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

Türekliliğine ve ticari açıdan Tıbbi türleri, çok önemlidirler ve ilginç doğal maddeler içerikler. Bununla birlikte, literatürde Gypsophila türlerinin uçucu yağ ve yağ asidi bileşimi hakkında herhangi bir çalışma bulunmamaktadır. Bu nedenle Gypsophila laricina Schreb. türünün uçucu yağ ve yağ asidi bileşiminin araştırılmasına karar verilmiştir.

Gercek ve Yöntemler: Bitki materyali çiçeklenme döneminde toplanmıştır. G. laricina Schreb. türünün uçucu yağ bileşiminin araştırılması için gaz kromatografi ve gaz kromatografi-kütle spektrometresi araçlarıyla analiz edilmiştir. Ayrıca uçucu yağın yağ asidi bileşiminin araştırılması da gaz kromatografi-kütle spektrometresi yöntemi kullanılarak gerçekleştirilmişdir.

Bulgular: G. laricina Schreb. uçucu yağının analizinde 66 bileşik tanınmıştır. Bu bileşiklerden en önemli olanlar; heksadekanoik asit (27.03%) ve hentriacontane (12.63%) tespit edilmiştir. Ayrıca yağ asidi bileşiminde de (Z,Z)-9,12-oktadekadienoik asit metil ester (%40.4), (Z)-9-oktadecenoik asit metil ester (18:1) (%35.0) ve heksadekanoik asit metil ester (16:0) (%13.0) bileşikleri tespit edilmiştir.

Sonuç: Bu çalısmada tespit edilen uçucu yağ ve yağ asidi bileşiminin, Gypsophila türlerinin kimyası üzerine daha ileri çalışmalar yapmak için önemlidir. 

Anahtar kelimeler: Gypsophila laricina, uçucu yağ, yağ asidi
INTRODUCTION
The family Caryophyllaceae has about 85 genera and 2630 species worldwide and is distributed mainly in Mediterranean and Irano-Turanian areas. 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. Some Gypsophila species are used in folk medicine as remedies for coughs, colds, and ailments of the upper respiratory tract and also used for medical treatment such as an expectorant and diuretic, and for hepatitis, gastritis, and bronchitis. The underground parts of the genus Gypsophila have triterpenoid saponins as a main component. Gypsophila species are used in industrial, medicinal, and decorative applications. The commercial Merck saponin, which has been widely utilized as a standard for hemolytic tests, was obtained from the roots of several Gypsophila species. The genus was reported to have cytotoxic activity, α-glucosidase activity, an immune-modulating effect, and cause normalization of carcinogen-induced cell proliferation. The saponins obtained from the genus Gypsophila are interesting in terms of their applications in vaccines. 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. 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.

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.

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%), tricinolane (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). 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 Üçpinar, 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. 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*. 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               | 20         |
| 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               | 28         |
| 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-nononal             | 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               | 28         |
| 15 | 1632 | 1638             | β-cyclocitrinal           | 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             | Tetradecan                | 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         |
was contained in very low amounts in *G. eriocalyx.* 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; 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 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.

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             | Docosanoic 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             | Docosanol | 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.
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. 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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