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EXPERIMENTAL PAPER

Biometric and phytochemical variability of roseroot (*Rhodiola rosea* L.) from field cultivation

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Summary

Roseroot (*Rhodiola rosea* L.) is an adaptogenic plant, widely used in the traditional medicine of Scandinavia, Russia, China and Mongolia. The aim of the study was to determine the biometric and phytochemical co-variability of this species under field cultivation in western Poland (Plewiska near Poznań). The plant material originated from four-year-old cultivation established twice by rhizome division in autumn 2007 and 2008. In the study, 46 individuals (23 plants in 2011 and in 2012) were used. The biometric analysis included measurements of the basic characteristics of plants related to the size (luxuriance) of clumps, shoots, leaves and rhizomes with roots (raw material). The amounts of total polyphenols (expressed as gallic acid), tannins (expressed as pyrogallol) and flavonoids (expressed as quercetin) were determined spectrophotometrically. The obtained results showed high variation of *Rh. rosea*, especially in the level of flavonoids (0.01–0.20% *DM*) and in the weight of raw material (113–1156 g *FM*/plant). There were observed correlations between the phytochemical (flavonoid and total phenolic content) and biometric (water content, leaf number, shoot and clump size) features.

Key words: *Rhodiola rosea*, *medicinal plants*, *adaptogenic plants*, *polyphenols, tannins, flavonoids*

INTRODUCTION

Roseroot (*Rhodiola rosea* L.), also called "golden root" and "arctic root", is widely known in the traditional medicine of Scandinavia, Russia, China and Mongolia. Rhizomes with roots and sometimes herb of this species have been used to increase physical strength and resistance to high altitude sickness as well as to treat tiredness, depression, anemia, infections and others [1-4]. Modern research has confirmed the antioxidative, immunomodulatory, antitumor and adaptogenic activities of this plant [5-12]. Approximately 140 chemical compounds have been isolated from *Rh. rosea* rhizomes and roots [13]. The most important biologically active constituents are phenolics [14]. There are mainly: phenylpropanoids (rosavin, rosin and rosarin), phenyletanoids (salidroside and *p*-tyrosol), flavonoids, proanthocyanidins as well as tannins [15-20].

Rh. rosea from the *Crassulaceae* family is an arctic-alpine plant species widely distributed in Europe, Asia and North America. In Poland, it occurs in the Giant Mountains, Babia Góra, Tatra and Bieszczady Mountains. This taxon grows on wet rocks, screes and rocky grasslands in the dwarf-pine belt and higher, occupying different substrates: limestone, granite or sandstone [21-23]. Due to its wide range, roseroot is characterized by the high morphological, phytochemical and genetic variability [4, 14, 21, 23-28]. Field investigations from the western, central and south-eastern Poland [4, 14, 26, 29-30] show that roseroot can be cultivated in the climatic and soil conditions of this country. They also provide interesting data on the biology and variability of *Rh. rosea*, but further detailed studies are required.

The aim of the present work was to determine the biometric and phytochemical co-variability of roseroot under field cultivation. Our research hypothesis assumed that the level of the main active compounds in *Rh. rosea* rhizomes with roots (total phenolics, tannins and flavonoids) might correlate with the size of plants (their above-ground and underground parts).

MATERIAL AND METHODS

Plant material and biometric analysis

Rh. rosea originated from four-year-old field cultivation in Plewiska near Poznań (Institute of Natural Fibres and Medicinal Plants) established twice by rhizome division in autumn 2007 and 2008. Plants have been grown at 45 x 45 cm spacing, without pesticides (cultivation was weeded by hand). In the study, morphologically diverse and well-developed plants: 46 specimens of the same age (23 plants in 2011 and in 2012) were used. The biometric analysis was performed in July (above-ground plant parts) and in October (underground plant parts). It included measurements of the basic characteristics of plants related to the size (luxuriance) of the clumps, shoots, leaves and rhizomes with roots (raw material). The clump diameter was an average of two measurements taken in a horizontal plane at

a 90° angle. The clump height was determined from the ground to the top of the highest shoot. From each plant, three fertile shoots of the 1st generation [4] were collected to determine the mean length and diameter of shoots, number of leaves per shoot, and foliage density. From these shoots (from their upper part with the largest leaves), three successive leaves were taken (9 leaves per plant). The length and width of leaves were measured using digiShape software after scanning [31]. After the harvest of raw material, the fresh weight was determined. Roseroot rhizomes with roots were cut into small pieces, dried at 40°C and relative humidity of 20% (UZ-108 heating chamber), and then used for phytochemical analysis. Dry weight of the raw material and water content in it [%] were measured after drying it at 105°C in a HR73 Halogen Moisture Analyzer (Mettler, Toledo).

Phytochemical analysis

The levels of the investigated groups of active compounds were determined spectrophotometrically, after water extraction (total polyphenols and tannins) or with acetone (flavonoids). The amounts of total polyphenols (expressed as gallic acid) and tannins (expressed as pyrogallol) were measured with the Folin-Ciocalteu reagent for approximately 0.1 g and 1.5 g of powdered raw material, respectively. For total phenolics, the modified method described by Singleton and Rossi [32] was used. The determination of tannins was conducted according to Polish and European Pharmacopoeias [33-35]. The flavonoid content (expressed as quercetin) was quantified for approximately 2.5 g of sample, using Christ-Müller's method [36].

The absorbance was measured on a Cintra 20 UV-VIS spectrometer (GBC) at $\lambda = 760.0$ nm (total phenolics and tannins) and at $\lambda = 425.0$ nm (flavonoids). The obtained results were calculated for dry matter (*DM*) of raw material (rhizomes with roots).

Statistical analysis

To determine the statistical significance of differences between harvesting years, we applied parametric and non-parametric tests: Student's and Cochran-Cox tests as well as Mann-Whitney test, respectively. To check the normality of variable distribution, the Shapiro-Wilk test was used. For the skewed distribution of variables, square root and logarithmic transformations of data were performed. The relations between the phytochemical and biometric features were analyzed using Pearson's and Spearman's rank correlations. For the statistical analysis, Statistica 7.1 software was used [37].

RESULTS

Our research shows a high variability in the content of phenolic compounds (especially flavonoids) as well as in the weight of raw material obtained from

individual plants of *Rh. rosea* (tab. 1). Similarly, a large variation was observed in other biometric features describing the size of roseroot specimens: clumps, shoots and leaves (tab. 2). The level of variability was increased by differences in two years of field cultivation. These differences between 2011 and 2012 concerned the content of all investigated active compounds as well as fresh weight of raw material and water content (tab. 3). Also, some of the parameters of above-ground plant parts of roseroot showed statistically significant differences (tab. 4).

Table 1.

Content of the polyphenols and the weight of raw material from *Rhodiola rosea* in field cultivation (2011–2012)

Variables	Mean ± SD	Min.	Max.	V [%]
Total polyphenols [%]	5.83 ± 1.89	2.54	10.57	32
Tannins [%]	2.05 ± 0.74	0.95	4.24	36
Flavonoids [%]	0.07 ± 0.04	0.01	0.20	62
Fresh weight of raw material [g]	451 ± 221	113	1156	49
Dry weight of raw material [g]	119 ± 59	34	299	49
Water content in raw material [%]	73.2 ± 3.4	67.1	79.5	5

Total polyphenols – expressed as gallic acid equivalent; Tannins – expressed as pyrogallol equivalent; Flavonoids – expressed as quercetin equivalent; SD – standard deviation; V – variability coefficient; n=46. The content of all compounds – in dry matter (*DM*) of raw material (rhizomes with roots).

Table 2.

Interspecimen size variability of the above-ground plant parts of *Rhodiola rosea* in field cultivation (2011–2012)

Variables	Mean ± SD	Min.	Max.	V [%]
Diameter of clump [cm]	48 ± 11	23	72	23
Height of clump [cm]	24 ± 6	12	40	26
Index of clump size [cm^2]	1181 ± 539	276	2880	46
Length of shoot [cm]	20 ± 5	10	32	24
Diameter of shoot [cm]	0.5 ± 0.1	0.3	0.7	18
Index of shoot size [cm^2]	9.6 ± 3.8	3.6	21.7	39
Number of leaves per shoot	56 ± 11	30	81	20
Number of living leaves per shoot	41 ± 11	19	67	27
Foliage density	2.9 ± 0.6	1.7	3.8	19
Length of leaf [cm]	2.8 ± 0.5	1.9	4.6	18
Width of leaf [cm]	1.5 ± 0.3	1.1	2.3	17
Index of leaf size [cm^2]	4.3 ± 1.6	2.0	10.7	36
Index of size of photosynthetic area [cm^2]	179 ± 77	51	408	43

Index of clump size – diameter × height of clump; Index of shoot size – length × diameter of shoot; Foliage density – number of leaves per 1cm of shoot; Index of leaf size – length × width of leaf; Index of size of photosynthetic area – number of living leaves per shoot × (length × width of leaf); SD – standard deviation; V – variability coefficient; n=46.

Table 3.

Differentiation of the polyphenol content and the raw material weight of *Rhodiola rosea* in two years of field cultivation (mean ± SD)

Variables	2011	2012	*p*-value
Total polyphenols [%][a]	**6.38** ± 1.56	5.29 ± 2.06	*
Tannins [%][a]	**2.33** ± 0.85	1.78 ± 0.49	*
Flavonoids [%][b]	0.04 ± 0.02	**0.09** ± 0.04	***
Fresh weight of raw material [g][c]	**520** ± 202	381 ± 220	*
Dry weight of raw material [g][b]	126 ± 54	112 ± 64	N.S.
Water content in raw material [%][b]	**76.0** ± 2.4	70.4 ± 1.4	***

Total polyphenols – expressed as gallic acid equivalent; Tannins – expressed as pyrogallol equivalent; Flavonoids – expressed as quercetin equivalent; SD – standard deviation. The content of all compounds – in dry matter (*DM*) of raw material (rhizomes with roots). Statistical tests: a – Cochran-Cox test; b – Mann-Whitney test; c – Student's test; *p*-value: *** – $p<0.001$, ** – $p<0.01$, * – $p<0.05$, N.S. – not significant, n=46.

Table 4.

Size differentiation of the above-ground plant parts of *Rhodiola rosea* in two years of field cultivation (mean ± SD)

Variables	2011	2012	*p*-value
Diameter of clump [cm][a]	49 ± 10	46 ± 11	N.S.
Height of clump [cm][a]	**26** ± 6	22 ± 5	**
Index of clump size [cm^2][a]	**1333** ± 609	1028 ± 417	*
Length of shoot [cm][b]	20 ± 5	20 ± 5	N.S.
Diameter of shoot [cm][a]	0.5 ± 0.1	0.5 ± 0.1	N.S.
Index of shoot size [cm^2][a]	9.7 ± 4.1	9.5 ± 3.5	N.S.
Number of leaves per shoot[b]	51 ± 8	**61** ± 12	**
Number of living leaves per shoot[c]	39 ± 8	43 ± 13	N.S.
Foliage density[c]	2.6 ± 0.6	**3.1** ± 0.4	**
Length of leaf [cm][a]	3.0 ± 0.6	2.7 ± 0.4	N.S.
Width of leaf [cm][a]	1.5 ± 0.3	1.5 ± 0.3	N.S.
Index of leaf size [cm^2][a]	4.6 ± 1.8	4.1 ± 1.3	N.S.
Index of size of photosynthetic area [cm^2][a]	182 ± 77	177 ± 80	N.S.

Index of clump size – diameter × height of clump; Index of shoot size – length × diameter of shoot; Foliage density – number of leaves per 1 cm of shoot; Index of leaf size – length × width of leaf; Index of size of photosynthetic area – number of living leaves per shoot × (length × width of leaf); SD – standard deviation; V – variability coefficient. Statistical tests: a – Student's test; b – Mann-Whitney test; c – Cochran-Cox test; *p*-value: *** – $p<0.001$, ** – $p<0.01$, * – $p<0.05$, N.S. – not-significant, n=46.

In the present study, we analyzed the relations between phytochemical and biometric variations in *Rh. rosea*. A relatively strong correlation was detected only

between flavonoid content in dry matter of rhizomes with roots and water content in fresh weight of this raw material. It was interesting that the number of leaves per shoot and the size of shoots correlated, although not excessively, with the amount of flavonoids as well as the size of roseroot clumps correlated with the level of total phenolics. In the case of tannins, we found no effect of the investigated parameters describing the size of *Rh. rosea* plants (tab. 5).

Table 5.

Correlation between the polyphenol content and the biometric parameters of *Rhodiola rosea* plants

Variables	Flavonoids	Total phenolics
Water content in raw material[a]	-0.68***	
Number of leaves per shoot[b]	0.48***	
Number of living leaves per shoot[b]	0.31*	
Index of shoot size[b]	0.31*	
Height of clump[b]		0.38**
Index of clump size[b]		0.36*

Index of shoot size – length × diameter of shoot; Index of clump size – diameter × height of clump; a – Spearman's rank correlation; b - Pearson's correlation; *p*-value: *** – $p \leq 0.001$, ** – $p < 0.01$, * – $p < 0.05$, n=46.

DISCUSSION

Roseroot has a wide distribution, but intensive harvest of raw material significantly reduces the natural resources of this species [3, 38-39]. In Poland, *Rh. rosea* occurs only in the national parks and it cannot be collected [4, 22-23]. Additionally, in its natural stands in arctic and mountain conditions, roseroot grows very slowly and blooms late, often after over a dozen years [4, 40-42]. Therefore, studies on field cultivation of this species have been conducted in many countries, especially in Russia, but also in Poland, Finland, Bulgaria, Germany, Denmark, Sweden and Canada [3-4, 25-26, 29-30, 38-39, 43-44].

The weight of *Rh. rosea* rhizomes with roots can be up to 3.5 kg [16, 40]. In Plewiska near Poznań, the fresh weight of the raw material after six years of growing from seeds reached up to 1.7 kg [29], and similarly in Finland – more than 2 kg [3]. Our later investigations of four-year-old plants propagated by rhizome division show that the mean fresh weight of underground roseroot parts is 451 g, ranging from 113 to 1156 g (tab. 1). It was much more than in the case of four-year field cultivation obtained from seeds, in the conditions of south-eastern Poland [4]. In this work, four-year-old *Rh. rosea* plants provided raw material with an average fresh weight of 100 g. The differences are in agreement with the observation of Kim [41] that plants from rhizome division grow more intensively than individuals obtained from seeds. These plants had a much greater height as well as weight of above- and underground parts, at least in the first three years

of growth. Platikanov and Evstatieva [39] indicate that raw material yield from four-year-old plants grown from seeds is about half of the crop of vegetatively propagated roseroot.

According to Revina et al. [38], vegetatively propagated roseroot at the end of the second year of cultivation is not inferior to long-term individuals from natural sites in terms of rhizome weight and salidroside content, and it can be used as a source of raw material. Under these Siberian conditions (Tomsk), the mean rhizome weight of plants increased by 10 g in the first year of cultivation and by 90 g in the second year, and the average total weight of raw material was 112 g. In the foreland of the Altai Mountains, roseroot reached a mean weight of raw material of 27, 98 and 168 g in the first, second and third year of cultivation from rhizome division, respectively [41]. In turn, in the Rhodopes Mountains (Bulgaria), the mean fresh weight of underground plant parts in the third year of cultivation from rhizomes was 338 g, with the maximum weight of 600 g [39]. Agricultural studies of *Rh. rosea* in the conditions of western Poland [30] show that the fresh weight of raw material increased significantly, sometimes more than twofold, in the third year of plant growth from rhizomes in comparison to the previous year. According to Galambosi [3] as well as Kołodziej and Sugier [4], roseroot should be harvested after four-five years of field cultivation from seeds. In the case of vegetatively propagated plants, raw material can be obtained after the third year of *Rh. rosea* growth [39].

The investigations of Przybył et al. [26] show that roseroot in field cultivation is characterized by high variability of the weight of rhizomes with roots as well as in the content of the main active compounds (especially salidroside) and others (*trans*-cinnamic alcohol, caffeic acid). The level of examined constituents was not correlated with raw material weight. In our research, no relationships were found between the amount of phenolics (total polyphenols, tannins, flavonoids) and the weight of underground plant parts, either (tab. 5). Field experiments conducted in Finland [3] indicate that organic fertilization affects the growth of vegetative shoots, the fresh weight of raw material and water content in it, while at the same time it influences the content of active compounds, such as salidroside, rosavin, and flavonoids. These observations point to the possibility of the existence of some correlations between the amount of chemical constituents and the parameters describing the size of roseroot plants. Therefore, these biometric features could be a simple indicator for the quality of raw material. According to our research, the level of flavonoids is negatively correlated with water content in the underground plant parts. The present study also indicates some relationships, but not strong, between the amounts of flavonoids, total phenolics and plant size (tab. 5).

Rh. rosea is a species that shows significant phytochemical variation, and it is associated not only with genetic factors, but also with growth stage and age of plants as well as with climatic and soil conditions [14, 38, 41, 45-46]. Our research indicates seasonal variation of the level of active compounds (tab. 3), and this phytochemical variability in two investigated years was greater than the variation in

some biometric parameters (tab. 4). The highest variability coefficient was found for flavonoids (tab. 1). Their amount ranged from 0.01 to 0.20% of dry matter (mean 0.07%). These results are comparable with those obtained by Galambosi [3] who reported about 0.1% flavonoid content in roseroot rhizomes. In turn, the level of tannins varied from 0.95 to 4.24% *DM* (tab. 1). A large content variability of this group of compounds was also shown by the investigations of Revina et al. [38]. They reported the amount of tannin, depending on the age of cultivation, from 6.4 to 10.9% *DM*, and even 16.9% for plants at natural sites. Unfortunately, a comparison of these results is difficult because different analytical procedure was used (the permanganate method of tannin determination, described in the 1940's).

In conclusion, our preliminary studies indicate high seasonal variation of *Rh. rosea* as well as the co-variability of phytochemical (flavonoid and total phenolic content) and biometric (water content, leaf number, shoot and clump size) features. The observed correlations are statistically significant, but usually not strong, and they require confirmation by further research. On the other hand, it would be interesting to investigate the relation between the size of roseroot plants and the level of individual compounds from the group of phenylpropanoids and phenyletanoids. The results of such investigations are being processed.

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ZMIENNOŚĆ BIOMETRYCZNA I FITOCHEMICZNA RÓŻEŃCA GÓRSKIEGO (*RHODIOLA ROSEA* L.) W UPRAWIE

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Streszczenie

Różeniec górski (*Rhodiola rosea* L.) jest rośliną o działaniu adaptogennym, szeroko stosowaną w tradycyjnej medycynie Skandynawii, Rosji, Chin i Mongolii. Celem prezentowanych badań było opisanie współzmienności biometrycznych i fitochemicznych cech tego gatunku, w warunkach uprawy polowej, prowadzonej w zachodniej Polsce (Plewiska koło Poznania). Materiał do analiz pochodził z czteroletnich upraw, założonych jesienią 2007 i 2008 r., przez podział kłączy macierzystych roślin. W latach 2011 i 2012 zebrano po 23 osobniki omawianego gatunku. Biometria obejmowała pomiary podstawowych cech roślin, opisujących wielkość (bujność) tworzonych kęp, pędów, liści oraz kłączy z korzeniami (surowca zielarskiego). Badania fitochemiczne wykonano metodą spektrofotometryczną, oznaczając zawartość polifenoli ogółem (w przeliczeniu na kwas galusowy), garbników (w przeliczeniu na pirogalol) i flawonoidów (w przeliczeniu na kwercetynę). Otrzymane wyniki wskazują na dużą zmienność *Rh. rosea*, szczególnie w przypadku poziomu flawonoidów (0,01–0,20% s.m.) i masy surowca (113–1156 g św.m./roślina). Stwierdzono również istotne statystycznie korelacje między cechami fitochemicznymi (zawartość flawonoidów i fenoli ogółem) a biometrycznymi (zawartość wody, liczba liści, wielkość pędów i kęp).

Słowa kluczowe: *Rhodiola rosea*, *rośliny lecznicze*, *rośliny adaptogenne*, *polifenole, garbniki, flawonoidy*