Antioxidant activity and content of salidroside in ethanolic extracts of *Rhodiola rosea*

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**Abstract** Rhodiola rosea L. is an important medicinal plant, grown in the Arctic regions. Recently, many investigations are devoted to the development of new methods of extraction bioactive components from *R.*rosea. One of the valuable and common methods of extraction is maceration in the water-ethanol mixture to obtain tincture. At the same time, a certain drawback of systematic studies of the dependence of such extraction efficiency on the concentration of ethanol is noticeable in the literature. In this work data of systematical study of maceration efficiency of water-ethanol mixtures with ethanol content 0 – 97 vol.% at 25°C. Salidroside content and total antioxidant activity were estimated. It was obtained that the most useful for salidroside as well as other antioxidants extraction is maceration with 50 % water-ethanol mixture rot 3 days. These data can be used to organize the extraction process and for further studies in the field of optimization of the processes of extraction of bioactive compounds from *R.*rosea.

1. **Introduction**
Rhodiola rosea L. (according to the www.theplantlist.org database, the accepted name is Sedum roseum (L.) Scop.) is a well-known medicinal plant, grown in the Arctic regions (Mountain Altai, Murmansk region, Polar Urals, etc.), Europe, and North America. Bioactive substances of *R.*rosea have such pharmacological effects as adaptogenic, antidepressive, cardioprotective, anticancerogenic, antioxidant effects and some others. The most valuable parts of this plant are the roots and rhizomes. These parts contain glycosides of tyrosol and cinnamic alcohol, flavonoids, terpenes, essential oils, etc. [1–3].

For the extraction of bioactive components various methods are used. They include extraction using methanol, ethanol, and other organic solvents at various conditions (heating, high pressure, microwave or ultrasound treatment) [4–6]. In our previous works, deep eutectic solvents were applied for the extraction of cinnamic alcohol and salidroside from *R.*rosea [7,8]. Applying deep eutectic solvents is also described in the work [9]. At the same time, a certain drawback of systematic studies of the dependence of the extraction efficiency with water-ethanol solutions, as the most often used for the preparation of tinctures, on the concentration of ethanol, is noticeable in the literature. It should be noted that systematic data on the effectiveness of widely used extractants are necessary for a comparative evaluation of the effectiveness of the newly developed extraction methods, including using alternative solvents, for example, deep eutectic solvents.

Thus, the purpose of this study was to assess the effectiveness of the extraction of bioactive components from *R.*rosea with using various water-ethanolic mixtures. One of the simplest and the most applicable for a commonly used method of extraction is maceration, which lets to obtain ethanolic
tinctures. Total antioxidant capacity as one of important characteristic of extracts and salidroside content were estimated to estimate extraction efficiency.

2. Materials and methods

2.1. Materials

Plant material was dried roots and rhizomes of R. Rosea (Company "Travy i Korni", Russia). Ammonium molybdate, potassium dihydrogenphosphate, lead acetate, potassium carbonate, sodium sulfate, sodium nitrite (all with > 99% purity, Vekton, Russia), sulfacetamide (medical grade), and concentrated hydrochloric and sulfuric acids (Lenreactiv, Russia) Distilled water and ethanol (EtOH, 95.0%, Vekton) were used for solvent preparation.

2.2. Extraction procedure

Powdered dried rhizomes of R. rosea were placed in the sealed vials, the solvent was added in the mass: volume ratio 1:10. 0.2 g of plant material was mixed with 2 ml of solvent. Ethanol – water mixtures with a volume fraction of ethanol 0 – 97 % (with step 10 %) were used as solvents. Vials were placed in the thermostat at 25 °C for 1 – 4 days. Samples were periodically shacked. Obtained extracts were filtrated through the filter paper.

2.3. Salidroside content estimation

The concentration of salidroside was estimated due to the method, described in [10]. The reactive solution is prepared as follows: 8.75 ml of 20 % sulfacetamide and 2.25 ml of concentrated hydrochloric acid are poured into 25 ml volumetric flask and water is added to reach appropriate volume. 1 ml of the obtained solution is poured into 100 ml cooled volumetric flask, 25 ml of water, and 0.2 ml of 10 % sodium nitrite are added, and then more water is added to this mixture to reach 100 ml.

0.5 ml of row extract is mixed with 0.6 ml of 10 % lead acetate solution and 0.2 ml of a saturated solution of sodium sulfate. Water is added to reach a volume of 5 ml. White precipitated forms in the mixture. The mixture is filtrated, and 2 ml of the obtained solution is mixed in the graduated tube with 0.25 ml of sodium carbonate, 0.25 ml of the reactive solution, and water is added to reach 5 ml. After 5 minutes absorbance at 486 nm is measured. For the measurements, KFK-3-01 “ZOMZ” spectrophotometer with 0.517 cm cuvette was used. The reference solution was distilled water.

The concentration of salidroside in row extract is calculated following the equation

\[ C = \frac{V_4 \times V_2 \times A}{V_3 \times V_1 \times E \times l} \]

where \( C \) – concentration of salidroside, \( V_1 \) – the volume of the sample of row extract (0.5 ml), \( V_2 \) – the volume of the mixture after addition of lead acetate and sodium sulfate (5 ml), \( V_3 \) – the volume of the aliquot of treated extract (2 ml), \( V_4 \) – the final volume of the mixture after addition sodium carbonate and reactive solutions (5 ml), \( E \) – specific absorbance, 25.3 mg/ml×cm, \( l \) – spectrophotometric cuvette length (0.517 cm).

Total antioxidant capacity was estimated using the method suggested in [11] and described in our previous work [12]. For this row extracts were diluted 50 times.

3. Results and discussion

The results of the assessment of salidroside concentration and total antioxidant activity are presented in Figures 1 and 2, respectively. 44 extracts were obtained, differing in the ethanol content in the extractant and the maceration time. It can be noted that both values increase during the first 3 days of extraction, and then fall. The highest content of salidroside and TAC is observed for extracts obtained using 40, 50 and 60% ethanol, while 50% ethanol contains the highest amounts of salidroside and other antioxidant substances. Pure solvents (water and ethanol) recover the target substances in general worse than their mixtures, however, for pure ethanol there are higher salidroside contents than for water.
The tendency of the change in the concentration of salidroside and the total antioxidant activity depending on the composition of the extractant and the time of maceration generally coincide. It should be noted that the antioxidant activity of dilute and concentrated ethanol solutions is approximately the same, while the content of salidroside from diluted ethanol solutions is much lower than in more concentrated ones. This discrepancy may be due to the antioxidant activity of other compounds contained in extracts of R. rosea (for example, flavonoids, cinnamic alcohol glycosides) that are not detected by the azo coupling reaction.

**Figure 1.** Concentration of salidroside in row water-ethanolic extracts of *Rhodiola rosea*.

**Figure 2.** Total antioxidant activity (TAC) in the equivalent content of ascorbic acid in row water-ethanolic extracts of *Rhodiola rosea*.

4. **Conclusions**
The paper presents data on the assessment of salidroside content and total antioxidant activity in water-ethanol extracts of *Rhodiola rosea*. 
Based on the data obtained, it can be concluded that despite the common method of maceration of Rhodiola rosea rhizomes on 40% ethanol for a week or brewing it with water, the most effective way to extract salidroside is to use 50% ethanol and maceration at room temperature for 3 days. The data obtained can be used to organize the extraction of biologically active substances from Rhodiola rosea and further studies in the field of optimization of extraction processes using both ethanol extractants and other solvents.

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