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Research Article

Essential oil profile of six spontaneous hybrids from male sterile *Salvia officinalis* L.

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ABSTRACT

Herbal medicines and beverages have started to take an essential place in our daily lives. *S. officinalis* is one of the most used herbal tea species in the sage family. Secondary metabolites, especially essential oils, plays an important role in its biological properties. *S. officinalis* essential oil is mostly rich in camphor and thujone, which of these compounds could be toxicological. In the present study, six spontaneous hybrid plants and their parents were analyzed for their essential oil contents. Male sterile *S. officinalis* were used as maternal plant, *S. fruticosa* and *S. aramiensis* were probable paternals where they were cultivated nearby. Grown plants were analyzed by gas chromatography-mass spectrometry. Essential oil compounds were used to identify their relation to each other. The main components of *S. officinalis* were thujone (40.97%), 1,8-cineole (24.65%) and camphor (19.37%). 1,8-cineole content of hybrid genotypes were varied between 35.13-64.92%. Camphor level of hybrids were varied in lower levels as between 2.92-26.35% while thujone content were very low compared to the maternal *S. officinalis* as 0.95-6.83%.

Keywords: Anatolian sage, Breeding, Greek sage, Hatay sage, Hybrid, Principle component

Introduction

Essential oils are complex combinations of volatile, organic compounds that provide the flavor and fragrance of a plant (Tisserand and Yound, 2014). Essential oils had proficiency in the prevention and cure of various diseases and worked as an antiviral, antibacterial, antioxidant, antidiabetic, and anticancer agent (Tanu and Harpreet, 2016). Many of the herbal products contain essential oils besides other biological constituents.

The genus *Salvia*, with approximately 1000 species, is an important genus regarding secondary metabolite contained species in the Lamiaceae family. The genus is widely distributed from the Far East, through Europe and across to the New World (Kintzios, 2000). Flora of Turkey represented by 100 species, seven varieties of the genus *Salvia* (Kusaksiz, 2019). Secondary metabolites of the genus have been studied to determine its antioxidant, antimicrobial, anti-Alzheimer, anticancer and insecticidal properties (Pavlidou et al., 2004; Senel et al., 2010; Exarchou et al., 2015; Sarrou et al., 2016). *Salvia* species has a great value in cosmetic, food and pharmaceutical industries (Carović-Stanko et al., 2016). The amount of trade for nature collected medicinal and aromatic plants are difficult to find, especially in underdeveloped countries. Commercially used *Salvia* species from Turkey are *S. coccinea*, *S. farinacea*, *S. microphylla*, *S. officinalis*, *S. officinalis* 'Incterina', *S. officinalis* 'Purpurascens', *S. officinalis* 'Tricolor', *S. splendens*, *S. x superba* and *S. transylvanica* (Karabacak, 2009). In Turkey, total sage cultivation (species not mentioned) is nearly 412 ha (Karik and Tunctürk, 2019). *S. fruticosa* and *S. officinalis* are the main species that cultivated and exported. Most of the *S. fruticosa* still collect from nature; an also small amount of cultivation has been producing for both *S. officinalis* and *S. fruticosa* (Arslan, 2014). *S. fruticosa*, in 2019, was exported that the amount of 500 tonnes (Kusaksiz, 2019). Three *S. officinalis* varieties (Erada TJ, Güripek and Elif) and one *S. fruticosa* variety (Karık) were recorded (Anonymous, 2020). Cultivation of registered varieties are essential to obtain standardized leaf and essential oil. Essential oil standards of *S. officinalis* were published in ISO 9909:1997. This report dedicated that essential oil composition of *S. officinalis* L. should contain α-thujone (18.0-43.0%), camphor (4.5-24.5%), 1,8-cineole (5.5-13.0%), *β*-thujone (3.0-8.5%), *α*-humulene (≤12.0%), *α*-pinene (1.0-6.5%), camphene (1.5-7.0%), limonene (0.5-3.0%), bornyl acetate (≤2.5%), linalool and bornyl acetate (≤1.0%). Herbal monograph of *Salvia officinalis* was reported from the European Medicines Agency (EMA, 2016). Extensive ranges for compounds could be seen in the report. *S. officinalis* know with its high content of thujone, and thujone reported to be neurotoxic. In the European Union herbal monograph on *S. officinalis* L. suggested that chemotypes with low content of thujone should be preferred (EMA, 2016). New varieties of sage with a high leaf and essential oil yields, also resistant to diseases should be developed.

Salvia species from Turkey's flora are insect-pollinated and outcrossing. There are several studies revealed hybridization in nature (Hedge, 1982). In the Flora of Turkey, Davis (1982) stated that many *Salvia* species create hybrids in the natural flora of Turkey. Hybrids between *S. suffruticosa* × *S. bracteata* named *S.×spireaefolia*; and also, from Iranian flora hybridization between *S. suffruticosa×S. hydrangea* were reported (Davis, 1982). Furthermore, hybrids between S. *ceratophylla* and *S. aethiopis, S. cyanescens* and *S. candidissima* were reported (Davis, 1982). Flower type (pin, thrum and homestyle) seen as the biggest obstacle for interspecific crossing (Haque and Ghoshal, 1981). In that study, during three years, fourteen *Salvia* species (*S. coccinea*, *S. splendens*, *S. farinacea*, *S. hispanica*, *S. grahamii*, *S. pratensis*, *S. taraxacifolia*, *S. aegyptica*, *S. tilifolia*, *S reflexa*, *S. glutinosa*, *S. verbenaca*, *S. hormium*, *S. lucantha*) were crossed and only in three species positive results were obtained.

S. officinalis, *S. fruticosa* and *S. aramiensis* present in the same section of the genus (Dogan et al., 2008). Natural hybrids from the flora of Croatia were recorded and analyzed with molecular markers (Radosavljevic et al., 2019; Rivera et al., 2019). Spontaneous hybridization between *Salvia officinalis* and *S. lavandulifolia* and *S. officinalis* and *S. fruticosa*, *S. fruticosa* and *S. tomentosa* in the cultivated areas were reported in different researches (Sanchez Gomez et al., 1995; Evropi-Sofia, 2013; Herraiz-Penalver et al., 2015; Bahtiyarca Bagdat et al., 2017). Male sterility of *S. officinalis* sourced from partially and completely undeveloped microspores were reported from the study of Linnert (1955). Essential oils and herb yield were the primary purposes of these studies. Artificial hybridization between *S. officinalis*, *S. fruticosa* and *S. tomentosa* were done by Putiesky et al. (1990), and cultivar called Neve Ya'ar No:4 were recorded (Dudai et al., 1999). Furthermore, artificial hybridization between *S. fruticosa*, *S. officinalis* and *S. aramiensis* were studied (Bahadirli and Ayanoglu, 2019). In these studies, essential oil content and rate of compounds were found in the middle of the parent plants while in some of them higher contents were observed.

The aim of this study was to identify essential oil content and compounds of the spontaneous hybrids from the seeds of male sterile *S. officinalis* that cultivated nearby *S. fruticosa* and *S. aramiensis*. Furthermore, to reveal their relations with parents by principle component analysis.

Materials and Methods

Plant Material

The seeds of the plant materials used for this study came from experimental field from Department of Field Crops, Hatay Mustafa Kemal University where *S. officinalis*, *S. fruticosa* and *S. aramiensis* were cultivated in nearby plots. Flowering started at the late of March in *S. fruticosa*, beginning of April for *S. officinalis* and mid of April for *S. aramiensis*. In both *S. officinalis* and *S. fruticosa* flowering continue almost two months and flowering overlap in three of the species in the study. To prove the male sterility of *S. officinalis* firstly anthers were removed and examined with triphenyl tetrazolium for vitality of pollens. Secondly, some of the flower stems were covered with net to detect if there is any self-pollination. *S. officinalis* seeds were collected during summer season in 2018. Most of the collected seeds were empty (without any embryo). Selected seeds primed in 500 ppm GA_3 solution for 24 hours before placing in petri-dishes. The germination gen-erally starts in seven days to one month. After germinations of the seeds (3-5 cm), the plants were sown in a plastic viol and placed in a green house. Planting material comprise peat and perlite mix (1/3). When the seedlings grow up to 20 cm, the seedlings planted in plastic pots. During summer time seedlings were placed outside of the green house and watered when needed. Grown hybrid plants were harvested in late July and air dried in drying oven at 35 °C.

Essential Oil Extraction

Dry leaves were hydro-distilled for 3 hours with using Clevenger-type apparatus. Essential oil ratio was calculated as the mean value from dry plant material weight and expressed in g/100 g dry weight (%). Essential oils were kept in amber vials at +4 °C for further analysis.

Essential Oil Analyses

The essential oils were determined according the method described by Bahadirli and Ayanoglu (2019). Seperations and determination of the essential oil components were done by GC-MS (Gas Chromatography Mass Spectrometry) device Thermo Scientific ISQ Single Quadrupole. Approximately 5 µl of essential oil was dissolved in a 2 ml cyclohexane for GC-MS injection. Separation of the essential oils were carried out by a TG-Wax MS (5% Phenyl Polysilphenylene-siloxane, 0.25 mm inner diameter * 60 m length, 0.25 µm film thickness) column. The ionization energy was calibrated as 70 eV, and the mass interval was m/z 1.2- 1200 amu. The scan mode was used as the screening more in data collection. MS transfer line temperature was 250°C, MS ionization temperature was 220°C, and whereas colon temperature was 50°C at the beginning, then it was increased up to 220°C with 3°C/min rate. The structure of each component was defined using mass spectrums (Wiley 9) with Xcalibur software. Retention indices were determined using retention times of n-alkanes (C8-C40) that were injected after the plants essential oil under the same chromatographic conditions.

Principal Component Analysis (PCA)

Comparison of Essential oils between parent species and hybrids were analyzed with PCA using XLSTAT (2009) statistics program. The compounds (PCA) that appeared in an amount higher than 1% in at least one sample were used.

Results and Discussion

The essential oil content of hybrids and their parent plants was determined. *S. officinalis* essential oil (EO) content was 2.5%, *S. fruticosa* EO content was 3.5% and *S. aramiensis* EO content was 2.14%. Essential oil rates of hybrid plants were found as follows H-1 was 2.10%, H-2 was 3.40%, H-3 was 3.0%, H-4 was 3.20%, H-5 was 1.60% and H-6 was 2.5%. *S. officinalis* × *S. lavandulifolia* hybrid essential oil content found in the middle of the parent species and essential oil ranged between 0.9-2.8% (Herraiz-Penalver et al., 2015).

Essential oil compounds were determined by GC-MS analysis and results were given in Table 1. The main components of *S. officinalis* were thujone 40.97%, 1,8-cineole 24.65% and camphor 19.37%. Thujone levels of all hybrid plants were found much lower than maternal plant *S. officinalis*, the range was 0.95-6.83%. All of the hybrid plants' 1,8-cineole range were higher than *S. officinalis* and ranged between 35.13-64.92%. Both of the *S. fruticosa* (50.27%) and *S. aramiensis* (57.76%) had higher 1,8-cineole rate than *S. officinalis*. All of the hybrids 1,8-cineole content were in the middle of the parents except H-2, 1,8-cineole was 64.92%.

Spontaneous hybrid between *S. officinalis* and *S. lavandulifolia* were investigated for essential oil composition (Sanchez Gomez et al., 1995). In the study, hybrid plants' essential oil content found as same as *S. officinalis* 0.60%. Major compounds of *S. officinalis* essential oil were α-thujone 22.82%, 1,8-cineole 15.71%, viridiflorol 10.92%, β-thujone 4.32% and camphor 4.99%, while hybrid plants' essential oil composition found as 1,8-cineole 18.01%, β-pinene 14.11%, camphor 10.80%, α-thujone 3.04% and β-thujone 0.56% (Sanchez Gomez et al., 1995).

Table 1. Essential oil compounds of hybrid genotypes and their parents

Compound Name	RI*	M	F-A	F-F	H-1	H-2	H-3	H-4	H-5	H-6
α-Pinene	1034	0.90	4.34	6.99	5.69	4.05	6.48	5.79	4.47	5.65
Camphene	1098	0.97	0.08	6.82	4.22	0.52	5.56	4.80	5.24	3.12
β-Pinene	1135	1.16	20.03	2.94	4.72	8.09	4.13	4.92	9.57	5.15
α-Myrcene	1160	1.44	1.78	1.38	2.03	2.55	1.63	2.48	2.41	3.15
α-Phellandrene	1179	0.02	1.51	Nd	0.17	0.27	0.07	0.16	0.21	0.19
α-Terpinene	1197	0.04	nd	0.07	0.06	0.15	0.12	0.08	nd	nd
Limonene	1205	0.97	2.67	1.31	1.08	0.98	1.23	1.09	1.03	1.08
γ-Terpinene	1245	0.26	0.13	0.08	0.35	0.73	0.38	0.37	0.12	0.57
1,8-cineole	1278	24.65	57.76	50.27	53.73	64.92	44.17	48.45	35.13	52.92
p-Cymene	1302	0.30	nd	1.00	0.20	0.24	0.21	0.14	0.07	0.36
1-Octen-3-ol	1457	0.06	0.07	0.06	0.06	nd	0.04	0.03	0.21	nd
Sabinene hydrate	1496	0.54	1.24	0.07	0.55	0.67	0.16	0.66	0.37	0.45
Linalool	1535	0.12	nd	0.42	0.35	0.19	0.40	0.2	0.56	0.21
Thujone	1587	40.97	nd	1.06	1.70	6.83	2.47	2.05	0.95	3.30
Valencene	1631	nd	nd	Nd	0.02	nd	0.07	0.08	nd	0.27
Caryophyllene	1662	0.88	4.80	1.07	3.01	2.04	1.38	4.40	1.14	6.67
Bornyl acetate	1700	1.17	nd	0.27	0.38	1.81	0.92	1.45	0.41	nd
α-Terpineol	1701	nd	nd	3.95	1.58	nd	3.21	nd	nd	nd
Camphor	1714	19.37	nd	18.44	15.55	2.92	23.68	16.22	26.35	11.29
α-Humulene	1721	0.14	0.55	0.28	0.31	0.46	0.47	nd	nd	nd
Borneol	1740	1.97	nd	Nd	1.96	0.79	1.70	3.19	0.58	2.43
Geranyl acetate	1760	nd	nd	Nd	nd	nd	nd	0.03	0.35	nd
Viridiflorol	2048	2.18	nd	0.67	0.12	0.06	0.34	0.60	7.24	0.27
Spathulenol	2089	nd	1.13	0.05	nd	nd	nd	nd	nd	nd
Caryophyllene oxide	2084	0.49	0.96	0.92	0.10	nd	0.15	0.45	1.03	0.60
Junipene	2365	nd	nd	0.04	0.03	nd	0.02	0.08	0.73	0.08
Total		98.60	97.05	98.16	97.97	98.27	98.91	97.72	98.17	97.76

nd= not detected, *RI= Retention Indices were calculated according to the *n*-alkanes
M=Mother plant (*S. officinalis*); F-A=Father plant (*S. aramiensis*); F-F=Father plant (*S. fruticosa*); H=hybrid plant

First artificial hybridization between *S. officinalis* and *S. fruticosa* was reported from Putievsky et al. (1990). In the study, thujone levels of hybrids found close to *S. officinalis,* while 1,8-cineole and camphor found in the middle of the parent plants. *S. officinalis* essential oil major compounds found as α-thujone 55.0%, 1,8-cineole 13.0%, β-thujone 10.0% and camphor 2.0%, *S. fruticosa* essential oil major compounds found as 1,8-cineole 48.0%, β-pinene 11.0% and camphor 8.0%. The major compounds of hybrids when *S. officinalis* used as maternal determined as 1,8-cineole 30.0%, α-thujone 27.0%, β-thujone 7.0% and β-pinene 7.0%. When *S. fruticosa* used as maternal plant hybrid, essential oils found as thujone 29.0%, 1,8-cineole 24.0%, β-thujone 7.0% and β-pinene 7.0%. Later that research, artificial hybrid between *S. officinalis* and *S. fruticosa* named Neve Ya'ar No:4 was studied for yield and essential oil characteristics (Dudai et al., 1999). Their results showed that major compounds of essential oil components were camphor (28.19%), thujone (22.20%) and 1,8-cineole (13.67%) (Dudai et al., 1999). Moldavian infraspecific hybrid *S. officinalis* cv. Miracol was analyzed and the major compounds of essential oil were found as α-thujone 21.24%, camphor 19.14% and 1,8-cineole 10.37% (Gonceariuc, 2014). A spontaneous hybrid from the cultivated area of *S. officinalis* and *S. lavandulifolia* subsp. *lavandulifolia* essential oil compounds were found as estimated for essential oil compounds. 1,8-cineole rates of *S. lavandulifolia* subsp. *lavandulifolia* found between 15.5-55.1%, in *S. officinalis* 3.3-11.1% and in hybrid 12.0-34.7%. α-thujone rates in of *S. lavandulifolia* subsp. *lavandulifolia* found between 0-0.2%, in *S. officinalis* 25.4-57.2% and in hybrid 13.6-23.6%. Camphor rate in *S. lavandulifolia* subsp. *lavandulifolia* 0.8-6.8%, in *S. officinalis* 1.6-10.8% and in hybrid 1.5-4.1% (Herraiz-Penalver et al., 2015). Another spontaneous hybrid were studied for essential oil content (Bahtiyarca Bagdat et al., 2017). Main components of essential oils showed wide variations such as α-thujone 8.32-42.46 %, β-thujone 2.02-21.39 %, 1,8-cineole 4.66-29.34 %, borneol 0.91-16.73 % and camphor 4.22-30.77%.

Principle Component Analysis (PCA) on essential oil compounds of all genotypes resulted in that high correlation was observed between EO compounds and genotypes. Thujone and camphor compounds had a negative correlation with 1,8-cineole, *α-β* pinene and camphene. Variables (F) F1 and F2 that explains 95.15% of the variations were chosen to create two-dimensional graphic and results were given in Figure 1. The figure shows the distribution of hybrids and their parents according to their essential oil compounds. S. officinalis placed far from all of the other genotypes in the figure. H-1 and H-2 were in the middle of the *S. fruticosa* while H-3, H-4, H-5 and H-6 were between *S. fruticosa* and *S. officinalis*, but close the *S. fruticosa*.

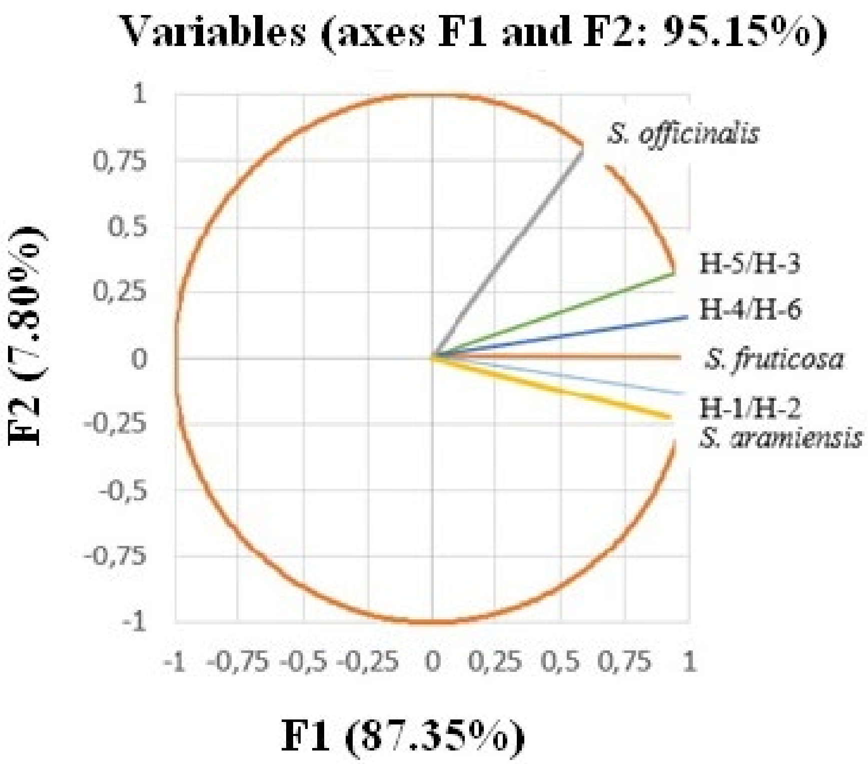

Figure 1. Hybrids and their parents' distribution by PCA according to essential oil compounds

According to the results, genotypes were distributed according to their 1,8-cineole, thujone and camphor content. Results were compatible with other studies. Jug-Ducakovic et al. (2012) were found a high negative correlation between thujone and camphor content in *S. officinalis* genotypes. Cvetkovikj et al. (2015), were analyzed 25 *S. officinalis* population according to their essential oil compounds and genotypes were distributed by high Thujone high trans-caryophyllene content. In the study of Herraiz-Penalver et al. (2015), PCA analysis separated the genotypes regarding of their thujone and 1,8-cineole rate.

Conclusion

The findings clearly illustrate that spontaneous hybridization has been occurred between *S. officinalis*, *S. fruticosa* and *S. aramiensis*. Male sterility of *S. officinalis* helped to identify the hybridization. Developing new cultivars still remains its importance, especially in medicinal and aromatic plants. New cultivars of sage with high yield, low camphor and thujone levels needed in medicinal and aromatic plant market. In the study, high 1,8-cineole with low camphor and thujone content were observed. However, camphor levels were not varied as thujone and 1,8-cineole content. *S. fruticosa* has been used mostly from collected materials from nature. Besides that, *S. officinalis* already has great value for trading. In the study, cultivated plants of *S. fruticosa* and *S. aramiensis* from the flora of Hatay were used. It is important to use existing diversity from flora. The further field trial will have established to obtain yield and patent of the genotypes.

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

Ethics committee approval: Author declare that this study does not include any experiments with human or animal subjects.

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Disclosure: -

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