Examination Of The Change in The Components Of Volatile Oil Of Medical Sage (*Salvia Officinalis L.*) Which is Grown in Different Locations

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***Abstract*—This study was conducted simultaneously in Çanakkale, Balıkesir and Kütahya locations in order to define the effect of location on the volatile oil components, volatile oil rate and volatile oil quality in Medical Sage (Salviaofficinalis L.) plant in 2015 growing season. Field experiments were repeated in 3 replicates according to randomized block design. The seedlings were planted by a horizontal distance of 30 cm and vertical distance of 50 cm. The volatile oils of the parts of Salviaofficinalis L. Which are over the soil were examined. These plants' volatile oils were obtained by hydro distillation method (GC_MS/FID) and the volatile oil rates in three different locations were measured as %0.99, %1.23, %1.85, respectively. The basic components of the volatile oil were determined as follows; α-thujone % 26,35, % 38,39, % 29,84, β-thujone % 29,20, % 12,26, % 9,07, camphor %5,60,%13,90, % 23,24, 1,8-cineole % 7,75, % 7,81, %6,57, viridiflorol%5,55, %4,38, %2,65. The highest volatile oil rate of the Medical Sage was reached in Kütahya location by a %1.85 rate. As a result of the study it was found out that volatile oil components are comparatively richer in terpenes and the amount of volatile oil differs according to the ecological factors.**

Keywords—Salviaofficinalis L.,volatileoil,α-thujone, GC-MS/FID

1- Introduction

The Salvia genus, which belongs to the Lamiaceae (Labiatae) family, contains over 98 species of both species in the world, spread to the Mediterranean and Central Europe,especially in the tropics and subtropics. 106 of these species are naturally distributed in our country and 58 of them are endemic. Turkey is ranked 13th in the world in terms of species richness of Salvia species[1]. Salvia is an important part of useful plants that have been known since ancient times and have never lost their significance to this day. The first records of medical features and the use of this plant in the treatment of various diseases are found in the fancy writing and paintings of tombs and monuments from ancient times. It was named as Salvia which origins from the word Salveo, which means to save and heal Latin because of its healing nature. Monoterpenes and their oxygenated derivatives in the composition of the essential oils of the genus have antiseptic effect. In the last 4 years, studies on this plant have revealed the presence of compounds that slow down cell DNA synthesis [17]. This finding is important for cancer research and treatment.

The largest group of chemical structures of essential oil compositions are terpenes.Besides, There are also small amounts of alcohols, aldehydes, esters, phenols, nitrogen and sulfur containing compounds. The oxygenated derivatives which are formed by the oxidation of terpenes are the fragrance, taste and therapeutic properties[14]. The refractive indices of volatile essential oils are generally high and optically active. Due to the effects of light and oxygen, they must be kept sealed in dark bottles for long periods of storage. In Roman, Greek and especially Egyptian civilizations, essential oils have been widely used. Interest in aroma therapy, seen as a branch of alternative medicine in recent years, has also increased the use of essential oils. Etheric oils are used in massage applied in therapies or in relaxing baths[13].

Salvia species are used in the treatment of various diseases. Salvia species are not only used on the medical field but also as a sweetener in perfumery and food industry[6]. Many Salvia species are used in liquor making [10-11]. Most Salvia species are grown as decorative ornamental plants in parks and gardens due to their beautiful appearance[4-18].

As a raw material, volatile oil (Oleum Salviae) obtained from the leaves and its own leaves (Folia Salviae, T.K.) are used [3]. Sage leaves contain % 0.5-2.5 volatile oil. At least % 1.5 of volatile oil is required in the codex [5]. The medically accepted oil contains α, β Thujon, 1,8 Cineol, Campher, Borneol, Bornylacetate. Some essential oils are reported to carry Thymol and Carvacrol [30]. In the essential oil, thujon ratio is % 30-50, cineol ratio is % 15, borneol ratio is % 10 [3].

Salvia plants have been an important part of human health for many years due to the effects of digestive facilitator, sedative, diuretic, antiperspirant, painkiller and so on. The field of use of medical sage

is quite extensive. It is used in medicine, food, drink, perfume and cosmetics industry. Nowadays it is used in the production of mouthwash and mouthwash preparations, in the production of hair strengthening shampoos, in deodorants, in hair coloring and skin creams, and is consumed as tea with its healing and relaxing effect [28]. Turkey is quite remarkable in terms of the current plant variety and has a rich fluoride. This richness is due to the existence of three phytogeographical regions, being the bridge between Southern Europe and Southwest Asia, the high level of species endemism with respect to possible ecological and phytogeographical changes in Anatolia, and being the origin of many breeds and segregations.

The fact that synthetic materials produced in recent years have more side effects and resistance of organisms to synthetic raw materials used as antimicrobials have increased the importance of natural plant sources and medical plants carrying these substances[17]. Today, the majority of the world's population also uses plants as pharmaceutical raw materials. Especially in developing countries, % 80 of the population provides health care from traditional medical plants in the first place. If % 80 of the world population is thought to be living in developing countries, % 64 of the total population uses plants for treatment [9]. Approximately % 25 of prescription raw materials in developed countries are herbal origin chemicals [21]. Preservation of genetic resources of sage varieties is of great importance for the continuity of our natural wealth and for future research [18]. This research has been carried out at different locations to determine the changes in the essential component of essential oil and volatile essential oil.

This research is a study to determine the essential oils of Salvia L. we grow in the Aegean and Marmara region, to compare with the composition of essential oil of Salvia L. and to evaluate essential oils. In this study, it was aimed to compare the essential oil of salvia officinalis L. species with other Salvia species, to determine the appropriate tribe according to the region, to contribute to product variety, taste and food industry.

2- Material and Method

2.1 Climatic and Soil Properties of Trial Sites

Climate data is available on the internet at www.mgm.gov.tr/iklim/clim-limitirmalari (Table 1).

Table 1. Meteorological Observation Table for the year 2015 of the test sites

Months	Kütahya		Çanakkale		Balıkesir	
	Monthly Total Rainfall Average (kg / m2)	Average monthly temperature (C^0)	Monthly Total Rainfall Average (kg / m2)	Average monthly temperature (C^0)	Monthly Total Rainfall Average (kg / m2)	Average monthly temperature (C^0)
April	53.5	16.2	47.0	17.2	51.3	19.3
May	54.9	21.2	32.0	22.6	41.2	24.5
June	36.5	25.1	22.4	27.8	24.6	29.2
July	18.7	28.2	11.7	30.7	7.9	31.2
August	15.4	28.5	6.5	30.6	5.8	31.3
September	23.1	24.7	24.2	26.4	23.3	27.8
October	41.7	18.9	57.0	20.7	45.4	22.1

* State Meteorology General Directorate Monthly Climatology Survey Sheet

In Kütahya province, summer is hot and dry, winter is cold and rainy. The annual temperature average in Kütahya is 10,5 ºC. The hottest months are July and August, and the coldest months are January and February. The lowest measured temperature is -28,1 ºC. The average annual rainfall is 565 mm. The most rainy month is December, the driest month is August month [1]. Çanakkale climate; It is generally considered mild. It shows a transition climate characteristic between the Mediterranean climate and the Black Sea climate. The annual rainfall is between 600-1200 mm. Frost events are over. It lasts for about a month in a year. Temperatures range from -10 ° C to + 30 ° C [1]. The Edremit district of Balıkesir is located in the Mediterranean climate zone where the summers are hot and arid and the winters are warm and rainy. The average annual rainfall is 723.6 mm and the average number of rainy days is 34,82. There is very little snowfall in winter months. Minimum temperature is in February with -4.2 ° C and maximum temperature is in August with +31.3 [1].

Table 2. Some chemical analysis results of soil samples from experimental sites

Analysis Type -**Balıkesir**	Result	Condition	Analysis Type -**Çanakkale**	Result	Condition	Analysis Type - **Kütahya**	Result	Condition
Potassium (K2O) kg/da	20.6072	Medium	Potassium (K2O) kg/da	13.467	Little	Potassium (K2O) kg/da	20.0348	Medium
Phosphorus (P2O5) Kg/da	4.9846	Little	Phosphorus (P2O5) Kg/da	3.1502	Little	Phosphorus (P2O5) Kg/da	6.819	Medium
Lime (%)	0.8348	Little lime	Lime (%)	2.5044	lime	lime(%)	4.0222	lime
Organic Substance (%)	0.7955	Very Little	Organic Substance (%)	3.178	Fine	Organic Substance (%)	0.8944	Very Little
Total Salt (%)	0.0032	Without Salt	Total Salt (%)	0.0034	Without Salt	Total Salt (%)	0.0037	Without Salt
PH	7.07	Neutral	PH	7.14	Neutral	PH	7.21	Neutral
Saturation (%)	50	Lined	Saturation (%)	52	Clayey-Lined	Saturation (%)	54.5	Clayey-Lined

Soil analyzes were carried out in Kütahya Agricultural Chamber soil-plant-irrigation water analysis laboratory. Soil analysis of Balıkesir - Edremit location is given in Table 2. According to Table 2, it is not very rich in potassium, it seems to be poor in phosphorus maintenance. The lime ratio was low. It is determined that it is poor in terms of organic matter and the soil is lined. The soil analysis of the location in Kütahya is given in Table 2. According to Table 2 it is not rich in potassium and phosphorus. Lime ratio is high and Soil saturation was determined as clayey – lined and rich in organic matter in the analysis. The Mediterranean climate in the Marmara region (Canakkale, Balıkesir) and the continental climate in the Inner Aegean region (Kütahya) are dominant. This has also affected the volatile oil composition among the locations.

2.2 Establishment of Plant Material and Experiments

The seeds used as material in this study were obtained from the Department of Field Crops of the Faculty of Agriculture of Ankara University. Seeds were grown in Kütahya Municipality in December 2014 by planting seedlings at Hekim Sinan Medicinal Plants Botanical Garden. Studies were carried out simultaneously in these 3 different locations: In 2015 Çanakkale Onsekiz Mart University Faculty of Agriculture Dardanos Campus test site, Balıkesir Edremit Kale Natürel A.Ş. Medical Plants Garden and Kütahya Municipality Physician Sinan Medical Plants Botanical Garden. Since 180 plants were needed at each locality, 216 plants were moved to the trial site, taking into consideration the greenhouse conditions and subsequent failures in the field. The planting seedlings were watered at regular intervals with a strainer bucket. Seed germination lasted 20-25 days. The roots of fiddling began to be transferred to the field from April of 2015. Seedlings was given water of life right after the transfer.

Field trials were carried out in 3 replications, according to random block trial design. Plant planting frequency is arranged according to 50 cm horizontal, 30 cm vertical distances and each parcel consists of 3 rows. The same order was established in all locations and it is preferred to start flowering, which has the highest proportion of essential oil as harvest time.

2.3 Essential Oil Production

The amount of essential oil in the determination of the amount of essential oil of spice medicines, condiments and medicinal plants is all substances which are vaporized by the conditions specified in the method and expressed in milliliters per 100 g of dry matter. T he reagents used must be in analytical purity and the pure water used must be distilled or equivalent purity. This method is based on the principle of distilling the aqueous suspension of the sample, collecting the discrete volatile oil in the distillate fraction from the aqueous phase, collecting the volatile oil collected, and then calculating the percent volatile oil. At the beginning of the test volatile oil analysis 20 g dry material was weighed. The material was taken into a 500 ml balloon with a round base and a shaved neck. 200 ml (may vary depending on the amount of sample, about 10 times), added with pure water and shaken. The balloon is placed on the heated jacket of the Neo-Cleaver system, the lower part of the vertical glass tube is connected to the glass balloon, and the upper part is connected to the cooling system. After filling the tube and the slant pipe with pure water, the coolant system is started and the balloon is heated. It is hydrodesticated for 2 hours. After the system has cooled down and the volatile oil collected in the graduated fraction has separated from the aqueous phase, the amount (ml) is determined. The amount of essential volatile oil in 100 g is calculated as the volatile oil content (%) according to the amount of sample (g) to be weighed [26].

2.4- Determination of essential oil composition by GC-MS

Analyzes of essential oil components were carried out at the Research Laboratory of the Western Mediterranean Agricultural Research Institute. Samples were diluted with 1% hexane and injected in 1 µl with 40:1 split ratios to Gas chromatography (Agilent 7890A). Capillary columns (HP InnowaxCapillary; 60.0 m x 0.25 mm x 0.25 µm) were used to separate the components. The column was split into two fractions at a rate of 1:1 using a splitter to the FID and mass spectrometry detector (Agilent 5975C). In the analysis, helium was used as carrier gas at a flow rate of 0.8 ml / min. The injector temperature was maintained at 250 ° C, the column temperature program was 10 minutes at 60 ° C, 4 ° C / minute (40 minutes) at 60 ° C and 220 ° C and 10 minutes at 220 ° C It is set to be 60 minutes. The scan range (m / z) for the mass detector is 35-450 atomic mass units and the electron bombardment ionization energy is 70 eV. The diagnosis of volatile oil components is based on the data from OIL ADAMS, WILEY and NIST libraries. The data of the FID detector were used for the volatile oil component ratios [20].

3. Findings and Discussion

Variation of volatile oil content and composition of Salvia species examined in this study according to their locations Salvia officinalis L. total essential oil yield was measured as % 0,99 in Canakkale, % 1,23 in Balıkesir - Edremit and % 1,85 in Kütahya respectively. The amounts of essential oil components are listed in Table 1. In the oil from salvia officinalis L, 19 - 23 and 21 components were analyzed and this constituted % 100 - %100 and %100 of total fat. The main components of essential oil have been identified according to locations as; Salvia officinalis L., α-thujone % 26,35, %38,39, %29,84, β-thujone %29,20, %12,26, %9.07, Camphor %5,60, %13, 90,% 23,24, 1,8-cineole %7.75, %7,81,% 6,57, Viridiflorol % 5,55, %4,38,% 2,65. The proportion of these major componnts constitue %74.45 of the total fat (Salvia officinalis L.) in Çanakkale, %76.74 in Balıkesir-Edremit and %71.37 in Kütahya.

Some investigators found that volatile oil in Salvia officinalis L. plant differ in ratioas %0.85-2.13 of (Ceylan et al., 1979); (Bernard et al., 1991), %0.83-1.63 (Yenikalaycı and Özgüven, 2001), %1-3 (Bayram and Sonmez, 2006), %1.5-1.7 on average (Haban and Otepka, 2007). According to the German pharmacopoeia (DAB 8), it is desirable to have a volatile oil content of %1-2.5 (average %1.5).

When the composition is examined, it is seen that the main substance of the composition is Thujon. The proportions of the major constituent constituents were in these range; α-pinen % 0.92-3.76, β-pinen %1.05-5.54, myrecene %0.61-1.25, 1-8 cineole %3.23-12.35, thujon %12.62-39.29, camphor %5.06-30.97, linalool %0.39-0.74, borneol %1.11-10.56, trans-caryophyllene %2.44-13.68 [8].

In a study conducted by Miladinovic and Miladinovic (2000), it was found that the main components of volatile oil were α-thujon (%24.88), camphor (%16.03), and camphor (%16.03) and that volatile oil content and composition were influenced by ecological factors such as climate and soil in Salvia officinalis L. -8 cineole (%9.79). Salameh and Dordevic (2000) found 29 compounds in the essential oil composition of Salvia officinalis L. strain originating in Jordan. They found that the main constituents were α-thujon (%29.9), β-thujon (%13.68), camphor (%15.74) and 1-8 cineole (%12.31).

Sagareishvili et al., (2000) identified 11 different components in the same plant, and as main constituents α Thujone (%31.56), β Thujone (%17.55), Camphor (%16.48) and 1,8 Cineol (%17.53) . Delamare, Ana Paula Longaray, et al. (2007) determined that the major constituents of S. officinalis oil were α-thujone, 1,8-cineole, camphor, borneol and β-pinene.

We see that the data obtained for the combination of essential oil and essential oil is in accordance with the literature. It has been determined that these differences may be due to both harvesting time and location, climate and environmental factors [25].

Table 3. Analysis results of Salvia officinalis L in terms of essential oil components according to locations

Amount of volatile oil(%)				
Salvia officinalis L. (Çanakkale)		*Salvia officinalis* L. Balıkesir (Edremit)		*Salvia officinalis* L. (Kütahya)
0,99		1,23		1,85
No	**Componentname**	**Component amount (%)**		
		*Salvia officinalis*L. (Çanakkale)	*Salvia officinalis*L. Balıkesir (Edremit)	*Salvia officinalis* L. (Kütahya)
1	α-pinene	4,32	3,62	2,34
2	Camphene	2,37	3,16	3,91
3	β-pinene	2,00	2,55	1,81
4	Sabinene	-	0,47	-
5	Myrcene	1,25	1,34	1,14
6	Limonene	1,29	1,47	1,66
7	1,8-cineole	7,75	7,81	6,57
8	γ –terpinene	0,41	0,44	0,37
9	α-terpinolene	-	-	0,41
10	Cymene	-	0,32	-
11	α-thujone	26,35	38,39	29,84
12	β-thujone	29,20	12,26	9,07
13	Trans-sabinene hydrate	0,31	0,32	-
14	Camphor	5,60	13,90	23,24
15	Linalool	-	0,28	0,52
16	Bornyl acetate	-	0,32	1,19
17	Terpinen-4-ol	0,29	0,30	-
18	β-caryophyllene	3,39	2,24	3,59
19	α –humulene	3,77	2,39	4,20
20	α-terpineol	-	**-**	0,68
21	Borneol	0,72	0,84	2,04
22	Caryophyllene oxide	1,18	0,63	0,35
23	Humulene epoxide II	1,14	0,60	0,46
24	Viridiflorol	5,55	4,38	2,65
25	Manool	3,11	1,98	3,98

4- Results

Differences were found compared to other studies in terms of ratios of plant species tested to essential oils according to the work carried out. Flowering time of salvia plant shows that volatile oil composition varies according to the Geographic and climatic factors. The diversity and amount of bioactive substances present in medical and aromatic plants also depend on the usable part of the plant, and methods of obtaining and analyzing essential oils used in post-harvest processes [24]. Salvia officinalis L., which is grown in regions with different soil structure and climatic characteristics in studies conducted in Çanakkale, Balıkesir, Kütahya locations varied in terms of essential oil components. The main constituents of the highest essential oil of salvia officinalis L plant were identified as α-thujone %38,39 Balikesir, %29,84 Kütahya, %26,35 Çanakkale. The highest volatile oil component was identified in Balıkesir (Salvia officinalis L.) location with 23 components. In order to determine the chemical profile of the Salvia species when it is considered that the essential oil components differ according to environmental factors, further studies on different Salvia species than the different locations of our country which is rich in Salvia species should be carried out [28]. Changes in the amount of volatile oil and volatile essential oil in the study compared to other studies differed in terms of genetic and environmental factors.

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