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Phytochemical variations of *Rhodiola rosea* L. wild-grown in Bulgaria

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ABSTRACT

Rhodiola rosea L. is a plant species with highly recognized adaptogenic properties and hence with an intensive application in traditional medicine, as well as, in clinical practice. The plant is characterized by a high phytochemical variability, depending on the geographical location among others. In this study, we report on the application of NMR-based metabolomics (1D and 2D) combined with multivariate data analysis and an HPLC method development for quantitative determination of the metabolic differences in *R. rosea* rhizomes, roots and aerial parts from its natural habitat in Bulgaria.

In the rhizomes the content of salidroside, rosarin, rosavin and rosin was 2.67%, 0.37%, 1.97% and 0.04%, respectively, while their respective amounts in the roots were 0.31%, 0.06%, 0.39% and 0.01%.

1. Introduction

Rhodiola rosea L., family Crassulaceae (synonyms: *Sedum rhodiola* – DC., *Sedum rosea* (L.) Scop., Golden Root, Arctic Root, Roseroot) is amongst the most popular plants, acting as adaptogens. In excess of 140 compounds have been identified in *R. rosea* so far (Panossian et al., 2010), with the most important from a pharmaceutical standpoint – being the phenylethanoids salidroside and *p*-tyrosol, as well as, the phenylpropanoids rosin, rosavin and rosarin (Marchev et al., 2016). They are known to be the biologically active molecules in the developed commercial products based on *R. rosea*. Characteristic feature and marker compound for the species, used as a diagnostic sign is the relative high content of rosavin (Panossian et al., 2010).

Nuclear magnetic resonance (NMR)-based metabolomics gives the opportunity for simultaneous detection of diverse groups of primary and secondary metabolites in complex natural extracts (Georgiev et al., 2015). So far this analytical technique has been applied for identification of the marker constituents in *R. rosea*, detection of novel compounds (Ioset et al., 2011; Ma et al., 2014), investigation of the metabolite variations between different plant organs and plant species according to the geographical location, plant age and sex, harvest time, as well as, some production parameters (Ioset et al., 2011; Peschel et al., 2016) and quality assessment of raw materials and commercial products (Booker et al., 2016).

In Bulgaria, the natural habitats of *R. rosea* are found in rivulet valleys in the alpine and subalpine zone of Rila, Pirin and Stara Planina Mountains between 2 000 and 2 600 m a.s.l. and is introduced for cultivation in the Rhodopes Mountains in the experimental garden Beglica at 1 525 m a.s.l. (Platikanov and Evstatieva, 2008). The plant is critically endangered an included in the Red Data Book of the Republic of Bulgaria (Meshinev, 2015).

The aim of our study was to investigate the metabolic differentiations from particular morphological plant parts (rhizomes, roots and aerial parts) from *R. rosea* collected from its natural habitat in Bulgaria. For the aim of the study we used NMR-based metabolomics (1D and 2D) combined with multivariate data analysis and an HPLC method for quantification of the major metabolites identified. To the best of our knowledge this is the first systematic phytochemical study of *R. rosea* from a wild population in Bulgaria.

2. Results and discussion

The plant material was collected in October 2014 at the end of the vegetation period when the highest amounts of salidroside and total rosavins are expected according to Platikanov and Evstatieva (2008). For the chemical experiments, the sample consisted of 10 male plants, pooled together. Male rhizomes are supposed to accumulate higher amounts of salidroside and rosavins than the

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female ones (Platikanov and Evstatieva, 2008). All individuals were of approximately the same size, *i.e.* 20–25 cm in length of the aerial part. According to Węglarz et al. (2008) rhizomes have a cylindrical shape, 2–10 cm in diameter with sparse roots. In the current case the underground parts with diameter ≥2 cm were recognized as rhizomes, while the parts with diameter <2 cm as roots.

The obtained results revealed qualitative and quantitative variations among the different plant parts of *R. rosea* (Fig. 1). The chemical structures of salidroside and rosavins were identified according to their NMR spectra and compared to published spectral data (Table 1). Although the signals of the aromatic region (δ 5.5–9.0 ppm) were dominated by the major markers salidroside and rosavins, the presence of other aromatic constituents were detectable as well. The ^{1}H NMR spectra assigned the characteristic signals of *p*-tyrosol and salidroside in the aromatic region at δ 7.16 (d, *J* = 8.6 Hz, H-2/6) and 6.8 (d, *J* = 8.6 Hz, H-3/5), indicating a *para*-substituted phenyl ring and triplet was observed at δ 2.87 (t, *J* = 7.4 Hz, H-7; Mudge et al., 2013; Varejão and Demuner 2013; Vasileva et al., 2016). The structure of rosavins was identified by the signals of the symmetric carbons in the aromatic ring: ^{1}H NMR: δ 7.47 (d, *J* = 7.7 Hz, H-2/6), δ 7.36 (t, *J* = 7.4 Hz, H-3/5), δ 7.22 (t, *J* = 7.5 Hz, H-4), δ 6.72 (d, *J* = 16.0 Hz, H-7), δ 6.37 (dt, *J* = 16.0; 6.0 Hz, H-8; Mudge et al., 2013). The presence of salidroside and rosavins was recognized in the *rhizome* and *radix*, while their typical signals were not identified in the aerial parts of *R. rosea* or were below the threshold of detection. Along with that we were able to assign the specific signals of herbacetin in all of the analyzed plant parts: δ 8.19 (d, *J* = 9.0 Hz, H-2/6), δ 6.9 (d, *J* = 9.0 Hz, H-3/5) and δ 6.29 (s, H-6) (Ma et al., 2014). Herbacetin is unusual flavonoid with highly potential anti-inflammatory and anticancer activity (Kim et al., 2016), identified so far in the rhizomes of *R. rosea* from Finland (Petsalo et al., 2006), Korea and China (Jeong et al., 2009; Ma et al., 2014).

The carbohydrate region (δ 3.0–5.5 ppm) was highly clustered due to the presence of many glycosidic constituents. Nevertheless, in this region α-, β- glucose and sucrose were identified. Signals from the aliphatic (δ 0.5–3.0 ppm) and aromatic regions corresponded to the amino acids alanine, glutamine, leucine, threonine and valine; phenolic acids such as gallic acid and some organic acids, *e.g.* acetic, succinic, formic, fumaric and malic acid (Table 1).

In order to highlight the differences or similarities in the investigated samples the ^{1}H NMR data was subjected to a principal component analysis (PCA; Fig. 2). In the PCA model based on the total spectral region, the first two principal components (PC1 and PC2) explained 72.3% of the total variance in the data, clearly clustering and dividing the *R. rosea* samples into three groups (Fig. 2A). The two-component PCA model for the PC2 revealed that *R. rosea* rhizomes accumulate predominantly phenylethanoids and phenylpropanoids (δ 5.5–8.0 ppm), amino acids and organic acids, while most of the carbohydrate signals were shifted towards the roots, where the presence of other non-identified aromatic compounds (δ 8.0–9.8 ppm) was detected as well (Fig. 2B). Rhizomes, compared to aerial parts also showed that the signals of salidroside and rosavins were again assigned in the rhizomes, while aerial parts accumulate mostly amino acids, some organic acids and also have the signals of other aromatic compounds in the region of δ 7.58–8.7 ppm (Fig. 2C). According to the loading column plot for the PC1 salidroside and rosavins, as well as, amino acids

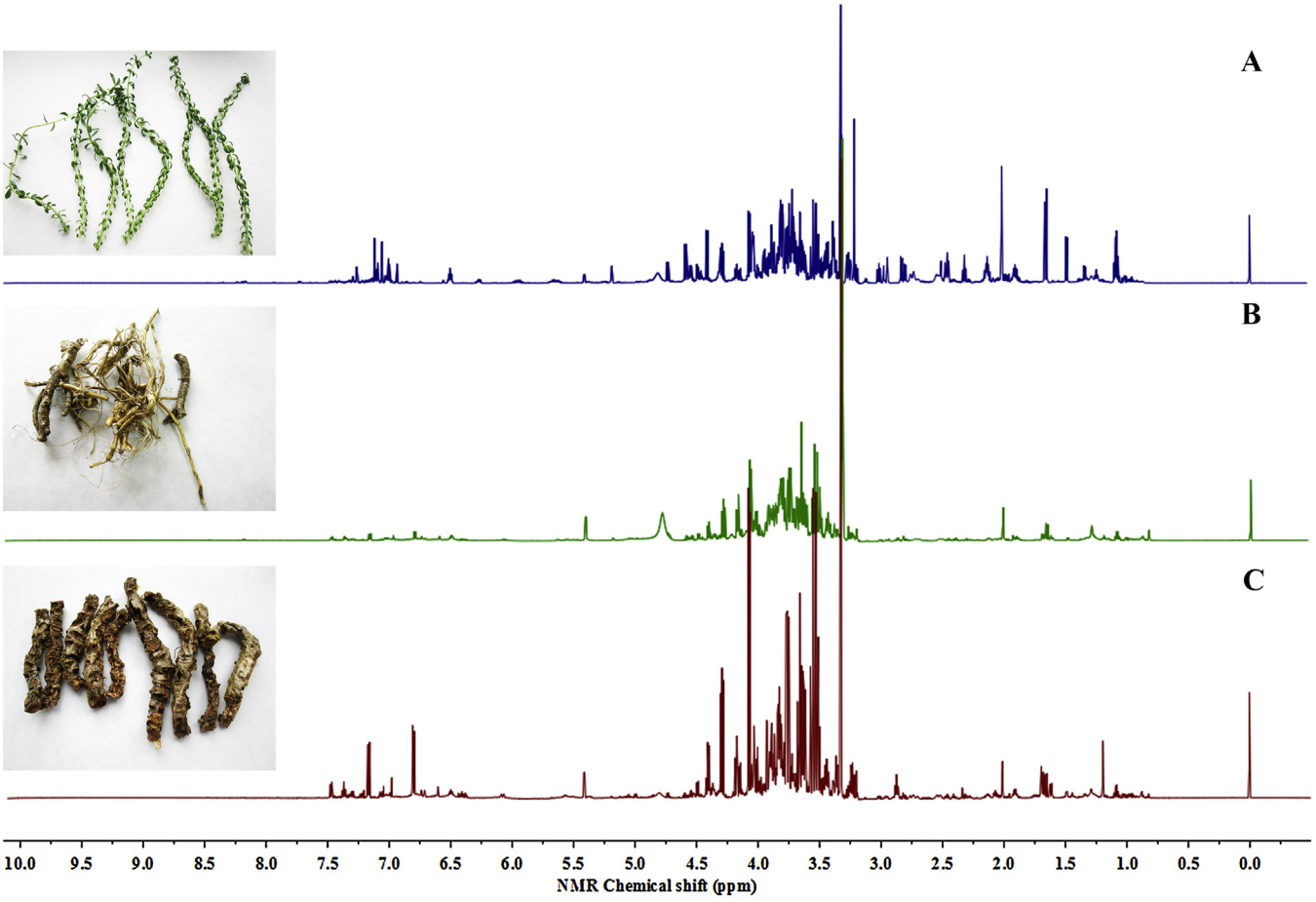

Fig. 1. 600 MHz ^{1}H NMR spectra of *R. rosea* extracts from: A – aerial parts; B – roots; C – rhizomes.

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