Volatile and Phenolic Contents, Antimicrobial and Tyrosinase activities of Two Endemic Species *Scorzonera pisidica* and *Scorzonera sandrasica* L. Grown in Turkey

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Abstract: Phytochemical analysis of two endemic *Scorzonera pisidica* Hub.-Mor. and *Scorzonera sandrasica* Hartvig & Strid species have not been mentioned before. In this work, volatile organic compounds, phenolic contents, antimicrobial, and tyrosinase inhibition activities of two endemic *S. pisidica* and *S. sandrasica* grown in Turkey were investigated. Aldehydes were the primary chemical class for the volatile organic compounds in the essential oils (EOs, 49.5%, and 44.9%) and SPME (85.8% and 56.9%) of *S. pisidica* and *S. sandrasica*, and aromatic compounds were the main class for the SPME of the *n*-hexane extracts of *S. pisidica* (86.9%) and *S. sandrasica* (86.3%), respectively. The phenolic constituent analysis for the methanol extract of *S. pisidica* and *S. sandrasica* gave gallic acid (6.33 mg/g and 2.63 mg/g) as the primary compound. The antimicrobial activity of the EOs and solvent extract (methanol and *n*-hexane) of *S. pisidica* and *S. sandrasica* were tested against nine microorganisms. Furthermore, the inhibitory potential for the methanol extract of the *S. pisidica* and *S. sandrasica* showed tyrosinase activity, and IC$_{50}$ values were found as 0.495±0.0073 µg/mL and 0.699±0.86 µg/mL, respectively.

Keywords: *Scorzonera pisidica*; *Scorzonera sandrasica*; volatile constituents; phenolic compounds; biological activities. © 2021 ACG Publications. All rights reserved.

1. Introduction

*Scorzonera* L., a member of the Asteraceae, grows mainly in dry areas throughout the Mediterranean and central Asia and includes 175 species. The genus includes up to 52 species in Turkey, and 32 of them are endemics [1-4]. *Scorzonera* species have been widely used as a traditional herbal medicine to treat lung diseases, colds, wounds, gastrointestinal disorders, stomach, diuretic, antipyretic and appetizing effects in Europe [5] and Chinese [5-7] traditional medicine. The extract of *Scorzonera austriaca* has been used as general medicine to treat hepatitis [6].

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In the literature, the neurobiological effects of Scorzonera taxa were investigated from the aerial parts of S. pisidica displayed the highest tyrosinase inhibition among the other Scorzonera species [8]. Besides, the antimicrobial activity of the n-hexane, chloroform, ethyl acetate, and ethanol extracts obtained from the aerial parts of S. sandrasica has been mentioned, and ethanol and chloroform extracts exhibited significant activity against multi-resistant strains of Stenotrophomonas maltophilia [9]. The major compounds of the chloroform extract of the S. sandrasica were reported to be caryophyllene oxide (19.7%), manoyl oxide (16.5%), and manool (11.3%) [9]. Furthermore, inhibition of quorum sensing-regulated behaviors of S. sandrasica was reported [10]. Generally, EOs of the Scorzonera species have not been mentioned in the literature. The previous search for the Scorzonerae taxa had shown the identification of phenolic compounds [11-17] by high-performance liquid chromatography (HPLC) analysis. Phenolic constituents have many beneficial effects on human health. Thus, investigations of the phenolic compounds in natural plants have become a topic of interest. The various phytochemical composition and biological activities of Scorzonera species (Scorzonera hispanica L., S. judaica Eig., S. latifolia (Fisch. & Mey.), S. laciniata (L.), S. acuminata, S. cana var. alpina, S. cana var. radicosa, S. eriophora, S. laciniata ssp. laciniata, S. suberosa ssp. suberosa, S. sublanata, S. crispatula Boiss., S. papposa DC., S. suberosa C. Koch, S. trachysperma Guss., S. acuminata, S. veratrifolia, S. cana var. jacquiniana, S. cretica, S. parviflora, S. cana var. radicosa, S. eriophora, Scorzonera incisa, S. mollis ssp. szovitsii, S. cinerea, and S. tomentosa) have been mentioned [18-27]. However, many studies in the field have shown that the plants' volatile and phenolic components can be influenced by environmental factors such as land, altitude, and temperature [11-31].

To date, no evaluation of the qualitative and quantitative content of volatile organic compounds for the S. pisidica and S. sandrasica is available in the literature. The purpose of this study is to evaluate the extent of the variations for the VOCs/phenolic compounds and biological activities for the EOs and solvent extracts (methanol and n-hexane) obtained from two endemic Scorzonera taxa distributed in North-Eastern Anatolia. This article presents the first report on volatile chemical evaluations and biological activities (antimicrobial and tyrosinase) for the S. pisidica and S. sandrasica grown in Turkey.

2. Materials and Methods

2.1. Plant Material

Scorzonera pisidica Hub.-Mor., and Scorzonera sandrasica Hartvig & Strid were collected (250 g, each) on June 23\textsuperscript{th}, 2017, in the flowering stage from two different localities of Muğla-Turkey. S. pisidica collected from Muğla (Köyceğiz, Sandras Mountain, beside the Topuklu-Fire tower under the Pinus species at heights of 1655 m). S. sandrasica was also collected from Muğla (Köyceğiz village, Beşparmak mountain-Fire tower near the rocky slopes-forest border at the heights of 2025m) [1-4]. The plants were authenticated by Prof. Kamil Coşkunçelebi. Voucher specimens (Coşkunçelebi-Makbul 231 and 232) deposited in the Herbarium of the Department of Forest Botany (KATO), Karadeniz Technical University (KATO-22400 and 22401).

2.2. Hydrodistillation (HD) Procedure for the Isolation of EOs

EOs of S. pisidica and S. sandrasica obtained from the dried plant (3x, 70 g, 65 g, and 60 g each, respectively) by hydrodistillation in a modified Clevenger-type apparatus with a cooling bath (-10 \textdegree C) system (3 h) [average yields: 0.12\% and 0.023\% (w/w), respectively]. The obtained oils dissolved in HPLC grade n-hexane (1 mL), dried over anhydrous sodium sulfate, and stored at 4-6 \textdegree C in a sealed brown vial.
Phytochemical composition and biological activity of *S. pisidica* and *S. sandrasica*

2.3. Solvent Extractions (Methanol and n-Hexane) of *S. pisidica* and *S. sandrasica*

The dried plants of *S. pisidica* and *S. sandrasica* (2.5 g, each) were grounded and extracted (x3 times) with HPLC grade MeOH (5 mL, each) and n-hexane (5 mL each) at room temperature. The crude methanol and n-hexane solutions were filtered through a 0.45 μm filter and concentrated under reduced pressure using a rotary evaporator to give crude methanol (58.2 mg and 82.3 mg) and n-hexane (30 mg and 44 mg) extracts, respectively.

2.4. Solid Phase Micro Extraction (SPME)

The fiber coating was placed to the headspace for temperature and times (incubation and extraction times) values set according to the experiment. A polydimethylsiloxane/carboxen/divinylbenzene coating fiber (PDMS/Carboxen/DVB, 50/30 μm, Supelco, USA) was used for the extraction of the volatile components. Before the SPME analysis, the fibers were conditioned for 5 min at 250 °C in the GC injector. Fresh plants (*S. pisidica* and *S. sandrasica*, 1.00 g each) were transferred into a 10 mL vial. SPME was done at 50 °C with an incubation time of 5 min and an extraction time of 10 min. Each sample was analyzed, and means were reported. The fiber-containing the extracted volatile organic compounds were then injected into the GC-MS injector (split mode, 1:30). The sample was analyzed and reported. The temperature, incubation, and extraction time were set according to the reported experiment [28, 29].

2.5. Gas Chromatography-Mass spectrometry (GC-FID/MS)

EO analysis was carried out using a Shimadzu QP2010 ultra GC-FID/MS, Shimadzu 2010 plus FID, fitted with a PAL AOC-5000 plus autosampler a Shimadzu Class-5000 Chromatography Workstation software. The separation was analyzed using a Restek Rxi-5MS capillary column (30 mm x 0.25 mm x 0.25 μm) (USA). GC-FID/MS analysis of EOs were performed in split mode (1:30) at 230 °C. The essential oil solutions (1 μL) in n-hexane (HPLC grade) were injected and analyzed with the column held initially at 60 °C for 2 min and then increased to 240 °C with a 3 °C/min heating ramp. The oven program was as follows: the initial temperature was 60 °C for 2 minutes, which was increased to 240°C at 3 minutes, the final temperature of 250 °C was held for 4 minutes. Helium (99.999 %) was used as carrier gas with a constant flow-rate of 1 mL/min. Detection was implemented in electronic impact mode (EI); ionization voltage was fixed at 70 eV, scan mode (40-450 m/z) was used for mass acquisition.

2.6. Identification of Volatile Constituents

Retention indices of the volatile components of *S. pisidica* and *S. sandrasica* were determined by the Kovats method using n-alkanes (C₆-C₃₂) as standards. Volatile compounds were identified by comparisons with literature RI and authentic compounds (α-pinene, β-pinene, linalool, undecane, dodecane, tridecane, tetradecane, pentadecane, hexadecane, eicosane, heneicosane, docosane, and tricosane) and with the literature [28-32]. MS compared to existing analytical standards and matching mass spectral libraries (NIST, Wiley7NL, FFNSC1.2, and W9N11).

2.7. HPLC analyses of *S. pisidica* and *S. sandrasica*

HPLC chromatographic analysis of phenolic compounds of *S. pisidica* and *S. sandrasica* carried out at Shimadzu Prominence series HPLC instrument using Zorbax Eclipse Plus-C18 (150 mm x 4.6 mm, 5 μm) analytical column. The mobile phase was formed from methanol (A) and 2% acetic acid solution (A, pH: 2.65) and ultra-pure water (B). The gradient applied is as follows: 0 min, 80% B; 4 min, 70% B; 7 min, 60% B; 10 min, 55% B; 12 min, 50% B; 14 min, 40% B; 16 min, 20% B. The sample injection volume is 20 μL, and the flow rate is 1.5 mL/min. The column furnace temperature is set at 25 °C. The photodiode array was detected at a wavelength of 270 nm.
2.8. Antimicrobial Activity

All test microorganisms were obtained from the Hifisizhia Institute of Refik Saydam (Ankara, Turkey). They were as: Escherichia coli ATCC35218, Yersinia pseudotuberculosis ATCC911, Pseudomonas aeruginosa ATCC43288, Enterococcus faecalis ATCC29212, Staphylococcus aureus ATCC25923, Bacillus cereus 709 Roma, Mycobacterium smegmatis ATCC607, Saccharomyces cerevisiae RSKK 251, and Candida albicans ATCC60193 [33-34]. EOs, methanol, and n-hexane extract were weighed, and stock solutions (31.3-16.4 μg/mL) were prepared in n-hexane and methanol, respectively. The experimental condition was described as before [30, 31].

2.9. Tyrosinase Inhibition Assay

The mushroom tyrosinase inhibition of S. pisidica and S. sandrasica was investigated by monitoring dopachrome formation via 3,4-Dihydroxy-L-phenylalanine (L-DOPA) oxidation. Kojic acid was used as a standard. Extracts (80 μL each) of S. pisidica and S. sandrasica in phosphate buffer (100 mM, pH 6.8) was incubated with 10 μL mushroom tyrosinase (500 U/mL) at 30 °C for 20 minutes, then 15 μL of L-DOPA (5 mM) added and the mixture was incubated for an additional 30 min. at 30 °C. Subsequently, the absorbance was measured at 470 nm using a 96-well microplate reader. Tyrosinase inhibition was calculated with the known equation [35, 36]. The extracts concentration giving 50% (IC50) of the original tyrosinase activity was determined.

3. Results and Discussion

The EOs, SPME, and SPME of n-hexane extract of the S. pisidica and S. sandrasica were analyzed by GC-MS with Rxi-SMS column. Identification of the VOCs made by a typical library search (NIST, Wiley7NL, FFNSC1.2, and W9N11) and literature comparison [17, 28-31]. The general chemical profile of the EOs, the percentage content, and retention indices of the constituents are summarized in Table 1.

In total, 44, 13, and 37 constituents in S. pisidica and 43, 12, and 33 compounds in S. sandrasica were identified and represented an average of 99.0% to 99.4% of the EOs, SPME, and SPME of n-hexane extracts in S. pisidica and S. sandrasica, respectively (Table 1). The present study's data demonstrated that the component of the EOs in S. pisidica and S. sandrasica varied significantly with elevation, as seen in Table 1. Predominant compounds were found to be monoterpenes (12.8%) and monoterpenoids (15.1%) among all terpenes in the EOs, SPME, and SPME of n-hexane extract of S. pisidica and S. sandrasica, respectively. Indeed, aldehyde type of compounds was the major class with the greatest number of compounds in the EOs (49.5%, 18 comp. and 44.9%, 22 comp.) and SPME (85.8%, 9 comp. and 56.9%, 7 comp.) of both S. pisidica and S. sandrasica. Aromatic type of compounds was the main group of the SPME of n-hexane extract obtained from S. pisidica and S. sandrasica (Table 1). The main component of the EOs of S. pisidica and S. sandrasica varies depending on the extraction technique. In general, hexanal (1.6% and 4.7%; 5.8% and 20.7%), 2-(E)-hexenal (1.3% and 14.2%; 2.8% and 13.2%), 2-(Z)-hexenol (0.1% and 0.7%; 0.2% and 0.1%), 2,4-hexadienal (1.1% and 0.9%; 0.1% and 3.2%), benzaldehyde (23.3% and 46.3%; 0.8% and 6.3%), caprylaldehyde (2.0% and 2.6%; 4.1% and 3.4%), phenylacetaldehyde (4.0% and 7.3%; 3.5% and 3.4%), and nonanal (5.5% and 5.3%; 6.3% and 6.7%) were found both in the EOs and SPME of S. pisidica and S. sandrasica, respectively. Hexanal (1.6%, 4.7%, and 0.1%), 2-(Z)-hexenol (0.1%, 0.7%, and 0.1%), nonanal (5.5%, 5.3%, and 0.1%) were found only in all three EOs, SPME and SPME of n-hexane extract of S. pisidica, respectively. The results showed that no regular increase or decrease in the amounts of components depends on the used techniques and species. Caryophyllene oxide (19.7%), manoyl oxide (16.5%), and manool (11.3%) were reported to be major compounds for the chloroform extract of the S. sandrasica [9]. We also observed caryophyllene oxide (1.2%) in the EOs of S. pisidica. The observed chemovariation of S. pisidica and S. sandrasica are in good agreement with the published data obtained from other plants [28-31, 62-64]. It is known that there are many environmental factors, such as land, altitude, growing conditions, temperature, and season, which can lead to qualitative and quantitative differences in the volatile organic compounds produced in the plant.
### Table 1. Volatile organic compounds identified from *S. pisidica* and *S. sandrasica*

| Compounds                        | RI<sup>a</sup> | RI<sup>b</sup> | Area (%)<sup>b</sup> |
|---------------------------------|-----------------|-----------------|----------------------|
| 2-Vinylfuran                    | 761<sup>[32]</sup> | 761             | 1.3                  |
| Methylbenzene                   | 782<sup>[32]</sup> | 782             | 8.7                  |
| Hexanal                         | 801<sup>[32]</sup> | 802             | 1.6                  |
| Butyl acetate                   | 814<sup>[32]</sup> | 815             | 1.0                  |
| (2E)-Hexanal                    | 846<sup>[32]</sup> | 852             | 1.3                  |
| (2Z)-Hexenol                    | 859<sup>[32]</sup> | 865             | 0.1                  |
| n-Hexanol                       | 863<sup>[32]</sup> | 864             | 1.3                  |
| Ethylbenzene                    | 871<sup>[32]</sup> | 872             | 0.9                  |
| p-Xylene                        | 883<sup>[32]</sup> | 884             | 0.9                  |
| \(\alpha\)-Xylene\              | 894<sup>[32]</sup> | 893             | 5.6                  |
| Cyclohexanone                   | 903<sup>[32]</sup> | 904             | 0.5                  |
| Heptanal                         | 901<sup>[32]</sup> | 905             | 0.9                  |
| (2E,4E)-Hexadienal              | 907<sup>[32]</sup> | 908             | 0.9                  |
| 2-Butoxy ethanol                | 909<sup>[32]</sup> | 912             | 0.1                  |
| Cumene                          | 924<sup>[32]</sup> | 930             | 0.1                  |
| \(\alpha\)-Pinene<sup>c</sup>   | 932<sup>[32]</sup> | 929             | 11.2                 |
| Propylbenzene                   | 950<sup>[32]</sup> | 948             | 3.1                  |
| Benzaldehyde                    | 952<sup>[32]</sup> | 962             | 23.3                 |
| Verbenene                       | 961<sup>[32]</sup> | 960             | 0.8                  |
| Hexanoic acid                   | 967<sup>[32]</sup> | 963             | 1.8                  |
| 1-Ethyl-3-methylbenzene         | 968<sup>[32]</sup> | 966             | 18.3                 |
| 1-Ethyl-4-methylbenzene         | 970<sup>[32]</sup> | 972             | 7.2                  |
| \(\beta\)-Pinene<sup>c</sup>    | 974<sup>[32]</sup> | 981             | 0.8                  |
| psi-Cumene (1,2,4-              | 985<sup>[32]</sup> | 984             | 33.6                 |
| trimethylbenzene)               | 2-Pentylfuran    | 984<sup>[32]</sup> | 993             |
| Mesitylene                      | 994<sup>[32]</sup> | 996             | 5.2                  |
| Caprylaldehyde                  | 998<sup>[32]</sup> | 1002            | 2.0                  |
| (2E,4E)-Heptadienal             | 1005<sup>[32]</sup> | 1012            | 0.6                  |
| \(p\)-Cymene                    | 1020<sup>[48]</sup> | 1020            | 1.3                  |
| \(m\)-Cymene                    | 1027<sup>[49]</sup> | 1027            | -                    |
| Benzyl alcohol                  | 1026<sup>[32]</sup> | 1029            | 2.8                  |
| Phenylacetaldelyde              | 1033<sup>[30]</sup> | 1036            | 4.0                  |
| Hemellitol                      | 1035<sup>[38]</sup> | 1037            | 5.8                  |
| Indane                          | 1034<sup>[31]</sup> | 1041            | 0.8                  |
| (2E)-Octen-1-al                 | 1049<sup>[32]</sup> | 1046            | -                    |
| 1-Methyl-3-propylbenzene        | 1058<sup>[49]</sup> | 1053            | 0.9                  |
| (2E)-Octen-1-ol                 | 1060<sup>[32]</sup> | 1056            | 0.4                  |
| \(p\)-Diethylbenzene            | 1056<sup>[44]</sup> | 1057            | 0.2                  |
| 1-Ethyl-3,5,                    | 1058<sup>[48]</sup> | 1060            | -                    |
| dimethylbenzene                 | 2-Ethyl-1,2-      | 1062<sup>[32]</sup> | 1061            |
| dimethylbenzene                 | 3-Methyldecane    | 1071<sup>[32]</sup> | 1067            |
| 1-Methyl-2-propylbenzene        | 1074<sup>[49]</sup> | 1069            | 0.4                  |
| \(p\)-Tolualdehyde              | 1077<sup>[32]</sup> | 1080            | 1.5                  |
| 4-Ethyl-1,2-                    | 1078<sup>[45]</sup> | 1079            | 0.1                  |
| dimethylbenzene                 | 1-Ethyl-2,4-      | 1083<sup>[48]</sup> | 1081            |
| dimethylbenzene                 | 2-Ethyl-1,4-      | 1085<sup>[48]</sup> | 1087            |
| dimethylbenzene                 | Linalool<sup>d</sup> | 1095<sup>[32]</sup> | 1097            |
| Undecane<sup>c</sup>            | 1100<sup>[32]</sup> | 1099            | 1.4                  |

<sup>a</sup> RI values are calculated from RI<sub>0</sub> values and RI<sub>90</sub> values at 90°C.

<sup>b</sup> Area values are given as percentages of the total area.

<sup>c</sup> Data from previous studies.

<sup>d</sup> Data from a different species of *Salvia*.
| Compounds                        | RI<sup>a</sup> | RI<sup>b</sup> | A1 | A2 | A3 | B1 | B2 | B3 |
|---------------------------------|----------------|---------------|----|----|----|----|----|----|
| Nonanal                         | 1100<sup>[12]</sup> | 1101           | 5.5| 5.3| 0.1| 6.3| 6.7| -  |
| 1-Ethyl-2,3-dimethylbenzene     | 1113<sup>[49]</sup> | 1109           | -  | -  | 0.1| -  | -  | 0.5|
| α-Campholenal                   | 1122<sup>[32]</sup> | 1128           | 1.3| -  | -  | -  | -  | -  |
| 1,2,4,5-Tetramethylbenzene      | 1131<sup>[53]</sup> | 1132           | -  | -  | 0.3| -  | -  | 1.7|
| (2E)-Nonenal                    | 1157<sup>[32]</sup> | 1157           | 0.4| -  | -  | 1.5| -  | -  |
| Nonanol                         | 1165<sup>[32]</sup> | 1170           | -  | -  | 0.3| -  | -  | -  |
| (2E)-Decanal                    | 1171<sup>[55]</sup> | 1176           | -  | -  | 0.5| -  | -  | -  |
| (2E,4Z)-Decadienal              | 1292<sup>[32]</sup> | 1291           | -  | -  | 0.7| -  | -  | -  |
| Dihydroedulan I                 | 1289<sup>[52]</sup> | 1292           | 0.9| -  | -  | -  | -  | -  |
| Tridecane<sup>c</sup>           | 1300<sup>[32]</sup> | 1300           | 3.3| 3.7| -  | 14.1| -  | -  |
| Theaspirane                     | 1300<sup>[58]</sup> | 1301           | -  | -  | 0.2| -  | -  | 1.0|
| Undecanal                       | 1305<sup>[32]</sup> | 1302           | -  | -  | 1.1| -  | -  | -  |
| (2E,4E)-Decadienal              | 1315<sup>[32]</sup> | 1314           | 1.2| 1.6| -  | -  | -  | 3.3|
| (2E)-Undecenal                  | 1357<sup>[32]</sup> | 1360           | 1.2| -  | -  | -  | -  | 3.3|
| (E)-β-Damascenone               | 1383<sup>[32]</sup> | 1386           | 1.6| -  | -  | 1.2| -  | -  |
| Tetradecane<sup>c</sup>         | 1400<sup>[32]</sup> | 1402           | -  | -  | 0.1| 1.9| -  | 0.5|
| Dodecanal                       | 1408<sup>[32]</sup> | 1403           | 0.3| -  | -  | 1.0| -  | -  |
| (E)-Caryophyllene               | 1417<sup>[32]</sup> | 1416           | 1.6| -  | 0.2| 1.2| -  | -  |
| Neryl acetone                   | 1434<sup>[32]</sup> | 1438           | 0.6| -  | -  | 0.9| -  | -  |
| α-Humulene                      | 1452<sup>[32]</sup> | 1463           | -  | -  | 0.1| -  | -  | -  |
| Geranyl acetone                 | 1453<sup>[32]</sup> | 1451           | -  | -  | 0.1| -  | -  | -  |
| (E)-Ethyl cinnamate             | 1465<sup>[32]</sup> | 1468           | 8.9| -  | -  | 37.9| -  | -  |
| 1-Dodecanol                     | 1469<sup>[32]</sup> | 1477           | -  | -  | -  | 2.6| -  | -  |
| Germacrene D                    | 1484<sup>[41]</sup> | 1482           | -  | -  | 0.1| -  | -  | -  |
| (E)-β-ionone                    | 1487<sup>[32]</sup> | 1488           | 2.0| -  | -  | 1.8| -  | -  |
| Pentadecane<sup>c</sup>         | 1500<sup>[32]</sup> | 1501           | 0.1| -  | -  | 1.2| -  | -  |
| Tridecanal                      | 1509<sup>[32]</sup> | 1507           | -  | -  | -  | 0.1| -  | -  |
| β-Bisabolene                    | 1505<sup>[32]</sup> | 1509           | -  | -  | 0.3| -  | -  | 0.4|
| δ-Cadinene                      | 1511<sup>[59]</sup> | 1510           | -  | -  | 0.1| -  | -  | -  |
| Caryophyllene oxide             | 1582<sup>[32]</sup> | 1582           | 1.2| -  | -  | -  | -  | -  |
| Viridiflorol                    | 1592<sup>[32]</sup> | 1591           | -  | -  | 0.6| -  | -  | -  |
| Hexadecane<sup>c</sup>          | 1600<sup>[32]</sup> | 1601           | -  | -  | -  | 1.3| -  | -  |
| Tetradecanal                    | 1611<sup>[32]</sup> | 1609           | 0.6| -  | -  | 0.1| -  | -  |
| Heptadecane<sup>c</sup>         | 1700<sup>[32]</sup> | 1701           | -  | -  | 0.7| -  | -  | -  |
| Pentadecanal                    | 1710<sup>[60]</sup> | 1708           | 0.3| -  | -  | 1.7| -  | -  |
| Tetradecanoic acid              | 1763<sup>[42]</sup> | 1763           | 0.4| -  | -  | 0.5| -  | -  |

<sup>a</sup> Relative retention index.

<sup>b</sup> Area percentage.
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Table 1 continued.

| Compounds                        | RI* | RIa | A1  | A2  | A3  | B1  | B2  | B3  |
|----------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|
| Hexahydrofarnesyl acetone        | 1844 | 1848 | 0.1 | -   | -   | 0.9 | -   | -   |
| n-Hexadecanoic acid              | 1959 | 1963 | 0.6 | -   | -   | 0.5 | -   | -   |
| Eicosanea                        | 2000 | 1999 | -   | -   | -   | 0.1 | -   | -   |
| Methyl linoleate                 | 2095 | 2104 | 1.3 | -   | -   | -   | -   | -   |
| Heneicosanee                     | 2100 | 2099 | 2.8 | -   | -   | 8.1 | -   | -   |
| Docosanee                        | 2200 | 2198 | -   | -   | -   | 0.3 | -   | -   |
| Tricosane                     | 2300 | 2299 | 2.3 | -   | -   | 5.1 | -   | -   |

Chemical classes: A1: Monoterpenes; A2: Monoterpeneoids; A3: Sesquiterpenes; B1: Sesquiterpenoids; B2: Terpene related; B3: Aromatics; B4: Aliphatic; B5: Hydrocarbons; B6: Aldehydes; B7: Ketones; B8: Esters; B9: Acids; B10: Other.

Percentages obtained by FID peak-area normalization.

Retention Index calculated from retention times relative to that of n-alkane series (C₈-C₃₀) on the non-polar Rxi-5MS column.

Retention index of references.

Compounds determined by GC-FID/MS, and analytical reference standard.

A1: S. pisidica, HD; A2: S. pisidica, SPME; A3: S. pisidica, SPME of n-hexane extract. B1: S. sandrasica, HD; B2: S. sandrasica, SPME; B3: S. sandrasica SPME of n-hexane extract.

The polyphenolic profile of S. pisidica and S. sandrasica was obtained through high-performance liquid chromatography (HPLC) analysis. The results of this study revealed that each species possesses a specific phenolic fingerprint based on its composition that is indicated by HPLC data using gallic acid, protocatechuic acid, protocatechuic aldehyde, p-hydroxybenzoic acid, chlorogenic acid, vanillic acid, caffeic acid, p-coumaric acid, ferulic acid, and benzoic acid as phenolic standards. Gallic acid, p-hydroxybenzoic acid, and vanillic acid were major phenolic constituents of S. pisidica, and gallic acid and caffeic acid were the main phenolic compounds of S. sandrasica. Recently, polyphenolic compounds of Scorzonera hispanica L., S. judaica Eig., S. latifolia (Fisch. & Mey.), S. lasiaca (L.), S. acuminata, S. cana var. alpina, S. cana var. radicosa, S. eriophora, S. lasiaca ssp. lasiaca, S. suberosa ssp. suberosa, S. sublanata, S. crispata Boiss., S. papposa DC., S. suberosa C. Koch, S. trachysperma Guss., S. acuminata, S. cana var. jacquiniana, S. cretica, S. parvislora, S. parviflora, S. cinerea, S. cana var. radicosa, S. eriophora, Scorzonera incisa, S. mollt ssp. szowitsii, and S. tomentosa have been mentioned, and they were mainly phenolic and glycosidic flavonoid type compounds [18-27].

The antimicrobial activities of EOs, methanol, and n-hexane extracts obtained from S. pisidica and S. sandrasica were assayed against nine bacterial species. They generally showed an anti-tuberculosis activity for M. smegmatis for the methanol extract and EOs with the inhibition zones values varying from 10.0 mm to 12.0 mm, respectively (Table 2) [33, 34].
Table 2. Antimicrobial activities for the solvent extracts and essential oils of *S. pisidica* and *S. sandrasica*

| Samples              | Stock Solution (µg/mL) | Microorganisms and inhibition zone (mm) |
|----------------------|------------------------|-----------------------------------------|
|                      |                        | Ec | Yp | Pa | Sa | Ef | Bc | Ms | Ca | Sc |
| Methanolic extracts  |                        |    |    |    |    |    |    |    |    |    |
| *S. pisidica*        | 116.4                  | -  | -  | 8  | 6  | -  | 6  | 10 | -  | -  |
| *S. sandrasica*      | 164.4                  | -  | -  | -  | 6  | -  | -  | -  | -  | -  |
| n-Hexane extracts    |                        |    |    |    |    |    |    |    |    |    |
| *S. pisidica*        | 31.5                   | -  | -  | -  | -  | 6  | -  | 12 | -  | -  |
| *S. sandrasica*      | 43.3                   | -  | -  | -  | -  | -  | -  | -  | -  | -  |
| Essential oils       |                        |    |    |    |    |    |    |    |    |    |
| *S. pisidica*        | 77.3                   | -  | -  | 6  | 6  | -  | -  | 12 | -  | -  |
| *S. sandrasica*      | 18.1                   | -  | -  | 6  | -  | -  | -  | -  | -  | -  |
| Amp.                 |                        | 10 | 10 | 10 | 18 | 10 | 35 | 15 |    |    |
| Strep.               |                        | 10 |    |    |    |    |    |    |    | 35 |
| Flu                  |                        | 5  |    |    |    |    |    |    |    | 25 |

Ec: *Escherichia coli*, Yp: *Yersinia pseudotuberculosis*, Pa: *Pseudomonas aeruginosa*, Sa: *Staphylococcus aureus*, Ef: *Enterococcus faecalis*, Bc: *Bacillus cereus* 702 Roma, Ms: *Mycobacterium smegmatis*, Ca: *Candida albicans*, *Saccharomyces cerevisiae* Amp.: Ampicillin, Str.: Streptomycin (-): Flu.: Fluconazole, (-): No activity.

In general, antimicrobial assays showed methanol extract of *S. pisidica* was more active against four microorganisms (*Pseudomonas aeruginosa*, *S. aureus*, *Bacillus cereus*, and *M. smegmatis*). However, the n-hexane extracts evaluated in this screening did not cause the inhibition of tested bacteria. The observed activities for the tested microorganisms could be explained by the high concentration of aldehydes and aromatic compounds present in the EOs and SPME of n-hexane extract and phenolic contents in the methanol extracts of these species, respectively.

The tyrosinase inhibition for the menthol extract of *S. pisidica* and *S. sandrasica* was expressed as the extract concentration that causes 50% inhibition [35, 36, 65]. IC₅₀ values for *S. pisidica* and *S. sandrasica* were found to be 0.495±0.073 µg/mL and 0.699±0.86 µg/mL, respectively, which were lower than kojic acid value (1.26±0.142 µg/mL) (Table 3). The EOs and n-hexane extracts of *S. pisidica* and *S. sandrasica* did not show tyrosinase inhibition. The tyrosinase activity of 80% methyl alcohol extract of the aerial part of *S. pisidica* was reported as 40.25 ± 0.74 µg/mL compared to kojic acid (78.89 ± 0.09 µg/mL) [8]. The tyrosinase activity result of the methanol extract of *S. pisidica* showed similar activity.

Table 3. Tyrosinase inhibition for the methanol extract of *S. pisidica* and *S. sandrasica*

| Samples              | IC₅₀ µg/mL  |
|----------------------|------------|
| *S. pisidica*        | 0.495 ± 0.073 |
| *S. sandrasica*      | 0.699 ± 0.860 |
| Kojic acid           | 1.126 ± 0.142 |

4. Conclusions

The type of extraction methods and different species indicates different volatile organic compounds and phenolic profiles for *S. pisidica* and *S. sandrasica*. The primary compound in the EO and SPME of *S. pisidica* was benzaldehyde (23.3% and 46.3%). The psi-cumene (33.6%) and 1 ethyl-3-methyl benzene (18.3%) were the major compounds in the SPME of n-hexane extract obtained from *S. pisidica*. α-Terpineol (14.1%), (E)-ethyl cinnamate (37.9%), and 1-ethyl-3-methylbenzene (26.1%)
were the main compounds in the EO, SPME, and SPME of n-hexane extract obtained from \textit{S. sandrasica}, respectively. Gallic acid (86.33 mg/mL and 2.63 mg/mL) was the major phenolic in methanol extracts of \textit{S. pisidica} and \textit{S. sandrasica}. The antimicrobial assay revealed methanol extract and essential oils exhibited significant activity against \textit{M. smegmatis} with the inhibition zone values varying from 10.0 to 12.0 mm, and tyrosinase activity for the methanol extract of the \textit{S. sandrasica} and \textit{S. pisidica} were found as 0.699±0.86 µg/mL and 0.495±0073 µg/mL, respectively. Due to the biological activities, these plants could be evaluated for further phytochemical search.

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