Clonal and Seasonal Variation of Major Essential Oil Components of *Salvia fruticosa* Clones

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Abstract

Anatolian sage (Salvia fruticosa MILL.) is widely used in many sectors such as food, cosmetics, and pharmaceuticals. The components in an essential oil species are important criteria to determine the usage area. This study was carried out to determine the differences in essential oil components and seasonal changes in the components of six clonally selected C-clones and one natural population. Samples were collected monthly from C-clones for two years, and essential oil ratios were determined. Assessment of all clones revealed that the time of monthly harvests had a significant effect on essential oil components. The highest β-caryophyllene content of clones was recorded in December or February and for essential oil in August. The clone and harvest time interaction caused significant differences in essential oil components, and clones reached the highest values in different months. The highest 1.8 cineole content of clones was recorded between March and June. High variation in the components, the differences in harvest times, and high correlations between components indicated that clones developed by selection have a significant production potential.

Introduction

The genus Salvia, belonging to the Lamiaceae family, includes medicinal, aromatic plant species that have been used for a long time and have maintained their importance. The most important Salvia species is *S. fruticosa*, often known as Anatolian sage or sage, gray chalba or apple sage. The cultivation of this species has increased lately. The Anatolian sage, of which leaves are consumed as tea and the essential oil obtained from the leaves is called 'apple oil,' is good for respiratory tract infections, nervous diseases, and diarrhea and has a pain relief effect (Baytop, 1999). The sage is one of the important species used in essential oil production in Turkey (Karık and Öztürk, 2010), and a significant part of the essential oil is exported. Therefore, the sage species used in essential oil production has increased over the years (BÜGEM, 2018).

Yield is the most important trait determining the commercial value of Salvia species, while chemical quality indicators have also gained importance recently. The type and ratio of components that determine the sector where the essential oil can be used determine the quality. The type and ratio of components that determine essential oil quality can be used. The smell of *S. fruticosa* species, which is widespread in the natural flora of Turkey, is more intense than *S. officinalis* L., known as medicinal sage. Although the essential oil components of both sages are similar, the essential oil component ratios are different. The *S. fruticosa*, which has a lower thujone ratio that has a toxic effect over a certain dose, is more suitable for tea (Zeybek and Zeybek 1994).

The market volume of medicinal, aromatic plants has increased recently with the demand for natural products. The medicinal and aromatic plants in Turkey are mostly collected from nature, but the quality standardization of the products collected from nature is complex. In addition, the collection of these plants from nature should be supervised to conserve the natural flora, producers should be supported, and products that do not comply with the standards should not be marketed (Metin et al. 2012; Erol, 2015).

The market demands cannot be meet in long term by collecting the medicinal and aromatic plants from nature. In addition, world markets and pharmaceutical industry demand “standard” products with high amount and quality of active ingredient. Meeting the growing demand and obtaining sufficient standard and quality products is not possible by collecting natural plants and can only be achieved by the cultivation of medicinal and aromatic plants obtained through selection and breeding studies (Bayram et al. 2010, Leontaritou et al. 2020). Therefore, breeding studies can be carried out according to producer and market demand (Franzel et al. 1996). Standard products can only be obtained using standard raw materials, and the most convenient procedure to obtain standard raw materials is breeding or selecting related varieties. The studies aimed to eliminate the deficit in natural products obtained from medicinal and aromatic plants have reached a certain point. The essential oil content of plants can vary depending on genetic structure, plant species and variety, plant age, ecological conditions of the growing region, harvest time, and abiotic stress conditions exposed (Saharkhix et al. 2009). New cultivars have been developed by evaluating herb yield and essential oil ratio (Bayram, 2001; Telci and Şahbaz, 2005; Telci et al. 2006; Arslan et al. 2014, Lal et al. 2021). However, the research on main component ratios of essential oils, clonal differences in essential oil components, harvest times, clone harvest time interactions and correlations between components are limited. In this context, the study results aimed to determine productive clones, appropriate harvest times for clones, and correlations between components offer many options to producers and consumers and provide economic opportunities. In addition, different harvest times should be investigated for the market's sustainability.

This study aimed to reveal a) the differences in essential oil content and essential oil components of 6 C-clones (variety candidates) determined by the selection, b) the effect of monthly harvest time on the clones, and c) the interaction between clone components. In addition, providing different options to the producer and the consumer regarding clones and harvest times was also the purpose of this study.

Material And Method

In this study, six C-clones selected among 1250 clones from 15 different populations were determined in the projects titled 'Selection Breeding in Some Sage (*Salvia* spp.) Species Growing in the Antalya Flora' and 'Identification of Genotypes with Superior Characteristics in the Anatolian Sage (*Salvia fruticosa* MILL.) Populations from Antalya Flora' were used. In addition, the seedlings obtained from the seeds of the Antalya-Gelidonia population were included in the control study. The information on the materials used is given in Table 1. Many studies have been carried out on genotypes from seeds. In this study, clonal material was used since more convenient in standard production.

Table 1
The locations of materials used in the study

Population No	Location	Altitude (m)	Coordinate
fk3-16	Kemer	31	36 34 59 N 30 34 33 E
fk4-9	Kemer	137	36 32 06 N 30 32 35 E
fk4-14	Kemer	137	36 32 06 N 30 32 35 E
fk5-7	Kemer	20	36 40 96 N 30 33 43 E
fd2-9	Demre	68	40 10 49 N 35 75 57 E
fd4-13	Demre	5	40 10 18 N 35 75 49 E
Standard	Kumluca	57	36 14 11 N 30 24 41 E

The experiment was carried out in the Aksu experimental fields of the West Mediterranean Agricultural Research Institute. The summers are hot and dry, and the winters are warm and rainy in Antalya, where the field experiments were conducted. The data obtained from Antalya Meteorology Regional Directorate revealed that the average precipitation, temperature, and relative humidity values during the experiment were close to the long-term averages. During the experiment (24 months), the average temperature, precipitation, and relative humidity were 19°C, 60 mm, and 73%, respectively (Table 2).

Table 2
Climatic data of Antalya-Aksu during the experiment

Years	**2017**							**2018**				
Months	June	July	August	September	October	Nov.	Dec.	Jan.	Feb.	March	April	May
Precipitation (mm)	0.0	0.0	0.0	0.0	29.0	48.0	74.0	93.0	91.0	94.0	2.0	19.0
Mean Rel. Hum. (%)	66.4	62.0	72.3	72.4	64.9	74.0	81.8	72.2	83.0	78.9	68.7	66.2
Mean Temp. (°C)	25.8	29.4	27.9	25.2	19.7	14.4	12.0	10.8	12.8	15.0	18.5	23.2
Max. Temp. (°C)	44.5	44.8	40.3	36.9	19.7	32.2	25.9	20.9	21.2	25.8	35.2	35.6
Min. Temp. (°C)	15.5	18.3	19.0	14.7	19.7	3.1	0.8	1.7	3.4	6.8	6.7	11.9
Years	2018							2019				
Months	June	July	August	September	October	Nov.	Dec.	Jan.	Feb.	March	April	May
Precipitation (mm)	65.0	18.0	0.0	13.0	24.0	57.0	156.0	300.0	127.0	72.0	149.0	7.0
Mean Rel. Hum. (%)	72.8	65.8	71.2	65.1	67.3	72.5	78.0	85.1	80.1	76.7	75.6	71.9
Mean Temp. (°C)	25.5	28.5	28.0	25.9	20.4	15.7	11.5	9.6	11.4	13.4	15.8	21.3
Max. Temp. (°C)	38.0	43.3	40.8	40.7	35.5	31.5	21.6	17.6	20.6	27.4	27.6	36.3
Min. Temp. (°C)	16.3	18.2	17.2	15.2	7.2	7.2	0.0	0.8	3.6	2.5	5.6	9.9

The experimental layout was randomized blocks with three replications. The drip irrigation system was installed in the experimental field, and mulch was laid on the soil surface. Selected C-clones were planted in four rows with ten plants in each row on March 28, 2017. Between and inter-row spacing in planting were 70 and 40 cm, respectively. All cultural practices, such as fertilization, irrigation, weed control, spraying, harvesting, were carried out during the growing seasons. The images of the field studies are given in Fig. 1. The essential oil content and components were determined as three replications in the samples taken every month starting from June 2017 (three months after planting) for 24 months.

The essential oil ratio (%) was determined by the hydrodistillation method in the Clevenger apparatus. Distillation was carried out for three hours by adding 200 ml of distilled water to the 20 g sample, and the essential oil ratio (ml/100 g dry sample, %) was calculated (Anonymous 2011).

The component ratios of essential oils obtained from sage samples were determined using a GC-MS/FID (Gas chromatography (Agilent 7890A)-mass detector (Agilent 5975C)/flame ionization detector) device and capillary column (HP Innowax Capillary; 60.0 mx 0.25 mm x 0.25 µm). The samples were initially diluted with n-hexane at a ratio of 1:100. High purity helium was used as the carrier gas at a 0.8 ml/min flow rate. Samples were injected into the device as one µl with a split ratio of 40:1. Injection block temperature was set at 250°C, column temperature was at 60°C (10 minutes) and increased from 60°C to 220°C at four °C/minute and kept at 220°C for 10 minutes. The total analysis time was 60 minutes, in line with the temperature mentioned above schedule. Scanning range (m/z) of 35–450 atomic mass units and electron bombardment ionization of 70 eV was used for the mass detector. MS detector data was used to identify the essential oil components, and Wiley7n and Oil Adams libraries were used for this purpose. In addition, the C8-40 alkane series data and the component retention index data were also used to identify the components. The component ratios were determined using the FID detector. Images of laboratory studies are given in Fig. 2.

The following mixed model was used to statistically analyze the measured and observed component data recorded during the two years of the experiment.

$$y_{ijklm} = T_i + P_j + B_k + C_l + BC_{kl} + e_{ijklm}$$

In the model;

In y_{ijklm}; *i* represents a year, *j*, harvest time, *k* block, *l* clone, and *m* observation,

T_i is the randomized effect of year *i* (*i* = 1, 2),

P_j is the constant effect of j. harvest time (month) (j = 1, 2, … 12),

B_k is the effect of k. block (k = 1, 2, 3),

C_l = randomized effect of *l*. Clone (genotype) (l= 1, 2… 7),

BC_{kl} is interaction of block and clone (plot),

e_{ijklm} is the experimental error.

Firstly, the data's normal distribution was tested using Shapiro–Wilk test, and variables like *β*-caryophyllene, *β*-thujone, and Camphor had non-normal distributions. These variables were normalized by square root transformation. All the other variables had normal distributions. Student-Newman-Keuls (SNK) multiple comparison test, which has a higher sensitivity, was applied for the factors that differed indicated in the variance analysis. The SAS 9.0 statistical software was used in the analysis of the data (SAS Institute Inc. 2002).

Results And Discussion

Anatolian sage clones' mean essential oil content and main components of 1,8-cineol, camphor, *β*-pinene, *β*-caryophyllene and *β*-thujone were 3.09 50.21, 11.29, 8.92, 5.74 and 1.87, respectively (Table 3). The values were compatible with those reported in other studies (Skoula et al. 2000; Leontaritou et al. 2020; Karık and Sağlam, 2018).

Table 3
Essential oil ratio and mean, minimum, maximum values and standard deviations of essential oil main components

Parameter/Traits	Essential oil	1,8 Cineol	Camphor	*β*-Pinene	*β*-Caryophyllene	*β*-Thujone
Mean	3.09 ± 0.04	50.21 ± 0.3	11.29 ± 0.4	8.92 ± 0.1	5.74 ± 0.2	1.87 ± 0.06
Minimum	1.67	34.69	0.44	3.66	0.55	0.32
Maximum	6.00	67.85	33.29	18.40	15.68	8.18
Standard deviation	0.76	5.81	7.51	2.77	2.77	1.24
Coefficient of variation (%)	24.58	11.56	66.51	31.04	52.74	66.09

Table 4
Variance analysis for the traits

Traits	Source of Variation	Degrees of Freedom	Mean Squares	F Value	P Value
Essential oil	Clone	6	0.21	0.74	0.626
	Harvest Time	11	16.67	168.22	< .0001
	Clone*Harvest time	66	0.14	1.63	0.0030
	Year	1	16.54	166.89	< .0001
	Block	2	0.17	0.66	0.5331
	Clone*Block	12	0.28	2.83	0.001
	Error	392	0.10		
1,8-cineol	Clone	6	540.04	76.87	< .0001
	Harvest Time	11	430.97	28.74	< .0001
	Clone*Harvest time	66	27.74	2.23	< .0001
	Year	1	500.86	33.40	< .0001
	Block	2	16.59	2.20	0.1416
	Clone*Block	12	6.89	0.46	0.9372
	Error	392	15.00		
Camphor	Clone	6	2.70	28.07	< .0001
	Harvest Time	11	42.10	117.74	< .0001
	Clone*Harvest time	66	0.730930	2.59	< .0001
	Year	1	2.22	6.22	0.013
	Block	2	0.18	1.64	0.2176
	Clone*Block	12	0.90	0.26	0.9947
	Error	392	0.36		
β-pinene	Clone	6	157.54	130.05	< .0001
	Harvest Time	11	115.02	44.14	< .0001
	Clone*Harvest time	66	7.26	4.37	< .0001
	Year	1	20.08	7.71	0.0058
	Block	2	0.67	0.52	0.6061
	Clone*Block	12	1.19	0.46	0.9391
	Error	392	2.60		
β-caryophyllene	Clone	6	2.16	11.07	0.0003
	Harvest Time	11	10.24	103.93	< .0001
	Clone*Harvest time	66	0.13	1.37	0.0416
	Year	1	17.88	181.50	< .0001
	Block	2	0.10	2.00	0.6144
	Clone*Block	12	0.20	2.00	0.0234

Traits	Source of Variation	Degrees of Freedom	Mean Squares	F Value	P Value
	Error	392	0.10		
β-thujone	Clone	6	6.67	64.11	< .0001
	Harvest Time	11	0.55	10.08	< .0001
	Clone*Harvest time	66	0.10	2.23	< .0001
	Year	1	0.16	2.95	0.0864
	Block	2	0.09	0.95	0.413
	Clone*Block	12	0.10	1.93	0.0291
	Error	392	0.05		

The results of variance analysis are given in Table 4. The differences between clones, harvest times, clone harvest x time interaction and years (except β-thujone) were statistically significant, except essential oil content. The essential oil content and essential oil components of medicinal and aromatic plants may vary depending on ecological conditions, climate and soil characteristics, genotypic structure, part of the plant used and harvest time (Saharkhix et al. 2009). The significant difference ($P < 0.01$) in the traits (except essential oil) among the clones shows the importance of genetic structure (Table 4). The mean values of traits for the clones are given in Table 5. The difference between the lowest and the highest ratio in the β-pinene component was nearly two-fold, while the difference between these values was nearly 4-fold for β-thujone. The highest 1,8-cineol, one of the main components in essential oil, content (56.53%) was recorded in fd4-13, followed by fd2-9. The lowest β-thujone ratio (0.99%) was obtained from fk3-16, while the highest ratio (3.57%) was obtained from Bk4-9. The clones with the highest component in each trait varied, while the fd4-13 clone had the highest 1.8-cineol and β-pinene ratio. The results revealed that the clonal selection is successful, and the differences are still maintained in the clones obtained at the end of the selection.

Table 5
Mean (%), standard errors and overall mean of essential oil content, and essential oil main components of genotypes (no beta thujone)

Clone	Essential oil	1,8 Cineol	Camphor	β-Pinene	β-Caryophyllene	β-Thujone
fd4-13	3.00 ± 0.10	56.53 ± 0.96	8.28 ± 0.21	10.26 ± 0.31	4.20 ± 0.08	1.61 ± 0.03
fd2-9	3.09 ± 0.09	51.96 ± 0.67	8.71 ± 0.17	10.36 ± 0.33	4.57 ± 0.75	1.03 ± 0.02
fk3-16	3.08 ± 0.09	50.77 ± 0.78	8.99 ± 0.18	10.29 ± 0.35	4.84 ± 0.07	0.99 ± 0.02
fk4-14	3.16 ± 0.10	50.71 ± 0.73	9.66 ± 0.16	9.13 ± 0.36	5.25 ± 0.07	1.46 ± 0.03
standard	3.06 ± 0.09	49.15 ± 0.46	12.02 ± 0.11	7.29 ± 0.17	5.46 ± 0.09	1.81 ± 0.03
Fk4-9	3.15 ± 0.09	48.00 ± 0.40	9.94 ± 0.10	8.45 ± 0.20	6.14 ± 0.07	3.57 ± 0.05
fk5-7	2.97 ± 0.12	44.94 ± 0.54	11.42 ± 0.15	5.68 ± 0.21	7.25 ± 0.11	2.34 ± 0.03
Overall Mean	3.07	50.29	9.86	8.78	5.39	1.83

Many researchers carried out studies on several aspects of Anatolian sage (Bayram et al. 1999, Baydar et al. 1999, Skoula et al. 2000, Aydın et al. 2019, Dinçer et al. 2012, Karık and Sağlam 2017, Karık and Sağlam 2018, Leontaritou et al., 2020). The essential oil ratios and main components obtained from 6 clones in the current study are similar to the previous studies. However, previous studies were carried out using Anatolian sage produced from seeds, while this study was carried out using the clones. Clonal studies are important to show the superiority of clones to obtain sustainable products at desired quality and quantity compared to the individuals produced from seeds. In addition, the findings obtained from the clones also indicated that clones could be selected according to the desired component.

The essential oil ratio at the harvest times statistically differed for all of the clones (Table 4), and the mean essential oil ratios of the different clones are given in Table 6. The highest essential oil ratio (3.93%) was obtained in August, while the lowest oil ratio

(2.10%) was recorded in February. The highest percentage of β-thujone (2.12%) was obtained in October and the lowest (1.05%) was in March. The ratios of other components also significantly varied with the harvest time. The relationship between harvest time and essential oil content was associated with temperature and light intensity, and the decrease in temperature and light intensity caused a decrease in the essential oil ratio (Kargiolaki et al. 1994). This study showed that essential oil and camphor reached the highest values in August when light and temperatures were higher. The results are consistent with the findings of Çiçek et al (2011), who obtained the highest essential oil content (4.58%) in Salvia officinalis in August. Therefore, the plants should be harvested in August to produce essential oil and camphor.

Determining the highest essential oil ratio for medicinal plants is not sufficient; however, the variation of main components of essential oil with the harvest time also is of great importance. Therefore, the present study determined the effect of harvest time on essential oil components. The results indicated that the ratio of some components significantly differed with the harvest time.

Table 6
Monthly variation of essential oil contents and main components of essential oil

	Essential oil	**1,8-cineol**	**Camphor**	**β-pinene**	**β-caryophyllene**	**β-thujone**
January	2.36 h	51.22 c	5.31 f	10.18 b	7.95 a	1.89 ab
February	2.10 i	51.74 bc	4.02 f	10.60 b	**8.09 a**	1.68 ab
March	2.11 i	52.65 abc	2.44 g	**12.33 a**	7.88 a	1.05 c
April	2.44 h	53.80 ab	4.05 f	10.53 b	7.75 a	1.25 c
May	3.72 bc	**54.71 a**	10.37 d	7.68 d	5.15 c	1.68 ab
June	3.37 ef	52.69 abc	13.21 c	7.69 d	4.37 d	1.63 b
July	3.60 cd	48.48 d	18.55 ab	6.85 d	2.45 f	1.94 ab
August	**3.93 a**	44.05 e	**21.36 a**	6.90 d	2.46 f	1.81 ab
September	3.82 ab	44.78 e	19.68 ab	7.29 d	2.66 f	1.96 ab
October	3.48 de	46.86 d	17.55 b	7.68 d	3.65 f	**2.12 a**
November	3.30 f	51.06 c	8.64 e	9.26 c	6.28 b	1.89 ab
December	2.80 g	50.94 c	5.60 f	10.96 bc	8.20 a	1.80 ab

The lowest and highest ratios of the camphor are approximately nine times different (Table 6). The β-binene, one of the important main components, reached its highest level (12.33%) in March, while it decreased by 1.7 times in July and reached its lowest level. İn contrary, β-caryophyllene reached the highest level (8.20%) in December, whereas it decreased by 3.4 times in July to 2.45%. the 1,8-cineol ratio was the lowest (44.05%) in August, when the essential oil was high, on the contrary, camphor, which was stated to have a toxic effect by Narayan and Singh (2012), had the highest ratio (21.04%) in August. Similarly, Sarrou et al. (2016) found that essential oil was the highest (5.60%) in the summer, while the ratio of 1.8 cineoles was the lowest (44.70%). On the other hand, β-thujone, which is among the unwanted components in some sectors (Hold et al. 2000), was the lowest in March (Table 6). In addition, Hold et al. (2000) reported the highest values for camphor (14.93%) and β-thujone (2.79%) in summer. There are seasonal similarities between the findings of Hold et al (2000) and the current study, while the results are slightly different monthly. The differences may be attributed to rain or harvest or collection time temperatures. Similarly, the ratio of α-pinene and β-caryophyllene harvested in April and May in Greece was reported as 4.19 and 9.74%, respectively (Leontaritou et al. 2020). Sarrou et al. (2016) conducted a study between April and October and found the highest ratio of β-pinene (14.07%) and β-caryophyllene (7.17%) in April. The differences in findings suggest that the harvest time is an important factor. In addition to harvest time, Sarrou et al. (2016) stated that water is influences essential oil yield and components, while Kargiolaki et al. (1994) stated that temperature and light were effective. The content and composition of essential oil in the plants depend on the plant parts collected or harvested (Bellomaria et al., 1992), different development stages of the plant (Porres-Martínez et al. 2014, Kara, 2020), post-harvest drying temperature (Venskutonis, 1997, Aydın et al. 2019) and storage conditions (Dinçer et al. 2012).

The ratio of essential oil components for each clone significantly differed with the months (clone harvest time interaction) (Annex 1). The relevant tables are presented as an appendix since all tables will occupy too much space in the text. The highest 1,8-cineol ratio (63.80%) was detected in fd4-13 samples harvested in March, followed by the Fd2-9 clone harvested in April (58.52%). The lowest 1.8 cineol ratio (39.77%) was obtained in fk3-16 harvested in August. Although the 1,8-cineol ratio differed with the clones, the highest ratio was obtained in plants harvested in March, April, May, and June (Annex 2). While the lowest camphor rate (0.92%) was obtained from fk3-16 clone in March, the highest camphor rate was detected in the same clone in August (Annex 3). According to the months, the *β*-pinene content among the clones varied between 4.75% and 14.95%. In general, *β*-pinene was detected at a high rate in the spring months, while it was highest in January in the fd4-13 clone (Annex 4). According to the harvest time of the clones, the highest content of *β*-caryophyllene (12.18%) was determined in the fk5-7 clone in April (Annex 5). The results revealed that the component ratios of each cultivar and/or cultivar candidate developed may differ according to the harvest time. Different harvesting period for each of clones enables to obtained higher yields.

A correlation test was also carried out between the ratio and essential oil components. The highest negative correlation (-0.80) was recorded between camphor and *β*-binene, and followed by the correlation between essential oil and *β*-caryophyllene (-0.72). The *β*-pinene and *β*-caryophyllene, which are essential oil components, have various biomedical properties. Leite et al. (2007) demonstrated the antibacterial effect of α and *β*-pinene components. In addition, Astani and Schnitzler (2014) reported that *β*-pinene has an antiviral effect and reduces viral infectivity by 100%. Koyama et al. (2019) determined the flavoring and wound healing properties of *β*-caryophyllene. The findings showed that camphor increases while *β*-pinene decreases, and *β*-caryophyllene decreases while essential oil increases. The results revealed that sage clones could be determined according to the production of targeted components, and the essential oil yield and the ratio of components can be increased together.

Table 7
Correlation between essential oil ratio and essential oil main components

Trait	Essential oil	1,8-cineol	Camphor	*β*-pinene	*β*-caryophyllene	*β*-thujone
Essential oil	1					
1,8-cineol	-0.23	1				
	< .0001					
Camphor	0.69	-0.62	1			
	< .0001	< .0001				
β-pinene	-0.50	0.54	-0.80	1		
	< .0001	< .0001	< .0001			
β-caryophyllene	-0.72	0.06	-0.67	0.34	1	
	< .0001	0.193	< .0001	< .0001		
β-thujone	0.20	-0.29	0.26	-0.47	-0.02	1
	< .0001	< .0001	< .0001	< .0001	0.6801	

The components in the essential oil can be beneficial, while they can also have many toxic effects depending on the dose. Many studies have been carried out on the toxicology of the thujone component. Lachenmeier et al. (2006) stated that thujone acts on the central nervous system, while Olsen (2000) reported that thujone is toxic but has a stimulating effect on the brain instead of affecting the central nervous system. In another study, Nikolić et al. (2015) revealed that camphor has a mutagenic effect. In addition, Narayan and Singh (2012) reported that camphor may have a toxic effect, especially on young children, while Chen et al. (2013) reported that camphor could cause miscarriages. Therefore, production planning should be carried out considering the correlations determined in our study for the compounds as mentioned earlier.

The results revealed that the clones with the desired components could be selected among six by considering essential oil and components. In case of production for 1.8 cineol, the fd4-13 clone can be selected (Table 5), and this clone should be harvested in March to obtain the highest 1.8 cineol ratio (Annex 2). The negative correlation (-0.62) between 1.8 cineol and camphor in March harvested plants indicates that the camphor ratio will be low, in contrast, the positive correlation (0.54) between 1.8 cineol and β-pinene indicates that β-pinene will be at a moderate level (Table 7).

The essential oil component ratios of clones at the harvest time are taken into account for the selection of Anatolian sage clones, which are used for many purposes in different sectors. Selecting the clones only for their essential oil ratio at harvest or collection time may cause problems in terms of the presence of some toxic substances in the essential oil and also the desired components in the essential oil may be lower at harvest time. The difference in the content and components of essential oil between clones reveals the importance of the standard raw material for the standard product. The standard raw materials can be obtained by developing suitable varieties on a sectoral basis. The current study reveals different options in this respect. For example, Valussi et al. (2021) stated that a camphor ratio higher than 10–23% might cause mucosal irritation and gastrointestinal problems. The fd4-13 clone with low camphor content can be recommended for the cosmetic industry. In addition, for thujone, which is stated to have a toxic effect above a certain dose Zeybek and Zeybek (1994) and Hold et al. (2000), the fd2-9 and fk3-16 clones with trace amounts of thujone can be recommended for the tea industry. Major component 1,8 cineol has antitussive (Fischer and Dethlefsen, 2013), mucolytic (Juergens et al., 2020), anti-inflammatory (Juergens et al., 2003) and antimicrobial (Juergens et al., 2020) characteristics (Valussi et al. 2021). In this context, the fd4-13 clone can be recommended. The fd2-9 clone with high 1,8-cineol and low thujone has market demanded essential oil component ratios.

The results revealed that the component ratios vary according to the clones, the component contents of clones differ according to the harvest time, and there are significant correlations between the components. In addition, the clones were rich in some components and low in some toxic components. The correlations between components on a seasonal basis have been ignored in many breeding studies. The correlations between harvest time and essential oil components of clones offer the opportunity to evaluate different options for producers and consumers. Proper use of options by the producers will contribute to more sustainable production and consumption of sage components.

Declarations

Competing interests: The authors declare no competing interests.

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Figures

Figure 1

Images of the area where the study was carried out. a) rooted seedlings, b) seedlings ready for planting, c) 3 months after planting, d) 24 months after planting.

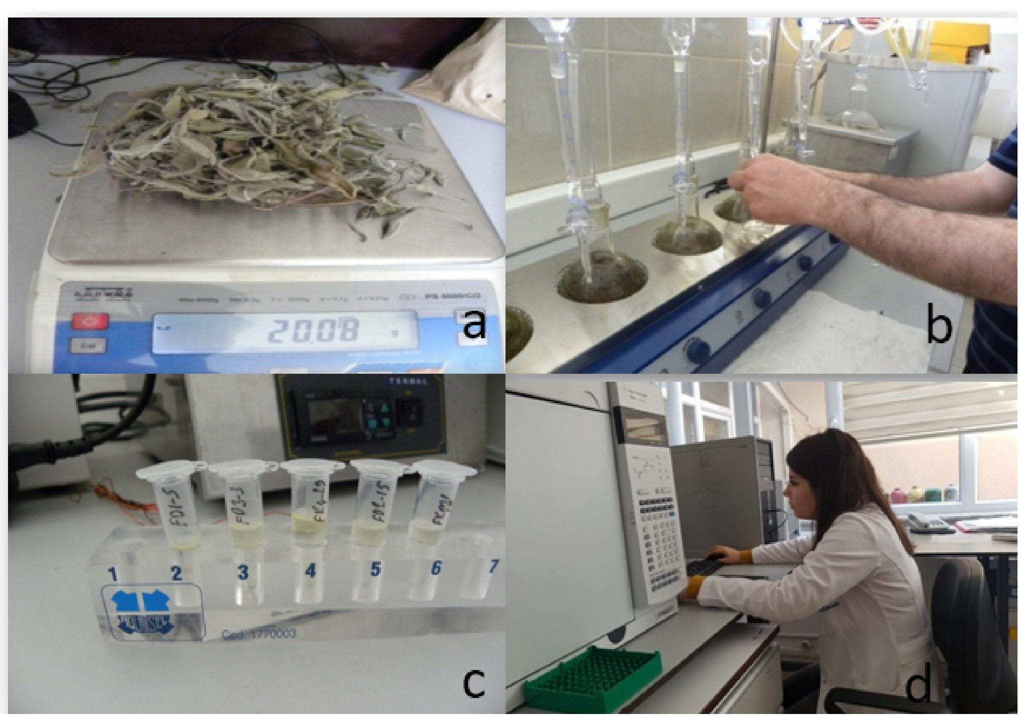

Figure 2

Images of laboratory studies. a) herbal sample, b) Clevenger apparatus, c) essential oils examples, d) GC-MS/FID (Gas chromatography).