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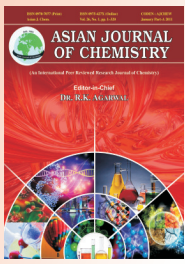

SPME/GC-MS Analysis of Volatile Organic Compounds from three Lamiaceae Species (*Nepeta conferta* Hedge & Lamond, *Origanum onites* L. and *Satureja cuneifolia* Ten.) Growing in Turkey

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Solid phase microextraction of *Nepeta conferta* Hedge & Lamond, *Origanum onites* L. and *Satureja cuneifolia* Ten., three Lamiaceae species to Turkey, were analyzed by GC-FID/MS. The main components of the volatile organic components were: *p*-cymene (25.5 %), eucalyptol (9.8 %), limonene (5.0 %), sabinene (4.8 %), carvacrol (3.7 %), *E*-linalool oxide (3.3 %), Z-linalool oxide (3.0 %) in *N. conferta*; carvacrol (47.3 %), *p*-cymene (15.8 %), γ-terpinene (8.6 %), myrecene (8.6 %), caryophyllene (2.0 %) in *O. onites* and carvacrol (32.6 %), *p*-cymene (22.2 %), γ-terpinene (15.1 %), myrecene (5.5 %) and caryophyllene (3.3 %) in *S. cuneifolia*. Carvacrol was the most abundant component in volatile organic compounds of *O. onites* (47.3 %), *S. cuneifolia* (32.6 %) and *N. conferta* (3.7 %).

Keywords: Volatile organic compounds, *Nepeta conferta*, Origanum onites, *Satureja cuneifolia*.

INTRODUCTION

The Lamiaceae family consists of more than 236 genus and 7173 species[1]. They are known for the wealth of species with medicinal properties, which have been used since early times and many of these species are common in Mediterranean area and Asia[2].

The genus *Nepeta* L. (Lamiaceae) comprises nearly 300 species that are distributed all over the world[3]. The genus *Nepeta* L. is represented in Turkey by 39 species including 18 endemic plants[4]. *Nepeta conferta* is endemic to Turkey and it is assessed as CR (Critically endangered) in the Red Data Book of Turkish plant[5]. The genus *Origanum* L. (Lamiaceae) represented 41 species that are mainly distributed thoroughly Mediterranean basin especially East Mediterranean region[6]. In the literature, essential oil analysis of *N. conferta*, which was obtained by steam distillation were mentioned[6,7].

The genus *Origanum* L. is represented in Turkey by 30 taxa and 15 of them are endemic to Turkey[8], endemism rate of *Origanum* genus in Turkey is 50 % and 5 taxa are hybrid[4]. *Origanum onites* is a non-endemic plant and naturally grows west of Turkey at Aegean and Mediterranean region[9]. Literature data of *O. onites* showed the chemical components of the essential oil which were obtained by steam distillation[10-12].

The genus *Satureja* L. (Lamiaceae) consists of 284 aromatic species and widely distributed in Mediterranean basin, Asia and boreal America[13-14]. The genus *Satureja* is represented by 15 taxa in Turkey[4] and 5 of them are endemic and endemism rate of *Satureja* genus is 33 %. *Satureja* species including *Satureja cuneifolia* mostly grows in the south and west Anatolia regions in Turkey[15-16]. In the literature, steam distillated essential oil analyses of *S. cuneifolia* were reported[17-20].

The Lamiaceae family has known to be rich in volatile organic compounds. The aim of this study was to determine semi quantitative differences in volatile organic compounds of three Lamiaceae species (*N. conferta*, *O. onites* and *S. cuneifolia*) by using SPME-GC-FID/MS analysis. Solid phase microextraction is an alternative technique for the extraction of organic volatiles from different sample sources, compared with conventional methods[21-22]. Solid phase microextraction[23] represents a reliable method for the analyzing very complex mixtures of volatile organic compounds. This is the first detailed report on the volatile organic compound composition for the *N. conferta*, *O. onites* and *S. cuneifolia* based on SPME and capillary GC-FID/MS analyses.

EXPERIMENTAL

Nepeta conferta, *Origanum onites* and *Satureja cuneifolia* were collected in Antalya-Finike, Turkey, (at heights of 1370 m; Üçkuzuluk region, 530 m; Asarönü village and 2010 m; Eren mountain, respectively), southeastern part of Turkey in 3 August, 26 June and 2 August, 2012, respectively. The plants

were authenticated by Prof.Dr.Salih Terzioglu[1,5,8,9,13,14]. Voucher specimens were deposited in the Herbarium of the Faculty of Forestry (KATO: 8728, KATO: 8754 and KATO: 8761, respectively), Karadeniz Technical University, Turkey.

Solid-phase microextraction/Gas chromatography-mass spectrometry (SPME-GC-MS): A manual SPME device including the fiber was obtained from Supelco company (USA). The fiber used for the extraction of the volatile components was polydimethylsiloxane/divinyl-benzene (PDMS/DVB, 65 μm-blue hub plain). The SPME fibers were conditioned for 5 min at 250 °C in the GC injector. For the following analyses, 4 min of desorption after each extraction was used as conditioning time. Extractions were done at 50 °C with incubation time of 5 min and extraction time of 10 min. Each sample was analyzed and mean reported. For SPME procedure, about 1 g of the tree plant was transferred to a 10 mL vial. The fiber coating was placed to the head space for temperature and times (incubation and extraction times) values set according to the experiment. Extractions were achieved with shaking.

The fiber containing the extracted aroma compounds were then injected into the GC injector (split mode) and kept during 4 min for thermal desorption at 250 °C. GC analysis was carried out using a Shimadzu 2010 Plus gas chromatograph coupled to a Shimadzu QP2010 Ultra mass selective detector. The separation was performed by means of a Restek Rxi-5MS capillary column, 60 m length, 0.25 mm i.d. and a 0.25 μm phase thickness. The split mode was used. The oven program was as follows: Initial temperature was 60 °C for 2 min, which was increased to 240 °C at 3 °C min^{-1}, 250 °C was maintained for 4 min. Helium (99.999 %) was used as carrier gas with a constant flow-rate of 1 mL min^{-1}. Detection was carried out in electronic impact mode (EI); ionization voltage was fixed to 70 eV. Scan mode (40-450 *m/z*) was used for mass acquisition. The volatile compounds were identified by comparison of their retention indices (relative to C7-C30 alkane standards) and by comparison with the mass spectra of the two libraries (FFNSC1.2 and W9N11).

RESULTS AND DISCUSSION

Three species (*N. conferta*, *O. onites* and *S. cuneifolia*) of the Lamiaceae family were collected in the different localities of the northwest part of Turkey. The volatile organic compounds were obtained by SPME method[21-22], analyzed with GC-FID-MS and identified by comparing their GC Kovats retention indexes (RI), determined with reference to a homologous series of *n*-alkanes and by comparing their mass spectral fragmentation patterns with literature data[24-33]. The principal components ratios of volatile organic compounds have shown a wide diversity within each species and variety of Lamiaceae family. The compositions and relative percentages of the volatile organic compounds from *N. conferta*, *O. onites* and *S. cuneifolia* were listed in table. Volatile organic compounds of *N. conferta*, *O. onites* and *S. cuneifolia* revealed 38, 29 and 31 compounds and the major fractions were monoterpene compounds 43.1, 39.6 and 53.9 %, respectively (Table). *Nepeta* volatile organic compounds were characterized by the presence of *p*-cymene (25.5 %), eucalyptol (9.8 %), limonene (5.0 %), sabinene (4.8 %), carvacrol (3.7 %), *E*-linalool oxide (3.3 %), *Z*-linalool oxide (3.0 %), which composed 77.5 % of the total volatile. In origanum volatile the most abundant compounds were also carvacrol (47.3%), *p*-cymene (15.8%), γ-terpinene (8.6 %), myrecene (8.6 %), caryophyllene (2.0 %), which participated in the mixture at 97.6 %. Along with carvacrol (32.6 %), *p*-cymene (22.2 %), γ-terpinene (15.1 %), myrecene (5.5 %) and caryophyllene (3.3 %) constituted 98.2 % of *Satureja* volatile organic compounds. Carvacrol was the most abundant component in volatile organic compound of *O. onites* (47.3 %) and *S. cuneifolia* (32.6 %). In the volatile organic compound of *N. conferta*, *O. onites* and *S. cuneifolia*, *p*-cymene (25.5, 15.8 and 22.2 %, respectivelly) was found to be the major constituents. α-Thujene, α-pinene, β-pinene, myrecene, α-phellandrene, α-terpinene, *p*-cymene, E-sabinene hydrate, 4-terpineol, α-terpineol, carvacrol, caryophyllene and β-bisabolene were the command compounds in all three Lamiaceae families (*N. conferta*, *O. onites* and *S. cuneifolia*).

The Nepetoideae comprise the majority of the essential oil rich genera of the Lamiaceae and particularly tend to accumulate monoterpenoid-rich essential oils as in our case[25]. Literature search revealed that caryophyllene oxide (16 %) and linalool 11.4 % were the major compounds of the essential oil of *N. conferta*[6-7]. In our case, linalool (0.2 %) and caryophyllene oxide (% 2.0) were the minor components of the volatile organic compound of *N. conferta*.

High amounts of carvacrol (78.3-79.5 %) and thymol (11.6 %) have been reported from *Origanum* majorana from Turkey and Cuba, respectively[26,27]. In the literature, carvacrol (62-64 %) was found to be major compound in the essential oil of *O. onites*[10-12]. Linalool (13.4 %) was the second major components of the essential oil of *O. onites*[10]. But, in our case, we did not observed linalool using SPME-GC/MS analysis of *O. onites*. Generally, the *Origanum* species is characterized by the presence of two major groups; the aromatic monoterpenes (*p*-cymene, carvacrol and thymol) and the thujanes (sabinene, sabinene hydrate and their derivatives)[28-32] which we observed similar result in our case for the volatile organic compounds of it.

The main constituents of *Satureja* montana were carvacrol (50.2 %) thymol (11 %), γ-terpinene (5.8 %), carvacrol methyl ether (4.6 %) and *p*-cymene (4.8 %)[33]. But, we did not observed thymol and carvacrol methyl ether in *S. cuneifolia*. Literature search revealed that carvacrol (45-59 %) was the major compound of the essential oil of *S. cuneifolia*[17-20]. But, seasonal variation study of the essential oil of *S. cuneifolia* showed that linalool (17-19 %) was the main compound and there was no carvacrol in the essential oil of *S. cuneifolia*[19].

In our results, we generally observed similar monoterpene compounds with different ratios. But, thymol was not determined in volatile organic compounds of *N. conferta*, *O. onites* and *S. cuneifolia*. A comparison with literature data on the chemical composition of volatile organic compound is difficult because of the great variability of the volatile compositions, which depends on several parameters such as locality, the climatic conditions, season, extraction technique and analytical methods[34-35].

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TABLE
VOLATILE ORGANIC COMPOUNDS FROM THREE LAMIACEAE SPECIES
(A: *Nepeta conferta*, B: *Origanum onites* AND C: *Satureja cuneifolia*)

No	Compounds	A (%)[a]	B (%)[a]	C (%)[a]	RT	RI[b]
1	α-Thujene	1.7	1.0	0.5	13.12	938
2	α-Pinene	2.0	0.6	0.4	13.46	946
3	2,4(10)-Thujadien	0.1	-	-	13.83	956
4	Camphene	-	0.3	0.2	14.09	962
5	Verbenene	0.3	-	-	14.28	966
6	Sabinene	4.8	-	-	15.00	984
7	1-Octen-3-ol	-	-	0.7	15.00	984
8	β-Pinene	1.2	0.1	0.2	15.22	989
9	3-Octanone	0.1	-	-	15.35	993
10	Myrecene	0.1	8.6	5.5	15.54	998
11	Octanol	-	-	0.9	15.66	1000
12	3-Octanol	0.2	-	-	15.69	1001
13	α-Phellandrene	0.3	0.4	0.3	16.25	1014
14	δ-3-Carene	-	0.1	0.1	16.52	1020
15	α-Terpinene	0.4	1.4	1.8	16.76	1026
16	*p*-Cymene	25.5	15.8	22.2	17.09	1033
17	Limonene	5.0	1.8	1.6	17.27	1038
18	β-Phallandrene	-	0.2	-	17.40	1439
19	Eucalyptol	9.8	-	-	17.44	1041
20	*E*-β-Ocimene	-	0.2	5.3	17.47	1042
21	γ-Terpinene	0.9	8.6	15.1	18.54	1067
22	*E*-Sabinene hydrate	1.5	1.3	0.6	18.92	1076
23	*E*-Linalool oxide	3.3	-	-	19.11	1080
24	1-Nonen-3-ol	-	-	0.1	19.24	1083
25	*Z*-Linalool oxide	3.0	-	-	19.79	1096
26	Terpinolene	-	0.4	0.4	19.84	1097
27	Linalool	2.0	-	0.5	20.15	1104
28	3-Tridecene	0.1	-	-	20.75	1118
29	α-Thujone	0.8	-	-	21.10	1126
30	Neo-allo-Ocimene	-	-	0.3	21.44	1134
31	α-Campholenal	0.6	0.2	-	21.50	1135
32	Phellandral	0.1	-	-	21.61	1138
33	*E*-Pinocarveol	2.2	-	-	22.12	1150
34	Verbenol	0.6	-	-	22.33	1154
35	Pinocarvone	1.0	-	-	23.18	1165
36	Endo-Borneol	-	2.0	1.6	23.29	1177
37	Phallandrol	-	0.3	-	23.46	1180
38	4-Terpineol	2.2	0.9	0.8	23.74	1187
39	α-Terpineol	0.2	0.1	0.2	24.27	1199
40	Z-Dihydrocarvone	-	0.1	0.3	24.58	1207
41	(R)-(-)-Myrtenal	1.0	-	-	24.63	1208
42	*E*-Dihydrocarvone	-	0.1	0.1	24.93	1215
43	Verbenone	0.7		-	25.18	1221
44	*E*-Carveol	0.1		-	25.44	1227
45	Cuminaldehyde	0.5		-	26.41	1250
46	(-)-Carvone	-	0.2	-	26.58	1254
47	Thymoquinone	-	-	1.2	26.75	1258
48	Linalyl acetate	-	1.8	-	26.77	1259
49	Carvacrol	3.7	47.3	32.6	28.75	1306
50	α-Copaene	0.9	-	-	32.04	1388
51	Tetradecane	0.1	-	-	32.51	1400
52	Caryophyllene	0.1	2.0	3.3	33.84	1435
53	α-*E*-Bergamotene	-	0.1	-	34.24	1445
54	Aromadendrene	-	0.3	0.2	34.59	1455
55	α-Humulene	-	-	0.1	35.16	1469
56	β-Bisabolene	0.2	1.4	1.0	36.95	1517

No	Compounds	A (%)[a]	B (%)[a]	C (%)[a]	RT	RI[b]
57	Caryophyllene oxide	0.2	-	0.1	40.02	1601
					Num. of compounds	
	A (%)	B (%)	C (%)	A	B	C
Monoterpene hydrocarbons	43.1	39.6	53.9	13	14	14
Monoterpenoids	30.0	52.4	37.9	15	10	9
Sesquiterpene hydrocarbons	1.2	3.8	4.6	3	4	4
Sesquiterpenoids	0.2	-	0.1	1	-	1
Terpene related compound	-	1.8	-		1	
Aldehyde	0.5	-	-	1	-	-
Hydrocarbones	0.2	-	-	2	-	-
Others	2.3	-	1.7	3	-	3
Total	77.5	97.6	98.2	38	29	31

[a] %Area obtained by FID peak-area normalization. [b]RI calculated from retention times relative to that of *n*-alkanes (C_7-C_{30}) on the non-polar HP-5 column

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