The chemical composition, antimicrobial and antiradical properties of the essential oil of Achillea grandifolia aerial parts from Serbia

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ABSTRACT:
Aromatic plants and essential oils have many applications in medicine, pharmaceuticals, cosmetics, and the food industry. The essential oil of the flowering aerial parts of Achillea grandifolia, obtained by hydrodistillation, was analyzed for its constituents and investigated for antimicrobial and radical scavenging activity.

The essential oil was characterized by a high amount of oxygenated monoterpenes (72.7%) with 1,8-cineole (29.2%) and camphor (23.4%) being the most abundant. Sesquiterpenes were present in smaller quantities (4.8%). Antimicrobial activity was tested against eight ATCC bacterial strains and two ATCC strains of Candida albicans. The essential oil exhibited highly pronounced antimicrobial activity against Micrococcus luteus with a MIC value of 3.50 µg/mL, as well as significant antimicrobial activity (<100 μg/mL) against Staphylococcus aureus, S. epidermidis and Bacillus subtilis. Gram-negative bacteria Escherichia coli and Pseudomonas aeruginosa were resistant. Achillea grandifolia essential oil exhibited concentration-dependent antiradical activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical with an SC50 value of 5.4 mg/mL. The TLC-DPPH assay revealed two main light yellow spots indicating components with anti-DPPH activity, which after isolation were identified as 1,8-cineole and camphor.

INTRODUCTION
Essential oils, as complex mixtures of natural compounds, possess various biological activities including antimicrobial, antioxidant, anti-inflammatory, analgesic, immunomodulatory, antithrombotic and insecticidal activity. The interest in aromatic plants and essential oils has grown substantially in the previous decades due to their widespread use in medicine, pharmacy, agriculture, cosmetics and the food industry (Carson & Hammer 2011).

In recent years, many studies have focused on research into essential oils and their components as potential antimicrobial and antioxidant agents (Yang et al. 2018; Valdivieso-Ugarte et al. 2019).

The genus Achillea L., one of the most important genera of the family Asteraceae, includes 110-140 species widely distributed in Europe, Asia, and North Africa, with centers of diversity in Southeast Europe and Southwest Asia (Baltisberger & Widmer 2016). The taxa vary broadly in morphology, life cycle, and ecology. The genus exhibits a complex phyletic structure due to excessive hybridization and polyploidy (Guo et al. 2004; Nedelcheva 2008).

Achillea species are important and frequently used medicinal plants. Among them, the species within the Mille-
A. grandifolia section are of the greatest significance. Due to their antiinflammatory, antispasmodic, stomachic and choleretic properties, Achillea species are traditionally used for the treatment of spasmodic gastrointestinal complaints, hepato-biliary disorders, minor spasms associated with menstrual periods as well as for temporary loss of appetite and wound healing (Saeidnia et al. 2011; Mohammadhosseini et al. 2017; Aminkhani et al. 2019, 2020).

Considering their vast distribution and various traditionally known applications, the Achillea species have attracted great interest among scientists regarding their chemical constituents, pharmacological activities and therapeutic applications (Saeidnia et al. 2011; Mohammadhosseini et al. 2017). The majority of investigated Achillea essential oils exhibit substantial antimicrobial and antioxidant effects (Mohammadhosseini et al. 2017).

Achillea grandifolia Friv. is one of the 19 Achillea species recorded in the flora of Serbia (Gajić 1975). This perennial is a relict and Balkan endemic species which grows in rocky and shaded places on limestone or siliceous bedrocks. It is also distributed in Albania, North Macedonia, Greece, Bulgaria and western Anatolia (Richardson 1976). This species is phylogenetically isolated within the Millefolium section (Nedelcheva 2008).

Research has shown that an infusion prepared from the flowerheads of A. grandifolia, originating from Turkey, exhibited antioxidant activity, which was correlated with the total phenol and flavonoid contents (Konyalioglu et al. 2016b). This species is phylogenetically isolated within the Millefolium section (Nedelcheva 2008).

For the determination of the minimal inhibitory concentration (MIC) a broth microdilution assay was used (CLSI 2014). The test strains were suspended in the medium (Müller-Hinton broth for bacterial strains and Sabouraud dextrose broth for C. albicans) to give a final concentration of 5 × 10⁵ cfu/mL. Twofold serial dilutions of essential oil in dimethylsulfoxide were prepared in 96-well microtiter plates to obtain final concentrations of the essential oil in 22.175822°C), during the period of full flowering. A voucher specimen is deposited at the Herbarium of the Natural History Museum, Belgrade (BOE, K05012018/16).

The essential oil was isolated from the air-dried plant material. The flowering aerial parts were cut and subjected to hydrodistillation for 2.5 h, using a Clevenger-type apparatus, according to the European Pharmacopoeia (Ph. Eur. 2017). The hydrodistillation was performed three times. The essential oil was dried over anhydrous sodium sulfate and kept at 4°C until analysis.

**GC-FID/MS analysis.** Gas chromatographic analysis (GC-FID/MS) was carried out using an Agilent 6890N gas chromatograph equipped with a flame-ionization detector (FID) combined with an Agilent 5975C MS detector. The chromatographic separation was performed on an HP-5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm). The carrier gas was He (1.0 mL/min), the temperature of the injector was 200°C and the oven temperature programmed from 60°C to 280°C at a rate of 3°C/min. The injected volume was 1 µL (1% solution of oil in absolute ethanol), the split ratio 10:1. FID and the MSD transfer line temperatures were set at 300 and 250°C, respectively. The EI mass spectra (70 eV) were obtained over the m/z range of 35-550.

The identification of the compounds was based on the comparison of their retention indices (RI), their retention times and mass spectra with those from the NIST/NBS and Wiley libraries and the literature (Adams 2007). Additionally, the identification of the compounds was confirmed by co-injection of available authentic standards. The linear RIs were determined in relation to a homologous series of n-alkanes (C₈-C₄₀) under the same operating conditions (Adams 2007). The relative percentages of the compounds were calculated based on the peak areas from the FID data.

**Antimicrobial activity.** The antimicrobial activity was tested against the laboratory control strains from the American Type Culture Collection (ATCC): Gram (+) bacteria Staphylococcus aureus (ATCC 25923), Staphylococcus epidermidis (ATCC 12228), Bacillus subtilis (ATCC 6633), Micrococcus luteus (ATCC 9341), Enterococcus faecalis (ATCC 29212), Gram (–) bacteria Escherichia coli (ATCC 25922), Klebsiella pneumoniae NCIMB 9111, Pseudomonas aeruginosa ATCC 27853 and two strains of yeast Candida albicans (ATCC 10231 and ATCC 24433). The microorganisms were provided by the Institute for Immunology and Virology, Torlak, Belgrade.

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**MATERIALS AND METHODS**

**Plant material and the isolation of the essential oil.** The aerial parts of A. grandifolia were collected in May 2018, in southeastern Serbia (Sićevo Gorge, N 43.316623°, E 22.175822°), during the period of full flowering. A voucher specimen is deposited at the Herbarium of the Natural History Museum, Belgrade (BOE, K05012018/16).

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the range of 3.5-1925 μg/mL. Microbial growth was determined after incubation for 24 h at 37°C for the bacteria and 48 h at 26°C for the yeast. All determinations were performed in triplicate. The MICs of standard antibiotics, ampicillin and nystatin were determined in parallel experiments and positive controls of growth were included.

**Antiradical activity.** The radical scavenging ability was determined by the DPPH assay (Cuendet et al. 1997). Different aliquots of the essential oil were mixed with 0.4 mL of 0.5 mM DPPH in absolute ethanol and adjusted up to 2 mL. The mixtures were shaken vigorously and left in the dark for 30 min. Absorbance was measured at 517 nm to 2 mL. The mixtures were shaken vigorously and left in the dark for 30 min. Absorbance was measured at 517 nm using ethanol as a blank. One milliliter of 0.5 mM DPPH diluted in 4 mL of absolute ethanol was used as a control. The DPPH radical scavenging activity was calculated using the equation: S(%) = 100 × (A0 - As)/A0, where A0 is the absorbance of the control (containing all the reagents except the tested sample), and As is the absorbance of the tested sample. The SC50 value represented the concentration of the tested sample, which caused the scavenging of 50% of the DPPH radicals. The activity was compared with some well-known antioxidant compounds (ascorbic acid, rutin and quercetin).

**TLC-DPPH test.** The thin layer chromatography (TLC)-DPPH assay was employed to determine the active compounds. Fifty microliters of essential oil diluted in absolute ethanol (1:5) were applied to two TLC plates (Silica gel 60 F254, Merck) and developed in toluene-ethyl acetate (93:7) as the mobile phase. One part of the plate was sprayed with vanillin-sulphuric acid reagent and another part with 0.2% DPPH reagent in absolute ethanol. The plates were left in the dark at room temperature and observed after 30 min, 1 and 2 hours. The yellow spots formed from the bleaching of DPPH were considered as the active zones, which were extracted with absolute ethanol. The isolated compounds were identified by GC-FID/MS analysis.

**Preparative TLC.** After identification of the active zones on the TLC-DPPH plate, in order to isolate the active compounds, preparative TLC analysis was performed. One hundred microliters of essential oil diluted in absolute ethanol (1:5) were applied to two TLC plates (Silica gel 60 F254, Merck). After development in toluene-ethyl acetate (93:7), one part of both plates was sprayed with 0.2% DPPH solution (in absolute ethanol), while the rest of the plates were used to scratch off the corresponding active zones, which were extracted with absolute ethanol. The isolated compounds were identified by GC-FID/MS analysis.

**RESULTS**

**Chemical composition of the essential oil.** The average essential oil yield, determined from three hydrodistillations, was 0.35% (w/w). The isolated oil was yellow, with

### Table 1. The composition of the essential oil of *Achillea grandifolia*

<table>
<thead>
<tr>
<th>Compound</th>
<th>RI&lt;sup&gt;lit&lt;/sup&gt;</th>
<th>RI&lt;sup&gt;exp&lt;/sup&gt;</th>
<th>%&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Pinenene</td>
<td>936</td>
<td>932</td>
<td>1.0</td>
</tr>
<tr>
<td>Camphene</td>
<td>950</td>
<td>946</td>
<td>2.4</td>
</tr>
<tr>
<td>Sabine</td>
<td>973</td>
<td>969</td>
<td>2.2</td>
</tr>
<tr>
<td>β-Pinenene</td>
<td>977</td>
<td>974</td>
<td>0.9</td>
</tr>
<tr>
<td>dehydro-1,8-Cineole</td>
<td>991</td>
<td>988</td>
<td>0.6</td>
</tr>
<tr>
<td>p-Mentha-1(7),8-diene</td>
<td>1002</td>
<td>1003</td>
<td>1.4</td>
</tr>
<tr>
<td>α-Terpine</td>
<td>1015</td>
<td>1014</td>
<td>4.1</td>
</tr>
<tr>
<td>p-Cymene</td>
<td>1023</td>
<td>1020</td>
<td>0.5</td>
</tr>
<tr>
<td>1,8-Cineole</td>
<td>1028</td>
<td>1026</td>
<td>29.2</td>
</tr>
<tr>
<td>γ-Terpine</td>
<td>1057</td>
<td>1054</td>
<td>0.8</td>
</tr>
<tr>
<td>cis-Sabinine hydrate</td>
<td>1067</td>
<td>1065</td>
<td>1.4</td>
</tr>
<tr>
<td>Terpinolene</td>
<td>1087</td>
<td>1086</td>
<td>0.3</td>
</tr>
<tr>
<td>trans-Sabinine hydrate</td>
<td>1101</td>
<td>1098</td>
<td>1.7</td>
</tr>
<tr>
<td>Isopentyl-2-methyl butanoate</td>
<td>1102</td>
<td>1100</td>
<td>0.3</td>
</tr>
<tr>
<td>cis-Thujone</td>
<td>1102</td>
<td>1101</td>
<td>1.0</td>
</tr>
<tr>
<td>Isopentyl isovalerate</td>
<td>1103</td>
<td>1102</td>
<td>0.6</td>
</tr>
<tr>
<td>trans-Thujone</td>
<td>1114</td>
<td>1112</td>
<td>tr</td>
</tr>
<tr>
<td>cis-p-Menth-2-en-1-ol</td>
<td>1121</td>
<td>1118</td>
<td>0.6</td>
</tr>
<tr>
<td>Chrysanthenone</td>
<td>1126</td>
<td>1124</td>
<td>0.6</td>
</tr>
<tr>
<td>cis-p-Menta-2,8-dien-1-ol</td>
<td>1135</td>
<td>1133</td>
<td>0.6</td>
</tr>
<tr>
<td>Camphor</td>
<td>1143</td>
<td>1141</td>
<td>23.4</td>
</tr>
<tr>
<td>Pinocarvone</td>
<td>1162</td>
<td>1160</td>
<td>0.6</td>
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<tr>
<td>Borneol</td>
<td>1166</td>
<td>1165</td>
<td>4.6</td>
</tr>
<tr>
<td>Terpinen-4-ol</td>
<td>1175</td>
<td>1174</td>
<td>2.0</td>
</tr>
<tr>
<td>α-Terpineol</td>
<td>1187</td>
<td>1186</td>
<td>3.6</td>
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<tr>
<td>Myrtenol</td>
<td>1197</td>
<td>1194</td>
<td>0.7</td>
</tr>
<tr>
<td>Myrtenal</td>
<td>1197</td>
<td>1195</td>
<td>0.3</td>
</tr>
<tr>
<td>Bornyl acetate</td>
<td>1290</td>
<td>1287</td>
<td>1.7</td>
</tr>
<tr>
<td>Lavandulyl acetate</td>
<td>1291</td>
<td>1288</td>
<td>0.6</td>
</tr>
<tr>
<td>p-Cymen-7-ol</td>
<td>1292</td>
<td>1289</td>
<td>0.5</td>
</tr>
<tr>
<td>Eugenol</td>
<td>1357</td>
<td>1356</td>
<td>tr</td>
</tr>
<tr>
<td>β-Bourbonene</td>
<td>1388</td>
<td>1387</td>
<td>tr</td>
</tr>
<tr>
<td>(E)-Jasnone</td>
<td>1392</td>
<td>1390</td>
<td>1.2</td>
</tr>
<tr>
<td>(E)-Caryophyllene</td>
<td>1419</td>
<td>1417</td>
<td>tr</td>
</tr>
<tr>
<td>α-Humulene</td>
<td>1453</td>
<td>1452</td>
<td>tr</td>
</tr>
<tr>
<td>(E)-β-Farnesene</td>
<td>1455</td>
<td>1454</td>
<td>0.3</td>
</tr>
<tr>
<td>ar-Curcumene</td>
<td>1481</td>
<td>1479</td>
<td>tr</td>
</tr>
<tr>
<td>Germacrene D</td>
<td>1482</td>
<td>1484</td>
<td>1.3</td>
</tr>
<tr>
<td>(E)-β-Ijonone</td>
<td>1489</td>
<td>1487</td>
<td>tr</td>
</tr>
<tr>
<td>Bicyclogermacrene</td>
<td>1502</td>
<td>1500</td>
<td>0.1</td>
</tr>
<tr>
<td>(E,E)-α-Farnesene</td>
<td>1507</td>
<td>1505</td>
<td>0.8</td>
</tr>
<tr>
<td>β-Sesquiphellandrene</td>
<td>1522</td>
<td>1521</td>
<td>0.3</td>
</tr>
<tr>
<td>(E)-Nerolidol</td>
<td>1563</td>
<td>1561</td>
<td>0.6</td>
</tr>
<tr>
<td>Spathulenol</td>
<td>1579</td>
<td>1577</td>
<td>0.9</td>
</tr>
<tr>
<td>Caryophyllene oxide</td>
<td>1583</td>
<td>1582</td>
<td>0.5</td>
</tr>
<tr>
<td>Caryophylla-4(12),8(13)-dien-5β-ol</td>
<td>1642</td>
<td>1639</td>
<td>tr</td>
</tr>
<tr>
<td>β-Eudesmol</td>
<td>1651</td>
<td>1649</td>
<td>tr</td>
</tr>
<tr>
<td>Identified</td>
<td>93.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Grouped components:**
- Monoterpene hydrocarbons: 13.6
- Oxygenated monoterpenes: 72.7
- Sesquiterpene hydrocarbons: 2.8
- Oxygenated sesquiterpenes: 2.0
- Others: 2.1

* The retention indices relative to C<sub>8</sub>-C<sub>40</sub> n-alkanes experimentally determined on the HP-5MS column; b The retention indices obtained from the literature (Adams 2007); c The relative area percentage-values are the mean of the three analyses; d trace (< 0.1%)
Table 2. The antimicrobial activity of the investigated *Achillea grandifolia* essential oil and standard antibiotics

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>A. grandifolia essential oil MIC&lt;sup&gt;a&lt;/sup&gt; (μg/mL)</th>
<th>Antibiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ampicilin</td>
<td>Nystatin</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 25923</td>
<td>64.17</td>
<td>0.5</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em> ATCC 12228</td>
<td>64.17</td>
<td>0.25</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em> ATCC 6633</td>
<td>96.25</td>
<td>0.8</td>
</tr>
<tr>
<td><em>Micrococcus luteus</em> ATCC 9341</td>
<td>3.50</td>
<td>2.8</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> ATCC 29212</td>
<td>770.00</td>
<td>0.5</td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 25922</td>
<td>&gt; 1540.00</td>
<td>2.0</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em> NCIMB 9111</td>
<td>224.58</td>
<td>2.0</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> ATCC 27853</td>
<td>&gt; 1925.00</td>
<td>3.0</td>
</tr>
<tr>
<td><em>Candida albicans</em> ATCC 10231</td>
<td>160.42</td>
<td>n.t.</td>
</tr>
<tr>
<td><em>Candida albicans</em> ATCC 24433</td>
<td>525.00</td>
<td>n.t.</td>
</tr>
</tbody>
</table>

<sup>a</sup> Minimal inhibitory concentration (μg/mL); <sup>b</sup> not tested

Table 3. Classes of terpenes and the most abundant components of the investigated and previously analyzed *A. grandifolia* essential oils

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Origin</th>
<th>Monoterpenes (%)</th>
<th>Sesquiterpenes (%)</th>
<th>Main components</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Flowering aerial parts</td>
<td>NW Greece, Vikos Gorge</td>
<td>68.0</td>
<td>14.76</td>
<td>camphor (25.6%), 1,8-cineole (12.8%), α-thujone (11.9%), β-thujone (9.2%), p-cymene (6.7%)</td>
<td>Hanlidou et al. 1992</td>
</tr>
<tr>
<td>Flowering aerial parts</td>
<td>E Serbia, Jerma Gorge</td>
<td>65.9</td>
<td>0.3</td>
<td>camphor (15.6%), ascaridole (15.5%), α-thujone (7.5%), (Z)-jasmone (6.4%), borneol (5.2%)</td>
<td>Radulović et al. 2010</td>
</tr>
<tr>
<td>Leaves and flowers</td>
<td>Turkey, Izmir</td>
<td>57.5</td>
<td>5.4</td>
<td>piperitone (34.0%), carvacrol (7.0%), p-cymene (5.0%)</td>
<td>Küçükbay &amp; Çetin 2012</td>
</tr>
<tr>
<td></td>
<td>Turkey, Aydin</td>
<td>64.9</td>
<td>10.6</td>
<td>1,8-cineole (32.0%), piperitone (18.7%), p-cymene (10.0%)</td>
<td></td>
</tr>
<tr>
<td>Flowering aerial parts</td>
<td>E Serbia, Jerma Gorge (Trnski Odorovci)</td>
<td>89.15</td>
<td>7.19</td>
<td>camphor (45.4%), 1,8-cineole (16.4%), α-thujone (15.1%), borneol (8.13%)</td>
<td>Stanković et al. 2016b</td>
</tr>
<tr>
<td>Flowering aerial parts</td>
<td>Turkey, Antalya province</td>
<td>76.2</td>
<td>20.6</td>
<td>α-terpinyl acetate (54.1%), p-cymene (17.7%), cis-piperitone-oxide (7.5%)</td>
<td>Özek 2018</td>
</tr>
<tr>
<td>Flowering aerial parts</td>
<td>E Serbia, Sićevo Gorge</td>
<td>72.7</td>
<td>13.6</td>
<td>1,8-cineole (29.2%), camphor (23.4%), borneol (4.6%), α-terpinene (4.1%)</td>
<td>This research</td>
</tr>
</tbody>
</table>

an aromatic, and pleasant smell. The chemical composition of the oil is summarized in Table 1. GC-FID/MS analysis resulted in the identification of 47 components which accounted for 93.2% of the total oil.

The essential oil obtained from the flowering aerial parts of *A. grandifolia* was characterized by a high amount of oxygenated monoterpenes (72.7%), with 1,8-cineole (29.2%) and camphor (23.4%) being the most abundant components. Among the remaining compounds, which were present in less than 5%, borneol (4.6%) and α-terpinene (4.1%) were in higher amounts. Sesquiterpenes were present in much smaller quantities (4.8%).

**Antimicrobial activity.** The antimicrobial activity of the *A. grandifolia* essential oil was evaluated against 10 microorganisms, including five Gram-positive bacteria, three Gram-negative bacteria, and two strains of yeast *Candida albicans*. The results of the antimicrobial activity, ex-
pressed as minimal inhibitory concentrations (MIC), are presented in Table 2.

The essential oil exhibited a very pronounced antimicrobial activity against Micrococcus luteus with a MIC value of 3.50 μg/mL. The tested oil also showed significant antimicrobial activity (MICs<100 μg/mL) (Cos et al. 2006) against Staphylococcus aureus (MIC 64.17 μg/mL), S. epidermidis (MIC 64.17 μg/mL) and Bacillus subtilis (MIC 96.25 μg/mL), as well as moderate activity against Candida albicans ATCC 10231 (MIC 160.42 μg/mL). The Gram-negative bacteria Klebsiella pneumoniae was less sensitive with a MIC of 224.54 μg/mL, whereas Escherichia coli and Pseudomonas aeruginosa were resistant, which is in line with the accepted opinion that essential oils are generally more active against Gram-positive than against Gram-negative bacteria (Kozics et al. 2019).

**Antiradical activity.** In the DPPH test, the essential oil of *A. grandifolia* exhibited concentration-dependent anti-radical activity, with a SC₅₀ value of 5.4 mg/mL. Ascorbic acid and flavonoids rutin and quercetin showed substantial antiradical activities, with SC₅₀ values of 4.09, 5.75 and 2.75 μg/mL, respectively. The antiradical capacity of the investigated essential oil was lower than that of standard antioxidants, but was much more pronounced compared to previous research on the anti-DPPH activity of *A. grandifolia* essential oils from the Jerma Gorge (IC₅₀ =33.6 mg/mL) (Stanković et al. 2016b) and Antalya province (10 mg/mL) (Özek 2018).

The TLC-DPPH test revealed four DPPH radical neutralizing zones, which were extracted and identified by GC-FID/MS analysis. The zones corresponding to 1,8-cineole (Rf=0.5) and camphor (Rf=0.59) were the most active; they appeared shortly after spraying with DPPH reagent. Two more zones (Rf=0.44 and Rf=0.9), which were revealed during the following 2 hours, corresponded to components with lower anti-DPPH activity: α-terpinene and α-pinene, respectively.

**DISCUSSION**

A large number of *Achillea* species have been investigated in terms of the content, composition and pharmacological activities of their essential oils. Studies on the composition of essential oils of different *Achillea* species have shown that their main ingredients are primarily oxygenated monoterpenes, with camphor, 1,8-cineole, sabine, borneol, thujones, linalool and α-terpineol as the most dominant components. The sesquiterpenoid fraction is usually less prominent, mainly represented by germacrene D, β-caryophyllene, cadinol derivatives and α-bisabolol (Kindlovits & Németh 2012; Mohammadhosseini et al. 2017).

The results obtained in our study are partly in line with previous investigations of *A. grandifolia* essential oils. Our sample was rich in oxygenated monoterpenes (72.7%) as were the previously analyzed samples (Table 3). In addition, similarities were also observed in terms of the dominant components. 1,8-cineole and camphor were also the most abundant compounds in previously analysed *A. grandifolia* essential oils from Vikos (Hanlidou et al. 1992) and the Jerma Gorge (Stanković et al. 2016b), but in contrast to our sample where 1,8-cineole prevailed, camphor was more dominant in the mentioned oils. Analysis of the essential oil from the leaves and flowers of *A. grandifolia* originating from the Aydin province revealed that the major compounds were 1,8-cineole (32.0%), piperitone (18.7%) and p-cymene (10.0%) (Küçükbaş & Çetin 2012), while in our sample piperitone was not detected and p-cymene was present in low quantities (0.5%). In another study of *A. grandifolia* essential oil, also originating from the Jerma Gorge, camphor (15.6%) was one of the most dominant components similar to our sample, but the other abundant components, i.e. ascaridole (15.5%), α-thujone (7.5%) and (Z)-jasmine (6.4%) were quite different. This sample also contained a considerable amount of sesquiterpenes (14.0%) (Radulović et al. 2010).

On the other hand, two essential oil samples of *A. grandifolia* from Turkey showed quite different chemical compositions, one (from Antalya) (Özek 2018) with α-terpinyl acetate (54.1%), p-cymene (17.7%) and cis-piperitoxone-oxide (7.5%) and another (from Izmir) (Küçükbaş & Çetin 2012) with piperitone (34.0%) and carvacrol (7.0%) as the main constituents (Table 3).

It should be noted that some previously analysed *A. grandifolia* essential oils contain substantial amounts of certain compounds that may pose risks to human health such as thujones (8.9-22.1%) (Hanlidou et al. 1992; Radulović et al. 2010; Stanković et al. 2016b), as well as ascaridole (15.5%) (Radulović et al. 2010). In our sample, thujones were present in traces, whereas ascaridole was not detected.

The qualitative and quantitative differences in the chemical composition of *A. grandifolia* essential oils suggest that environmental factors and probably different periods of plant material collection strongly influence its chemical composition.

In a previous investigation of antimicrobial activity, the essential oil of *A. grandifolia* demonstrated inhibitory and bactericidal effects against various microbial strains isolated from human material, although in much a higher range of MICs from 5.77-46.15 mg/mL (Stanković et al. 2016b) than in this study.

The demonstrated antimicrobial activity of the investigated *A. grandifolia* essential oil might be related to the high content of 1,8-cineole and camphor, which constitute more than 50% of the investigated essential oil. Both compounds have known antimicrobial properties (Hammer & Carson 2011; Bill et al. 2014). In addition, the synergistic activity of 1,8-cineole and camphor against some bacteria have already been observed (Viljoen et al. 2003; Koroch et al. 2007). The components present
in lower amounts such as borneol (Santoyo et al. 2005), α-terpinene, α-terpineol and terpinen-4-ol (Koroch et al. 2007), could contribute to the antimicrobial activity since their inhibitory effects on several microorganisms have been previously reported. Also, these minor components could be included in some type of synergism with other active compounds (Marino et al. 2001).

Literature data indicate different antiradical capacities of DPPH radical neutralizing components revealed in the TLC-DPPH assay (Kim et al. 2004; Koroch et al. 2007; Wang et al. 2019). It was previously reported that 1,8-cineole, as a constituent of Myrtus communis essential oil, shows considerable DPPH scavenging activity in the TLC-DPPH assay (Mimica-Dukić et al. 2010). Both 1,8-cineole and camphor were detected and isolated from Curcuma wenyujin essential oil, as components with moderate and weak antiradical activity, respectively (Wang et al. 2020). In addition, essential oils of Achillea millefolium subsp. millefolium (Candan et al. 2003) and A. vermicularis (Polatoğlu et al. 2013), which share a similar chemical pattern as the investigated A. grandifolia essential oil (1,8-cineole and camphor as the main constituents), also exhibited significant anti-DPPH activity. Our results point out that the observed antioxidative activity of A. grandifolia essential oil can be attributed to its main compounds, 1,8-cineole and camphor. Still, the fact already observed by some researchers that minor compounds and synergistic effects may significantly contribute to the expressed activity, should not be neglected (Candan et al. 2003; Koroch et al. 2007).

**CONCLUSION**

The chemical analysis of the essential oil from the flowering aerial parts of Achillea grandifolia showed that 1,8-cineole and camphor were the most abundant compounds. Although some previously analyzed A. grandifolia essential oils contain significant amounts of certain components of concern, in the investigated sample thujones were present in traces, whereas ascaridole was not detected.

The essential oil exhibited very strong antimicrobial activity against Micrococcus luteus, as well as significant antimicrobial activity against Gram-positive bacteria Staphylococcus aureus, S. epidermidis, and Bacillus subtilis, whereas Gram-negative bacteria Escherichia coli and Pseudomonas aeruginosa were resistant.

In the DPPH assay, the essential oil exhibited concentration-dependent antiradical activity, which can be attributed to its main components, 1,8-cineole, and camphor.

The essential oil of the flowering aerial parts of A. grandifolia originating from Sîcevo Gorge from Serbia, with its advantageous chemical profile and observed antimicrobial and radical scavenging activities, is a promising candidate for further research in order to define its potential use in the pharmacy, food, and cosmetic industries.

Since A. grandifolia is an endemic plant, for further research and potential application it is necessary to consider the use of cultivated plants in order to obtain a larger amount of plant material and to protect natural habitats.

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Hemijski sastav, antimikrobna i antiradikalska svojstva etarskog ulja nadzemnih delova *Achillea grandifolia* iz Srbije

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Aromatične biljke i etarska ulja nalaze primenu u medicini, farmaciji, kozmetičkoj, parfimerijskoj i prehrambenoj industriji. Etarsko ulje iz nadzemnih delova u cvetu *Achillea grandifolia* izolovano je destilacijom vodenom parom i ispitivano u pogledu hemijskog sastava, antimikrobne i antiradikalske aktivnosti. Etarsko ulje se odlikovalo velikom količinom oksidovanih monoterpena (72,7%) sa 1,8-cineolom (29,2%) i kamforom (23,4%) kao dominantnim jedinjenjima. Seskviterpeni su bili prisutni u manjoj količini (4,8%). Antimikrobna aktivnost je ispitivana prema osam ATCC sojeva bakterija i dva ATCC soja *Candida albicans*. Etarsko ulje je pokazalo veoma izraženu antimikrobnu aktivnost prema *Micrococcus luteus* sa MIC vrednošću od 3,50 µg/mL, kao i značajnu aktivnost prema *Staphylococcus aureus*, *S. epidermidis* i *Bacillus subtilis*. Gram-negativne bakterije *Escherichia coli* i *Pseudomonas aeruginosa* bile su rezistentne. Etarsko ulje je ispoljilo dozno-za-visnu antiradikalsku aktivnost prema DPPH radikalu sa SC50 vrednošću od 5,4 mg/mL. U TLC-DPPH testu uočene su dve glavne žute zone koje odgovaraju anti-DPPH aktivnim jedinjenjima, a koja su nakon izolovanja identifikovana kao 1,8-cineol i kamfor.

Ključne reči: *Achillea grandifolia*, etarsko ulje, 1,8-cineol, kamfor, antimikrobna aktivnost, antiradikalska aktivnost