The genus *Sideritis* numbers more than 150 species occurring mainly in the Mediterranean area (1). Two species occur in Macedonia (*S. scardica* Griseb. and *S. raeseri*). *S. scardica* is an endemic of the Balkan Peninsula, distributed in Macedonia, Bulgaria, Southwest Albania and Greece. Heywood in Flora Europaea (2) says that *S. raeseri* and *S. taurica* are synonyms of *S. syriaca* L., while Baden in Mountain flora of Greece (3) describes *S. raeseri* separately from *S. syriaca*. The populations of *S. raeseri* in Macedonia are located mainly on the Galičica mountain, whereas *S. scardica* is more widespread in the mountains of central and western Macedonia.

**Acta Pharm. 57 (2007) 371–377 Short communication**

**Assay of flavonoid aglycones from the species of genus *Sideritis (Lamiaceae)* from Macedonia with HPLC-UV DAD**

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Flavonoids obtained from *Sideritis* species (*Lamiaceae*), *S. raeseri* and *S. scardica*, grown in Macedonia were studied. Qualitative and quantitative analyses of the flavonoid aglycones were performed using high-performance liquid chromatography (HPLC) with a UV diode array detector. Extracts were prepared by acid hydrolysis in acetone, re-extraction in ethyl acetate and evaporation to dryness; the residue dissolved in methanol was subjected to HPLC analysis.

Isoscutellarein, chryseriol and apigenin were identified in the extracts. Also, a 4′-methyl ether derivative of isoscutellarein was found, together with hypolaetin and its methyl ether derivative, which were identified according to previously isolated glycosides and literature data. Quantitation was performed using calibration with apigenin.

According to this screening analysis, the samples of the genus *Sideritis* from Macedonia are rich in polyhydroxy flavones and analogous with the previously studied Mediterranean *Sideritis* species from the Ibero-North African and Greek *Sideritis* species with respect to the presence of 8-OH flavones and their derivatives.

**Keywords**: flavonoids, polyhydroxy flavones, *Sideritis*, HPLC-UV DAD

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Over the years, the phytochemistry of the genus *Sideritis* (*Lamiaceae*) has been studied and various terpenoids, sterols, coumarins, and especially flavonoid aglycones and glycosides have been identified. The flavonoids from *Sideritis* species grown in the Mediterranean (4–6) and Atlantic regions (7) and North Africa (8) have been extensively studied. The species from the Balkan Peninsula, including several interesting endemic species, have also been studied and found to be rich in phenolic compounds, especially flavonoids, which have been proved to possess a valuable antioxidant activity (9, 10). This fact is especially important considering the pharmacological interest and the traditional use of »mountain tea« in folk medicine for its anti-inflammatory and anti-rheumatic properties.

However, data on the chemical composition of the *Sideritis* species growing in Macedonia are very poor. Hence, identification of the flavonoids in wild growing populations of *S. scardica* and *S. raeseri* in this central Balkan region was the aim of our investigation.

**EXPERIMENTAL**

*Plant material.* – Aerial parts of *Sideritis raeseri* L. (Galičica, Macedonia) and *Sideritis scardica* L. (Stogovo, Solunska Glava and Karadžica, Macedonia) were collected at flowering in the summer of 2005.

*Reagents and authentic samples.* – Reagents of HPLC purity were purchased from Sigma Chemical Co. (Germany). Authentic substances: apigenin and chryseriol were the products of Extrasynthese (France), isoscutellarein was kindly donated by Dr. Milena Nikolova of the Institute of Botany, Bulgarian Academy of Sciences, Sofia, Bulgaria. Authentic samples of glycosides of hypolaetin, 3’-OCH₃ hypolaetin and isoscutellarein were previously isolated from *Sideritis* species and identified in our laboratory using NMR (¹H and ¹³C) (11, 12).

*Preparation of plant extracts.* – Ground plant material (0.5 g) was extracted twice with 15 mL acetone and 1 mL of concentrated HCl. Extraction was performed in an Erlenmeyer flask with reflux in a water bath for 40 min at 60 °C. The extract was then cooled, filtered and transferred to a separating funnel. Water (50 mL) was added and extraction with ethyl acetate was repeated 3 times with 20 mL each. The ethyl acetate fractions were collected and washed three times with 50 mL of water each, then dried with anhydrous Na₂SO₄, filtered, and evaporated to dryness under low pressure. The residue was dissolved in 1 mL methanol and the solution was used for analyses of flavonoid aglycones by HPLC.

*HPLC analysis.* – Flavonoid aglycones in the extracts were identified by the HPLC method, using a Varian HPLC system equipped with a ternary pump Model 9012 and UV diode-array detector Model 9065. A reverse phase column C18 (250 x 4.6 mm, 5 μm particles) was used. The mobile phase consisted of H₂O with pH adjusted to 3 with H₃PO₄ (A) and CH₃CN (B), and the elution program for extracts screening was the following: 0–5 min 70% A; 10–20 min 65% A; 25–30 min 55% A; 40–48 min 35% A. The flow rate was 1 mL min⁻¹, the temperature was set to 35 °C and the injection volume was 20 μL.
The elution was monitored in the whole UV range and the chromatograms for flavones screening were best seen at 348 nm, which is in the region where flavones exhibit an absorption maximum. Identification was made according to the retention times and UV spectra of the components compared to those of authentic samples of flavonoids.

Quantification of flavones was performed using calibration with standard solutions of apigenin in the range from 0.1 to 1.0 mg mL\(^{-1}\) at 348 nm (dependence of the peak area vs. concentration of apigenin).

RESULTS AND DISCUSSION

An HPLC method has been developed for analysis of flavonoids after acid hydrolysis, using a UV diode array detector. Flavone aglycones were identified using authentic substances, UV-spectra and results from our previous research and published data (11–13). The results were compared with the ones published for Sideritis flavonoids in the Balkans and the Mediterranean region. Four samples of wild growing populations (one of S. raeseri and three of S. scardica) were studied. Extraction with prior hydrolysis of the glycosides is often used as the first step in studying the profile flavonoid because it enables identification of the flavones present without the need to study the various glycoside forms, which are not readily available as authentic samples.

The chromatograms obtained for the analyzed samples are given in Fig. 1 and the peaks of the identified flavones are numbered from 1 to 6. The structures of the identified flavones are given in Fig. 2. Identification of the flavones present was performed using authentic samples of isoscutellarein-2, apigenin-4, and chryseriol-6. The 4'-methyl ether of isoscutellarein-5 was identified according to its UV-spectrum and our previous studies of quantitative structure-retention relationships (14), which allow the conclusion that the peak at 19.40 min with a UV-spectrum almost identical to the one of isoscutellarein is due to the presence of its 4'-methyl ether derivative.

Similarly, two peaks at around 5.40 and 9.80 min with almost identical UV-spectra have been identified as due to the presence of hypolaetin-1 and its methyl ether derivative-3. The position of methylation is probably 3', which is supported by the data from the isolation and spectroscopic identification of the corresponding glycosides of these flavones (in progress), and also by the literature data (10–13).

The screening results of these extracts relating to the quantity of the aglycones identified are presented in Table 1. From the results shown in Table I, the differences between the two analyzed Sideritis species can be seen with respect to their flavones composition. Hypolaetin and 4'-methyl ether of isoscutellarein were identified only in the S. raeseri extracts, whereas chryseriol was found in all samples of S. scardica from different locations. It can also be seen that the total flavonoids content was higher in S. raeseri than in the samples of S. scardica.

Extensive chemosystematic studies of the genus Sideritis species using their flavonoid patterns have been performed by Barberan and co-workers (4, 5, 8). They analyzed the Spanish and north African Sideritis species and found the 8-OH flavones hypolaetin and isoscutellarein to be characteristic and most abundant in the section Sideritis, whereas 5,7-OH flavones were found only in a few studied specimens of Ibero-North African Sideritis. Barberan et al. (8) have broadly divided the species of the genus Sideritis into...
two groups based on accumulation of the glycosides of 8-OH flavones and 5,7-OH flavones. In the first group, the 8-OH flavones isoscutellarein and/or hypolaetin are dominant whereas in the section *Hesiodia* the 5,7-OH flavones chryseriol, luteolin and apigenin are
most abundant. In the studied Macedonian Sideritis samples, the 8-OH flavones are the main flavone compounds in the extracts, but significant amounts of 5,7-OH flavones, notably apigenin, are present as well, which can broadly put them into the first group.

As regards the Balkan Sideritis representatives, the phytochemical studies of Sideritis samples from Bulgaria have revealed the presence of glycosides of isoscutellarein and hypolaetin-4′-methyl ether (9). Studies of Greek S. raeseri (10) have also revealed the presence of 7-O-glycosides of hypolaetin and its 3′ and 4′-methyl ether as well as isoscutellarein and its 4′-methyl ether and their potential antioxidant activity. In our study, the presence of five flavonoid aglycones in S. raeseri has been confirmed. Four of them belong to the group of 8-OH flavones (hypolaetin and isoscutellarein and their methyl ether derivatives).

Comparing the flavone composition of the two studied species of S. scardica and S. raeseri reveals greater variety of the 8-OH flavones in S. raeseri (hypolaetin; 3′-methyl ether of hypolaetin; isoscutellarein and 4′-methyl ether of isoscutellarein), whereas only isoscutellarein and 3′-methyl ether of hypolaetin were found in S. scardica. Additionally, chryseriol was found only in the S. scardica extracts, indicating the possibility of their chemosystematic distinction. These results imply further investigation directed towards testing the possibility of chemosystematic distinguishing between these two Sideritis species based on the presence of 5,7-OH flavones (chryseriol) in Sideritis scardica and 8-OH flavones (hypolaetin, 4′-methyl ether of isoscutellarein) in Sideritis raeseri. These analyses can help in solving the dilemma if S. raeseri is a synonym of S. syriaca or not, which is still under discussion (2, 3).

There are not much data on the quantification of the flavonoid compounds in Sideritis species. Palomino et al. (6) have analyzed the flavonoids content in Spanish Sideritis species using HPLC. They have identified and quantified glycosides of isoscutellarein and hypolaetin, and luteolin, chryseriol and apigenin as aglycons, along with the less polar flavones sideritoflavone, xantomicrol, gardenin D, 8-methoxy cirsilineol and desmethylnobiletine.

This study is the first report on the flavonoids content of the Balkan Sideritis species, which shows their valuable chemical composition and justifies their popular use in the traditional medicine. The species S. raeseri has been shown to be richer in variety as well as in quantity of these flavones, ranging from 0.07 to 0.26% (m/m) in dry plant material. The relatively high total content of flavones explains the strong antioxidant activity determined for Sideritis extracts (9, 10).

Table I. Content of flavones in the studied Macedonian Sideritis species

<table>
<thead>
<tr>
<th>Plant material (location)</th>
<th>Hypolaetin</th>
<th>Isoscutellarein</th>
<th>3'-OCH₃ hypolaetin</th>
<th>Apigenin 4'-OCH₃ isoscutellarein</th>
<th>Chryseriol</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. raeseri (Galičica)</td>
<td>0.07</td>
<td>0.12</td>
<td>0.26</td>
<td>0.08</td>
<td>0.07</td>
</tr>
<tr>
<td>S. scardica (Stogovo)</td>
<td>–</td>
<td>0.16</td>
<td>0.16</td>
<td>0.12</td>
<td>–</td>
</tr>
<tr>
<td>S. scardica (Solunska Glava)</td>
<td>–</td>
<td>0.19</td>
<td>0.20</td>
<td>0.10</td>
<td>–</td>
</tr>
<tr>
<td>S. scardica (Karadžica)</td>
<td>–</td>
<td>0.08</td>
<td>0.07</td>
<td>0.05</td>
<td>traces</td>
</tr>
</tbody>
</table>
CONCLUSIONS

The presence of two types of flavones 8-OH (hypolaetin and isoscutellarein and their methoxy derivatives) and 5,7-OH (apigenin and chryseriol) has been confirmed and the possibility of distinguishing between the two studied species (S. scardica and S. raeseri) is suggested. This hypothesis should be further explored and tested with the Sideritis species from the Balkans in order to establish their chemosystematic distinction. Moreover, the relatively high content of individual polyhydroxy flavones determined in the extracts (up to 0.26%) justifies the need for further investigations in order to correlate their biological activities with their chemical composition.

REFERENCES

Određivanje aglikona flavonoida iz vrsta roda *Sideritis* (*Lamiaceae*)
iz Makedonije pomoću HPLC-UV DAD

**BISERA JANESKA, MARINA STEFOVA i KALINA ALIPIEVA**

U radu su proučavani flavonoidi dobiveni iz vrsta *Sideritis* (*Lamiaceae*), *S. raeseri* i *S. scardica*, podrijetlom iz Makedonije. Kvalitativna i kvantitativna analiza aglikona flavonoida provedena je pomoću tekućinske kromatografije visoke učinkovitosti (HPLC) s UV detektorom. Ekstrakti su pripravljeni kiselom hidrolizom u acetonu, te ponovnom ekstrakcijom etil-acetatom. Ostatak nakon uparavanja je otopljen u metanolu i analiziran pomoću HPLC.

Usporedbom s ranije izoliranim glikozidima i s literaturnim podacima u ekstraktima su identificirani izoskutelarein, krizeriol, apigenin, 4’-metil eterski derivat izoskutelareina, hipolaetin te njegov metil eter. Kvantifikacija je provedena pomoću kalibracijske krivulje za apigenin.

Rezultati ukazuju da su uzorci *Sideritis* roda iz Makedonije bogati polihidroksiflavonima kao i ranije proučavane mediteranske vrste *Sideritis* iz sjeverne Afrike i iz Grčke.

**Ključne riječi:** flavonoidi, polihidroksiflavoni, *Sideritis*, HPLC-UV DAD

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