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Research Article

The Classification of the *Salvia* L. (*Labiatae*) Species Distributed in West Anatolia According to Phenolic Compounds

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Abstract: The seven species of *Salvia* L. growing naturally in West Anatolia (*Salvia tomentosa* Mill., *Salvia fruticosa* Mill., *Salvia smyrnaea* Boiss., *Salvia argentea* L., *Salvia horminum* L., *Salvia verbenaca* L., and *Salvia virgata* Jacq.) and a cultivated form (*Salvia officinalis* L.) were selected as the study materials. The phenolic compounds extracted from the leaves of the species were separated by two- dimensional thin-layer chromatography. On the basis of the distribution of phenolic spots in the species, the matching coefficients and the coefficient of similarity were calculated in order to determine the relationship between the species. As a result of the group analysis in consideration of those coefficients, the species were categorized into two groups.

Key Words: *Salvia*, *Labiatae*, Phenol, Chemotaxonomy, Turkey.

Batı Anadolu'da Yayılış Gösteren *Salvia* L. (*Labiatae*) Türlerinin Fenolik Bileşiklerine Göre Sınıflandırılması

Özet: Araştırma materyali olarak Batı Anadolu'da doğal olarak yetişen yedi *Salvia* L. türü (*Salvia. tomentosa* Mill., *Salvia fruticosa* Mill., *Salvia smyrnaea* Boiss., *Salvia argentea* L., *Salvia horminum* L., *Salvia verbenaca* L., *Salvia virgata* Jacq.) ve bir kültür formu (*Salvia officinalis* L.) seçildi. Türlerin yapraklarından özütlenen fenolik bileşikler iki yönlü ince-tabaka kromatografisi yöntemi ile ayrıldı. Türlerdeki fenolik lekelerin dağılımı esas alınarak, türler arasındaki akrabalık ilişkilerini ortaya koymak üzere uygunluk ve benzerlik katsayıları hesaplandı. Bu katsayılar dikkate alınarak yapılan küme analizleri sonucunda türler iki kümeye ayrıldı.

Anahtar Sözcükler: *Salvia*, *Labiatae*, Fenol, Kemotaksonomi,Türkiye.

Introduction

The family *Labiatae* includes 200 genera and 3000 species. Since most of them are important in medical and economic terms, the members of the family need to be revised in terms of their systematic positions. Therefore, some new revisions and studies have been carried out to elucidate the morphological, anatomical and chemical characters of the family members. According to Metcalfe and Chalk (1950), the structure of the conductive bundles in the cross-section of the leaf petioles of the *Labiatae* members may be useful taxonomically. Nakiboğlu and Oğuz (1990) reported that the numbers of conductive bundles in the cross-sectioned petioles of the *Salvia* L. species varied among the species.

Chemotaxonomical studies date back to the works of De Candolle (Hegnauer, 1967). Erdtman (1956), studying the phenolics from *Pinus* L. species, used them in determining the taxonomy of the species and showed that they supported the data from the conventional taxonomy. Nakiboğlu (1988) classified the species of the genus *Vicia* L. on the basis of the phenolic compounds in their leaves. Haque (1981) stated that in the species of the genus *Salvia* with high hybridization rate it is worth studying the chemical features as well as other characteristics such as morphology and anatomy. Nakiboğlu (1992) studied the morphological properties of the *Salvia* species. Alston and Turner (1963) determined the relationship between the hybrid species of the genus *Baptisia* Vent. by examining the phenolics in their leaves. The chemical substances that are used in plant classification can be recognized as alkaloids, phenolics, glucosinolates, terpenoids, oils and waxes, and carbohydrates. Phenolic compounds are of great use in chemotaxonomic research. The taxonomically most important phenolics are the flavonoids, which have a relatively common nucleus with a great variety of types, and patterns of side-groups characterise the individual compounds. There is usually a considerable diversity of

flavonoids in any one species; some of these are widespread, others very rare, and the pattern and combination of occurrences have on many occasions proved valuable as taxonomic evidence in the flowering plants at all levels from order downwards. The following characteristics show that they are appropriate compounds for taxonomic studies: they vary from simple to complex types, they can be isolated easily from the plant, they are not affected by environmental conditions and they remain more stable (Bate-Smith, 1962; Mabry et al., 1970).

Phenolic substances were used by Erdtman (1956), Bate-Smith (1958), Alston and Turner (1963), Mabry et al. (1970), Barberan et al. (1988, 1990), Thomas-Lorante et al. (1989), Voirin et al. (1994), Nakiboğlu (1988, 1995), and Apaydın and Bilgener (2000) to solve taxonomical problems. The aim of this research was to show whether there is any correlation between the classical taxonomy and chemical taxonomy. If there is strong correlation between the classical taxonomy and thin layer chromatographic evidence, this question can be considered to be answered positively.

Materials and Methods

The seven species of *Salvia* growing in West Anatolia and a cultivated form were selected as the study materials (Table 1). Taxonomical description of the species has been made according to Davis (1982). Voucher specimens are deposited in the Herbarium of the Faculty of Sciences of Ege University, in Izmir, Turkey (Acronym:EGE).

A. Chemical Studies

1. Extraction and Thin-Layer Chromatography

Young leaves were dried at a temperature of 25°C. The leaves (0.5 g) were crushed and extracted with 2 ml methanol containing 0.3% hydrochloric acid. This extract was filtered and refrigerated before chromatography (Nybom, 1964; Bose and Fröst, 1967). Two-dimensional thin-layer chromatography methods were employed in chemical studies. For this purpose, prepared plastic cellulose plates (Sigma Cell Type 100 Cellulose on Polyester, 20x20 cm) were used as an adsorbent. The plates were run in the first direction in a solvent system consisting of butanol, acetic acid and water (BAW 3:1:1). Then they were run in the second direction in 15% acetic acid in water (Harborne, 1973).

2. Differentiation of the Phenolic Spots

In order to distinguish phenolic spots in the chromatography from the other spots, the polarities of the activated benzene ring and of the OH group bound to the aromatic ring were considered and the ability of this benzene ring to react with various reagents to form the differentiating colours and the quality of this ring to absorb in ultraviolet spectrum area were used, respectively (Harborne, 1975). The spots were not

Table 1. The localities of plant species.

Plant Species	Locality and Collector	Herbarium No
Salvia fruticosa Mill.	B2 İzmir: Ödemiş, Bozdag, 1250 m, Nakiboğlu 1988	EGE 31941
Salvia tomentosa Mill.	B1 İzmir: Kemalpaşa, Mount Mahmut 750 m, Nakiboğlu, 1987.	EGE 31949
Salvia smyrneae Boiss.	B1 İzmir: Kemalpaşa, top of Mount Nif, 1350 m, Nakiboğlu,1987.	EGE 31945
Salvia horminum L.	B1 İzmir: Gaziemir, rocky place, 250 m, Nakiboğlu, 1987.	EGE 31948
Salvia virgata Jacq.	B1 İzmir: Kemalpaşa, roadside, 275 m, Nakiboğlu,1988.	EGE 31942
Salvia verbenaca L.	B1 İzmir: Buca, roadside, 25 m, Nakiboğlu, 1987.	EGE 31947
Salvia argentea L.	B1 İzmir: Mount Yamanlar 700 m, Nakiboğlu 1987.	EGE 31946
	B1 İzmir: Ödemiş, Bozdag, Kılıç plateau, 1100 m, Nakiboğlu, 1989.	EGE 31943
Salvia officinalis L.	Cultivated form	

identified chemically; but even without this information, the spots themselves have value as specific characters in systematic classification (Harney and Grant, 1964).

The four chromatograms were prepared for each species sample in order to separate the spots in the chromatograms. The first was examined under ultraviolet light (256-236 nm) and NH_3 vapour and observable spots were outlined with a pencil. $FeCl_3$ with $K_3Fe\ (CN)_6$ mixture (1:1) and Na_2CO_3 and $AlCl_3$ solutions were used as reagents (Mabry et al., 1970). Spots were separated with respect to Rf values and spot colours. The Rf values of spots established under ultraviolet light were counted and the spots that gave the same colour reaction to the reagents as specific Rf values were marked with the same number, and the spots that gave different colour reactions were marked with different numbers. The number of spots in the chromatograms and their colours obtained under ultraviolet light were marked with the related numbers and the colour signs respectively on every spot. The spots on the chromatogram belonging to each species were transferred to tracing paper without undergoing any change. All the spots occurring in the material were given consecutive numbers, and a "master key" containing most of the spots found in the eight collections was constructed.

B) Numerical Analyses

The total number of spots and their distribution in the species are shown in Table 2. Considering the distribution of the spots in the species, the coefficient of similarity (Cs) and the matching coefficient (Cm) were calculated with the following formulas:

$Cs= p / (p+d)$

$Cm=(p+n) / N$

Cs: The coefficient of similarity

Cm: The matching coefficient

p: The number of common spots in both species

d: The number of spots present only in one species

n: The number of spots absent in the two compared species

N: Total numbers of spots considered in these species

Using the coefficient of similarity and the matching coefficient obtained in these formulas, the dendrograms of the species were drawn and the degrees of their relativity established (Sokal and Sneath, 1963; Runemark, 1968).

Results

A total of 24 spots were obtained in the species. The spots on the chromatogram belonging to each species were transferred to tracing paper without undergoing any changes. The spots are numbered from 1 to 24 and the colours shown as BF=Bright fluorescent, PF=Pale flourescent, BV=Blue violet, P=Pink, PB=Pale blue. A "master key" combining most of the spots is presented in Figure 1. All 24 spots and their distribution in the species are listed in Table 2. In this table, the presence of the spots in the species is represented by (•) and the absence of the spot by (o) marks.

The presence or absence of spot numbers was used in the calculation of the matching coefficient (Cm) and coefficient of similarity (Cs). The matching coefficient and coefficient of similarity values are shown in Table 3.

The dendrograms of the species were drawn and the degrees of their relativity established according to the coefficient of similarity and matching coefficient values (Figure 2).

The systematic position of the *Salvia* species was studied chemotaxonomically according to the presence or absence of the phenolic spots, which were rearranged on the basis chemical findings as a result of the numerical analyses. The plant specimens belonging to the same species formed the same spots although they were collected from different areas. For example *S. argentea* specimens collected from different places did not show a clear chemical variation (Table 1). The species were categorized into two groups. These groups are shown as follows:

Group A. *S. fruticosa* Mill, *S. tomentosa* Mill, *S. smyrnaea Boiss.*, *S. officinalis* L. Group B. *S. argentea* L., *S. horminum* L., *S. verbenaca* L., *S. virgata* Jacq.

Discussion

The *Salvia* species in Turkey have been divided taxonomically into three groups according to stamen type: A, B and C (Davis, 1982). In this research, the plant specimens were categorized into the A and B groups according to their phenolic substances. These groups are the same as Davis' classification.

Ten spots (nos.1, 2, 3, 4, 5, 8, 10, 11, 12, 13) are found on the chromatograms from *S. fruticosa* (Table 2). Nine of these are observed in common with *S. officinalis.*

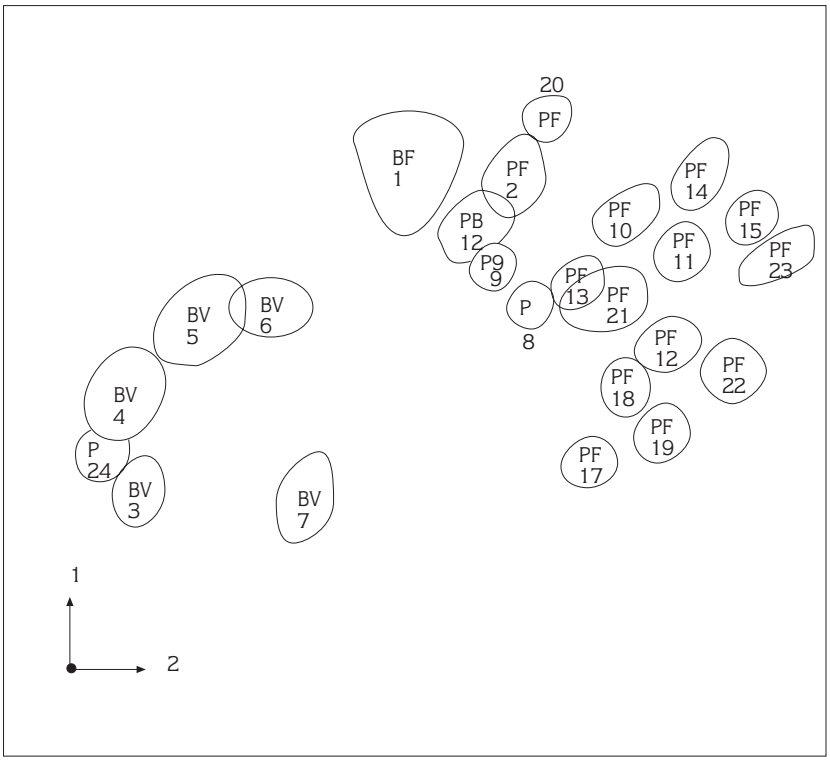

Figure 1. The "master key" of the phenolic compounds in the species. BF=Bright fluorescent, PF=Pale fluorescent, BV=Blue-violet, P=Pink, PB=Pale blue

Table 2. All 24 spots and their distribution in the species (F : fruticosa, O: officinalis, T: tomentosa, S: smyrnaea, H: horminum, A: argentea, Vr: virgata; Ve: verbenaca)

Species/Spot No	F	O	T	S	H	A	Vr	Ve
1	•	•	•	•	•	•	•	•
2	•	•	•	•	•	•	o	•
3	•	•	•	o	o	o	o	o
4	•	•	•	•	•	•	o	o
5	•	•	•	o	•	•	o	o
6	o	•	o	•	•	o	o	o
7	o	•	o	•	o	o	o	o
8	•	•	•	•	o	•	o	o
9	o	o	o	o	o	•	o	o
10	•	•	o	o	•	o	o	•
11	•	•	o	•	o	o	o	o
12	•	•	o	o	•	o	o	o
13	•	o	o	•	o	o	o	o
14	o	o	o	•	•	o	o	o
15	o	o	o	o	o	o	o	o
16	o	o	o	o	o	o	o	o
17	o	o	o	o	o	•	o	o
18	o	o	o	o	o	•	o	o
19	o	o	o	o	o	•	o	o
20	o	o	o	•	o	o	o	o
21	o	o	o	o	•	o	•	•
22	o	o	o	o	o	o	•	•
23	o	o	o	o	•	o	•	•
24	o	o	o	o	•	o	o	o

Table 3. The coefficient of similarity (A), and the matching coefficient (B) values.
F: fruticosa, O: officinalis , T: tomentosa, S: smyrnaea, H: horminum, A: argentea, Vr: virgata, Ve: verbenaca.

A							
	O	T	S	H	A	Vr	Ve
F	0.64	0.53	0.42	0.40	0.35	0.07	0.23
O		0.58	0.50	0.42	0.33	0.07	0.21
T			0.28	0.38	0.41	0.10	0.16
S				0.31	0.28	0.07	0.14
H					0.25	0.68	0.41
A						0.08	0.15
Vr							0.66

B							
	O	T	S	H	A	Vr	Ve
F	0.87	0.83	0.66	0.66	0.62	0.54	0.58
O		0.79	0.70	0.58	0.58	0.45	0.50
T			0.54	0.62	0.70	0.58	0.58
S				0.54	0.54	0.50	0.50
H					0.50	0.62	0.70
A						0.54	0.50
Vr							0.87

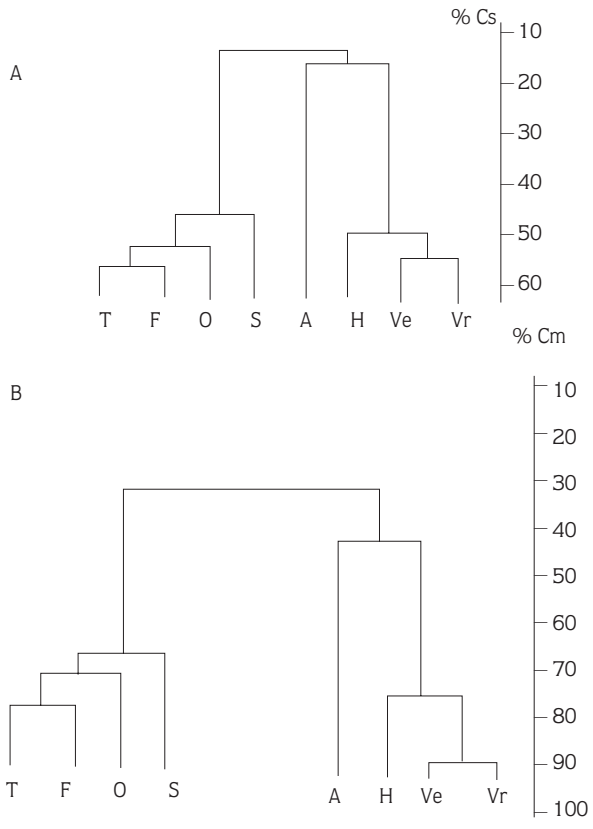

Figure 2. Dendrograms constructed from the different coefficients (A. From the coefficient of similarity, B. From the matching coefficient).
F: fruticosa, O: officinalis, T: tomentosa, S: smyrnaea, H: horminum, A: argentea, Vr: virgata, Ve: verbenaca.

Six of these are observed in common with *S. tomentosa* and *S. smyrnaea*. According to Davis, these species are members of group A. Phenolic spots (nos. 21, 22 and 23) belonging to *S. virgata* and *S. verbenaca* are seen to differ from those of *S. tomentosa, S. fruticosa, S. officinalis* and *S. smyrnaea*. According to Davis, *S. virgata* and *S. verbenaca* species are members of group B. Some phenolic spots (nos. 9, 17, 18 and 19) are found only in *S. argentea*. One phenolic spot (no. 20) is observed only in *S. smyrnaea.* Another spot (no. 24) is observed only in *S. horminum*. These spots can be characteristic spots of the *S. argentea, S. smyrnaea* and *S. horminum* species (Table 2). Cm and Cs values belonging to the species show clearly the degrees of these species' relativity (Table 3). For example, Cm (0.87), Cs (0.66) values were determined in *S. verbenaca* and *S. virgata*, and Cm (0.87), Cs (0.64) values in *S. fruticosa* and *S. officinalis*.

A good agreement between classical taxonomy and chromatographical data was found for the phenolic spots when the coefficient of similarity, and the matching coefficient values were correlated with classical taxonomy.

In conclusion, chemotaxonomy can be suggested as a useful tool in the taxonomy of *Salvia* species. However, it is necessary to study all the species considering all their properties in order to obtain a definitive result in this matter. We can add that such studies should be performed at the population level as well. In this study a correlation was apparent in studies at population level.

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