Metals content in deep eutectic solvents-based extracts of Koenigia Weyrichii growing in the Kola Peninsula

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Abstract. Koenigia Weyrichii is a potentially valuable plant for the extraction of flavonoids. Growing in the Polar region, in particular in the Kola Peninsula, this plant accumulates high amounts of flavonoids. However, industrial pollution can lead to an increased content of various metals in plant material and extracts obtained from it. One of the methods of extraction bio-active components from plants is applying deep eutectic solvents. These solvents can extract not only bio-active components but also metals, including toxic ones. In this research extracts of K. Weyrichii were obtained with a mixture of choline chloride + glycerol with the addition of 10 – 50 wt.% of water. 60% v/v ethanol + water mixture was reference extractant. Various metals concentrations in extracts were estimate with mass-spectrometer with inductively coupled plasma. It is obtained that extracts based on deep eutectic solvents are characterized by low concentrations of toxic and rare metals, and relatively high concentrations of macro- and micro-nutrient metals.

1. Introduction

Koenigia Weyrichii (early Polygonum Weyrichii Fr.Schmidt [1]) is a perspective plant for the extraction of flavonoids. This species was naturalized on the Kola Peninsula in early XX-th. This plant quickly accumulates biomass, is frost-resistant, and easy to cultivate. During the vegetation period, relatively large amounts of flavonoids accumulate in the inflorescences [2–4]. Furthermore, extreme conditions of the Polar region stimulate the plants to generate more flavonoids, and this may be significant for developing medical plant cultivation in this region. Due to many important effects of flavonoids for human health, including antioxidant activity, cardioprotective, anti-inflammatory, and anti-cancer effects [5–7], K. Weyrichii can be considered a potentially valuable source of flavonoids.

The promising direction of research of the extraction of bioactive components, particularly flavonoids, from plants relates to the using of deep eutectic solvents (DES)[8]. DESs are the mixtures of two (rarely three) substances, one of them is a hydrogen bond donor (HBD) and the other is a hydrogen bond acceptor (HBA). It may be a mixture of choline chloride with glycerol, urea, carboxic acids, sugars, etc. DESs have low toxicity, high biodegradability, and high efficiency for bioactive
component recovery. But also, DESs can extract not only bioactive organic molecules but various inorganic compounds include metals [9].

Due to the fact that mining and mining enterprises operate on the Kola Peninsula, in some areas the soil and, hence, plants may contain an increased amount of various metals. These metals can be incorporated into extracts obtained from plants growing in open areas. Therefore, the content of macro-, micro-elements, as well as toxic elements in plant extracts should be assessed. There are relatively few works on the analysis of the elemental composition of DES-based extracts. Thus, the aim of this work was to estimate the content of various metals in the extracts of *K. Weyrichii* based on DES.

2. Materials and methods

2.1. Materials
Inflorescence of *K. Weyrichii* cultivated in Polar Alpine Botanical Garden and Institute (Apatity) were collected and dried and stored in accordance with the rules for drying and storage of vegetable medicinal raw materials [10]. Before extraction plant material was powdered, and a fraction of 1 mm was taken.

Choline chloride (99%, RONGSHENG BIOTECH) was used as an HBA for DES, glycerol (99.0%, Vekton) was used as HBDs. Distilled water and ethanol (EtOH, 95.0%, Vekton) were used for reference solvent preparation (60% v/v ethanol-water mixture).

2.2. Extraction procedure
To prepare DES-based extractants choline chloride and glycerol were mixed in molar ration 1:2 in chemical glass and were kept during the day in an air thermostat at 50°C. As a result, the homogeneous liquid had been obtained. To decrease viscosity and estimate the effect of water addition, DES was mixed with 50%, 30%, and 10% of distill water by mass.

Extraction was performed under ultrasound assistant treatment. Powdered dried inflorescences of *K. Weyrichii* and the extractants were mixed in mass/volume ratio 1:10 in the sealed glass vials and were hold in an ultrasound bath for 3 at 50°C. After these extracts were centrifuged using ELMI Multi Centrifuge CM 6M and filtered.

2.3. ICP-MS measurements
The mass concentration of elements in the samples was measured using a mass spectrometer with an inductively coupled argon plasma model ELAN 9000 DRC-e, (Perkin Elmer, USA) with quadrupole reactive cell. Standard sample Multi-element ICP-MS Calibration Standard STD 1 (SCP Science, USA) was used for equipment settings. Calibration was made using standard solutions ICP-MS Calibration Standard IV-STOCK-21, IV-STOCK-26, IV-STOCK-28, IV-STOCK-29 (Inorganic Ventures, USA) with detected elements concentration 10 mg/l, ARD is less then 0.5% at $p=0.95$.

2% of nitric acid was used for the dilution of the samples. This acid was previously purified by isothermal distillation system Berghof-BSB-939-IR (Berghof, Germany), deionized water (with conductivity 18.2 MΩ cm) was obtained with water purification system «Millipore Element» (Millipore, USA).

3. Results and discussion
The content of various metals in original dried and powdered plant material and the recovery of these metals in DES-based extractants with three different water additions and 60% ethanol are presented in table 1. Also, correlations between the recovery of metals and water content in DES-based extractants were estimated using MS Excel. Recoveries of metals that play a role in human metabolism and which are macro- or micro-nutrients, such as sodium, magnesium, calcium, magnesium, ferrum, cobalt, copper, zinc, and molybdenum are presented in figure 1. Rare elements, such as lanthanides, some
actinides, etc. have relatively similar behavior in the extraction process. The recovery of some of them is presented in Figure 2.

It may be noted, that for the most of elements there is a positive correlation with water content in DES-based extractant. Exceptions are potassium, lead, nickel, molybdenum, zinc, and tungsten. Most of the toxic elements, like thallium, germanium, tin, have a relatively low recovery, but the recovery of cadmium riches more than 90%. It should be taking into account for the further application of DES for the extraction processes. Relatively strange behavior was obtained for the lead: There is no lead in the DES-based extracts contained 50 and 30 % of water, but 100% of lead recovers into DES-based extractant contained 10% of water. These results should be refined in future work. Among micro-nutrients, zinc should be noted, since its recovery reaches almost 100%. Rare elements recovery is less than 10%, and it can be noted, that the lower the water content in DES-based extractant, the less the rare elements are extracted.

Table 1. The content of metals in plant material (\(w^0\)), recovery of metals to various extractants, and correlation between recovery and water content in DES-based extractants.

| Element | \(w^0\), ppm | Recovery (%) | Correlation |
|---------|---------------|--------------|-------------|
|         |               | Water content in DES, % | EtOH |             |
|         |               | 50.00 | 30.00 | 10.00 |             |
| Li      | 0.710         | 8.2   | 3.1   | 1.3   | 5.0   | 0.965      |
| Na      | 2680          | 0.0   | 47.8  | 18.2  | 30.3  | -0.376     |
| Rb      | 52.2          | 76.2  | 66.6  | 54.0  | 51.5  | 0.997      |
| Be      | 0.380         | 15.3  | 5.0   | 0.0   | 1.6   | 0.981      |
| Mg      | 3804          | 72.0  | 61.4  | 25.8  | 47.3  | 0.955      |
| Ca      | 9666          | 23.7  | 1.5   | 4.1   | 0.6   | 0.806      |
| Sr      | 265.4         | 4.7   | 7.5   | 4.4   | 0.3   | 0.078      |
| Ba      | 80.1          | 9.8   | 10.7  | 5.9   | 0.0   | 0.759      |
| B       | 14.7          | 15.2  | 17.2  | 11.0  | 17.4  | 0.666      |
| Al      | 6707          | 10.4  | 7.2   | 1.8   | 4.5   | 0.990      |
| Ga      | 1.31          | 15.6  | 10.5  | 2.7   | 3.5   | 0.993      |
| In      | 0.002         | 58.8  | 11.8  | 0.0   | 0.0   | 0.945      |
| Tl      | 0.002         | 0.0   | 0.0   | 0.0   | 28.2  | -          |
| Ge      | 0.100         | 18.0  | 7.0   | 5.0   | 2.0   | 0.929      |
| Sn      | 0.230         | 9.1   | 0.0   | 0.9   | 0.0   | 0.818      |
| Pb      | 0.371         | 0.0   | 0.0   | 100.0 | 0.0   | -0.866     |
| Sb      | 0.024         | 12.5  | 0.0   | 9.9   | 0.0   | 0.196      |
| Te      | 0.007         | 18.6  | 22.5  | -5.1  | 0.0   | 0.793      |
| Ti      | 911.5         | 0.3   | 0.3   | 0.2   | 0.0   | 0.602      |
| V       | 9.38          | 0.0   | 0.0   | 0.0   | 0.0   | -          |
| Cr      | 17.0          | 0.0   | 0.0   | 0.0   | 0.0   | -          |
| Mn      | 98.5          | 33.3  | 26.5  | 7.1   | 8.1   | 0.964      |
| Fe      | 2018          | 6.5   | 7.2   | 4.6   | 3.8   | 0.698      |
| Co      | 1.18          | 11.2  | 12.5  | 5.2   | 0.0   | 0.773      |
| Ni      | 7.98          | 0.0   | 21.7  | 7.9   | 0.0   | -0.361     |
| Cu      | 9.81          | 31.6  | 37.0  | 20.1  | 16.8  | 0.666      |
|  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|
| Zn | 31.4 | 86.6 | 82.5 | 98.4 | 61.1 | -0.713 |
| Mo | 1.27 | 0.0 | 20.1 | 21.2 | 19.3 | -0.888 |
| Cd | 0.026 | 88.5 | 96.2 | 65.2 | 36.5 | 0.722 |
| Y | 3.08 | 8.5 | 7.0 | 1.3 | 0.7 | 0.950 |
| Zr | 16.4 | 4.3 | 0.9 | 0.6 | 0.0 | 0.902 |
| Nb | 6.71 | 4.0 | 2.4 | 0.6 | 0.0 | 0.999 |
| La | 11.4 | 7.0 | 5.3 | 0.8 | 0.1 | 0.969 |
| Ce | 18.9 | 6.4 | 5.1 | 0.8 | 0.1 | 0.956 |
| Pr | 1.95 | 6.4 | 5.0 | 0.7 | 0.1 | 0.960 |
| Nd | 6.164 | 6.5 | 5.3 | 0.8 | 0.1 | 0.949 |
| Sm | 1.00 | 6.8 | 5.4 | 0.7 | 0.2 | 0.954 |
| Eu | 0.297 | 6.8 | 6.1 | 1.2 | 0.3 | 0.912 |
| Gd | 0.971 | 7.4 | 5.7 | 0.9 | 0.3 | 0.965 |
| Tb | 0.136 | 6.9 | 5.6 | 0.7 | 0.4 | 0.949 |
| Dy | 0.610 | 6.8 | 5.8 | 1.0 | 0.4 | 0.938 |
| Ho | 0.112 | 7.2 | 5.5 | 0.7 | 0.5 | 0.962 |
| Er | 0.245 | 7.7 | 6.0 | 0.7 | 0.6 | 0.962 |
| Tm | 0.035 | 6.2 | 4.9 | 0.8 | 0.8 | 0.961 |
| Yb | 0.172 | 7.4 | 5.6 | 1.0 | 0.8 | 0.970 |
| Lu | 0.020 | 9.6 | 5.4 | 2.1 | 0.8 | 0.998 |
| Hf | 0.296 | 6.4 | 2.4 | 0.5 | 0.0 | 0.980 |
| Ta | 0.801 | 4.6 | 2.2 | 2.1 | 1.5 | 0.879 |
| W | 0.008 | 0.0 | 0.0 | 4.5 | 11.4 | -0.866 |
| Th | 0.714 | 7.2 | 3.6 | 0.8 | 0.2 | 0.997 |
| U | 0.262 | 9.7 | 4.8 | 1.9 | 1.8 | 0.989 |

Figure 1. The recovery of some important for human health metals from *K. Weyrichii*. On the X-axis type of extractant is placed: Labels “50%”, “30%”, “10%” related to water content in the DES-based extractant, EtOH – 60% ethanol.
Figure 2. The recovery of some rare metals from *K. Weyrichii*. On the X-axis type of extractant is placed: Labels “50%”, “30%”, “10%” related to water content in the DES-based extractant, EtOH – 60% ethanol.

4. Conclusions
Analysis of various metals content in extracts of *K. Weyrichii* obtained with DES choline chloride + glycerol (in molar ratio 1:2) with 10 – 50% of the water in comparison to 60% ethanol + water mixture is presented in this work.

Most elements have a positive correlation between recovery and water content in DES-based extractant. Toxic elements have a relatively low recovery, with the exception of cadmium, which is pretty much 100% extracted. High recovery is typical for zinc. Rare elements are extracted with low recovery, and almost not extracted with ethanol.

Obtained data may be important for the organization of the extraction processes and production of the extracts of plants growing in the Kola Peninsula.

Acknowledgements
This work was financially supported by Grant of President of Russian Federation (MK-566.2020.11), and has been carried out in the framework of Scientific Research Contracts №№ 0226-2019-0032 and 0186-2019-0009.

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