Chemical Composition of the Essential Oil and Antimicrobial Activity of *Scaligeria* DC. Taxa and Implications for Taxonomy

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Abstract: Six different *Scaligeria* DC. taxa (Apiaceae) essential oils (EOs) obtained by hydrodistillation from herba with the flowers collected from different sites from Turkey. The oils were analyzed and characterized by gas chromatography-flame ionization detector (GC-FID) and gas chromatography–mass spectrometry (GC-MS) simultaneously. A total of 133 different compounds were identified and relative qualitative and quantitative differences were observed among the evaluated samples. Analytical profiles of the *Scaligeria* EOs showed characteristic differences in terms of different main chemical constituents, between the two taxa *S. lazica* Boiss. and *S. tripartita* (Kal.) Tamamsch.; and *S. napiformis* (Sprengel) Grande, *S. meifolia* (Fenzl) Boiss., *S. capillifolia* Post, *S. hermonis* Post, *S. glaucescens* (DC.) Boiss. taxa, respectively. The main component germacrene D can be utilized as marker for the chemical discrimination of the *Scaligeria* genus. In addition, *Scaligeria* EOs were evaluated in vitro for their antimicrobial activity against pathogenic Gram positive (*Staphylococcus aureus* and *Bacillus cereus*), Gram negative (*Pseudomonas aeruginosa*) and yeast (*Candida albicans*, *C. parapsilosis*, and *C. krusei*) standard strains by using a micro-dilution assay. As a general result, the oils showed moderate inhibitory range when compared with standard antimicrobial agents.

Keywords: *Scaligeria* sp.; chemo-taxonomy; essential oil; antimicrobial activity, Turkey. © 2017 ACG Publications. All rights reserved.

1. Introduction

Aromatic plants are often used in traditional medicine for their antimicrobial activities due to their essential oils (EOs), volatile compounds since ancient times. Several components such as

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lipophilic compounds including terpenoid derivatives of a variety of essential oils showed significant in vitro antimicrobial activity against bacteria, yeasts, dermatophyte, and fungal strains such as Aspergillus [1-4], and also have therapeutic potential in different infectious diseases. When elaborating the mode of action, the components in essential oils have been demonstrated to disrupt cellular membranes in bacteria and fungi, hence inhibiting cellular respiration and ionic transport [5-8].

The Apiaceae family products are among the prominent EOs, which are rich in terpenoids and phenylpropanoids [8-13]. This family is represented by 101 genera comprising 485 species of which 181 are endemic species in Turkey [14]. Scaligeria DC. genus is represented by 7 species of which two are endemic in Turkey. The species recorded in Turkish Flora are listed as follows: S. napiformis (Sprengel) Grande, S. tripartita (Kalen.) Tamamsch, S. lazica Boiss. (endemic), S. meifolia (Fenzl) Boiss., S. glaucescens (DC.) Boiss., S. hermonis Post, S. capillifolia Post (endemic) [15, 16]. Stevens stated that Scaligeria DC. spp. is an annual (which is not clear), biennial and perennial plant with white flowers and smooth fruit with globose shape [15]. Historically and traditionally Scaligeria species from Turkey have been associated with anise, and their local names were given inspired from this similarity both from the morphology and aromatic characteristics. Thus, Scaligeria species are generally named using “Kıl anason”, “Puslu anason”, "Laz anasonu", "Uzun anason” etc. referring to anise [16].

Previous studies and phytochemical investigations showed that the chemical composition profiles of Scaligeria and Pimpinella species are surprisingly similar [17]. For the initial characteristic phytochemical discrimination of Scaligeria genus from Turkey, the chemical composition and antimicrobial effects of EOs from 6 taxa were evaluated comparatively in this present study, to the best of our knowledge for the first time.

2. Materials and Methods

2.1. Plant Materials

The herbal parts of Scaligeria species were collected in the flowering period (2014-2016) from different regions of Turkey. The plant specimens were deposited in the Science Faculty of Erciyes University, Kayseri-Turkey. In this regard, the voucher numbers of the studied species are listed in Table 1.

2.2. Isolation of the Essential Oil

The plant materials were air dried at room temperature and were subjected to hydrodistillation for 3 hrs using a Clevenger-type apparatus to extract essential oils. The percentage yields were calculated on a dry weight basis as given in Table 1. The oils were dried over anhydrous sodium sulfate to remove moisture and stored at +4 °C until analyzed and tested further.

2.3. Analysis of Essential Oil

2.3.1. GC-FID Condition

The GC analysis was carried out using an Agilent 6890N GC system. Innowax FSC column (60 m x 0.25 mm, 0.25 µm film thickness) was used as described for the GC-MS. FID detector temperature was 300 °C. To obtain the same elution order with the GC-MS system, simultaneous auto-injection was performed on a duplicate of the same column applying the same operational conditions. Relative percentage amounts (%) of the separated compounds were calculated from FID chromatograms. The analysis results are given in Table 2.

The individual essential oil components were identified by comparison of their relative retention times with those of the authentic samples or by comparison of their relative retention index
Chemical composition of the essential oil and antimicrobial activity of *Scaligeria sp.*

(RRI) to series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library, MassFinder3 Library) [18, 19] and in-house “Başer Library of Essential Oil Constituents” built up by genuine compounds and components of known oils, as well as MS literature data [20, 21], was used for the identification.

2.3.2. GC-MS Condition

The analyses were carried out using an Agilent 5975 GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 µm film thickness) was used with helium as carrier gas (0.8 mL/min). GC oven temperature was kept at 60 °C for 10 min and programmed to 220 °C at a rate of 4 °C/min, and kept constant at 220 °C for 10 min and then programmed to 240 °C at a rate of 1°C/min. Split ratio was adjusted at 40:1. The injector temperature was set at 250 °C. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 35 to 450.

Table 1. *Scaligeria* species collection sites, yields and codes.

<table>
<thead>
<tr>
<th>Species</th>
<th>Collection site</th>
<th>Code</th>
<th>Collection date</th>
<th>Herbarium number</th>
<th>Oil yield* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. lazica</em></td>
<td>Artvin: Borçka, Maral forest, roadside</td>
<td>SL-1</td>
<td>July 2014</td>
<td>ERCH 5101</td>
<td>0.19</td>
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<tr>
<td><em>S. lazica</em></td>
<td>Trabzon: Köprübaşi, Beşköy, Büyükdoğanli village 800 m.</td>
<td>SL-2</td>
<td>July 2014</td>
<td>ERCH 5102</td>
<td>0.08</td>
</tr>
<tr>
<td><em>S. lazica</em></td>
<td>Trabzon: Altundere, Sümela monastery surroundings</td>
<td>SL-3</td>
<td>July 2014</td>
<td>ERCH 5103</td>
<td>0.38</td>
</tr>
<tr>
<td><em>S. napiformis</em>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Antalya: Akseki, Çukur village, Istarlas located, 1050 m.</td>
<td>SN-1</td>
<td>May 2014</td>
<td>ERCH 5104</td>
<td>0.10</td>
</tr>
<tr>
<td><em>S. napiformis</em>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Muğla: Köyceğiz, Ekincik village, Sandallitepe located, 480 m.</td>
<td>SN-2</td>
<td>May 2014</td>
<td>ERCH 5105</td>
<td>0.10</td>
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<tr>
<td><em>S. meifolia</em></td>
<td>Siirt: Pervari, between Tandırköy and Göl village gateway, in among oaks</td>
<td>SM-1</td>
<td>June 2014</td>
<td>ERCH 5106</td>
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<tr>
<td><em>S. meifolia</em></td>
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<td><em>S. capillifolia</em></td>
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<tr>
<td><em>S. tripartita</em></td>
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<td>June 2016</td>
<td>ERCH 5109</td>
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<tr>
<td><em>S. glaucescens</em></td>
<td>Van: between Van and Muradiye, 7 km before Karahan, steppe slopes, 1685 m.</td>
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<td>June 2016</td>
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</tr>
<tr>
<td><em>S. hermonis</em></td>
<td>Konya: between Seydişehir and içeri kısla, 1200 m.</td>
<td>SH-1</td>
<td>June 2016</td>
<td>ERCH 5111</td>
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</tbody>
</table>

<sup>a</sup>Calculated on moisture-free basis.
<sup>b</sup>Essential oil components of *S. napiformis* were reported by Baldemir et al. (2016) [22].

2.4. In vitro Antimicrobial Evaluation

*In vitro* antimicrobial activity of the *Scaligeria* sp. EOs (SC-1, SL-1, SL-2, SL-3, SH-1, ST-1) were evaluated using the microdilution method according to Clinical and Laboratory Standards institute (CLSI) guidelines [23, 24]. Chloramphenicol, Ciprofloxacin, Amphotericin B and Ketoconazole were used as standard antimicrobial agents, where applicable. All experiments were repeated in triplicate and average MIC results are listed in Table 3.
3. Results and Discussion

The initial EO yields of Scaligeria species (S. tripartita, S. lazica, S. meifolia, S. glaucescens, S. hermonis, S. capillifolia) were variable and ranged from 0.01 to 0.38%, collected from different regions of the Turkey (Table 1). To the best our knowledge, this is the first report on the comparative study of Scaligeria taxa and their subsequent essential oil chemical compositions.

EOs were analyzed both by GC-FID and GC-MS using a polar column, and constituents were compared to known compounds using the in-house Baser Library and Wiley GC/MS Library, MassFinder3 Library. As a result; one hundred and thirty three different compounds were identified in Scaligeria EOs, which constituted 59.7-100 % of the total oil. These compounds are listed in Table 2 with their relative percentages.

The use and utilization of EOs bearing plants in traditional medicine systems is being practiced since ancient times in human history [33]. Aerial parts of S. cretica (Mill.) Boiss. is used for splenomegaly and for jaundice in the Southwest Balkan, Peninsula and Asia Minor [34]. The Scaligeria species essential oil composition, various biological activity studies were reported in previous studies [17, 22, 35-39]. However, comprehensive studies on the Scaligeria genus are still very limited and not enough to clarify their existing taxonomic problems. In previous studies, some chemical components characteristic to Pimpinella spp. were also detected in S. tripartita and S. lazica species. For instance, S. tripartita fruit oil was rich in geijerenes and its root oil contained epoxypseudoisoeugynelangelate and geijerene; which were identified as antibacterial compounds by bioautography method [17]. It is well known that C12 sesquiterpenes (geijerene, pregeijerene) and phenylpropanoids (pseudoisoeugenyl 2-methylbutyrate, epoxypseudoisoeugenyl 2-methylbutyrate) are phytochemical markers for the Pimpinella genera [40-42]. In another study, while fruit oil of Scaligeria lazica was found as a rich source of (Z)-β-farnesene (89,2%), the oil of the aerial parts was rich in (E)-anethole (49.7%). In this present study, these substances were determined in SL-1, SL-2 and SL-3 oils. Also, geijerene, pregeijerene B, isogeijerene, cogeijerene known as C12 sesquiterpenes could be characterized in ST-1 oil, especially geijerene and pregeijerene B were the main constituents (Figure 1). Interestingly, geijerenes were previously detected in different families such as Asteraceae, Lamiaceae, Rosaceae, Rutaceae and Fabaceae. However, the geijerenes were present only in the Pimpinella genus of the Apiaceae family [41, 42-46]. The stems and leaves, fruit and root essential oil components of S. tripartita confirmed our results that ST-1 oil contained 36.8%, 54.6% and 28.5% geijerene, respectively [17]. Furthermore, (E)-anethole was identified as the main component in S. lazica essential oil of the aerial parts both in our study and in previously reported studies [35, 36]. Rowshan and Tarakemeh (2013) reported that main constituents of S. meifolia essential oil of the aerial parts collected from Iran were germacrene D, germacrene B and limonene. In our study, while α-copaene, cubebol, hexadecanoic acid were the major components for SM-1 oil, caryophyllene oxide, hexadecanoic acid, α-copaene and cubebol are components were the main components for the SM-2 oil, respectively (Table 3). However, the main components identified in S. meifolia collected from two different localities in our work, were different from the components reported in a previous work [39].
### Table 2. Relative percentages (%) of the essential oils of herb with flowering of *Scaligeria* species gathered from Turkey.

<table>
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<th>KI²</th>
<th>RRI³</th>
<th>Compound</th>
<th>SH-1</th>
<th>SC-1</th>
<th>SL-1</th>
<th>SL-2</th>
<th>SM-1</th>
<th>ST-1</th>
<th>SL-3</th>
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Table 2. (Continued).

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<th>KI&lt;sup&gt;a&lt;/sup&gt;</th>
<th>RRI&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Compound</th>
<th>SH-1</th>
<th>SC-1</th>
<th>SL-1</th>
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<th>SM-1</th>
<th>ST-1</th>
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a KI, b RRI, c Table 1 continued, d Eudesma-4(15)-7-dien-1β-ol, e Ref. 20, f Ref. 21

Table 2. (Continued).

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<th>SL-1</th>
<th>SL-2</th>
<th>SM-1</th>
<th>ST-1</th>
<th>SL-3</th>
<th>SM-2</th>
<th>SG-1</th>
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<td>(E)-Pseudoisoeugenyltiglate</td>
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<td>3.6</td>
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Total identified (%)  

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<th></th>
<th>SH-1</th>
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<th>SL-1</th>
<th>SL-2</th>
<th>SM-1</th>
<th>ST-1</th>
<th>SL-3</th>
<th>SM-2</th>
<th>SG-1</th>
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<tr>
<td></td>
<td>82.7</td>
<td>59.7</td>
<td>100.0</td>
<td>96.9</td>
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<td>90.6</td>
<td>97</td>
<td>88.9</td>
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</table>

* KI Kovats indices from literature c) [25], d) [26], e) [27], f) [28], g) [29], h) [30], j) [31], k) [32], * RRI: Relative retention indices calculated against n-alkanes for a polar column, % calculated from FID data, t Trace (< 0.1 %), *from Adams library
The EOs of the leaf and fruit of S. assyriaca Freyn & Bornm. were analysed. Germacrene D, β-Sesquiphellandrene and kessane were detected in both oil and identified ranging (21.1% and 13.7%), (10% and 8.7%) and (7.4% and 9.5%) in leaf and fruit oils, respectively [37]. Main components of the EO of S. cretica (Miller) Boiss. are α-pinene, β-farnesene and germacrene D, which were also detected in previous Scaligeria EOs studies [38]. In our earlier study on the EOs of the flowering aerial parts of S. napiformis, which was collected from two localities in Turkey were analyzed resulting in the characterization of (E)-β-farnesene, germacrene D, α-zingiberine and spathulenol as the main constituents. Overall, 46 compounds were reported from SN-1 and SN-2 aerial part EOs and spathulenol (10.6%) was identified as the main compound in Scaligeria sp. previously [18]. In another study it was suggested that the distribution of pseudoisoeugenol and propenylphenol derivatives could be used as a taxonomic markers for Pimpinella sp. [41]. In this present study, propenylphenol derivatives and also (E)-anethole was found as the major constituent in SL-1, SL-2 and SL-3 oils, respectively. However, S. lazica differed from the other Scaligeria species due to its phytochemical constituents and converged rather to the Pimpinella genus. In addition, geijerene derivatives were identified as the main compounds in the S. tripartita oil, which distinguished them from other Scaligeria species on account of this finding. In the other Scaligeria oil analysis, δ-cadinene was characterized as the main component, however only in the SC-1 oil, as seen in Tables 2-4.

Compared to previous studies of the herba and fruit of S. lazica oil collected from Rize-Camlıhemşin analyzed [35, 36], our present study on the flowering herba of S. lazica collected from three different localities were characterized as different in phytochemical volatile constituents, as seen in Table 1. The S. tripartita root, fruit, stem and leaf essential oil from Ordu was previously studied reported [17]. In this present study, the flowering herbal parts of S. tripartita was collected from Rize and compared to both oil contents as seen in Tables 2 and 4. In a recent report, S. napiformis flowering herba essential oil chemistry was published by the Baldemir and co-authors [22]. The essential oil of S. meifolia from Turkey were evaluated and samples were collected from two different localities for essential chemical analysis (Table 1). The chemistry of S. meifolia essential oil collected from Iran was reported [39], where the results were different from our present study in terms of main components (Table 2, Table 4). To the best of our knowledge there was no information on the oil analysis both of S. hermonis and S. glaucescens, which is the first chemo-systematic study among accepted Scaligeria species from Turkey.

### Table 3. Antimicrobial activity results of Scaligeria essential oils (mg/mL).

<table>
<thead>
<tr>
<th>Test sample</th>
<th>Bacillus cereus NRRL B-3711</th>
<th>Staphylococcus aureus ATCC 1026</th>
<th>Pseudomonas aeruginosa ATCC 10145</th>
<th>Candida albicans ATCC 90028</th>
<th>C. parapsilosis ATCC 22019</th>
<th>C. krusei ATCC 6258</th>
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</thead>
<tbody>
<tr>
<td>SC-1</td>
<td>0.32</td>
<td>0.32</td>
<td>0.64</td>
<td>0.64</td>
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<tr>
<td>SL-1</td>
<td>0.64</td>
<td>0.64</td>
<td>0.64</td>
<td>0.64</td>
<td>0.64</td>
<td>0.64</td>
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<tr>
<td>SL-2</td>
<td>0.64</td>
<td>0.64</td>
<td>0.64</td>
<td>0.64</td>
<td>0.64</td>
<td>0.64</td>
</tr>
<tr>
<td>SL-3</td>
<td>0.64</td>
<td>0.64</td>
<td>0.64</td>
<td>0.64</td>
<td>0.64</td>
<td>0.64</td>
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<tr>
<td>SH-1</td>
<td>0.32</td>
<td>&gt;2.56</td>
<td>2.56</td>
<td>2.56</td>
<td>2.56</td>
<td>2.56</td>
</tr>
<tr>
<td>ST-1</td>
<td>2.56</td>
<td>&gt;8.96</td>
<td>&gt;8.96</td>
<td>2.56</td>
<td>8.96</td>
<td>8.96</td>
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<tr>
<td>Amphoterin B</td>
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<td>-</td>
<td>8</td>
<td>64&gt;</td>
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<tr>
<td>Chloramphenicol</td>
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<tr>
<td>Ciprofloxacin</td>
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<td>16</td>
<td>2&gt;</td>
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</table>

**The quantitative amounts of SN-1, SN-2, SM-1, SM-2 and SG-1 essential oil were insufficient.**
Table 4. Previous reports of Scaligeria essential oils.

<table>
<thead>
<tr>
<th>Plant name</th>
<th>Part used</th>
<th>Main Compounds (%)</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td><em>S. tripartita</em></td>
<td>stems and leaves</td>
<td>Geijerene (36.8) (Z)-β-farnesene (9.4) β-Bisabolene (9)</td>
<td>[15]</td>
</tr>
<tr>
<td></td>
<td>fruit</td>
<td>Geijerene (54.6) Geijerene isomer not identified (12) β-Bisabolene (7.1)</td>
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</tr>
<tr>
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<td>root</td>
<td>4-methoxy-2-(3-methyl-oxiranyl) phenyl angelate (36.9) Geijerene (28.5) Dictamnol (9.4)</td>
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<tr>
<td><em>S. napiformis A</em></td>
<td>aerial parts</td>
<td>(E)-β-farnesene (22.2) Germacrene D (19.7) α-Zingiberene (5)</td>
<td>[18]</td>
</tr>
<tr>
<td><em>S. napiformis B</em>*</td>
<td>aerial parts</td>
<td>Germacrene D (32.7) Spathulenol (10.6) (E)-β-farnesene (7.4)</td>
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</tr>
<tr>
<td><em>S. lazica</em></td>
<td>aerial parts</td>
<td>(E)-Anethole (49.7) β-caryophyllene (19.3) (Z)-β-farnesene (10.2)</td>
<td>[27]</td>
</tr>
<tr>
<td><em>S. lazica</em></td>
<td>fruit</td>
<td>(Z)-β-farnesene (89.2) (E)-Anethole (1.6) δ-2-carene (0.9)</td>
<td>[28]</td>
</tr>
<tr>
<td><em>S. assyriaca</em></td>
<td>leaf</td>
<td>Germacrene D (21.1) β-caryophyllene (13.4) α-copaene (10.2)</td>
<td>[37]</td>
</tr>
<tr>
<td></td>
<td>fruit</td>
<td>Myristicin (24.3) Germacrene D (13.7) Elemicine (11)</td>
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<tr>
<td><em>S. cretica</em></td>
<td>aerial parts</td>
<td>Sabinene (13.7) β-farnesene (29.7) Germacrene D (28.4)</td>
<td>[38]</td>
</tr>
<tr>
<td><em>S. meifolia</em></td>
<td>aerial parts</td>
<td>Germacrene D (24.2) Germacrene B (14.8) Limonene (14.2)</td>
<td>[39]</td>
</tr>
</tbody>
</table>

*A: from Muğla (Köyceğiz) province **B: from Antalya (Akseki) province*

According to literature, EO and their constituents display a broad antibacterial activity spectrum, and could therefore be used in pharmaceutical industries against microbial resistance versus conventional antibiotics [1, 3–4, 8]. In this present study, according to the in vitro antibacterial results the tested oils except for ST-1 showed moderate antimicrobial activity but also SH-1 oil was only effective oil sample against *B. cereus*. SC-1 oil was more effective against Gram positive bacteria compared to other Scaligeria samples (Table 3). Possibly the antimicrobial effects of SL-1, SL-2 and SL-3 oils can be linked to the main component (E)-anethole (Figure 1). Also, this compound was used as chemical precursor of paramethoxyamphetamine in the clandestine manufacture of amphetamines [47]. It is well known that anethole is therapeutically an important compound, which was reported to have strong anti-inflammatory, antifungal, antibacterial, and antiplatelet activities among others [48-53].
4. Conclusion

Overall, GC-FID and GC-MS profiles of the investigated *Scaligeria* taxa exhibited important differences between the two taxa (*S. lazica* and *S. tripartita*) and other *Scaligeria* taxa (*S. napiformis*, *S. meifolia*, *S. capillifolia*, *S. hermonis*, *S. glaucescens*) highlighting the existence of different main chemical constituents. Thus, the results of this study certainly contributed to the taxonomy of the genus *Scaligeria* via essential oil chemistry. On the other hand, while germacrene D was determined as main component in the other *Scaligeria* species it can be accepted mainly to the discrimination on the *Scaligeria* genus, due to characteristic chemical compositions *S. lazica* and *S. tripartita*’s placement in *Scaligeria* is questionable. The status of *Scaligeria* taxa may become more evident, when other targeted studies such as on molecular, chemical analysis of the root and fruit, anatomical and morphological levels are completed.

Acknowledgments

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Supporting Information

Supporting information accompanies this paper on [http://www.acgpubs.org/RNP](http://www.acgpubs.org/RNP)

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**References**

Chemical composition of the essential oil and antimicrobial activity of Scaligeria sp.


