Estimation of extraction efficiency of salidroside from
Rhodiola rosea using deep eutectic solvents

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Abstract. This article is devoted to the new possibilities of extraction of biologically active substances (BAS) from Rhodiola rosea L. This plant contains such BAS as salidroside, rhodiosin, rosavin, rosarin, rosin, which are glycosides of cinnamon alcohol and tyrosol. Pharmacological effects Rhodiola rosea include adaptogenic and stress-protective, cardioprotective, antioxidant effect, stimulating the central nervous system, anti-fatigue, antidepressive, anxiolytic, endocrine activity normalizing and life-span increasing effects. One of the promising methods for extracting biologically active substances is extraction using deep eutectic solvents. The aim of this work is to assess the applicability of three deep eutectic solvents for the extraction of biologically active substances, in particular, salidroside, from Rhodiola rosea. In this work the maceration method is used to obtain extracts and the HPLC method is used for the semi-quantitative analysis of the salidroside content. It has been established that the most suitable extractant for salidroside extraction is a mixture of choline chloride + malonic acid + methanol. Extractants containing choline chloride and urea, in contrast, do not extract salidroside from Rhodiola rosea.

1. Introduction
Rhodiola rosea (Crassulaceae) is a valuable medicinal plant, common in the Arctic zones, on the coasts and sea cliffs of Russia (Mountain Altai, Murmansk region, Polar Urals), North America, Europe, and also in the mountainous parts of Mongolia and China. This plant has such pharmacological effects as adaptogenic and stress-protective, cardioprotective, antioxidant effect, stimulating the central nervous system, anti-fatigue, antidepressive, anxiolytic, endocrine activity normalizing and life-span increasing effects [1]. One of the main biologically active components of Rhodiola Rosea is salidroside, which exhibits anticancer and life-span increasing activity [2,3]. The accumulation of biologically active substances occurs mainly in the underground parts of the plant, and it is the roots and rhizomes that are used for further extraction. Being a common plant in the coastal zone of the White Sea, Rhodiola Rosea is an important natural resource for this Murmansk region.

The promising extractants of biologically active substances from plant material are deep eutectic solvents, DES [4–7]. They are a mixture of a hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA). There is evidence of high efficacy of such solvents for the extraction of rutin - quercetin glycoside. Some similarity between the structures of rutin and salidroside (both are
glycosides, aglycones contain hydroxyl groups) suggests a potentially high efficiency of extraction of salidroside using DES.

The purpose of this study was to assess the applicability of deep eutectic solvents for the extraction of salidroside from the underground parts of Rhodiola Rosea.

2. Materials and methods

2.1. Materials
Dried roots and rhizomes of Rhodiola Rosea (Company "Travy i Korni", Russia) were initial raw material. Choline chloride (99%, RONGSHENG BIOTECH) was used as an HBA for DES, malonic acid (99.5%, Chemical Line), glycerol (99.0%, Vekton) and urea (99.5%, Vekton) were used as HBDs. Distilled water, methanol (MeOH, 99.0%, Vekton) ethanol (EtOH, 95.0%, Vekton) were used as reference solvents.

2.2. Extraction procedure
Three DES were used for the extragents preparation: the mixtures of choline chloride + malonic acid in mol. ratio 1:1 (DES1), choline chloride + glycerol 1:2 (DES2), and choline chloride + urea 1:2 (DES3). Components of DES were mixed in weighing bottles and were kept during the day in air thermostat at 50°C. As a result, homogeneous viscous liquids had been obtained. Pure DES have fairly high viscosity, this is why they were mixed with reference solvents (water, methanol or ethanol) in volume ratio 1:1.

Extraction was performed by the maceration. Powdered dried rhizomas of Rhodiola rosea and extragent were mixed in mass/volume ratio 1:20 in the sealed glass vials and were kept during the day at 50°C. Obtained extracts were centrifuged using ELMI Multi Centrifuge CM 6M. 5 ml plastic syringes were used for filtration. When the piston was taken out, a filter paper was placed inside the syringe, the extract was poured, then the piston was inserted back and the liquid was forced through the filter paper.

2.3. HPLC analysis
Analysis was performed with using Agilent 1200 and Agilent 6538 (LC-ESI-qTof). Chromatography was performed on a reverse phase column Zorbax SB C-18 (Agilent) 150mm x 3.1mm, 1.8 μm with gradient elution with system water-acetonitrile (from 0 to 40% acetonitrile). Sample volume was 1 μl. Registration was made with UV-detector at 220, 254 and 320 nm. Substances were identified using MS or collision fragmentation MS results.

3. Results and Discussion
To estimate amount of salidroside in obtained extracts chromatogram peaks were integrated (figure 1) and peaks area for each extract were compared. The results of HPLC analysis is presented in table 1.

| Extragent            | S, mV×min |
|----------------------|-----------|
| Water                | 181.51    |
| MeOH                 | 39.07     |
| EtOH                 | 23.71     |
| DES1 + Water         | 45.40     |
| DES1 + MeOH          | 199.94    |

Table 1. The results of a semi-quantitative analysis of extracts of Rhodiola rosea.
DES2 + Water 40.16
DES2 + MeOH 156.10
DES2 + EtOH 126.98
DES3 + Water -
DES3 + MeOH -
DES3 + EtOH 16.88

**Figure 1.** The HPLC analysis of DES1 + MeOH extract. The first peak relates to salidroside.

Results in table 1 demonstrate that the extracts with DES1 + methanol, DES2 + methanol, DES2 + ethanol and also with water contain greatest amount of salidroside. Extracts with DES3 contain no salidroside. Commonly used for extraction of various bioactive substance methanol and ethanol demonstrate relatively low efficiency for extraction of salidroside using simple maceration. DES1 + methanol based extract contains 5-8 times more salidroside than methanol and ethanol based ones.

4. **Conclusions**

This paper demonstrates data of comparing the efficiency of extraction of salidroside from the rhizomes of Rhodiola rosea using water, methanol, ethanol and extractants containing three types of deep eutectic mixtures: choline chloride + malonic acid, choline chloride + glycerol, choline chloride + urea.

From the data of the semi-quantitative analysis, it can be concluded that the most efficient extraction of salidroside was achieved using a mixture of DES1 + methanol. On the contrary, DES3, containing urea, failed to extract salidroside.

The data obtained in the work will be used for the further development of research on the using of deep eutectic solvents for the extraction of biologically active substances from medicinal plants.

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