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Original article

Essential oils and fatty acids of *Stachys* L. taxa, a chemotaxonomic approach

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Summary. The aerial parts and seeds of ten *Stachys* L. taxa were investigated for their essential oil and fatty acid composition. Linanyl acetate, α-terpineol, germacrene D, β-myrcene, α-pinene, linalool, caryophyllene oxide, β-caryophyllene, spathulenol were detected as the major components of the essential oils. Almost all *Stachys* taxa have the same fatty acid pattern, except for minor differences. The most abundant components were linoleic, oleic and palmitic acid. A cluster analysis was carried out for comparison and characterisation of the seed and essential oil from the *Stachys* taxa.

Key words: chemotaxonomy, essential oil, fatty acid, *Stachys*, Lamiaceae

Introduction

Stachys L. is a subcosmopolitan genus, contains approximately 300 species and is considered as one of the largest genera of the Lamiaceae family. Most of these species occur in the warm temperate regions of the Mediterranean, south-West Asia, North and South America and southern Africa (1). In recent years, phytochemical investigations on different *Stachys* taxa have shown that extracts or isolated constituents of *Stachys* taxa exert various pharmacological effects, such as anti-inflammatory, antitoxic, hypoazotemic, antihepatitis, antibacterial and antioxidant (2, 3).

In Turkey, some *Stachys* taxa are used as tonic and stomachic (4) and have been reported in folk medicine to treat genital tumors, sclerosis of the spleen, inflammatory tumors and cancerous ulcers (5). Besides, whole plants are used in phytotherapy, possessing sedative, antispasmodic, diuretic and emmenagogue activities as a tea preparations (6). Like many other representatives of the Lamiaceae, *Stachys* taxa produce essential oils, but in spite of the large size of this genus, the composition of volatile compounds is known only for a small number of taxa. Studies on essential oils and fatty acids, as well on the phylogenic and chemotaxonomy of *Stachys* and other Lamiaceae species have been reported in the literature (7-12). "Anxiety is affecting one-eighth of the total population of the world and became a very important area of research interest in psychopharmacology during the last decade. Anxiety disorders are among the most prevalent of all mental disorders that exist in many forms and could have a huge impact on the quality of life. Some *Stachys* taxa have attracted attention for their anxiolytic effects. Particularly, *S. lavandulifolia*, which has been used as an anxiolytic and sedative in Iranian folk medicine, demonstrated to possesses an anxiolytic activity accompanied by a decrease in the locomotor activity, i.e., sedation. A recent study evidenced the anxiolytic effects of the essential oil of *S. tibetica*, characterized by the sesquiterpene hydrocarbon aciphyllene as dominant constituent followed by fenchyl alcohol, α-pinene, caryophyllene oxide, menthol, and geraniol" (11). "In view of the recognised use of plants of this genus in folk medicine for centuries to

treat genital tumors, sclerosis of the spleen, inflammatory diseases, cough, and ulcers (13), a clear definition if the taxonomy and chemotaxonomy of these plants is desirable. Several studies have demonstrated anti-inflammatory, cytotoxic, antitoxic, antibacterial and antioxidant activities (14-17) of extracts from *Stachys* spp." (18). The objective of present study was to determine essential oil and fatty acid composition of ten *Stachys* taxa growing in the eastern part of Turkey. In the Flora of Turkey, *Stachys* is represented by 86 species (109 taxa), 37 of which are endemic and divided into fourteen sections; *S. cretica* subsp. *mersinaea* and *S. spectabilis* are in the Eriostomum section; *S. sylvatica* is in the *Stachys* section; *S. mardinensis* is in the Fragilicaulis section; *S. lavandulifolia* var. *lavandulifolia* is in the Zietenia section; *S. iberica* subsp. *stenostachya, S. atherocalyx, S. angustifolia* and *S. annua* subsp. *anuua* var. *annua* are in the Olisia section; *S. ramosissima* var. *ramosissima* is in the Sideritopsis section (19-22). Their chemical composition is then related to their chemotaxonomic significance. For some of the studied *Stachys* taxa, this is the first report on their chemical composition.

Materials and Methods

Plant materials

Plant materials were identified based on the Flora of Turkey and East Aegean Islands, volume seven (Davis, 1982). Information on collection of specimens of *Stachys* taxa; *S. cretica* subsp. *mersinaea*; vicinity of Yahyalı district (Elazığ-Keban), slopes, 1200-1300 m, 30.06.2012, voucher number: Kilic 4579. *S. spectabilis*; vicinity of Çırak district (Elazığ-Keban), steppe, 1200-1300 m, 30.06.2012, Kilic, 4577. *S. sylvatica*; north of Aşağıköy village (Bingöl), slopes, 1450-1500 m., 10.07.2012, Kilic 4801. *S. mardinensis*; Alatepe village (Bingöl) road 1. km, edge of stream, 14.06.2013, 1300-1350 m., Kilic 4841. *S. lavandulifolia* var. *lavandulifolia*; vicinity of Aşağıçakmak village (Elazığ-Keban), slopes, 1250-1300 m, 29.06.2012, Kilic 4576. *S. iberica* subsp. *stenostachya*; vicinity of Yahyalı district (Elazığ-Keban), slopes, 1200-1300 m, 01.07.2010, Kilic 3402. *S. atherocalyx*; vicinity of Dikme village (Bingöl), *Quercus* forest, 1550-1600 m., 27.06.2013, Kılıç, 5390. *S. angustifolia*; north of Aşağıköy village (Bingöl), slopes, 1450-1500 m., 23.06.2012, Kilic 4562. *S. annua* subsp. *anuua* var. *annua*; entrance of Örnek village (Elazığ-Keban), 1150-1200 m, 29.06.2012, Kilic 4575. *S. ramosissima* var. *ramosissima*; vicinity of Dikme village (Bingöl) district, steppe, 1550-1600 m., 24.06.2013, Kilic 5385. The voucher specimens have been deposited at the FUH, herbarium of Yildirimi and department of Park and Garden Plants of Bingol University. The seeds were collected from the respective habitats in the fruiting period of the plants. The plant materials dried at the room temperature and powdered with a blender device.

HS-SPME procedure

5 g. of the powdered aerial parts of the plants were subjected to headspace solid phase microextraction (HS-SPME) using a DVB/CAR/PDMS fiber, with 50/30 mm film thickness (Supelco, Bellafonte, PA, USA); Prior to analysis, the fiber was preconditioned in the injection port of the GC as indicated by the manufacturer. For each sample 5 g of plant samples, previously homogenized were weighed into a 40 ml vial; the vial was equipped with a "mininert" valve (Supelco, Bellafonte, PA, USA). The vial was kept at 35^{0}C with continuous internal stirring and the sample was left to equilibrate for 30 min. Then, the SPME fiber was exposed for 40 min to the headspace while maintaining the sample at 35^{0}C. After sampling, the SPME fiber was introduced into the GC injector and left for 3 min to allow thermal desorption of the analytes. In order to optimize the technique, the extraction efficiency as a functionof various parameters, such as sample volume, sample headspace volume, sample heating temperature and extraction time was determined as reported by Verzera *et al.,* 2004 (23).

GC-MS analysis

A Varian 3800 gas chromatograph directly interfaced with a Varian 2000 ion trap mass spectrometer (Varian Spa, Milan, Italy) was used. Injector temperature, 260^{0}C; injection mode, splitless; column, 60 m, CP-Wax 52 CB 0.25 mm i.d., 0.25 mm film thickness (Chrompack Italy s.r.l., Milan, Italy). The oven temperature was programmed as follows: 45^{0}C held for 5 min, then increased to 80^{0}C at a rate of 10^{0}C/

min, and to 240^{0}C at 2^{0}C/min. The carrier gas was helium at a constant pressure of 10 psi; transfer line temperature, 250^{0}C; ionisation mode, electron impact (EI); acquisition range, 40-200 m/z; scan rate, 1 μs^{-1}. The compounds were identified using the NIST (National Institute of Standards and Technology) mass spectral library and verified by the retention indices, which were calculated as described by Van den Dool and Kratz, 1963 (24).

Extraction of the seed oils and fatty acid analysis

2 g. of seed material were homogenized, mixed with hexane/isopropanol, 2 v/v according to Hara and Radin, 1978 (25). The mixture was filtreted, and most of the solvents were removed by rotary evaporation. The remaining lipid residues were taken up in in hexane-isopropanol, and nonlipid contaminants were removed by washing with 0.88% KCl solution. Fatty acids in the lipid extracts were converted into their methyl esters by means of 2% sulphuric acid (v/v) in methanol (26).

Analysis of mixtures of fatty acid methyl esters

The methyl esters were separated and quantified by gas chromatography and flame-ionization detection. (Shimadzu GC 17 Ver.3, Tokyo, Japan) coupled to a Glass GC 10 software computing recorder Chromatography was performed with a capillary column (25 m in length and 0.25 mm in diameter, Permabound 25, Machery-Nagel, city Germany) using nitrogen as a carrier gas (flow rate 0.8 ml/min). The temperature of the column, detector and injection valve were 150-220, 240, 280˚C, respectively. Fatty acids were determined and calculated based on standards.

Cluster analysis

The statistical software Cropstat (IRRI 2005) was used to perform the ANOVA and pattern analysis. Standard analyses of variance (anova) were used to analyze the data obtained.

Results

The specimens of ten *Stachys* taxa from the eastern Anatolian region were found to contain between 35 and 45 volatile compounds in their essential oils, making up between 90% and 96% of the total compounds present (Table 1). Germacrene D was present in the essential oils of all taxa, its proportion ranging from 4.6%-23.9%, but was conspicuously absent from *S. mardinensis*. This compound had previously also been identified as a major component of the essentials oils of *S. cretica* subsp. *lesbiaca* Rech.fil. and *S. cretica* subsp. *trapezuntica* Rech.fil. (20.3% and 12.9% respectively) (27). It is noteworthy that in the composition of *S. mardinensis* Germacrene D was not determined (Table 1). *S. iberica* subsp. *stenostachya, S. atherocalyx, S. angustifolia* and *S. annua* subsp. *anuua* var. *annua* are in the Olisia section and these four *Stachys* taxa are including germacrene D and linanyl acetate as major components. *S. mardinensis* showed a very different chemical behavior from all the other studied *Stachys* taxa, in respect to no percentage of germacrene D (Table 1). α-Pinene (20.1%) was found as the major component in the essential oil of *S. lavandulifolia* Vahl. from Iran as well (28); α-Terpineol was absent from the essential oil of *S. persica* or present only in low percentages in the essential oil of *S. byzantina* (hydrodistillation 0.2% - steam distillation 0.7%) (29), but it was among the major components of *S. iberica* subsp. *stenostachya* (19.8%), *S. ramosissima* var. *ramosissima* (20.6%), *S. annua* subsp. *anuua* var. *annua* (21.6%), *S. angustifolia* (19.3%) (Table 1). The essential oils of *S. lavandulifolia* var. *lavandulifolia*, *S. atherocalyx*, *S. spectabilis* and *S. angustifolia* had a chemical composition different from that of all the other species, producing high amounts of β-myrcene (15.2%, 16.3%, 15.6%, 12.3%, respectively), whereas β-myrcene was not detected as major component in the other six studied *Stachys* taxa (Table 1) and *S. oblique* from Turkey (30). Linalool was one of the major constituents of the essential oils of *S. ramosissima* var. *ramosissima* (16.6%), *S. atherocalyx* (12.4%) (Table 1) and *S. iberica* subsp. *stenostachya* (18.9%) growing in Turkey (31). In the essential oil of *S. aleutites* from Turkey linalool was determined in low percentage (0.8%) (32). The essential oil obtained from the aerial parts of *S.officinalis* from Serbia contained sesquiterpene hydrocarbons (69.1%) as the main compounds; cadinanes, germacranes, and cadinane-related sesquiterpenoids, andcaryophyllanes represent the most abundant constituents (33). In another recent

Table 1. Chemical composition of studied *Stachys* taxa (%).

Compounds	**RRI***	***S.iberica* subsp. *stenostachya***	***S.lavandulifolia* var. *lavandulifolia***	***S.ramosissima* var. *ramosissima***	***S.atherocalyx***	***S.spectabilis***	***S.annua* subsp. *annua* var. *annua***	***S.mardinensis***	***S.angustifolia***	***S.sylvatica***	***S.cretica* subsp. *mersinaea***
(E)-2-Hexenal	847	-	0.1	0.6	0.1	-	0.3	0.2	-	0.3	-
α-thujene	931	0.1	0.6	-	-	0.3	-	-	0.2	0.5	-
α-pinene	936	0.1	13.3	11.4	0.5	-	-	1.1	-	19.6	0.1
Camphene	954	-	0.6	2.1	0.3	0.5	0.4	-	0.6	-	-
Benzaldehyde	960	0.2	-	0.3	-	-	-	-	-	0.1	0.3
Sabinene	977	-	0.4	-	0.2	-	0.6	1.3	2.6	-	0.7
β-pinene	980	0.3	-	0.4	-	0.2	-	0.3	0.4	-	-
β-mrycene	989	0.6	15.2	3.9	16.3	15.6	2.7	-	12.3	1.2	2.2
α-phellandrene	1005	-	0.1	-	0.4	-	0.1	0.4	-	0.3	-
δ-3-Carene	1010	-	0.2	0.9	-	0.9	-	0.6	0.5	1.9	-
α-terpinene	1015	0.2	0.5	-	0.6	0.7	0.3	-	-	-	0.3
p-cymene	1026	0.4	-	1.3	-	-	-	0.3	0.1	0.4	-
β-ocimene	1037	1.5	3.6	-	5.7	1.4	2.3	0.5	-	-	-
α-terpinolene	1134	0.6	0.4	-	-	-	-	-	0.4	1.2	0.3
Limonene	1046	0.4	-	3.6	-	0.5	-	0.7	1.9	-	0.4
Linalool	1097	0.8	2.8	16.6	12.4	3.1	6.7	0.9	2.8	0.7	-
1,8-cineole	1100	0.6	-	0.4	-	-	2.8	-	4.6	0.1	0.3
γ-terpinene	1102	0.3	0.3	0.3	0.4	2.2	-	1.1	-	1.4	0.6
Cis-sabinol	1125	-	-	0.2	0.1	0.4	0.3	1.2	0.7	-	-
Verbenol	1140	-	0.2	0.3	0.3	-	0.4	1.3	-	-	-
Cis-Sabinenehydrate	1151	-	0.5	-	0.2	1.3	0.1	-	-	2.5	-
Terpinen-4-ol	1175	0.1	0.6	-	-	-	-	0.6	0.3	-	0.1
α-terpineol	1187	19.8	0.3	20.6	1.5	0.6	21.6	-	19.3	0.2	-
n- decanal	1203	-	-	0.3	0.1	0.8	0.7	1.3	-	-	0.3
Citronellol	1224	0.5	-	0.2	-	-	-	0.3	0.4	-	-
Pulegone	1237	0.8	0.1	-	-	0.6	0.6	-	-	0.5	3.6
Carvone	1250	-	-	0.2	0.5	-	-	-	0.7	-	0.1

Continued

Compounds	RRI*	*S.iberica* subsp. *stenostachya*	*S.lavandulifolia* var. *lavandulifolia*	*S.ramosissima* var. *ramosissima*	*S.atherocalyx*	*S.spectabilis*	*S.annua* subsp. *annua* var. *annua*	*S.mardinensis*	*S.angustifolia*	*S.sylvatica*	*S.cretica* subsp. *mersinaea*
Geraniol	1252	5.3	0.4	-	2.7	0.3	3.4	0.8	-	-	-
Linanyl-acetate	1258	24.2	-	5.2	10.8	1.9	13.5	5.2	0.5	0.1	-
Thymol	1293	0.1	1.0	1.6	-	0.4	-	-	2.3	-	1.9
Carvacrol	1299	-	-	0.7	0.3	-	0.5	0.3	-	-	2.6
γ-elemene	1338	0.2	1.2	-	-	0.2	-	-	0.8	2.0	0.3
α-terpinyl acetate	1345	-	-	0.1	0.1	-	0.1	0.3	-	-	-
Eugenol	1352	-	-	-	0.3	0.8	-	-	1.2	0.2	0.4
Piperitenone oxide	1363	-	0.1	-	0.1	1.7	0.2	-	-	-	-
α-copaene	1374	0.3	6.5	0.5	-	0.8	-	1.9	1.3	0.8	2.3
Geranyl acetate	1380	5.8	-	-	0.5	-	0.6	1.1	-	-	-
β-bourbonene	1387	-	0.6	0.3	0.4	0.4	-	10.9	0.2	-	0.9
α-cubebene	1389	-	2.5	0.3	-	1.3	0.4	-	0.3	1.5	0.3
β-elemene	1390	0.6	1.3	-	0.2	-	-	0.3	-	-	-
β-caryophyllene	1415	4.9	-	-	0.1	13.7	0.3	25.4	3.2	20.8	12.9
Cedrene	1419	-	0.5	0.1	-	0.1	0.1	-	0.4	-	0.4
α-gurjunene	1432	0.1	0.3	-	0.2	-	-	-	-	-	-
α-humulene	1438	-	0.2	-	-	0.2	-	0.8	-		0.5
Aromadendrene	1445	0.7	-	0.2	0.5	-	0.9	-	1.1	1.4	0.1
Trans-β-Farnesene	1460	0.4	2.3	3.9	-	0.1	-	-	0.6	-	0.7
Germacrene D	1480	15.6	19.7	4.6	25.3	13.2	22.2	-	22.6	23.9	12.4
β-selinene	1485	-	-	0.1	0.1	2.3	0.1	2.7	-	-	-
α-amorphene	1490	-	-	0.4	-	0.4	0.3	-	0.1	-	0.2
α-muurolene	1496	0.2	0.1	-	-	-	-	1.4	-	0.7	2.8
Bicyclogermacrene	1502	-	4.2	1.1	1.3	0.3	0.2	1.7	-	-	0.3
β-bisabolene	1509	-	-	-	0.2	0.4	-	-	0.1	-	-
α-cadinene	1520	1.8	0.1	0.3	-	0.5	-	0.3	0.7	1.7	6.9
δ-cadinene	1523	2.3	-	-	1.4	0.2	0.4	3.6	-	0.9	1.7
β-sesquiphellandren	1529	-	0.5	0.3	-	0.3	0.1	-	0.3	-	-

Continued

Compounds	RRI*	*S.iberica* subsp. *stenostachya*	*S.lavandulifolia* var. *lavandulifolia*	*S.ramosissima* var. *ramosissima*	*S.atherocalyx*	*S.spectabilis*	*S.annua* subsp. *annua* var. *annua*	*S.mardinensis*	*S.angustifolia*	*S.sylvatica*	*S.cretica* subsp. *mersinaea*
(E)-Nerolidol	1562	0.2	-	0.2	-	-	-	0.4	-	-	-
Dodecanoic acid	1567	0.3	-	-	0.5	0.7	1.6	1.3	2.1	1.5	5.2
Spathulenol	1575	-	0.1	5.2	0.1	-	0.2	12.6	-	1.4	1.8
Caryophyllene oxide	1582	-	-	0.9	6.1	14.6	1.5	2.2	-	3.2	12.1
Globulol	1585	0.1	0.3	-	-	0.1	-	-	0.7	-	-
Cedrol	1598	-	-	1.2	-	-	0.1	0.1	-	-	0.7
Humulene epoxide	1605	1.3	1.5	-	-	2.1	0.3	2.3	0.5	-	-
Cubenol	1636	-	-	0.6	0.1	0.1	-	1.3	-	0.1	-
τ-muurolol	1641	0.5	0.4	0.2	-	0.9	1.1	1.2	0.4	0.4	-
α-cadinol	1651	0.7	1.1	-	-	1.9	-	1.0	-	-	4.6
β-eudesmol	1659	-	-	0.4	0.6	-	0.4	-	-	0.2	-
α-bisabolole	1680	-	0.7	-	-	-	0.2	-	0.3	-	2.9
Heptadecane	1701	-	-	0.9	-	0.3	-	0.3	-	-	-
Farnesyl acetate	1720	0.1	-	0.3	0.2	1.2	-	0.1	1.9	-	1.2
Tetradecanoic acid	1770	-	0.4	-	-	-	0.3	-	-	0.1	0.9
Octadecane	1795	-	-	0.7	-	0.3	-	0,1	0.3	-	-
Hexadecanoic acid	1975	1.2	0.5	-	0.9	0.3	0.5	0.2	3.5	-	3.7
Octadecanoic acid	2175	-	0.1	-	-	0.3	0.1	0.2	-	-	3.4
Carene	2102	-	-	0.3	-	0.1	-	-	0.6	0.1	-
Trans-phytol	2115	-	2.9	-	-	-	0.3	0.3	-	-	-
Octadecanal	2154	-	-	0.2	0.1	-	-	-	0.8	-	0.6
Tricosane	2295	0.2	0.2	0.8	-	-	-	-	0.7	0.2	-
Eicosane	2395	-	0.6	-	0.2	0.4	-	0.1	-	-	-
Nonacosane	2455	0.1	-	0.3	-	-	1.2	-	0.4	-	0.2
	Total	94.3	90.1	95.5	92.9	93.1	91.2	92.5	95.7	92.1	94.1

Continued

Table 2. Fatty acid composition of the *Stachys* taxa (%).

Fatty acids	***S.iberica* subsp. *stenostachya***	***S.lavandulifolia* var. *lavandulifolia***	***S.ramosissima* var. *ramosissima***	***S.atherocalyx***	***S.spectabilis***	***S.annua* subsp. *anuua* var. *annua***	***S.mardinensis***	***S.angustifolia***	***S.sylvatica***	***S.cretica* subsp. *mersinaea***
Capric acid (C10:0)	-	t	-	-	2.5	-	t	t	-	1.2
Lauric acid (C12:0)	1.4	-	0.5	t	t	0.8	-	-	t	-
Myristic acid (C14:0)	t	1.8	t	0.6	-	t	0.8	1.4	-	t
Palmiteloic acid (C16:1)	-	-	-	t	0.3	0.1	t	-	0.1	0.2
Palmitic acid (C16:0)	8.2	9.5	10.3	7.8	5.8	6.9	9.1	8.8	3.9	5.6
Pentadecanoic acid (C15:0)	t	0.4	-	-	t	-	0.2	t	-	t
Linoleic acid (C18:2)	52.6	37.3	30.8	45.4	54.8	52.3	32.6	46.8	32.0	51.8
Oleic acid (C18:1)	27.4	35.2	30.1	35.1	21.7	29.1	31.7	34.6	25.3	23.3
Linolenic acid (C18:3)	-	t	1.1	-	-	t	-	-	1.3	0.2
6-octadecanoic acid	5.8	13.8	21.0	5.1	6.8	7.5	16.8	3.8	34.1	12.1
Stearic acid (C18:0)	2.3	-	3.8	4.2	2.1	t	5.4	3.3	2.6	3.3
11,13-Eicosadienoic acid (C20:2)	0.2	t	-	0.4	-	0.2	0.3	-	t	t
11-Eicosenoic acid (C20:1)	-	0.2	0.3	-	0.4	-	t	t	0.2	-
Eicosanoic acid (C20:0)	0.5	-	1.3	t	2.2	0.5	-	0.4	-	t
Behenic acid (C22:0)	-	0.1	-	0.2	-	t	t	-	t	0.1
Saturated FA	10.5	10.0	8.1	12.2	7.9	6.9	14.7	12.1	6.5	9.0
Unsaturated FA	82.9	79.8	91.1	86.6	88.7	90.5	81.7	87.0	93.0	88.8
Total FA	98.4	98.3	99.2	98.8	96.6	97.4	96.9	99.1	99.5	97.8

work ten wild populations of *S. lavandulifolia*, collected from different geographical regions of Iran, were studied for the essential oils composition and the most abundant essential oil compounds were myrcene, limonene, germacrene D, bicyclogermacrene, δ-cadinene, pulegone, (*Z*)-hex-3-enyl tiglate, (*E)*-caryophyllene and spathulenol (34).

Discussion

The principal fatty acid components were found to be linoleic (32.0-54.8%), oleic (21.7-35.1%), palmitic (3.9-9.5%), 6-octadecanoic acid (3.8-34.1%) and stearic (trace to 4.2%) acids. The most important chemotaxonomic marker of *Stachys* taxa was found to be 6-octadecynoic acid (Table 2). In a study 18:3/18:2 was used ratio of the seed oil of plant species for chemotaxonomic evaluation (35). However, here in, linolenic acid content of *Stachys* species was determined to be in trace, except *S. ramosissima* var. *ramosissima* (1.0%) and *S. sylvatica* (1.3%) (Table 2). In this study the reported taxa of *Stachys* also have same linolenic and linoleic acid ratios comparable with previous report in the literature. In addition, apart from linolenic acid, the most important chemotaxonomical marker in the genus *Stachys* is 6-octadecynoic acid (Table 2). A previous study of *Stachys* taxa found that palmitic acid (3.0-7.6 %) and stearic acid (0.6-2.5 %) were the major saturated fatty acids (36). The current study detected that unsaturated fatty acid amount was greater than saturated fatty acids. This is a characteristics of the seed oils of the Lamiaceae taxa (36). Hierarchical cluster analysis seed of ten studied *Stachys* taxa is seen in Figure 2. It is seen that 4,8 are oleic acid; 1,6,5,10 are linoleic; 2,7,3 are palmitic and 9 is 6-octadecanoic chemotypes of studied plants.

Hierarchical cluster analysis essential of ten studied *Stachys* taxa is seen in Fig. 1. According to these results, germacrene D and linanyl acetate are the chemotype of Olisia section. *S. spectabilis,* and *S. cretica* subsp. *mersinaea* contained high concentrations of β-caryophyllene (13.7%-12.9%) and caryophyllene oxide (14.6%-12.1%, respectively) and these taxa are belongs to Eriostomum section. So caryophyllene oxide and β-caryophyllene are the chemotaxonomic marker for section Eriostomum. *S.*

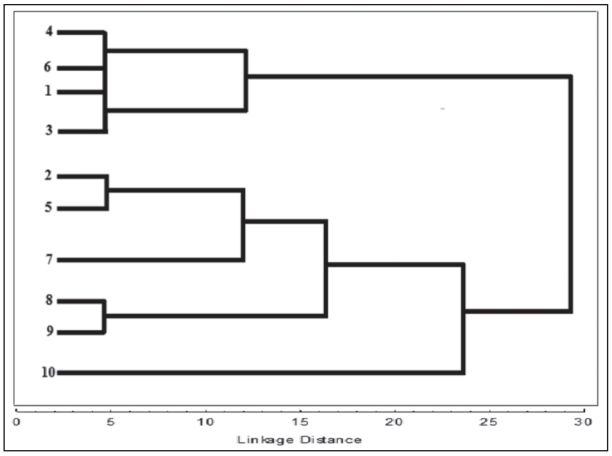

Figure 1. Hierarchical cluster analysis essential oil of *Stachys* taxa. 1. *S. iberica* subsp. *stenostachya*, 2. S. *lavandulifolia* var. *lavandulifolia*, 3. *S. annua* subsp. *annua* var. *annua*, 4. *S. atherocalyx* 5. *S. ramosissima* var. *ramosissima*, 6. *S. angustifolia*, 7. *S. sylvatica*, 8. *S. spectabilis*, 9. *S. cretica* subsp. *mersinaea*, 10. *S. mardinensis*

lavandulifolia var. *lavandulifolia, S. ramosissima* var. *ramosissima* and *S. sylvatica* contained high concentrations of α-pinene (13.3%, 11.4% and 19.6%, respectively). Results of cluster analysis based on the distribution of essential oil compounds show two main groups. One of them 1.2.3.4.5.6.7.8. and 9. samples. The other group is 10. sample which is only in the Fragilicaulis section and was very far apart from all the other taxa. We can seperate first main group (1.2.3.4.5.6.7.8. and 9.) in four groups. First 4-6-1-3, second 2-5-6, third 7 and fourth 8,9 samples. In fact, in the first main group *S. sylvatica* which is only in *Stachys* section was very far apart from all the other taxa (1. 2. 3. 4. 5. 6. 8. 9.). *S. spectabilis* and *S. cretica* subsp. *mersinaea* which were in the same section (Eriostomum), are very close in the dendogram in terms of major chemical components. So relationships of *S. spectabilis* and *S. cretica* subsp. *mersinaea* which were determined with morphological charachters, stay up with chemical charachters too (Figure 1). *S. iberica* subsp. *stenostachya, S. atherocalyx*, *S. annua* subsp. *anuua* var. *annua* and *S. angustifolia* which were in the same section (Olisia), are very close in the dendogram in terms of major chemical components. So relationships of these four *Stachys* taxa which were determined with morphological charachters, also stay up with chemical charachters. *S. lavandulifolia* var. *lavandulifolia* and *S. ramosissima* var. *ramosissima* which are in the different sections in

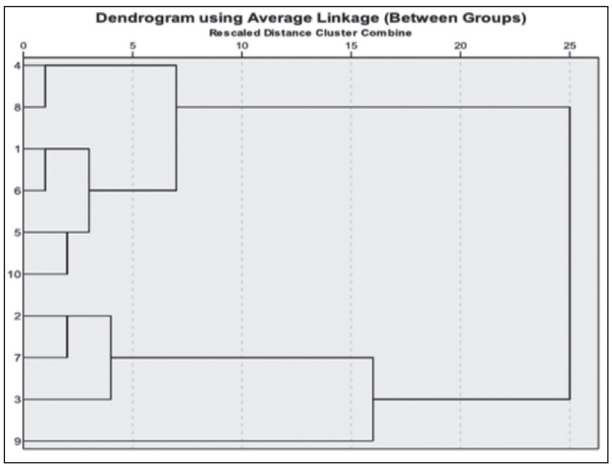

Figure 2. Hierarchical cluster analysis fatty acid of *Stachys* taxa. 1. *S. iberica* subsp. *stenostachya*, 2. S. *lavandulifolia* var. *lavandulifolia*, 3. *S. annua* subsp. *annua* var. *annua*, 4. *S. atherocalyx* 5. *S. ramosissima* var. *ramosissima*, 6. *S. angustifolia*, 7. *S. sylvatica*, 8. *S. spectabilis*, 9. *S. cretica* subsp. *mersinaea*, 10. *S. mardinensis*

the Flora of Turkey; are seen close in terms of chemical relationships (Figure 1). According to these results, morphological relationships of studied *Stachys* taxa are stay up in this study with with our chemical results. "It is also important to note that different geographic localities, seasons, harvest periods, properties of soils and climatic conditions strongly affect the secondary metabolite composition of plant species especially essential oil composition" (37). The essential oil chemotypes of studied taxa were detected as; linanyl acetate/α-terpineol in *S. iberica* subsp. *stenostachya*; germacrene D/β-myrcene in *S. lavandulifolia* var. *lavandulifolia*; α-terpineol/ linalool in *S. ramosissima* var. *ramosissima*; germacrene D, β-myrcene in *S. atherocalyx*; β-myrcene/caryophyllene oxide in *S. spectabilis*, germacrene D/α-terpineol in *S. annua* subsp. *anuua* var. *annua*; β-caryophyllene / spathulenol in *S. mardinensis*; germacrene D/α-terpineol in *S. angustifolia*; germacrene D/β-caryophyllene in *S. sylvatica*; β-caryophyllene/germacrene D in *S. cretica* subsp. *mersinaea* (Table 1). In addition, the present study showed that palmitic acid (C 16:0) was the major saturated fatty acid was detected in all studied taxa, and that stearic acid (C 18:0) was the second major saturated fatty acid in the *Stachys* taxa. The *Stachys* taxa had the highest unsaturated fatty acid amount (79.8-93.0%) and low saturated fatty acid amount (6.5-14.7%). *S. sylvatica* had the highest unsaturated fatty acid amount (93.0%) while *S. mardinensis* had the highest saturated fatty acid amount (14.7%) among ten *Stachys* taxa. The fatty acid chemotypes of studied taxa were determined as; linoleic acid in *S. iberica* subsp. *stenostachya*; *S. spectabilis*, *S. annua* subsp. *anuua* var. *annua*, *S. cretica* subsp. *mersinaea*; oleic acid in *S. lavandulifolia* var. *lavandulifolia*, *S. atherocalyx*, *S. angustifolia*; 6-octadecanoic acid in *S. ramosissima* var. *ramosissima, S. sylvatica*; 6-octadecanoic acid and linoleic acid in *S. mardinensis* (Table 2). In conclusion, while seed extracts of *Stachys* taxa have mainly linoleic and oleic acids, palmitic acid content of the genus is seen in a very small amount. The *Stachys* taxa in this study have an ability to synthesise unusual fatty acid, 6-octadecanoic acid and palmitic acid, which is the main chemotaxonomical marker of taxa together with oleic and linoleic acids. The taxa of *Stachys* could be a source of 6-octadecanoic acid and palmitic acids. The fatty acid profiles and essential oil compositions of some of the species could also be same chemotaxonomic cluster. The chemical results from this study might be helpful chemotaxonomy and potential usefulness of *Stachys* taxa. Besides, due to their various bioactivities, further researchs should be carried out on the drug development of *Stachys* extracts and their constituents.

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