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Chemical Composition and Antimicrobial Activity of the Essential Oils of Five *Scrophularia* L. Species from Turkey

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Abstract: The essential oils of the five *Scrophularia* species; *Scrophularia chrysantha* Jaub. et Spach, *Scrophularia kotschyana* Benth., *Scrophularia olympica* Boiss., *Scrophularia cinerascens* Boiss. and *Scrophularia zuvandica* Grossh. were obtained by hydrodistillation (HD) with a range of 0.10% to 0.16% yield and analazsed by GC-FID/MS. In the meantime, the volatile organic compounds (VOCs) of *S. chrysantha*, *S. kotschyana*, *S. olympica*, *S. cinerascens* and *S. zuvandica* were also identified with the technique of SPME GC-FID/MS and the phytochemical results were evaluated. The experimental results of this study showed that the major compounds of essential oils which were taken by the hydrodistillation were carvacrol (52.4%), 2-pentadecanone (26.7%), 2-pentadecanone (12.2%), (Z)-2-nonenal (11.2%) and carvacrol (69.1%) respectively. Isovaleraldehyde (37.1%, 27.9%), eucalyptol (13.8%), 2-ethyl furan (14.8%) and 3(Z)-hexenol (91.3%) were respectively found as the main constituents of the five *Scrophularia* species with SPME method. Also antimicrobial activities of the essential oils of the five *Scrophularia* species were screened by using agar well diffusion method. It was identified that *S. olympica*, *S.chrysantha*, and *S. kotschyana*, have anti-tuberculosis activity, whereas, the essential oils gained from *S. zuvandica* and *S. cinerascens* have anti-fungal activity.

Keywords: *Scrophularia;* SPME GC-FID/MS; anti-tuberculosis activity; agar well diffusion.

1. Introduction

The genus *Scrophularia* L. (Scrophulariaceae) which comprise of 310 species worldwide, is represented by 66 species (83 taxa) in Turkey [1-4]. *Scrophularia* species grow mountainous regions, forests, and riversides [1]. Species of the genus *Scrophularia* are commonly known as figworts. Additionally, the name of the genus is reported to come from "scrofula" which is also the name of a form of tuberculosis and given to the genus because of its reported usage in scrofulous swellings [4].

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Some of the *Scrophularia* species are used as traditional medicine in Turkey for the treatment of vesicles on the skin, warts, and inflammatory skin diseases like abscess, lichen infections wounds, urticaria, bacterial-viral infections, as analgesic, antirheumatic, and antipyretic [5-10].

Despite the existence of 23 endemic species belongs to the genus of *Scrophularia* in Turkey, studies on the essential oil components of *Scrophularia* species are limited. In a study conducted in 2013, the essential oil components of the aerial parts of *Scrophularia oxysepala* Boiss. were determined by GC-MS analysis and phytol (25.3%), methyl benzyl alcohol (9.3%), dehydrodieugenol (6.7%), methyl benzaldehyde (5.3%) and eugenol (1.3%) were found as the main compounds. Additionally the low level of free radical scavenging activity (RC50 value 1.852 mg/mL) and low insecticidal activity of the volatile oil were reported [11]. In addition, the essential oil analysis on the radix of *Scrophularia ningpoensis* Hemsl. was also mentioned and palmitic acid (25.4%), linoleic acid (10.04%) and α-linolenic acid (6.06%) were identified as the main constituents of essential oil [12]. Seasonal variation for the essential oil of *Scrophularia frigida* Boiss. was compared and the essential oil of autumn sample was found to be composed of fatty acid derivatives mainly, while the essential oil of summer sample contained oxygenated monoterpenes as the major constituents [13]. Chemical composition of the essential oil of *Scrophularia amplexicaulis* Benth., which is a native species to Turkey, was also investigated by GC-FID/MS and the main constituents were found to be eugenol (53.8%) and eugenol acetate (24.5%) [14].

Our literature survey showed that no research has been performed on the volatile organic compounds of *S.chrysantha, S. kotschyana S. olympica, S.cinerascens* and *S. zuvandica*. In the light of this evidence, we planned to analyze the volatile constituents of the aerial parts of these five *Scrophularia* species from the flora of Turkey and to determine the possible antimicrobial effects of the essential oils of these species.

2. Materials and Methods

2.1. Plant Materials

The aerial parts of plants were collected from different locations of Turkey during the flowering stages. Voucher specimens were identified by Dr Gülin Renda and have been deposited in the Herbarium of Hacettepe University, Faculty of Pharmacy (HUEF) (Table 1). Plant materials cleaned to remove impurities and stored in air-tight container until use.

Table 1. Examined species, voucher numbers, collection data and localities of the *Scrophularia* species in Turkey.

Species	Voucher No. (HUEF)	Collection Date	Localities
S. chrysantha (A)	15007	12.07.2015	Trabzon Caykara, Mogalakamboz plateau
S. kotschyana (B)	15002	11.05.2014	Trabzon Macka, Altindere village, Sumela Monastery
S. olympica (C)	15020	11.07.2015	Rize Camlihemsin, Vercenik village, Cicekliyayla plateau
S. cinerascens (D)	15004	20.06.2015	Erzurum-Pasinler road 10. Km
S. zuvandica (E)	15003	20.06.2015	Erzurum Cesme, roadsides of the way to pond

2.2. Isolation of the Essential Oils

The aerial parts of the fresh *Scrophularia* samples (120 g, each) were hydrodistilled in a Clevenger-type apparatus using cooling bath (-15ºC) system (4 h). The essential oils were taken in HPLC grade n-hexane (0.5 mL) and kept at 4ºC in a sealed brown vial until the use. 1μL of the essential oils were separately injected to GC-FID/MS instrument.

2.3. SPME Analysis

The flowered fresh plant materials (1.0 g, each) of five *Scrophularia* species were analyzed with a SPME device (Supelco, USA). Plant materials were crumbled and placed in a 10 mL vial sealed with a silicone-rubber septum cap. A polydimethylsiloxane/divinyl-benzene 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. Extractions were achieved with magnetic stirring at 50 °C using an incubation time of 5 min and an extraction time of 10 min. Fibers with extract of aroma compounds were subsequently injected into the GC injector. Each sample was analyzed and reported. Conditioning time for subsequent assays was set at 4 min of desorption after each extraction. The temperature, incubation and extraction times were set according to the reported experiment [15,16].

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) 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. The fibers containing the extracted volatiles (SPME) were injected into the GC-MS injector. Split mode was employed and split ratio was 1:20. 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. 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 [16].

2.5. Compound Identification

Retention indices of the components were determined by Kovats method using n-alkanes (C6-C32) as standards. The volatile compounds were identified by comparison of their retention indices (relative to C6-C32 alkane standards) and mass spectra with those of the mass spectra of the two libraries (FFNSC1.2 and W9N11) and also confirmed by comparing the retention indices with the data published in the literature.

2.6. Antimicrobial Activity

All test microorganisms; *Escherichia coli* (*E. coli*) ATCC35218, *Yersinia pseudotuberculosis* (*Y. pseudotuberculosis*) ATCC911, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC43288, *Enterococcus faecalis* (*E. faecalis*) ATCC29212, *Bacillus cereus* (*B. cereus*) 709 Roma, *Mycobacterium smegmatis* (*M. smegmatis*) ATCC607, *Candida albicans* (*C. albicans*) ATCC60193 and *Saccharomyces cerevisiae* (*S. cerevisia*) RSKK 251 were obtained from Refik Saydam Hifzissihha Institute (Ankara, Turkey). All of the essential oils were weighed and dissolved in hexane to prepare extract stock solutions.

2.7. Agar Well Diffusion Assay

The antimicrobial effects of the substances were tested quantitatively in respective broth media by using double microdilution [17-19]. The antibacterial and antifungal assays were performed in Mueller-Hinton broth (MH) (Difco, Detroit, MI) at pH.7.3 and buffered Yeast Nitrogen Base (Difco, Detroit, MI) at pH 7.0, respectively. The micro dilution test plates were incubated for 18-24 h at 35°C. Brain Heart Infusion broth (Difco, Detriot, MI) was used for *M. smegmatis*, and incubated for 48-72 h at 35°C [20]. Antimicrobial activity was assessed by comparing the inhibition zone generated by

essential oils against the test microorganisms with the inhibition zone generated by standard drugs. Ampicillin (10 mg/mL), streptomycin (10 mg/mL) and fluconazole (2 mg/mL) were used as standards. Dimethylsulphoxide with dilution of 1:10 was used as solvent control. The results were interpreted in terms of diameter of inhibition zone and given in Table 4.

3. Results and Discussion

The essential oils of *S. chrysantha*, *S. kotschyana*, *S. olympica*, *S. cinerascens* and *S. zuvandica* obtained by hydrodistillation in a Clevenger type apparatus produced light orange oils with a yield of 0.10%, 0.15%, 0.11%, 0.16% and 0.11% (w/w), respectively. The identity, retention time, and the percentage of composition of oils obtained from the *Scrophularia* species are presented in table 2. The percentages of the composition are presented as relative peak area. A total of 77 compounds from the five *Scrophularia* species were identified and quantified, accounting from 98.6% to 99.9% ratio. Among the species studied, *S. cinerascens* and *S. olympica* were found to be the most volatile oil containing species (0.16%), while the least volatile oil containing species was found to be *S. chrysantha* (0.10%). The highest numbers of compound diversity were observed in the essential oils of both *S. cinerascens* and *S. olympica* species with 28 compounds (Table 2).

Composition of volatile organic compounds for the same five *Scrophularia* species were also identified by SPME with GC-FID/MS. Identifications were made on the basis of comparison of GC Kovats retention indexes (RIs) with reference to a homologous series of n-alkanes. The results of SPME analysis were compared with those obtained by HD and all of the results were given in Table 2. According to the results of the study, the major compounds identified in the SPME extracts of the five *Scrophularia* species were isovaleraldehyde (37.1% and 27.9%, in *S. chrysantha* and *S. kotschyana*), eucalyptol (13.8%, in *S. olympica*), 2-ethylfuran (14.8%, in *S. cinerascens*) and (Z)-3-hexenol (91.3%, in *S. zuvandica*) respectively. Whereas, the most abundant component in the investigated essential oils of five *Scrophularia* species, were (Z)-2-nonenal (11.2%, in *S. cinerascens*), carvacrol (69.1% and 52.4 %, in *S. zuvandica* and *S. chrysantha*), and 2-pentadecanone (12.2% and 26.7%, in *S. olympica* and *S. kotschyana*), respectively as seen in table 2.

The chemical class distribution of the volatile organic compounds for the five *Scrophularia* species were listed in table 3 and they were classified into eleven classes, namely monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, oxygenated diterpenes, terpen related compounds, aliphatic hydrocarbons, aldehydes, ketones, alcohols and others (Table 3). Comparative chemical class evaluation in the essential oils of the studied *Scrophularia* species gave that aldehydes (36.2% and 19.2%) in *S. olympica* and *S. cinerascens*, oxygenatede monoterpenes (84.4% and 76.6%) in *S. chrysantha* and *S. kotschyana*, and ketones (29.2%) in *S. zuvandica* were the main class of organic compounds, respectively. However, SPME GC-FID/MS analysis of the *Scrophularia* species revealed that *S. zuvandica* was mainly composed of alcohols which were consisting of (Z)-3-hexenol with in ratio of 91.3% and the major class of organic compounds for the *S. olympica, S. kotschyana*, and *S. zuvandica* was aldehydes (61.0%, 61.9%, and 80.3%), respectively. But, the SPME result of *S.cinerascens* gave the monoterpene hydrocarbons (47.6%) as the main group of volatile organic compounds. The essential oil compositions of the *Scrophularia* species were evaluated and carvacrol was found to be remarkable in the essential oil of *S. zuvandica* and *S. chrysantha* within the ratio of 69.1% and 52.4%, respectively.

Table 2. Identified volatile organic compounds from five *Scrophularia* species growing in Turkey.

Compunds	References	RI*	RI[a]	RT	A1 (%)[b]	A2 (%)[b]	B1 (%)[b]	B2 (%)[b]	C1 (%)[b]	C2 (%)[b]	D1 (%)[b]	D2 (%)[b]	E1 (%)[b]	E2 (%)[b]
Monoterpene hydrocarbons														
α-Thujene	[22]	931	931	12.829	-	-	-	-	-	4.1	-	-	-	0.9
α-Pinene	[23]	939	939	13.156	-	-	-	-	-	1.6	-	-	-	0.1
Sabinene	[24]	978	977	14.695	-	2.7	-	-	-	10.8	-	-	-	-
β-Pinene	[23]	979	981	14.891	-	5.3	-	-	-	11.7	-	-	-	0.2
Myrcene	[23]	991	991	15.200	-	-	-	-	-	-	-	-	0.4	-
p-Cymene	[25]	1022	1020	16.820	3.9	-	-	-	0.9	5.0	-	-	3.4	0.2
Limonene	[23]	1029	1030	16.967	-	-	0.1	-	-	10.7	-	-	-	0.3
γ-Terpinene	[23]	1062	1061	18.271	2.9	-	-	-	-	1.4	-	-	3.1	-
(E)-Sabinene	[22]	1074	1069	18.648	-	-	-	-	-	2.3	-	-	-	-
α-Terpinolene	[26]	1086	1086	19.066	-	-	-	-	-	-	6.4	-	-	-
Nerol	[27]	1228	1228	25.635	-	-	-	-	1.8	-	-	-	-	-
Geraniol	[22]	1255	1256	26.495	-	-	-	-	0.9	-	-	-	-	-
Oxygenated monoterpenes														
Eucalyptol	[28]	1034	1035	17.780	-	3.8	-	-	-	13.8	-	-	0.6	0.4
Linalool	[29]	1097	1096	19.910	19.0	-	-	-	9.3	-	-	-	9.6	-
Camphor	[30]	1150	1149	22.124	-	-	-	-	-	0.2	-	-	-	-
Terpinen-4-ol	[31]	1192	1191	23.479	2.3	-	-	-	-	-	-	-	1.0	-
α-Terpineol	[29]	1189	1192	23.981	-	-	-	-	2.8	4.6	2.3	-	0.6	0.2
β-Cyclocitral	[32]	1220	1222	25.056	-	-	-	-	1.4	-	0.4	-	-	-
Carvone	[33]	1246	1247	26.288	1.2	-	-	-	-	-	-	-	0.9	-
Thymol	[34]	1290	1290	28.084	1.7	-	-	-	-	-	-	-	2.6	-
Carvacrol	[28]	1299	1301	28.574	52.4	-	-	-	-	-	-	-	69.1	-
Sesquiterpene hydrocarbons														
(E)-Caryophyllene	[23]	1419	1418	33.572	1.6	-	-	-	-	-	-	-	1.0	-
β-Bisabolene	[24]	1509	1509	36.695	1.8	-	-	-	-	-	-	-	1.3	-
Oxygenated sesquiterpenes														
(Z)-Nerolidol	[35]	1533	1532	37.362	-	-	-	-	2.1	-	5.4	-	-	-
Spathulenol	[32]	1579	1577	39.504	-	-	-	-	-	-	-	-	0.3	-
α-Muurolol	[32]	1645	1649	41.600	-	-	-	-	-	-	-	-	0.3	-
Oxygenated diterpenes														

Phytol	[28]	1950	1949	51.149	-	-	11.5	-	12.0	-	7.9	-	-	-
Terpene related compounds														
Neryl acetone	[26]	1435	1438	34.390	-	-	0.3	-	-	-	-	-	-	-
Geranyl acetone	[29]	1455	1448	35.216	-	-	-	-	1.4	-	2.2	-	-	-
β-Ionone	[29]	1489	1490	35.801	1.0	-	0.8	-	4.0	-	2.8	-	-	-
(Z,Z)-Farnesylacetone	[26]	1860	1860	45.139	-	-	1.6	-	1.0	-	1.9	-	-	-
Aliphatic hydrocarbons														
1-Octene	[22]	794	796	8.349	-	-	-	-	-	2.9	-	-	-	-
Tetradecane	[29]	1400	1403	32.229	-	0.6	-	-	-	-	-	1.7	-	-
Eicosane	[29]	1997	1996	53.173	-	-	0.2	-	-	-	0.8	-	-	-
Heneicosane	[29]	2101	2104	56.036	-	-	8.6	-	2.3	-	2.2	-	-	-
Docosane	[29]	2200	2197	58.620	-	-	-	-	0.2	-	-	-	-	-
Tetracosane	[26]	2400	2400	61.155	-	-	9.8	-	7.1	-	4.6	-	-	-
Pentacosane	[28]	2500	2502	61.745	-	-	-	-	2.0	-	-	-	-	-
Aldehydes														
Isovaleraldehyde	[36]	700	697	5.932	-	37.1	-	27.9	-	3.9	-	13.6	-	1.4
Pentanal	[36]	715	720	6.505	-	-	-	5.0	-	-	-	2.3	-	-
Tiglic aldehyde	[22]	748	747	7.365	-	-	-	-	-	-	-	9.4	1.3	1.2
(*E*)-2-Pentenal	[26]	754	756	7.584	-	-	-	2.2	-	0.6	-	-	-	-
Hexanal	[23]	802	802	8.610	2.3	8.8	-	18.0	-	5.4	-	11.1	-	0.6
(*E*)-2-Hexenal	[37]	850	849	10.181	-	0.1	-	5.3	-	4.6	-	2.0	-	0.1
Heptenal	[26]	899	896	11.662	-	-	-	-	5.3	-	-	-	-	-
Heptanal	[38]	906	905	11.756	-	1.6	-	2.6	-	0.3	-	1.5	-	0.1
2,4-Hexadienal	[26]	907	906	12.141	-	-	-	0.4	-	-	-	-	-	-
(*E*)-2-Heptenal	[39]	959	959	13.878	-	-	-	-	-	0.1	-	-	-	-
Benzaldehyde	[29]	960	960	14.422	-	5.6	3.5	9.5	2.8	2.5	3.5	11.0	1.1	0.9
Octanal	[26]	998	999	15.723	-	-	0.8	3.2	-	1.6	4.2	5.8	-	0.1
(*E,E*)-2,4-Heptadienal	[29]	1013	1013	16.112	-	-	-	0.3	-	0.2	-	-	-	-
Benzene acetaldehyde	[29]	1045	1047	17.135	-	8.1	0.5	0.8	2.9	1.3	5.8	2.2	0.6	0.4
(*E*)-2-Octenal	[22]	1049	1048	18.191	-	-	0.3	-	-	-	-	-	-	-
Nonanal	[29]	1101	1101	20.037	-	0.6	2.7	5.0	5.5	3.0	7.8	2.1	-	0.3
(Z)-2-Nonenal	[26]	1142	1142	21.485	-	-	-	-	-	-	11.2	-	-	-
Capraldehyde	[26]	1201	1196	24.371	-	-	-	0.1	0.5	-	1.8	-	-	-
(*E*)-2-Decanal	[26]	1260	1260	26.705	-	-	0.4	-	0.4	-	0.8	-	-	-

Undecanal	[40]	1305	1302	28.728	-	-	0.2	-	0.4	0.1	-	-	-	-
(*E*,*E*)-2,4-Decadienal	[23]	1314	1312	31.744	-	-	-	-	1.4	-	-	-	-	-
Tetradecanal	[23]	1611	1613	40.576	-	-	1.6	-	-	-	1.1	-	-	-
Ketone														
1-Octen-3-one	[40]	977	974	14.650	-	-	-	-	0.1	-	0.1	-	-	-
3-Octanone	[26]	979	981	15.000	-	-	-	-	-	-	0.9	-	-	-
Undecen-2-one	[26]	1293	1295	28.418	-	-	-	-	-	-	3.1	-	-	-
(Z)-β-Damascenone	[22]	1347	1345	31.944	-	-	2.5	-	4.3	-	-	-	-	-
(E)-β-Damascenone	[29]	1383	1385	32.019	1.6	-	-	-	-	-	3.0	-	-	-
2-Pentadecanone	[41]	1696	1692	42.843	-	-	26.7	-	12.2	-	7.2	-	-	-
Alcohols														
2-Methyl-1-butanol	[37]	733	736	7.110	-	0.3	-	-	-	-	-	-	-	-
(*Z*)-3-Hexenol	[40]	864	861	10.262	-	7.7	-	-	-	-	-	-	-	91.3
n-Hexanol	[40]	870	872	10.610	-	-	-	-	-	-	-	12.0	-	0.1
1-Octen-3-ol	[29]	979	978	14.765	6.6	-	26.1	-	11.8	-	4.0	5.7	2.3	0.1
Octan-3-ol	[22]	993	995	15.450	-	-	0.5	-	-	-	5.3	-	0.3	-
Nonanol	[40]	1168	1169	23.188	-	-	-	-	-	-	1.4	-	-	-
Others														
2-Ethyl furan	[42]	728	724	6.572	-	17.6	-	19.5	-	7.2	-	14.8	-	1.0
1-Ethyl-3-methylbenzene	[43]	968	967	14.511	1.2	-	-	-	-	-	-	-	-	-
2-Pentylfuran	[29]	993	992	15.265	-	-	0.6	0.1	1.8	-	1.8	4.7	-	-
n-Hexadecanoic acid	[26]	1959	1955	51.591	0.1	-	-	-	-	-	-	-	0.1	-
				TOTAL	**99.6**	**99.9**	**99.3**	**99.9**	**98.6**	**99.9**	**99.9**	**99.9**	**99.9**	**99.9**

* Retention Index of references; [a]Retention Index calculated from retention times relative to that of n-alkane (C_6-C_{32}) series. [b]Percentages obtained by FID peak-area normalization; A1: Clevenger of *S. chrysantha*; A2: SPME of *S. chrysantha;* B1: Clevenger of *S. kotschyana*; B2: SPME of *S. kotschyana*; C1: Clevenger of *S. olympica*; C2: SPME of *S. olympica*; D1: Clevenger of *S. cinerascens* ; D2: SPME of *S. cinerascens*; E1: Clevenger of *S. zuvandica*; E2: SPME of *S. zuvandica*.

Table 3. The chemical class distribution of the volatile organic components of the five *Scrophularia* species.

Chemical class	A1		A2		B1		B2		C1		C2		D1		D2		E1		E2	
	NC[a]	%[b]	NC[a]	%[b]	NC[a]	%[b]	NC[a]	%[b]	NC[a]	%[b]	NC[a]	%[b]	NC[a]	%[b]	NC[a]	%[b]	NC[a]	%[b]	NC[a]	%[b]
Monoterpene hydrocarbons	2	6.8	2	8.0	1	0.1	-	-	3	3.6	8	47.6	1	6.4	-	-	3	6.9	5	1.7
Oxygenated monoterpene	5	76.6	1	3.8	-	-	-	-	3	13.5	3	18.6	2	2.7	-	-	7	84.4	2	0.6
Sesquiterpene hydrocarbons	2	3.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	2.3	-	-
Oxygenated sesquiterpenes	-	-	-	-	-	-	-	-	1	2.1	-	-	1	5.4	-	-	2	0.6	-	-
Oxygenated diterpenes	-	-	-	-	1	11.5	-	-	1	12.0	-	-	1	7.9	-	-	-	-	-	-
Terpene related compounds	1	1.0	-	-	3	2.7	-	-	3	6.4	-	-	3	6.9	-	-	-	-	-	-
Aliphatic hydrocarbons	-	-	1	0.6	3	18.6	-	-	4	11.6	1	2.9	3	7.6	1	1.7	-	-	-	-
Aldehyde	1	2.3	7	61.9	8	10.0	13	80.3	8	19.2	12	23.6	8	36.2	10	61.0	3	3.0	9	5.1
Ketone	1	1.6	-	-	2	29.2	-	-	3	16.6	-	-	4	13.4	-	-	-	-	-	-
Alcohols	1	6.6	2	8.0	2	26.6	-	-	1	11.8	-	-	4	11.6	2	17.7	2	2.6	3	91.5
Other	2	1.3	1	17.6	1	0.6	2	19.6	1	1.8	1	7.2	1	1.8	2	19.5	1	0.1	1	1.0
Total	15	99.6	14	99.9	21	99.3	15	99.9	28	98.6	25	99.9	28	99.9	15	99.9	20	99.9	20	99.9

[a] NC: Number of compounds. [b]% Area of compounds; A1: Clevenger of *S. chrysantha*; A2: SPME of *S. chrysantha;* B1: Clevenger of *S. kotschyana*; B2: SPME of *S. kotschyana*; C1: Clevenger of *S. olympica*; C2: SPME of *S. olympica*; D1: Clevenger of *S. cinerascens* ; D2: SPME of *S. cinerascens*; E1: Clevenger of *S. zuvandica*; E2: SPME of *S. zuvandica*.

Investigations on the other *Scrophularia* species have shown that the oxygenated terpene and monoterpene compounds are abundant and the results are consistent with the reported other species of the genus [11-14]. Fatty acids were not found in the species that we studied, however they were abundant in the essential oil composition of *S. ningpoensis* which has been intensively used and has patented preparations in Chinese folk medicine [12]. *S. kotschyana* was described as "strongly foetid" in the monograph of flora of Turkey [1]. Its unpleasant smell was also observed by us during the collection of these plants. As a result of this study, isovaleraldehyde was found to be the major compound in the SPME of the *S. cinerascens*, *S. chrysantha*, and *S. kotschyana* in a ratio of 13.6%, 37.1%, and 27.9%, respectively (Table 3). Since the significant odor of isovaleraldehyde was reported before, it could be responsible for the characteristic fragrance that was indicated in the flora of Turkey [1,21].

The antimicrobial activity for the essential oils was tested in vitro using the agar-well diffusion method against the microorganisms seen in Table 4. None of the essential oils showed antibacterial activity against Gram-negative bacteria. The essential oils of *S.chrysantha, S. kotschyana S. olympica, S.cinerascens* and *S. zuvandica* showed weak antimicrobial activity against *B. cereus* and all of the tested essential oils found to have moderate activity against *M. tuberculosis* within the Gram-positive bacteria. Tuberculosis of the lymphatic glands which was caused by the bacterium *M. tuberculosis*, has affected humans for thousands of years [43]. Considering the remarkable activity of all of the studied species against *M. tuberculosis*, it is noteworthy that the name of the genus *Scrophularia* is reported to come from its usage in scrofulous swellings which are the results of a type of tuberculosis [4,44].

A few studies conducted on the antimicrobial activities of essential oils of *Scrophularia* species. The essential oil of *S. amplexicaulis* has previously been shown to have comparable antibacterial activity with the positive control ampicillin against *Staphylococcus aureus* [14]. The insecticidal activity of the essential oil of *S. oxysepala* was studied and found to be time and concentration-dependent [11]. 3(ζ)-hydroxy-octadeca-4(E),6(Z)-dienoic acid, ajugoside and scropolioside B which were isolated from *S. desertii*, were reported to have moderate antibacterial activity against strains of multidrug and methicillin-resistant *S. aureus* (MRSA) and a panel of rapidly growing mycobacteria (MIC values ranging from 32 to 128 μg/ml) [45].

The comparative analysis of the volatile chemical profiles of the five *Scrophularia* species proved that they differ markedly in their volatile chemical profiles and in their antimicrobial activity.

Table 4. Screening for antimicrobial activity of the essential oils from *Scrophularia* species (μg/μL).

Plant species	Stoc. Sol. (μg/μL)	Microorganisms and Inhibition Zone (mm)								
		Ec	Yp	Pa	Sa	Ef	Bc	Ms	Ca	Sc
S. chrysantha	62/800	-	-	-	-	-	8	10	-	-
S. kotschyana	145/800	-	-	-	-	-	-	13	-	-
S. olympica	89/100	-	-	-	-	-	6	14	-	-
S. cinerascens	100/500	-	-	-	-	-	6	14	6	-
S. zuvandica	44/200	-	-	-	-	-	8	12	8	6
Ampicillin	10000	10	10	18	10	35	15	-	-	-
Streptomycin	10000	-	-	-	-	-	-	35	-	-
Fluconazole	2000	-	-	-	-	-	-	-	25	25

Ec: *E. coli* ATCC 35218, Yp: *Y. pseudotuberculosis* ATCC 911, Pa: *P. aeruginosa* ATCC 27853, Sa: *S. aureus* ATCC 25923, Ef: *E. faecalis* ATCC 29212, Bc: *B. cereus* 709 Roma, Ms: *M. smegmatis* ATCC607, Ca: *C. albicans* ATCC 60193, Sc: *S. cerevisiae* RSKK 251, (-): activity not detected at tested concentration.

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