Comparison of phenylethanoids content in *Rhodiola kirilowii* and *Rhodiola rosea* roots applying a newly developed UPLC-MS/MS method

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Summary

A concentration of two phenylethanoids in the roots of two species: *Rhodiola kirilowii* and *rosea* were compared, aqueous and hydroalcoholic extracts from those plants were also analyzed. In order to determine the content of *p*-tyrosol and salidroside, the ultra performance liquid chromatography connected with a tandem mass spectrometry (UPLC-ESI MS/MS, Waters) was used. The obtained results shown that content of measured phenylethanoids depends on *Rhodiola* species. Roots of *R. kirilowii* contain more *p*-tyrosol, while *R. rosea* roots are reacher in salidroside. Our results indicate that the application of UPLC MS/MS method allows to determine the phenylethanoids content in tested samples with satisfactory precision.

Key words: *Rhodiola kirilowii, Rhodiola rosea, phenylethanoids, UPLC-MS/MS*

INTRODUCTION

The history of use of *Rhodiola* species is very interesting. In 1885 nearly 15 species of *Rhodiola* were characterised. *R. rosea, R. quadrifida,* and *R. kirilowii* are of the widest popularity. They usually grow in cold climate in China [1], Europe and USA [2, 3] at an altitude of 1000–5600 m. Plants of this family have been used for centuries in traditional Eastern medicine to treat many diseases [4] and have been applied increasingly in Europe and USA [5]. Oral administration of *R. kirilowii* extract has a protective effect on people with circulatory system disorders living at high altitudes [6]. Preparations containing *Rhodiola kirilowii* affect the properties of human adaptive mechanism [7, 8]. Preparations from *R. rosea* influence central nervous system [2, 3], have an antioxidant [9–11] and immunological [12] activity.

The roots of *R. kirilowii* contain many bioactive compounds: phenylethanoids (*p*-tyrosol and salidroside) [13–15], phenylopropanoids [14, 15], catechins [8, 14, 16–19], coumarins [20], phenolic acids [8, 14–17], phytosterols [14], tannins [8], cyanogenic glycosides [19], arbutin [19] and terpenoids [18].

The roots of *R. rosea* contain: phenylpropanoids – rosavin, rosarin, rosin [21], phenylethanoids – salidroside, *p*-tyrosol [11, 21], flavonoids – rhodionin, rhodiolin, rhodiosin, acetylrodalgin and tricin [22-24], phenolic acids [25], monoterpenes [25], phytosterols [26], tannins [25], cyanogenic glucoside – lotaustralin [27] and essential oils – n-decanol, geraniol [28]. The most typical compounds present in *Rhodiola* species are salidroside and *p*-tyrosol [4].

The aim of this research was to investigate a selective and specific analythical method to designate contents of individual compounds of phenylethanoids.

MATERIAL AND METHODS

Plant material

In this research, *Rhodiola kirilowii* and *Rhodiola rosea* roots were used. The plants were collected in October 2009 from field crops of the Institute of Natural Fibres and Medicinal Plants, Plewiska near Poznań, Poland. Roots were dried in a room temperature (22–24ºC).

Preparation of plant extracts

Subsequently, dry roots were powdered (0.315) and 2 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 hours at 90ºC (material to solvent ratio 1:10). After filtering, the extracts were frozen at –55ºC and than lyophilised [29]. The dry plant extracts were stored at a temperature of 20–25ºC.

Preparation of 50% *(v/v)* ethanol extract

The powdered dry roots were extracted with 50% (*v/v)* ethanol using the percolation method at plant material to solvent ratio 1:10. After the evaporation of the alcohol in reduced pressure at a temperature of 40–45ºC the extracts were frozen at –55ºC and than lyophilised [29]. Dry plant extracts were stored at a temperature of 20–25ºC.

Standard substances

The following comparison substances were used in the experiment: *p*-tyrosol, salidroside (ChromaDex) and D-(-)-salicine (SIGMA).

Standard solution

Standards of phenylethanoids were purchased from ChromaDex. Internal standard in this analytical method was D-(-)-salicine (Sigma Aldrich). Methanolic solutions of substances in the range of 5 different levels of concentration 100–1000 ng/ml were used to prepare calibration curves.

Sample preparation

Roots of *Rhodiola*

1.0 g of plant material (ca. 1.0 of dried powdered (0.315)) *Rhodiola kirilowii* or *Rhodiola rosea* root was weighed out and placed in a 100 ml round-bottom flask. To 19.0 ml of 10% (*v/v*) methanol a methanolic solution of D-(-)-salicine (IS) was added. This sample was heated under a reflux condenser in the boiling point of solvent for 45 min. Next, the sample was filtrated and the extraction of sample was performed twice. The filtrate was concentrated to evaporate the methanol up to a volume of about ¼ in a rotary evaporator in vacuum. Sample was transferred quantitatively to 20 ml volumetric flask. Subsequently, the solution was made up to the mark with the 10% (*v/v)* methanol. The sample was filtered through a membrane filter with a diameter of 0.20 μm.

Extracts of *Rhodiola*

0.5 g of *R. kirilowiii* or 0.1 g *Rhodiola rosea* extract was weighed out and placed in a 100 ml round-bottom flask. To 19.0 ml of 10% (*v/v*) methanol, a methanolic solution of D-(-)-salicine (IS) was added. This sample was heated under a reflux condenser in the boiling point of the solvent for 45 min. Next, the sample was filtrated and the extraction of sample was repeated one more time. The filtrate was concentrated to evaporate the methanol up to a volume of about ¼ in a rotary evaporator in vacuum. Sample was transferred quantitatively to 20 ml volumetric flask. Subsequently, the solution was then made up to the mark with the 10% (*v/v)* methanol. The sample was filtered through a membrane filter with a diameter of 0.20 μm.

LC-MS/MS assay

All analyses were conducted by ultra performance liquid chromatography connected with a tandem mass spectrometry (UPLC-ESI MS/MS; Waters). The separation of analytes was performed on an Acquity UPLC BEH C18 column, 1.7 μm 2.1 × 50 mm (Waters). Mobile phase: phase A: methanol, phase B: acetonitrile. Mobile phase flow rate was: 0.45 ml/min. The assay was performed in gradient elution: 0.0 min. – 97% of phase A, 4.8 min. – 82% of phase A, 4.9 min. – 97% of phase A. Column temperature was 30ºC; ion source temperature: 100ºC; desolvation temperature: 300ºC. Gas flow rate: desolvation gas: 700 l/h; cone gas: 10 l/h. All the substances were analyzed in the negative-ions source.

Method extraction of the phenylethanoids (fig. 1) was subjected to optimization determining the content of: *p*-tyrosol, salidroside, using D-(-)-salicine as an internal standard. To determine the content of several compounds fragmentation was used as follows: for salidroside MRM of m/z 299→89 Da and SIR of *p*-tyrosol: m/z 137→137 Da. Figure 1 gives the structure of individual phenylethanoids. The salidroside was identified by fragmentation of parent ion, *p*-tyrosol was identified as a parent ion, because this substance does not fragmentate.

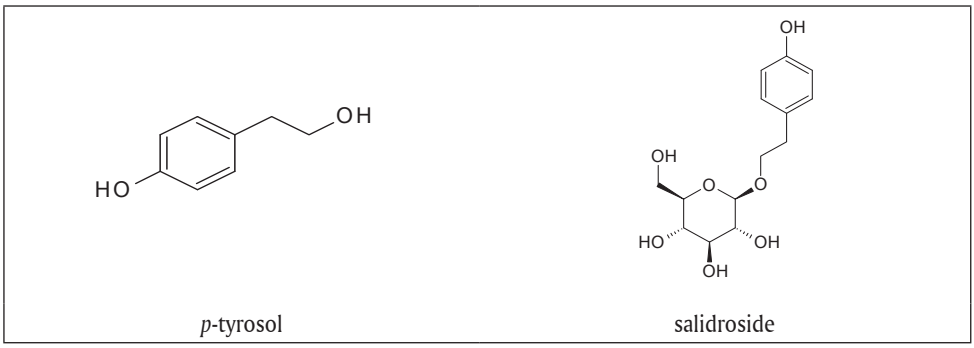

Figure 1.
Structures of phenylethanoids

Statistical method

For the phenylethanoids and internal standard, the regression analysis was performed at 5 concentration levels. Concentration of phenylethanoids was carried out for all samples in 6 repeats. The average and relative standard deviations (RSD) for those results were determined.

Validation

That method of extraction of phenylethanoids was validated with ICH rules. In a first step, linearity of calibrations curves was checked. Recurrence of time retention and peak area for all compounds were analyzed. The analytical method was characterized by a high recurrence and precision. The data for the roots and extract samples were itemised in 6 repeats. The accurancy of this method was conducted for 3 different levels. Percentage of recovery ranged from 89.72 to 101.38%.

RESULTS

In table 1 the analytical and statistical parameters of phenylethanoids are shown. The analytical method employed was evaluated for precision, linearity and accuracy. Precision and linearity were evaluated with use of regression analysis for each compared substance (tab. 2). The recovery of analytical method was analysed for for *R. kirilowii* roots using the enrichment method by the addition of dry hydroalcoholic extract of *R. rosea* with the determined level of phenylethanoids to the sample. Figure 2 gives the MRM chromatogram showing the fragmentation of phenylethanoids in *Rhodiola kirilowii* and Fig. 3 showing the fragmentation of *Rhodiola rosea* roots.

Table 1.

Characteristic parameters of phenylethanoids detection

Compound	Retention time [min]	RSD_t (n=15) [%]	RSD_p (n=5) [%]	Fragmentation m/z [Da]
p-Tyrosol	1.73	0.00	5.41	137→137
Salidroside	1.66	0.36	4.86	299→89
D-(-)-salicine (IS)	1.44	0.39	1.72	285→123

Table 2.

Method of recovery (n=3)

Sample	*p*-Tyrosol	Salidroside
20%	95.71±3.26	89.72±4.89
40%	93.51±4.85	96.62±5.21
80%	101.38±2.59	93.29±3.68

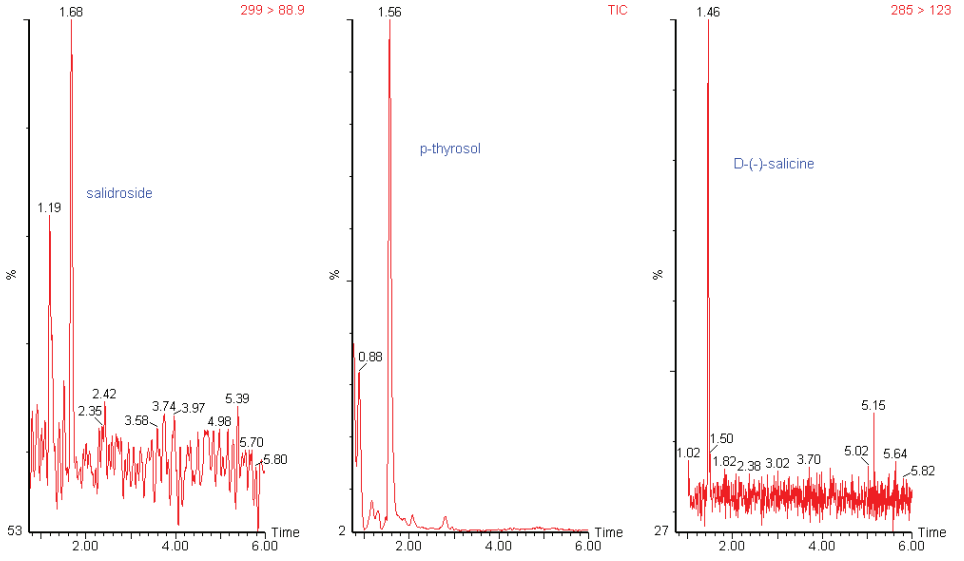

Figure 2.

The MRM chromatogram showing the fragmentation of phenylethanoids from *Rhodiola kirilowii* roots

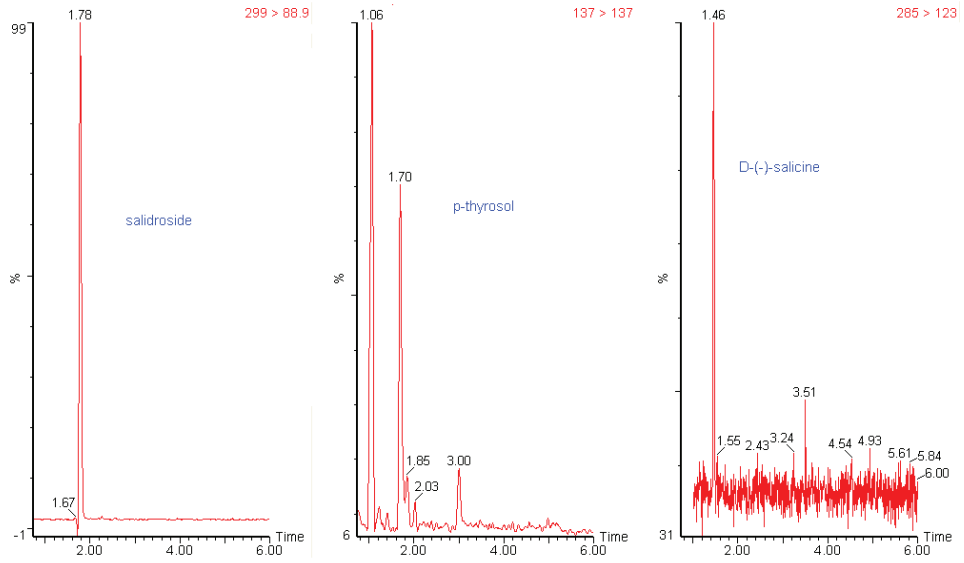

Figure 3.

The MRM chromatogram showing the fragmentation of phenylethanoids from *Rhodiola rosea* roots

The detection of each analysed compound was conducted in negative ions source. The signals visible in the chromatogram come from the parent ion fragmentation $[M-H]^-$. Among tested substances, only p-tyrosol does not fragmentate into daughter ions because it is not a part of the binding which could be cleaved in the structure of the compound.

DISCUSSION

The novel method of detection UPLC-MS/MS allows the identification of compounds with parent ions. The ESI detection allows the determination of very small amounts of the analyte. Additional advantage of this method, as compared to the HPLC-DAD is that fragmentation of the relevant parameters allows more accurate identification of tested substances, reducing the error in the determination of the content of particular component in the analysed sample.

In the comparison of the contents of 2 compounds (tab. 3) in the roots of 2 species of *Rhodiola* it can be seen that *R. rosea* has a lower content of individual phenylethanoids than *R. kirilowii*. Total amount of phenylethanoids in *R. rosea* is 5.506 mg/100 g of dry powdered material and 25.921 mg/100 g of dry powdered material in *R. kirilowii* root. In both cases, the content of phenylethanoids in the extracts was significantly higher than in the raw material. In *R. kirilowii,* extract

richest in these two compounds was an aqueous extract. However, in *R. rosea,* a richer source of *p*-tyrosol and salidroside was hydroalcoholic extract. In roots of *R. kirilowii*, the main phenylethanoid in the extract was p-tyrosol (22.891 mg/100 g of dry powdered material). However, a large concentration of salidroside (646.991 mg/100 g of dry powdered material) was found in the hydroalcoholic extract from *R. rosea*.

Table 3.

Content of phenylethanoids in *Rhodiola kirilowii* and *Rhodiola rosea* roots and extracts

Sample	*p*-Tyrosol		Salidroside	
	Content [mg/100 g of dry powdered material]	RSD [%]*	Content [mg/100 g of dry powdered material]	RSD [%]*
Rhodiola kirilowii root	**25.682**	6.35	0.239	4.18
50% Ethanol extract	18.800	2.66	0.054	2.38
Aqueous extract	***22.891***	5.55	***0.133***	2.86
Rhodiola rosea root	0.791	2.53	**4.715**	5.18
50% Ethanol extract	***236.025***	2.67	***646.991***	1.98
Aqueous extract	2.651	4.15	92.015	2.92

* RSD – relative standard deviation (n=6)

CONCLUSION

In summary, all the validation tests undertaken show that the analytical method confirmed that ultra performance liquid chromatography connected with a tandem mass spectrometry (UPLC-ESI MS/MS) can be successfully used for the determination of this group of components.

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REFERENCES

1. Wolski T, Baj T, Ludwiczuk A, Głowniak K, Czarnecka G. *Rhodiola* genus taxonomy chemical composition, activity and use also phyochemical analysis of roots of two species of *Rhodiola: Rhodiola rosea* L. and *Rhodiola quadrifida* (Pall.) Fish et Mey, Postępy Fitoterapii 2008; 11(1):2-14.

2. Saratikov A, Marina TF, Fisanova LL. Effect of golden root extract on processes of serotonin synthesis in CNS. J Biol Sci 1978; 6:142.
3. Krajewska-Patan A, Mikołajczak PŁ, Okulicz-Kozaryn I, Bobkiewicz-Kozłowska T, Buchwald W, Łowicka A et al. *Rhodiola rosea* extracts from roots and callus tissues – study on relationship between their chemical contents and CNS affecting pharmacological activity. Herba Pol 2005; 51(Suppl. 1):105-6.
4. Cui S, Hu X, Chen X, Hu Z. Determination of p-tyrosol and salidroside in three samples of *Rhodiola crenulata* and one of *Rhodiola Kirilowii* by capillary ion electrophoresis. Anal Bioanal Chem 2003; 377:370-374.
5. Yousef GG, Grace MH, Cheng DM, Belolipov IV, Raskin I, Lila MA. Comparative phytochemical characterization of three *Rhodiola* species. Phytochemistry 2006; 67:2380-2391.
6. Zych M, Furmanowa M, Krajewska-Patan A, Łowicka A, Dreger M, Mendlewska S. Micropropagation of *Rhodiola Kirilowii* plants using encapsulated axillary buds and callus. Acta Biologica Cracoviensia Series Botanica 2005; 47(2):83-87.
7. Wójcik R, Siwicki AK, Skopińska-Różewska E, Buchwald W, Furmanowa M. The *in vitro* influence of *Rhodiola Kirilowii* extracts on blood granulocytes potential killing activity (PKA) in pigs. Centr Eur J Immunol 2009; 34(3):158-161.
8. Zuo G, Li Z, Chen L, Xu X. Activity of compounds from Chinese herbal medicine *Rhodiola Kirilowii* (Regel) Maxim against HC V NS 3 serine protease. Antiviral Research 2007; 76:86-92.
9. Furmanowa M, Skopińska-Różewska E, Rogala E, Hartwich M. *Rhodiola rosea* L. *in vitro* culture – phytochemical analysis and antioxidant action. Acta Soc Bot Pol 1998; 67:69.
10. Furmanowa M, Kędzia B, Hartwich M, Kozłowski J, Krajewska-Patan A, Mścisz A et al. Phytochemical and pharmacological properties of *Rhodiola rosea* L. Herba Pol 1999; 45:108-13.
11. Battistelli M, De Sanctis R, De Bellis R, Cucchiarini L, Dacha M, Gobbi P. *Rhodiola rosea* as antioxidant in red blood cells: ultrastructural and hemolytic behaviour. Eur J Histochem 2005; 49(3):243-54.
12. Udintsev SN, Shakhov VP. Decrease of cyclophosphamide haematotoxity by *Rhodiola rosea* root eextract in mice with Ehrlich and Lewis transplantable tumors. Eur J Cancer 1991; 27:1182.
13. Krasnov EA, Kuvaiev VB, Chorużaya TG. Chemotaksonomic investigations of *Rhodiola* sp. Rast Res 1978; 14(2):153-160.
14. Krajewska-Patan A, Furmanowa M, Derger M, Łowicka A, Górska-Paukszta M, Mścisz A et al. Zawartość związków biologicznie czynnych w hodowlach kalusa i w hodowlach zawiesinowych *Rhodiola Kirilowii* (Regel.) Maxim. Herba Pol 2006; 52(3);47-8.
15. Wong YC, Zhao M, Zong YY, Chan CY, Che CT. Chemical constituents and anti-tuberculosis activity of root of *Rhodiola kirilowii*. China J Chin Mat Med 2008; 33(13):1561-5.
16. Mścisz A, Mielcarek S, Buchwald W, Krajewska-Patan A, Furmanowa M, Skopińska-Różewska E et al. Phytochemical study of *Rhodiola rosea, Rhodiola quadrifida* and *Rhodiola kirilowii* extracts. Basic and Clinical Pharmacology and Toxicology 2005; 97(suppl I):41.
17. Buchwald W, Mścisz A, Krajewska-Patan A, Furmanowa M, Przybylak J, Luczkowska T et al. Contents of biological active compounds of *Rhodiola kirilowii* roots during the vegetation. Herba Pol 2005; 51(suppl 1):105-6.
18. Mielcarek S, Mścisz A, Buchwald W, Krajewska-Patan A, Furmanowa M, Skopińska-Różewska E et al. Phytochemical investigation of *Rhodiola* sp. roots. Herba Pol 2005; 51(Suppl. 1):159-160.
19. Wiedenfeld H, Zych M, Buchwald W, Furmanowa M. New compounds from *Rhodiola kirilowii*. Sci Pharm 2007; 75:29-34.
20. Zhang S, Wang J, Zhang H. Chemical constituents of Tibetan medicinal herb *Rhodiola kirilowii* (Reg.) Reg. China J Chin Mat Med 1991; 16(8):483,512.
21. Kir'yanov A, Bondarenko L, Kurkin V, Zapesochnaya G et al. Determination of biologically active consistuents of *Rhodiola rosea* rhizomes. Kim-Prir Soedin 1991; 3:320.
22. Kurkin V, Zapesochnaya G, Klyaznika V. *Rhodiola rosea* rhizome flavonoids. Khim Prir Soedin 1982; 5:581.
23. Kurkin V, Zapesochnaya G, Shchavlinskii A. Flavonoids of rhizomes of *Rhodiola rosea*. III. Khim Prir Soedin 1984; 5:657.
24. Zapesochnaya G, Kurkin V. Flavonoids of *Rhodiola rosea* rhizomes. I. Khim Prirod Soed 1983; 1:23.
25. Kurkin V, Zapesochnaya G. Chemical composition and pharmacological properties of *Rhodiola sp.* Plants Review. Khim-Farm Zh 1986; 20(10):1231.
26. Kurkin V, Zapesochnaya GG, Kir'yanov AA et al. Quality of raw *Rhodiola rosea* L. material. Khim-Farm Zh 1989; 23:11.

27. Akgul Y, Ferreira D, Abourashed EA, Khan IA. Lotaustralin from *Rhodiola rosea* roots. Fitoterapia 2004; 75(6):612-4.
28. Rohloff J. Volatiles from rhizomes of *Rhodiola rosea* L. Phytochemistry 2002; 59:655:61.
29. Gryszczyńska A, Mielcarek S, Buchwald W. The determination of flavan-3-ol content in the root of *Rhodiola Kirilowii*. Herba Pol 2011; 51(1): 27-37.

PORÓWNANIE ZAWARTOŚCI FENYLOETANOIDÓW W KORZENIACH *RHODIOLA KIRILOWII* I *RHODIOLA ROSEA* ZA POMOCĄ METODY UPLC-MS/MS

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

Przedmiotem badań było zwalidowanie metody analitycznej oznaczenia zawartości fenyloetanoidów. Do detekcji *p*-tyrozolu i salidrozydu w analizowanych dwóch gatunkach różeńca wykorzystano wysokosprawny chromatograf cieczowy sprzężony z tandemowym spektrometrem mas (UPLC-MS/MS). Analizie poddano *Rhodiola kirilowii* oraz *Rhodiola rosea* uzyskane z hodowli gruntowej w 2009 r. w Instytucie Włókien Naturalnych i Roślin Zielarskich w Poznaniu. Dodatkowo z surowca przygotowano dwa wyciągi suche: wyciąg wodny i wyciąg wodnoalkoholowy (50% wyciąg etanolowy). Zawartość poszczególnych składników różni się w zależności od analizowanej matrycy.

Słowa kluczowe: *Rhodiola kirilowii, Rhodiola rosea, zawartość fenyloetanoidów, UPLC-MS/MS, p-tyrozol, salidrozyd*