

The Comparison of Volatile Components of *Salvia ceratophylla* L. Collected from Different Regions in TURKEY

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The genus *Salvia* is represented in Turkey by 95 species, of which 48 are endemic. *Salvia ceratophylla* L. was collected from Kayseri, Elazığ and Adıyaman in Turkey. The volatile components obtained from three samples by using microdistillation were analyzed by GC and GC/MS systems simultaneously. 30, 35 and 27 components of *S. ceratophylla* from Kayseri, Elazığ and Adıyaman were identified representing 94.5%, 95.5% and 92.0% of the samples, respectively. The major components of the Kayseri sample were α -pinene (27.0%), β -pinene (16.3%) and β -caryophyllene (10.6%). The major components of the Elazığ sample were α -pinene (24.6%) and β -pinene (10.3%). The major components of the Adıyaman sample were α -pinene (23.7%), 1,8-cineole (8.9%) and borneol (7.0%). Pinenes were observed as main constituents in all samples.

Key words: *Salvia ceratophylla*, Microdistillation, Pinene, GC/MS.

Türkiye’de Farklı Bölgelerden Toplanan *Salvia ceratophylla*’nın Uçucu Bileşiklerinin Karşılaştırılması

Salvia cinsi 48’i endemik olmak üzere 95 tür ile Türkiye’de temsil edilmektedir. *Salvia ceratophylla* L. türüne ait örnekler Kayseri, Elazığ ve Adıyaman’dan toplanmıştır. Mikrodistilasyon yöntemi kullanılarak bu üç örneğin uçucu bileşikleri elde edilmiş, eş zamanlı olarak gaz kromatografisi (GK) ve gaz kromatografisi/kütle spektroskopisi (GK/KS) sistemleri ile analiz edilmiştir. Kayseri, Elazığ ve Adıyaman örneklerinden sırasıyla 30, 35, 27 bileşik %94.5, %95.5 ve %92.0 verimle tanımlanmıştır. Kayseri örneğinin ana bileşikler α -pinen (%27.0), β -pinen (%16.3) ve β -karyofillen (%10.6); Elazığ örneklerinin ana bileşikler α -pinen (% 24.6) ve β -pinen (%10.3); Adıyaman örneklerinin ana bileşenleri ise α -pinen (%23.7), 1,8-sineol (%8.9) ve borneol (%7.0) olarak bulunmuştur. Pinenler, tüm örneklerde ana bileşik olarak bulunmuştur.

Anahtar kelimeler: *Salvia ceratophylla*, Mikrodistilasyon, Pinen, GK/KS.

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INTRODUCTION

Salvia is one of the most important and the largest genus of Lamiaceae family. Lamiaceae family has worldwide distribution and includes over 250 genera and about 7000 species. Also this family is known for its fine herbs like lavender, sage, basil, oregano,

thyme, mint, rosemary and is a rich source of essential oils for the perfume and flavoring industry. Lamiaceae is the third largest family in Turkish Flora (1-3).

The genus *Salvia* includes nearly 900 species spread throughout the world and Turkey is a diversity centre for *Salvia* in Asia.

The genus *Salvia* is represented in Turkey by 95 species, of which 48 are endemic (4-7).

Salvia species are commonly used in traditional medicine all around the world, possessing antibacterial, antioxidant, positive effects on memory, antitumor, astringent and spasmolytic properties (8-11). In addition, many of the herbs and essential oils are often used in the food, drug, cosmetic and perfumery industries. They are well known among people and widely used as flavour or fragrance and for medicinal purposes (12-16).

Salvia ceratophylla L. is a biennial herb. The aerial parts of *Salvia* species usually yield triterpenic compounds and flavonoids, while the roots contain diterpenoids. The identified diterpenoids in Turkish *Salvia* species include mainly abietane, rarely pimarane and labdane type structures (17). Gören et al. (2002), have obtained four known and two new diterpenoids from the roots of *S. ceratophylla*. In addition, ursolic acid and oleanolic acid,

sitosterol and the flavone salvigenin were obtained from acetone extract of the roots (17). In previously studies, composition of essential and fixed oils, and also antioxidant, anticholinesterase and antimicrobial activities of *S. ceratophylla* were reported (14, 18-20). At the present work, we determined the volatile compounds of *S. ceratophylla* and compared the samples collected from different regions in Turkey.

EXPERIMENTAL

Plant material

Aerial parts of the plants were collected from the following regions of Turkey by the authors. Voucher specimens were deposited at the Laboratory of Plant Systematics & Taxonomy, Department of Biology, ODTU. Detailed information on the plant materials used is given in Table 1.

Table 1. Information on of *Salvia ceratophylla* samples

Sample Code	Collection place	Altitude(m)	Collection date
A	Develi to Bakırdağ, between Kılıçkaya to Bakırdağ ca. 2km, Kayseri	1421	09.06.2006
B	Elazığ to Baskil, above Kayabeyi	1600	07.07.2006
C	3 km from Gerger to Kaşyazı, Adıyaman	876	19.05.2007

Isolation of the volatile components

The volatile components were obtained by microdistillation of the dried, ground plant materials (50 mg) using an Eppendorf MicroDistiller® with 10 mL distilled water per sample vial. The sample vial was heated to 108°C at a rate of 20°C/min and kept at this temperature for 90 min, then heated to 112°C at a rate of 20°C/min and kept at this temperature for 30 min. The sample was subjected to a final post-run for 2 min under the same conditions. The collecting vial, containing a solution of NaCl (2.5 g) and water (0.5 mL) plus *n*-hexane (350 µL) to trap

volatile components, was cooled to -5°C during distillation. After the distillation, the organic layer in the collection vial was separated and analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) systems, simultaneously.

GC analysis

GC analyses were performed using 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 GC/MS

analyses. Simultaneous auto injection was done to obtain equivalent retention times. Relative percentages of the separated compounds were calculated from integration of the peak areas in the GC/FID chromatograms

GC/MS analysis

The GC/MS analyses were done with an Agilent 5975 GC/MSD system. An Innowax fused silica capillary (FSC) column (60 m × 0.25 mm, 0.25 µm film thickness) was used with helium as the carrier gas (0.8 mL/min). Oven temperature was kept at 60 °C for 10 min, then programmed to 220 °C at a rate of 4 °C/min, then adjusted to 220 °C for 10 min, and finally programmed to 240 °C at a rate of 1 °C/min. Injector temperature was set at 250 °C. Split flow was adjusted at 50:1. Mass spectra were recorded at 70 eV with the mass range m/z 35 to 450.

Identification of volatile components

Identification of the volatile components was carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of *n*-alkanes. Computer matching against Wiley GC/MS Library, Adams Library, MassFinder 3 Library (21, 22) and “Başer Library of Essential Oil Constituents” built up by genuine compounds and components of known essential oils, as well as MS literature data (23-25) were used for the identification.

RESULTS AND DISCUSSION

The volatile components were obtained by microdistillation from air dried aerial parts of *S. ceratophylla* collected from different regions in Turkey. The volatile components were subsequently analyzed by GC and GC/MS and the individual identified components and their relative percentages are given in Table 2.

30 volatile components of the Kayseri sample were identified representing 94.5% of the sample and the major compounds were α -pinene (27.0%), β -pinene (16.3%), β -caryophyllene (10.6%). Other major

compounds were bornyl acetate (5.9%), linalool (4.0%), borneol (3.7%), carvacrol (3.6%) and camphene (3.6%).

35 volatile components of the Elazığ sample were detected representing 95.5% of the total components. The major compounds were α -pinene (24.6%), β -pinene (10.3%) and other major compounds were 1,8- cineole (6.6%), α -terpineol (6.4%), camphene (4.7%), spathulenol (4.4%) and sabinene (3.7%).

27 volatile components of the Adıyaman sample were identified representing 92.0% of the detected constituents and the major compounds were α -pinene (23.7%), 1,8 cineole (8.9%), borneol (7.0%), camphene (5.9%), β -pinene (5.3%) and spathulenol (5.1%). 1,8- cineole, camphor, thujenes and pinenes have previously been reported as main constituents of *Salvia* essential oils (26-30). According to our findings, pinenes were observed as the main components in all three samples. Pinene-rich oil containing *Salvia* species are also consumed in Turkey as herbal tea.

CONCLUSION

According to literature survey, there is only one study on the essential oil from aerial parts of *S. ceratophylla* collected from B5 Kayseri-Incesu highway in 2009 reported by Gürsoy et al (2012). The essential oil obtained by hydro-distillation using Clevenger type apparatus yielding 0.8%. 53 volatile compounds were identified in the essential oil representing 95.8% of the total oil. γ -Muurolene (11.4%) and α -pinene (7.6%) were found as major compounds (31).

In our present study, we aimed to evaluate the volatile compositions of three samples of *S. ceratophylla* collected from different regions in Turkey. According to our results, Kayseri sample was found to be richer in pinenes than the other samples. Başer reported in 2002, *Salvia* species rich in pinenes: *S. tomentosa* essential oil contains α -pinene (6-29%) and β -pinene (5-33%), *S. wiedemannii* essential oil α -pinene (23-33%) and β -pinene (14-30%), *S. potentillifolia* essential oil α -pinene (10%) and β -pinene (8%) (32).

Table 2. The composition of the volatile compounds of *Salvia ceratophylla* samples

RRI	Compounds	A (%)	B (%)	C (%)	Identification
1032	α -Pinene	27.0	24.6	23.7	t_R , MS
1076	Camphene	3.6	4.7	5.9	t_R , MS
1118	β -Pinene	16.3	10.3	5.3	t_R , MS
1132	Sabinene	<0.1	3.7	2.6	t_R , MS
1203	Limonene	0.9	1.5	1.8	t_R , MS
1213	1,8-Cineole	2.9	6.6	8.9	t_R , MS
1255	γ -Terpinene	<0.1	<0.1	-	t_R , MS
1280	<i>p</i> -Cymene	<0.1	1.3	1.9	t_R , MS
1360	Hexanol	<0.1	<0.1	-	MS
1497	α -Copaene	-	0.9	-	MS
1532	Camphor	-	0.9	3.6	t_R , MS
1553	Linalool	4.0	2.8	3.1	t_R , MS
1586	Pinocarvone	-	0.9	<0.1	t_R , MS
1591	Bornyl acetate	5.9	3.2	1.7	t_R , MS
1611	Terpinen-4-ol	1.0	1.3	0.8	t_R , MS
1612	β -Caryophyllene	10.6	2.8	1.6	t_R , MS
1648	Myrtenal	<0.1	<0.1	0.9	MS
1670	<i>trans</i> -Pinocarveol	1.0	1.3	1.6	t_R , MS
1683	<i>trans</i> -Verbenol	0.2	1.7	2.7	t_R , MS
1687	α -Humulene	1.2	1.0	-	t_R , MS
1706	α -Terpineol	0.7	6.4	-	t_R , MS
1718	<i>p</i> -Menth-4-en-3-one	-	0.5	0.9	MS
1719	Borneol	3.7	1.0	7.0	t_R , MS
1725	Verbenone	<0.1	0.9	0.9	t_R , MS
1763	Naphthalene	2.1	<0.1	1.5	MS
1804	Myrtenol	<0.1	0.8	0.5	MS
1868	(<i>E</i>)-Geranyl acetone	1.0	<0.1	1.3	MS
1953	Palustrol	1.4	1.3	1.4	MS
1958	(<i>E</i>)- β -Ionone	3.1	2.9	-	MS
2008	Caryophyllene oxide	0.6	2.9	3.6	t_R , MS
2071	Humulene epoxide-II	-	0.2	-	MS
2104	Viridiflorol	-	1.3	1.9	MS
2131	Hexahydrofarnesyl acetone	1.5	<0.1	-	MS
2144	Spathulenol	2.2	4.4	5.1	MS
2239	Carvacrol	3.6	3.4	1.8	t_R , MS
2308	Methyl Dihydrojasmonate	<0.1	-	-	MS
	Total	94.5	95.5	92.0	

RRI, Relative retention indices calculated against *n*-alkanes % calculated from FID data; Identification method, t_R , identification based on the retention times of genuine compounds on the HP Innowax column; **MS**, identified on the basis of computer matching of the mass spectra with those of the Wiley, Adams and MassFinder libraries and comparison with literature data. (A), Kayseri sample; (B), Elazığ sample; (C), Adıyaman sample.

REFERENCES

1. Thorne RF, Classification and geography of the flowering plants, Bot Rev 58, 225-348, 1992.
2. Wagstaff SJ, Hickerson L, Spangler R, Reeves PA, Olmstead RG, Phylogeny in *Labiatae* s. 1, inferred from cpDNA sequences, Plant Syst Evol 209, 265-274, 1998.
3. Davis PH (ed.), 1965-1985: Flora of Turkey and the East Aegean Islands, 1-9, Edinburg Univ Press, Edinburg, 1982.
4. Habibvash FN, Rajamand MA, Anatomical observations on nutlets of some *Salvia* Species (Lamiaceae) from West Ajarbaijan in Iran, Pakistan J Bio Sci 10(19), 3385-3389, 2007.
5. Bağcı E, Koçak A, Essential oil composition of the aerial parts of two *Salvia* L. (*S. multicaulis* Vahl. Enum and *S. tricochlada* Benth) species from East Anatolian region (Turkey), Int J Sci Technol 3(1), 13-18, 2008.
6. Celep F, Doğan M, Duran A, A new record for the Flora of Turkey: *Salvia viscosa* Jacq. (Labiatae), Turk J Bot 33, 57-60, 2009.
7. Hamzaoglu E, Duran A, Pinar NM, *Salvia anatolica* (Lamiaceae), a new species from East Anatolia, Turkey, Ann Bot Fenn 42, 215-220, 2005.
8. Gali-Muhtasib H, Anticancer and medicinal properties of essential oil and extracts of East Mediterranean sage (*Salvia triloba*), Adv Phytomed 2, 169-180, 2006.
9. Erdemoğlu N, Turan NN, Çakıcı I, Sener B, Aydın A, Antioxidant activities of some Lamiaceae plant extracts, Phytother Res 20(1), 9-13, 2006.
10. Kennedy DO, Pace S, Haskell C, Okello E, Milne A, Scholey AB, Effects of cholinesterase inhibiting sage (*Salvia officinalis*) on mood, anxiety and performance on a psychological stressor battery, Neuropsychopharmacol 31, 845-852, 2006.
11. Kintzios SE (ed.), Sage: The Genus Sage, Harwood Academic Publishers, Amsterdam, 2000.
12. Chalchat JC, Michet A, Pasquier B, Study of the clones of *Salvia officinalis* L. yields and chemical composition of essential oil, Flavour Fragr J 13, 68-70, 1998.
13. Ozcan M, Tzakou O, Couladis M, Essential oil composition of Turkish herbal tea (*Salvia aucheri* Benth var. *canescens* Boiss. et Heldr.), Flavour Fragr J 18, 325-327, 2003.
14. Demirci B, Başer KHC, Yildiz B, Bahcecioglu Z, Composition of essential oils of six endemic *Salvia* spp. from Turkey, Flavour Fragr J 18, 116-121, 2003.
15. Ulubelen A, Cardioactive and antibacterial terpenoids from some *Salvia* species. Phytochem 64, 395-399, 2003.
16. Dulger B, Hacıoğlu N, Antifungal Activity of Endemic *Salvia tigrina* in Turkey, Trop J Pharm Res 7(3), 1051-1054, 2008.
17. Gören A, Topcu G, Öksüz S, Kökdil G, Voelter W, Ulubelen A, Diterpenoids from *Salvia ceratophylla*, Nat Prod Res 16(1), 47-52, 2002.
18. Kocabaş YZ, Karaman S, Essential oils of Lamiaceae family from South East Mediterranean region (Turkey), Pakistan J Bio Sci 4(10), 1221-1223, 2001.
19. Gören AC, Kılıç A, Dirmenci T, Bilsel G, Chemotaxonomic evaluation of Turkish species of *Salvia*: Fatty acid compositions of seed oils, Biochem Syst Ecol 34(2), 160-164, 2006.
20. Orhan I, Kartal M, Naz Q, Ejaz A, Yilmaz G, Kan Y, Konuklugil B, Şener B, Choudhary MI, Antioxidant and anticholinesterase evaluation of selected Turkish *Salvia* species, Food Chem 103(4), 1247-1254, 2007.
21. McLafferty FW, Stauffer DB, The Wiley/NBS Registry of Mass Spectral Data, J. Wiley and Sons, New York, 1989.
22. Koenig WA, Joulain D, Hochmuth DH, Terpenoids and Related Constituents of Essential Oils. MassFinder 3. Hochmuth DH (ed). Convenient and Rapid Analysis of GCMS. Hamburg, Germany, 2004.
23. Joulain D, König WA, The Atlas of Spectra Data of Sesquiterpene Hydrocarbons, EB-Verlag, Hamburg, 1998.
24. ESO 2000, The Complete Database of Essential Oils, Boelens Aroma Chemical Information Service, The Netherlands, 1999.
25. Jennings WG, Shibamoto T, Quantitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary GC, Academic Press, New York, 1980.
26. Rajabi Z, Ebrahimi M, Farajhour M, Mirza M, Ramshini H, Compositions and yield variation of essential oils among and within nine *Salvia* species from various areas of Iran, Ind Crops Prod 61, 233-239, 2014.
27. Özek G, Demirci F, Özek T, Tabanca N, Wedge DE, Khan SI, Başer KHC, Duran A, Hamzaoglu H, Gas chromatographic-mass spectrometric analysis of volatiles obtained by four different techniques from *Salvia rosifolia* Sm., and evaluation for biological activity, J Chrom A 1217, 741-748, 2010.
28. Delamare APL, Moschen-Pistorello IT, Artico L, Atti-Serafini L, Echeverrigaray S,

- Antibacterial activity of the essential oils of *Salvia officinalis* L. and *Salvia triloba* L. cultivated in South Brazil, Food Chem 100, 603-608, 2007.
29. Kelen M, Tepe B, Chemical composition, antioxidant and antimicrobial properties of the essential oils of three *Salvia* species from Turkish flora, Biores Technol 99, 4096-4104, 2008.
 30. Bayrak A, Akgül A, Composition of essential oils from Turkish *Salvia* species, Phytochem 26(3), 846-847, 1987.
 31. Gürsoy N, Tepe B, Akpulat HA, Chemical composition and antioxidant activity of the essential oils of *Salvia palaestina* (Bentham) and *S. ceratophylla* (L.), Rec Nat Prod 6(3), 278-287, 2012.
 32. Başer KHC, Aromatic biodiversity among the flowering plant taxa of Turkey, Pure Appl Chem 74(4), 527-545, 2002.

Received: 03.07.2014

Accepted: 04.12.2014