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Elżbieta Romanowska, Faculty of Biology, University of Warsaw, Poland

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## ORIGINAL RESEARCH PAPER

# Biometric features and content of phenolic compounds of roseroot (*Rhodiola rosea* L.)

Artur Adamczak<sup>1\*</sup>, Waldemar Buchwald<sup>1</sup>, Agnieszka Grysczyńska<sup>2</sup><sup>1</sup> Department of Botany, Breeding and Agricultural Technology of Medicinal Plants, Institute of Natural Fibres and Medicinal Plants, Kolejowa 2, 62-064 Plewiska, Poland<sup>2</sup> Department of Pharmacology and Phytochemistry, Institute of Natural Fibres and Medicinal Plants, Kolejowa 2, 62-064 Plewiska, Poland\* Corresponding author. Email: [artur.adamczak@iwnirz.pl](mailto:artur.adamczak@iwnirz.pl)**Abstract**

Roseroot (*Rhodiola rosea* L.) belongs to important herbs in folk medicine of Scandinavia, Russia, Mongolia, and China. Its therapeutic usage is mainly associated with the adaptogenic properties of this species. Roseroot is characterized by high morphological, phytochemical, and genetic differentiation. The aim of the present work was to determine the biometric and phytochemical co-variability of this taxon. Samples of *Rh. rosea* were collected from 4-year-old experimental field cultivation established by rhizome division in western Poland. For each plant, the biometric measurements of the clumps, shoots, leaves, and rhizomes with roots were carried out. In the underground plant parts (raw material), the contents of the main active compounds (phenylpropanoids, phenylethanoids, phenolic acids, and catechins) were determined by the HPLC-DAD method. *K*-means clustering analysis showed three well-separated plant groups of *Rh. rosea* that differed significantly in the level of most of the investigated components. It was interesting that in the raw material with a high content of phenylethanoids, a low level of phenylpropanoids was found, and vice versa. These chemical groups clearly differed in luxuriance of plants, too. The important diagnostic feature was also the degree of leaf serration. The morphological and phytochemical co-variability of roseroot was confirmed by the correlations detected between some active compounds (especially catechins and rosavin) and biometric traits describing the size and serration of leaves, the size of clumps and shoots as well as the weight of the raw material.

**Keywords***Rhodiola rosea*; medicinal plants; biometric traits; HPLC; chemotypes**Introduction**

*Rhodiola rosea* L. (Crassulaceae) is a herbaceous perennial plant with fleshy leaves and thick rhizomes. This arctic-alpine species has a wide range of distribution, from the mountains of Western and Central Europe, Siberia, Mongolia to Far East and North America [1]. Its largest resources are in the Altai and Sayan Mountains where *Rh. rosea* occurs in subalpine meadows along the rivers and streams as well as in low thickets [2,3]. In Poland, roseroot grows in the Giant Mountains, Babia Góra, Tatra and Bieszczady Mountains [4–6]. For centuries, rhizomes of this species, also known as golden root, arctic root, and *Hongjingtian* in Chinese, have been an important raw material in folk medicine of Scandinavia, Russia, Mongolia, and China [7–10]. Traditional use was associated with the adaptogenic properties of this taxon. In the last decades, many research studies have found that roseroot increases mental and physical strength as well as it shows anti-stress, cardioprotective, antioxidative, immunomodulatory, and anticancer activities [7,11–14]. The above-described features of *Rh. rosea* are associated with the presence of phenolics, especially phenylpropanoids, so-called rosavins and phenylethanoids – salidroside and *p*-tyrosol [15].

Roseroot is characterized by high morphological, phytochemical, and genetic differentiation [16–21]. Our previous investigations showed biometric and phytochemical co-variability of this species. We found correlations between flavonoid and total phenolic contents in the underground plant parts and biometric features, such as: water content in rhizomes with roots, leaf number per shoot as well as shoot and clump size [22]. These relationships were statistically significant, but usually not strong, and they require further, more detailed research. Therefore, in this present study we took into account new phytochemical and morphological characteristics. In particular, the analyses included the individual chemical compounds from the phenylpropanoids, phenylethanoids, phenolic acids, and catechins. Our current hypothesis was that it is possible to separate homogeneous groups of roseroot samples differing in the content of the main active compounds and some biometric features. We made an assumption that the parameters characterizing the size and habit of plants as well as the leaf shape might correlate with the phytochemical traits of *Rh. rosea*. The above-described methodological approach and more precise data allow find new interesting relationships in the case of this species.

## Material and methods

### Plant material and biometric analysis

Roseroot samples were collected from 4-year-old field cultivation established by rhizome division in Plewiska near Poznań (Institute of Natural Fibres and Medicinal Plants, Poland). For the study, 25 morphologically diverse and well-developed plants were selected. The basic biometric measurements of plants concerning the size (luxuriance) of the clumps, shoots, leaves, and rhizomes with roots were carried out according to the methods described in the previous work [22]. From each plant, three fertile shoots of the first generation [21] were collected, and then from their upper part with the largest leaves three successive leaves were taken (nine leaves per specimen). After scanning by the digiShape software [23], the roseroot leaves were used to determine leaf size and shape. In comparison with earlier research [22], leaf width in 1/4, 1/2, and 3/4 of the leaf length, leaf area, leaf skeleton length, skeleton end length, and skeleton end node number were additionally measured. Shape of leaves was described using the proportion between leaf length and width as well as the comparison of three leaf width values. Leaf skeleton was obtained in digiShape according to the method of symmetry axis transform of the shape [23,24]. In this algorithm, the number of skeleton branches depends on the adopted threshold value, and for the level  $N = 15$  the analyzed characteristics (especially end node number) were a good indicator of the degree of serration of roseroot leaves.

After the harvest of underground plant parts, the fresh, air-dry, and dry weight of them as well as the share of rhizomes and roots in fresh weight of raw material were determined. The obtained plant material was cut into small pieces and dried at 40°C and relative humidity of 20% in a GoBest UZ-108 heating chamber (Poznań, Poland). Water content (%) and dry weight of samples were measured after drying at 105°C in a HR73 Halogen Moisture Analyzer, Mettler Toledo (Switzerland) [22]. Air-dried and powdered rhizomes with roots of *Rh. rosea* were used for the phytochemical analysis.

### Phytochemical analysis

The analytical procedures have been described in the earlier work [25]. Briefly, 1.0 g of a sample with 20 mL of 70% (v/v) methanol was refluxed for 15 min. The procedure was repeated three times using 15 mL of methanol. The combined extracts were evaporated to dryness, and then dissolved in 5.0 mL of 70% (v/v) methanol. The obtained samples were filtered through a 0.45 µm membrane filter and injected into the HPLC-DAD system (Agilent 1100, USA). The separation of analytes was performed on a LiChrospher C18 column, 250 × 4.0 mm, 5 µm (Merck, Germany) at 24°C. Phase

A: 0.2% (v/v) phosphoric acid solution in water, phase B: acetonitrile, and mobile phase flow rate: 1.0 mL min<sup>-1</sup> were used. The assay was carried out in the following gradient elution: 0–30 min – 95% of phase A, 35 min – 80% A, 40 min – 20% A, 56 min – 20% A, 60 min – 95% A. Rosavins, salidroside, and *p*-tyrosol were detected at a wavelength of 205 nm, gallic acid – at 220 nm, while chlorogenic and caffeic acids – at 330 nm. Peaks were identified by comparison of their retention times and UV-spectra with parameters of chemical standards (ChromaDex, USA).

### Statistical analysis

The plant samples were characterized by the basic statistics (mean, standard deviation, minimum, maximum, and variability coefficient) describing the biometric and phytochemical features of the raw material: rhizomes with roots. The chemotypes of roseroot were distinguished on the basis of *k*-means clustering analysis for the standardized contents of the active compounds. To determine the statistical significance of differences between these plant groups, Pillai's trace test for MANOVA and *F*-test were used. The chemical differentiation of the above-mentioned chemotypes of *Rh. rosea* were confirmed by the Kruskal–Wallis and post-hoc tests. The roseroot groups were also described by the morphological traits with the statistical analysis of the differences between them in respect of the size and habit of plants as well as their leaf shape. The normality of variable distribution was checked using the Shapiro–Wilk test. For the skewed distribution of variables, square root, logarithmic and inverse proportion transformations of data were performed. Pearson's and Spearman's rank correlations were applied to evaluate the relationships between variables.

## Results

### Raw material characteristics

The investigated specimens of *Rh. rosea* from 4-year-old field cultivation were characterized by high variability of raw material yield, from 113.4 to 961.7 g fresh weight (FW) per plant. Rhizomes constituted the main part of the underground organs, with an average share of 82.2% FW. In the fresh weight of raw material, the water content ranged from 67.1 to 73.6%, and after drying at 40°C it was reduced to 6.4–8.8% (Tab. 1).

**Tab. 1** Characteristic of the raw material (rhizomes with roots) of *Rhodiola rosea* from 4-year-old field cultivation.

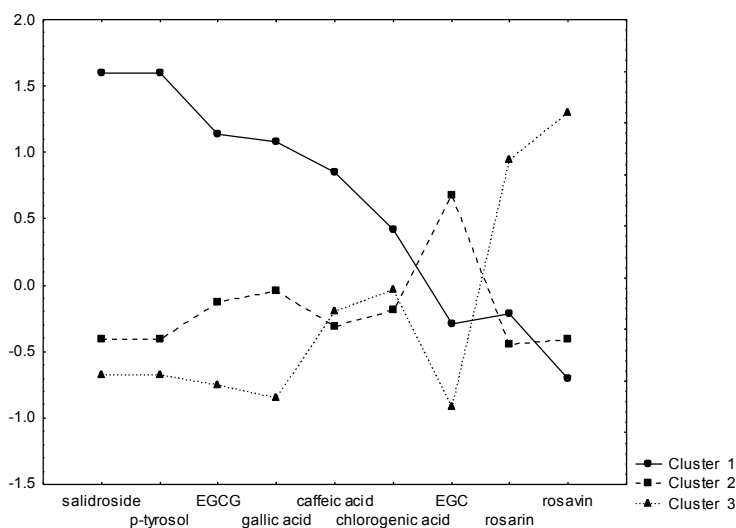
Variables	Mean ±SD	Min	Max	V (%)
Fresh weight of raw material per plant (g)	369.5 ±215.9	113.4	961.7	58.4
Air-dry weight of raw material per plant (g)	117.0 ±67.0	36.3	295.2	57.3
Dry weight of raw material per plant (g)	108.5 ±62.5	33.8	276.2	57.6
Water content in fresh raw material (%)	70.5 ±1.4	67.1	73.6	1.9
Water content in air-dry raw material (%)	7.3 ±0.7	6.4	8.8	9.0
Fresh weight of rhizomes per plant (g)	302.9 ±178.4	94.4	798.4	58.9
Fresh weight of roots (g)	66.5 ±40.2	13.4	163.2	60.5
Share of rhizomes in fresh weight of raw material (%)	82.2 ±4.1	74.0	89.2	4.9

SD – standard deviation; V – variability coefficient; *n* = 25.

**Tab. 2** Content of the active compounds in the rhizomes with roots of *Rhodiola rosea*.

Variables	Mean $\pm$ SD	Min	Max	V (%)
Rosavin	2.08 $\pm$ 0.92	0.74	3.61	44.0
Salidroside	1.55 $\pm$ 1.07	0.42	3.79	69.0
Epigallocatechin gallate	0.92 $\pm$ 0.22	0.43	1.27	23.6
<i>p</i> -Tyrosol	0.77 $\pm$ 0.53	0.20	1.89	69.4
Epigallocatechin	0.74 $\pm$ 0.17	0.49	1.23	23.1
Gallic acid	0.47 $\pm$ 0.15	0.28	0.84	30.9
Rosarin	0.25 $\pm$ 0.15	0.05	0.63	58.8
Caffeic acid	0.10 $\pm$ 0.03	0.05	0.17	34.0
Chlorogenic acid	0.09 $\pm$ 0.03	0.06	0.19	28.5
Phenylpropanoids	2.33 $\pm$ 1.02	0.86	4.11	43.6
Phenylethanoids	2.32 $\pm$ 1.60	0.62	5.68	69.1
Catechins	1.65 $\pm$ 0.26	1.07	2.06	15.9
Phenolic acids	0.67 $\pm$ 0.16	0.41	1.09	23.5
Total	6.97 $\pm$ 1.56	5.00	10.37	22.4

Compound content – in mg g<sup>-1</sup> dry matter, *n* = 25. Phenylpropanoids – sum of rosavin and rosarin (rosin – not detected); phenylethanoids – sum of salidroside and *p*-tyrosol; catechins – sum of epigallocatechin gallate and epigallocatechin; phenolic acids – sum of gallic, caffeic, and chlorogenic acids; total – sum of all investigated active compounds; SD – standard deviation, V – variability coefficient.



**Fig. 1** K-means clustering of 25 samples of the roseroot raw material: graph of means for the standardized contents of the active compounds. Pillai's trace test for MANOVA: 1.78,  $p < 0.001$ ; *F*-test – salidroside: 62.64,  $p < 0.001$ , *p*-tyrosol: 62.65,  $p < 0.001$ , EGCG: 10.84,  $p < 0.001$ , gallic acid: 10.97,  $p < 0.001$ , caffeic acid: 3.46,  $p < 0.05$ , chlorogenic acid: 0.71,  $p > 0.05$ , EGC: 10.87,  $p < 0.001$ , rosarin: 6.39,  $p < 0.01$ , rosavin: 26.05,  $p < 0.001$ ; Cluster 1: *n* = 6, Cluster 2: *n* = 12, Cluster 3: *n* = 7. EGCG – epigallocatechin gallate; EGC – epigallocatechin.

## Content of phenolic compounds

The obtained results showed a wide range in the level of the main active compounds (phenylpropanoids and phenylethanoids) in the roseroot raw material. Depending on the individual plant, the phenylpropanoid (sum of rosavin and rosarin) content varied from 0.86 to 4.11 mg g<sup>-1</sup> dry matter (DM), and for phenylethanoids (salidroside and *p*-tyrosol) – from 0.62 to 5.68 mg g<sup>-1</sup> DM. The mean content of these two groups of compounds was similar: 2.33 and 2.32 mg g<sup>-1</sup> DM, respectively. Catechins (epigallocatechin gallate and epigallocatechin) and phenolic acids (especially gallic acid) were also an important chemical component. The variability coefficient (V%) for these groups of compounds was significantly lower than for phenylpropanoids and phenylethanoids. The amount of catechins ranged from 1.07 to 2.06 mg g<sup>-1</sup> DM, and for phenolic acids – from 0.41 to 1.09 mg g<sup>-1</sup> DM. The minimum total content of the investigated active compounds was 5.00, and the maximum content reached 10.37 mg g<sup>-1</sup> DM of *Rh. rosea* rhizomes with roots (Tab. 2).

## Chemical groups of roseroot

High differentiation in the content of some chemical compounds and distribution type of some variables suggested the possibility of the presence of chemical groups of *Rh. rosea*. K-means clustering analysis showed three well-separated groups of plant specimens that differed significantly in most of the investigated compounds (Fig. 1, Tab. 3). The first cluster was distinguished by a high level of phenylethanoids, epigallocatechin gallate (EGCG), and phenolic acids as well as by a low mean content of phenylpropanoids, especially rosavin. In the raw material of the second plant group, only a high amount of epigallocatechin (EGC) was detected. In the third cluster, primarily a high level of phenylpropanoids drew attention. It was interesting that in the raw material with the high phenylethanoid content (Cluster 1), a low level of phenylpropanoids was recorded, and vice versa (Cluster 3). This relationship was confirmed by the negative correlation found between phenylethanoids and the main component of phenylpropanoids – rosavin ( $r = -0.52$ ,  $p = 0.008$ ). The above-described chemical groups of roseroot plants also distinguished in the total content of the active compounds. The average sum of all investigated components in the clusters varied from 5.95 to 9.24 mg g<sup>-1</sup> DM (Tab. 3).

**Tab. 3** Chemical differentiation of *Rhodiola rosea* plant groups (mean  $\pm$ SD).

Variables	Plant groups			p-value
	Cluster 1	Cluster 2	Cluster 3	
Salidroside	<b>3.25</b> $\pm$ 0.57 <sup>a</sup>	1.12 $\pm$ 0.45 <sup>b</sup>	0.82 $\pm$ 0.21 <sup>b</sup>	***
p-Tyrosol	<b>1.62</b> $\pm$ 0.28 <sup>a</sup>	0.55 $\pm$ 0.23 <sup>b</sup>	0.41 $\pm$ 0.11 <sup>b</sup>	***
Phenylethanoids	<b>4.87</b> $\pm$ 0.85 <sup>a</sup>	1.67 $\pm$ 0.67 <sup>b</sup>	1.23 $\pm$ 0.32 <sup>b</sup>	***
Epigallocatechin gallate	<b>1.16</b> $\pm$ 0.07 <sup>a</sup>	0.89 $\pm$ 0.20 <sup>b</sup>	0.75 $\pm$ 0.13 <sup>b</sup>	**
Gallic acid	<b>0.63</b> $\pm$ 0.16 <sup>a</sup>	0.47 $\pm$ 0.10 <sup>ab</sup>	0.35 $\pm$ 0.06 <sup>b</sup>	**
Caffeic acid	<b>0.13</b> $\pm$ 0.02 <sup>a</sup>	0.09 $\pm$ 0.04 <sup>b</sup>	0.09 $\pm$ 0.01 <sup>ab</sup>	*
Chlorogenic acid	<b>0.10</b> $\pm$ 0.04	0.09 $\pm$ 0.02	0.09 $\pm$ 0.01	n.s.
Phenolic acids	<b>0.86</b> $\pm$ 0.13 <sup>a</sup>	0.64 $\pm$ 0.10 <sup>b</sup>	0.53 $\pm$ 0.05 <sup>b</sup>	***
Total	<b>9.24</b> $\pm$ 1.12 <sup>a</sup>	5.95 $\pm$ 0.78 <sup>b</sup>	6.77 $\pm$ 0.59 <sup>ab</sup>	***
Epigallocatechin	0.69 $\pm$ 0.06 <sup>ab</sup>	<b>0.85</b> $\pm$ 0.17 <sup>a</sup>	0.58 $\pm$ 0.07 <sup>b</sup>	***
Rosarin	0.22 $\pm$ 0.06 <sup>ab</sup>	0.18 $\pm$ 0.08 <sup>b</sup>	<b>0.39</b> $\pm$ 0.20 <sup>a</sup>	*
Rosavin	1.44 $\pm$ 0.54 <sup>a</sup>	1.71 $\pm$ 0.60 <sup>a</sup>	<b>3.28</b> $\pm$ 0.33 <sup>b</sup>	***
Phenylpropanoids	1.65 $\pm$ 0.60 <sup>a</sup>	1.89 $\pm$ 0.63 <sup>a</sup>	<b>3.67</b> $\pm$ 0.47 <sup>b</sup>	***

Kruskal–Wallis test: \*\*\*  $p < 0.001$ ; \*\*  $p < 0.01$ ; \*  $p \leq 0.05$ ; n.s. – not significant;  $n = 25$ . The highest values are shown in bold. Values with the same letter are not significantly different (post-hoc test,  $p > 0.05$ ). Groups of roseroot plants – according to  $k$ -means clustering (Fig. 1). Compound content – in  $\text{mg g}^{-1}$  dry matter. Phenylethanoids – sum of salidroside and  $p$ -tyrosol; phenolic acids – sum of gallic, caffeic, and chlorogenic acids; phenylpropanoids – sum of rosavin and rosarin; total – sum of all investigated active compounds; SD – standard deviation.

### Biometric parameters of chemical groups

Our research indicated some morphological features differentiating the separated chemical groups (Tab. 4, Tab. 5). Roseroot plants from the first cluster developed the largest above- and underground organs, while individuals in the third group were the smallest. In the case of characteristics such as clump height, shoot diameter, and number of leaves per shoot, the differences between the described clusters were statistically significant (Tab. 4). Plant habit was not a clearly differentiating factor of the groups in question, but leaf shape served this role (Tab. 5). Individuals from the first cluster were characterized by the most strongly serrated leaves (high values for leaf skeleton length and number of skeleton end nodes). Plants collected in the other two groups had the margin of the leaf blade visibly more entire. In turn, other parameters describing leaf shape (the ratio of leaf length to width and the proportions of leaf width in 1/4, 1/2, and 3/4 of its length) were not statistically significant.

### Morphological and phytochemical co-variability

Biometric differentiation of plants from the individual clusters (Tab. 4, Tab. 5) found confirmation in the correlations between morphological and chemical features (Tab. 6). The leaf skeleton length (index of serration) was significantly related to the total content of the investigated active compounds as well as the amount of phenolic acids and phenylethanoids. On the other hand, negative correlations were observed between the main component of phenylpropanoids – rosavin and the parameters describing the size of the aboveground plant parts. Most of the relationships were detected in the case of catechins. The level of the sum of epigallocatechin and

**Tab. 4** Differentiation of plant size in *Rhodiola rosea* chemical groups (mean  $\pm$ SD).

Variables	Plant groups			p-value
	Cluster 1	Cluster 2	Cluster 3	
Diameter of clump (cm)	<b>52.1</b> $\pm$ 9.8	45.2 $\pm$ 9.6	41.0 $\pm$ 14.7	n.s.
Height of clump (cm)	<b>26.5</b> $\pm$ 3.7 <sup>a</sup>	21.3 $\pm$ 4.6 <sup>ab</sup>	17.9 $\pm$ 3.5 <sup>b</sup>	**
Length of shoot (cm)	<b>22.8</b> $\pm$ 4.4	19.4 $\pm$ 4.5	16.7 $\pm$ 5.4	n.s.
Diameter of shoot (mm)	<b>5.0</b> $\pm$ 0.6 <sup>a</sup>	4.8 $\pm$ 1.0 <sup>ab</sup>	4.0 $\pm$ 0.4 <sup>b</sup>	*
Number of leaves per shoot	<b>70.1</b> $\pm$ 9.5 <sup>a</sup>	62.5 $\pm$ 11.5 <sup>a</sup>	47.6 $\pm$ 9.5 <sup>b</sup>	**
Leaf area (cm <sup>2</sup> )	<b>4.0</b> $\pm$ 1.4	3.4 $\pm$ 1.2	3.0 $\pm$ 0.7	n.s.
Fresh weight of rhizomes with roots (g)	<b>412.9</b> $\pm$ 161.9	352.6 $\pm$ 188.1	261.1 $\pm$ 180.6 <sup>1)</sup>	n.s.
Dry weight of rhizomes with roots (g)	<b>126.6</b> $\pm$ 49.4	101.4 $\pm$ 53.5	76.4 $\pm$ 51.5 <sup>1)</sup>	n.s.

Kruskal–Wallis test: \*\*\*  $p < 0.001$ ; \*\*  $p < 0.01$ ; \*  $p \leq 0.05$ ; n.s. – not significant;  $n = 25$ . <sup>1)</sup> Without one outlier observation. SD – standard deviation. The highest values are shown in bold. Values with the same letter are not significantly different (post-hoc test,  $p > 0.05$ ). Groups of roseroot plants – according to  $k$ -means clustering (Fig. 1).

**Tab. 5** Differentiation of plant habit and leaf shape in *Rhodiola rosea* chemical groups (mean  $\pm$ SD).

Variables	Plant groups			p-value
	Cluster 1	Cluster 2	Cluster 3	
Length of leaf skeleton (cm)	<b>7.80</b> $\pm$ 0.84 <sup>a</sup>	5.17 $\pm$ 0.92 <sup>b</sup>	5.27 $\pm$ 0.96 <sup>b</sup>	**
Skeleton end length (cm)	<b>5.67</b> $\pm$ 0.84 <sup>a</sup>	3.26 $\pm$ 0.90 <sup>b</sup>	3.63 $\pm$ 0.97 <sup>b</sup>	**
Skeleton end node number	<b>14.18</b> $\pm$ 1.88 <sup>a</sup>	10.80 $\pm$ 1.92 <sup>b</sup>	11.37 $\pm$ 1.51 <sup>b</sup>	**
Length / Diameter of shoot	<b>45.13</b> $\pm$ 6.46	40.73 $\pm$ 6.65	37.50 $\pm$ 8.10 <sup>1)</sup>	n.s.
Foliage density	3.12 $\pm$ 0.29	<b>3.26</b> $\pm$ 0.35	2.95 $\pm$ 0.40	n.s.
Length / Width of leaf	1.71 $\pm$ 0.12	<b>1.90</b> $\pm$ 0.22	1.75 $\pm$ 0.17	n.s.
Diameter / Height of clump	1.96 $\pm$ 0.26	<b>2.16</b> $\pm$ 0.51	2.04 $\pm$ 0.40 <sup>1)</sup>	n.s.

Kruskal–Wallis test: \*\*\*  $p < 0.001$ ; \*\*  $p < 0.01$ ; \*  $p \leq 0.05$ ; n.s. – not significant;  $n = 25$ . <sup>1)</sup> Without one outlier observation. SD – standard deviation. Foliage density – number of leaves per 1 cm of shoot. The highest values are shown in bold. Values with the same letter are not significantly different (post-hoc test,  $p > 0.05$ ). Groups of roseroot plants – according to  $k$ -means clustering (Fig. 1).

epigallocatechin gallate was strongly correlated not only with the roseroot clump and shoot size, but with the mean leaf area and the number of leaves per shoot as well as fresh and dry weight of raw material.

## Discussion

*Rhodiola rosea* is a slow-growing and long-lived plant occurring in the harsh climate of the polar tundra and high mountains. According to calculations of Nukhimovskii [2], the total age of roseroot polycormons in some cases can be up to about 300 years. In the Altai Mountains, the first symptoms of plant ageing are usually observed in the 15th–20th or even just in the 40th year of vegetation. The fresh weight of the largest specimens was 7.8 kg, and the weight of living rhizomes – 3.5 kg [2]. However, plant

**Tab. 6** Main correlations between morphological and chemical features of *Rhodiola rosea*.

Variables	Total	Phenolic acids	Phenylethanoids	Rosavin	Epigallocatechin	Catechins
Leaf skeleton length	0.68***	0.62***	0.58**			
Index of clump size			0.42*	-0.56**		0.59**
Shoot leaf number				-0.67***	0.49*	0.74***
Index of shoot size				-0.55**	0.54**	0.64***
Diameter of shoot				-0.51**	0.65***	0.55**
FW of raw material						0.51**
DW of raw material						0.51**
Leaf area <sup>1)</sup>						0.50*

Pearson's correlation: \*\*\*  $p \leq 0.001$ ; \*\*  $p < 0.01$ ; \*  $p < 0.05$ ;  $n = 25$ . <sup>1)</sup> Without two outlier observations. Total – sum of all investigated active compounds; phenolic acids – sum of gallic, caffeic, and chlorogenic acids; phenylethanoids – sum of salidroside and *p*-tyrosol; catechins – sum of epigallocatechin gallate and epigallocatechin. Index of clump size – Diameter  $\times$  Height of clump; index of shoot size – Length  $\times$  Diameter of shoot. FW – fresh weight; DW – dry weight.

growth in natural stands is very slow. For example, the yield of underground organs of 30–50-year-old individuals found in the Altai Mountains was similar to that of 5-year-old roseroot from field cultivation in the Moscow region. On the other hand, plants growing under these conditions showed some signs of ageing already after 6–8 years of cultivation. The following were noted: a decrease in the number of flowering shoots and in shoot height, necrosis foci in the rhizomes, weaker growth of rhizomes, and others [26]. In the plantations established from seeds in the milder climate of Poland, the yield of raw material decreased already in the sixth year of cultivation [15,21]. In the fifth year of vegetation, the mean weight of air-dry rhizomes with roots of *Rh. rosea* was 120.4 g plant<sup>-1</sup> in the case of a field experiment located in Warsaw [15] and 209.9 g plant<sup>-1</sup> for Lublin [21]. In our investigations, the air-dry weight of underground organs of 4-year-old roseroot growing in a plantation established by rhizome division ranged from 36.3 to 295.2 g plant<sup>-1</sup>, with an average value of 117.0 g plant<sup>-1</sup> (Tab. 1). The mean share of rhizomes in the total weight of the underground part amounted to 82.2%, and it was similar to the results obtained by Przybył et al. [15]: 81 and 83% in the case of the 4th and 5th year of cultivation, respectively. In the next year, a decrease in the percentage share of rhizomes to 69% of the total weight of raw material was detected. This was due to the decay of the oldest, central parts of rhizomes and the division of them into many smaller pieces [18].

Observations conducted by Kim [27] in natural stands in the Altai Mountains showed that, depending on the habitat type, the mean height of plants varies from 19.4 to 26.3 cm, leaf number per shoot: 39.4–58.6, leaf length: 1.2–3.1 cm, and the mean rhizome weight: from 13.4 to 54.8 g plant<sup>-1</sup>. According to other investigations from this region, the weight of rhizomes ranged from 50 to 840 g per plant, and the weight of aboveground parts: from 20 to 300 g plant<sup>-1</sup> [28]. Our previous research [22] also indicated large variation of the biometric features describing the size of roseroot specimens: clumps, shoots, and leaves. For example, the height of 4-year-old plants varied from 12 to 40 cm, leaf number per shoot: 30–81, mean leaf length: 1.9–4.6 cm, and the fresh weight of raw material: from 113 to 1156 g plant<sup>-1</sup>. In addition, comparative studies showed statistically significant differences between plant material collected in two investigated years. In the present work, the shape of leaves was also described and high variation in the degree of leaf serration was noted (Tab. 5). On the basis of the serration, size, and color of leaves, Kurkin et al. [3] distinguished six morphotypes of *Rh. rosea* with varying yield and rosavin content. These plants had green or less silvery-green leaves, with serrate or entire margin. The length of the leaf lamina ranged from less than 1.5 to 5.0 cm, and the width from 0.5 to more than 1.5 cm.

The raw material of roseroot originating from separate populations is characterized by high phytochemical variation [10,16,18,29]. The results obtained by Wiedenfeld et al. [17] indicate that, besides the content of substances, also their composition can change in a broad range. Additionally, large intrapopulation variability of the level of the main active compounds and the yield of underground parts of *Rh. rosea* was also noted. However, no correlation was found between the raw material weight and the amount of analyzed components (phenylethanoids, *trans*-cinnamic alcohol, rosavins, and caffeic acid) [30]. In our previous work, no significant relationships were detected between the level of total polyphenols, tannins, flavonoids and the weight of rhizomes with roots, either. On the other hand, a relatively strong correlation ( $r_s = -0.68$ ,  $p < 0.001$ ) between flavonoid content in dry matter of roseroot underground organs and water content in fresh weight of this plant material drew attention [22]. In the present investigations, we described relationships between the weight of raw material and catechin content (Tab. 6). In addition, the water content in fresh raw material correlated with the level of phenylethanoids ( $r = -0.43$ ,  $p = 0.031$ ), caffeic acid ( $r = -0.45$ ,  $p = 0.024$ ), and chlorogenic acid ( $r = -0.44$ ,  $p = 0.029$ ).

Interesting data about the morphological and phytochemical co-variability of *Rh. rosea* were provided by the studies of Kurkin et al. [3]. They showed a clearly higher content of rosavin in the morphotypes with entire or slightly serrated leaf margin compared with plants with strongly serrated leaves. It was consistent with our results where individuals with the highest amount of rosavin in the underground organs (Cluster 3) were distinguished by a low level of the leaf skeleton parameters describing the degree of leaf serration (Tab. 3, Tab. 5). Additionally, plants belonging to the above-mentioned group were characterized by the lowest mean leaf area (Tab. 4), which also confirms the previous observations of Kurkin et al. [3]. According to these authors, the small-leaved morphotype had the highest amount of rosavin, but it gave a low yield of raw material. In our investigations, plants from the third cluster reached the smallest size of above- and underground parts: clumps, shoots, and rhizomes with roots (Tab. 4). Some relationships between phytochemical and biometric features of roseroot can be found in the field experiments conducted in southern Finland [31]. They showed the effect of organic fertilization on the growth of vegetative shoots, the fresh weight of raw material and water content, and at the same time on the content of salidroside, rosavin, and flavonoids. A similar conclusion arose from a field experiment which was carried out in Poland [25]. In this case, organic fertilization significantly influenced the yield of fresh and air-dry matter of *Rh. rosea* rhizomes with roots and the level of phenylpropanoids, too.

In summary, roseroot exhibits high morphological and phytochemical differentiation. Literature data concerning the co-variability of these two groups of features are limited. However, they largely confirm our observations of the occurrence of *Rh. rosea* chemotypes well-characterized in terms of morphology. In the present study, the groups of individuals which were distinguished on the basis of quantitative analysis of the chemical composition of the raw material clearly differed in luxuriance of plants. The important diagnostic feature was also the degree of leaf serration. These relationships were consistent with the correlations found between the individual compounds or groups of active compounds and the analyzed biometric traits.

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