INTRODUCTION

Caryophyllaceae family has about 85 genera and 2630 species in the world and distributed mainly in Mediterranean and Iran-Turan areas.[1] *Gypsophila* is the 3th biggest genus in family of Caryophyllaceae to Turkey. *Gypsophila* species are annual, biennial or perennial herbaceous plants. Stem length of the plant is about 1 m and its flowering time is June and July.[2]

Some *Gypsophila* species are used in folk medicine as remedies for cough, cold, ailments of the upper respiratory tract[3] and also used for medical treatment such as expectorant, diuretic, hepatitis, gastritis and bronchitis.[4] The underground parts of *Gypsophila* genus have triterpenoid saponins as a main component. *Gypsophila* genus are used in industrial, medicinal and decorative application.[5] The commercial *Merck* saponin which has been widely utilized as a standard for hemolytic test was obtained from the roots of several *Gypsophila* species.[3] The genus were reported to have cytotoxic activity, α-glucosidase activity, immune-modulating effect and normalization of carcinogen induced cell proliferation.[4,6] The saponins that get from *Gypsophila* genus are interest in terms of their applications in vaccines.[7] Biological activities of the genus seems to be associated with triterpene saponins. Due to the various beneficial biological activities, *Gypsophila* was the focus of studies that described the phytochemistry of the genus extensively.

Previously, antioxidant and antibacterial acitivities of chloroform extracts of underground parts of *Gypsophila eriocalyx* and *Gypsophila sphaerocephala* var. *sphaerocephala* were investigated. The chloroform extracts of both species had high antioxidant properties but showed low antibacterial activity.[8]

Additionaly, the toxic boron levels of some plant species (*Gypsophila sphaerocephala* var. *sphaerocephala*, *Gypsophila perfoliata*, *Puccinellia distans* subsp. *distans* and *Elymus elongates*) were reported. Among these plant species, *G. sphaerocephala* contained considerably higher boron concentrations in its above-ground parts compared to the roots and organs of the other species. This study shows that *G. sphaerocephala* was not only able to grow on heavily boron contaminated soils, but was also able to accumulate extraordinarily high concentrations of boron.[9]

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.[10]

Another study from Turkey, essential oil composition and fatty acid profile of *Gypsophila tuberculosa* and *Gypsophila eriocalyx* were reported. The main components of essential oils were determined hexadecanoic acid (25.3%) and hentriacontane (13.0%) for *G*. *tuberculosa* and octacosane (6.83%), eicosanal (6.19%), triacontane (6.03%) and heneicosane (5.78%) for *G*. *eriocalyx*. The major compounds of fatty acids of *G*. *tuberculosa* and *G*. *eriocalyx* were (*Z*)-9-octadecenoic acid methyl ester (42.0%, 36.0%), (*Z*,*Z*)-9,12-octadecadienoic acid methyl ester (19.6%, 10.5%) and hexadecanoic acid methyl ester (17.7%, 25.2%), respectively.[11]

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 and fatty acid composition of *Gypsophila laricina* Schreb. This prompted us to investigate the essential oil and fatty acid composition of *Gypsophila laricina* Schreb*.* Here we report for the first time on the essential oil composition and fatty acid profile of *Gypsophila laricina* Schreb*.*

EXPERIMENTAL

Plant Materials

Plant materials were collected during the flowering period; *Gypsophila laricina* Schreb. was collected from 1740 m–1800 m height in Ucpinar, Sarkisla, Sivas,

Turkey in July 2015 by Celik and Budak. Voucher specimen has been deposited in the Herbarium of Bozok University (Voucher no. Bozok HB 3302).

Fatty Acids Analyses

The aerial parts of the collected specimen, dried separately in shadow and ground with an electric mill (Retsch SM 100). Aerial part of the plant (400 g) was extracted with hexane for three days at room temperature. After filtration through a filter paper, the extract was concentrated by rotary evaporator and 4 g crude hexane extract were obtained from the aerial parts of *Gypsophila laricina* Schreb. The crude extract was stored at 4°C. In this study we used hexane extract for fatty acid compositions. Methyl-ester derivatives of fatty acids (FAME), found in hexane extract was obtained by transesterification method.[11] In this method 1 g dried extract was dissolved in 5 mL hexane and then extracted with 2 M methanolic KOH at room temperature. The mixture was shaken for 2 min and allowed to stand for 10 min. The upper phases were removed. *G*. *laricina* Schreb. afforded fixed oil from the hexane extract with 0.07% (v/w) yields. The fixed oil was analysed by GC-MS system.

Essential Oils Analyses

Aerial parts (200 g) 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. *G*. *laricina* Schreb. afforded oils from the aerial parts with 0.01% (v/w) yields. The oils were recovered with 1 mL *n*-hexane and preserved in amber vials under -20°C until the day they were analysed.

Gas chromatography/mass spectrometry for fatty acids

The fatty acid compositions of the hexane extracts were investigated by means of Gas Chromatography-Mass Spectroscopy (GC-MS) system. The fatty acid methyl esters were analyzed using Agilent 5975C GC-MSD system with Innowax FSC polar column (30m x 0.25 mm, 0.25 µm). The inlet temperature was set at 250°C. Helium was the carrier gas at a constant flow rate of 1 ml/min. Split ratio was set to 50:1. The oven temperature was programmed from 40°C to 210°C at rate of 5°C/min and kept constant at 210°C for 10 min. EI/MS was taken at 70 eV ionization energy. Mass range was from *m/z* 35-450 amu (atomic mass unit). Relative percentage amounts of the separated compounds were calculated from integration of the peaks in MS

chromatograms. Identification of fatty acid components were carried out by comparison of their retention indices (RI) obtained by series of *n*-alkanes (C5 to C30) to the literature and with mass spectra comparison.[13-19] Mass spectra comparison was done by computer matching with commercial Wiley 8th Ed./NIST 05 Mass Spectra library. Analysis was completed in 50 minutes.

Gas Chromatography/Mass Spectrometry for essential oils

The GC-MS analysis was performed with an Agilent 5975C GC-MSD system operating in EI mode. Essential oil samples were diluted 1/100 (v/v) with *n*-hexane. 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.

Gas Chromatography for essential oils

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.[20-40] 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.

RESULT AND DISCUSSION

Fatty acid composition of *Gypsophila laricina* Schreb. were analysed by GC-MS. Ten compounds were identified in the fatty acid that represent 98.9% of the fatty acid.

The extract consisted of six saturated fatty acids (21.8%) and four unsaturated fatty acids (77.2%). The major components of the fatty acid were (*Z*,*Z*)-9,12-octadecadienoic acid methyl ester (Linoleic acid) (18:2) 40.4%, (*Z*)-9-octadecenoic acid methyl ester (Oleic acid) (18:1) 35.0% and hexadecanoic acid methyl ester (Palmitic acid) (16:0) 13.0%. The fatty acid composition of *Gypsophila laricina* Schreb. are represented in Table 1.

Essential oil composition of *Gypsophila laricina* Schreb. were analysed by GC and GC-MS. The essential oils of aerial parts of *Gypsophila laricina* Schreb. afforded very low oils yields 0.03% (v/w) yield). Sixty-six compounds were identified in the essential oil of *G*. *laricina* Schreb. by GC system that represent 76.0% of the oil. The major components of the oil were hexadecanoic acid (27.03%) and hentriacontane (12.63%). The essential oil composition of *Gypsophila laricina* Schreb. are given in Table 2.

The essential oil composition of *Gypsophila laricina* showed similar chemical behaviour to *G*. *tuberculosa*.[11] Both species had hexadecanoic acid and hentriacontane as major components in essential oils. But hexadecanoic acid contained at 4.64% levels in *G*. *eriocalyx* and in a nearly six amount in *G*. *tuberculosa* and *G*. *laricina*. And also hentriacontane contained at very low amounts in *G*. *eriocalyx*.[11] Three *Gypsophila* species had linoleic acid, oleic acid and palmitic acid as main components in different percentage in fatty acids.

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[10] however these compounds were not detected in the oil of *Gypsophila laricina* Schreb. *Gypsophila laricina* Schreb. showed very different chemical behaviour from *G*. *bicolor*. 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 or volatile composition 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.

CONCLUSION

The essential oil composition and fatty acid profile of *G*. *laricina* Schreb. are investigated for the first time. The major components of the fatty acid were oleic acid, linoleic acid and palmitic acid. The unsaturated fatty acids content were higher than their saturated fatty acids content. The essential oils of *G. laricina* Schreb. were dominated by fatty acid derivatives and *n*-alkanes. Hexadecanoic acid and hentriacontane are found as major components in essential oil. The high hexadecanoic acid content might be explained about the collection time of plant materials at late flowering period. *Gypsophila laricina* exhibited important differences *Gypsophila bicolor* and *Gypsophila eriocalyx* highlighting the existence of different main chemical constituents. Thus, the results of this study certainly contributed to the taxonomy of the genus *Gypsophila* via essential oil chemistry. We believe the results obtained from this research will stimulate further research on the chemistry of *Gypsophila* species.

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Table 1. The fatty acid composition of *Gypsophila laricina* Schreb.

RI[1]	**Compound**	**Mean(%)[2]**	**Id. Met.[3]**
1299	Dodecanoic Acid ME (Lauric acid)	0.3	RI, MS
1499	Tetradecanoic Acid ME (Myristic acid)	1.2	RI, MS
1678	(*Z*)-9-Hexadecenoic Acid ME* (Palmitoleic acid)	0.6	RI, MS
1699	Hexadecanoic Acid ME (Palmitic acid)	13.0	RI, MS
1867	(*Z*,*Z*)-9,12-Octadecadienoic Acid ME* (Linoleic acid)	40.4	RI, MS
1873	(*Z*)-9-Octadecenoic Acid ME* (Oleic acid)	35.0	RI, MS
1899	Octadecanoic Acid ME (Stearic acid)	2.3	RI, MS
1984	(*Z*)-11-Eicosenoic Acid ME (Gondoic acid)	1.2	RI, MS
1999	Eicosanoic Acid ME (Arachidic acid)	3.4	RI, MS
2299	Docosanoic Acid ME (Behenic acid)	1.5	RI, MS
	Total saturated acid	**21.8**	
	Total unsaturated acid	**77.2**	
	Total	**98.9**	
	Unsaturated/saturated	**3.6**	

*: Fatty acids with *cis* (Z) configuration, ME: Methyl ester,

[1]RI: Retention indices,

[2]The results of the analysis,

[3]Identification method: RI: identification based on the retention times (RI) 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.

Table 2. The essential oil composition of *Gypsophila laricina* Schreb.

No	RRI[1]	RRI Lit.[2]	Compound	Mean (%)[3]	Id. Met.[4]	Lit.[5]
1	1233	1244	2-pentyl furan	0.27	RI, MS	[20]
2	1397	1399	Nonanal	0.29	RI, MS	[20]
3	1400	1400	Tetradecane	0.16	RI, MS, Ac	
4	1442	1443	Dimethyl-tetradecane	0.06	RI, MS	[27]
5	1499	1505	Dihydroedulan II	0.15	RI, MS	[27]
6	1502	1500	Pentadecane	0.15	RI, MS, Ac	
7	1504	1505	Decanal	0.47	RI, MS	[28]
8	1510	1516	Theaspirane B	0.7	RI, MS	[28]
9	1525	1532	Camphor	0.04	RI, MS	[22]
10	1529	1535	Dihydroedulan I	0.14	RI, MS	[28]
11	1543	1548	(*E*)-2-nonenal	0.12	RI, MS	[28]
12	1549	1553	Theaspirane A	0.64	RI, MS	[27]
13	1558	1549	1-Tetradecene	0.08	RI, MS	[28]
14	1602	1600	Hexadecane	0.29	RI, MS, Ac	
15	1632	1638	β-cyclocitral	0.13	RI, MS	[28]
16	1635	1644	Thujopsene	0.04	RI, MS	[32]
17	1652	1655	(*E*)-2-decanal	0.25	RI, MS	[28]
18	1660	1664	Nonanol	0.1	RI, MS	[28]
19	1693	1685	6,10-dimethyl-2-undecanone	0.1	RI, MS	[39]
20	1702	1700	Heptadecane	0.28	RI, MS, Ac	
21	1717	1722	Dodecanal	0.29	RI, MS	[28]
22	1761	1763	Naphthalene	0.32	RI, MS	[28]
23	1775	1779	(*E*,*Z*)-2,4-Decadienal	0.13	RI, MS	[28]
24	1804	1779	Octadecane	0.21	RI, MS, Ac	

Table 2. Continued

No	RRI[1]	RRI Lit.[2]	Compound	Mean (%)[3]	Id. Met.[4]	Lit.[5]
25	1824	1827	(*E*,*E*)-2,4-decadienal	0.4	RI, MS	[28]
26	1831	1823	(*E*)-α-Damascenone	0.2	RI, MS	[20]
27	1836	1838	(*E*)-β-Damascenone	0.36	RI, MS	[28]
28	1865	1864	(*E*)-Geranyl acetone	1.12	RI, MS	[28]
29	1879	1871	Undecanol	0.17	RI, MS	[33]
30	1886	1864	*p*-Cymene-8-ol	0.08	RI, MS	[28]
31	1931	1933	Tetradecanal	0.38	RI, MS	[28]
32	1953	1958	(*E*)-β-Ionone	1.03	RI, MS	[28]
33	1968	1973	Dodecanol	0.63	RI, MS	[28]
34	2002	2000	Eicosane	0.29	RI, MS, Ac	
35	2005	2007	Caryophyllene oxide	0.29	RI, MS	[23]
36	2037	2036	Pentadecanal	0.26	RI, MS	[21]
37	2043	2050	(*E*)-Nerolidol	0.05	RI, MS	[24]
38	2051	2056	13-Tetradecanolide	0.35	RI, MS	[37]
39	2135	2131	Hexahydro farnesyl acetone	1.65	RI, MS	[21]
40	2138	2142	Spathulenol	0.05	RI, MS	[20]
41	2145	2136	Hexadecanal	0.3	RI, MS	[27]
42	2170	2192	Nonanoic acid	0.5	RI, MS	[22]
43	2276	2282	Decanoic acid	1.03	RI, MS	[20]
44	2304	2300	Tricosane	0.55	RI, MS, Ac	
45	2315	2315	2,4-bis(*tert*-butyl)phenol	0.35	RI, MS	[40]
46	2354	2353	Octadecanal	0.28	RI, MS	[36]
47	2382	2384	Farnesyl acetone	1.41	RI, MS	[20]
48	2407	2400	Tetracosane	0.31	RI, MS, Ac	

Table 2. Continued

No	RRI[1]	RRI Lit.[2]	Compound	Mean (%)[3]	Id. Met.[4]	Lit.[5]
49	2448	2471	Nonadecanal	0.2	RI, MS	30
50	2488	2492	Dodecanoic acid	3.51	RI, MS	20
51	2508	2500	Pentacosane	1.4	RI, MS, Ac	
52	2585	2582	Eicosanal	2.07	RI, MS	30
53	2590	2617	Tridecanoic acid	0.23	RI, MS	28
54	2606	2600	Hexacosane	0.31	RI, MS, Ac	
55	2615	2614	Phytol	1.76	RI, MS	20
56	2671	2676	Heneicosanal	1.97	RI, MS	30
57	2701	2704	Tetradecanoic acid	4.7	RI, MS	21
58	2708	2700	Heptacosane	0.7	RI, MS, Ac	
59	2775	2783	1-Docosanol	0.31	RI, MS	30
60	2795	2800	Octacosane	0.25	RI, MS, Ac	
61	2803	2809	Pentadecanoic acid	1.4	RI, MS	20
62	2838	2857	Palmito-γ-lactone	0.21	RI, MS	37
63	2921	2931	Hexadecanoic acid	27.03	RI, MS	25
64	2982	2990	Docosanal	0.22	RI, MS	30
65	3108	3100	Hentriacontane	12.63	RI, MS, Ac	
			Total	**76.0**		

In addition to the above data, diisobutyl phthalate is a common plasticizer contaminant and it was detected as a considerable component as 2.15 percentage for *G. laricina* Schreb.

[1]RRI(FID): Relative retention time indices calculated against *n*-alkanes (C5-C30) in FID chromatograms

[2]RRI Lit.: Relative retention time given in the literature for the compound in similar columns and analysis conditions.

[3]The result of the analysis in FID chromatograms.

[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.

Lit.[5]: Literature