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RESEARCH PAPER

Volatile Constituents of Three *Stachys* L. Species from Turkey

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ABSTRACT

Volatile compounds of three *Stachys* species; *Stachys macrantha* (C. Koch) Stearn, *Stachys sylvatica* L. and *Stachys annua ssp. annua var. annua* L. were analyzed by a solid phase micro extraction (SPME) method coupled with gas chromatography-flame ionization detector (GC–FID) and gas chromatography-mass spectrometry (GC-MS). A total of 38 (99.2%), 39 (99.0%) and 33 (98.8%) volatile compounds were identified from *S. annua* ssp. *annua* var. *annua, S. sylvatica* and *S. macrantha* respectively. The major volatile constituents of the investigated three *Stachys* species were; α-pinene (11.2%), *p*-cymene (18.2%), and carvacrol (28.8%) in *S. macrantha*, γ-muurolene (10.2%), α-cedrene (11.2%), and limonene (37.0%) in *S. sylvatica* and α-pinene (11,4%), β-pinene (23.1%), and *(Z)*-β-ocimene (24.8%) in *S. annua* ssp. *annua* var. *annua.* Comparison of volatile organic compounds of all three species showed that monoterpene hydrocarbons were the major constituents in *S. annua* ssp. *annua* var. *annua* and *S. sylvatica* (65.8% and 49.8% respectively), whereas oxygenated monoterpenes were the main components (42.1%) of *S. macrantha.*

Keywords: *Stachys macrantha, Stachys sylvatica, Stachys annua ssp. annua var. annua,* Lamiaceae, volatile compounds, SPME.

1. INTRODUCTION

The genus *Stachys* L. (Lamiaceae) is represented by 93 species and 57 of the 118 taxa are endemic in Turkey. The genus is distributed all over the world, especially in warm temperate regions of the Mediterranean and Southwestern Asia, North America, South America and Southern Africa [1-6]. Plants of this genus have been used for the treatment of cold, cough, diarrhea, urinary system disorders, hypertension, headache, throat pain and as an antipyretic or stomachic in folk medicine [7-10]. Also they have been used as tea which is made from whole plant or leaves. The decoction prepared from herb of *S. annua* subsp. *annua* L. is used as antipyretic against common cold [7] and the infusion prepared from the aerial parts of *S. sylvatica* is used against cardiac disorders in Turkey [10]. *Stachys* species are also used for the problems of skin and for the treatment of wounds at veterinary medicine [11-13].

Since the volatile organic compounds are complex mixtures, there are varied identification methods for the characterization of organic compounds [14]. SPME which is one of the sensitive, selective and cheap methods of evaluating volatile compounds, has been applied with gas chromatography and mass spectrometry [15].

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A review of the literature showed that the chemical composition of essential oils from *S. macrantha* (C. Koch) Stearn, *S. sylvatica* L. and *S. annua* ssp. *annua* var. *annua* L. had been reported before [16-22]. Germacrene D, spathulenol and phytol were found to be the main components of *S. annua* ssp. *annua* var. *annua* grown in central Italy [22]. Bulgaria, Kosova, Croatia, Italy and Hungary originated *S. sylvatica* samples together with *S. macrantha* samples which were collected from Hungary were previously studied by different groups and sesquiterpene lactones were reported to be the major group of substances at all of researches. Essential oils were obtained by hydrodistillation at all of these previous studies [16-21]. However the head space volatile organic compounds of the same species grown in Turkey has not been investigated previously. Therefore we wanted to determine the relative difference of these three *Stachys* species. The aim of this study is to investigate and compare the volatile organic composition of the three native *Stachys* species of Turkey mentioned before.

The present work deals with comparative evaluation of the volatile chemical profiles of the three native *Stachys* species grows in Turkey, by SPME coupled with GC-FID and GC-MS.

2. MATERIALS AND METDODS

2.1. Plant Materials

Plant samples were collected from Black Sea Region of Turkey during the flowering stages. Voucher specimens were authenticated by Dr. Gülin Renda. The voucher specimens of *S. annua* ssp. *annua* var. *annua, S. sylvatica* and *S. macrantha* were deposited at the Herbarium of the Faculty of Pharmacy, Hacettepe University, Ankara, Turkey (HUEF 13027, 13028, 13029 respectively). Impurities like other plants, soil or waste were removed and plant material was stored in air-tight container until use.

2.2. SPME Analysis

The samples of three *Stachys* species were separately analyzed by a SPME device (Supelco, USA). The flowered fresh plant materials (1.00 g, each) were crumbled and placed in a 10 mL vial sealed with a silicone-rubber septum cap. The fiber coating was placed to the head space. The temperature, incubation and extraction times were set according to experiment. Extractions took place with magnetic stirring. Fibers with extracted aroma compounds were subsequently injected into the GC injector [23-24].

A polydimethylsiloxane/divinyl-benzene coating fiber was placed to the head space and used to obtain volatile components. The SPME fibers were conditioned for 5 min at 250 °C in the GC injector. Conditioning time for subsequent assays was set at 4 min of desorption after each extraction. Extractions were performed at 50 °C using an incubation time of 5 min and an extraction time of 10 min. Extractions were achieved with magnetic stirring. Each sample was analyzed and reported [23].

2.3. Gas Chromatography-Mass Spectrometry (GC/MS)

The gas chromatography-flame ionization detector (GC-FID) analysis was carried out on a Shimadzu QP2010 plus gas chromatography equipped with a flame ionization detector (FID) and a Rtx-5MS capillary column (30 m x 0.25 mm, film thickness, 0.25 μm). Shimadzu QP2010 Plus gas chromatograph was coupled to a Shimadzu QP2010 Ultra mass selective detector. The fiber containing the extracted volatiles (SPME) were injected into the GC-MS injector. Split mode was employed and split ratio was 1:20. The oven program was as follows: initial temperature was 60 °C for 2 min, which was increased to 240 °C at 3 min, final temperature 250 °C was held for 4 min. The injector and mass transfer line temperatures were set at 280 °C and 250 °C, respectively. Helium (99.999%) was used as carrier gas with a constant flow-rate of 1 mL/min. Detection was carried out in electronic impact mode (EI); ionization voltage was fixed to 70 eV and scan mode (40-450 m/z) was used for mass acquisition [25].

2.4. Compound Identification

Retention indices of the components were determined by Kovats method using *n*-alkanes (C6-C32) as standards. The volatile compounds were identified by comparison of their retention indices (relative to C_6-C_{32} alkane standards) and mass spectra with those of the mass spectra of the two libraries (FFNSC1.2 and W9N11) and also confirmed by comparing the retention indices with the data published in the literature [26-42].

3. RESULTS

Composition of the essential oils of three *Stachys* species; *S. macrantha*, *S. sylvatica* and *S. annua* ssp. *annua* var. *annua* were identified with SPME with GC-MS. Identifications were made on the basis of comparison of GC Kovats retention indexes (RIs) with reference to a homologous series of *n*-alkanes and results were given in Table 1. A total of 70 compounds were identified and different volatile compounds were determined within all three species. The chemical class distributions of the volatile constituents were also given in Table 1, under the classes of terpenoids (monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes), aldehydes, esters, terpene related compounds and others.

According to the results of our study, monoterpene hydrocarbons were found as the major group of compounds in *S. annua* ssp. *annua* var. *annua* and *S. sylvatica* with in the 65.8%, and 49.8% area, respectively. Oxygenated monoterpenes were the main constituents of *S. macrantha* (with a total of 42.1%). Among them, the major volatile constituents of the investigated three *Stachys* species were; *α*-pinene (11,4%), *β*-pinene (23.1%), and *(Z)*-*β*-ocimene (24.8%) in *S. annua* ssp. *annua* var. *annua*; *γ*-muurolene (10.2%), *α*-cedrene (11.4%), and limonene (37%) in *S. sylvatica* and *α*-pinene (11.2%), *p*-cymene (18.2%), and carvacrol (28.8%) in *S. macrantha* (Table 1).

4. DISCUSSION

The chemical composition of the essential oils from *S. macrantha, S. sylvatica* and *S. annua* ssp. *annua* var. *annua* had been reported before [16-22], but there is no literature data for the comparative evaluation of the volatile chemical composition obtained from *S. macrantha, S. sylvatica* and *S. annua* ssp. *annua* var. *annua* grown in Turkey.

According to data presented in Table 1; 38, 39, and 33 compounds were identified as representing 99.2%, 99.0%, and 98.8% area of the volatile organic constituent obtained from *S. macrantha, S. sylvatica* and *S. annua* ssp. *annua* var. *annua*, respectively. SPME/GC-MS analysis of all three *Stachys* species revealed that monoterpene hydrocarbons were the main group of constituents of *S. sylvatica* and *S. annua* ssp. *annua* var. *annua* which had a higher content of monoterpene hydrocarbons with in the area of 49.8% and 65.8% respectively. Whereas, oxygenated monoterpenes (42.1%) were the major group of volatile compounds of *S. macrantha.*

SPME/GC-MS analysis of *S. macrantha* showed that there is no aldehyde type organic compound. Carvacrol (28.8%), *β*-cymene (18.2%) and α-pinene(11.2%) were found to be the abundant components of *S. macrantha.*

All three *Stachys* species were also rich in sesquiterpene hydrocarbons within the ratio of 17.6%, 32.4% and 15.7%, respectively.

In *S. sylvatica*, 38 components were identified, 7 monoterpene hydrocarbons of which represented the 49.8% of the total oil. Limonene (37%), *α*-cedrene (11.2%), and *γ*-muurolene (10.2%) were found to be the major components of the volatile compounds in *S. sylvatica.*

Bulgaria, Kosova, Croatia, Italy and Hungary originated *S. sylvatica* samples were previously studied by different groups and sesquiterpene lactones were reported as the major group of substances at all of the works [16-21]. Germacrene D was found to be the major component at Hungary, Kosovo and Italy originated samples. In our case, germacrene D was in small amount (0.1%) and monoterpene hydrocarbons were the main constituents. We would like to point out that there are important qualitative and quantitative differences between our results and previously reported content.

The chemical composition of the organic volatile compounds from *S. annua* ssp. *annua* var. *annua* of which 98.8 % was identified, contained a high proportion of monoterpene hydrocarbons (65.8%) followed by aldehydes (13.4%) and sesquiterpene hydrocarbons (11.5%). *α*-Pinene, *β*-pinene and *(E)*-*β*-ocimene were found as the major compounds within this species grown in Turkey. Whereas sesquiterpenoids (42.5% area) were found to be the main components of the same species grown in central Italy [22]. Phytol which is used in the fragrance industry, was found to be the major component of the plant grown in Central Italy [22] although could not be found in the same species grown in Turkey.

All three *S. macrantha, S. sylvatica* and *S. annua* ssp. *annua* var. *annua* species contained *α*-pinene (11.2%, 4.3%, 11.4%), *β*-pinene (0.4%, 0.4%, 23.1%), myrcene (1.9%, 1.4%, 0.8%), limonene (1.6%, 37.0%, 0.8%), *α*-copaene (0.3%, 0.7%, 0.2%), *β*-bourbonene (0.5%, 0.9%, 7.4%), γ-muurolene (0.1%, 10.2%, 0.6%), *α*-amorphene (0.6%, 0.1%, 0.1%), and germacrene D (1.6%, 0.1%, 0.5%) in different ratio, respectively (Table 1). These results are compatible with the results of the *Stachys* species which were collected from Turkey and previously studied [43]. Concerning all over investigated members of the genus *Stachys* grown in Turkey, it is noteworthy that caryophyllene is present in large quantities in most of the species studied whereas it is totally absent in the essential oil of *S. annua* ssp. *annua* var. *annua* and also it is found in small proportions in *S. sylvatica* and *S. macrantha* (Table 1) [43-44].

Table 1. Identified volatile organic compounds of three *Stachys* L. species from Turkey.

Compund	RRI Lit.	References	RRI	*S. macrantha* (%)	*S. sylvatica* (%)	*S. annua* ssp. *annua* var. *annua*(%)
Monoterpene hydrocarbons (MT HC)						
α-Thujene	930	[30]	928	0.6	-	-
α-Pinene	939	[30]	936	11.2	4.3	11.4
Sabinene	971	[31]	971	0.2	4.0	-
β-Pinene	979	[30]	976	0.4	0.4	23.1
Myrcene	990	[30]	987	1.9	1.4	0.8
α-Phellandrene	1002	[32]	1001	0.1	2.0	-
α-Terpinene	1014	[32]	1013	0.6	-	-
p-Cymene	1022	[30]	1020	18.2	0.7	-
Limonene	1029	[30]	1027	1.6	37.0	0.8
(Z)-*β*-Ocimene	1037	[32]	1039	1.0	-	24.8
(*E*)-*β*-Ocimene	1050	[32]	1053	0.1	-	3.9
γ-Terpinene	1064	[33]	1064	2.0	-	-
neo-allo-Ocimene	1146	[34]	1147	-	-	1.0
Oxygenated monoterpenes (Oxy. MT)						
Linalool	1097	[35]	1101	-	1.1	-
Pinocarvone	1164	[30]	1164	-	-	0.1
Thymol, methyl ether	1235	[32]	1238	0.7	-	-
Carvacrol, methyl ether	1245	[32]	1248	3.4	-	-
Carvone	1243	[30]	1241	-	0.1	-
Thymoquinone	1252	[36]	1257	9.2	-	-
Carvacrol	1298	[32]	1296	28.8	-	-
Sesquiterpene hydrocarbons (ST HC)						
α-Cubebene	1351	[35]	1349	-	0.1	0.1
Cyclosativene	1371	[32]	1367	0.1	-	-
α-Copaene	1377	[35]	1378	0.3	0.7	0.2
β-Bourbonene	1388	[35]	1388	0.5	0.9	7.4
β-Elemene	1391	[35]	1390	0.3	-	0.8
α-Cedrene	1412	[32]	1416	-	11.2	-
(E)-Caryophyllene	1419	[35]	1422	8.8	0.3	-
β-Ylangene	1421	[32]	1423	-	-	0.5
(Z)-*β*-Farnesene	1440	[32]	1441	-	-	0.6
Aromadendrene	1441	[37]	1442	0.3	-	-
α-Guaiene	1445	[38]	1446	0.3	-	-
(E)-*β*-Farnesene	1458	[32]	1458	0.4	-	-
α-Humulene	1455	[35]	1456	2.5	4.6	-
γ-Muurolene	1478	[32]	1474	0.1	10.2	0.6
α-Amorphene	1483	[32]	1477	0.6	0.1	0.1
Germacrene D	1485	[35]	1483	1.6	0.1	0.5
β-Selinene	1490	[30]	1489	0.2	-	-
α-Zingiberene	1493	[32]	1490	-	3.2	-
α- Selinene	1498	[37]	1498	0.6	-	-
Bicyclogermacrene	1500	[32]	1499	-	0.6	-
β-Bisabolene	1506	[35]	1505	0.2	-	-
γ-Cadinene	1514	[35]	1515	0.2	0.1	-

Compund	RRI Lit.	References	RRI	*S. macrantha* (%)		*S. sylvatica* (%)		***S. annua*** **ssp.** ***annua*** **var.** ***annua***(%)	
δ-Cadinene	1524	[37]	1526	0.5		-		0.5	
(E)-γ-Bisabolene	1531	[32]	1538	0.3		-		-	
α-Cadinene	1539	[32]	1540	-		-		0.2	
Oxygenated sesquiterpenes (Oxy. ST)									
Spathulenol	1578	[32]	1584	-		-		0.3	
Caryophyllene oxide	1583	[35]	1588	0.2		2.3		-	
Viridiflorol	1593	[35]	1597	-		0.3		-	
Terpene related compounds									
Perhydro farnesyl acetone	1848	[32]	1845	-		0.2		-	
Aldehydes									
Hexanal	802	[39]	807	-		1.4		1.0	
(2*E*)-Hexenal	855	[39]	857	-		7.3		1.1	
Heptanal	902	[40]	902	-		0.1		0.1	
(2*E*,4*E*)-Hexadienal	912	[41]	911	-		0.1		0.2	
(2*E*)-Heptenal	958	[40]	954	-		0.5		0.2	
Benzaldehyde	960	[32]	962	-		0.6		10.5	
(2E,4E)-Heptadienal	1005	[32]	1008	-		0.4		0.1	
Benzene acetaldehyde	1042	[35]	1040	-		0.7		-	
Nonanal	1101	[35]	1108	-		0.1		0.1	
(*E*)-Cinnamaldehyde	1270	[37]	1270	-		-		0.1	
Undecanal	1307	[35]	1307	-		0.1		-	
Dodecanal	1408	[32]	1409	-		0.1		-	
Esters									
Methyl salicylate	1195	[38]	1194	-		-		0.1	
α-Terpinyl acetate	1349	[32]	1345	0.1		-		-	
Others									
3-Methyl-4-heptanone	924	[32]	925	-		0.7		-	
Octen-3-ol	979	[35]	974	-		0.1		1.4	
3-Octanone	988	[42]	989	1.1		-		-	
n-Octanol	1071	[42]	1071	-		0.5		-	
Octan-3-ol acetate	1120	[32]	1113	-		-		2.0	
Naphthalene	1181	[34]	1184	0.1		-		-	
(E)-β-Ionone	1486	[35]	1486	-		0.4		4.2	
				Area %	NC	Area %	NC	Area %	NC
Monoterpene hydrocarbons (MT HC)				37.9	12	49.8	7	65.8	7
Oxygenated monoterpenes (Oxy. MT)				42.1	4	1.2	2	0.1	1
Sesquiterpene hydrocarbons (ST HC)				17.8	18	32.1	12	11.5	11
Oxygenated sesquiterpenes (Oxy. ST)				0.2	1	2.6	2	0.3	1
Terpene related compounds				-	-	0.2	1	-	-
Aldehydes				-	-	11.4	11	13.4	9
Esters				0.1	1	-	-	0.1	1
Others				1.2	2	1.7	4	7.6	3
Total				99.3	38	99.0	39	98,8	33

RRI calculated from retention times relative to that of *n*-alkane series. Percentages obtained by FID peak-area normalization. Percentages obtained by FID peak-area normalization. NC: Number of compounds.

(*E*)-*β*-Ocimene which is the main component of *S. annua* ssp. *annua* var. *annua*, is not present in *S. sylvatica* and present in small amounts in *S. macrantha* (Table 1). Also limonene is the major component of *S. sylvatica* but it is present in small quantities in the oils of the other two species (Table 1). *S. macrantha* appears to be the most differentiated species with the major component carvacrol which is a medically important constituent found even in a ratio of 28.8 % in this species but not found at the other two species (Table 1) [45-46].

In the literature, phytol (9.8%), germacrene D (9.2%), and spathulenol (8.5%) was found as the major compounds of the essential oil of flowering aerial parts of *Stachys annua* L. subsp. *annua* [29]. In our case, volatile compounds of *S. annua* ssp. *annua* var. *annua* revealed *α*-pinene (11.4%), *β*-pinene (23.1%) and benzaldehyde (10.5%) as main components and germacrene D (0.5%) and spathulenol (0.3%) were found to be in fewer amounts. γ-Muurolene (16.82%, 17.85%) phytol (9.82%, 11.98%), and benzaldehyde (7.93%, 14.36%) were mentioned to be main compounds in the leaf and flower oils of *S. sylvatica* from Bulgaria [21]. But limonene (37%), *α*-cedrene (11.2%) and *γ*-muurolene (10.2%) was found as the main constituents of *S. sylvatica* grown in Turkey. Volatile constituents of three *Stachys* species which grown in Turkey are defined by this study for the first time. The current results revealed that volatile organic compounds of these three *Stachys* species grown in Turkey contains relatively different components when compared with the results of the same species grown in different places of the world. Chemical differentiation within the same species can occur generally as a result of the varied ecological or geographic origin as well as the genetic differentiation, collection time, climate or method of analysis.

Conflict of Interest

The authors declare they have no conflict of interest.

Türkiye'den Üç *Stachys* L. Türünün Uçucu Bileşenleri

ÖZ

Stachys macrantha (C. Koch) Stearn, *Stachys sylvatica* L. ve *Stachys annua* ssp. *annua* var. *annua* L. türlerine ait uçucu bileşenler Katı Faz Mikro Ekstraksiyon (SPME) metoduna ek olarak Alev İyonizasyon Dedektörü (GC-FID) ve Gaz Kromotografisi-Kütle Spektrometresi (GC-MS) ile analiz edildi. *S. annua* ssp. *annua* var. *annua, S. sylvatica* ve *S. macrantha* türlerinde sırasıyla toplam 38 (%99.2), 39 (%99.0) ve 33 (%98.8) uçucu bileşen tanımlandı. İncelenen bu üç türdeki major uçucu bileşenler *S. macrantha* türünde *α*-pinen (%11.2), *p*-simen (%18.2) ve karvakrol (%28.8), *S. sylvatica*'da *γ*-muurolen (%10.2), *α*-sedren (%11.2) ve limonen (%37.0) ve *S. annua* ssp. *annua* var. *annua*'da *α*-pinen (%11.4), *β*-pinen (%23.1) ve *(Z)*-*β*-osimen (%24.8) olarak bulundu. Türler üzerinde yapılan karşılaştırma çalışması *S. annua* ssp. *annua* var. *annua* ve *S. sylvatica* türlerinin ana monoterpen hidrokarbon yapısında olduğunu bileşenlerinin (sırasıyla %65.8 ve %49.8) ve *S. macrantha*'nın ana bileşenlerinin ise oksijenlenmiş monoterpenler (%42.1) olduğunu gösterdi.

Anahtar kelimeler: *Stachys macrantha, Stachys sylvatica, Stachys annua ssp. annua var. annua,* Lamiaceae, uçucu bileşikler, SPME.

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