The Chemical Composition of the Essential oil, SPME and Antimicrobial Activity of *Rhododendron caucasicum* Pall.

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Abstract: The aim of this research was to investigate the effect of different extraction methods and chemical composition of the essential oil and solid-phase microextraction (SPME) from *Rhododendrum caucasicum* Pall. The volatiles of *R. caucasicum* have been isolated by hydro distillation (HD) and SPME. The compositions of the volatiles were characterized by GC-FID/MS. A total of twenty-five and thirty-one compounds were identified constituting over 89.25%, and 90.33% of volatiles obtained with HD and SPME, respectively. The main volatile constituents of *R. caucasicum* were found to be calarene (46.13% (HD) and 54.91% (SPME)) and sandaracopimaradiene (25.93% (HD) and 8.16% (SPME)). Furthermore, the obtained essential oil (EO) and solvent extracts (n-hexane and methanol) of *R. caucasicum* were tested against the following nine bacteria: *Escherichia coli*, *Yersinia pseudotuberculosis*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus cereus*, *Mycobacterium smegmatis*, *Candida albicans*, and *Saccharomyces cerevisiae*. The EO showed moderate antimicrobial activities with the inhibition zone from 6 to 18 mm against *E. faecalis*, *S. aureus*, *B. cereus* and *M. smegmatis*, respectively. Methanol extract gave better antimicrobial activity against the *P. aeruginosa*, *E. faecalis*, *S. aureus*, and *B. cereus* with the almost 15 mm inhibition zones.

Keywords: *Rhododendron caucasicum*; hydrodistillation; SPME; essential oil; antimicrobial activity; GC-FID/MS. © 2019 ACG Publications. All rights reserved.

1. Introduction

Euro-Siberian, Irano-Turanian, and Mediterranean are three major regions of Turkey flora. Rize city exists at the Colchis part of Euro-Siberian flora region. *R. caucasicum* Pall, *Rhododendron ponticum* L., *Rhododendron smirnovii* Trautv., *Rhododendron luteum* Sweet., and *Rhododendron ungerii* Trautv. plants were determined also in the plant group of Colchis by the researches [1-3]. *R. caucasicum* is an endemic plant of Ericaceae evergreen bush of family that is native to the Caucasus.

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Rhododendron is believed to be the most diverse genus with more than 1000 species in worldwide [4] and more than 400 of them are in Asia. The smaller percentage grows in high, cool, and rainy regions of Europe, North America, and Australia. Rhododendrons, commonly known as rosage or by the folk names ‘black poison or komar’, are members of Ericaceae family. There are nine Rhododendron species growing naturally in Turkey and especially in East Blacksea Region, namely R. luteum, R. ponticum, R. smirnovii, R. caucasicum, R. ungerii [1] and R. x rosifaciensis, R. x davisiunum, R. x filidactylis, R. x sochadzeae [5]. They are evergreen small trees with green leaves and have flowers of different colors and an aesthetically important role in landscape. Although popularly known to be toxic among public, previous studies on the pharmacological activities of Rhododendron species indicated that they contain potent antioxidative compounds [3-4, 6-7]. The sap obtained from young parts of R. ponticum is dropped into tooth cavity against toothache in Turkish people prescription [2, 7-8]. The blossoms of another Rhododendron species have likewise been recorded in old and present-day monographs as pain relieving and bug sprays in Chinese customary medication [9]. Dissimilar to different sorts of Rhododendron; R. caucasicum is one of a kind in light of the fact that among the more than 1200 Azalea/Rhododendron species around the world, almost every one of them are dangerous and ought to not be devoured by people. Clinical preliminaries have demonstrated R. caucasicum to be protected and compelling for human utilization. The R. caucasicum is a moderately obscure weight reduction and life span health supplement that has been affirmed safe and incredibly successful. It is one of only a handful couple of Rhododendrons that has a background marked by territorial utilize spreading over 400 years. Essential oils in the plant are complex volatile mixtures obtain at low concentrations. Prior to investigating the oils, they must be obtained from the plant. A few extraction forms have been utilized to get the high return of parts [10-17].

The EO composition of R. caucasicum has been recently mentioned. The main constituent was identified as 8(14),15-pimaradiene (27%, sandaracopimaradiene) [18]. But, the antimicrobial activity for the EO and solvent extracts (n-hexane and methanol) of R. caucasicum have not been investigated [18]. Result of this work showed quantitative differences for the volatile compositions of R. caucasicum due to number of factors that are; geographical location, part of the plant that was used, degree of ripeness and age, production method, etc.

The present work aims at investigation of volatiles compounds of EO and SPME and find out antimicrobial activity behavior for the EO and solvent extracts (n-hexane and methanol) obtained from of R. caucasicum

2. Materials and Methods

2.1. Plant Material

R. caucasicum was harvested from Uzungöl, Trabzon-Turkey in September 2014. The plant was authenticated by Prof. S. Terzioglu [19-21]. Voucher specimen was deposited in the Herbarium of the Faculty of Forestry, KATO (KATO: 12171), Karadeniz Technical University, Turkey.

2.2. Hydro Distillation (HD) Procedure

The fresh aerial part of plant material (100 g) was grounded into small pieces and submitted to hydrodistillation (HD) using a Clevenger-type apparatus with cooling bath (-15 °C) system (4h) (yield (v/w): 0.035%). The obtained oil was extracted with n-hexane (0.5 mL) and dried over anhydrous sodium sulphate and stored at -5 °C in a sealed brown vial.

2.3. Solid Phase Micro Extraction (SPME) Procedure

The fresh plant material (2g) were grounded into small pieces and place to a sealed vial (10 mL) with a silicone-rubber septum cap then submitted to head space solid-phase micro extraction
device (Supelco, USA). A DVB/Carboxen/PDMS coating fiber was placed to the head space and used to obtain volatile components. The SPME fibers were conditioned for 5 min at 250 °C in the GC injector. Extraction were achieved with magnetic stirring at 80 °C using an incubation time of 5 min and an extraction time of 10 min. Fiber with extract of volatile compounds were subsequently injected into the GC injector. The carrier gas used was helium at a flow rate of 1 mL/min. The injection was performed in split mode (1:30) at 250 °C. Sample was analyzed and reported. The temperature, incubation and extraction time were set according to the reported experiment [12].

2.4. Gas Chromatography-Mass Spectrometry (GC-FID/MS)

The gas chromatography-flame ionization detector (GC-FID) analysis was carried out on a Shimadzu QP2010 plus gas chromatography equipped with a flame ionization detector (FID, 70 eV) using a Rtx-5MS capillary column (30 m x 0.25 mm, film thickness, 0.25 μm). Shimadzu QP2010 Plus gas chromatograph was coupled to a Shimadzu QP2010 Ultra mass selective detector. Split mode was employed, and split ratio was 1:30. The oven program was as follows: initial temperature was 60°C for 2 min, which was increased to 240°C at 3 min, final temperature 250°C was held for 4 min and total analysis time of 62 minutes. The injector and mass transfer line temperatures were set at 280 °C and 250 °C, respectively. Helium (99.999%) was used as carrier gas with a constant flow-rate of 1 mL/min. Detection was carried out in electronic impact mode (EI); ionization voltage was fixed to 70 eV and scan mode (40-450 m/z) was used for mass acquisition [12].

2.5. Solvent Extraction

20 Grams of wrapped plant in filter paper were refluxed in Soxhlet apparatus with n-hexane and methanol separately. The extraction was continued for 5 hours. Then, the solvents in the flask were evaporated, and the amounts of crude extract were calculated [22].

2.6. Identification of Constituents

Retention indices of all of the components were determined by the Kovats method, using n-alkanes (C<sub>6</sub>-C<sub>36</sub>) as standards. The constituents of the oils were identified by comparison of their mass spectra with those of mass spectral libraries (NIST and Wiley 7NL), authentic compounds (linalool, α-terpineol, β-selinene, copaene, aromadendrene, aristolene, calarene, decane, tridecane, tricosane, heneicosane) and data published in the literature [2,12,19-28].

2.7. Antimicrobial Activity Assessment

All test microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: Escherichia coli ATCC 25922, Klebsiella pneumoniae ATCC 13883, Yersinia pseudotuberculosis ATCC 911, Serratia marcescens ATCC 13880, Enterococcus faecalis ATCC 29212, Staphylococcus aureus ATCC 25923, Bacillus subtilis ATCC 6633, Candida albicans ATCC 60193, Candida tropicalis ATCC 13803. Essential oil was weighed and dissolved in acetone to prepare extract stock solution of 1000 µg/mL.

2.8. Agar-well Diffusion Method

Simple susceptibility screening test using agar-well diffusion method as adapted earlier [23, 24] was used. Each microorganism was suspended in Brain Heart Infusion (BHI) (Difco, Detroit, MI) broth and diluted approximately 10<sup>6</sup> colony forming unit (cfu) per ml. They were “flood-inoculated” onto the surface of BHI agar and Sabouraud Dextrose Agar (SDA) (Difco, Detroit, MI) and then dried. For C. albicans, C. tropicalis, SDA were used. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 100 µL of the extract substances were delivered into the wells. The plates were incubated for 18 h at 35°C. Antimicrobial activity was evaluated by measuring the zone of
inhibition against the test organism. Ceftazidime (Fortum) (10 µg) and Triflucan (5 µg) were standard drugs. Acetone was used as solved control. The tests were carried out in duplicate. Results were interpreted in terms of the diameter of the inhibition zone: (-): < 5.5 mm; (+): 5.5-10 mm; (++): 11-15 mm; (+++): ≥ 16 mm. The results are shown in Table 4.3.

3. Results and Discussion

*R. caucasium* is a naturally growing plant in Trabzon province of Turkey. The EO that is obtained by HD (Clevenger-type apparatus) and SPME of *R. caucasium* were analyzed by GC-FID/MS. The RTX-5M column was used in GC and the volatile compounds of *R. caucasium* were identified based on a typical library search match exceeding 80% [13-17, 23]. The identity, retention time, and the percentage of composition of the EO and SPME obtained from the *R. caucasium* is presented in Table 1. A total of twenty-five and thirty-one compounds from *R. caucasium* were identified and quantified, accounting 89.25% and 90.33% ratio, respectively. The first step in our study was to compare the volatile contents of two different extraction techniques, namely as EO and SPME methods, obtained from the crude plant material.

Calerene (46.13%), sandaracopimaradiene (25.93%), *_epi*-globulol (1.35%) were found as the major compounds in the EO of *R. caucasium*. The chemical class distribution of the EO and SPME of *R. caucasium* components were separated into eight classes, which were monoterpenoids, sesquiterpenes, sesquiterpenoids, diterpene, aldehydes, alcohols, hydrocarbons and others (Table 1). Monoterpenoids constituted 1.37% and the major compound of the monoterpenoids was linalool (0.40%), the ratio of the sesquiterpenes was 48.75% and the main component of the sesquiterpenes was calerene (46.13%) and the sesquiterpenoids constituted 2.2% and the major representative of sesquiterpenoids was *_epi*-globulol (1.35%), the ratio of diterpenes was 26.44% and the main component of the sesquiterpenes was sandaracopimaradiene (25.93%). The ratio of the other compounds was 0.6% in the EO of *R. caucasium*. The results of the terpene analyses showed that sesquiterpenoids are the main constituents (48.75%) for the EO of *R. caucasium*.

In the second part of the work, solid phase micro-extraction GC-FID/MS analysis gave thirty-one volatile components. HS-SPME GC-MS analysis allowed the examination of all the plant material under the comparable conditions in spite of the fact that it’s far reaching piece. This is not constantly practicable with other extraction techniques. Utilizing an apolar poly (dimethylsiloxane) (PDMS) stage, various terpenoid hydrocarbons, together with alcohols, cyclic ethers, and esters were extracted. The usability and the high goals of the chromatographic profiles got make HS-SPME appropriate to the quick portrayal of the primary segments of the unpredictable division of plants [31]. Interestingly, calerene and sandaracopimaradiene were found to be in high amount for all two methods. The general chemical profile of the SPME of *R. caucasium* was summarized in Table 1. These volatile components consist of 3 and 4 monoterpenoids, 5 and 8 sesquiterpenes, 3 and 0 sesquiterpenoids, 1 and 2 diterpenes, 7 and 8 aldehydes, 1 and 5 alcohol, 3 and 1 hydrocarbons, and 2 and 3 other compounds in the EO and SPME of *R. caucasium*, respectively (Table 1).

Sesquiterpenes (46.13% and 60.57%) and diterpene (26.44% and 8.1%) were found to be the major groups of all compounds in the EO and SPME of *R. caucasium*, respectively (Table 2). Some classes and hybrids of plants were noted to be valuable sources of EOs, from which sesquiterpenes, aldehydes, phenols, carbohydrates and lipids and such individual compounds as carbazol, citronellol, engenol, geraniol, coumarin, linalool, citral, nerol, safrole, linalyl acetate, terpineol, lavandulol, patchouline, isomenthol, borneol, methyl anthranilate, benzoic acid, citronellic acid and camphor were isolated [2]. In our case, we detected similar result with different ratios, which could be owing to geographical origins and the climates [2].

Additionally, SPME GC-MS analysis gave the calerene (54.91%), sandaracopimaradiene (8.1%), *n*-nonanal (4.39%) and phenethyl alcohol (3.18%) were found as main components, respectively.
### Table 1. Identified volatile components in the EO and SPME of *R. caucasicum*

<table>
<thead>
<tr>
<th>Compounds</th>
<th>HD Area (%)</th>
<th>SPME Area (%)</th>
<th>Ex. °RI</th>
<th>Lit. RI</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Monoterpenoids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>trans</em>-Linalool Oxide</td>
<td>0.25</td>
<td>0.33</td>
<td>1080</td>
<td>1073</td>
<td>[2]</td>
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<tr>
<td><em>Endo</em>-Borneol</td>
<td>-</td>
<td>0.38</td>
<td>1170</td>
<td>1169</td>
<td>[1]</td>
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<td><em>α</em>-Terpineol</td>
<td>0.34</td>
<td>0.46</td>
<td>1190</td>
<td>1189</td>
<td>[1]</td>
</tr>
<tr>
<td>Linalool</td>
<td>0.40</td>
<td>1.01</td>
<td>1100</td>
<td>1097</td>
<td>[2]</td>
</tr>
<tr>
<td><strong>Sesquiterpenoids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copaene</td>
<td>-</td>
<td>0.36</td>
<td>1380</td>
<td>1377</td>
<td>[2]</td>
</tr>
<tr>
<td>Aristolene</td>
<td>1.73</td>
<td>2.36</td>
<td>1431</td>
<td>1427</td>
<td>[5]</td>
</tr>
<tr>
<td>Calarene</td>
<td>46.13</td>
<td>54.91</td>
<td>1447</td>
<td>1444</td>
<td>[5]</td>
</tr>
<tr>
<td>Aromadendrene</td>
<td>0.35</td>
<td>0.43</td>
<td>1461</td>
<td>1461</td>
<td>[6]</td>
</tr>
<tr>
<td>Valencene</td>
<td>0.36</td>
<td>0.94</td>
<td>1465</td>
<td>1464</td>
<td>[18]</td>
</tr>
<tr>
<td>γ-Murolene</td>
<td>-</td>
<td>0.65</td>
<td>1482</td>
<td>1480</td>
<td>[3]</td>
</tr>
<tr>
<td>β-Selinene</td>
<td>0.18</td>
<td>0.52</td>
<td>1492</td>
<td>1490</td>
<td>[3]</td>
</tr>
<tr>
<td>α-Murolene</td>
<td>-</td>
<td>0.40</td>
<td>1511</td>
<td>1507</td>
<td>[4]</td>
</tr>
<tr>
<td><strong>Diterpenes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kaur-16-ene</td>
<td>-</td>
<td>0.51</td>
<td>2067</td>
<td>2070</td>
<td>[9]</td>
</tr>
<tr>
<td><strong>Aldehydes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caproaldehyde</td>
<td>-</td>
<td>0.57</td>
<td>806</td>
<td>806</td>
<td>[16]</td>
</tr>
<tr>
<td>2(E)-Hexenal</td>
<td>0.40</td>
<td>-</td>
<td>854</td>
<td>854</td>
<td>[24]</td>
</tr>
<tr>
<td><em>n</em>-Heptanal</td>
<td>0.36</td>
<td>-</td>
<td>903</td>
<td>903</td>
<td>[12]</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>0.25</td>
<td>0.88</td>
<td>961</td>
<td>961</td>
<td>[12]</td>
</tr>
<tr>
<td><em>n</em>-Octanal</td>
<td>0.23</td>
<td>1.41</td>
<td>1004</td>
<td>1004</td>
<td>[12]</td>
</tr>
<tr>
<td>Phenyl acetaldehyde</td>
<td>-</td>
<td>0.25</td>
<td>1047</td>
<td>1046</td>
<td>[12]</td>
</tr>
<tr>
<td><em>n</em>-Nonanal</td>
<td>6.34</td>
<td>4.39</td>
<td>1103</td>
<td>1103</td>
<td>[12]</td>
</tr>
<tr>
<td><em>n</em>-Decanal</td>
<td>-</td>
<td>0.92</td>
<td>1208</td>
<td>1207</td>
<td>[19]</td>
</tr>
<tr>
<td>2(E)-Decenal</td>
<td>0.33</td>
<td>0.26</td>
<td>1264</td>
<td>1265</td>
<td>[13]</td>
</tr>
<tr>
<td><em>n</em>-Undecanal</td>
<td>0.65</td>
<td>0.46</td>
<td>1309</td>
<td>1309</td>
<td>[12]</td>
</tr>
<tr>
<td><strong>Alcohols</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1-Octen-3-ol</td>
<td>0.36</td>
<td>1.45</td>
<td>984</td>
<td>981</td>
<td>[12]</td>
</tr>
<tr>
<td>Benzyl alcohol</td>
<td>-</td>
<td>1.94</td>
<td>1040</td>
<td>1039</td>
<td>[12]</td>
</tr>
<tr>
<td>1-Octanol</td>
<td>-</td>
<td>0.33</td>
<td>1072</td>
<td>1074</td>
<td>[12]</td>
</tr>
<tr>
<td>Phenethyl alcohol</td>
<td>-</td>
<td>3.18</td>
<td>1113</td>
<td>1107</td>
<td>[3]</td>
</tr>
<tr>
<td>Lilac alcohol</td>
<td>-</td>
<td>0.28</td>
<td>1218</td>
<td>1220</td>
<td>[17]</td>
</tr>
<tr>
<td><strong>Hydrocarbons</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decane</td>
<td>0.33</td>
<td>-</td>
<td>1004</td>
<td>1000</td>
<td>[19]</td>
</tr>
<tr>
<td>Tridecane</td>
<td>-</td>
<td>0.27</td>
<td>1299</td>
<td>1300</td>
<td>[19]</td>
</tr>
<tr>
<td>Heneicosane</td>
<td>0.68</td>
<td>-</td>
<td>2099</td>
<td>2100</td>
<td>[2]</td>
</tr>
<tr>
<td>Tricosane</td>
<td>0.89</td>
<td>-</td>
<td>2299</td>
<td>2300</td>
<td>[2]</td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-Methyl-5-Heptene-2-one</td>
<td>0.22</td>
<td>-</td>
<td>985</td>
<td>985</td>
<td>[20]</td>
</tr>
<tr>
<td>3-Octanone</td>
<td>-</td>
<td>0.35</td>
<td>991</td>
<td>988</td>
<td>[12]</td>
</tr>
<tr>
<td>γ-Hexalactone</td>
<td>-</td>
<td>0.31</td>
<td>1060</td>
<td>1057</td>
<td>[21]</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>-</td>
<td>1.72</td>
<td>1163</td>
<td>1165</td>
<td>[19]</td>
</tr>
<tr>
<td>Nonanoic acid</td>
<td>0.34</td>
<td>-</td>
<td>1270</td>
<td>1273</td>
<td>[14]</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>89.25%</td>
<td>90.33%</td>
<td></td>
</tr>
</tbody>
</table>

*°RI calculated from retention times relative to that of n-alkanes (C₆-C₃₂) on the non-polar RTX-5M column.*
Table 2. Classification and total area of volatile compounds in the EO and SPME of *R. caucasicum*

<table>
<thead>
<tr>
<th></th>
<th>HD</th>
<th>SPME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoterpenoids</td>
<td><em>endo</em>-borneol, linalool, α-terpineol, trans-linalool oxide.</td>
<td>linalool, α-terpineol, trans-linalool oxide.</td>
</tr>
<tr>
<td>Total</td>
<td>1.37%</td>
<td>1.08%</td>
</tr>
<tr>
<td>Sesquiterpenes</td>
<td>β-selinene, copaene, aromadendrene, aristole, calarene, valencene</td>
<td>β-selinene, α-muurolene, γ-muurolene, copaene, aromadendrene, aristole, calarene, valencene</td>
</tr>
<tr>
<td>Total</td>
<td>48.75%</td>
<td>60.57%</td>
</tr>
<tr>
<td>Sesquiterpenoids</td>
<td>valerenal, epi-globulol, 10-epi-γ-eudesmol</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>2.2%</td>
<td>-</td>
</tr>
<tr>
<td>Diterpene</td>
<td>Kaur-16-ene, sandaracopimaradiene</td>
<td>Sandaracopimaradiene</td>
</tr>
<tr>
<td>Total</td>
<td>26.44%</td>
<td>8.1%</td>
</tr>
</tbody>
</table>

Calarene (46.13% and 54.91%) and sandaracopimaradiene (25.93% and 8.1%) were identified as major components in the EO and SPME of *R. caucasicum*, respectively. Their chemical formulas are shown in Figure S1 (see supporting information) and they could provide a chemotaxonomic marker for the EO and SPME obtained from *R. caucasicum* grown in Turkey.

In conclusion, in both techniques of HD and SPME were richest in sesquiterpenes (especially, calarene) and diterpenes (sandaracopimaradiene), followed by monoterpenoids. And also, the amount of identified components and the number of compounds looks almost equal (Table 2).

In the literature, the EO contents of *R. caucasicum* were reported and 66 constituents were mentioned with a total of 91.6% ratio. 8(14), 15-pimaradiene (27%) was found as main compounds [18]. Indeed, we found the calarene (46.13% in the EO and 54.91% in SPME) as major compound in both EO and SPME of *R. caucasicum*. However, second major compound in our work was the same as previous report [18]. Identification of different major compound could be explained by the location and so on.

The antimicrobial activity of the essential oil of *R. caucasicum* was tested in vitro using the agar-well diffusion method with the microorganisms as seen in Table 3. The essential oil of *R. caucasicum* showed the antimicrobial activity against gram-positive bacteria (*S. aureus, B. cereus*), acid-alcohol resistant bacterium *M. smegmatis* and *E. faecalis*. The antimicrobial activity of the *n*-hexane extract of *R. caucasicum* showed the antimicrobial activity only against *M. smegmatis* while methanol extract was active against *P. aeruginosa, E. faecalis, S. aureus*, and *B. cereus*.

Table 3. Screening for the antimicrobial activity of essential oil, *n*-hexane and methanol extracts of *R. caucasicum* (μg/μL).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Stock Solution (μg/μL)</th>
<th>Microorganisms and inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ec</td>
</tr>
<tr>
<td>Essential oil</td>
<td>29380</td>
<td>-</td>
</tr>
<tr>
<td><em>n</em>-Hexane extract</td>
<td>38400</td>
<td>-</td>
</tr>
<tr>
<td>Methanol extract</td>
<td>40000</td>
<td>-</td>
</tr>
<tr>
<td>Amp.</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Strep.</td>
<td>35</td>
<td>25</td>
</tr>
<tr>
<td>Flu</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/rec-nat-products

References


