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Determination of phenolic compounds and diterpenes in roots of *Salvia miltiorrhiza* and *Salvia przewalskii* by two LC–MS tools: Multi-stage and high resolution tandem mass spectrometry with assessment of antioxidant capacity

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ABSTRACT

Comparative phytochemical analyses of hydroalcoholic (50% EtOH) extracts from roots of *S. miltiorrhiza* (SM) and *S. przewalskii* (SP) were performed using two complementary LC–MS systems: the first system HPLC-DAD-MS^n an ion trap mass spectrometer and the second system consisted high resolution MS/MS Orbitrap mass spectrometer. The individual compounds were identified using a previously published approach via comparison of the exact molecular masses, mass spectra and retention times to those of standard compounds, online available databases and literature data. Moreover, the determination of antioxidative activities of extracts by DPPH and FRAP methods was carried out. Analysis allowed to identify 39 chemical compounds in extracts from both species. Extract from root of SP differs from SM in the presence of several metabolites such as: przewalskinic acid and their derivatives, przewaquinone C, przewaquinonate A, glycosides of rosmarinic acid, methyltanshinonate, whereas tanshinones, salvianolic acids and lithospermic acids occurred in both species. Moreover, it was shown that hydroalcoholic extract from roots of SM exerted stronger antioxidant properties in a FRAP test (max. 323.92 μM Fe^{2+}/L) and in DPPH test (max. 78.64 nM TE) in comparison with SP extract.

1. Introduction

Salvia miltiorrhiza Bunge (SM) and *S. przewalskii* Maxim. (SP) belong to the *Lamiaceae* family. These plants grown on all continents, from America to Asia (Li et al., 2010). *S. miltiorrhiza* is considered as a one of the most important plant in traditional Chinese medicine (Xu et al., 2007) and is mentioned not only in the Chinese Pharmacopoeia (2005) but also in European Pharmacopoeia (2013). Roots and rhizome of SM include two groups of bioactive compounds. The first group consists of numerous

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diterpenes such as tanshinone I, tanshinone IIA, tanshinone IIB, cryptotanshinone, 15, 16-dihydrotanshinone, isotanshinone and salvinorin A. The other group comprises polyphenols in which i.e. rosmarinic acid, caffeic acid, salvianolic acid, litospermic and ursolic acid are included (Zhong et al., 2009). SM is widely used for treating cardio-cerebrovascular diseases, exerts multiple neuroprotective effects and recently its attractiveness increased in the area of the Alzheimer's disease treatment potential due to its anti-amyloid-β, antioxidant, anti-apoptotic or anti-inflammatory properties and acetylcholinesterase inhibition (Zhang et al., 2016).

The second species is insufficiently investigated so far. According to Jiang et al. (2013) SP is used to treat chronic renal failure, liver cirrhosis and coronary heart disease. This endemic plant is called as a big Danshen or Gansu Danshen in Chinese medicine, it can be found in western China (Li et al., 2010). Its content of lipophilic and hydrophilic compounds makes it significantly different from SM (Zhong et al., 2009).

Knowledge of the chemical composition of the SP extract can provide information about the similarity related to SM. Such comparative study may explain the use of raw materials interchangeably in phytotherapy. To the best of our knowledge, there is not any data on the complete phytochemical profile of the extracts from roots of SM and SP growing in the same conditions. Moreover, in our point of view very interesting seems to be the chemical composition of extracts from these plants growing in East-Central European term conditions and the relationship between the presence of active compounds in the extracts and their antioxidative activities (*in vitro*).

The usage of high throughput and sensitive methods, such as combination of mass spectrometry and advanced liquid chromatography is the most selective technique for the rapid qualitative determination of known compounds as well as the identification of unknown compounds from extracts of natural products (Piasecka et al., 2015). Excellent complementation and confirmation of data obtained from the ion trap analyzer is measurement of accurate masses of analyzed ions in a high resolution mass spectrometer, which entails the ability to generate chemical formula of particular ions (Piasecka et al., 2015).

The aim of this study was to carry out the comparative phytochemical analyses of hydroalcoholic (50% EtOH) extracts from roots of SM and SP by using two complementary LC–MS systems. The first system was based on an ion trap mass analyzer and the second system was a tandem high resolution quadrupole – Orbitrap mass spectrometer. Furthermore, the antiradical scavenger activity of two extracts by using DPPH and FRAP tests was also assessed.

2. Materials and methods

2.1. Plant material

Raw plant material (root of *Salvia miltiorrhiza* Bunge and *S. przewalskii* Maxim., *Lamiaceae*) was obtained from field crops of the Institute of Natural Fibres and Medicinal Plants in Poznań (Plewiska), Poland.

The plant species were identified by Assoc. Prof. Waldemar Buchwald, and voucher specimens were deposited in the Herbarium of the Department of Botany, Breeding and Agricultural Technology of Medicinal Plants of the Institute of Natural Fibres & Medicinal Plants, under numbers 1903/2011 (*Salvia miltiorrhiza* Bunge) and 1904/2011 (*Salvia przewalskii* Maxim.). The field cultivation was established in 2009 in two repetitions. The rhizomes with roots were harvested in the end of October of 2011 after the second year of plant growth and dried at room temperature (23–25 °C, 50–55% relative humidity). Dry raw materials were stored for 6 months at room temperature in paper bags.

2.2. Chemicals and reagents

All reagents for phytochemical (HPLC, UPLC: acetonitrile, ethanol, formic acid, methanol, trifluoroacetic acid) (determination of total hydroxycinnamic acid derivatives: sodium molybdate, sodium nitrite, sodium hydroxide) and antioxidant capacity (acetic acid, DPPH, ferric chloride, ferrous sulfate, hydrochloric acid, sodium acetate, TPTZ, Trolox) analyses were purchased from Sigma–Aldrich (Germany), Merck (Germany) and POCh (Poland). Standards of caffeic acid and protocatechuic acid were purchased from Sigma–Aldrich (Germany), tanshinones from ChromaDex (USA). Ultrapure water for LC–MS was obtained from a Millipore Direct Q3 device.

2.3. Preparation of the extracts

The dry roots were extracted with using 50% (v/v) ethanol by percolation (24 h) method at a 1:10 ratio of plant material to solvent at room temperature (22 ± 1 °C) and filtrated. After concentration of solution to 1/3 vol under reduced pressure, the extract was frozen and lyophilized at −55 °C. Extraction yields were 41.0% (DER 2.44:1) for S. *miltiorrhiza*, and 40.3% (DER 2.48:1) for *S. przewalski*.

For HPLC-DAD-MS^n analysis lyophilized material was diluted in 2 mL of 50% (v/v) ethanol and then filtered through 0.45 μm syringe filters GHP Acrodisc 13 (Waters, Milford, USA). Prior to the UPLC–MS/MS analysis, 200 μL of the obtained extracts were diluted with 50% (v/v) ethanol (800 μL).

2.4. Determination of total hydroxycinnamic acid derivatives

Determination of total hydroxycinnamic acid (HCA) derivatives calculated on rosmarinic acid was performed according to the procedure in European Pharmacopoeia 6.0 (2007).

To 0.1 g of extracts was added 190 mL of ethanol (50%, V/V). It was boiled in a water-bath under a reflux condenser for 30 min. The solution was filtered with ethanol (50%, V/V) after cooling. Next, the filtrate and the rinsings were combined in a volumetric flask and it was diluted to 200.0 mL with ethanol (50%, V/V). To this stock solution (1 mL) in a test-tube was added 2 mL of 0.5 M hydrochloric acid, 2 mL of a solution (prepared by dissolving 10 g of sodium nitrate and 10 g of sodium molybdate in 100 mL of water) and 2 mL of dilute sodium hydroxide solution, and then this solution was diluted 10.0 mL with water and it was mixed (test solution). In another test-tube place, to 1 mL stock solution was added 2 mL of 0.5 M hydrochloric acid and 2 mL of dilute sodium hydroxide solution, next, it was diluted to 10.0 mL with water (compensation liquid). The absorbance of the test solution at 505 nm was measured by comparison with the compensation liquid. The percentage content of total hydroxycinnamic derivatives, expressed as rosmarinic acid, was calculated using the following expression:

$$[\%] = A \times 5/m,$$

where, A – absorbance at 505 nm; m – mass of the extracts, in grams.

2.5. HPLC-DAD quantitative analysis

2.5.1. Sample preparation

Approximately 100.0 mg of the dry extract was weighted in test-tube and 5.0 mL of methanol was added. Sample was

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