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CHEMICAL COMPOSITION OF THE ESSENTIAL OILS OF *SALVIA* SPP. LEAVES

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Keywords: Sage, Essential oil, Chemical composition, *Salvia aramiensis*, *Salvia fruticosa*, *Salvia tomentosa*, *Salvia officinalis*

Abstract

The chemical composition of the hydrodistilled essential oils of four *Salvia* spp. were analysed by GC-MS. Three of them (*Salvia aramiensis* Rech. fil., *Salvia fruticosa* Mill., *Salvia tomentosa* Mill.) analyzed in this study grow naturally in the Hatay flora. On the other hand, *S. aramiensis* is an endemic plant in Hatay flora. Fourth species (*Salvia officinalis* L.) is not growing in the flora of Turkey, but is only cultivated. The highest essential oil content (5.31%) was found in *S. aramiensis* and the least 1.68% was detected in *S. officinalis*. Eucalyptol was the main constituent for *S. aramiensis*, *S. fruticosa* and *S. tomentosa*. While this component was 58.65% in *S. aramiensis*, it was determined as 44.70 and 34.97% in *S. tomentosa* and *S. fruticosa*, respectively. In *S. officinalis*, the main constituent was determined as δ-Thujone (33.83%) and camphor (21.46%). Eucalyptol has been identified as the main composition in sage species which is grown in flora.

Introduction

Salvia genus with about 900 species is one of the most largest members of *Lamiacea* in the world (Askun *et al*. 2010) The species of *Salvia* were represented in Turkey by 89 species and 94 taxa and 45 which are endemic (Davis 1982, Baser 2002). Davis (1982) reported that there are 24 species in Hatay flora and 4 of these species are endemic.

Salvia species are commonly known as ‘‘Adacayi’’, ‘‘Salba’’ and/or ‘‘Dadirak’’ by the local people in Anatolia and Mediterranean region and used in folk medicine for the treatment of various diseases since ancient times (Jimenez *et al.* 1986, Askun *et al*. 2010). *Salvia tomentosa* Mill. poured onto the open cuts, *S. fruticosa* Mill. tea is widely used to cure colds and stomach aches (Yesilada *et al.* 1995, Demirci *et al.* 2002). *Salvia officinalis* L. is one of the most important culinary herbs and its essential oil is important for the pharmaceutical industry (Kelen and Tepe 2008). *Salvia aramiensis* Rech. fil. is widely consumed as an important tea and spice plant (Davis 1982).

It is reported that essential oils of these *Salvia* species contain many useful secondary metabolites such as terpenes, phenolic compounds and their derivatives (Tepe *et al.* 2007). Essential oils represent a rich potential source for alternative and natural control agents due to their antimicrobial, insecticidal, repellent and/or nutritional deterrent effects (Sener *et al.* 2009). However, the high content of camphor and thujone of these sage species, except *Salvia aramiensis* Rech. fil., limits their use as tea and spice in the food industry due to their toxic and carcinogenic effects (Manoguerra *et al*. 2006, Böszörmenyi *et al*. 2009, Shahabi *et al.* 2012, Pelkonen *et al.* 2013). It was reported the thujone ratio of *S. fruticosa* Mill. and both camphor and thujone ratio of *Salvia aramiensis* Rech. fil. is low (Lamaison *et al.* 1991, Cuvelier *et al.* 1994, Lawrence 1998, Demirci *et al.* 2002, Karaman. *et al*. 2007). It is clear that the composition of essential oil, oil and

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protein can be affected by factors such as varieties and location (Mert *et al.* 2005). However, no comprehensive study has been found in which the essential oil contents and compositions of *S. aramiensis* Rech. fil., *S. fruticosa* Mill., *S. tomentosa* Mill. and *S. officinalis* L. have been studied. Therefore, the present study was aimed to investigate essential oil content and chemical composition of these four *Salvia* spp.

Materials and Methods

Sage plant samples used in the study were collected from different natural flora in Hatay province of Turkey. The plant identification was done by Dr. I. Üremis (Head of the Herbology's Department). Herbarium specimen was deposited for preservation at the Herbarium Collection of Field Crops Department at Hatay Mustafa Kemal University. The sage plants (*S. aramiensis*, *S. fruticosa*, *S. tomentosa*), which are naturally found at an altitude of 170-400 m in the flora of Hatay region (Turkey), were collected and dried at 35°C. On the other hand, *S. officinalis* was collected from the experimental fields in Hatay Mustafa Kemal University. Essential oil was obtained from dried leaves and flowers. A total of 25 g of each of the ground plant samples was used for the separate hydrodistillation experiment. A weighed sample weight was individually and carefully placed into a 1 L flask. Distilled water was then added until it covered the sample completely. The essential oils were then obtained by water- distillation for 3 hr by using a Clevenger type apparatus according to European Pharmacopoeia method. The trial was repeated three times. Percentage essential oil yield was calculated according to dry weight of plant materials and amount of essential oils obtained. The Essential oils were dried over anhydrous sodium sulfate and stored in dark vial bottles at +4°C until analysis (Türkmen and Mert 2020, Kara *et al.* 2021, Türkmen *et al.* 2021).

The components of the essential oils of the plants were determined by gas-chromatographic (GC-MS) method. Determination of essential oil components was carried out with Thermo Scientific ISQ Single Quadrupole model gas chromatographic device under the following conditions. TR-FAME MS model, 5% Phenyl Polysilphenylene-siloxane, 0.25 mm inner diameter x 60 m length, 0.25 µm film thickness column was used. Helium (99.9%) was used as the carrier gas for Peer Review only at a flow rate of 1 ml/min. Mass spectra were recorded at 70eV, the mass range was from 1.2-1200 m/z. Scan Mode was used for data collection. The MS transfer line temperature was 250°C, the MS ionization temperature was 220°C, the injection port temperature was 220°C, the column temperature was initially 50°C and the temperature was raised to 220°C with a rate of heat increase of 3°C/min. The structure of each compound was identified using mass spectra with the Xcalibur program (Wiley 9). The individual compositions were determined by comparing their retention index and with Wiley Library (Wiley Interscience, New York). The relative quantities of individual compounts were calculated with Xcalibur Report programme. The compounds were identified from the GC/MS spectra by comparison of their retention indices (RI) with homologous series of n-alkanes. Retention indices were determined using retention times of n-alkanes (C8-C40) injected under the same chromatographic conditions, co-injection with standards compared with those data from Wiley 9 comparison of fragmentation pattern in the mass spectra of each constituent with those data from Wiley 9 libraries. RI compared with the reported values. Identification of each individual compound was made by comparison of their retention times with those of authentic samples and by computer searching and matching with mass spectral data held in computer libraries (Asil 2018, Arpag *et al.* 2020, Türkmen 2021).

Data obtained from essential oil contents of *Salvia* spp. were subjected to ANOVA and means were compared using Duncan's multiple range test by using a SPSS statistical program (Version 24.0, IBM, USA).

Results and Discussion

The essential oil content of *S. aramiensis* Rech. fil., *S. fruticosa* Mill., *S. tomentosa* Mill. and *S. officinalis* L. is presented in Table 1 and in Figs 1,2,3 and 4. Among the plants, *S. aramiensis* had significantly higher ($p < 0.001$) essential oil content with 5.31% followed by *S. fruticosa*, *S. officinalis* and *S. tomentosa* with the ratio of 2.04, 1.68 and 1.40%, respectively (Table 1). The essential oil content of *S. aramiensis* was reported to be 3.00, 2.20 and 1.30% by Sarer (1987), Karaman *et al.* (2007) and Arslan (2016), respectively. In the present study, in *S. aramiensis* essential oil content was detected highest compared to previous studies. Similarly, the essential oil content (1.40%) of *S. tomentosa* was determined and found to be slightly higher than the findings of Arslan (2016).

Table 1. Essential oil content of *Salvia* species.

Name of plants	Essential oil content (%)
S. aramiensis Rech. fil.	5.31 ± 0.90^{a}
S. fruticosa Mill.	2.04 ± 0.23^{b}
S. tomentosa Mill.	1.40 ± 0.05^{b}
S. officinalis L.	1.68 ± 0.11^{b}
P	***

Data are given as mean values ± standard deviation. [a,b] Means in the same column showing different small letters are significantly different (***$p < 0.001$).

Chemical composition of the essential oils of four *Salvia* spp. leaves are presented in Table 2. The chromatograms were obtained and they are shown in Fig. 1,2,3 and 4. As expected, terpenes were detected as the dominant chemical class among all essential oils. Because, it is known that terpenes are the main components of essential oils (Aldred *et al.* 2009).

GC/MS analysis revealed that 29 (99.31%), 33 (99.54%), 28 (97.69%) and 29 (99.45%) components were detected for the essential oils of *S. aramiensis*, *S. fruticosa*, *S. tomentosa* and *S. officinalis*, respectively (Table 2). Terpenes constituted more than 90% the ratio of essential oil components. While eucalyptol was the main constituent for *S. aramiensis* (58.65%), *S. fruticosa* (34.97%) and *S. tomentosa* (44.70%), δ-thujone was dominant component for *S. officinalis* (33.83%) (Figs 1,2,3,4). It is known that eucalyptol, a terpenoid oxide, has a significant role in human health due to anti-inflammatory and antioxidant properties in various diseases (Seol and Kim 2016).

It is reported that *S. aramiensis* is located only in Hatay flora in Turkey (Davis 1982). Therefore it is important to evaluate this *Salvia* species in terms of being endemic to Hatay. In the essential oil of *S. aramiensis*, eucalyptol (58.65%) and β-pinene (11.07%) were identified as major components. These results are more or less compatible with the findings of Demirci *et al.* (2002), Kelen and Tepe (2008) and Askun *et al.* (2010). Other major components after eucalyptol and β-pinene were borneol (5.44%), α-pinene (4.58%) and camphene (4.53%), respectively but camphor and thujone were not detected in the essential oil of *S. aramiensis* (Table 2).

In the case of *S. fruticosa* essential oil, eucalyptol (34.97%) and camphor (24.20%) determined as the major components (Table 2). These findings are in agreement with the previous studies (Putievsky *et al.* 1992, Askun *et al.* 2010). It was reported that camphor has toxic properties while eucalyptol and camphor are significant chemicals in terms of their antimicrobial

activities (Tirillini *et al.* 1996, Pattnaik *et al.* 1997, Tzakou *et al.* 2001). In *S. fruticosa* α-terpineol (5.73%), isobornyl acetate (5.31%), camphene (4.47%) and caryophyllene oxide (4.47%) were identified as other major components in the essential oil (Table 2).

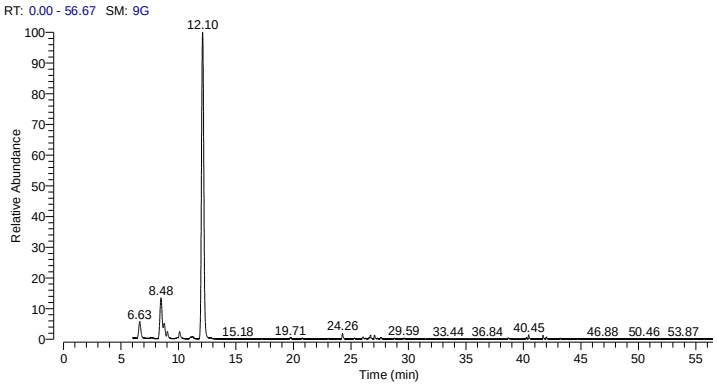

Fig. 1. GC-MS chromatogram of essentials oil of *S. aramiensis* Rech. fil

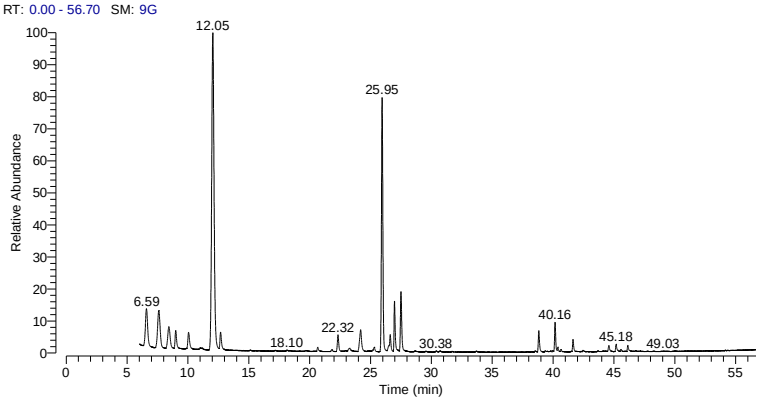

Fig. 2. GC-MS chromatogram of essentials oil of *S. fruticosa* Mill.

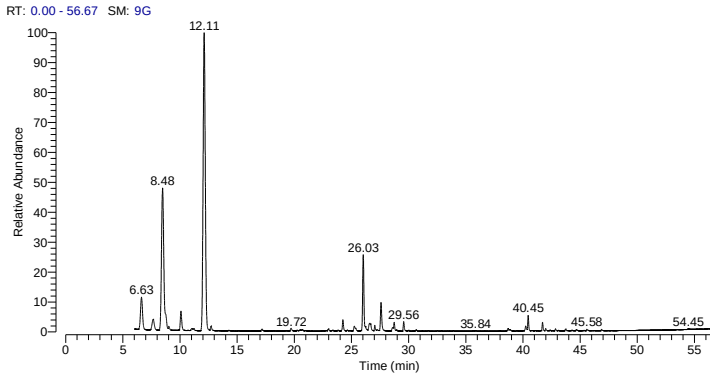

Fig. 3. GC-MS chromatogram of essentials oil of *S. tomentosa* Mill.

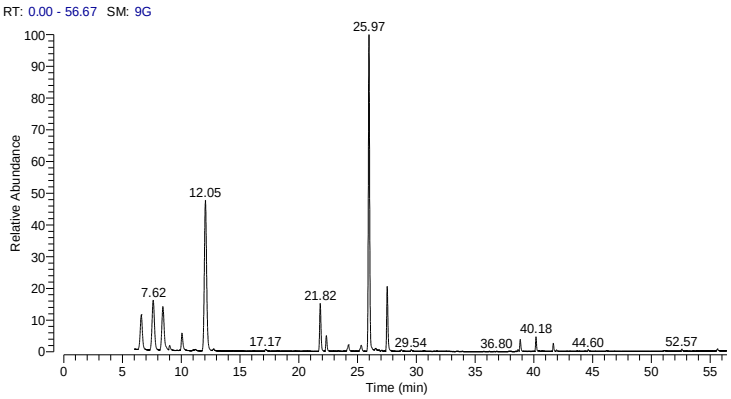

Fig. 4. GC-MS chromatogram of essentials oil of *S. officinalis* L.

Table 2. Chemical composition of the essential oil of *Salvia* species.

RT	Name of compounds	RI	Area (%)			
			S. aramiensis Rech. fil.	*S. fruticosa* Mill.	*S. tomentosa Mill.*	*S. officinalis* L.
6.59	α-Pinene†	1069	4.58	3.91	4.43	0.76
7.61	Camphene†	1137	4.53	4.47	1.54	0.98
8.43	β-Pinene†	1190	11.07	2.45	21.34	0.85
8.99	Myrcene†	1224	1.14	1.65	0.32	0.7
10.05	Limonene†	1283	1.44	1.53	2.27	1.07
10.98	Sabinene†	1330	1.47	0.19	0.34	nd
11.13	Terpinene†	1338	0.18	0.13	0.23	0.17
12.04	Eucalyptol†	1381	58.65	34.97	44.70	16.12
12.68	Cymene†	1410	0.06	1.53	0.5	1.06
18.1	1-Octen-3-ol§	1682	0.08	0.09	0.18	0.06
19.65	cis-Sabinene hydrate†	1764	0.15	0.15	0.56	0.15
20.52	β-Bourbonene†	1808	nd	nd	0.07	nd
20.66	Linalool†	1815	0.27	0.36	0.11	0.1
22.32	β-Thujone†	1893	nd	1.7	nd	nd
21.79	δ-Thujone†	1869	nd	nd	nd	33.83
22.89	trans-Sabinene hydrate†	1919	nd	nd	nd	0.17
23.27	Alloaromadendrene†	1936	0.12	0.32	nd	nd
23.34	α-Campholenal*	1939	nd	nd	0.07	nd
24.19	trans-Caryophyllene†	1975	nd	2.13	nd	2.21
24.25	Terpinen-4-ol§	1978	nd	nd	1.14	nd
25.25	Sabinyl acetateδ	2020	nd	nd	0.82	nd
25.3	Bornyl acetateδ	2023	0.51	0.33	nd	0.4

Contd.

RT	Name of compounds	RI	Area (%)			
			S. aramiensis Rech. fil.	*S. fruticosa* Mill.	*S. tomentosa Mill.*	*S. officinalis* L.
25.95	Camphor†	2051	nd	24.2	8.67	21.46
26.24	Pinocarvone‡	2063	nd	nd	0.07	nd
26.66	Elemol†	2080	nd	nd	0.58	nd
26.77	cis-Thujanol†	2085	nd	nd	nd	0.11
26.97	α-Terpineol†	2093	1.15	5.73	0.53	0.13
27.0	Borneol†	2094	5.44	nd	3.13	4.21
27.5	Isobornyl acetateδ	2113	0.38	5.31	nd	nd
28.6	Geranyl acetateδ	2151	1.02	0.08	nd	nd
28.59	Bicyclogermacrene†	2151	nd	nd	0.37	nd
28.73	Myrtenal*	2155	nd	nd	0.95	nd
29.53	Myrtenol†	2182	0.23	0.06	1.06	0.04
30.39	exo-2-Hydroxycineole§	2211	0.12	0.08	nd	nd
30.7	cis-Calamenene†	2221	0.1	0.08	nd	0.07
31.68	p-Cymen-8-ol§	2254	0.18	0.05	nd	0.06
33.66	Gurjunenepoxide†	2340	0.24	0.07	nd	nd
38.51	Thymol†	2602	nd	0.12	nd	nd
38.82	Veridiflorol†	2613	0.35	1.79	0.35	7.65
39.35	Globulol†	2633	0.68	0.11	nd	0.05
39.79	Carvacrol†	2650	nd	0.14	nd	0.15
40.16	Caryophyllene oxide†	2663	2.04	4.47	1.1	3.98
40.39	Spathulenol†	2672	1.92	0.29	1.43	0.3
41.64	Humuladienone‡	2717	0.75	0.98	nd	nd
43.66	Humulene†	2786	0.46	0.07	0.83	0.53
52.55	Manool†	3057	nd	nd	nd	2.08

† Terpene. ‡ Ketone. δ Ester. § Alcohol. * Aldehyde.

Similar to *S. aramiensis*, eucalyptol (44.70%) and β-pinene (21.34%) constituted the main components for *S. tomentosa* (Table 2). On the other hand, camphor was not identified in *S. aramiensis* unlike the essential oil of *S. tomentosa.* Other major components for *S. tomentosa.* were followed by camphor (8.67%), α-pinene (4.43%) and borneol (3.13%), respectively. These components are compatible with the results reported by Askun *et al.* (2010). However, researchers reported that α-pinene and camphor were main compounds followed by borneol and eucalyptol.

Terpenes were found to be the major chemical class for *S. officinalis* (97%). However, δ-thujone (33.83%) and camphor (21.46%) were determined the main components for the essential oil of *S. officinalis.* As mentioned before, due to their toxic effects, camphor and thujone limits using of the sages as tea and spice in the food industry. In the essential oil obtained from the *S. officinalis*, other major compunds were eucalyptol (16.12%), veridiflorol (7.65%) and borneol (4.21%), respectively. These results are in accordance with the findings of Putievsky *et al.* (1992).

The essential oils of medicinal and aromatic plants contain a wide range of beneficial metabolites such as terpenoids, phenolic compounds vitamins and their derivatives. On the other hand, various toxic and carcinogenic substances can be found in the plants. In this respect, content and chemical composition of essential oil of some sage species consumed as tea and spice plants are important. In this study, the essential oil composition and content of *S. officinalis* L. as growing cultivated and 3 *Salvia* species growing naturally in Hatay province was investigated. Among these species, *S. aramiensis*, which has the highest essential oil content and does not contain toxic components such as camphor and thujone, is recommended to be cultivated and to expand its cultivation. Further studies should be done on the selection and cultivation of this plant.

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