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Essential oil composition and antibacterial activities of *Gypsophila* species

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Abstract: Essential oil composition of *Gypsophila turcica* Hamzaoğlu, *Gypsophila pinifolia* Boiss. & Hausskn., *G. tuberculosa* Hub.-Mor., *G. eriocalyx* Boiss. and *G. laricina* Schreb. were analyzed by means of gas chromatography-mass spectrometry (GC-MS). Thirty six, fourty four, sixty six, forty one and sixty one compounds were identified in the essential oils of *G. turcica*, *G. pinifolia*, *G. laricina*, *G. tuberculosa* and *G. eriocalyx* respectively. The major components were determined hentriacontane (12.93 ± 0.4%), 1-octadecanol (8.97 ± 0.1%), hexahydrofarnesyl acetone (6.9 ± 0.09%) and pentacosane (6.63 ± 0.08%) in *G. turcica* oil, hexadecanoic acid (17.6 ± 0.4%), 1-tetradecanol (7.6 ± 0.1%) and phytol (5.63 ± 0.05%) in *G. pinifolia* oil, octacosane (6.83%), eicosanal (6.19%), triacontane (6.03%) and heneicosane (5.78%) for *G. eriocalyx*, hexadecanoic acid (25.3%, 27.0%) and hentriacontane (13.0%, 12.6%) for *G. tuberculosa* and *G. laricina*, respectively. Antibacterial activity of *G. eriocalyx*, *G. tuberculosis* and *G. laricina* were investigated against Gram negative (*Escherichia coli*) and Gram positive (*Staphylococcus aureus*) bacteria.

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1. INTRODUCTION

Gypsophila is the 3th biggest genus in family of Caryophyllaceae to Turkey. *Gypsophila* species are annual, biennial or perennial herbaceous plants. This genus are distributed mainly in Mediterranean and Iran-Turan areas in Turkey. *Gypsophila* has 56 species in 10 sections and 33 species are endemic to Turkey [1]. By this way, it has made a significant contribution to the biodiversity of Turkey [2]. *Gypsophila turcica* is perennial plant and it was described as a new species in 2012 [3].

Gypsophila species are rich source of triterpene saponin especially in root parts [4,5]. Triterpene saponin from this genus are used commercially as medicines, detergent, adjuvants and cosmetics [5,6]. Root and barks of the genus used as analgesic, sedative, antipyretic,

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antiinflammatory, emetic and insecticidal in Turkey [7]. Biological activities of the genus seem to be associated with triterpene saponin. Due to the various beneficial biological activities, *Gypsophila* was the focus of studies that described the phytochemistry of the genus extensively.

According to study from Iran, antimicrobial activity and chemical constituents of the essential oils from flower, leaf and stem of *Gypsophila bicolor* were investigated. The main components of the essential oil from flower were germacrene-D (21.2 %), *p*-cymene (20.6 %), bicyclogermacrene (17.6 %), γ-dodecadienolactone (13.7%) and terpinolene (9.4 %). The main components of the essential oil from leaves were germacrene-D (23.4 %), terpinolene (14.5 %), bicyclogermacrene (7.5 %), γ-dodecadienolactone (6.8 %), *p*-cymene (6.7 %) and *cis*-β-ocimene (6.3 %). The main components of the essential oil from stems were γ-dodecadienolactone (28.5 %), bicyclogermacrene (14.8 %), germacrene-D (12.6 %), *p*-cymene (12.5 %), terpinolene (11.6 %) and *trans*-β-ocimene (4.2 %). The essential oils had moderate effect on Gram-positive and Gram negative bacteria, but had significant effect on the fungi [8].

As summarized above *Gypsophila* species have very high medicinal and commercial importance and also contains interesting natural substances. However, according to our literature survey we have not encountered any reports on the essential oil composition of *Gypsophila* species from Turkey. Additionaly, there is no report on antibacterial activity of essential oils of *G. eriocalyx*, *G. laricina* and *G. tuberculosa*. This prompted us to investigate the essential oil composition and antibacterial activity of *Gypsophila* genus. To the best of our knowledge this is the first report on the essential oil composition and antibacterial activity of *Gypsophila* genus.

2. MATERIALS AND METHODS

2.1. Plant Materials

Plant materials were collected during the flowering period; *G. pinifolia* on 17.07.2016 from Aşağı Ulupınar town between Darende and Malatya (1300 m), *G. turcica* on 17.07.2016 from Jipsli Hills Zara-Baglama village in Sivas (1760 m), *G. laricina* on 17.07.2017 from Ucpinar, Sarkisla in Sivas (1740-1800 m), *G. tuberculosa* on 16.07.2015 from Aşağı Ulupınar town between Darende and Malatya (1480 m) and *G. eriocalyx* on 20.07.2015 from Jipsli Hills Soğuk Çermik way in Sivas (1440 m) in Turkey by Çelik and Budak. Voucher specimens have been deposited in the Herbarium of Bozok University (Voucher no: Bozok HB 3310 and Bozok HB 3309 for *G. pinifolia* and *G. turcica* respectively), Turkey.

2.2. Isolation of the Essential Oils

Aerial parts of the air dried plants subjected to hydrodistillation for 3 h, using a Clevenger-type apparatus to produce essential oils. Condenser of the Clevenger was attached to a microchiller that set to 4°C. Essential oil yields obtained from *G. pinifolia*, *G. turcica*, *G. laricina*, *G. eriocalyx* and *G. tuberculosa* 0.03;0.01;0.01;0.01;0.03% (v/w), respectively. The oils were recovered with 1 mL *n*-hexane and preserved in amber vials under -20°C until the day they were analyzed.

2.3. Gas Chromatography/Mass Spectrometry Analysis

The GC-MS analysis was performed with an Agilent 5975C Inert XL EI/CI MSD system operating in EI mode. Essential oil of *G. pinifolia* and *G. turcica* were diluted 1/65 and 1/100 (v/v) with *n*-hexane, respectively. Injector and MS transfer line temperatures were set at 250˚C. Innowax FSC column (60 m (x) 0.25 mm, 0.25 µm film thickness) and helium as carrier gas (1 mL/min) were used in both GC/MS analyses. Splitless injection was employed. Oven temperature was programmed to 60˚C for 10 min. and raised to 220˚C at rate of 4˚C/min. Temperature kept constant at 220˚C for 10 min. and then raised to 240˚C at a rate of 1˚C/min. Mass spectra were recorded at 70 eV with the mass range *m*/*z* 35 to 425.

2.4. Gas Chromatography Analysis

The GC analyses were done with an Agilent 6890N GC system. FID detector temperature was set to 300ºC and same operational conditions applied to a duplicate of the same column used in GC-MS analyses. Simultaneous auto injection was done to obtain the same retention times. Relative percentage amounts of the separated compounds were calculated from integration of the peaks in FID chromatograms. Identification of essential oil components were carried out by comparison of their relative retention indices (RRI) obtained by series of *n*-alkanes (C5 to C30) to the literature and with mass spectra comparison [11-27]. Mass spectra comparison was done by computer matching with commercial Wiley 8th Ed./NIST 05 Mass Spectra library, Adams Essential Oil Mass Spectral Library and Pallisade 600K Complete Mass Spectra Library. The analysis was carried out in triplicate and the results were given as the mean ± standard deviation.

2.5. Antibacterial Assay

Antibacterial activities of the essential oils were tested against two strains; Gram positive *Staphylococcus aureus* (ATCC 25923) and Gram negative *Escherichia coli* (ATCC 25922). For the antimicrobial tests, Luria-Bertani broth was used as a growth medium for bacteria.

In order to evaluate antibacterial activity, minimum inhibition concentration (MIC_{50}) values were determined by using broth dilution method. DMSO was used in stock solutions to enhance solubility of the essential oils. Serial dilutions of the stock solutions were prepared on a 96 well plate. After incubation at 37°C for 24 h, bacterial suspension concentrations were standardized to McFarland No: 0.5. Essential oils and bacterial cultures were mixed in the range of 1000-1,95 µg/mL as final concentration. It was paid attention to not exceed 1% final concentration for DMSO. After treatment, the bacteria were incubated at 37°C for 24 h. As negative control, essential oil-free solutions were used. Each test was repeated for three times. Growth analysis was done by using spectrophotometric measurements for MIC determination. Minimum inhibitory concentrations (MIC_{50}) were detected as the minimum concentration at which at least 50% of bacterial growth was missing.

3. RESULTS

Essential oil composition of *Gypsophila turcica*, *G. pinifolia*, *G. tuberculosa*, *G. eriocalyx* and *G. laricina*. were analyzed by means of gas chromatography-mass spectrometry (GC-MS). In order, thirty six, fourty four, sixty six, forty one and sixty one compounds were identified in the essential oils of *G. turcica*, *G. pinifolia*, *G. laricina*, *G. tuberculosa* and *G. eriocalyx* that represent 69.1%, 71.7%, 78.1%, 71.7% and 85.6% of the oil, respectively. The major components were determined hentriacontane (12.93%), 1-octadecanol (8.97%), hexahydrofarnesyl acetone (6.9%) and pentacosane (6.63%) in *G. turcica* oil, hexadecanoic acid (17.6%), 1-tetradecanol (7.6%) and phytol (5.63%) in *G. pinifolia* oil, octacosane (6.83%), eicosanal (6.19%), triacontane (6.03%) and heneicosane (5.78%) for *G. eriocalyx*, hexadecanoic acid (25.3%, 27.0%) and hentriacontane (13.0%, 12.6%) for *G. tuberculosa* and *G. laricina*, respectively. The essential oil composition of five *Gypsophila* species are given in Table 1.

Antibacterial of the oils were evaluated for one Gram (+) and one Gram (-) bacteria by using a broth microdilution assay. *G. eriocalyx* essential oil showed mild activity on *S. aureus* (250 µg/mL) but the oil showed very low activity against *E. coli* (1000 µg/mL). However, *G. tuberculosa* and *G. laricina* essential oils did not show any significant activity against tested grains. The results of antibacterial activity of *Gypsophila* species are given in Table 2.

The essential oil of *G. pinifolia*, *G. tuberculosa* and *G. laricina* had hexadecanoic acid in high amount unlike *G. turcica* and *G. eriocalyx*. Essential oils of *G. turcica*, *G. tuberculosa* and

G. laricina were rich in hentriacontane. But hentriacontane contained at low amount in *G. eriocalyx* and not detected in *G. pinifolia*.

Table 1. The essential oil composition of five *Gypsophila* species

				G.laricina	*G.tuberculosa*	*G. turcica*	*G.eriocalyx*	*G. pinifolia*	
No	**RRI[1]**	**RRI Lit.[2]**	**Compound**	**Mean (%)[3]**	**Mean (%)**	**Mean (%)**	**Mean (%)**	**Mean (%)**	**Id. Met.[4]**
1	1200	1200	Dodecane	-	-	-	-	0.6	RI, MS, Ac
2	1233	1244	2-pentyl furan	0.27	-	-	0.08	-	RI, MS
3	1300	1300	Tridecane	-	-	-	0.09	-	RI, MS, Ac
4	1398	1399	Nonanal	0.29	0.33	-	0.32	0.33	RI, MS
5	1401	1400	Tetradecane	0.16	0.26	0.4	0.35	0.8	RI, MS, Ac
6	1442	1443	Dimethyl tetradecane	0.06	-	-	-	-	RI, MS
7	1498	1466	α-cubebene	-	-	-	-	0.1	RI, MS
8	1499	1505	Dihydroedulan II	0.15	-	-	-	-	RI, MS
9	1502	1500	Pentadecane	0.15	0.26	0.6	0.1	0.2	RI, MS, Ac
10	1505	1506	Decanal	0.47	1.05	0.5	1.79	2.53	RI, MS
11	1510	1516	Theaspirane A	0.70	-	0.5	0.04	0.1	RI, MS
12	1525	1532	Camphor	0.04	-	-	-	-	RI, MS
13	1529	1535	Dihydroedulan I	0.14	-	-	-	-	RI, MS
14	1536	1541	Benzaldehyde	-	-	-	0.07	-	RI, MS
15	1543	1548	(*E*)-2-nonenal	0.12	-	-	-	-	RI, MS
16	1549	1553	Theaspirane B	0.64	-	0.4	0.18	-	RI, MS
17	1550	1553	β-Linalool	-	0.34	-	-	2.03	RI, MS
18	1558	1549	1-Tetradecene	0.08	-	-	0.07	-	RI, MS
19	1580	1562	Longifolene	-	-	-	0.09	-	RI, MS
20	1602	1600	Hexadecane	0.29	0.34	0.77	0.45	0.80	RI, MS, Ac
21	1603	1605	2-undecanone	-	-	0.03	0.06	-	RI, MS
22	1608	1612	β-caryophyllene	-	-	-	0.13	0.1	RI, MS
23	1612	1617	Undecanal	-	-	-	-	2.57	RI, MS
24	1612	1613	β-cedrene	-	-	-	0.13	-	RI, MS
25	1633	1638	β-cyclocitral	0.13	-	-	0.06	0.17	RI, MS
26	1635	1644	Thujopsene	0.04	-	-	0.1	-	RI, MS
27	1649	1654	1-Hexadecene	-	-	0.3	-	-	RI, MS
28	1653	1655	(*E*)-2-Decanal	0.25	-	-	0.45	0.3	RI, MS
29	1660	1664	Nonanol	0.1	-	-	-	-	RI, MS
30	1672	1671	(*E*)-β-Farnesene	-	-	-	0.13	-	RI, MS
31	1683	1687	α-Humulene	-	-	-	0.07	-	RI, MS
32	1693	1685	6,10-dimethyl-2-undecanone	0.1	-	-	-	-	RI, MS
33	1701	1700	Heptadecane	0.28	0.33	0.50	0.39	-	RI, MS, Ac
34	1703	1706	α-terpineol	-	-	-	-	0.80	RI, MS
35	1718	1722	Dodecanal	0.29	0.28	-	0.67	0.53	RI, MS
36	1735	1742	β-Selinene	-	0.23	-	-	-	RI, MS
37	1761	1763	Naphthalene	0.32	-	-	-	-	RI, MS
38	1764	1766	Decanol	-	-	-	0.23	0.23	RI, MS
39	1775	1779	(*E,Z*)-2,4-Decadienal	0.13	0.13	-	0.11	-	RI, MS

Table 1. Continues

40	1785	1786	Ar-curcumene	-	-	-	0.03	-	RI, MS
41	1802	1800	Octadecane	0.21	0.29	0.3	0.51	0.3	RI, MS, Ac
42	1804	1820	Isogeraniol	-	-	-	-	0.3	RI, MS
43	1815	1815	2-tridecanone	-	-	0.1	-	-	RI, MS
44	1823	1823	(*E*)-α-Damascenone	0.2	-	-	-	0.6	RI, MS
45	1824	1827	(*E*,*E*)-2,4-Decadienal	0.4	0.56	-	0.13	-	RI, MS
46	1826	1830	Tridecanal	-	-	-	0.51	2.3	RI, MS
47	1830	1838	(*E*)-β-Damascenone	0.36	0.18	-	-	1.63	RI, MS
48	1850	1857	Geraniol	-	-	-	-	1.23	RI, MS
49	1863	1868	(*E*)-Geranyl acetone	1.12	1.17	1.03	0.6	1.43	RI, MS
50	1879	1871	Undecanol	0.17	-	-	-	-	RI, MS
51	1886	1864	*p*-cymene-8-ol	0.08	-	-	-	-	RI, MS
52	1901	1900	Nonadecane	-	0.73	-	1.3	-	RI, MS, Ac
53	1931	1933	Tetradecanal	0.38	-	-	0.96	0.5	RI, MS
54	1953	1958	(*E*)-β-Ionone	1.03	0.52	0.5	0.73	0.6	RI, MS
55	1969	1973	1-Dodecanol	0.63	0.88	-	-	0.6	RI, MS
56	2003	2000	Eicosane	0.29	0.74	0.3	1.13	0.23	RI, MS, Ac
57	2005	2007	Caryophyllene oxide	0.29	-	-	-	-	RI, MS
58	2028	2036	2-pentadecanone	-	0.41	0.3	-	-	RI, MS
59	2037	2036	Pentadecanal	0.26	-	-	-	-	RI, MS
60	2039	2036	Hexadecanal	-	-	-	-	2.00	RI, MS
61	2043	2050	(*E*)-Nerolidol	0.05	-	-	-	-	RI, MS
62	2051	2056	13-Tetradecanolide	0.35	-	-	-	-	RI, MS
63	2104	2100	Heneicosane	-	0.55	0.5	5.78	0.27	RI, MS, Ac
64	2135	2131	Hexahydro farnesyl acetone	1.65	1.9	6.9	4.44	2.73	RI, MS
65	2145	2136	Hexadecanal	0.3	-	-	1.01	-	RI, MS
66	2145	2148	(*Z*)-3-hexeneyl benzoate	-	-	-	-	1.36	RI, MS
67	2170	2192	Nonanoic acid	0.5	-	-	-	-	RI, MS
68	2173	2179	Tetradecanol	-	-	0.7	0.68	7.6	RI, MS
69	2184	2186	Eugenol	-	-	-	-	0.1	RI, MS
70	2190	2144	Spathulenol	0.05	-	-	-	1.1	RI, MS
71	2190	2198	1-Docosene	-	2.21	-	-	-	RI, MS
72	2202	2200	Docosane	-	0.6	0.4	1.6	-	RI, MS, Ac
73	2225	2226	Hexadecanoic acid methyl ester	-	-	-	-	0.2	RI, MS
74	2240	2242	2-Heptadecanone	-	0.19	-	0.12	-	RI, MS
75	2278	2282	Decanoic acid	1.03	1.56	-	-	1.37	RI, MS
76	2290	2296	Isophytol	-	-	0.4	-	-	RI, MS
77	2302	2300	Tricosane	0.55	0.81	2.2	4.5	-	RI, MS, Ac
78	2318	2315	2,4-bis-tert-butylphenol	0.35	-	2.23	-	-	RI, MS
79	2338	2345	Galaxolide I	-	-	-	0.13	-	RI, MS
80	2345	2353	Galaxolide II	-	-	-	0.09	-	RI, MS
81	2355	2353	Octadecanal	0.28	-	0.9	1.71	-	RI, MS
82	2381	2384	1-Hexadecanol	-	-	-	0.4	-	RI, MS

Table 1. Continues

83	2384	2381	Farnesyl acetone	1.41	1.5	1.87	0.8	2.1	RI, MS
84	2402	2400	Tetracosane	0.31	0.63	0.6	0.5	-	RI, MS, Ac
85	2448	2471	Nonadecanal	0.2	-	-	-	-	RI, MS
86	2489	2492	Dodecanoic acid	3.51	7.55	-	0.17	1.9	RI, MS
87	2504	2500	Pentacosane	1.4	3.06	6.63	2.32	-	RI, MS, Ac
88	2555	2592	Diisobutyl phthalate	2.15	4.23	4.48	1.42	2.91	RI, MS
89	2585	2582	Eicosanal	2.07	-	-	6.19	-	RI, MS
90	2589	2607	1-octadecanol	-	0.88	8.97	0.63	-	RI, MS
91	2590	2617	Tridecanoic acid	0.23	0.37	-	-	-	RI, MS
92	2606	2600	Hexacosane	0.31	0.58	-	0.32	-	RI, MS, Ac
93	2618	2622	Phytol	1.76	1.1	2.7	2.59	5.63	RI, MS
94	2671	2676	Heneicosanal	1.97	-	-	-	-	RI, MS
95	2701	2704	Tetradecanoic acid	4.7	6.53	-	0.26	1.33	RI, MS
96	2706	2700	Heptacosane	0.7	1.97	1.3	3.40	1.27	RI, MS, Ac
97	2775	2783	1-Docosanol	0.31	-	0.8	-	-	RI, MS
98	2796	2794	1-Eicosanol	-	-	3.63	-	-	RI, MS
99	2795	2800	Octacosane	0.25	-	-	6.83	-	RI, MS
100	2806	2822	Pentadecanoic acid	1.4	1.69	-	-	0.60	RI, MS
101	2838	2857	Palmito-γ-lactone	0.21	0.41	-	0.25	-	RI, MS
102	2904	2900	Nonacosane	-	-	1.37	1.65	-	RI, MS, Ac
103	2918	2931	Hexadecanoic acid	27.03	25.3	-	4.64	17.6	RI, MS
104	2984	2990	Docosanal	0.22	-	0.73	-	-	RI, MS
105	3003	3000	Triacontane	-	-	2.4	6.03	-	RI, MS, Ac
106	3098	3100	Hentriacontane	12.63	13.0	12.93	1.20	-	RI, MS, Ac
			Total	**78.1**	**85.6**	**69.1**	**71.7**	**71.7**	

[1]RRI: Relative retention time indices calculated against *n*-alkanes (C5-C30).
[2]RRI Lit.: Relative retention time given in the literature for the compound in similar columns and analysis conditions.
[3]The results of the analysis.
[4]Identification method: RI: identification based on the relative retention times (RRI) of genuine compounds on the HP Innowax column and the literature data; MS: identification based on MS comparison with the database or the literature data, Ac: Identification is done according to RRI and MS values of the authentic compounds.

Table 2. Antibacterial activity [MIC_{50} (µg/mL)] of the essential oils of *G. eriocalyx*, *G. tuberculosa* and *G. laricina*

Strain	*G. eriocalyx* (µg/mL)	*G. tuberculosa* (µg/mL)	*G. laricina* (µg/mL)
E. coli	1000	>1000	>1000
S. aureus	250	>1000	>1000

4. DISCUSSION and CONCLUSION

Only mild antibacterial activity is observed on *G. eriocalyx* essential oil against *S. aureus*. The main compounds of essential oil of *G. eriocalyx* contained low amount or not detected in other *Gypsophila* species. Eicosanal is one of the main compound of *G. eriocalyx*. Antibacterial activity could be correlated with this compound. According to a study from Iran, *Gypsophila bicolor* was reported to contain germacrene-D, *p*-cymene, bicyclogermacrene, γ-dodecadienolactone, terpinolene, *cis*-β-ocimene and *trans*-β-ocimene [8] however these compounds were not detected in the *G. turcica*, *G. pinifolia*, *G. eriocalyx*, *G. tuberculosa* and

G. laricina. These differences in the previous literature and present data could be related to different collection times, climatic and soil conditions, ecological factors, methods and instruments employed in analysis or different genotypes. There are very few reports on the essential oil of *Gypsophila* species therefore it is difficult to produce a comment on the chemo-systematic position of this species according to current findings and the existing reports. We believe the results obtained from this research will stimulate further research on the chemistry of *Gypsophila* species.

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