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ORIGINAL ARTICLE

Essential Oil and Fatty Acid Composition of Endemic *Gypsophila laricina* Schreb. from Turkey

Türkiye'de Yetişen Endemik *Gypsophila laricina* Schreb. Türünün Uçucu Yağ ve Yağ Asidi Bileşimi

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ABSTRACT

Objectives: *Gypsophila* species have very high medicinal and commercial importance and contain interesting natural substances. However, there is no report on the essential oil or fatty acid composition of any *Gypsophila* species. This prompted us to investigate the essential oil and fatty acid composition of *Gypsophila laricina* Schreb.

Materials and Methods: Plant materials were collected during the flowering period. The essential oil composition of the aerial parts of *G. laricina* Schreb. was analyzed by gas chromatography and gas chromatography-mass spectrometry. The fatty acid compositions were analyzed by gas chromatography-mass spectrometry.

Results: Sixty-six and ten compounds were identified in the essential oil and fatty acid of *G. laricina* Schreb., respectively. The major components of the essential oil were hexadecanoic acid (27.03%) and hentriacontane (12.63%). The main compounds of the fatty acid were (*Z,Z*)-9,12-octadecadienoic acid methyl ester (18:2) 40.4%, (*Z*)-9-octadecenoic acid methyl ester (18:1) 35.0%, and hexadecanoic acid methyl ester (16:0) 13.0%.

Conclusion: The results showed that the fatty acid composition is rich in polyunsaturated fatty acids. The essential oils of *G. laricina* Schreb. were dominated by fatty acid derivatives and *n*-alkanes. We think the results obtained from this research will stimulate further research on the chemistry of *Gypsophila* species.

Key words: *Gypsophila laricina*, essential oil, fatty acid

ÖZ

Amaç: *Gypsophila* türleri, tıbbi ve ticari açıdan çok önemlidirler ve ilginç doğal maddeler içerirler. Bununla birlikte, literatürde *Gypsophila* türlerinin uçucu yağ ve yağ asidi bileşimi hakkında herhangi bir çalışma bulunmamaktadır. Bu nedenle *Gypsophila laricina* Schreb.'nin uçucu yağ ve yağ asidi bileşiminin araştırılmasına karar verilmiştir.

Gereç ve Yöntemler: Bitki materyali çiçeklenme döneminde toplanılmıştır. *G. laricina* Schreb. türünün toprak üstü kısmının uçucu yağ bileşimleri gaz kromatografi ve gaz kromatografi-kütle spektrometresi aracılığıyla analiz edilmiştir. Yağ asit bileşimleri gaz kromatografi-kütle spektrometresi aracılığıyla analiz edilmiştir.

Bulgular: *G. laricina* Schreb. uçucu yağlarında altmış altı bileşik ve yağ asitlerinde on bileşik tespit edilmiştir. Uçucu yağın ana bileşenleri heksadekanoik asit (%27.03) ve hentriakontan (%12.63) olarak belirlenmiştir. Yağ asidinin ana bileşenleri ise (*Z,Z*)-9,12-oktadekadienoik asit metil ester (18:2) %40.4, (*Z*)-9-octadesenoik asit metil ester (18:1) %35.0 ve heksadekanoik asit metil ester (16:0) %13.0 olarak tespit edilmiştir.

Sonuç: Bitki yağ asidi bileşiminin çoklu doymamış yağ asitleri bakımından zengin olduğu saptanmıştır. Bitki uçucu yağının yüksek oranda *n*-alkan ve yağ aside türevleri içerdiği belirlenmiştir. Bu araştırmadan elde edilen sonuçların, *Gypsophila* türlerinin kimyası üzerine yapılacak daha ileri araştırmalara katkı sağlayacağı düşünülmektedir.

Anahtar kelimeler: *Gypsophila laricina*, uçucu yağ, yağ asidi

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INTRODUCTION

The family Caryophyllaceae has about 85 genera and 2630 species worldwide and is distributed mainly in Mediterranean and Irano-Turanian areas.[1] *Gypsophila* is the third biggest genus in the family Caryophyllaceae in 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 coughs, colds, and ailments of the upper respiratory tract[3] and also used for medical treatment such as an expectorant and diuretic, and for hepatitis, gastritis, and bronchitis.[4] The underground parts of the genus *Gypsophila* have triterpenoid saponins as a main component. *Gypsophila* species are used in industrial, medicinal, and decorative applications.[5] The commercial Merck saponin, which has been widely utilized as a standard for hemolytic tests, was obtained from the roots of several *Gypsophila* species.[3] The genus was reported to have cytotoxic activity, α-glucosidase activity, an immune-modulating effect, and cause normalization of carcinogen-induced cell proliferation.[4,6] The saponins obtained from the genus *Gypsophila* are interesting in terms of their applications in vaccines.[7] The biological activities of the genus seem 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 activities of chloroform extracts of the 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]

Additionally, the toxic boron levels of some plant species (*G. 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. That 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]

In a study from Iran, the antimicrobial activity and chemical constituents of the essential oils from the flower, leaf, and stem of *Gypsophila bicolor* were investigated. The main components of the essential oil from the 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 the leaf 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 the stem 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 a moderate effect on gram-positive and gram-negative bacteria, but had a significant effect on fungi.[10]

In another study from Turkey, the essential oil composition and fatty acid profile of *Gypsophila tuberculosa* and *G. eriocalyx* were reported. The main components of the essential oils were 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 the fatty acids of *G. tuberculosa* and *G. eriocalyx* were (*Z*)-9-octadecenoic acid methyl ester (42.0% and 36.0% respectively), (*Z*,*Z*)-9,12-octadecadienoic acid methyl ester (19.6% and 10.5% respectively), and hexadecanoic acid methyl ester (17.7% and 25.2% respectively).[11]

As summarized above, *Gypsophila* species have very high medicinal and commercial importance and contain interesting natural substances. However, during our literature survey we did not encounter any reports on the essential oil or fatty acid composition of *Gypsophila laricina* Schreb. This prompted us to investigate the essential oil and fatty acid composition of this species. Here we report for the first time on the essential oil composition and fatty acid profile of *G. laricina* Schreb.

EXPERIMENTAL

Plant materials

The plant materials were collected during the flowering period; *G. laricina* Schreb. was collected from 1740-1800 m altitude in Üçpınar, Şarkışla, Sivas, Turkey, in July 2015 by Çelik and Budak. The voucher specimen has been deposited in the Herbarium of Bozok University (Voucher no. Bozok HB 3302).

Fatty acid analyses

The aerial parts of the collected specimen were dried separately in the shade and ground with an electric mill (Retsch SM 100). The aerial parts of the plant (400 g) were extracted with hexane for 3 days at room temperature. After filtration through filter paper, the extract was concentrated by rotary evaporator and 4 g of crude hexane extract was obtained from the aerial parts. The crude extract was stored at 4°C. In the present study we used hexane extract for fatty acid compositions. Methyl-ester derivatives of fatty acids found in the hexane extract were obtained by transesterification.[12] In this method 1 g of dried extract was dissolved in 5 mL of hexane and then extracted with 2 M methanolic KOH at room temperature. The mixture was shaken for 2 min and left to stand for 10 min. The upper phases were removed. *G. laricina* Schreb. afforded fixed oil from the hexane extract in 0.07% (v/w) yields. The fixed oil was analyzed by gas chromatography-mass spectrometry (GC-MS).

Essential oil analyses

The aerial parts (200 g) of the air-dried plants were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus to produce essential oils. The condenser of the apparatus was attached to a microchiller set to 4°C. *G. laricina* Schreb. afforded oils from the aerial parts in 0.01% (v/w) yields. The oils were recovered with 1 mL of *n*-hexane and preserved in amber vials at -20°C until the day they were analyzed.

GC-MS for fatty acids

The fatty acid compositions of the hexane extracts were investigated by means of GC-MS. The fatty acid methyl esters were analyzed using an Agilent 5975C GC-MSD system with an Innowax FSC polar column (30 m×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 the 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 *m/z* 35-450 atomic mass unit. Relative percentage amounts of the separated compounds were calculated from integration of the peaks in the MS chromatograms. The identification of fatty acid components was carried out by comparison of their retention indices obtained by a series of *n*-alkanes (C5 to C30) to the literature and with mass spectra comparison.[13-19] The mass spectra comparison was done by computer matching with the commercial Wiley 8th Ed./NIST 05 Mass Spectra library. The analysis was completed in 50 min.

GC-MS 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. An Innowax FSC column (60 m×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. The oven temperature was programmed to 60˚C for 10 min and raised to 220˚C at the rate of 4˚C/min. The temperature was kept constant at 220˚C for 10 min and then raised to 240˚C at the rate of 1˚C/min. The mass spectra were recorded at 70 eV with the mass range *m/z* 35 to 425.

GC for essential oils

The GC analyses were done with an Agilent 6890N GC system. FID detector temperature was set to 300˚C and the same operational conditions were applied to a duplicate of the same column used in the GC-MS analyses. Simultaneous autoinjection was used to obtain the same retention times. The relative percentage amounts of the separated compounds were calculated from integration of the peaks in the FID chromatograms. The identification of the essential oil components was carried out by comparison of their relative retention indices obtained by series of *n*-alkanes (C5 to C30) to the literature and with mass spectra comparison.[20-40] The mass spectra comparison was done by computer matching with the commercial Wiley 8th Ed./NIST 05 Mass Spectra library, Adams Essential Oil Mass Spectral Library, and Pallisade 600K Complete Mass Spectra Library.

RESULTS AND DISCUSSION

The fatty acid composition of *G. laricina* Schreb. was analyzed by GC-MS. Ten compounds were identified in the fatty acid, making up 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 *G. laricina* Schreb. is represented in Table 1.

The essential oil composition of *G. laricina* Schreb. was analyzed by GC and GC-MS. The essential oils of the aerial parts of *G. laricina* Schreb. afforded very low oil yields (0.03% (v/w) yield). Sixty-six compounds were identified in the essential oil of *G. laricina* Schreb. by GC, representing 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 *G. laricina* Schreb. is given in Table 2.

The essential oil composition of *G. laricina* showed similar chemical behavior to *G. tuberculosa*.[11] Both species had hexadecanoic acid and hentriacontane as major components in their essential oils. However, hexadecanoic acid was contained at 4.64% levels in *G. eriocalyx* and nearly six times that amount in *G. tuberculosa* and *G. laricina*. Moreover, hentriacontane

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

RI	Compound	Mean (%)**	Identification method***
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**	

ME: Methyl ester, MS: Mass spectrometry, RI: Retention index
*Fatty acids with cis (Z) configuration, **The results of the analysis, ***Identification method: RI: identification based on the retention times 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*	RRI literature**	Compound	Mean (%)****	Identification method****	Literature
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	
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

Table 2. Continued

No	RRI*	RRI literature**	Compound	Mean (%)****	Identification method****	Literature
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	
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**		

MS: Mass spectrometry, RRI: Relative retention index, FID: Flame ionization detector, Ac: According
In addition to the above data, diisobutyl phthalate is a common plasticizer contaminant and it was detected as a considerable component (2.15%) for *G. laricina* Schreb. *RRI (FID): Relative retention time indices calculated against n-alkanes (C5-C30) in FID chromatograms, **RRI literature: Relative retention time given in the literature for the compound in similar columns and analysis conditions, ***The result of the analysis in FID chromatograms, ****Identification method: RI: identification based on the 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

was contained in very low amounts in *G. eriocalyx*.[11] The three *Gypsophila* species had linoleic acid, oleic acid, and palmitic acid as the main components in different percentages.

According to a study from Iran, *G. bicolor* contained 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 *G. laricina* Schreb. *G. laricina* Schreb. showed very different chemical behavior from *G. bicolor*. These differences in the previous literature and the present data could be related to different collection times, climatic and soil conditions, ecological factors, methods and instruments employed in the analysis, or different genotypes. There are very few reports on the essential oil or volatile composition of *Gypsophila* species and therefore it is difficult to comment on the chemo-systematic position of this species according to the current findings and the existing reports.

CONCLUSIONS

The essential oil composition and fatty acid profile of *G. laricina* Schreb. were investigated for the first time. The major fatty acid components were oleic acid, linoleic acid, and palmitic acid. The unsaturated fatty acids were higher in content than the saturated fatty acids. The essential oils of *G. laricina* Schreb. were dominated by fatty acid derivatives and *n*-alkanes. Hexadecanoic acid and hentriacontane were the major essential oil components. The high hexadecanoic acid content might be explained by the collection time of the plant materials in the late flowering period. *G. laricina* exhibited important differences from *G. bicolor* and *G. 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 think the results obtained from this research will stimulate further research on the chemistry of *Gypsophila* species.

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