ACTA SCIENTIARUM POLONORUM *Acta Sci. Pol., Hortorum Cultus 12(3) 2013, 147-160*

INFLUENCE OF PLANTS AGE ON THE CHEMICAL COMPOSITION OF ROSEROOT (*Rhodiola rosea* L.)

Barbara Kołodziej, Danuta Sugier
University of Life Sciences in Lublin

Abstract. Roseroot, *Rhodiola rosea L.* has been used in the traditional Asian, Scandinavian and Eastern European medicine for centuries as remedies for improvement of physical condition, treatment of anemia, depression, asthenia, impotence, gastro-intestinal and nervous system disorders and also as a immunostimulant and anti-inflammatory agent. This valuable plant grows naturally in Himalayas, Altai, Alps and the Carpatian mountains. Roseroot raw material contains phenylethanoids (salidroside and *p*-tyrosol) and cinnamic glycosides known as phenylpropanoids (rosin, rosavin and rosarin), that are considered the most important active substances identified in raw material. The objective of this experiment was to compare the content of phenolic compounds (salidroside, *p*-tyrosol) and cinnamic glycosides (rosarin, rosavin and rosin) determined by HPLC method, from particular morphological parts (roots, rhizomes, and for the first time – tips and above ground parts) of the raw material of *R. rosea* cultivated in Poland through seven following vegetation periods. In this study we found that significantly yearly increases in total phenylethanoids and phenylpropanoids concentrations occur with *R. rosea* grown in Poland. Rhizomes were characterized by highest amount of phenylpropanoids and phenylethanoids studied, in comparison to the other morphological parts of plants at the same age, whereas a certain amount of active substances were also found in the stems and leaves of *Rhodiola rosea* (on an average as twice as lower than in the under ground parts of plants). Thus, above ground parts of roseroot could be a potential source of phenylethanoids and phenylpropanoids for pharmacy. Roseroot harvested after only 3 year of vegetation contained significantly lower amounts of phenylethanoids and phenylpropanoids in under ground parts of plants than harvested after 4, 5 or 6 year. Since these phenolics and glycosides are the major active constituents of *Rhodiola rosea,* this change to an earlier harvest (before fourth or in appropriate cases in third year) may have an effect on the quality of the harvested raw material.

Key words: morphological parts, quality of raw material, phenylpropanoids and phenylethanoids content

Corresponding author: Barbara Kołodziej, Department of Industrial and Medicinal Plants, University of Life Sciences in Lublin, 15 Akademicka Str., 20-950 Lublin; Poland, phone (+48) 81 44 566 79, e-mail: barbara.kolodziej@up.lublin.pl

INTRODUCTION

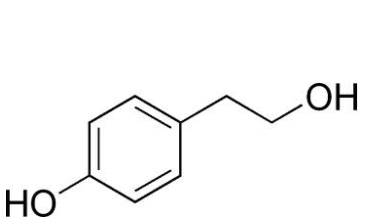
Rhodiola rosea L. from Crassulaceae family grows in crevices of mountain rocks of Arctic regions of Europe, Asia (Siberia) and North America [Galambosi 2006; Altantsetseg et al. 2007; Platikanov and Evstatieva 2008]. The main source of commercially available roots and rhizome are Mountain Altai and in south region of foothill Altai [Bykov et al. 1999; Ganzera et al. 2000; Przybył et al. 2004; Galambosi 2006; Pannosian et al. 2010]. The rapidly growing demand and also high prices for raw material for industry could cause increased pressure on natural habitats. Due to intensive collection, the natural populations are seriously threatened and nowadays roseroot is registered as an endangered plant in many European countries [Lange 1998; Galambosi 2006; Platikanov and Evstatieva 2008]. In Poland, its presence is limited to mountainous areas – the Sudeten and Carpathian Mountains – legally protected (Karkonosze National Park, Tatra National Park, Babia Góra National Park) – Krajewska-Patan et al. [2005]. The herbal raw material is the rhizome and roots, collected mainly from field cultivation at least from three year old plants [Revina et al. 1976; Bykov et al. 1999; Galambosi 2006; Platikanov and Evstatieva 2008] – Fig. 5.

Intensive research on *Rhodiola rosea* has been performed in the former Soviet Union, resulting in the isolation of several classes of compounds: essential oils, *trans*-cinnamic alcohol glycosides, flavonoids, organic acids, fats, phenolics including tannins, and proteins [Revina et al. 1976; Zapesochnaya and Kurkin 1983; Kiryanov et al. 1991; Bykov et al. 1999; Ganzera et al. 2000; Kurkin 2003; Przybył et al. 2004; Galambosi 2006; Ma et al. 2008; Węglarz et al. 2008; Pannosian et al. 2010]. Salidroside and its precursor *p*-tyrosol (belonging to phenylethanoids) and cinnamic glycosides known as phenylpropanoids (rosin, rosavin and rosarin) are considered most important active substances identified in raw material [Galambosi 2006; Linh et al. 2000; Przybył et al. 2004] – Fig. 1.

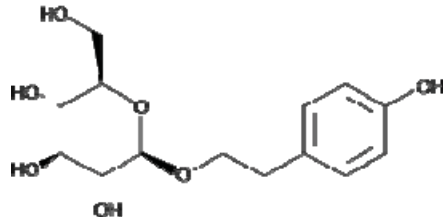
Salidroside (p-hydroxyphenethyl-ß-D-glucopyranoside), known as rhodioloside – Fig. 1, is one of the major phenolic glycoside of golden root and its content is often used as one of criteria to evaluate the quality of the crude drug [Linh et al. 2000, Kurkin 2003]. Characteristic feature of *R. rosea* is presence of cynnamic alcohol glucosides and relatively high content of phenylpropanoids rosavin (*trans*-cinnamyl O-(6'-O-α-L--arabinopyranosyl-ß-D-glucopyranoside) – Fig. 1, which was not detected in other 21 genus of Rhodiola species morphologically similar to *R. rosea* [Bykov et al. 1999; Kurkin 2003; Altantsetseg et al. 2007; Pannosian et al. 2010]. Thus, it was established that salidroside content does not provide an objective assessment of the identity; nor does it definitively characterize the quality of the *R rosea* rhizome [Bykov et al. 1999; Zapesochnaya and Kurkin 1982]. For this reason, special investigations were devoted to the development of methods capable of isolating phenylpropanoids (rosin, rosavin and rosarin). Usually ratio of phenylethanoids to phenylpropanoids in the plant rhizomes is approximately 1 : 3 [Brown et al. 2002].

Roseroot is a popular plant in traditional medicine in the Nordic countries, Eastern Europe and Asia, with a reputation for stimulating the nervous system, decreasing depression, enhancing work performance, eliminating fatigue, and preventing high altitude sickness [Kiryanov et al. 1991; Brown et al. 2002; Węglarz et al. 2008; Galambosi

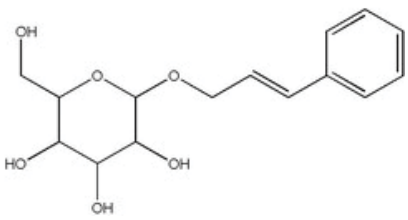
2006; Pannosian et al. 2010]. Salidroside, rosarin and rosavin showed antioxidant and neurostimulating properties [Kurkin et al. 1986]. Biologically active compounds have also antidepressive, anti-fatigue, cognitive-enhancing, anti-anoxia, hepatoprotective, anti-allergy, anti-inflammatory properties [Linh et al. 2000; Platikanov and Evstatieva 2008; Panossian et al. 2010]. *R. rosea* root extracts showed adaptogenic, stress protective (a.a. neuro- cardio- and hepato- protective), antifatigue and CNS stimulating effect [Panossian et al. 2010]. Preparations of *Rhodiola rosea* extracts are also used worldwide as a dietary supplement or component of functional food [Ma et al. 2008].



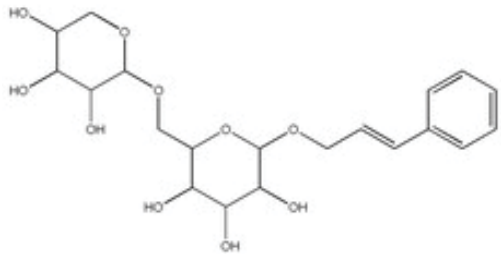
p-tyrosol



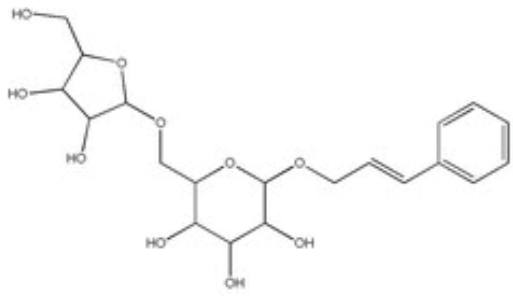
salidroside



rosin



rosavin



rosarin

Fig. 1. Structures of *Rhodiola rosea* phenylethanoids and phenylpropanoids

Previous studies have shown that phenylethanoids and phenylpropanoids content in golden root depend on the morphological part of plant [Przybył et al. 2008; Węglarz et al. 2008], its age [Revina et al. 1976; Platikanov and Evstatieva 2008; Węglarz et al. 2008; Kucharski et al. 2011], place of raw material harvesting (wild, cultivated or *in vitro*) [Revina et al. 1976; Kurkin et al. 1989; Bykov et al. 1999; Krajewska-Patan et al. 2005], time of harvesting [Buchwald et al. 2006; Platikanov and Evstatieva 2008], place of origin [Altantsetseg et al. 2007; Węglarz et al. 2008; Altanes, fertilization [Galambosi 2006; Kucharski et al. 2011] or cultivation methods [Galambosi 2006; Kucharski et al. 2011].

Although the phenylethanoids and phenylpropanoids are the main active principles of roseroot raw material, little information is available on the relationship between plants age and the amount of these constituents in under and above ground parts of plants grown in Poland. This is particular importance in understanding the significance of the change in the production system to a possible earlier schedule.

The aim of this study was to compare the content of main biologically active compounds (salidroside, *p*-tyrosol, rosarin, rosavin and rosin) from particular morphological parts of the raw material of *R. rosea* cultivated in Poland through seven following vegetation periods.

MATERIAL AND METHODS

In the years 2005–2011 an experiment was conducted on experimental fields at the University of Agriculture in Lublin (N 51 09'06.81", E 22 28'23.42"). Scheme of the experiment was showed in our previous paper [Kołodziej and Sugier 2012].

Plant material was obtained at the end of vegetation period (15th of September 2011) from 10 randomly chosen plants from each plot. After digging and cleaning underground parts of plants, the raw material was subjected to thermal drying – at 80ºC in a drying chamber. The samples of plant material (above ground parts as well as roots, rhizomes and tips – Fig. 5.) were taken of each object of plant material (by Polish standards PN-91/R-87019) for chemical analysis on the content of glycosides. For analysis of active substances – phenylpropanoids (expressed as sum of rosin, rosavin and rosarin) and phenylethanoids (salidroside and *p*-tyrosol) content HPLC method was used according to Przybył et al. [2008] and Węglarz et al. [2008].

Individual plant parts were pulverized in a homogenizer, and then 1.0 g of raw material was extracted with 100 ml of methanol under the reflux during 4 hours. After evaporation of solvent, the residue was dissolved in 5 ml of methanol, filtered through Captiva Polipropylene column 0.2 μm – SPE station (Varian Inc., USA) and subjected to HPLC. The analysis were carried out using Varian 920 LC chromatograph (Varian Inc, USA) with DAD detector equipped with Gemini 5 μm C_{18} 250 mm × 4.6 mm column (Phenomenex). The gradient of 0.2% phosphoric acid in HPLC grade water (A) and acetonitrile (B) was used as follows: 0 min, 4% B; 10 min, 13% B, 20 min, 15% B, 30 min, 20% B; 33 min, 25% B; 38 min, 30% B; held constant for another 22 min. The following analysis parameters were used: injection volume: 10 μl, flow rate: 1.2 ml·min^{-1}; time of analysis 60 min, recorded wave range: 190–450 nm, detection wave length:

275 nm. Peaks were identified by comparison the retention time and spectral data with adequate parameters of standards (*Rhodiola rosea* standards Kit by ChromaDex). Quantification was based on the peak area. The content of the determined compounds was calculated in $mg \cdot g^{-1}$ dry matter.

Numerical results concerning content of individual biologically active compounds in different morphological parts of plants were subjected to analysis of variance and t-Duncan's multiple range test (= 0.05% of significance level) was used for means separation. The results of the chemical analyses of various morphological parts of roseroot plants at different age were analyzed also by Multivariate Statistical Package (MVSP 3.1). Cluster analysis was performed by using UPGMA (Unweighted Pair Group Method Using Arithmetic Averages) complete linkage to group the plants by the similarity of chemical parameters. Mean values of active substances content are listed in Table 1–4. The result of the clustering was plotted as a hierarchical tree or dendrogram (Fig. 2–4).

RESULTS AND DISCUSSION

In the experiment there were observed a different content of phenylpropanoids and phenylethanoids examined in various morphological parts of plant (Fig. 5) and its significant changes along with roseroot aging (Tab. 1–4).

The total active substances content increased from approximately 6.55 $mg \cdot g^{-1}$ for the first year roots to almost 13.60 $mg \cdot g^{-1}$ (twice as much) for four-year-old roots and after that, like in Buchwald et al. [2006] experiment, decreased gradually from 12.58 $mg \cdot g^{-1}$ for 5-yr-old roots to 9.02 $mg \cdot g^{-1}$ for 7-year-old plants – Table 1. In accordance with Węglarz et al. [2008] results, increases in the concentrations of phenylpropanoids (rosavin, rosin) accounted for most of these increases. Rosavin content ranged from 2.53 $mg \cdot g^{-1}$ for 1-year-old plants to 5.39 $mg \cdot g^{-1}$ for 4-year-old roots, when rosin content varied between 1.54 $mg \cdot g^{-1}$ (3-year-old) and 4.60 $mg \cdot g^{-1}$ (4-year-old plants) and were lower than in Przybył et al. [2008] and Węglarz et al. [2008] studies, but comparable with Buchwald et al. [2006] and Ma et al. [2008] results. Phenylethanoids constituted a small part of the total amount of active substances in roseroot roots (from 0.68 $mg \cdot g^{-1}$ in the case of two year old plants to 1.92 $mg \cdot g^{-1}$ in the oldest, 7-year-old plants). It is worth to emphasize that *p*-tyrosol content was extremely low (form 0.11 $mg \cdot g^{-1}$ for 7-year-old roots to 0.25 $mg \cdot g^{-1}$ for 6-year-old plants) and was not obviously depended on age. Similar content of tyrosol was obtained in *Rhodila rosea* roots by Linh et al. [2000] and Buchwald et al. [2006]. Over the seven year period, these increases were much larger than those reported by Węglarz et al. [2008]. Results obtained in our study are different from the previous studies, which may be due to the difference of genetic, cultivation conditions as well as the geographic location of *R. rosea*.

As in Węglarz et al. [2008] studies rhizomes of *Rhodiola rosea* were characterized by higher amount of phenylpropanoids and phenylethanoids studied in comparison to the roots at the same age (Tab. 1, 2). The total active substances content in rhizomes were particularly rich and varied from 7.90 $mg \cdot g^{-1}$ for the first year roots to 15.50 $mg \cdot g^{-1}$ (almost twice as much) for five-year-old roots. Similarly as in the case of roots we ob-

Table 1. Content of biologically active compounds in *Rhodiola rosea* roots depending on plants age

Plants age (year)	(in mg·g^{-1})							
	salidroside	tyrosol	rosarin	rosavin	rosin	phenylethynoids	phenylpropanoids	total
1	0.94 ± 0.15a[b]	0.13 ± 0.06a	1.57 ± 0.25a	2.53 ± 0.40a	1.36 ± 0.31a	1.07 ± 0.17a	5.47 ± 0.44a	6.55 ± 1.57a
2	0.54 ± 0.11a	0.14 ± 0.06a	1.62 ± 0.32a	2.64 ± 0.53a	2.67 ± 0.47a	0.68 ± 0.35a	6.94 ± 0.44a	7.62 ± 0.47a
3	0.56 ± 0.33a	0.18 ± 0.02a	2.10 ± 0.39a	4.01 ± 0.45a	1.54 ± 0.76a	0.74 ± 0.43a	7.65 ± 0.26a	8.40 ± 1.57a
4	0.71 ± 0.20a	0.16 ± 0.05a	2.32 ± 0.52a	5.75 ± 0.83a	4.60 ± 0.84b	0.88 ± 0.25a	12.69 ± 1.72b	13.57 ± 1.78b
5	0.94 ± 0.22a	0.18 ± 0.11a	2.20 ± 0.20a	5.39 ± 0.50a	3.87 ± 0.28a	1.12 ± 0.28a	11.46 ± 0.67b	12.58 ± 2.53b
6	0.89 ± 0.25a	0.25 ± 0.02a	2.35 ± 0.68a	3.88 ± 0.34a	3.45 ± 0.20a	1.15 ± 0.21a	9.68 ± 1.14a	10.83 ± 1.30a
7	1.81 ± 0.30b	0.11 ± 0.05a	1.28 ± 0.23a	3.50 ± 0.22a	2.30 ± 0.66a	1.92 ± 0.29b	7.09 ± 0.42a	9.02 ± 1.41a
[a] (significance level)	**	n.s.	n.s.	n.s.	*	**	**	**

[a] n.s., *, ** – non significant or significant at p ≥ 0.05 or 0.1; a–b values within columns followed by the same letter are not significantly different from each other (ANOVA, Duncan multiple range test, P < 0.05); [b]Data are means ± SD

Table 2. Content of biologically active compounds in *Rhodiola rosea* rhizomes depending on plants age

Plants age (year)	(in mg·g^{-1})							
	salidroside	tyrosol	rosarin	rosavin	rosin	phenylethynoids	phenylpropanoids	total
1	1.65 ± 0.56a[b]	0.19 ± 0.04a	0.97 ± 0.42a	3.87 ± 1.31a	1.21 ± 0.77a	1.84 ± 0.60a	6.06 ± 1.03a	7.90 ± 1.62a
2	1.34 ± 0.44a	0.13 ± 0.02a	1.67 ± 0.52a	5.90 ± 1.11a	1.75 ±0.38a	1.47 ± 0.46a	9.33 ± 1.90a	10.80 ± 2.35a
3	1.26 ± 0.45a	0.31 ± 0.09ac	2.71 ± 0.47b	5.21 ± 0.89a	3.62 ± 0.86b	1.58 ± 0.54a	11.55 ± 1.65a	13.13 ± 1.92a
4	1.02 ± 0.37a	0.11 ± 0.01a	3.33 ± 0.33b	7.53 ± 0.77a	3.08 ± 0.57a	1.14 ± 0.37a	13.95 ± 2.63b	15.10 ± 2.25b
5	1.09 ± 0.19a	0.17 ± 0.05a	1.69 ± 0.15a	9.77 ± 0.76b	2.77 ± 0.53a	1.26 ± 0.14a	14.24 ± 1.44b	15.50 ± 1.35b
6	0.89 ± 0.23a	0.38 ± 0.11b	2.75 ± 0.16b	5.92 ± 0.73a	2.54 ± 0.29a	1.28 ± 0.13a	11.23 ± 0.77a	12.51 ± 1.96a
7	0.58 ± 0.35b	0.23 ± 0.12a	2.59 ± 0.63b	3.85 ± 0.21a	2.93 ± 0.38a	0.81 ± 0.24b	9.37 ± 1.47a	10.1 ± 2.30a
[a] (significance level)	**	**	**	*	**	*	**	*

[a] n.s., *, ** – non significant or significant at p ≥ 0.05 or 0.1; a–c values within columns columns followed by the same letter are not significantly different from each other (ANOVA, Duncan multiple range test, P < 0.05); [b]Data are means ± SD

Table 3. Content of biologically active compounds in *Rhodiola rosea* tips depending on plants age

Plants age (year)	(in mg·g^{-1})							
	salidroside	tyrosol	rosarin	rosavin	rosin	phenylethynoids	phenylpropanoids	total
1	1.23 ± 0.01a[b]	0.16 ± 0.02a	2.87 ± 0.02a	1,75 ± 0.03a	0.81 ± 0.02a	1.40 ± 0.02a	5.44 ± 0.06a	6.84 ± 0.07a
2	1.29 ± 0.09a	0.16 ± 0.01a	0.96 ± 0.08b	4.71 ± 0.05b	1.64 ± 0.14b	1.45 ± 0.08a	7.32 ± 0.10a	8.78 ± 0.19a
3	1.34 ± 0.11a	0.33 ± 0.01b	1.46 ± 0.21b	7.85 ± 1.40bc	1.58 ± 0.47b	1.67 ± 0.12b	10.91 ± 2.09b	12.58 ± 2.21b
4	1.97 ± 0.12b	0.20 ± 0.05a	3.19 ± 0.71a	9.14 ± 0.60bcd	1.57 ± 0.49b	2.17 ± 0.10bc	13.91 ± 1.35bc	16.08b ± 2.60c
5	1.35 ± 0.02b	0.22 ± 0.01ac	1.42 ± 0.02b	7.26 ± 0.45bc	2.40 ± 0.52bc	1.57 ± 0.02bc	11.09 ± 0.98b	12.66 ± 0.99b
6	0.81 ± 0.01bc	0.18 ± 0.01a	1.87 ± 0.04b	6.02 ± 0.10bcd	1.63 ± 0.08b	0.99 ± 0.01bcd	9.53 ± 0.12b	10.53 ± 0.47ab
7	0.69 ± 0.04bc	0.21 ± 0.02a	1.66 ± 0.14ac	8.53 ± 0.51bce	1.72 ± 0.10b	0.91 ± 0.06bcd	11.93 ± 0.75bd	12.84 ± 0.81b
[a] (significance level)	**	**	**	**	**	**	**	**

[a] n.s., *, ** – non significant or significant at p ≥ 0.05 or 0.1; [a] r.n.; a–d values within columns columns followed by the same letter are not significantly different from each other (ANOVA, Duncan multiple range test, P < 0.05,; [b]Data are means ± SD

Table 4. Content of biologically active compounds in *Rhodiola rosea* aboveground parts depending on plants age

Plants age (year)	(in mg·g^{-1})							
	salidroside	tyrosol	rosarin	rosavin	rosin	phenylethynoids	phenylpropanoids	total
1	0.16 ± 0.02a[b]	0.23 ± 0.02a	1.22 ± 0.01a	0.39 ± 0.02a	0.38 ± 0.01a	0.39 ± 0.04a	2.00 ± 0.02a	2.40 ± 0.02a
2	0.69 ± 0.02b	0.37 ± 0.02b	1.23 ± 0.02a	0.44 ± 0.03a	0.84 ± 0.03b	1.06 ± 0.04b	2.52 ± 0.02b	3.59 ± 0.06a
3	0.64 ± 0.04b	0.31 ± 0.01bc	1.47 ± 0.37a	1.47 ± 0.37b	1.87 ± 0.03bc	0.96 ± 0.37b	4.61 ± 0.53c	5.57 ± 0.91b
4	0.84 ± 0.02b	0.31 ± 0.02a	1.82 ± 0.01b	1.27 ± 0.02b	1.87 ± 0.02bc	1.16 ± 0.02b	4.96 ± 0.03d	6.12 ± 0.06b
5	1.18 ± 0.03bc	0.28 ± 0.04bc	1.47 ± 0.12a	1.04 ± 0.03bc	0.48 ± 0.14a	1.47 ± 0.05bc	3.00 ± 0.03e	4.48 ± 1.08bc
6	1.02 ± 0.11b	0.13 ± 0.02bcd	1.14 ± 0.22a	0.64 ± 0.02a	0.26 ± 0.03bcd	1.16 ± 0.19b	2.05 ± 0.12f	3.22 ± 0.90a
7	0.91 ± 0.07b	0.15 ± 0.03bcd	0.49 ± 0.12bc	0.43 ± 0.02a	0.37 ± 0.04a	1.07 ± 0.07b	1.30 ± 0.05g	2.37 ± 0.05a
[a] (significance level)	**	**	**	**	**	**	**	**

[a] n.s., *, ** – non significant or significant at p ≥ 0.05 or 0.1; a–g values within columns columns followed by the same letter are not significantly different from each other (ANOVA, Duncan multiple range test, P < 0.05); [b]Data are means ± SD

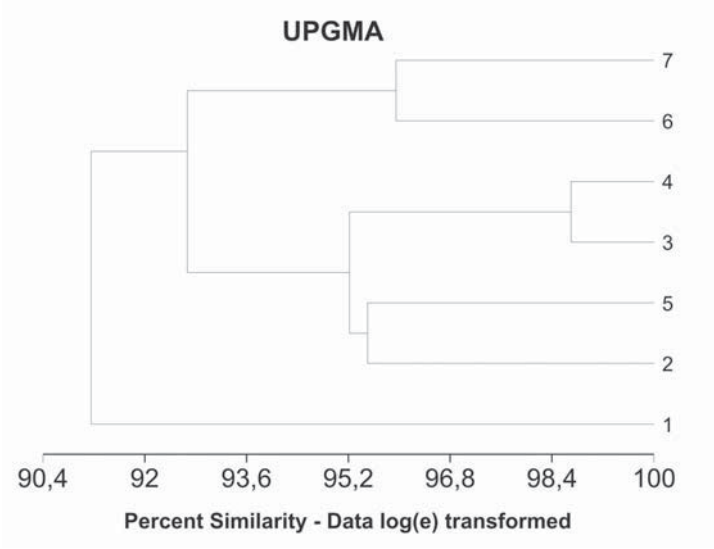


Fig. 2. Cluster analysis of chemical content of under ground parts of roseroot depending on the age of plants. Legend: 1 – one year old plants; 2 – two year old plants; 3 – three year old plants; 4 – four year old plants; 5 – five year old plants; 6 – six year old plants; 7 – seven year old plants

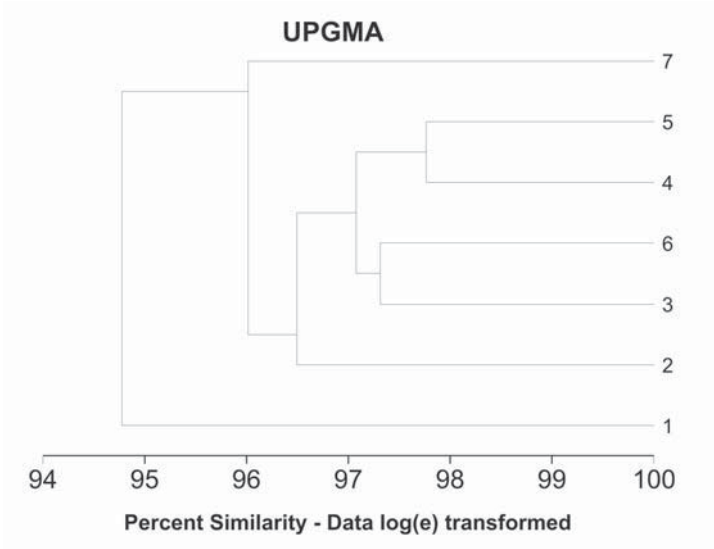


Fig. 3. Cluster analysis of chemical content of above ground parts of roseroot depending on the age of plants. Legend: 1 – one year old plants; 2 – two year old plants; 3 – three year old plants; 4 – four year old plants; 5 – five year old plants; 6 – six year old plants; 7 – seven year old plants

served active substances increment along with plants aging, which was in accordance with Węglarz et al. [2008] and Kucharski et al. [2011] results. The highest content of phenylpropanoids and phenylethanoids, as in Przybył et al. [2008] and Węglarz et al. [2008] examination, was found in five year old plants and in two following years we noted a distinct decrease of total active compounds content – Table 2. The level of active substances noted was comparable to Kucharski et al. [2011] studies, but lower than in Przybył et al [2008] and Węglarz et al. [2008] experiments. The highest content of phenylethanoids (mainly salidroside) was noted in younger plants (1 year old) – 1.65 $mg \cdot g^{-1}$ and along with plants aging content of these compounds decreased, whereas in the case of phenylpropanoids we observed a progressive increase of rosavin and rosarin content up to 5 or 4 year of vegetation (respectively 9.77 $mg \cdot g^{-1}$ and 3.33 $mg \cdot g^{-1}$) and after that its significant decline – Table 2. Similarly as in the case of roots, content of active substances in rhizomes obtained in our study differed from the previous study, probably due to the genetic differences, various cultivation and climatic conditions. The studies carried out by Kurkin et al. [1989] and Węglarz et al. [2008] showed that salidroside content in *Rhodiola rosea* rhizomes and roots increased along with aging respectively from 0.1% in two year old plants to 0.83% in seven years old and in Polish conditions from 0.18% in the first year to 0.54% in fifth year of vegetation. In our study we observed the same tendency, but salidroside content determined, like in Kucharski et al. [2011] study and as in Mongolian raw material determined by Altantsetseg et al. [2007], was significantly lower. Similarly, rosavins content in rhizomes and roots like in Altantsetseg et al. [2007] and Przybył et al. [2008] study was lower, but comparable to Ganzera et al. [2000], Buchwald et al. [2006] and Kucharski et al. [2011] results. This could be the result of genetic variability of roseroot plants, accurately described by Przybył et al. [2004].

In our experiment for the first time we examined chemical composition of tips (adventitious buds with part of rhizome that could be used for vegetative propagation of roseroot plants, that can be see at Figure 5). We found out that tips were rich in phenyl--propanoids and phenylethanoids (especially in fourth year of vegetation – Tab. 3). The total content of active compounds in tips increased annually from 6.84 $mg \cdot g^{-1}$ in the case of the youngest plants to 16.08 $mg \cdot g^{-1}$ in 4-year-old plants, while at the fifth, sixth and seventh years decreased gradually as it was found earlier in roots and rhizomes. Generally, chemical composition of roseroot tips was comparable to rhizomes. Cluster analysis of chemical content of under ground parts of roseroot depending on the age of plants showed that among the plants of all ages can be distinguished three groups: 1-year-old; six and seven year old; or including other age groups, with underground parts of plants of two year old were more similar to the five year old and four and three year old formed a separate group, supporting our observation that is in this age of the plants should be sourced from field plantations (Fig. 2).

For the first time we determined also selected active compounds content in roseroot above ground parts of plants (stems and leaves). The total active substances content increased from approximately 2.40 $mg \cdot g^{-1}$ for the first year roots to almost 6.12 $mg \cdot g^{-1}$ (two and a half times more than in the first growing season) for four-year-old roots and after that decreased gradually from 4.48 $mg \cdot g^{-1}$ for 5-yr-old roots to 2.37 $mg \cdot g^{-1}$ for 7-year-old plants – Tab. 4. Increases in the concentrations of phenylethanoids

(*p*-tyrosol) and phenylpropanoids (rosarin) accounted for most of these increases. Generally, we found higher than in the case of under ground organs content of phenylethanoids (especially *p*-tyrosol) in these morphological parts. It is likely that the stems and leaves are newly grown annually, therefore the content is conform in each year. Cluster analysis of chemical content of above ground parts of roseroot depending on the age of plants showed that plants at the age of three and six year old as well as four and five year old were similar to each other and create separate groups (Fig. 3).

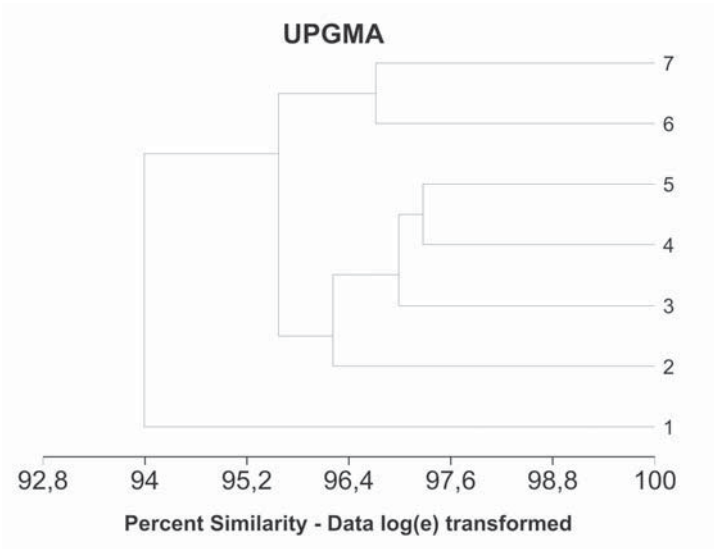


Fig. 4. Cluster analysis of chemical content of roseroot depending on the age of plants. Legend: 1 – one year old plants; 2 – two year old plants; 3 – three year old plants; 4 – four year old plants; 5 – five year old plants; 6 – six year old plants; 7 – seven year old plants

Our examination of five glycosides resulted in a wide variation in content depending on the morphological part of plant, not only its age. Taking into consideration four different morphological parts of roseroot plant under study, we found that rhizomes contained most of the active substances (with an exception of tips from four year old plants), which was in agreement with Platikanov and Evstatieva [2008], Węglarz et al. [2008] and Przybył et al. [2008] experiments. Independently form the age of plant, tips of roseroot contained on an average 5% lower, whereas roots approximately 20% lower amount of phenylpropanoids and phenylethanoids and above ground parts – three times less than in rhizomes. So the most valuable part of roseroot plant taking into account chemical composition, seems to be rhizomes with tips. What is more, stems and leaves of golden root could be a source of phenylethanoids and also phenylpropanoids for pharmacy, but it needs further examination.

A)

B)

Fig. 5. Selected morphological parts of roseroot under ground organs: A) tips (in the autumn period); B) from the left: tips with developing stems (in the spring time), rhizomes and roots

Independently form roseroot age, salidroside content was the highest in tips (form 0.69 mg·g^{-1} in 7-year-old to 1.97 mg·g^{-1} in 4-year-old) and rhizomes (from 0.58 mg·g^{-1} in 7-year-old to 1.65 mg·g^{-1} in 1-year-old plants) and the lowest in above ground parts (from 0.16 mg·g^{-1} in 1-year-old to 1.18 mg·g^{-1} in 5-year-old plants). According to Kurkin at al. [1989] and Bykov et al. [1999] the content of salidroside in *R. rosea* root ranges from 0.8 to 1.2% but in the raw material originating from culture near Tomsk ranged from 0.18 to 0.67% [Revina et al. 1976]. In Polish conditions, Przybył et al. [2008] and Węglarz et al. [2008] obtained significantly higher amounts of salidroside (c.a. 0.67%) in rhizomes and roots (c.a. 0.25%), like in Platikanov and Evstatieva [2008] in Bulgaria.

According to Russian Pharmacopoeia the content of salidroside in roseroot raw material should not be lower than 0.8% [Bykov et al. 1999]. However, the results of the studies of Kędzia et al. [2006] indicate that salidroside does not affect the immu-

nostimulating and tranquillizing activity of roseroot extracts. In the case of *p*-tyrosol the highest amount of this compound was found in roseroot stems and leaves (from 0.13 $mg \cdot g^{-1}$ after 6 years of vegetation to 0.37 $mg \cdot g^{-1}$ in two-year old plants), whereas the lowest in roots (from 0.11 $mg \cdot g^{-1}$ in 7-year-old to 0.25 $mg \cdot g^{-1}$ in 6-year-old plants).

The same level of *p*-tyrosol was found in Altantsetseg et al. [2007] and Węglarz et al. [2008] studies. We found that rosarin content was the highest in rhizomes (from 0.97 $mg \cdot g^{-1}$ in 1-year-old to 3.33 $mg \cdot g^{-1}$ in 4-year-old plants), while in roseroot tips and roots it remains almost similar. As in Węglarz et al. [2008] studies, rosavin formed the greatest part of the determined roseroot active ingredients. Its content was the highest in tips and rhizomes (varied from 1.75 $mg \cdot g^{-1}$ in 1-year-old tips to 9.77 $mg \cdot g^{-1}$ in 5-year--old rhizomes). Roots were characterized by on an average twice lower amount of this compound comparing to previously discussed ones, while above ground parts contained extremely low amount of rosavin (from 0.39 $mg \cdot g^{-1}$ in 1 year old to 1.47 $mg \cdot g^{-1}$ in 3-year-old plants). As far as rosin content it was the highest in the case of roots (from 1.36 $mg \cdot g^{-1}$ in 1 year old to 4.60 $mg \cdot g^{-1}$ in 4-year-old plants), and the lowest in above ground parts of roseroot (from 0.37 $mg \cdot g^{-1}$ in 7-years-old to 1.87 $mg \cdot g^{-1}$ in 3 and 4 years old plants). The content of rosavin – the dominant compound among *trans*-cinnamic alcohol derivatives in *R. rosea* root – ranges from 0.4 to 3.7% [Altantsetseg et al. 2007; Przybył et al. 2008; Węglarz et al. 2008].

In our studies the content of this compound was generally lower, but comparable with that found by Buchwald et al. [2006] and Kucharski et al. [2011]. Cluster analysis of chemical content of roseroot plants depending on their age indicated that plants at age of four and five years old were similar and create separate cluster, like six and seven years old, whereas the remaining plants at age 1, 2, or 3 years old were not similar to each other and create separate clusters (Fig. 4). On the basis of these changes we conclude that *Rhodiola rosea* should be harvested after 4 or 5 years (only in appropriate case after third year, as Platikanov and Evstatieva [2008] recommended in Bulgaria).

CONCLUSIONS

This study found that significantly yearly increases in total phenylethanoids and phenylpropanoids concentrations occur with *Rhodiola rosea* grown in Poland. In addition, the changes in the concentration of individual active substances differ from those reported previously. Roseroot harvested after only 3 year contained significantly lower amounts of phenylethanoids and phenylpropanoids in roots than roseroot harvested after 4, 5 or 6 year. In the case of rhizomes and tips, plants dug after 3 year was characterized by lower active constituents content in these parts than after 4 or 5 year. A certain amount of active substances were also found in the stems and leaves of *R. rosea* (on an average of twice lower than in the under ground parts of plants), thus above ground parts of golden root could be a potential source of phenylethanoids and phenylpropanoids for pharmacy. Since these glycosides are the major active constituents of *Rhodiola rosea*, this change to an earlier harvest (before fourth or in appropriate cases in third year) may have an effect on the desirability of the harvested raw material.

ACKNOWLEDGMENTS

The studies were financed by the Ministry of Science and Higher Education within grant No. N N310 448438

REFERENCES

Altantsetseg K., Przybył J.L., Węglarz Z., Geszprych A., 2007. Content of biologically active compounds in roseroot (*Rhodiola* sp.) raw material of different derivation. Herba Pol., 53 (4), 20–26.

Brown R.P., Gerbarg P.L., Ramazanov Z., 2002. *Rhodiola rosea*: a phytomedicinal overview. Herbal Gram, 56, 40–52.

Bykov V.A., Zapesochnaya G.G., Kurkin V.A., 1999. Traditional and biotechnological aspects of obtaining medicinal preparations from *Rhodiola rosea l.* (a review). Pharm. Chem. J. 33(1), 29–40.

Buchwald W., Mścisz A., Krajewska-Patan A., Furmanowi M., Mielcarek S., Mrozikiewicz P.M., 2006. Contents of biologically active compounds in *Rhodiola rosea* roots during the vegetation period. Herba Pol. 52, 39–43.

Galambosi B., 2006. Demand and availability of *Rhodiola rosea* L. raw material. Bogers R.,Cracer L., Lange D. (eds.) Medicinal and Aromatic Plants. Springer, 223–236.

Ganzera M., Yayla Y., Khan I.A., 2000. Analysis of the marker compounds of *Rhodiola rosea* L. (golden root) by reversed phase high performance liquid chromatography. Arch. Pharm. Res. 23(4), 349–352.

Kędzia B., Furmanowa M., Krajewska-Patan A., Hołderna-Kędzia H., Mścisz A., Wójcik J., 2006. Badania nad toksycznością oraz działaniem adaptogennym i przeciwdrobnoustrojowym wyciągów otrzymanych z podziemnych części wybranych gatunków *Rhodiola* L. Herba Pol., 52, 117–32.

Kiryanov, A.A., Bondarenko L.T., Kurkin V.A., Zapesochnaya G.G., Dubichev A.A., Vorontsov E.D., 1991. Determination of biologically active components of the rhizomes of *Rhodiola rosea*. Khim. Prir. Soedin. 3, 320–323.

Kołodziej B., Sugier D., 2012. Selected elements of biology and morphology of roseroot in southeastern Poland. Acta Sci. Pol. Ser. Hortorum Cultus 11(5), 127–142.

Krajewska-Patan A., Dreger M., Górska-Paukszta M., Łowicka A., Furmanowa M., Mrozikiewicz P.M., 2005. *Rhodiola rosea* L. – present status of biotechnology investigations. Herba Pol. 51(3/4), 51–64.

Kucharski W., Mordalski R., Buchwald W., Mielcarek S.: Roseroot – the comparison of tillage in conventional and ecological system. J. Res. Appl. Agric. Eng. 2011, 56(3), 232–235.

Kurkin V.A., 2003. Phenylpropanoids from medicinal plants: distribution, classification, structural analysis and biological activity. Chem. Nat. Comp. 39(2), 123–153.

Kurkin V.A., Zapesochanaya G.G., Gorbunov Y.N., Nukhimovskii E.L., Shreter A.I., Shchavlinskii A.N., 1986. Chemical investigations on some species of *Rhodiola* L. and *Sedum* L. genera and problemes of their chemotaxonomy. Rast. Res. 22 (3), 310–319.

Lange, D., 1998. Europe's Medicinal and Aromatic Plants: Their Use, Trade and Conservation. TRAFFIC International, Cambridge, United Kingdom.

Linh P.T., Kim Y.H., Hong S.P., Jian J.J., Kang J.S., 2000. Quantitative determination of salidroside and tyrosol from the underground part of *Rhodiola rosea* by HPLC. Arch. Pharm. Res., 23, 349–52.

Ma C.Y., Tang J., Wang X., Gu X., Tao G.J., 2008. Simultaneous determination of six active compounds in *Rhodiola rosea* L. by RP-LC. Chromatographia 67 (5/6), 383–388.

Panosian A., Wikman G., Sarris J., 2010. Roseroot (*Rhodiola rosea* L.): traditional use, chemical composition, pharmacology and clinical efficiacy. Phytomed, 17, 481–493.
Platikanov S., Evstatieva L., 2008. Introduction of wild golden root (*Rhodiola rosea* L.) as a potential economic crop in Bulgaria. Economic Botany 64(4), 621–627.
Przybył J., Węglarz Z., Pawełczak A., 2004. Zmienność w obrębie populacji różeńca górskiego (*Rhodiola rosea* L.) pod względem plonu surowca i zawartości związków biologicznie czynnych. Zesz. Probl. Post. Nauk Roln. 497, 525–31.
Przybył J., Węglarz Z., Geszprych A., 2008. Quality of roseroot (*Rhodiola rosea* L.) cultivated in Poland. Acta Hort., 765, 143–150.
Revina T.A., Krasnov E.A., Sviridova T.P., Stepanyuk G.Y., Surov Y.P., 1976. Biological characteristics and chemical composition of *Rhodiola rosea* grown in Tomsk. Rast. Res. 12(3), 355–360.
Węglarz Z., Przybył J., Geszprych A., 2008. Roseroot (*Rhodiola rosea* L.): Effect of internal and external factors on accumulation of biologically active compounds. In: Ramawat K.G., Merillon J.M. (eds.): Bioactive Molecules and Medicinal Plants. Berlin, Heidelberg., 16, 297–315.
Zapesochanaya G.G., Kurkin V.A., 1983. Glycosides of cinnamyl alcohol from the rhizomes of *Rhodiola rosea*. Chem. Nat. Comp. 18 (6), 685–688.
Zapesochnaya G. G., Kurkin V.A., 1982. Cinnamic glycosides of *Rhodiola rosea* rhizomes. Khim. Prir. Soed. 6, 723–727.

WPŁYW WIEKU ROŚLIN NA SKŁAD CHEMICZNY RÓŻEŃCA (*Rhodiola rosea* L.)

Streszczenie. Różeniec, *Rhodiola rosea* L. od wieków stosowany był w tradycyjnej azjatyckiej, skandynawskiej i wschodnioeuropejskiej medycynie jako środek poprawiający kondycję fizyczną, w przypadku anemii, depresji, astenii, impotencji, zaburzeń żołądkowo-jelitowych i układu nerwowego, a także jako immunostymulant i środek przeciwzapalny. Ta cenna roślina rośnie w Himalajach, górach Ałtaju, Alpach i Karpatach. Surowiec zielarski rożeńca zawiera fenyloetanoidy (salidrozyd i *p*-tyrozol) i glikozydy kwasu cynamonowego znane jako fenylopropanoidy (rozyna, rozawina i rozaryna), które uważane są za najważniejsze substancje aktywne w nim zawarte. Celem niniejszego eksperymentu było porównanie zawartości związków fenolowych (salidrozydu, *p*-tyrozolu, rozaryny, rozawiny i rozyny) metodą HPLC w poszczególnych częściach morfologicznych roślin (korzeniach, kłączach, i po raz pierwszy – tipsach i częściach nadziemnych) w surowcu uprawianym w Polsce w ciągu kolejnych siedmiu sezonów wegetacyjnych. W tych badaniach stwierdziliśmy coroczne istotne zwiększanie się ogólnej zawartości fenyletanoidów i fenylpropanoidów w *Rhodiola rosea* rosnącym w Polsce. Kłącza charakteryzowały się największą koncentracją fenyletanoidów i fenylpropanoidów, w porównaniu z pozostałymi częściami morfologicznymi roślin w tym samym wieku, podczas gdy pewną ilość substancji aktywnych znaleźliśmy także w łodygach i liściach *R. rosea* (przeciętnie dwukrotnie mniej niż w częściach podziemnych). Dlatego też wydaje się, że części nadziemne mogą być potencjalnym źródłem fenyletanoidów i fenylpropanoidów dla przemysłu farmaceutycznego. Różeniec zbierany po trzech latach uprawy zawierał istotnie mniejsze ilości fenyletanoidów i fenylpropanoidów w częściach podziemnych niż zebrany po 4, 5 lub 6 latach. Ponieważ glikozydy są najważniejszymi składnikami aktywnymi *Rhodiola rosea* zmiana polegająca na wcześniejszym zbiorze (przed czwartym lub w odpowiednich przypadkach w trzecim roku) może mieć wpływ na jakość zebranego surowca.

Słowa kluczowe: części morfologiczne, jakość surowca, zawartość fenyletanoidów i fenylpropanoidów

Accepted for print: 22.11.2012