c	176.54 cd	21.54 d	66.87 d
3	Kózki	0.75 b	108.7 b	398.87 b	50.54 d	348.87 b	248.02 c	48.28 b	163.40 c
4	Drohiczyn Wąwóz	0.54 d	56.87 d	165.54 c	6.54 f	9.87 f	256.54 c	20.54 d	236.54 b
5	Zajęczniki	0.88 a	13.54 f	12.54 f	121.54 b	63.54 e	304.45 b	5.64 f	125.54 c
6	Siemiatycze	0.90 a	216.88 a	673.50 a	153.71 a	442.23 a	660.54 a	61.54 a	456.81 a
7	Gródki	0.64 c	30.54 e	162.54 c	9.46 f	161.96 d	365.24 b	57.88 a	410.94 a
8	Czarnystok	0.79 b	105.4 b	129.54 c	36.54 fe	460.54 a	225.61 c	41.8 b	125.64 c
9	Radecznica	0.61 c	46.54 d	50.54 d	15.54 f	245.54 c	153.54 d	29.54 c	140.54 c
10	Czernięcin Pod.	0.43 e	36.55 e	46.54 d	20.54 e	58.77 e	150.54 d	12.54 e	105.88 c
11	Sędziejowice	0.47 e	201.54 a	56.87 e	121.54 b	475.21 a	151.44 d	20.77 d	69.23d
12	Chomontówek	0.46 e	82.4 c	32.54 a	48.87 d	176.54 d	75.64 e	21.56 d	35.94 e
13	Podłęże	0.40 e	96.54 c	44.87 c	46.58 d	323.54 b	107.93 de	21.25 d	69.75 d
14	Kozubów	0.60 c	89.87 e	203.60 bc	23.54 e	256.98 c	138.76 d	25.70 cd	57.95 de
15	Skowronno	0.39 e	55.87 d	65.54 d	20.54 e	81.55 e	213.54 c	30.54 c	93.54 cd
16	Bóbrka	0.62 c	33.54 e	50.54 d	13.54 f	159.54 d	193.54 cd	16.88 e	132.54 c
17	Bukowiec	0.38 e	105.54 b	189.87 c	93.54 c	223.54 c	130.54 d	20.54 d	40.05 e
18	Terka	0.52 d	111.54 b	89.87 cd	62.54 d	163.87 d	138.78 d	33.54 c	75.87 d
19	Wołkowyja	0.56 d	78.97 c	148.78 c	84.87 c	208.78 cd	140.54 d	25.48 cd	53.45 de
20	Krzemienna	0.55 d	62.56 d	92.78 cd	42.32 d	146.19 d	126.09 d	21.73 d	41.66 e
	Mean	0.58	83.73	142.4	54.93	226.2	206.38	28.13	130.38
	SD	0.15	51.65	152.25	41.78	132.92	127.64	14.27	114.92
	CV	0.26	0.62	1.07	0.76	0.59	0.62	0.51	0.88

Values in columns marked with different letters differ at $p < 0.05$, Tukey's test

Kohlmünzer [31] as well as Eghdami and Sadeghi [46] showed a higher level of tannins in yarrow herb (3–4%). When regards flavonoids, four compounds were identified, namely: luteolin, luteolin-3',7-diglucoside, apigenin and apigenin-7-glucoside. Their presence in yarrow herb was noted also by other authors [13-15, 47, 48]. Apigenin 7-glucoside was present here in the highest amount (from 9.87 to 475.21 mg × 100 g^{-1}; CV 0.59), while the highest differences among the populations concerned the content of luteolin-3',7-diglucoside (from 12.54 to 673.50 mg × 100 g^{-1}; CV 1.07). The level of aglycones ranged as following: luteolin from 13.54 to 216.88 mg × 100 g^{-1}; CV 0.62 and apigenin from 6.54 to 153.71 mg × 100 g^{-1}; CV 0.76. Population No. 6 was distinguished by the highest content of luteolin, luteolin-3',7-diglucoside and apigenin. Both luteolin, apigenin and their glycosides occur very often in plant raw material. Luteolin is characterized by high spasmolytic activity, while apigenin reveals diuretic effect [31]. Besides the pharmacological activity, the composition of flavonoid fraction may be also an important marker used for recognition of individual species within *Achilea millefolium* group, especially diploids and tetraploids [14].

In the present study, three phenolic acids were identified: 3-caffeoylquinic (chlorogenic) acid, 2,3-dicaffeoylquinic (cichoric) acid and rosmarinic acids. The dominant was rosmarinic acid (from 75.64 to 660.54 mg × 100 g^{-1}, CV 0.62), followed by cichoric acid (from 40.05 to 456.81 mg × 100 g^{-1}, CV 0.88). The content of chlorogenic acid varied from 5.64 to 61.54 mg × 100 g^{-1}, CV 0.51 (tab. 4). These compounds represent caffeic acid derivatives (depsides) known of choleretic activity [7, 19, 31]. The presence of chlorogenic acid in yarrow herb was shown earlier [14-16, 48]. According to Vitalini *et al.* [16], phenolic acid fraction in this raw material consists of dicaffeoylquinic acid isomers, namely: 1,3; 1,4; 3,4; and 3,5-dicaffeoylquinic acid, while Benedek *et al.* [14] mention also 1,5 and 4,5-dicaffeoylquinic acid.

The present work indicated a very high morphological and chemical diversity of investigated populations, which allows to suppose that they represent different genetic material. Despite the fact that there is no requirement in European Pharmacopeia regarding a recognition of individual yarrow species before harvest of herb, both collectors and consumers of this raw material should be aware of such variability.

CONCLUSIONS

- Examined yarrow populations growing in *ex situ* conditions differed distinctly in respect of developmental traits as well as accumulation and composition of essential oil, tannins, phenolic acids and flavonoids.
- The highest differences among the populations concerned the fresh mass of herb, number of shoots per m^2 and the length of the longest internode.
- In respect to essential oil composition, 3 chemotypes were selected within investigated populations: β-pinene , β-pinene + chamazulene, and 1,8-cineole.

- Identified flavonoids occurred predominantly in a form of glycosides. Apigenin-7-glucoside was present in the highest amount, while the highest differences among populations concerned the content of luteolin-3',7-diglucoside.
- Identified phenolic acids represented depsides, derivatives of caffeic acid. The rosmarinic acid was the dominant, while cichoric acid differentiated investigated populations at the highest degree.
- There was no clear relationship between geographical localization of investigated populations and chemical composition of herb.

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REFERENCES

1. Richardson IBK. *Achillea* L. in: Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA. Flora Europaea, v.4. Cambridge University Press, London, New York, Melbourne 1976.
2. Hofman L, Fritz D, Nits S, Kollmansberger H, Drawert F. Essential oil composition of three polyploids in the *Achillea millefolium* 'complex'. Phytochem 1992; 31:537-572.
3. Špinarová Š, Petříková K. Variability of the content and quality of some active substances within *Achillea millefolium* complex. Hort Sci 2003; 30(1):7-13.
4. Nemeth E. Essential oil composition of species in the genus *Achillea*. J Essent Oil Res 2005; 17:501-512.
5. Bimbiraitė K, Ragažinskienė O, Maruška A, Kornyšova O. Comparison of the chemical composition of four yarrow (*Achillea millefolium* L.) morphotypes. Biologija 2008; 54(3):208-212.
6. European Pharmacopoeia 8th ed. *Millefolii herba*. 01/2008:1382. European Directorate for the Quality of Medicines and Health Care (EDQM), Council of Europe, Strasbourg 2008.
7. Wichtl M. *Millefolii herba* in: Wichtl M. (ed.), Herbal Drugs and Phytopharmaceuticals, a Handbook of Practice on a Scientific Basis, third ed., CRC Press, Stuttgart 2004.
8. Committee on Herbal Medicinal Products. Community herbal monograph on *Achillea millefolium* L., herba. EMA/HMPC/290284/2009.
9. Mockute D, Judzentiene A. Variability of the essential oils composition of *Achillea millefolium* ssp. *millefolium* growing wild in Lithuania. Biochem Syst Ecol 2003; 31:1033–1045.
10. Gudaitytė O, Venskutonis PR. Chemotypes of *Achillea millefolium* transferred from 14 different locations in Lithuania to the controlled environment. Biochem Syst Ecol 2007; 35:582-592.
11. Rahimmalek M, Tabatabaei BES, Etemadi N, Goli SAH, Arzani A, Zeinali H. Essential oil variation among and within six *Achillea* species transferred from different ecological regions in Iran to the field conditions. Ind Crop Prod 2009; 29:348-355.
12. Glasl SP, Mucaji I, Werner A, Presser J, Jurenitsch J. Sesquiterpenes and flavonoid aglycones from a Hungarian taxon of the *Achillea millefolium* group. Zeitschrift für Naturforschung [C] 2002; 57:976-982.
13. Benedek B, Kopp B. *Achillea millefolium* L. s.l. revisited: Recent findings confirm the traditional use. Wiener Medizinische Wochenschrift 2007; 157:312-314.
14. Benedek B, Gjoncaj N, Saukel J, Kopp B. Distribution of phenolic compounds in middleeuropean taxa of the *Achillea millefolium* L. aggregate. Chem Biodivers 2007; 4:849-857.
15. Benetis R, Radušiene J, Janulis V. Variability of phenolic compounds in flowers of *Achillea millefolium* wild populations in Lithuania. Medicina 2008; 44:775-781.
16. Vitalini S, Beretta G, Iriti M, Orsenigo S, Basilico N, Dall'Acqua S. Phenolic compounds from *Achillea millefolium* L. and their bioactivity. Acta Biochim Pol 2011; 58:203-212.

17. Saeidnia S, Yassa Y, Razaeipoor R. Comparative investigation of the essential oils of *Achillea talagonica* Boiss. and *A. millefolium*, chemical composition and immunological studies. J Essent Oil Res 2004; 16:262-265.
18. Applequist WL, Moerman DE. Yarrow (*Achillea millefolium* L.): a neglected panacea? A review of ethnobotany, bioactivity, and biomedical research. Econ Bot 2011; 65(2):209-225.
19. Benedek B, Geisz N, Jäger W, Thalhammer T, Kopp B. Choleretic effects of yarrow (*Achillea millefolium* s.l.) in the isolated perfused rat liver. Phytomedicine 2006; 13:702-706.
20. Lemmens-Gruber R, Marchart E, Rawnduzi P, Engel N, Benedek B, Kopp B. Investigation of the spasmolytic activity of the flavonoid fraction of *Achillea millefolium* s.l. on isolated guinea-pig ilea. Arzneimittelforschung 2006; 56:582-588.
21. Kastner U, Jurenitsch J, Glasl S, Baumann A, Robien W, Kubelka W. Proazulenes from *Achillea asplenifolia*. Phytochemistry 1992; 31:4361-4362.
22. Kastner U, Sosa S, Tubaro A, Breuer J, Rücker G, Della Loggia R et al. Anti-edematous activity of sesquiterpene lactones from different taxa of the *Achillea millefolium* group. Planta Med 1993; 59:669.
23. Sosa S, Tubaro A, Kastner U, Glasl S, Jurenitsch J, Della Loggia R. Topical anti-inflammatory activity of a new germacrane derivative from *Achillea pannonica*. Planta Med 2001; 67:654-658.
24. Unlü MD, Daferera E, Dönmez M, Polissiou B, Tepe A, Sökmen I. Compositions and the *in vitro* antimicrobial activities of the essential oils of *Achillea setacea* and *Achillea teretifolia* (*Compositae*). J Ethnopharmacol 2002; 83:117-121.
25. Candan FM, Unlü B, Tepe D, Daferera M, Polissiou A, Sökmen H et al. Antioxidant and antimicrobial activity of the essential oil and methanol extracts of *Achillea millefolium* subsp. *millefolium* (*Asteraceae*). J Ethnopharmacol 2003; 87:215-220.
26. Radusiene J, Gudaityte O. Distribution of proazulenes in *Achillea millefolium* s.l. wild populations in relation to phytosociological dependence and morphological characters. Plant Gen Resour-C 2005; 3:136-143.
27. Matuszkiewicz W. Przewodnik do oznaczania zbiorowisk roślinnych Polski, third ed. Wydawnictwo Naukowe PWN, Warszawa 2012.
28. Bączek K, Kosakowska O, Przybył JL, Kuczerenko A, Pióro-Jabrucka E, Węglarz Z. Zróżnicowanie chemiczne dziko rosnących populacji krwawnika pospolitego (*Achillea millefolium* L.). Pol J Agron 2013; 15:89-94.
29. Węglarz Z, Geszprych A, Kosakowska O, Osińska E, Pelc M, Przybył JL. Intraspecific diversity of Polish wild growing medicinal plants being introduced into cultivation. Pamiętnik Puławski 2009; 151(1):381-392.
30. Wolfram K. Zielone Płuca Polski. Regionalny system ochrony tożsamości przyrodniczej i kulturowej północno wschodniej Polski. Wyd. NFOŚiGW, Białystok 2003.
31. Kohlmünzer S. Farmakognozja. Wydawnictwo Lekarskie PZWL, Warszawa 2013.
32. Danihelka J, Rotreklova O. Chromosome numbers within the *Achillea millefolium* and the *A. distans* groups in the Czech Republic and Slovakia. Folia Geobot 2001; 36:163-191.
33. Boskovic Z, Radulovic N, Stojanovic G. Essential oil composition of four *Achillea* species from the Balkans and its chemotaxonomic significance. Chem Nat Comp 2005; 41:674-678.
34. Judzentiene A, Mockute D. Essential oil composition of two yarrow taxonomic forms. Cent Eur J Biol 2009; 55:1-7.
35. Kubelka W, Kastner U, Glasl S, Saukel J, Jurenitsch J. Chemotaxonomic relevance of sesquiterpenes within the *Achillea millefolium* group. Biochem Syst Ecol 1999; 27:437-444.
36. Oświęcimska M. Chemotaxonomic investigations of an azulene in *Achillea*. Herba Pol 1973; 19:207-215.
37. Dąbrowska J. Systematic and geographic studies of the genus *Achillea* L. in Poland with special reference to Silesia. Acta Univ Wratislav Prace Bot 1982; 24:1-223.
38. Rumińska A. Rośliny lecznicze. Podstawy biologii i agrotechniki. Wyd. PWN, Warszawa 1983.
39. Giorgi A, Bononi M, Tateo F, Cocucci M. Yarrow (*Achillea millefolium* L.) growth at different altitudes in central Italian Alps: biomass yield, oil content and quality. J Herbs Spices Med Plants 2005; 11(3):47-58.
40. Orav A, Arak E, Raal A. Phytochemical analysis of the essential oil of *Achillea millefolium* L. from various European countries. Nat Prod Res 2006; 20:1082-1088.
41. Nadim MM, Malik AA, Javed A, Bakshi SK. The essential oil composition of *Achillea millefolium* L. cultivated under tropical condition in India. World J Agric Sci 2011; 7(5):561-565.
42. Kocak A, Bagci E, Bakoglu A. Chemical composition of essential oils of *Achillea teratifolia* Willd. and *A. millefolium* L. subsp. *millefolium* growing in Turkey. Asian J Chem 2010; 22:3653-3658.

43. Amin G, Sourmaghi MHS, Azizzadeh M, Yassa N, Asygari T. Seasonal variation of the essential oil composition of cultivated yarrow in Tehran-Iran. J Essent Oil Bear Plants 2008; 11:628-633.
44. Barghamadi A, Mehrdad M, Sefidkon F, Yamini Y, Khajeh M. Comparison of the volatiles of *Achillea millefolium* L. obtained by supercritical carbon dioxide extraction and hydrodistillation methods. J Essent Oil Res 2009; 21:259-263.
45. Michler B, Arnold CG. Predicting presence of proazulenes in the *Achillea millefolium* group. Folia Geobot 1999; 34:143-161.
46. Eghdami A, Sadeghi F. Determination of total phenolic and flavonoids contents in methanolic and aqueous extract of *Achillea millefolium*. Org Chem J 2010; 2:81-84.
47. Gudeon D, Abbe P, Lamaison JL. Leaf and flower head flavonoids of *Achillea millefolium* L. subspecies. Biochem Syst Ecol 1993; 21:607-611.
48. Dias MI, Barros L, Duenas M, Pereira E, Carvalho AM, Alves RC et al. Chemical composition of wild and commercial *Achillea millefolium* L. and bioactivity of the methanolic extract, infusion and decoction. Food Chem 2013; 141:4152-4160.

ZRÓŻNICOWANIE WEWNĄTRZGATUNKOWE KRWAWNIKA (*ACHILLEA MILLEFOLIUM* L. S.L.) POD KĄTEM CECH ROZWOJOWYCH I CHEMICZNYCH

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Streszczenie

Celem pracy było określenie zakresu zróżnicowania 20 populacji krwawnika rosnących w warunkach *ex situ*, pod kątem wybranych cech rozwojowych oraz zawartości i składu

chemicznego związków biologicznie czynnych (olejku eterycznego, garbników, flawonoidów i kwasów fenolowych). Eksperyment założono na polu doświadczalnym Katedry Roślin Warzywnych i Leczniczych. Obserwacje morfologiczne oraz zbiór surowca przeprowadzono w drugim roku wegetacji roślin, na początku kwitnienia. Badane populacje różniły się istotnie pod względem cech rozwojowych oraz zawartości i składu analizowanych związków aktywnych. Cechami najbardziej różnicującymi populacje były: świeża masa ziela (0,46–1,79 kg z rośliny), liczba pędów na m^2 (64–243) oraz długość najdłuższego międzywęźla (42–158 mm). Zawartość olejku eterycznego wahała się od 0,10 do 1,00%. Spośród 24 zidentyfikowanych związków *β*-pinen, 1,8-cineol, terpinen-4-ol, nerolidol i chamazulen były dominujące. Biorąc pod uwagę te składniki, wśród badanych populacji wyróżniono 3 następujące chemotypy: *β*-pinen, *β*-pinen + chamazulen i 1,8-cineol. Zawartość garbników wynosiła od 0,38 do 0,90%. W obrębie flawonoidów zidentyfikowano 4 związki, przy czym w największej ilości obecny był 7-glukozyd apigeniny (9,87–475,21 mg × 100 g^{-1}), podczas gdy związkiem najbardziej różnicującym populacje był 3',7-diglukozyd luteoliny. Wśród kwasów fenolowych zidentyfikowano 3 związki, tj. pochodne kwasu kawowego. Dominował tu kwas rozmarynowy (75,64–660,54 mg × 100 g^{-1}), a kwas cykoriowy różnicował populacje w najwyższym stopniu.

Słowa kluczowe: *krwawnik, populacje, chemotypy, olejek eteryczny, związki fenolowe*