Content of biologically active compounds in roseroot (*Rhodiola* sp.) raw material of different derivation

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S u m m a r y

Four populations of roseroot (*Rhodiola rosea* and *Rh. quadrifida*) originating from Poland and Mongolia were compared in respect of the content of main biologically active phenolic compounds (salidroside, p-tyrosol, *trans*-cinnamic alcohol, rosarin, rosavin and rosin) in the underground organs. Three solvents (75% ethanol, methanol and water) were used for the extraction of these phenolic compounds. *Rh. rosea* grown in Poland was characterised by similar content of salidroside and higher content of rosavin in comparison to the plants growing wild in Mongolia. The content of these compounds in *Rh. quadrifida* was lower than in *Rh. rosea*. Water appeared to be the best extraction medium for the isolation of salidroside and tyrosol. Cinnamic alcohol and its derivatives were more efficiently extracted with methanol and 75% ethanol.

Key words: Rhodiola rosea, Rhodiola quadrifida, salidroside, rosavin, extraction, solvent, HPLC

INTRODUCTION

The genus *Rhodiola* of the family *Crassulaceae* is represented by about 90 species distributed in mountainous regions of the Northern Hemisphere – in Asia (Siberia, Mongolia), Europe (Scandinavia, Iceland, Pyrenees, Alps, Carpathian Mountains) and North America [1]. The most thoroughly investigated species is *Rhodiola rosea* L. Traditional usage of its underground organs as a medicinal raw material has been proved and supported by contemporary pharmacological studies indicating immunostimulating, antioxidant and anti-stress activity, as well as ability to enhance memory and physical endurance [1-5]. About 20 other species of *Rhodiola* genus (e.g. *Rh. kirilovii*, *Rh. crenulata*, *Rh. sacra* and *Rh. quadrifida*) are used mainly in traditional Asian medicine and the data on their chemical composition and biological activity are fragmentary. Results of the first studies concerning comparison of the content of biologically active compounds in underground organs of different species of roseroot indicated that some constituents (namely *trans*-cinnamic alcohol derivatives) are specific for *Rh. rosea*, whereas others (e.g. salidroside and its aglycone tyrosol) occur more widely within the genus [6-8]. According to recent reports, there are some species of roseroot that contain the same biologically active compounds as *Rh. rosea* and that could be used in a similar way [3, 9, 10].

The aim of our study was to compare the content of main biologically active phenolic compounds (salidroside, p-tyrosol, *trans*-cinnamic alcohol, rosarin, rosavin and rosin) in the raw material of *Rh. rosea* and *Rh. quadrifida* coming from Mongolia and Poland, as well as to evaluate the usefulness of 75% ethanol, methanol and water for extraction of these phenolic compounds.

MATERIALS AND METHODS

Raw materials (air-dry underground organs) obtained from three populations of *Rh. rosea* and one of *Rh. quadrifida* were compared:

1 – raw material of *Rh. rosea* grown at the experimental field of the Department of Vegetable and Medicinal Plants WULS-SGGW in Warsaw and collected in October 2005,

2 – raw material of *Rh. rosea* collected from a natural site in Mongolian Altai in August 2005,

3 – raw material of *Rh. rosea* obtained from the Mongolian Traditional Medicinal Training Centre in Ulaanbaatar and originating from a natural site in Gorkhi Terelj (date of collection unknown),

4 – raw material of *Rh. quadrifida* collected from a natural site in Mongolian Altai in August 2005.

Three extraction media, i.e. 75% ethanol, methanol and water, were applied. Air-dry grounded raw material in amount of 1.0 g was extracted exhaustively with 100 ml of extrahent in Büchi B-811 Extraction System. After evaporation of sol-

vent, the residue was dissolved in 10 ml of methanol, filtered through a Supelco IsoDisc PTFE 25 mm × 0.45 μm filters and subjected to HPLC. The analysis was carried out using the Shimadzu chromatograph with SPD-M10A VP DAD detector equipped with Luna 5 μm C18 (2) 250 mm × 4.6 mm column (Phenomenex). The gradient of 0.2% phosphoric acid in HPLC grade water (A) and ACN (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 22 min. The following analysis parameters were used: injection volume: 20 μl, flow rate 1.2 ml × min^{-1}, oven temperature 31ºC, time of analysis 60 min., recorded wave range: 190–450 nm, detection wave length: 275 nm. Peaks were identified by comparison of 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/100 g of dry matter. The results were analysed with multifactor ANOVA Tukey's HSD test at the 0.05 significance level in Statgraphics Plus for Windows v. 4.1.

RESULTS AND DISCUSSION

In the beginning salidroside was regarded to be responsible for pharmacological activity of roseroot extracts and was used for the standardisation of the raw material [7, 11]. Later studies indicated that better markers of the quality of *Rh. rosea* root are *trans*-cinnamic alcohol derivatives – rosavin, rosarin and rosin, sometimes generally called rosavins. They were found to be specific for *Rh. rosea* only and thus indicated for identification of the raw material of this species. Nowadays, roseroot extracts are standardised for both salidrolide and rosavins [1]. However, it appeared that rosavin can also be found in other species of *Rhodiola* genus, e.g. *Rh. quadrifida*, although in lower amount [10]. The underground part of *Rh. quadrifida* is used in Chinese traditional medicine as a haemostatic, antibechic and tonic agent, and externally – for burns and contusions. Antiallergic activity of cyanoglycosides present in this raw material was also stated [12]. The studies of Kędzia et al. [3] proved that *Rh. quadrifida* extracts reveal adaptogenic activity expressed by affecting immune system, enhancing physical endurance, memory and learning ability as well as antioxidant action. In our studies all the determined compounds (salidroside, p-tyrosol, *trans*-cinnamic alcohol, rosarin, rosavin and rosin) were present in the underground organs of *Rh. quadrifida* (tab. 1). The content of majority of these compounds in this raw material was lower in comparison to the raw material of *Rh. rosea* grown in Poland and growing wild in Mongolia but higher than in *Rh. rosea* root obtained from the Mongolian Traditional Medicinal Training Centre. Very low content of all investigated compounds in the latter raw material may result from the method of drying (in open sunny space) and long-term storage. It is recommended to dry the underground organs of roseroot at 80ºC [13].

Table 1.

Content of biologically active compounds in roseroot raw material of different derivation (mg/100 g)

compound	derivation of raw material														
	Rh. rosea – Poland				*Rh. rosea* – Mongolian Altai				*Rh. rosea* – Mongolian Traditional Training Centre				*Rh. quadrifida* – Mongolian Altai			
tyrosol-derivatives																
p-tyrosol	15.93	±	6.15	c*	59.99	±	4.99	a	5.19	±	0.52	d	21.43	±	6.29	b
salidroside	140.98	±	8.05	a	143.96	±	25.30	a	74.81	±	23.03	c	109.99	±	40.57	b
trans-cinnamic alcohol derivatives																
trans-cinnamic alcohol	123.50	±	57.64	c	829.56	±	652.32	a	36.48	±	21.89	d	473.14	±	347.58	b
rosarin	323.07	±	16.36	b	471.59	±	39.96	a	55.09	±	21.64	d	261.38	±	44.33	c
rosavin	2785.76	±	325.34	a	1886.32	±	697.46	b	610.69	±	312.64	d	757.97	±	284.61	c
rosin	537.00	±	49.60	a	299.37	±	23.60	b	73.71	±	31.81	d	263.97	±	21.95	c

* mean values in rows marked with the same letter do not differ at $\alpha=0.05$

The studies carried out by Kurkin et al. [11] and Kir'yanov et al. [14] indicated that *Rh. rosea* plants growing wild in Altai mountains were characterised by higher content of biologically active compounds than those introduced into cultivation in Moscow, Novosibirsk or Irkutsk region. Kucinskaite et al. [15] reported that the content of rosavins (rosavin, rosarin and rosin) in extracts obtained from the plants growing wild in Altai region of Russia was lower than in extracts from the plants grown in Lithuania, whereas the content of salidroside in both extracts was similar. In our study the raw materials obtained from *Rh. rosea* plants grown in Poland and those growing wild in Mongolian Altai were also characterized by similar content of salidroside (c.a. 0.14%) but differed in respect of the content of other active compounds. According to Kurkin at al. [8, 13] the content of salidroside in *Rh. rosea* root ranges from 0.8 to 1.2% but in the raw material originating from different natural sites in China ranged from 0.13 to 1.11% [16]. Within a population grown in Poland the content of this compound was even more diverse (0.13–1.90%) [17]. According to Russian Pharmacopoeia XI [18], the content of salidroside in roseroot raw material should not be lower than 0.8%. However, the results of the studies of Kędzia et al. [3] indicate that salidroside does not affect the immunostimulating and tranquillizing activity of roseroot extracts.

The content of rosavin – the dominant compound among *trans*-cinnamic alcohol derivatives in *Rh. rosea* root – ranges from 0.4 to 3.7% [8, 11, 13, 17, 19]. In our studies the content of this compound in Polish and Mongolian raw material was 2.8% and 1.9%, respectively. Kurkin et al. [13] report that unsuitable method of stabilisation of plant material leads to the hydrolysis of rosavin to *trans*-cinnamic alcohol.

The content of rosin and rosarin in the raw materials obtained from plants grown in Poland and those growing wild in Mongolian Altai was higher in comparison with that reported in Russian literature – 0.1% and 0.05%, respectively [20, 21], but comparable (except from distinctly lower content of rosin in Mongolian raw material) to the results obtained earlier by Przybył et al. [17].

The content of particular compounds determined in the studied raw materials was significantly affected by the extraction medium used for the analysis (tab. 2). Water appeared to be the best extraction medium for salidroside and tyrosol isolation. Cinnamic alcohol and its derivatives were more efficiently extracted with methanol and 75% ethanol. These results correspond with the results obtained by Kucinskaite et al. [15] which demostrated that 70% ethanol extracts were characterised by higher content of rosavins and lower content of salidroside than 40% ethanol extracts.

Table 2.

Effect of extraction medium on the content of biologically active compounds in roseroot raw material (mg/100 g)

compound	extraction medium											
	75 % ethanol				methanol				water			
tyrosol-derivatives												
p-tyrosol	25.60	±	22.67	b*	21.26	±	20.77	c	30.04	±	22.04	a
salidroside	118.66	±	33.20	b	92.35	±	38.53	c	141.30	±	27.58	a
trans-cinnamic alcohol derivatives												
trans-cinnamic alcohol	337.75	±	278.55	b	687.77	±	658.59	a	71.49	±	49.81	c
rosarin	286.43	±	155.18	a	294.84	±	159.44	a	252.08	±	160.35	b
rosavin	1749.72	±	911.10	a	1783.20	±	1012.27	a	997.63	±	920.55	b
rosin	298.41	±	152.40	a	308.60	±	189.92	a	273.52	±	179.03	b

* mean values in rows marked with the same letter do not differ at $\alpha=0.05$

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ZAWARTOŚĆ ZWIĄZKÓW BIOLOGICZNIE CZYNNYCH W SUROWCU RÓŻEŃCA (*RHODIOLA* SP.) RÓŻNEGO POCHODZENIA

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Streszczenie

Celem pracy było porównanie trzech populacji *Rhodiola rosea* (z Polski i z Mongolii) i jednej *Rh. quadrifida* (z Mongolii) pod względem zawartości głównych biologicznie czynnych związków fenolowych (salidrozydu, p-tyrozolu, alkoholu *trans*-cynamonowego, rozaryny, rozawiny i rozyny) w organach podziemnych. Do ekstrakcji tych związków użyto trzech rozpuszczalników (75% etanol, metanol i woda). *Rh. rosea* uprawiana w Polsce charakteryzowała się podobną zawartością salidrozydu, ale wyższą zawartością rozawiny w porównaniu z roślinami dziko rosnącymi w Mongolii. Zawartość tych związków w *Rh. quadrifida* była niższa niż w *Rh. rosea*. Najlepszym medium ekstrakcyjnym do izolacji salidrozydu i tyrozolu okazała się woda. Alkohol cynamonowy i jego pochodne lepiej ekstrahowane były przy użyciu metanolu i 75% etanolu.

Słowa kluczowe: Rhodiola rosea, Rhodiola quadrifida, salidrozyd, rozawina, ekstrakcja, rozpuszczalnik, HPLC