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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±0073 µg/mL and 0.699±0.86 µg/mL, respectively.

Keywords: *Scorzonera pisidica; Scorzonera sandrasica;* volatile constituents; phenolic compounds; biological activities.

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 has 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 *Scorzonerinae* 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. *szowitsii, 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 23th, 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 ^{o}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 ^{o}C in a sealed brown vial.

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 ºC 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 × 0.25 μm) (USA). GC-FID/MS analysis of EOs were performed in split mode (1:30) at 230 ºC. The essential oil solutions (1 μL) in *n*-hexane (HPLC grade) were injected and analyzed with the column held initially at 60 ºC for 2 min and then increased to 240 ºC with a 3 ºC/min heating ramp. The oven program was as follows: the initial temperature was 60 ºC for 2 minutes, which was increased to 240ºC at 3 minutes, the final temperature of 250 ºC 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 (C_6-C_{32}) 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 × 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 Hifzisihha 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% (IC_{50}) 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-5MS 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 comps. and 44.9%, 22 comps.) and SPME (85.8%, 9 comps. and 56.9%, 7 comps.) 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 the *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*	RI[a]	A1	A2	A3	B1	B2	B3
			Area (%)[b]					
2-Vinylfuran	761[37]	761	1.3	-	-	-	-	-
Methylbenzene	782[38]	782	8.7	-	4.4	9.0	-	0.1
Hexanal	801[32]	802	1.6	4.7	0.1	5.8	20.7	-
Butyl acetate	814[39]	815	-	-	0.2	-	-	-
(2*E*)-Hexanal	846[32]	852	1.3	14.2	-	2.8	13.2	-
(2*Z*)-Hexenol	859[32]	865	0.1	0.7	0.1	0.2	0.1	-
n-Hexanol	863[32]	864	-	1.3	-	-	-	-
Ethylbenzene	871[40]	872	-	-	0.9	-	-	0.3
p-Xylene	883[38]	884	-	-	-	-	-	2.5
o-Xylene	894[41]	893	-	-	5.6	-	-	1.1
Cylohexanone	903[42]	904	-	-	6.0	-	-	-
Heptanal	901[32]	905	-	-	-	0.9	-	-
(2*E*,4*E*)-Hexadienal	907[32]	908	1.1	0.9	-	0.1	3.2	-
2-Butoxy ethanol	907[43]	912	-	-	-	-	-	0.1
Cumene	924[32]	930	-	-	0.1	-	-	0.1
α-Pinene[c]	932[32]	929	11.2	-	-	-	-	-
Propylbenzene	950[44]	948	-	-	3.1	-	-	4.0
Benzaldeyhde	952[32]	962	23.3	46.3	-	0.8	6.3	-
Verbenene	961[32]	960	0.8	-	-	-	-	-
Hexanoic acid	967[32]	963	-	-	-	-	0.1	-
1-Ethyl-3-methylbenzene	968[45]	966	-	-	18.3	-	-	26.1
1-Ethyl-4-methylbenzene	970[46]	972	-	-	7.2	-	-	-
β-Pinene[c]	974[32]	981	0.8	-	-	-	-	-
psi-Cumene (1,2,4-trimethylbenzene)	985[47]	984	-	-	33.6	-	-	8.7
2-Pentylfuran	984[32]	993	-	-	-	3.8	1.8	-
Mesitylene	994[44]	996	-	-	5.2	-	-	8.5
Caprylaldeyhde	998[32]	1002	2.0	2.6	-	4.1	3.4	-
(2*E*,4*E*)-Heptadienal	1005[32]	1012	0.6	2.3	-	-	-	-
p-Cymene	1020[48]	1020	1.3	-	-	-	-	0.8
m-Cymene	1027[49]	1027	-	-	-	-	-	14.7
Benzyl alcohol	1026[32]	1029	-	2.8	-		-	-
Phenylacetaldeyhde	1033[50]	1036	4.0	7.3	-	3.5	3.4	-
Hemellitol	1035[38]	1037	-	-	5.8	-	-	-
Indane	1034[51]	1041	-	-	0.8	-	-	2.2
(2*E*)-Octen-1-al	1049[32]	1046	-	-	-	1.3	-	-
1-Methyl-3-propylbenzene	1058[49]	1053	-	-	0.9	-	-	4.0
(2*E*)-Octen-1-ol	1060[52]	1056	0.4	-	-	-	-	-
p-Diethylbenzene	1056[44]	1057	-	-	0.2	-	-	2.6
1-Ethyl-3,5-dimetilbenzene	1058[45]	1060	-	-	-	-	-	3.2
o-Tolualdeyhde	1062[32]	1061	0.1	-	-	-	-	-
3-Methyldecane	1071[52]	1067	-	-	-	-	-	0.5
1-Methyl-2-propylbenzene	1074[49]	1069	-	-	0.4	-	-	1.1
p-Tolualdeyhde	1077[32]	1080	-	-	-	1.5	-	-
4-Ethyl-1,2-dimethylbenzene	1078[45]	1079	-	-	0.1	-	-	1.8
1-Ethyl-2,4-dimethylbenzene	1083[49]	1081	-	-	-	-	-	1.6
2-Ethyl-1,4-dimethylbenzene	1085[49]	1087	-	-	0.6	-	-	2.1
Linalool[c]	1095[32]	1097	-	-	-	1.0	-	-
Undecane[c]	1100[32]	1099	-	-	1.4	-	-	6.6

Table 1 continued..

Compounds	RI*	RI[a]	A1	A2	A3	B1	B2	B3
			Area (%)[b]					
Nonanal	1100[32]	1101	5.5	5.3	0.1	6.3	6.7	-
1-Ethyl-2,3-dimethylbenzene	1113[49]	1109	-		0.1	-	-	0.5
α-Campholenal	1122[32]	1128	1.3	-	-	-	-	-
1,2,4,5-Tetramethylbenzene	1131[53]	1132	-	-	0.3	-	-	1.7
1-(2-Methylphenyl) ethanone	1139[54]	1133	-	-	1.0	-	-	-
Pentyl cyclohexane	1130[51]	1134	-	-	-	-	-	0.2
trans-Pinocarveol	1135[32]	1134	0.1	-	-	-	-	-
1,2,3,4-Tetramethylbenzene	1159[38]	1155	-	-	0.1	-	-	0.7
(2*E*)-Nonenal	1157[32]	1157	0.4	-	-	1.5	-	-
Nonanol	1165[32]	1170	-	-	-	0.3	-	-
1-(3-Methyphenyl) ethanone	1171[55]	1176	-	-	0.5	-	-	-
1-(4-Methyphenyl) ethanone	1182[56]	1186	-	-	-	-	-	0.5
α-Terpineol	1186[32]	1192	3.3	-	-	14.1	-	-
Dodecane[c]	1200[32]	1198	-	-	0.2	-	-	1.0
Decanal	1201[32]	1201	5.4	2.2	-	3.3	-	-
β-Cyclocitral	1217[32]	1224	0.1	-	-	-	-	-
Thymoquinone	1248[32]	1252	-	-	0.1	-	-	0.1
(2*E*)-Decenal	1260[32]	1260	0.1	-	-	1.8	-	-
Vitispirane	1286[57]	1284	0.5	-	-	-	-	-
Thymol	1289[32]	1290	-	-	0.1	-	-	0.1
(2*E*,4*Z*)-Decadienal	1292[32]	1291	-	-	-	0.7	-	-
Dihydroedulan I	1289[52]	1292	0.9	-	-	-	-	-
Tridecane[c]	1300[32]	1300	-	-	-	-	-	0.1
Theaspirane	1300[58]	1301	3.7	-	-	-	-	-
Undecanal	1305[32]	1302	-	-	-	1.1	-	-
(2*E*,4*E*)-Decadienal	1315[32]	1314	1.4	-	-	3.0	-	-
(2*E*)-Undecenal	1357[32]	1360	1.2	-	-	1.8	-	-
(*E*)-*β*-Damascenone	1383[32]	1386	1.6	-	-	1.2	-	-
Tetradecane[c]	1400[32]	1402	-	-	0.1	1.9	-	0.5
Dodecanal	1408[32]	1403	0.3	-	-	1.0	-	-
(*E)*-Caryophyllene	1417[32]	1416	1.6	-	0.2	1.2	-	-
Neryl acetone	1434[32]	1438	0.6	-	-	0.9	-	-
α-Humulene	1452[32]	1463	-	-	0.1	-	-	-
Geranyl acetone	1453[32]	1451	-	-	0.1	-	-	-
(*E*)-Ethyl cinnamate	1465[32]	1468	-	8.9	-	-	37.9	-
1-Dodecanol	1469[32]	1477	-	-	-	-	2.6	-
Germacrene D	1484[41]	1482	-	-	0.1	-	-	-
(*E*)-*β*-ionone	1487[32]	1488	2.0	-	-	1.8	-	-
Pentadecane[c]	1500[32]	1501	0.1	-	-	1.2	-	-
Tridecanal	1509[32]	1507	-	-	-	0.1	-	-
β-Bisabolene	1505[32]	1509	-	-	0.3	-	-	0.4
δ-Cadinene	1511[59]	1510	-	-	0.1	-	-	-
Caryophyllene oxide	1582[32]	1582	1.2	-	-	-	-	-
Viridiflorol	1592[32]	1591	-	-	-	0.6	-	-
Hexadecane[c]	1600[32]	1601	-	-	-	1.3	-	-
Tetradecanal	1611[32]	1609	0.6	-	-	0.1	-	-
Heptadecane[c]	1700[32]	1701	-	-	-	0.7	-	-
Pentadecanal	1710[60]	1708	0.3	-	-	1.7	-	-
Tetradecanoic acid	1763[42]	1763	0.4	-	-	0.5	-	-

Table 1 continued..

Compounds	RI*	RI[a]	A1	A2	A3	B1	B2	B3
			Area (%)[b]					
Hexahydrofarnesyl acetone	1844[61]	1848	0.1	-	-	0.9	-	-
n-Hexadecanoic acid	1959[32]	1963	0.6	-	-	0.5	-	-
Eicosane[c]	2000[32]	1999	-	-	-	0.1	-	-
Methyl linoleate	2095[32]	2104	1.3	-	-	-	-	-
Heneicosane[c]	2100[32]	2099	2.8	-	-	8.1	-	-
Docosane[c]	2200[32]	2198	-	-	-	0.3	-	-
Tricosane[c]	2300[32]	2299	2.3	-	-	5.1	-	-

Chemical classes	A1		A2		A3		B1		B2		B3	
	%[b]	NC[d]	%[b]	NC[d]	%[b]	NC[d]	%[b]	NC[d]	%[b]	NC[d]	%[b]	NC[d]
Monoterpenes	12.8	3	-	-	-	-	-	-	-	-	-	-
Monoterpeneoids	4.8	4	-	-	0.1	1	15.1	2	-	-	0.1	1
Sesquiterpenes	1.6	-	-	-	0.8	5	1.2	1	-	-	0.4	1
Sesquiterpenoids	1.2	1	-	-	-	-	0.6	1	-	-	-	-
Terpene related	9.3	5	-	-	0.1	1	3.9	3	-	-	-	-
Aromatics	11.3	3	-	-	87.0	19	12.8	2	1.8	1	86.3	22
Aliphatic hydroc.	4.9	2	-	-	1.7	3	17.0	7	-	-	8.7	5
Aldehydes	49.5	18	85.8	9	0.2	2	44.9	22	56.9	7	-	-
Alcohols	0.5	2	4.8	3	0.1	1	0.5	2	2.7	2	0.1	1
Ketones	0.1	1	-	-	7.5	3	0.9	1	-	-	0.5	1
Esters	1.3	1	8.9	1	0.2	1	-	-	37.9	1	-	-
Acids	1.0	2	-	-	-	-	1.0	2	0.1	1	-	-
Other	-	-	-	-	0.8	1	-	-	-	-	2.4	2
Total	98.3	44	99.5	13	98.5	37	97.9	43	99.4	12	98.5	33

*Retention index of references.
[a] Retention index calculated from retention times relative to that of *n*-alkane series (C_6-C_{30}) on the non-polar Rxi-5MS column.
[b] Percentages obtained by FID peak-area normalization.
[c] Compounds determined by GC-FID/MS, and analytical reference standard.
[d] NC: Number of compounds.
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. 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. cana* var. *jacquiniana*, *S. cretica*, *S. parviflora*, *S. parviflora*, *S. cinerea*, *S. cana* var. *radicosa*, *S. eriophora*, *Scorzonera incisa*, *S. mollis* 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	-	-	12	-	-
n-Hexane extracts										
S. pisidica	31.5	-	-	-	-	-	-	-	-	-
S. sandrasica	43.3	-	-	-	-	-	-	-	-	-
Essential oils										
S. pisidica	77.3	-	-	-	6	-	-	12	-	-
S. sandrasica	18.1	-	-	-	6	-	-	10	-	-
Amp.	10	10	10	18	10	35	15			
Strep.	10							35		
Flu	5								25	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]. IC_{50} 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	IC_{50} 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 *S. sandrasica*, respectively. Gallic acid (86.33 mg/mL and 2.63 mg/mL) was the major phenolic in methanol extracts of *S. pisidica* and *S. sandrasica.* The antimicrobial assay revealed methanol extract and essential oils exhibited significant activity against *M. smegmatis* with the inhibition zone values varying from 10.0 to 12.0 mm, and tyrosinase activity for the methanol extract of the *S. sandrasica* and *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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