EKSPERIMETINIS TYRIMAS

Evaluation of biologically active compounds in roots and rhizomes of *Rhodiola rosea* L. cultivated in Lithuania

Agnė Kučinskaitė, Loretta Pobłocka-Olech[1], Mirosława Krauze-Baranowska[1], Małgorzata Sznitowska[2], Arūnas Savickas, Vitalis Briedis

Department of Pharmaceutical Technology and Social Pharmacy, Kaunas University of Medicine, Lithuania, [1]Department of Pharmacognosy, [2]Department of Pharmaceutical Technology, Medical University of Gdansk, Poland

Key words: *salidroside; rosavins; solid-phase extraction and thin-layer chromatography; Rhodiola rosea L.*

Summary. *Salidroside and rosavins (rosavin, rosarin, and rosin), biologically active compounds of Rhodiola rosea L., were analyzed in dried roots and rhizomes of the plants cultivated in Lithuania and naturally growing in Altai geographical region in Russian Federation. The quantitative analysis of the aqueous-ethanolic extracts prepared by percolation was performed employing solid-phase extraction and thin-layer chromatography followed by densitometric determination. Similar amounts of salidroside were found in the extracts (1.35–1.62 mg/mL), irrespective of the origin of the crude plant; however, the differences in the profile of rosavins were substantial with higher content of total rosavins in the extracts obtained from the plant cultivated in Lithuania (7.4 vs. 4.2 mg/mL). It was demonstrated that extracts prepared with 70% (v/v) ethanol contained low concentration of salidroside (0.16–0.17 mg/mL), while the extraction of rosavins with 70% (v/v) ethanol was more efficient compared to 40% (v/v) ethanol.*

Introduction

The percentage of marketed pharmaceutical preparations containing active compounds of herbal origin is in the range of 35–45%. Therefore, the demand for the herbal raw materials is constantly increasing, and it is expected to maintain this tendency in the future. As the natural resources of some widely used medicinal plants are limited to quite specific areas of their natural growth, the possible solution could be introduction and cultivation of these plants in other geographical regions within the same or similar climatic zone. This is why the species of medicinal plants are introduced and cultivated under new ecological conditions (1). The application of this approach results in the expansion of species, subspecies, and varieties of plants that do not grow naturally in Lithuania and neighboring regions. The vegetative organs of such plants should be evaluated to determine their potential in using them as a raw material for the production of safe, efficient, and stable pharmaceutical preparations. Phytochemical analysis of vegetative and generative organs of introduced and cultivated plants allows determining qualitative and quantitative parameters of potential pharmaceutical raw material. The value of the introduced plant species could be unambiguously established based on these results, and further steps in quality management of medicinal plants could be determined. The climatic conditions, insolation/day length, or chemical composition of the soil usually have a significant effect on the phytochemical parameters of the raw material of herbal origin (2).

Rhodiola rosea L. (golden root) belongs to *Crassulaceae* family (3–8). The plant grows naturally in Asia, namely in China (3, 6–10), in the territory of Kazakhstan, Uzbekistan, and Mongolia (3), Altai, and Eastern Siberia (3, 4). The roots and rhizomes of *Rhodiola rosea* are used as medicinal herbal substance, and the main biologically active compounds are salidroside, tyrosol, rosavin, rosarin, and rosin. Numerous publications indicate that therapeutic activity could be attributed to the presence of these compounds (3–11). Russian Pharmacopoeia indicates that the raw material of *Rhodiola rosea* L. must contain not less than 0.8% of salidroside (12). *Rhodiola rosea* L.

Correspondence to A. Kučinskaitė, Department of Pharmaceutical Technology and Social Pharmacy, Kaunas University of Medicine, A. Mickevičiaus 9, 44307 Kaunas, Lithuania. E-mail: agnekucinskaite@yahoo.com

extracts used in most clinical studies were standardized to minimum 3% of rosavins and 0.8–1% of salidroside, because the naturally occurring ratio of these compounds in *Rhodiola rosea* L. roots and rhizomes is approximately 3:1 (3).

The raw material, roots and rhizomes, is used for the preparation of infusions, decoctions and in manufacturing the fluid and dry extracts. The ethanolic extract of golden root is registered in Russian Federation as a medicinal product for human use (3). The preparation is traditionally used as an adaptogenic agent (3–5, 8–11). It is also indicated for use in the treatment of somatic and infectious diseases, psychiatric and neurological conditions, also to improve the function of the memory and physical potential (3, 5, 7–9). In some Scandinavian countries, the preparations containing *Rhodiola rosea* L. are recommended as psychostimulant and tonic agents, for suppressing the stress or increasing mental work capacity (3, 11).

The main parameters to establish the quality of raw material of golden root are the quantities of biologically active compounds (salidroside, rosavin, rosarin, and rosin) in the processed material. The aim of this study was to apply newly developed method of solid-phase extraction and thin-layer chromatography coupled with densitometry for quantitative analysis of biologically active components in the fluid extracts from *Rhodiola rosea* L. plants that have been cultivated in Lithuania and naturally growing in Altai Mountains (Russian Federation). Moreover, the effect of concentration of ethanol on the content of active compounds in the fluid extracts from *Rhodiola rosea* L. was evaluated.

Materials and methods

Materials. The roots and rhizomes of *Rhodiola rosea* L. were collected at Kaunas Botanical Garden of Vytautas Magnus University in 2002 or received from a natural source in Altai Mountains (Russian Federation). The dried herbal raw material with no signs of external damage was used for phytochemical analysis.

Standards of salidroside, rosavin, rosarin, and rosin were purchased from ChromaDex (Santa Ana, CA, USA).

Extraction procedure. The herbal raw materials were comminuted to obtain particle fraction of 0.16–1.60 mm in size. The experimental fluid extracts were produced from *Rhodiola rosea* L. raw material by percolation, in the ratio of 1:2. Ethanol in concentrations 40% (*v/v*) (extracts L40 and R40) or 70% (*v/v*) (extracts L70 and R70) was used for extraction. The plant material was moistened with ethanol for 1 h and then extracted in a percolator for 24 hours with a flow of the solvent (3 drops per min). The produced fluid extracts were kept at 8°C for 72 hours and filtered through a paper filter. Before the analysis, samples of the extracts were freeze-dried, and depending on the concentration of ethanol used for extraction, the dry residue was dissolved in 40% or 70% (*v/v*) ethanol, in the ratio of 1:3 (*v/v*), and used for solid-phase extraction and thin-layer chromatography (SPE-TLC) analysis.

Analysis of active compounds. The content of the biologically active compounds (salidroside, rosavin, rosarin, and rosin) in *Rhodiola rosea* L. was determined by analysis of the fluid extracts using the SPE-TLC coupled with densitometry. The method was described in detail elsewhere (13).

The samples (100 μL) of fluid extracts were introduced onto Bakerbond SPE columns filled with silica gel RP-18 (300 mg) (J. T. Baker, Philipsburg, USA), and the elution was performed with a mixture of acetonitrile-water (30:70, *v/v*) (4 mL). Eluates were lyophilized and dissolved in 40% or 70% (*v/v*) (0.5 mL) ethanol for determination of salidroside and rosavins, respectively.

TLC separation was performed on silica gel F_{254} plates (10×20 cm) (Merck AG, Germany) with ethyl acetate-methanol-water (77:13:10, *v/v/v*) at a distance of 6 cm. Samples and standards (2 μL) were applied on plates using automatic sample applicator Desaga AS-30 (Desaga AG, Numbrecht, Germany). The densitograms were obtained by using Desaga CD 60 densitometer. UV detection of salidroside and rosavins was performed at λ=215 nm and at λ=245 nm, respectively.

Standard solutions of salidroside, rosavin, rosarin, and rosin in the concentration range of 0.2–0.45 mg/mL were prepared by dissolving standard substances in 96% (*v/v*) ethanol (13). The quantitative relationship between the peak areas and amounts of the applied substances was established for calculation of the concentrations of active components in the extracts.

Statistical analysis. The data were processed using Statistica 6.0. Values are expressed as arithmetic means ± standard errors.

Results and discussion

The reliable determination of biologically active compounds in the fluid extracts, produced from *Rhodiola rosea* L. roots and rhizomes cultivated in Lithuania and naturally growing in Altai Mountains (Russian

Federation), would supply objective criteria for the evaluation of qualitative and quantitative phytochemical composition of the medicinal raw material, originating from different geographical regions.

The roots and rhizomes of *Rhodiola rosea* L. cultivated in Lithuania and naturally growing in Altai were used for production of the fluid extracts (marked as L and R extracts, respectively). *Rhodiola Extract Liquid* has been registered in the former Soviet Union as medicinal preparation for human use (reg. No. 75/933/14). It was produced by extracting *Rhodiola rosea* L. with 40% (*v/v*) ethanol in the ratio of 1:1 (3). In our studies, extracts were produced using 40% or 70% (*v/v*) ethanol (2 parts for 1 part of the dried plant material).

The quantitative determination of main phenylpropanoids (salidroside, rosavin, rosarin, and rosin) in extracts was performed by developed SPE-TLC method. It was proven that preliminary separation of the investigated substances from the interfering substances using SPE was necessary to obtain reliable TLC densitograms and reproducible and accurate results (13).

Typical densitograms of the salidroside and rosavin standards recorded at λ=215 nm and at λ=245 nm are presented in Fig. 1. It is shown that determination of salidroside is possible if the densitometry is performed at λ=215 nm.

Figs. 2 and 3 present densitograms of four investigated extracts, recorded at 215 nm (Fig. 2) and 245 nm (Fig. 3). Determined concentrations of salidroside, rosavin, rosarin, rosin, and total amount of rosavins in the extracts are presented in Table.

The quantity of salidroside in the extracts produced from the raw *Rhodiola rosea* L. plant cultivated in Lithuania ranged from 0.163 to 1.352 mg/mL depending on the concentration of ethanol used for extraction. The lowest concentration of salidroside was determined in the extract L70, produced with 70% (*v/v*) ethanol, and the highest salidroside concentration was determined when the plant material was extracted with 40% (*v/v*) ethanol (extract L40). The highest total

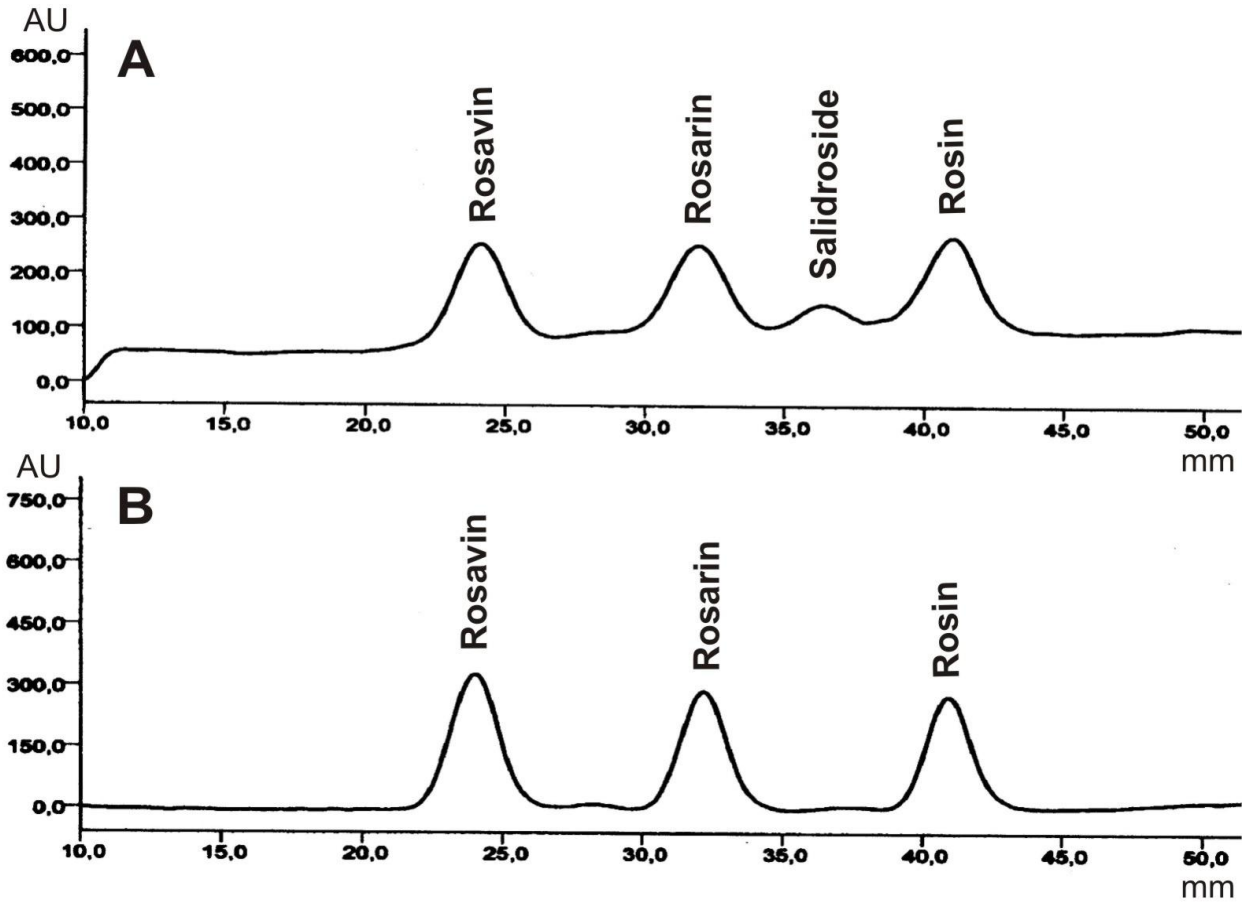

Fig. 1. **Densitograms of the mixture of standards – salidroside, rosavin, rosarin, and rosin – obtained at λ=215 nm (A) and at λ=245 nm (B)**

The concentration of each standard compound in the solution was 250 μg/mL, and the volume of the applied sample was 6 μL.

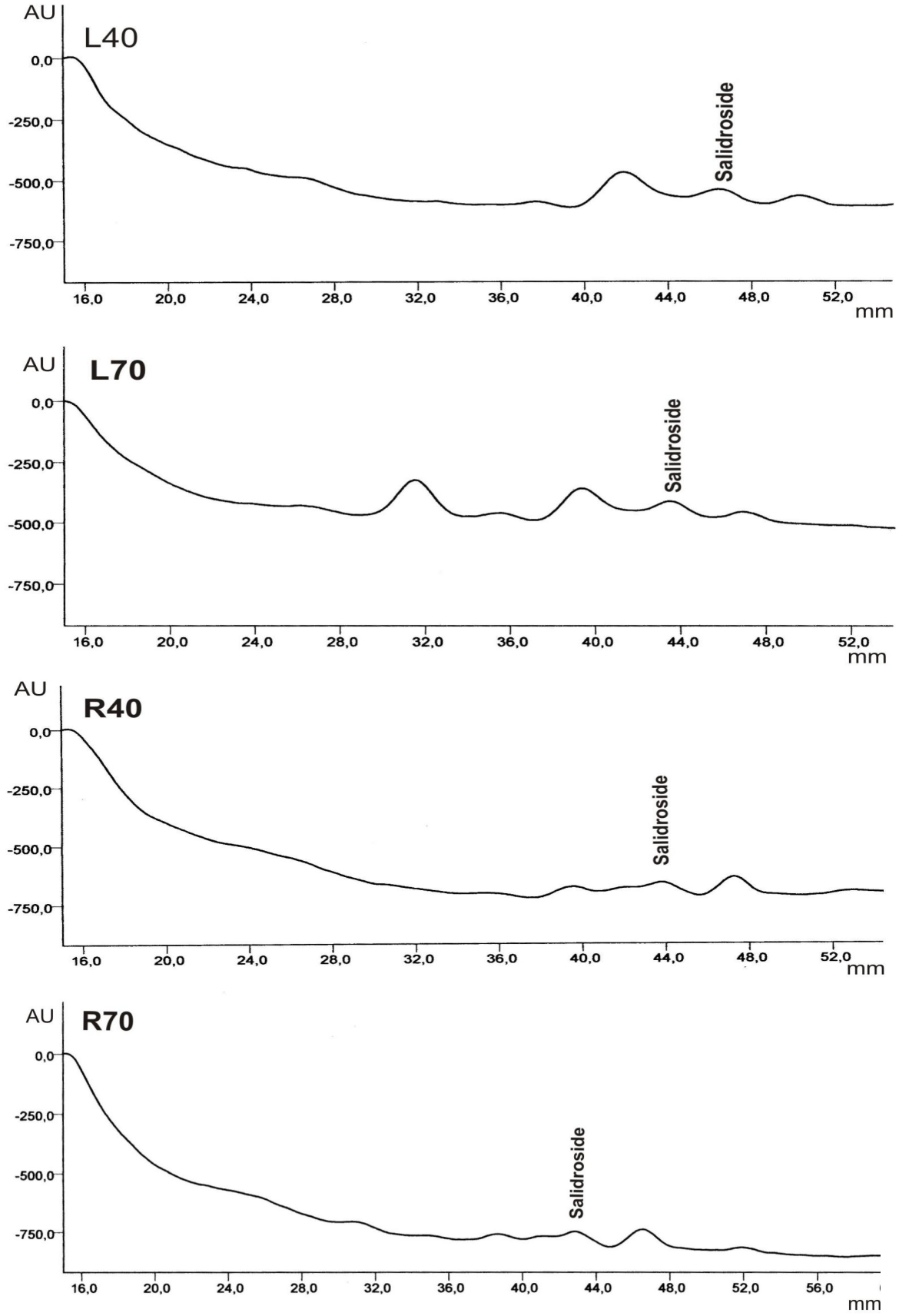

Fig. 2. **Densitograms of fluid extracts from *Rhodiola rosea* L. recorded at λ=215 nm**
For the extract symbols see Table.

Table. **Amounts of salidroside, rosavin, rosarin, and rosin in fluid extracts as determined by solid-phase extraction and thin-layer chromatography**

Extract symbol	Amounts of biologically active compounds (mg/mL)				
	Salidroside	Rosavin	Rosarin	Rosin	Total rosavins
L40	1.352±0.018	0.200±0.036	3.220±0.043	1.603±0.019	5.023±0.033
L70	0.163±0.024	3.688±0.044	2.621±0.050	1.120±0.035	7.429±0.043
R40	1.624±0.028	0.100±0.012	1.015±0.018	2.142±0.041	3.257±0.024
R70	0.176±0.020	0.562±0.023	1.146±0.052	2.574±0.016	4.282±0.030

Extracts L40 and L70 were produced from plants cultivated in Lithuania using 40% or 70% (*v/v*) ethanol, respectively. Extracts R40 and R70 were produced from naturally growing plants in Altai region (Russian Federation) using 40% or 70% ethanol (*v/v*), respectively.

concentration of rosavins (7.429 mg/mL) was determined when extracts were produced using 70% (*v/v*) ethanol (L70).

The analysis of fluid extracts produced from raw plant material originating from Altai region confirmed the above conclusion that the use of 40% (*v/v*) ethanol results in a higher content of salidroside, while 70% (*v/v*) ethanol is more efficient in the extraction of rosavins. The above data demonstrate the varying ability of aqueous ethanol solutions to extract biologically active compounds from *Rhodiola rosea* L. roots and rhizomes, which should be attributed to different solubility of salidroside, rosavin, rosarin, and rosin in ethanol and water. The efficacy of 40% and 70% (*v/v*) ethanol in extracting rosavin, rosarin, and rosin from *Rhodiola rosea* L. raw material is presented in Fig. 4.

The quantitative differences in the biologically active compounds were observed in the extracts produced from *Rhodiola rosea* L. roots and rhizomes of different origin. Fluid extract R40, produced from *Rhodiola rosea* L. originating from Altai region, contained 1.624 mg/mL salidroside that is by 20% higher if compared to 1.352 mg/mL in the extract L40 produced from the plant cultivated in Lithuania. The determined concentration of total rosavins in the L70 extract was approximately by 73% higher as compared to the R70 extract. The composition of rosavins in the extracts also varied significantly when the plant raw materials of different origin were used. These data are presented in the Table and Fig. 4. It is evident that higher amounts of rosavin were present in the L70 extract from *Rhodiola rosea* L. cultivated in Lithuania. Rosarin amounts in this extract were also 2–3 times higher than in R70 extract. In contrast, the content of rosin was about 2 times higher in the R70 extract.

Table and Fig. 4 demonstrate that the change of extracting solvent from 40% to 70% (*v/v*) ethanol resulted in a significant increase in the amount of extracted rosavin from both types of the investigated material, while the changes in rosarin and rosin concentrations were insignificant.

These results suggest that the composition of the extract can be considerably influenced by regional special features of *Rhodiola rosea* L. origin and by techniques employed in the process of extraction. The differences in determined phytochemical composition can be explained mainly by the differences in climatic conditions and chemical components of the soil. This is, however, the first report on the differences, based upon the analysis of one batch of each plant material, and further monitoring of the plants collected throughout the following years should be performed.

The quality of *Rhodiola rosea* L. could be established after unambiguous definition of biological activities of each individual biologically active compound extracted from the dried plant raw material. The possible way to produce the extracts containing specific quantities of salidroside and rosavins is to apply a sequence of extracting agents with varying extraction capacity for these biologically active compounds. Alternatively, 40% (*v/v*) ethanol is also suitable for extraction from *Rhodiola rosea* L. In the extracts produced with 40% (*v/v*) ethanol, the amount of total rosavins is only about 1.5 times lower than in the extracts prepared with 70% (*v/v*) ethanol. On the other hand, if 70% (*v/v*) ethanol is used for extraction, the amount of salidroside in the extract is considerably decreased. SPE-TLC densitometric analysis may be used for quality control of the herbal raw material of different origin, especially when HPLC method is not available.

Conclusions

1. Solid-phase extraction coupled with thin-layer chromatography and densitometry can be used for the qualitative and quantitative analysis of biologically active compounds in the roots and rhizomes of *Rhodiola rosea* L.

2. The quantity of total rosavins extracted by 70%

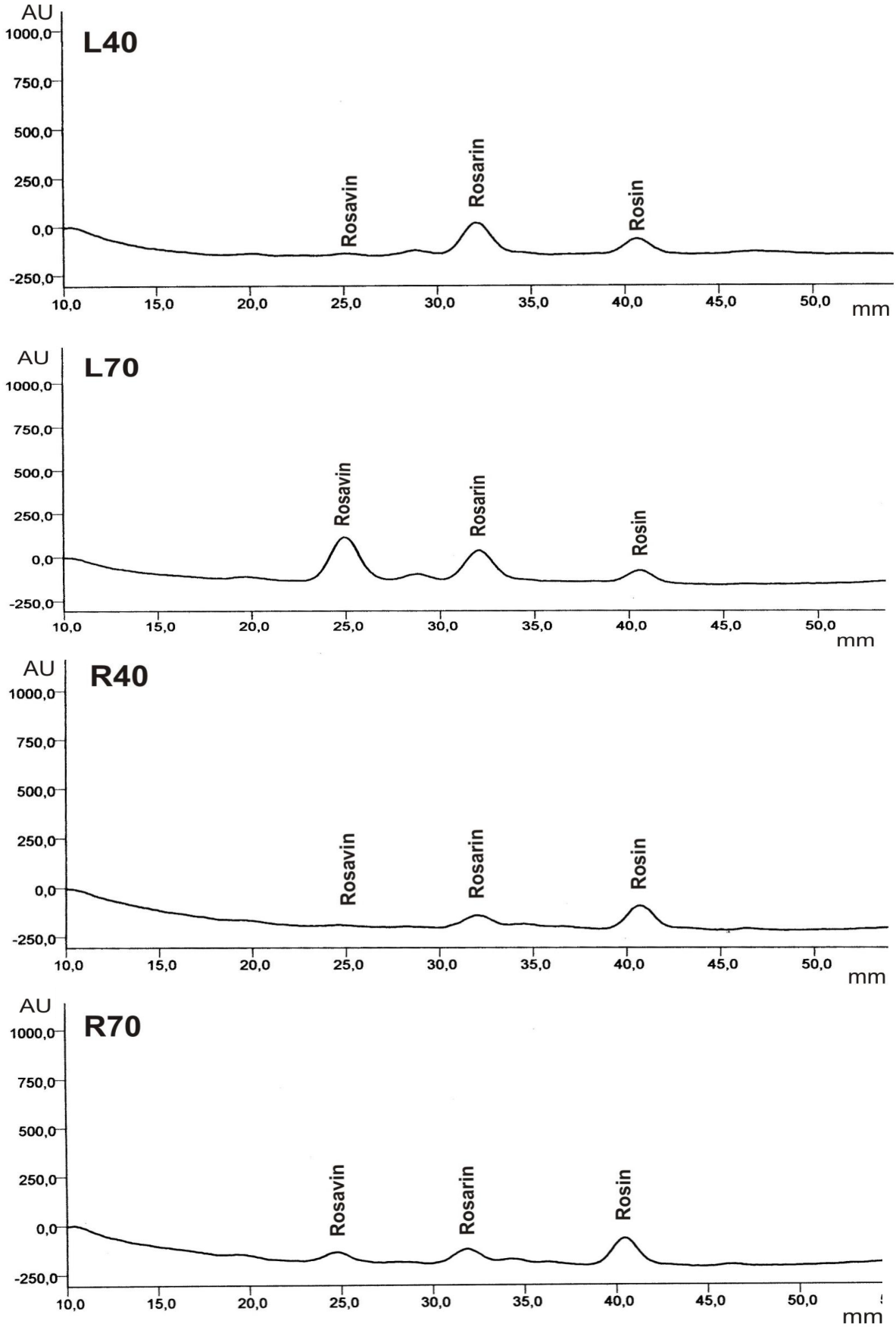

Fig. 3. **Densitograms of fluid extracts from *Rhodiola rosea* L. recorded at λ=245 nm**
For the extract symbols see Table.

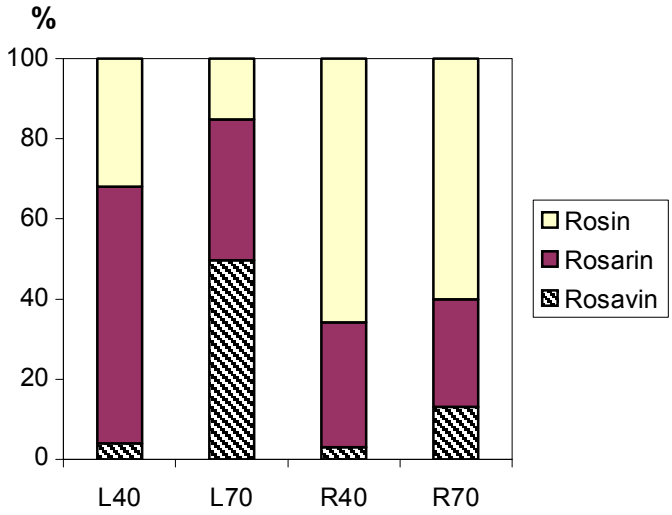

Fig. 4. **The efficacy of 40% and 70% (*v/v*) ethanol in extracting different rosavins from the roots and rhizomes of *Rhodiola rosea* L.**

For the extract symbols see Table.

(*v/v*) ethanol was up to 1.4-fold higher than in extracts produced with 40% (*v/v*) ethanol. On the other hand, extraction of salidroside with 40% (*v/v*) ethanol was significantly more efficient, and it can be concluded that two-step extraction from the roots and rhizomes of *Rhodiola rosea* L. with these two solvents should be recommended.

3. Ethanol at a concentration of 40% (*v/v*) can be recommended for efficient extraction from *Rhodiola rosea* L. in a single-step process because of relatively high amounts of the active substances, particularly salidroside, in the resulting extract.

4. *Rhodiola rosea* L. cultivated in Lithuania and naturally growing in Altai region of Russian Federation demonstrated diverse ability to accumulate salidroside and rosavins. The difference in the content of salidroside was small but was substantial in the case of rosavins. The plant cultivated in Lithuania was a richer source of rosavin and rosarin but higher concentration of rosin was found in the extracts produced from the material collected in Altai region.

5. The raw plant material of *Rhodiola rosea* L. cultivated in Lithuania could be used as a substitute for naturally growing plant after the reliable quantitative determination of the biologically active compounds in the dried plant.

Veikliųjų medžiagų vertinimas Lietuvoje auginamų *Rhodiola rosea* L. šaknyse ir šakniastiebiuose

Agnė Kučinskaitė, Loretta Pobłocka-Olech[1], Mirosława Krauze-Baranowska[1], Małgorzata Sznitowska[2], Arūnas Savickas, Vitalis Briedis

Kauno medicinos universiteto Vaistų technologijos ir socialinės farmacijos katedra, Lietuva, Gdansko medicinos universiteto [1]Farmakognozijos katedra, [2]Vaistų technologijos katedra, Lenkija

Raktažodžiai: salidrozidas, rozavinai, SPE-TLC, *Rhodiola rosea* L.

Santrauka. Nustatytos veikliosios medžiagos – salidrozido ir rozavinų (rozavinas, rozarinas, rozinas) auginamų Lietuvoje ir Altajaus kalnuose (Rusijos Federacija) natūraliai augančių *Rhodiola rosea* L. šaknyse ir šakniastiebiuose. Perkoliacijos metodu pagamintų vandeninių-etanolinių skystųjų ekstraktų kiekybinė analizė atlikta ekstrakcijos kietąja faze – plonasluoksnės chromatografijos (SPE-TLC) – densitometrijos analizės metodu. Nepriklausomai nuo žaliavos kilmės, ištraukose nustatyti panašūs salidrozido kiekiai (1,35–1,62 mg/ml). Taip pat nustatyti rozavinų kiekių skirtumai: didesnis rozavinų kiekis rastas ištraukose, pagamintose iš Lietuvoje auginamos žaliavos (7,4 ir 4,2 mg/ml). Tyrimai parodė, jog, ekstrahuojant žaliavas etanoliu 70 proc. (V/V) ištraukose rasti maži salidrozido kiekiai (0,16–0,17 mg/ml); daugiau rozavinų rasta ekstrakcijai vartojant etanolį 70 proc. (V/V) palyginus su etanoliu 40 proc. (V/V).

Adresas susirašinėti: A. Kučinskaitė, KMU Vaistų technologijos ir socialinės farmacijos katedra, A. Mickevičiaus 9, 44307 Kaunas. El. paštas: agnekucinskaite@yahoo.com

References

1. Ragažinskienė O, Povilaitytė V. Vaistinių augalų bioįvairovės išsaugojimas šiuolaikinei farmacijai. Tarptautinė mokslinė konferencija „Farmacija šiuolaikinėje visuomenėje“. (Preservation of biodiversity of medicinal herbs for modern pharmacy. International conference.) Kaunas: Medicina; 2003. p. 119-21.
2. Jakštas V, Janulis V, Labokas J, Bernatonienė J, Brusokas V, Malinauskas F, et al. Lietuvoje introdukuotų gudobelės rūšių žiedų ir lapų fitocheminis tyrimas. (Phytochemical research of hawthorn species introduced in Lithuania.) Medicina (Kaunas) 2003;39:80-4.
3. Brown RP, Gerbarg PL, Ramazanov Z. *Rhodiola rosea*: a phytomedicinal overview. Herbal Gram. American Botanical Council 2002;56:40-52.
4. Ganzera M, Yayla Y, Khan IA. Analysis of the marker compounds of *Rhodiola rosea* L. (golden root) by reversed phase high performance liquid chromatography. Chem Pharm Bull 2001;49(4):465-7.
5. Rohloff J. Volatiles from rhizomes of *Rhodiola rosea* L. Phytochemistry 2002;59:655-61.
6. Tolonen A, Pakonen M, Hohtola A, Jalonen J. Phenylpropanoid glycosides from *Rhodiola rosea*. Chem Pharm Bull 2003;51(4):467-70.
7. Abidov M, Crendal F, Grachev S, Seifulla R, Ziegenfuss T. Effect of extracts from *Rhodiola rosea* and *Rhodiola crenulata* (*Crassulaceae*) roots on ATP content in mitochondria of skeletal muscles. Bull of Experimental Biology and Medicine 2003;136(6):585-7.
8. György Z, Tolonen A, Pakonen M, Neubauer P, Hohtola A. Enhancing the production of cinnamyl glycosides in compact callus aggregate cultures of *Rhodiola rosea* by biotransformation of cinnamyl alcohol. Plant Science 2004;166:229-36.
9. Kelly GS. *Rhodiola rosea*: a possible plant adaptogen. Altern Med Rev 2001;6(3):293-302.
10. *Rhodiola rosea* [monograph]. (Overview of medicinal plant known as golden root and arctic root). Altern Med Rev 2002. Available from: URL: http://www.findarticles.com/cf_dls/m0FDN/5_7/94159012/p1(2.3.4)/article.jhtml
11. Shevtsov VA, Zholus BI, Shervarly VI, Volskij VB, Korovin YP, Khristich MP, et al. A randomized trial of two different doses of a SHR-5 *Rhodiola rosea* extract versus placebo and control of capacity for mental work. Phytomedicine 2003;10:95-105.
12. State Pharmacopoeia of the USSR. Vol. 2. Moscow: Medicina; 1990. p. 364-6.
13. Kučinskaitė A, Pobłocka-Olech L, Krauze-Baranowska M, Briedis V, Savickas A, Sznitowska M. Use of SPE-TLC method for quality control of *Rhodiola rosea* L. extract. J Planar Chromatogr 2007;20(2):121-5.

Received 23 February 2007, accepted 11 June 2007
Straipsnis gautas 2007 02 23, priimtas 2007 06 11