INTRODUCTION

The only representative of the genus Telekia in the flora of Bosnia and Herzegovina (BiH) is *Telekia speciosa* (Schreb.) Baumg. It inhabits wet and shady positions in mountain woodlands. It is a perennial herbaceous plant with alternating, wide, whole leaves and large heterogeneous heads which can be individual or in cluster blooms. It is widespread in Eastern and Central Europe and the Balkan Peninsula [1]. The root of *T. speciosa* is traditionally used as a remedy for bronchial asthma in Balkan countries. In BiH, root smoke of this plant is used in inhalations for bronchial asthma [2].

The root of *T. speciosa* contains essential oil, bitter compounds and inulin [3]. Phytochemical investigations have revealed *T. speciosa* as a rich source of sesquiterpene lactones, especially in its underground parts [4]. Isoalantolactone is almost exclusively contained in the essential oil of *T. speciosa* roots. It is a sesquiterpene lactone – an eudesmanolide with an antiproliferative and anti-inflammatory activity [5]. Sesquiterpene lactones have been found to be active bactericidal principles, as well [6].

The aerial part extracts have been found to contain fatty acids, namely palmitic, linoleic, oleic and capric acids [7]. Reports concerning the sterols of *T. speciosa* extracts can also be found in the literature [8]. Pseudoguaianolide - 2,3-dihydroaromaticin and three thymol derivatives have been isolated as major secondary metabolites from the aerial parts of methanol extract of *T. speciosa*.

Phenol acids derivatives in earlier investigations were isolated from an extract of *T. speciosa* flowers. Those compounds were: one derivative of ferulic acid [(E)-ferulic acid 4-O-β-(6-O-2-hydroxyisovaleryl)-glucopyranoside] and five caffeic acid derivatives [(E)-caffeic acid 4-O-β-(6-O-2-hydroxyisovaleryl)-glucopyranoside, (E)-caffeic acid 4-O-β-(6-O-3-hydroxy-2-methylpropanoyl)-glucopyranoside, 6-O-(E)-caffeoyl-glucopyranose, (E)-caffeic acid 4-O-β-glucopyranoside] and 5-caffeoylquinic acid (chlorogenic acid) [9].

The aim of the present study was to identify and quantify phenol acids in the methanol extracts of *T. speciosa* aerial and underground parts and determine the antioxidant capacity of the extracts. To the best of our knowledge, the chemical composition of the extracts of underground parts as well as the antioxidant capacity of the plant extracts had never been studied before. In addition, we analyzed the volatiles of both, aerial and underground parts.

MATERIAL AND METHODS

PLANT MATERIAL

Aerial and underground parts of *T. speciosa* were collected at their specific location of Zlača, Banovići
municipality (BiH) during the flowering period in August 2015. Geographical coordinates of the location were N44°20'22.3" E18°33'39.6". The plant material was identified according to Flora Croatia by authors [10] and voucher specimen was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, the University of Tuzla. The plant material was cleaned, cut, and dried.

REA AGENTS AND CHEMICALS

All the reagents used were of analytical grade. Folin-Ciocalteu’s phenol reagent, sodium carbonate, sodium acetate anhydrous, and ferric (III) chloride were obtained from Merck (Germany). HPLC-grade acetonitrile and formic acid were purchased from Merck. Water for HPLC was prepared by Milli-Q Water Purification System. Methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), ferrous (II) sulfate heptahydrate, hydrochloric acid, glacial acetic acid, HPLC-grade chlorogenic acid, caffeic acid, ellagic acid, gallic acid, and o-coumaric acid were purchased from Sigma-Aldrich (USA). Rutin and ferulic acid were obtained from Carl Roth (Germany).

PLANT EXTRACTS

The dried plant material was crushed in a grinder until powder formation. The samples were extracted with 98% methanol on a magnetic stirrer under reflux at 50 °C for 1 hour. The mixtures were filtered through a filter paper (Whatman No. 1). The methanol was removed by evaporation. The dried extracts were stored in the fridge at 4 °C, in glass bottles for further investigations.

HPLC ANALYSES

HPLC analyses of extracts (3 mg/mL in methanol) were carried out using an Agilent 1260 Infinity (Agilent Technologies, USA) system equipped with a Agilent 1260 Infinity Quaternary Pump, Agilent 1260 Infinity Standard Autosampler, Agilent 1260 Infinity Diode Array Detector, and Agilent 1260 Infinity Thermostatted Column Compartment. The separations were performed on a Merck LiChroCAT R 250-4 C18 RP analytical column (250x4.6mm i.d., 5 µm). The mobile phase consisted of 0.1% formic acid in water (solvent A) and acetonitrile (solvent B). The following gradient was applied: 0-15 min, linear gradient from 10% to 20% B; 15-30 min, linear gradient from 20% to 30% B; 30-35 min, linear gradient from 30% to 40% B; 35-40 min, linear gradient from 40% to 90% B; 40-45 min, then returned to the initial conditions. The injection volume was 10 µL; the flow rate was 0.8 mL/min. The detection wavelength was 325 nm, and the column thermostat was set at 30 °C [11]. Component identification was performed comparing their retention times and UV spectra with those obtained from standards. The calibration curve for chlorogenic acid was obtained by the external standard method in the concentration range of 15.6-500 µg/mL (y=28.93x-220.2, R²=0.9996).

TOTAL PHENOL CONTENT AND ANTIOXIDANT CAPACITY

The total phenol content was determined by the Folin Ciocalteu spectrophotometric method [12]. The in vitro antioxidant capacity was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical assays [13] and the ferric reducing antioxidant potential (FRAP) [14].

ISOLATION AND GC-FID/MS ANALYSES OF ESSENTIAL OILS

The dried aerial and underground parts of T. speciosa were subjected to hydrodistillation for 3 h using a Cleaver-type apparatus, according to the European Pharmacopoeia. The obtained oils were separated, dried over anhydrous sodium sulfate, and stored at -20 °C until the analysis.

The volatile constituents were determined by the GC-FID/MS analyses using an Agilent 6890N GC system coupled with an Agilent 5975 MSD, FID, and equipped with a HP-5 MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm). The oven temperature was programmed linearly rising from 60 to 280 °C at 3 °C min⁻¹ and then isothermal at 280 °C for 5 min; injector 200 °C; FID 300 °C; transfer-line 250 °C; carrier gas He (1.0 mL min⁻¹, constant flow mode); injection volume 1 µL of essential oil dissolved in ethanol; split ratio 10:1. EI Mass spectra (70 eV) were acquired over the m/z range of 35-550.

The identification of the individual compounds was based on the comparison of their retention times (tR), retention indices (RIs), and mass spectra with those obtained from authentic samples and/or listed in the NIST, Wiley mass spectral libraries, and the literature [15]. For the quantification, the relative area percentages obtained by FID were used.

RESULTS AND DISCUSSION

The yields of the methanol extracts of T. speciosa were 4.31% and 11.52% for the aerial and underground parts, respectively.

The qualitative and quantitative analysis of the extracts was performed by the RP-HPLC method. The chlorogenic acid (CGA) and caffeic acid deriva-
tives (CA derivatives) were present in the extracts. In the aerial parts of *T. speciosa* except CGA, two CA derivatives (Rt - 22.464 min and Rt - 35.631 min) were detected. In the underground parts, except CGA, four CA derivatives (Rt - 22.464 min, Rt - 25.433 min, Rt - 35.627 min and Rt - 37.694 min) were also present. Caffeic acid and other reference substances used in the analyses (gallic acid, o-coumaric acid, ferulic acid, ellagic acid and rutin) were not detected.

The content of chlorogenic acid was determined in the extracts (Table 1). In the extracts from the underground parts of *T. speciosa* the CGA content was on average three and a half times higher than the CGA content in the extracts from the aerial parts of *T. speciosa*.

Table 1. Chlorogenic acid content in the methanol extracts of *T. speciosa* aerial and underground parts

<table>
<thead>
<tr>
<th>Extract</th>
<th>Chlorogenic acid (mg per 100 g extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. speciosa</em> aerial parts</td>
<td></td>
</tr>
<tr>
<td>summer Zlača</td>
<td>272.0±18</td>
</tr>
<tr>
<td><em>T. speciosa</em> underground</td>
<td></td>
</tr>
<tr>
<td>parts summer Zlača</td>
<td>977.8±85</td>
</tr>
</tbody>
</table>

*a mean ± SD (n=3)*

There were no available literature data about phenol acids in *T. speciosa* underground and aerial parts except some derivatives of phenol acids from a fraction of an extract from *T. speciosa* flowers. Research studies have shown that chlorogenic acid demonstrates a wide range of pharmacological activities including antioxidant, antiobesity, anti-edematogenic and antinociceptive activities [16], [17].

Phenol compounds contribute to the overall antioxidant capacity of plants, which is why the extracts of *T. speciosa* were analyzed for their total phenol content by the Folin Ciocalteu method. DPPH and FRAP assays were used to evaluate the antioxidant capacity of those extracts (Table 2).

Table 2. Total phenol content, IC₅₀ and FRAP values of *T. speciosa* methanol extracts

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total phenol content (mg GAE g⁻¹ extract)</th>
<th>IC₅₀ (µg mL⁻¹)²</th>
<th>FRAP µmol Fe²⁺ g⁻¹ extract²</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH</td>
<td>63.0±2.17</td>
<td>497.79±31.86</td>
<td>384.32±9.54</td>
</tr>
<tr>
<td>TSR</td>
<td>162.8±4.76</td>
<td>129.57±3.93</td>
<td>1337.48±48.03</td>
</tr>
</tbody>
</table>

*a mean ± SD (n=3)*

To the best of our knowledge, the data about the antioxidant capacity of this plant have not been found by now. The extract of the underground parts of *T. speciosa* had on average three and a half times higher antioxidant capacity in comparison with the extract of the plant aerial parts. IC₅₀ for rutin used as standard was 12.42 µg mL⁻¹. The content of chlorogenic acid indicates positive correlation existence between its content and antioxidant capacity in analysed extracts. The obtained results also confirm the well-known positive correlation between the total phenol content and antioxidant capacity.

The aerial parts of *T. speciosa* contained 0.04% (v/w) of yellow, liquid fragrant essential oil. The identified 69 constituents from the aerial parts of *T. speciosa* accounting for 87.9% of the oil are presented in Table 3. The oil of *T. speciosa* was characterized by the presence of a high concentration of oxygenated sesquiterpenes (51.8%). The major components were (E)-nerolidol (10.3%), caryophyllene oxide (8.2%), (Z,E)-farnesol (7.7%) and prenopsan-8-ol (4.9%). Sesquiterpene hydrocarbons constituted (5.8%) of the oil. Thymol derivative 10-isobutyryloxy-8,9-epoxythymyl isobutyrate (3.4%) was the major representative of oxygenated monoterpenes (12.0%). Non-terpene compounds presented an appreciable amount of essential oil (18.3%) with dominant (E)-phytol (4.9%) and hexadecanoic acid (3.3%).

Table 3. Chemical composition of essential oils from *T. speciosa* aerial and underground parts

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>RIE²</th>
<th>Aerial parts (%)</th>
<th>Undergr. parts (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>linalool</td>
<td>1101.4</td>
<td>1.4</td>
<td>0.2</td>
</tr>
<tr>
<td>2</td>
<td>nerol oxide</td>
<td>1155.3</td>
<td>0.4</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>α-terpineol</td>
<td>1192.5</td>
<td>0.3</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>δ-decanal</td>
<td>1206.2</td>
<td>0.6</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>nerol</td>
<td>1229.5</td>
<td>0.7</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>geraniol</td>
<td>1255.1</td>
<td>0.3</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>dihydrolinalool</td>
<td>1294.1</td>
<td>1.3</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>silphiperfol-5-ene</td>
<td>1326.2</td>
<td>-</td>
<td>0.3</td>
</tr>
<tr>
<td>9</td>
<td>7-epi-silphiperfol-5-ene</td>
<td>1345.0</td>
<td>-</td>
<td>0.4</td>
</tr>
<tr>
<td>10</td>
<td>decanoic acid</td>
<td>1371.9</td>
<td>-</td>
<td>0.2</td>
</tr>
<tr>
<td>11</td>
<td>silphiperfol-6-ene</td>
<td>1377.3</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>12</td>
<td>modheph-2-ene</td>
<td>1381.0</td>
<td>-</td>
<td>0.2</td>
</tr>
<tr>
<td>13</td>
<td>α-isocoumarone</td>
<td>1387.8</td>
<td>-</td>
<td>0.2</td>
</tr>
<tr>
<td>14</td>
<td>β-elemene</td>
<td>1394.0</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>15</td>
<td>β-ionol</td>
<td>1406.2</td>
<td>0.7</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>β-isocoumarone</td>
<td>1407.2</td>
<td>-</td>
<td>0.2</td>
</tr>
<tr>
<td>17</td>
<td>(E)-β-caryophyllene</td>
<td>1421.4</td>
<td>1.3</td>
<td>0.5</td>
</tr>
<tr>
<td>18</td>
<td>α-β-santalene</td>
<td>1448.2</td>
<td>-</td>
<td>0.2</td>
</tr>
<tr>
<td>19</td>
<td>geranyl acetone</td>
<td>1453.5</td>
<td>0.6</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>α-humulene</td>
<td>1455.7</td>
<td>0.2</td>
<td>-</td>
</tr>
<tr>
<td>21</td>
<td>4,5-di-epi-aristolochene</td>
<td>1472.9</td>
<td>0.2</td>
<td>-</td>
</tr>
<tr>
<td>22</td>
<td>β-chamigrene</td>
<td>1477.6</td>
<td>0.3</td>
<td>0.1</td>
</tr>
</tbody>
</table>

No. | Compound | RIEa (%) | Aerial parts (%) | Undergr. parts (%)
---|-----------|----------|------------------|------------------
23 | thymol isobutyrate | 1485.6 | 0.3 | 0.3
24 | (E)-β-ionone | 1487.8 | 0.7 | -
25 | neryl isobutanolate | 1491.4 | 1.7 | 1.3
26 | α-selinene | 1498.3 | 0.7 | 0.2
27 | β-bisabolene | 1509.3 | - | 0.2
28 | cameroonan-7α-ol | 1512.1 | 0.7 | -
29 | modhephen-8β-ol | 1515.5 | 0.3 | 0.4
30 | (E)-dihydro-apofarnesal | 1521.0 | 0.3 | -
31 | δ-cadinene | 1525.8 | 1.1 | -
32 | cis-calamenene | 1536.7 | 0.1 | -
33 | α-calacorene | 1545.6 | 0.6 | -
34 | isocaryophyllene oxide | 1556.5 | 1.5 | -
35 | epo-longipinanol | 1562.1 | 0.2 | -
36 | (E)-nerolidol | 1567.8 | 10.3 | -
37 | neryl (5S)-2-methylbutyrate | 1576.3 | - | 0.5
38 | prenopsan-8-ol | 1577.7 | 4.9 | -
39 | caryophyllene oxide | 1587.6 | 8.2 | 1.3
40 | humulene epoxide II | 1612.4 | 0.9 | 0.2
41 | cis-isogalactol | 1614.3 | 0.5 | -
42 | murola-4,10(14)-diene-1β-ol | 1631.6 | 0.4 | -
43 | α-acorenl | 1634.3 | - | 0.1
44 | caryophylla-4(12),8(13)-dien-5-α-ol | 1636.6 | 1.1 | 0.1
45 | caryophylla-4(12),8(13)-dien-5-β-ol | 1640.6 | 2.6 | -
46 | β-eudesmol | 1645.8 | 2.8 | 1.6
47 | α-eudesmol | 1653.1 | 0.3 | -
48 | atracylone | 1656.3 | - | 0.4
49 | selin-11-en-4α-ol | 1657.7 | 0.8 | 0.4
50 | 14-hydroxy-(Z)-caryophyllene | 1667.9 | 0.7 | 0.2
51 | trans-calamenen-10-ol | 1670.2 | 0.3 | -
52 | 14-hydroxy-9-epi-(E)-caryophyllene | 1674.5 | 2.0 | 0.7
53 | cadalene | 1677.7 | 0.6 | -
54 | epi-cadabrol | 1685.2 | 0.5 | -
55 | 6-methoxythymyl isobutirate | 1687.5 | 0.6 | 0.4
56 | n-heptadecane | 1698.4 | 0.7 | 0.2
57 | δ-dodecalactone | 1707.8 | 0.5 | 0.7
58 | (E,Z)-farnesal | 1716.3 | 0.7 | -
59 | 3-methyl-cumynyl isobutirate | 1719.7 | - | 0.3
60 | 6-isopropenyl-4,8a-dimethyl-1,2,3,5,6, 7,8,8a-octahydro-naphtalen-2-ol | 1723.0 | - | 0.3
61 | (Z,E)-farnesal | 1725.0 | 7.7 | -
62 | (E,E)-farnesal | 1743.8 | 1.1 | -
63 | fukinone | 1749.1 | 0.4 | -
64 | cyclocolorone | 1753.8 | 0.4 | -
65 | tetradecanoic acid | 1765.0 | 1.1 | -
66 | α-costa | 1775.4 | 0.6 | -
67 | n-octadecane | 1798.4 | 0.3 | -
68 | hexahydrofarnesyl acetone | 1845.3 | 1.9 | -
69 | benzyl salicylate | 1870.1 | 0.4 | -
70 | alantolactone | 1902.1 | - | 2.4

RIEa – experimental retention indices
n/a – not available
”-” - not detected

In the previous investigations, aerial parts collected during the flowering time of *T. speciosa* from Serbia contained 0.06% (v/w) of essential oil. Dominant compounds were sesquiterpenes, non-terpene components and oxygenated monoterpenes [4]. Furthermore, leaf, stem, flower and root essential oils were also studied. The qualitative composition of the essential oils from the examined plant material was similar, whereas the quantities of individual components of the oils varied widely depending on the kind of the plant material [18]. The results of essential oils components obtained from the literature were similar to our results.

The amount of the essential oil found in the underground parts of *T. speciosa* (0.29% (v/w)) was higher than in the aboveground parts. The oil was yellowish with needle crystals, and of aromatic odor. The identified 35 constituents from the underground parts of *T. speciosa* accounting for 96.9% of the oil are presented in Table 3. The oil was characterized by the presence of high concentration of oxygenated sesquiterpenes (85.6%) with isoalantolactone being the major component (77.2%), β-Eudesmol (1.6%) and caryophyllene oxide (1.3%) were also the representatives of oxygenated sesquiterpenes in the oil of the underground parts. Sesquiterpene hydrocarbons constituted only (3.2%) of the oil. Thymol derivatives [10-isobutyroxy-8,9-epoxymethyl isobutyrat (3.7%), 9-isobutyroxythymyl isobutyrat (2.4%)] and neryl isobutanoate (1.3%) were the oxygenated
monoterpenes found in appreciable amounts. They constituted (8.1%) of the oil.

In the previous investigations, the underground parts collected during the flowering time of *T. speciosa* from Poland and Montenegro contained 0.4% - 1.7% of essential oils. The dominant compound of those oils was also isosalantolactone (62.3% - 95%) [1], [18].

**CONCLUSION**

To the best of our knowledge, the extracts from the aerial and underground parts of *Telekia speciosa* (Schreb.) Baumg. were analysed on the presence of phenol acids by HPLC for the first time. The chlorogenic acid and caffeeic acid derivatives were detected in the extract from the aerial and underground parts, with a higher amount of caffeic acid derivatives and three and a half times higher amount of the chlorogenic acid in the underground parts.

The presence of phenol compounds contributes to the antioxidant capacity, which was also evaluated in the extracts for the first time, as we know. On average the three and half times higher antioxidant capacity was determined for the underground parts than for the aerial parts of *T. speciosa* extracts. The results for the chemical composition of the essential oils of *T. speciosa* were similar to the previously published data.

The obtained results contribute to better knowledge of phytochemical properties of *T. speciosa*, which is traditionally used in bronchial asthma therapy. The recommendation for further investigations refers to the isolation of caffeic acid derivatives using the column chromatography and the determination of their structures as known or new compounds by comparison of their spectral data (1H NMR, UV) with those found in the literature.

**REFERENCES**


**ACKNOWLEDGEMENT**

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