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Classification and Identification of Wild Populations of *Salvia fructicosa* Mill. of Eastern Mediterranean of Turkey

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The morphological characterization and contents of essential oil in ten wild populations of *Salvia fructicosa* Mill. from Aydin Province in Turkey were studied in present studies. Essential oils content and composition of populations were determined. The essential oil content of 10 populations ranged from 1.99-3.04 % and mean value was 2.47 %. A cluster analysis was applied for essential oil content. Two groups were defined by cluster analysis. The correlation coefficients among characters indicated that drug herb ratio and drug leaf ratio are negatively correlated with the leaf morphological characters such as plant height and plant size. In all populations predominant component was 1,8-cineole.

Key Words: *Salvia fructicosa* Mill., Morphological characters, Essential oil, Cluster analysis, 1,8-Cineole.

INTRODUCTION

Salvia genus has been referred to as an herbaceous, suffructicosa or shrubby perennials, rarely biennial or annual, often strongly aromatic. The plant grows mainly in mild and hot climates. Approximately 900 species have been recorded widespread throughout the world. This genus is represented, in Turkish flora, by 88 species and 93 taxa, 45 of which are endemic[1]. In other words, Anotolia is a major center for the genus in Asia[2]. Some members of this genus are of economic importance, since they have been used as a flavouring agent in perfumery and cosmetics.

Salvia fructicosa Mill., restricted to Eastern Mediterranean, is one of the most commercially exploited sage plant as a food flavouring in Turkey. The collection of this species from wild population should be avoided; otherwise this species will be endangered in naturally growing area. Despite its economic importance, *S. fructicosa*, its genetic resources and variability and, its wild populations have not yet been fully explored. *S. fructicosa*, growing in Mediterranean region and having economical values, was listed among the aromatic and medicinal plants and was focused at the meeting organized by International Plant Genetic Research Institution (IPGRI) in Bari-Valenzo[3].

S. fructicosa (Turkish sage), formerly *Salvia triloba* L. is known with the vernacular names Anadolu adaçayi, Dagelmasi and Elma Çalpasi in Turkey. Turkish sage is traditionally used for cardiac symptoms, lung complaints, colds, coughs, nervousness and digestive problems[4-6]. The essential oil content of *S. fructicosa* ranges from 0.91 to 3.7 %[4,5]. The essential oil is dominated by 1,8-cineole (> 60 %)[7] and contains small amounts of thujone (5 %)[7,8] and camphor (3.6-13.6 %)[9,10]. Previous studies[11-13] indicated a high variability in quantity and composition essential oils depending on different populations. Environmental factors such as light, nutrient and season[14-16] effect on variation at quantity and contents of essential oils in the *Lamiaceae*. Skoula *et al.*[17] showed that differences in quantity and contents of essential oil in *S. fructicosa* Mill. resulted from genetic sources.

There are few documented references on position of *S. fructicosa* in Turkey[3,10,18]. This study will be contributed to primary knowledge of *S. fructicosa* populations from an area not previously explored and additionally, the morphological characterizations of collected populations from all locations in Aydin, except Aydin-Kusadasi-Yavansu, were not previously studied. Therefore, in this study morphological characterization and contents of essential oil in wild population of *S. fructicosa* and clustering at these populations from Aydin Province in Turkey are presented.

EXPERIMENTAL

The samples of *S. fructicosa* plant were collected from different locations in its native habitat in 2004. These locations are in the Aydin province of Aegean region in Turkey. Aydin province is located in the West of Turkey and has typical Mediterranean climatic conditions (latitude 37º44'-37º49' N and longitude 27º44'-27º50' E). The results of long-term observation showed that the monthly mean temperature and annual total rainfall in the meteorology station of Aydin is 17.5 ºC and 657.7 mm, respectively.

For each location, plants were sampled during the full flowering period, from the end of May to middle of June. Plant materials are identified according to Flora of Turkey and East Aegean Islands[2]. The population name, localities and altitudes are presented in Table-1. After measuring the plant height and plant size, the aboveground biomass of each individual plant was harvested. Then, plant material was weighed to calculate drug herb ratio and drug leaf ratio. The lower leaf and upper leaf were identified as low on the plant and near the main stem and top on the plant and outside the main stem, respectively. Before the drying process, lower leaf width, upper leaf width and lower leaf length, upper leaf length were measured on the most wide of leaf and distance from petiole to tip of leaf, respectively. Ten leaves were randomly selected for lower and upper leaf width and length. These leaves were weighed for lower and upper leaf weight. All samples were air-dried in the shade at room temperature for 10 d. Leaves were separated from stem. It was weighed to calculate drug leaf ratio and stored until analysis inside paper bags in the dark.

TABLE-1
LOCATION OF COLLECTED AREAS OF *S. fructicosa* IN AYDIN, TURKEY

Location	Altitude (m)
Soke-Tuzburgazi-Yavansu (A)	10
Soke-Eski Doganbey village (B)	30
Soke-Doganbey village-Karina toptepe (C)	0
Didim-Tasburnu (D)	0
Didim-Akbuk-Kirikici (E)	50
Kusadasi-Ilicabasi (F)	50
Kusadasi-Yavansu (G)	50
Kusadasi-Kalafat mountain (H)	60
Kusadasi-Kemeronu (I)	90
Kusadasi-Icmeler (J)	125

The essential oil was extracted from drug leaves by hydro-distillation for 3 h under continuous steam using Clevenger apparatus according to standard procedure described in European Pharmacopoeia[19] for determining the percentage of essential oil content.

The essential oil samples were analyzed using a Carlo Erba Fractovap series 2350 gas chromatograph equipped with a flame ionization detector at the Central Laboratory of the Aegean University. A glass column (3 m long, 3.18 mm internal diameter), packed with 3 % OV-1 50 chromosorb 80/100-mesh, was used. Carrier gas was N_2 at a flow rate of 25 mL/min. Each GC runs lasted for 20 min. Temperatures of injector, column and detector were 250, 110 and 250 ºC, respectively.

Peaks taken from GC were identified using the retention times obtained for reference standards of α-pinene, β-pinene, 1,8-cineole, thujone, camphor, borneol and terpineole. Relative content (%) of individual constituents of the oil was calculated proportionally on the basis of the peak area corresponding to each component.

Data on the subject of essential oil content were analyzed by using cluster analysis method (hierarchical clustering). The correlation coefficients between observed traits were estimated according to Pearson. The SPSS[20] (Version 9.0) was used for all analysis.

RESULTS AND DISCUSSION

The results of mean values and standard deviations concerning the morphological characters and essential oil contents are presented in Table-2. The mean values were determined 1.71 cm for lower leaf width, 3.80 cm for lower leaf length, 0.83 cm upper leaf width, 2.28 cm upper leaf length, 0.69 for upper leaf weight and 1.20 g for lower leaf weight. The standard deviations of these characters ranged from 0.188 to 0.684. The lower standard deviations indicated that these characters were non-variable and it may be explained that the heritability degrees of mentioned characters are higher than that of others (Table-2). The plant height and plant size had 76.94 and 89.54 cm average values, respectively. The standard deviations of both characters were very high (27.751 and 57.715, respectively). Especially, the

economic characters such as drug herb ratio and drug leaf ratio had 46.51 and 27.58 % average values. It was seen that the standard deviations of these characters were moderate level (8.694 and 4.667, respectively).

TABLE-2
MEAN VALUES OF INVESTIGATED CHARACTERS IN 10 POPULATIONS

Locations	LLWi (cm)	LLL (cm)	ULWi (cm)	ULL (cm)	ULW (g)
A	1.97±0.09	3.74±0.16	**1.12±0.08**	2.54±0.12	**0.93±0.08**
B	**2.04±0.13**	4.22±0.27	1.04±0.06	**2.65±0.03**	0.91±0.14
C	2.00±0.09	4.30±0.12	0.82±0.03	2.50±0.09	0.70±0.04
D	1.94±0.08	**4.34±0.19**	0.77±0.04	2.23±0.09	0.70±0.04
E	1.54±0.09	4.05±0.16	0.74±0.03	2.40±0.08	0.72±0.03
F	**1.32±0.08**	**3.04±0.12**	0.75±0.03	**1.84±0.09**	**0.42±0.02**
G	1.53±0.06	3.32±0.13	0.78±0.04	2.00±0.03	0.65±0.03
H	1.36±0.15	3.47±0.18	**0.71±0.04**	2.21±0.05	0.64±0.04
I	1.69±0.06	3.52±0.16	0.79±0.03	2.21±0.08	0.62±0.02
J	1.68±0.10	3.99±0.22	0.82±0.04	2.23±0.05	0.62±0.06
Mean	1.71	3.80	0.83	2.28	0.69
SD	0.387	0.684	0.188	0.330	0.227
Locations	LLW (g)	PH (cm)	PS (cm)	DHR (%)	DLR (%)
A	**1.60±0.18**	**48.90±4.41**	65.50±6.14	**38.14±1.82**	24.80±0.88
B	1.53±0.16	90.90±9.93	98.40±15.60	39.21±0.92	24.99±1.08
C	**1.60±0.10**	**100.00±6.95**	**161.40±24.80**	38.93±0.86	**23.82±0.66**
D	1.58±0.13	91.60±6.10	105.10±8.12	42.78±3.69	25.61±0.44
E	1.20±0.09	89.50±7.69	83.00±15.60	43.10±1.12	26.68±0.83
F	0.79±0.10	51.00±4.99	**51.50±6.10**	54.82±2.36	29.09±2.05
G	0.93±0.09	58.50±6.28	104.50±20.30	52.55±1.33	**32.93±1.66**
H	**0.77±0.05**	77.50±9.52	82.00±18.40	**56.60±1.37**	30.15±1.61
I	1.00±0.07	88.00±5.97	65.00±6.58	51.71±0.92	29.42±1.12
J	0.99±0.10	73.50±7.53	79.00±8.23	47.26±2.06	28.31±1.26
Mean	1.20	77.94	89.54	46.51	27.58
SD	0.477	27.751	52.715	8.694	4.667

In bold = Maximum and minimum measurements.
LLWi = Lower leaf width, LLL = Lower leaf length, ULWi = Upper leaf width, ULL = Upper leaf length, ULW = Upper leaf weight, LLW = Lower leaf weight, PH = Plant height, PS = Plant size, DHR = Drug herb ratio, DLR = Drug leaf ratio.

The lower leaf width varied between 1.32 (location-F) and 2.04 cm (location-B). The observations of collection from location-A showed that this location had lower plant height and drug herb ratio, but higher upper leaf width, upper leaf weight and lower leaf weight than that of the other. The lower leaf width, lower leaf length, upper leaf length, upper leaf weight and plant size were obtained as the least values whereas essential oil content (Fig. 1) in the plants of location-F were measured the highest values (3.04 %).

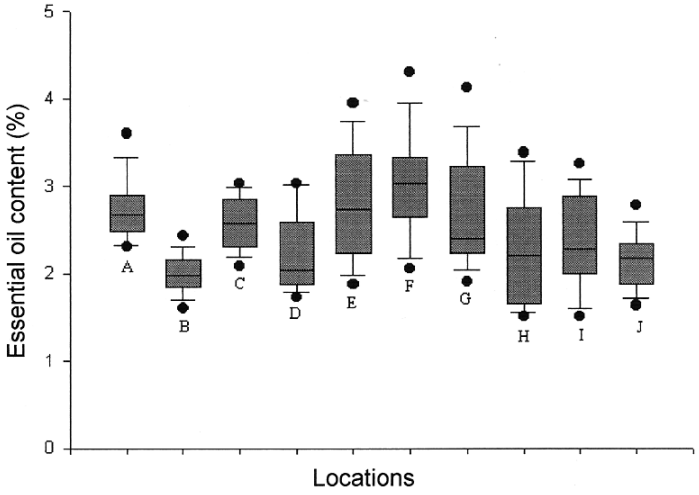

Fig. 1. Essential oil contents of *S. fructicosa* at different locations

The correlations among observed characters are given in Table-3. Especially, when the correlations among lower leaf width, lower leaf length, upper leaf width, upper leaf length, upper leaf weight, upper leaf length, upper leaf weight, lower leaf weight, plant height and plant size were examined, it was showed that these correlations were mostly positive and significant. These results indicated that these morphological characters were triggered each other. Drug herb ratio and drug leaf ratio negatively correlated with plant height and plant size (Table-3). However, the correlation between drug herb ratio and drug leaf ratio was positive and significant. With regards to Essential oil content, it must be said that essential oil content was only positive and significant correlated to upper leaf length.

TABLE-3
CORRELATION COEFFICIENT BETWEEN OBSERVED CHARACTERS

Characters	LLL	ULWi	ULL	ULW	LLW	PH	PS	DHR	DLR	EOC
LLWi	0.723‡	0.577‡	0.568‡	0.450‡	0.817‡	0.390‡	0.396‡	-0.585‡	-0.295‡	-0.142
LLL		0.305‡	0.572‡	0.303‡	0.727‡	0.535‡	0.438‡	-0.554‡	-0.350‡	-0.135
ULWi			0.555‡	0.624‡	0.476‡	-0.021	0.036	-0.395‡	-0.301‡	-0.072
ULL				0.659‡	0.624‡	0.265‡	0.260‡	-0.534‡	-0.349‡	-0.236†
ULW					0.438‡	0.031	0.038	-0.412‡	-0.194	-0.105
LLW						0.273‡	0.345‡	-0.555‡	-0.361‡	-0.132
PH							0.605‡	-0.240†	-0.146	-0.140
PS								-0.273‡	-0.112	-0.079
DHR									0.457‡	0.052
DLR										0.145

†0.05 and ‡0.01 significant probability level, respectively.
LLWi = Lower leaf width, LLL = Lower leaf length, ULWi = Upper leaf width, ULL = Upper leaf length, ULW = Upper leaf weight, LLW = Lower leaf weight, PH = Plant height, PS = Plant size, DHR = Drug herb ratio, DLR = Drug leaf ratio, EOC = Essential oil content.

The mean values concerning observed characters and the correlation coefficients indicated that leaf characters, plant height and plant size with low values, positively affected the essential oil content.

The essential oil content of the 10 locations ranged from 1.99-3.04 % and mean value was 2.47 %. Stahl and Schild[21] and DAB 9[8] stated that at least essential oil content of *Salvia fructicosa* Mill. must be 1.8 %. According to previous researchers, essential oil content varied between 0.80 and 2.60 % in Turkey[10,22]. Bayram[3] found 1.00-4.25 % essential oil content for Aydin-Kusadasi location.

The data of essential oil content concerning locations were used to compute a dendrogram showing Euclidian distance in order to define relationships among the locations (Fig. 2). Two groups (cluster I and II) were defined by cluster analysis. This evidenced moderately differences within 10 locations due to the low standard deviation of essential oil content (0.627). The locations A and E were the closest for essential oil content. These locations are characterized by long and heavy leaf, low drug herb ratio and drug leaf ratio. The location-G, which can be found close to A-E cluster of Fig. 2, showed comparable morphological characteristics, because of narrow and short leaves, lower leaf weights and short plant height, but larger plant size. Moreover the location-G had higher drug herb ratio value and the highest drug leaf ratio within the locations. In the dendrogram, the locations H and F, which are characterized by narrow-long and light leaf, have moderate plant height and plant size and higher drug herb ratio and drug leaf ratio closed to the A-E-G cluster.

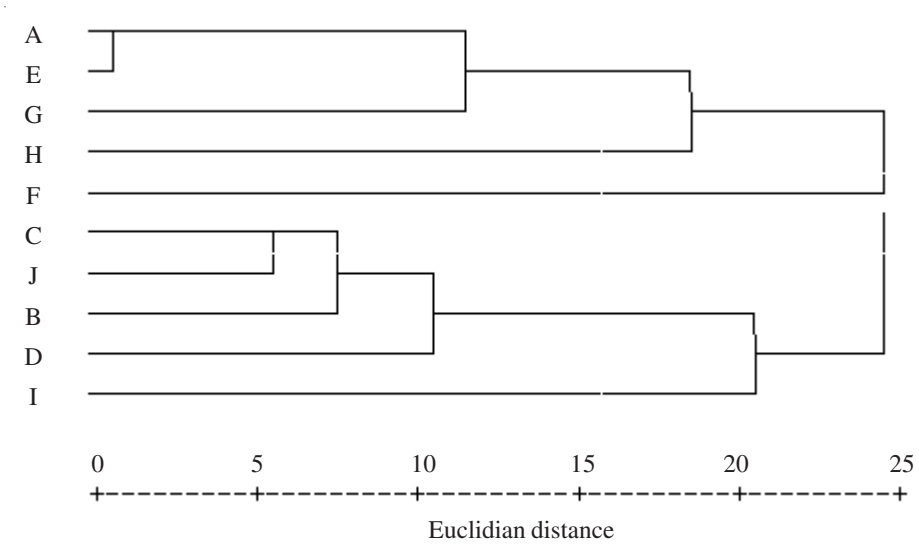

Fig. 2. Classification of locations of different *S. fructicosa* based on studied cluster analysis of variation essential oil content

The locations C and J cluster in the same branch of the dendrogram. The populations B, D and I, respectively neighbored to the locations C-J cluster. The cluster II locations are generally characterized by large-long and moderately heavy, higher plant height and plant size and moderate drug herb ratio, but low drug leaf ratio.

The chemical composition of the essential oil in *S. fructicosa* from 10 locations was determined by GC (Fig. 3). The essential oil compositions were examined for 10 plants from each location. There was considerable variation between locations with respect to the essential oil composition. Major components were identified 1,8-cineole. This component was followed by camphor, β-pinene, α-pinene, thujone, borneol and α-terpineol, respectively. These seven components represent over 90 % of the total essential oil content.

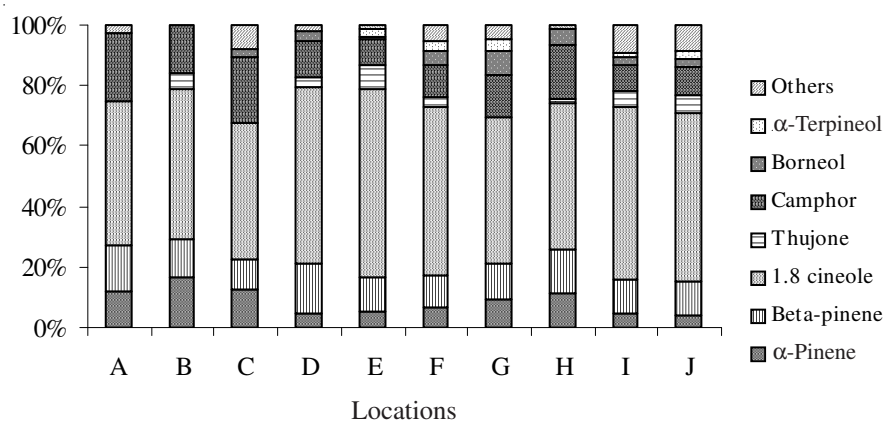

Fig. 3. Essential oil composition of 10 locations collected from Aydin, Turkey

1,8-Cineole was the predominant component in samples of all locations. 1,8-Cineole content in locations varied from 44.5-62.3 %. In location-E, the highest 1,8-cineole content was obtained. Camphor content, second component, was 8.0-23.1 %. In location-A, the highest camphor content was obtained. Previous researchers reported that 1,8-cineole content was varied between 4.0 and 80.8 % and camphor content was varied between 2.5 and 44.5 %[7,9,10,18,23]. Similar to our results, Gabriel[22] and Bayram[3] reported 1,8-cineole content between 15.96 and 74.03 % and camphor content between 3.19-49.10 % for clones in *S. fructicosa* from West Anatolia. Wichtl[7] and DAB 9[8] indicated 5 % thujon content. However, in samples of some locations were determined above 5 % and the highest content was obtained 8.18 %. Similar results are reported by previous researchers[3,10,18,22].

Conclusion

Ten populations of *S. fructicosa* collected from the wild were shown to exhibit some differences for essential oil content and morphological characters[24]. Concerning the morphological variability, relationships among characters and essential oil content

in *S. fructicosa*, there is no published data exists especially for Aydin Province of Turkey. As far as the mean values and correlation coefficients between characters, the essential oil content was positively affected by smaller leaf size, plant height and plant size[24].

The dendogram results from cluster analysis clustered locations into two main groups. The cluster I with higher essential oil content was defined as narrow and short leaves, with lower drug herb ratio and higher drug leaf ratio. The characters such as wide-long leaves, moderate drug herb ratio and low drug leaf ratio placed in cluster II with lower essential oil content.

In the plants containing essential oil, chemical composition is generally important. There is a wide range of variation in essential oil composition. Although essential oil content among the locations showed significant differences, chemical composition of the samples of different locations had similar values.

This primary classification showed that variability of the essential oil content of *S. fructicosa* in different locations might be related to mainly genetic diversity. If the habitat of collected locations considered, it was concluded that environmental factors such as light, nutrition and temperature had effects on the essential oil content.

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