Comparison of proanthocyanidins content in *Rhodiola kirilowii* and *Rhodiola rosea* roots – application of UPLC-MS/MS method

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S u m m a r y

The purpose of presented study was the comparison of flavan-3-ol content in the roots of *Rhodiola kirilowii* and *Rhodiola rosea* with use of UPLC-MS/MS method. Two kinds of extract were prepared: aqueous extract and 50% *v/v* ethanol extract. The worked out UPLC MS/MS method allowed to determine the content of five flavan-3-ols: (+)-catechin, (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin gallate (ECG) and (-)-epigallocatechin gallate (EGCG). The obtained results shown that the content of measured catechins was higher in *R. kirilowii* roots than in *R. rosea*. Both *Rhodiola* roots contain EGCG as a main proanthocyanidin compound – the content in *R. kirilowii* roots is about 0.14%. Our results indicate that application of ultra performance liquid chromatograph connected to a tandem mass spectrometer (UPLC MS/MS method) allows to determine the proanthocyanidins content in tested samples with satisfactory precision and can be used in the *Rhodiola* sp. phytochemical investigations.

Key words: *Rhodiola kirilowii, Rhodiola rosea, flavan-3-ol content, UPLC-MS/MS method, (-)-epigallocatechin gallate (EGCG)*

INTRODUCTION

Two species of *Rhodiola* L. genus (*Crassulaceae* family) are most frequently used in official and traditional medicine: *R. rosea* and *R. kirilowii*.

R. kirilowii (Regel.) Maxim grows in mountains at an altitude of 2000–5600 m in Asia: mainly Tien-Shan, Altaj, Pamir [1]. The main pharmacological activity of extracts from *R. kirilowii* is preventing high altitude reactions of human organism [2]. The roots contain: phenylethanoids as *p*-tyrosol and salidroside [3-5], phenylopropanoids [4, 5], catechins [4, 6-10], coumarins [11], phenolic acids [4-7, 10], phytosterols [4], tannins [10], cyanogenic glycosides [9], arbutin [9], terpenoids [8].

Rhodiola rosea L., roseroot (synonyms: Golden root, Arctic root), is a herbaceous perennial plant growing in Arctic and in the mountainous regions of Asia, North America and Europe. This plant shows some physiological and pharmacological properties: stimulates the central nervous system (CNS) [12-13], enhances physical and mental work performance [14-15], eliminates fatigue and possesses adaptogenic [16-17], cardioprotective [18], anticancer [19], antioxidant [20-22] and antimicrobial activities [23, 24]. Some activities of extracts have been proved in pharmacological and clinical studies [14, 15]. The roots of *R. rosea* contain: phenylpropanoids

– rosavin, rosarin, rosin [25], phenylethanoids – salidroside, p-tyrosol [21, 25], flavonoids – rodionin, rodiolin, rhodiosin, acetylrodalgin and tricin [26-28], phenolic acids [30], monoterpenes [30], phytosterols [31], tannins [30], cyanogenic glucoside – lotaustralin [32] and essential oils – n-decanol, geraniol [33].

Chemical constituents of both *Rhodiola* species, pharmacological activities of roots extracts and *in vitro* cultures have been investigated in the Institute of Natural Fibres and Medicinal Plants (previously: Research Institute of Medicinal Plants) for several years [4, 6-9, 13, 21, 23, 24, 34]. The purpose of present study was the comparison of flavan-3-ol content in the roots of these species using UPLC-MS/MS method.

MATERIAL AND METHODS

Plant material

The roots of *R. kirilowii* and *R. rosea* were harvested from field cultivation (in the Garden of Medicinal Plants, Institute of Natural Fibres and Medicinal Plants in Plewiska near Poznań, Poland) in October 2009. The roots were dried in the room temperature (about 22–24ºC).

Preparation of plant extracts

Two kinds of extract were prepared: aqueous extract and 50% (*v/v*) ethanol extract.

Preparation of aqueous extract

The powdered dry roots were extracted with purified water for 3 h at 90ºC (1:10 plant material to solvent ratio). After filtering, the extracts were frozen at –55ºC and then lyophilized.

Preparation of 50% (v/v) ethanol extract

The powdered dry roots were extracted with 50% (*v/v*) ethanol using the percolation method at a 1:10 ratio of plant material to solvent. After evaporation of the alcohol under reduced pressure at a temperature of 40–45ºC, the extracts were frozen at –55ºC and than lyophilized.

The dry plant extracts were stored at a temperature of 20–25ºC.

Standard substances

The following substances were used in the experiment for comparison: (+)-catechin, (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin gallate, (-)-epigallocatechin gallate (ChromaDex) and D-(-)-salicine (SIGMA).

Preparation of test samples: extraction of flavan-3-ols from dry plant materials and from plants extracts

The method of flavan-3-ol extraction from roots and extracts by P. Mammela [35] was used. An exact amount of ca. 0.5 g of dried and powdered (0.315) *R. kirilowii* roots, an exact amount of ca. 0.75 g of dried and powdered (0.315) *R. rosea* roots and ca. 0.1 g of dried and powdered extracts (aqueous and 50% *v/v* ethanol) from *R. kirilowii* and *R. rosea* roots were weighed out and placed in a 20 ml volumetric flasks. 15.0 ml of 80% *v/v* methanol was added and the solutions were subjected to ultrasounds for 60 min at a room temperature (20–25ºC). Subsequently, the solutions were made up to the mark with the same solvent and filtered on a quantitative filter paper. The filtrates were concentrated to evaporate the methanol up to a volume of about 1/5 in a rotary evaporator in vacuum. The residues were extracted with 4 × 16.0 ml of diethyl ether. The combined ether extracts were dried with anhydrous sodium sulphate and evaporated to dryness in a rotary evaporator in vacuum. The dry residues were dissolved in 4.0 ml of 10% *v/v* methanol and then transferred quantitatively to 5 ml volumetric flasks. 0.023 ml of 0.5 mg/ml D-(-)-salicine (IS) was added to every flasks and the solutions were made up to the mark with 10% *v/v* methanol. The samples were filtered through a membrane filter of a 0.20 μm diameter.

LC-MS/MS assay

The validated assay using ultra performance liquid chromatograph connected to tandem mass spectrometer (UPLC-ESI MS/MS; Waters) was worked out in the Institute of Natural Fibres and Medicinal Plants [34]. The preparation of calibration curves for flavan-3-ols, the evaluation of precision, linearity and accuracy of this analytical method is described by Gryszczyńska et al. [34].

Statistical analysis

The results of the study were statistically verified determining the relative standard deviation (RSD), n=6.

RESULTS AND DISCUSSION

Flavan-3-ols (so called proanthocyanidins) have the antioxidative activity, thus, they protect against harmful effects of free radicals and reactive oxygen forms. They also show anticarcinogenic, anti-inflammatory, antiallergenic,

antimutagenic and antiaging activity as well as improve the function of liver [36]. There are also reports that catechins can prevent obesity. Tests performed on animals have confirmed their activity in hypercholesterolaemia [38]. According to the fact that supplements containing *R. rosea* extract are recommended for protection against many pathogenic agents as well as that using of *R. kirilowii* extract in the Far East ethno-medicine is connected with the protection of human against high altitude reactions, the determination of proanthocyanidins in the *Rhodiola* genus could help explain the activities of the extracts.

The content of flavan-3-ols was investigated in *R. kirilowii* and *R. rosea* roots and in the extracts prepared according to the method described above. The UPLC MS/MS method worked out in the Institute of Natural Fibres and Medicinal Plants [34], allows to determine the contents of five flavan-3-ols: (+)-catechin, (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin gallate (ECG), (-)-epigallocatechin gallate (EGCG). The MRM chromatograms showing the fragmentation of flavan-3-ols from *Rhodiola kirilowii* and *R. rosea* roots are demonstrated in figures 1 and 2. The obtained quantitative results are presented in tables 1 and 2.

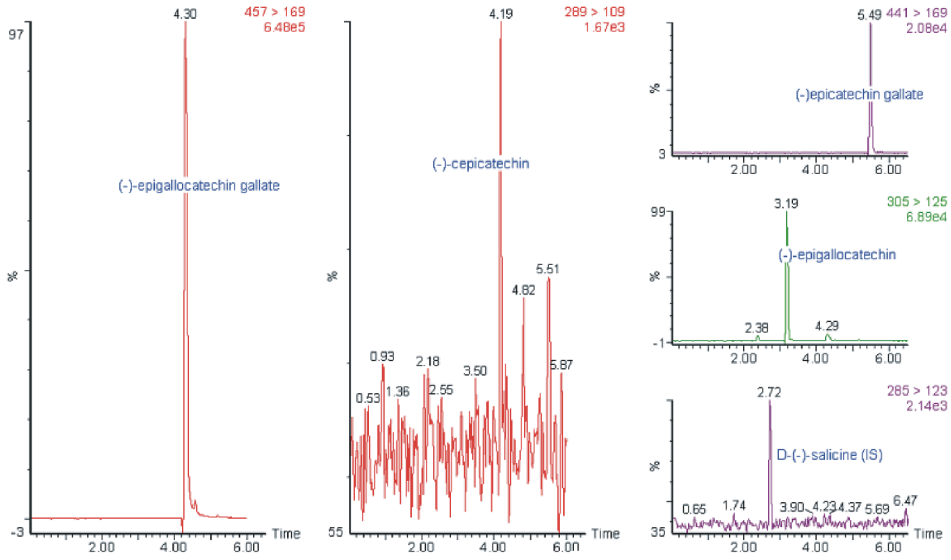

Figure 1.
MRM chromatogram showing fragmentation of flavan-3-ols from *Rhodiola kirilowii* roots.

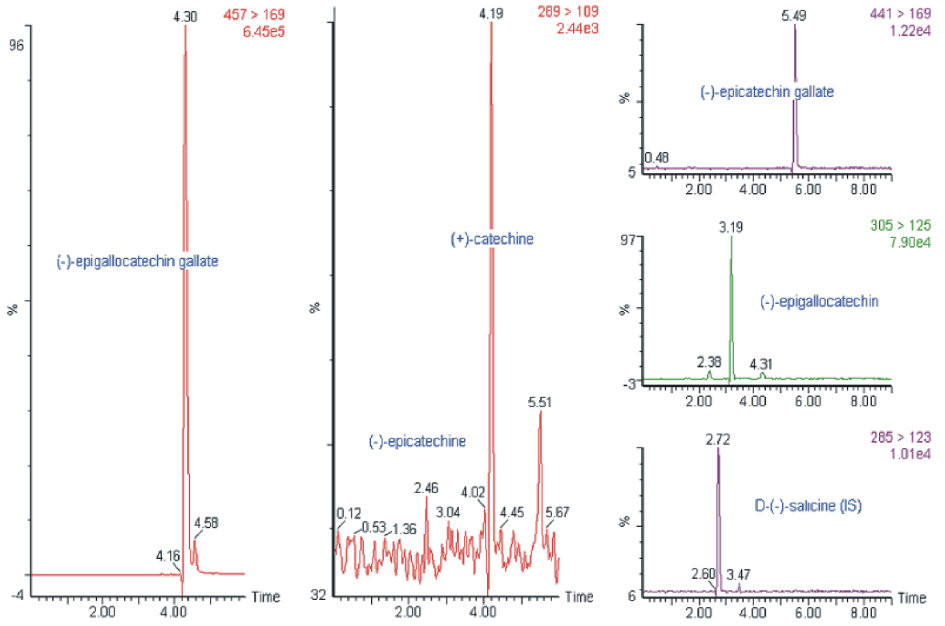

Figure 2.
MRM chromatogram showing fragmentation of flavan-3-ols from *Rhodiola rosea* roots

Table 1.

Content of catechins in *Rhodiola kirilowii* and *Rhodiola rosea* roots

Sample	(+)-Catechin		(-)-Epicatechin		(-)-Epigallo-catechin		(-)-Gallate epicatechin		(-)-Gallate epigallocatechin	
	Content [mg/100 g][1]	RSD [%]*	Content [mg/100 g][1]	RSD [%]*	Content [mg/100 g][1]	RSD [%]*	Content [mg/100 g][1]	RSD [%]*	Content [mg/100 g][1]	RSD [%]*
R. kirilowii root	**0.097**	2.06	**0.288**	2.08	**19.584**	7.53	**5.294**	4.55	**135.435**	2.00
R. rosea root	0.051	3.92	0.135	1.48	8.526	1.77	0.437	2.29	36.873	2.17

*- RSD – relative standard deviation (n=6)

[1] – the content in 100 g of powdered dry material

The obtained results show that the content of measured catechins was higher in *R. kirilowii* roots than in *R rosea*. Both *Rhodiola* roots contain (-)-gallate epigallocatechin as a main proanthocyanidin compound – the content in *R kirilowii* roots is about 0.14% (tab. 1).

(-)-Epicatechin and (-)-epigallo-catechin were found in the highest concentration in ethanol extract, whereas (+)-catechin and (-)-gallate epigallocatechin in the aqueous extracts (tab. 2).

Table 2.

Content of catechins in *Rhodiola kirilowii* and *Rhodiola rosea* extracts

Sample	(+)-Catechin		(-)-Epicatechin		(-)-Epigallo-catechin		(-)-Gallate epicatechin		(-)-Gallate epigallocatechin	
	Content [mg/100 g][1]	RSD [%][*]	Content [mg/100 g][1]	RSD [%][*]	Content [mg/100 g][1]	RSD [%][*]	Content [mg/100 g][1]	RSD [%][*]	Content [mg/100 g][1]	RSD [%][*]
R. kirilowii 50% ethanol extract	0.377	1.86	1.651	1.51	97.357	3.81	4.045	3.51	266.494	2.72
R. kirilowii aqueous extract	0.565	2.12	0.858	3.38	31.558	5.81	5.138	2.80	304.449	3.78
R. rosea 50% ethanol extract	<LOD	-	0.613	1.63	24.978	5.85	1.589	0.63	133.407	4.10
R. rosea aqueous extract	0.381	2.62	0.392	2.55	3.081	7.14	0.955	4.19	299.702	3.67

[*] – RSD – relative standard deviation (n=6)
[1] – the content in 100 g of powdered dry material

Searched proanthocyanidins were reported in *R. kirilowii* roots by Wiedenfeld et al. [9], Zuo et al. [10] and Wong et al. [5]. The presence of (-)-gallate epigallocatechin (EGCG) in *R. kirilowii* roots was also demonstrated in our previous investigations on biological activities of extracts [6-8] or searching on tissue cultures of *R. kirilowii* [38] – the content varied from 68 to 1734 mg/100 g d.w. according to harvest time (as was determined by HPLC method) [38].

Pronthocyanidins in *R. rosea* were searched by Yousef et al. [36]. They found that roots contains (-)-epigallocatechin and its 3-*O*-gallate esters in different degrees of polymerization. In our previous investigation on *R. rosea* we have found that the content of (-)-gallate epigallocatechin (EGCG) was different in various periods of vegetation and varied from 141 to 399 mg/100 g d.w. [39], as it was determined by HPLC method.

The above mentioned determination of EGCG content in *R. kirilowii* and *R. rosea* roots [38, 39] and our results presented above indicate that *R. kirilowii* can be a better source of catechin as compared with *R. rosea* roots, especially if the content of (-)-gallate epigallocatechin is taken into consideration. In this case, the water extraction is recommended. The application of ultra performance liquid chromatograph connected to a tandem mass spectrometer (UPLC MS/MS method) allows the determination of the proanthocyanidins content in tested samples with satisfactory precision and can be used in the *Rhodiola* sp. phytochemical investigations.

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PORÓWNANIE ZAWARTOŚCI PROANTOCYJANIDYN W KORZENIACH *RHODIOLA KIRILOWII* I *RHODIOLA ROSEA* Z WYKORZYSTANIEM METODY UPLC-MS/MS

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S t r e s z c z e n i e

Celem przedstawionych badań było porównanie za pomocą opracowanej metodyki wykorzystującej ultrasprawny chromatograf cieczowy sprzężony z tandemowym spektrometrem mas (Waters) zawartości flawan-3-oli w korzeniach *Rhodiola kirilowii i Rhodiola rosea*. Badano wodne i alkoholowo-wodne (50% EtOH) wyciągi z korzeni. Opracowana metoda UPLC MS/MS pozwoliła na określenie stężeń pięciu flawan-3-oli: (+)-katechiny, (-)-epikatechiny, (-)-epigalokatechiny, galusanu (-)-epikatechiny (ECG) oraz galusanu (-)-epigalokatechiny (EGCG). Otrzymane wyniki wskazują, że zawartość tych katechin jest wyższa w korzeniach *R. kirilowii* niż w korzeniach *R. rosea*. Oba surowce zawierają galusan (-)-epigallokatechiny jako główną proantocyjanidynę – jej zawartość w korzeniach *R. kirilowii* wynosi ok. 0,14%. Uzyskane przez nas wyniki wskazują, że metoda analityczna z wykorzystaniem ultrasprawnego chromatografu cieczowego sprzężonego z tandemowym spektrometrem mas pozwala z zadawalającą precyzją oznaczyć zawartości proantocyjanidyn w analizowanych próbkach i może być stosowana w badaniach rodzaju *Rhodiola*.

Słowa kluczowe: *Rhodiola kirilowii, Rhodiola rosea, zawartość flavan-3-oli, UPLC-MS/MS, galusan epigalokatechiny (EGCG)*