Chemical Composition of Four *Salvia* L. Species From Turkey, a Chemotaxonomic Approach

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Abstract: In this study the chemical composition of four *Salvia* L. species (*S. trichoclada* Bentham., *S. virgata* Jacq., *S. ceratophylla* L., *S. multicaulis* Vahl.) from Turkey, were analyzed by GC-MS (Gas chromatography / mass spectrometry) system. Forty, forty one, forty three and thirty nine compounds were identified representing 91.9%, 90.4%, 89.7%, 88.4% of the *S. trichoclada, S. virgata*, *S. ceratophylla* and *S. multicaulis* oils, respectively. Caryophyllene oxide (25.1%), spathulenol (15.4%) and β-pinene (12.3%) were identified to be the main constituents of *S. trichoclada.* 1,8-cineole (20.3%), α-copaene (18.6%) and germacrene D (17.6%) were determined the major compounds of *S. virgata.* Germacrene D (23.6%), α-copaene (19.4%) and 1,8-cineole (7.8%) were found to be the main constituents of *S. ceratophylla.* Caryophyllene oxide (22.5%), spathulenol (12.7%) and β-pinene (7.5%) were detected to be the main constituents of *S. multicaulis.* The results were discussed in view of chemotaxonomy and natural products.

Key words: *Salvia*; Essential oil; Chemotaxonomy; GC/MS.

Introduction

The genus *Salvia* L. includes more than 900 species and is mostly found in both subtropical and temperate parts of the world; the two largest gen centers of the *Salvia* are in America and South-West Asia[1]. In Turkey, endemism ratio of *Salvia* is 48 %, so Turkey is a major gen centre for the *Salvia* genus [2]. This genus is named "*Salvia*", derived from latin "Salveo", which means to "save, to recover" [3]. *Salvia* taxa is used in folk medicine from ancient times and find application in many commercial and medicinal products, particularly in essential or volatile oils and flavoring agents manufacture and is widely used in the food and cosmetic industries. The essential oils of *Salvia* plants have broad spectrum of beneficial for the human health characteristics, such as: antioxidant, antifungal analgesic and antiinflammatory [4]. These essential oils have been used also for treatment of asthma, eczema, psoriasis and tuberculosis deseases [5]. In addition, the antituber culous, antibacterial, and antiphlogistic activities of the *Salvia* species extracts are well known [6,7]. Also, there are several researchs on phytochemical analysis of *Salvia* [8] genus and Lamiaceae taxa [9-12]. Phytochemical studies conducted on plants of *Salvia* demonstrated the presence of many diterpenoids of the abietane, ictexane, labdane, neoclerodane and phenalenone types in the extracts [13,14]. Triterpenes and sterols were also found [15], in addition to anthocyanins, coumarins, polysaccharides, flavonoids and phenolic acids and their derivatives [16] However, the essential oils chemical composition is known for a number of *Salvia* taxa [17-19], but few data are found in chemotaxonomy studies of *Salvia* genus. On the other hand, essential oil composition of this genus has been proved particularly helpful in assessing taxonomic relationships of several genera in Lamiaceae [1].

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From the taxonomical and systematic point of view, the more a substance is deduced and found in the biosynthetic pathway the more distinct it is for certain taxa. For example monoterpenes are typical for the genus *Mentha* L., but menthol is characteristic for *M. piperita* L. and *M. arvensis* L. [20]. On the other hand, the phenylpropenoid eugenol, typical for cloves of *Syzygium aromaticum* (L.) Merr. & L.M.Perry. This compound can also be found in large amounts in *Cinnamomum zeylanicum* Breyne., *Ocimum basilicum* L.; as sources for anethole in *Pimpinella anisum* and fennel in *Foeniculum vulgare* both in Apiaceae family; eucalyptol (1,8-cineole) named after its occurrence in *Eucalyptus* sp. Taking the above facts into consideration, chemotaxonomically relevant are accepted or distinct pathways, typical fingerprints, and either major constituents or very specific even minor (δ-3-carene to separate *Citrus grandis* from other *Citrus* sp) or trace compounds [21]. Regarding the essential oil, there are many mono- and sesquiterpenes found in sage but, in *Ocimum* sp. and *Perilla* sp., no phenylpropenes were found. To understand species-specific differences within *Salvia* genus, the Mediterranean *S. officinalis* L., *S. fruticosa* Mill., and *S. lavandulifolia* Vahl. will be confronted with the *S. stenophylla* Burch. ex Benth., *S. repens* Benth., and *S. runcinata* L. indigenous to South Africa: in the Mediterranean group usually α- and β-thujones, 1,8-cineole, camphor, linalool, β-pinene, limonene, *cis*-sabinyl acetate are the prevailing compounds, whereas in the South Africa group caryophyllene and α-bisabolol are main constituents [22].

This study aims to investigate the essential oil compounds of four *Salvia* species (*S. trichoclada, S. virgata*, *S. ceratophylla*, *S. multicaulis*), to explain the chemotaxonomic significance; to determined chemotypes and to potential usefulness of studied samples.

Materials and methods

Plant Materials

Plant samples were collected from their natural habitats. *S. trichoclada* was collected from west of Dikme village (Bingöl) steppe, on 20.06.2013, at an altitude of 1400-1500 m., by O.Kilic., collection number; 4702. *S. virgata* was collected from vicinity of Dikme upland (Bingöl) slopes, on 20.06.2013, at an altitude of 1400-1500 m., by O.Kilic., collection number; 4774. *S. ceratophylla* was collected from west of Dikme village (Bingöl), rocky slopes, on 26.06.2013, at an altitude of 1350-1400 m., by O.Kilic, collection number; 4900. *S.multicaulis* was collected from vicinity of Yelesen village (north of Dikme upland) (Bingöl) steppe, on 30.06.2013, at an altitude of 1350-1400 m., by O.Kilic, collection number; 5149. All plant samples were identified by Kilic with Flora of Turkey and East Aegean Islands [3]. The voucher specimens have been deposited at the Technical Vocational Clollege, Department of Park and Garden Plants, Bingol University.

Isolation of the essential oil

Dried aerial parts of studied *Salvia* species were exposed to hydrodistillation using a Clevenger-type apparatus for three hour.

Gas chromatographic (GC) analysis

The essential oil of studied species was analyzed using HP 6890 GC equipped with and FID detector and an HP-5 MS column (30 m × 0.25 mm i.d., film tickness 0.25 μm) capillary column was used. The column and analysis conditions were the same as in GC-MS. The percentage composition of the essential oils was computed from GCFID peak areas without correction factors.

Gas chromatography/mass spectrometry (GC-MS) analysis

The essential oils of studied *Salvia* species were analyzed by GC, GC-MS, using a Hewlett Packard (HP) - Agilent 5973 N GC-MS system with 6890 GC in Plant Products and Biotechnology Laboratory in Firat University (Elazığ-Turkey). HP-5 MS column (30 m × 0.25 mm i.d., film tickness (0.25 μm) was used and helium as the carrier gas. Injector temperature was 250 ºC, split flow was 1 mL / min. The GC oven temperature was adjustment at 70 ºC for 2 min and programmed to 150 ºC at a rate of 10 ºC/min and then kept constant at 150 ºC for 15 min to 240 ºC at a rate of 5 ºC/min. Alkanes were used as reference points in the calculation of RRI (relative retention indices. MS were taken at 70 eV and a mass range of 35-425. Constituent identification was applied using WILEY and NIST spectrometric electronic libraries; detected compounds are showed in Table 1.

Results

In this study caryophyllene oxide (25.1%), spathulenol (15.4%) and β-pinene (12.3%) were in *S. trichoclada*; 1,8-cineole (20.3%), α-copaene (18.6%) and germacrene D (17.6%) in *S. virgata*; germacrene D (23.6%), α-copaene (19.4%) and 1,8-cineole (7.8%) in *S. ceratophylla*; caryophyllene oxide (22.5%), spathulenol (12.7%) and β-pinene (7.5%) in *S. multicaulis*, were identified as main compounds (Table 1). Caryophyllene oxide was determined as the main compounds of *S. trichoclada* (25.1%), *S. multicaulis* (22.5%) (Table 1) and *S. atropatana* Bunge (19.3%) [23]; whereas low amounts of this compound were detected in the esential oil of *S. virgata* (3.5%), and *S. ceratophylla* (2.9%) (Table 1).

Discussion

Caryophyllene oxide was not reported in *S. macrosiphon* Boiss. and *S. oligophylla* Aucher ex Benth. oils extracts [23]. Spathulenol was found to be the major compounds of *S. trichoclada* (15.4%) and *S. multicaulis* (12.7%). On the other hand, spathulenol was detected in very low amount in the esential oil of *S. virgata* (0.3%) and was missing in *S. multicaulis* oil (Table 1). Among the studied *Salvia* species, β-pinene was found to be the main constituents of *S. trichoclada* (12.3%) and *S. multicaulis* (7.5%) (Table 1), although literature data did not indicated the presence of this compound in the oil extracts of six different *Salvia* species [23]. 1,8-cineole was found to be the main compounds of *S. virgata* (20.3%), *S. ceratophylla* (7.8%) (Table 1) and *S. mirzayinii* (21.9%) [24]; whereas this compound was reported in low amounts in the esential oil of *S. multicaulis* (4.1%) (Table 1). Among the sesquiterpene hydrocarbons, α-copaeen was found to be the major constituent of *S. mexicana* L. [25], this compound also has been reported as main constituent in the *S. virgata* (18.6%) and *S. ceratophylla* (19.4%) (Table 1); whereas α-copaene was found to be in very low amount in the essential oils of *S. tirchoclada* (0.3%) (Table 1). In the *S. reuterana* essential oil 21 components were identified with (*E*)-β-ocimene (32.3%), α-gurjunene (14.1%) and germacrene-D (11.2%) were the main compounds [26]. In our study germacrene D was identified as the main compounds of *S. virgata* (17.6%) and *S. ceratophylla* (23.6%) (Table 1). In another study the essential oil composition of some *Salvia* species is reported: *S. canariensis*, *S. confertiflora*, *S. mexicana* and *S. microphylla* . Among the detected compounds, α-pinene, β-pinene, camphene, 8-3-carene, limonene (monoterpene hydrocarbons) were the main compounds of *S. canariensis*; 1,8-cineole, camphor, borneol, bornyl acetate were the major constituents of *S. confertiflora*; β-caryophyllene, γ-muurolene, germacrene B, α-copaene were the main compounds of *S. mexicana*; globulol, guaiol, spathulenol, and α-eudesmol (oxygenated sesquiterpenes) were the main constituents of *S. microphylla* [25]. In our study caryophyllene oxide, bornylacetate, β-pinene; 1,8-cineole, α-copaene, germacrene D; β-caryophyllene, linalool, β-pinene and caryophyllene oxide, spathulenol, β-pinene were the main constituents of *S.*

trichoclada; S. virgata; S. ceratophylla and *S. multicaulis* respectively (Table 1). 1,8-cineole or eucalyptol, is widely distributed in plants and is found in high concentrations in the essential oil of *Eucalyptus polybractea* R.T.Baker. It is broadly used in cosmetics industry, muscular pain, for cough treatment, neurosis, rheumatism, asthma, and urinary stones [27]. In this study 1,8-cineole was identified as main compounds of *Salvia virgata* (20.3%) and *Salvia ceratophylla* (7.8%) (Table 1).

One of the highest concentrations of α-pinene and β-pinene is in the essential oil fruit of *Juniperis communis* L. (Cupressaceae) include over 80% of these compounds. α-pinene is also found the main compounds in the essential oil of *Pinus resinosa* Sol. ex Aiton (12.96%), *Pinus flexilis* E. James (33.29%), *Pinus strobus* L. (32.96%), *Pinus parviflora* Siebold & Zucc (25.56%) and *Pinus mugo* Turra subsp. *mugo* (9.00%) [28]. In the industry, α- and β-pinene are used in the production of alcoholic beverages like gin [27]. In our research the β-pinene was identified as the main constituent of *S. trichoclada* and *S. multicaulis* (12.3% - 7.5% respectively) (Table 1). β-caryophyllene or caryophyllene derivatives are the main sesquiterpene of hops and are being used as cosmetic additives in soaps and fragrances [27]. In herbal medicine, the mild sedative properties of hops is due to the presence of β-caryophyllene [29]. Furthermore, by *in vitro* studies it was demonstrated the cytotoxic activity of the β-caryophyllene against breast cancer cells [30]. Our study demonstrated that the oil extracts of *S. trichoclada* and *S. multicaulis* contained 25.1% and 22.5% caryophyllene oxide, respectively (Table 1). The existence of different valuable compounds in the selected *Salvia* species (*S. trichoclada*, *S. virgata*, *S. ceratophylla*, *S. multicaulis*) was revealed by the detailed oil composition characterisation performed in this study, thus demonstrating their applicability for medicinal and pharmaceutical purposes; and in the cosmetic and beverages industry.

In conclusion, some of the *Salvia* species showed various chemotypes of essential oil including, β-caryophyllene/germacrene D/linalool/caryophyllene oxide in *S. palaestina*, and α-pinene/germacrene D/β-pinene in *S. tomentosa* [8]. Caryophyllene oxide/β-pinene/spathulenol were detected as the chemotypes of *S. trichoclada* and *S. multicaulis*; 1,8-cineole/α-copaene/germacrene D were found to be as the chemotypes of *S. virgata* and *S. ceratophylla* in eastern Anatolian region of Turkey. According to these results, studied *Salvia* species are overlapping with morphological classification with Flora of Turkey [3].

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

Compounds	***RRI**	***S. trichoclada***	***S. virgata***	***S. ceratophylla***	***S. multicaulis***
2-Hexenal	965	0.2	-	0.3	0.6
α-thujene	1012	0.2	0.4	0.1	-
α-pinene	1020	3.4	3.2	2.5	2.6
Camphene	1032	-	1.2	-	0.5
Benzaldehyde	1040	0.1	-	0.3	-
Sabinene	1052	-	0.4	-	1.3
7-octen-4-ol	1058	0.3	-	0.4	0.1
β-pinene	1061	**12.3**	1.5	2.6	**7.5**
3-octane	1063	-	0.6	0.3	-
β-mrycene	1067	2.2	1.3	1.5	1.1
3-octanol	1069	0.4	-	-	-
p-cymene	1082	-	1.1	0.3	0.3
β-ocimene	1089	0.5	0.1	0.2	1.6
Limonene	1095	1.2	-	2.1	3.5
1,8-cineole	1102	6.8	**20.3**	**7.8**	4.1
Benzeneacetaldehyde	1105	-	0.4	-	-
δ-3-carene	1108	0.3	-	0.1	0.3
γ-terpinene	1112	-	0.8	0.2	-
α-terpinolene	1125	1.2	0.2	0.3	0.2
Linalool	1140	0.5	0.7	1.1	4.1
Butanoic acid	1153	0.1	-	0.2	0.4
Cis-sabinenehydrate	1159	-	0.1	-	-
Terpinen-4-ol	1165	0.2	0.1	0.3	1.1
Acetaldehyde	1167	0.3	-	1.2	-
Myrtenal	1171	-	0.4	-	0.2
Pulegone	1175	0.7	0.2	-	1.0
Bicyclo (3.1.1) heptan-2-one	1178	0.3	-	0.2	-
Trans-pinocarveol	1180	-	0.1	0.1	0.3
Camphor	1183	3.4	4.8	5.6	2.6
Pinocarvone	1185	0.1	0.2	-	-
Borneol	1192	0.4	-	1.2	0.4
α-terpineol	1210	-	1.2	1.7	-
Linanyl-acetate	1235	0.1	0.3	0.2	3.3
Bornyl acetate	1247	-	1.2	0.9	2.1
Thymol	1282	0.2	-	0.3	-
Bicycloelemene	1320	-	0.2	-	0.1
α-copaene	1352	0.6	**18.6**	**19.4**	3.3
β-bourbenene	1360	0.2	-	0.6	0.5
β-elemene	1362	-	1.3	0.5	0.2
β-caryophyllene	1385	5.3	2.6	2.5	3.9
Aromadendrene	1407	-	0.3	-	0.8
Epi-bicyclophellandrene	1415	1.1	-	0.3	-
Naphtalene	1428	0.1	0.3	0.1	0.2
Germacrene D	1438	4.2	**17.6**	**23.6**	1.1
Germacrene B	1482	2.6	1.7	0.8	-
Spathulenol	1495	**15.4**	0.3	-	**12.7**
Caryophyllene oxide	1499	**25.1**	3.5	2.9	**22.5**
β-selinene	1503	0.2	-	0.1	-
Salvial-4 (14) - en -1-on	1506	0.1	0.2	3.8	0.3
δ-cadinene	1518	-	0.3	-	0.5
Nerolidol	1522	0.2	-	0.6	-
α-cadinol	1533	-	1.1	-	0.3
α-farnesene	1540	0.6	-	1.3	2.2
α-ylangene	1551	0.2	0.3	-	-
Azulene	1554	-	0.2	0.1	0.2
β-eudesmol	1612	0.1	-	0.4	-
α-cadinol	1656	0.4	1.0	-	-
Benzene	1683	-	-	0.7	0.1
	Total	**91.9**	**90.3**	**89.7**	**88.4**

***RRI:** Relative Retention Index