

# Determination of phenolic compounds in methanolic extracts of flowering stems and rosette leaves of *Sideritis raeseri*

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## Introduction

Aerial parts of *Sideritis raeseri* are widely utilized in Mediterranean folk medicine. Their use as a remedy is based on anti-inflammatory and antioxidant properties, antimicrobial effects, and possible immune-stimulant activity (Karapandzova et al., 2013). Although numerous data have been published about the chemical composition of phenolic compounds from various extracts in aerial parts of *S. raeseri* (Petreska et al., 2011, Karapandzova et al., 2013), there are no data about the phenolic compounds in the rosette leaves. Traditionally, rosette leaves of *S. raeseri* are not used (Qazimi et al., 2014).

The aim of this work was determination of the phenolic compounds in the methanolic extracts of spontaneous flowering stems and rosette leaves of *S. raeseri* using LC/DAD/ESI-MS<sup>n</sup>

## Materials and methods

*Plant material:* The flowering stems (S-f) and rosette leaves (S-r) of *Sideritis raeseri* were collected in 3 different localities from National Park

Galichica in R. N. Macedonia (Kazani, Krstec and Vojtino). The plant material was air dried, packed in paper bags and kept in a dark and cold place until analysis.

*Extraction of phenolic compounds:* 0.2 g of powder plant material of flowering stems (homogenized samples from flower, leaf and stem) (S-f) and rosette leaves (S-r) were extracted with 25 ml of 70% methanol, 30 min, using US bath. The supernatant was filtered through 0.45 µm pore-size polyethersulfone filter before analysis.

*LC/DAD/ESI-MS<sup>n</sup> analysis:* Chromatographic separations were carried out on 250 mm x 4.6 mm, 5 µm C18 Luna column (Phenomenix). The mobile phase consisted of two solvents: water - formic acid (1 % v/v) (A) and methanol (B). A linear gradient starting with 25% B was installed to reach 30% B at 7 min, 45% B at 30 min, 50% B at 50 min and 100 % B at from 55 to 60 min. The HPLC system was equipped with an Agilent 1100 series diode array and mass detector in series (Agilent Technologies, Waldbronn, Germany). It consisted of a G1312A binary pump, a G1329A autosampler, a G1379B degasser and G1315D photo-diode array detector,

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controlled by a ChemStation software (Agilent, v.08.03)

Spectral data from all peaks were accumulated in range 190-600 nm and chromatograms were recorded at 290 and 300 nm for glycosides and acylated derivatives and at 330 nm for phenylethanoid glycosides and hydroxycinnamic acid. The mass detector was a G2449A Ion-Trap Mass Spectrometer equipped with an electrospray ionisation (ESI) system and controlled by LCMSD software (Agilent, v.6.1.). Nitrogen was used as nebulising gas at pressure of 65 psi and the flow was adjusted to 12 L min<sup>-1</sup>. Maximum accumulation time of ion trap and the number of MS repetitions to obtain the MS average spectra were set at 300 ms and 5, respectively.

The identification and peak assignment of all phenolic compounds was based on comparison of their retention times and mass spectral data with those of standards and published data.

## Results and discussion

Phenolic compounds in the *Sideritis* extracts were identified by their UV spectra, their deprotonated molecular ions and their corresponding ion fragments, by using LC/DAD/ESI-MS<sup>n</sup>. Total of 28-29 and 26-28 components were identified in the methanolic extracts of spontaneous flowering stems (S-f) and rosette leaves (S-r) of *S. raeseri*, representing 78,98-104,76 mg/g DW and 131,64-157,20 mg/g DW of the total content, respectively. Phenolic compounds in S-f and S-r were classified into four groups: hydroxycinnamic acids derivatives (1-3 and 1-3), phenylethanoid glycosides (PHEG) (8-9 and 8-9), flavonoid 7-O-diglucosides (5 and 5) and flavonoid acetylglucosides aglycones (12-13 and 11-12), respectively.

The total amount of hydroxycinnamic acid derivatives in S-f and S-r extracts ranged from 1,89-3,13 mg/g and 1,56-2,34 mg/g, respectively. 5-caffeoylquinic acid was found in all samples and it was dominant hydroxycinnamic acid. PHEG were the abundant group of polyphenols in the studied samples with the content ranging from 43,84-62,55 mg/g in S-f and 70,29-106,40 mg/g in S-r. The most abundant compounds of PHEG in S-f and S-r samples

were: verbascoside (17,11-22,27 mg/g and 27,93-36,16 mg/g), lavandulfolioside (9,63-18,70 mg/g and 13,68-40,73 mg/g) and allysonoside (3,85-6,97 mg/g and 5,46-17,93 mg/g), respectively, and represent around 90% of total PHEG content. Total content of flavonoid glycosides (non acetylated and acetylated) in S-f and S-r ranged from 32,69-40,95 mg/g and 49,25-60,15 mg/g, respectively. The major components in S-f and S-r samples were isoscutellarein 7-O-[6'''-O-acetyl]-allosyl(1→2) glucoside (4,53-5,30 mg/g and 5,97-8,14 mg/g), 3'-O-methylhypolaetin 7-O-[6'''-O-acetyl]-allosyl(1→2)glucoside (8,91-11,89 mg/g and 12,61-16,49 mg/g) and 4'-O-methylhypolaetin 7-O-[6'''-O-acetyl]-allosyl-(1→2)-[6''-O-acetyl]-glucoside (5,73-6,58 and 6,82-11,83 mg/g), respectively.

## Conclusion

The differences in total phenolic content between S-f and S-r samples are directly correlated with differences in PHEG content. Rosette leaves exhibit very similar phenolic compounds profile with the stems, and accordingly can be recommended for utilization as an additional plant material source of this endemic aromatic plant. For complete assessment additional phytochemical analysis are required.

## References

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