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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Study of Some *Salvia L.* (Lamiaceae) Species Native to West Azarbaijan (Iran) Considering Their Phenolic Compounds

Fatemeh Nejad Habibvash[1], Mohammad Ali Rajamand[1], Reza Heidari[1],
Siavash Hosseini Sarghein[1] and Mahnaz Heidari Ricani[2]
[1]Department of Biology, Faculty of Science, Urmia University, Urmia, West Azarbaijan, Iran
[2]Agricultural Research Center of West Azarbaijan, Herbarium, Iran

Abstract: Nine species of *Salvia* L. growing naturally in West Azarbaijan in Iran were selected as the study materials. The phenolic compounds extracted from the leaves of these species, were separated by one-dimensional chromatography and total content of phenolic compounds was measured. In order to determine the relations among the species according to the phenolic spots distribution, matching coefficient and similarity coefficient were calculated and based upon these coefficients, species were categorized as two groups. Also it was found that total content of phenolic compounds is an important factor in the distinction of these species.

Key words: *Salvia*, phenolic compounds, Lamiaceae, Iran

Introduction

The genus *Salvia* L. has c.900 species throughout the world. *Salvia* species are an important group of useful plants. Some of them are well known for their medicinal, aromatic and antioxidant properties (Malenčić *et al.*, 2000). In addition *Salvia* species are grown in parks and gardens as ornamental plants (Nakiboğlu, 1993). Since most of the members of the Lamiaceae family are important in medical and economic terms, they need to be reviewed in terms of their systematic positions therefore some new studies have been carried out to determine the morphological, anatomical and chemical characters of this family. Haque (1981) stated that in the species of the genus *Salvia* with high hybridization rate, the chemical features as well as other characteristics such as morphological and anatomical studies are useful. Alston and Turner (1963) determined the relationship between the hybrid species of the genus *Baptisia* Vent. by examining the phenolics in their leaves. To solve taxonomical problems, phenolic substances were used by Erdtman (1956); Bate-Smith (1958); Alston and Turner (1963), Mabry *et al.* (1970), Barberan and and Wollenweber (1990), Thomas-Lorante *et al.* (1989), Voirin *et al.* (1994), Nakiboğlu (1995) and Apaydin and Bilgener (2000).

The most popular species of the genus *Salvia*, sage (*Salvia officinalis* L.) is a well-known medicinal plant. The sage acts as an antiphlogistic, stomachic, antiseptic, antiasthematic, astringent drug and used as spice (Chiej, 1988). However, the majority of wild-growing *Salvia* species have not been fully evaluated from their phytochemical point.

The aim of this study was to illustrate similarity among species of *Salvia* L. in West Azarbaijan also to provide evidence for efficiency of application of these data at species level of the genus. There are almost no reports about phenolic compounds of the species found in Iran.

Materials and Methods

We initiated the biochemical studies at the Laboratory of Biochemistry in the Science Faculty of Urmia University in West Azarbaijan in Iran, using living materials which were collected from their natural habitats during Jun and of Jul 2006.

Thin layer chromatography and Folin-Ciocalteu methods were employed in chemical studies.

For TLC purpose, Young leaves were dried at a temperature of 25°C. The leaves (0.5g) were crushed and extracted with methanol containing 0.3% hydrochloric acid. The extract was filtered and refrigerated before chromatography (Bose and FrÖst, 1967). For this purpose glass cellulose plates (Avicel, Merck: 2330, 20×20 cm) were used as an adsorbent. The plates were run in a solvent system containing butanol, acetic acid and water (BAW 3:1:1) (Harborne, 1973). Chromatographic plates were sprayed with NH3 vapour and examined under ultraviolet light [254nm-366nm]. Spots were separated with respect to Rf values (Nakiboğlu, 2002).

The Folin-Ciocalteu procedure is used for determination of the total content of plant phenolic compounds (Markantonuts *et al.*, 1993). The appropriate dilutions of extracts were oxidized with Folin-Ciocalteu reagent and the reaction was neutralized with 21% sodium carbonate. The absorbance of the resulting blue color was measured at 725 nm after 35 min. Tannic acid was used as a standard for the calibration curve. The total amount of phenolic compounds was calculated and expressed as TAE (mg/g). Data are reported as mean±SD for at least three replications. For statistical

Table 1: All 29 spots and their distribution in the species (s:syriaca, sc: sclarea, m: macrochlamys, v: vertisillata, c: candidissima, mu: multicaulis, l: limbata, n: nemorosa, h: hydrangea)

Species/Spot No	s	sc	m	v	c	mu	l	n	h
1	1	0	1	0	0	0	1	0	1
2	0	1	1	1	1	0	1	0	1
3	1	1	1	1	1	0	1	1	1
4	0	0	1	1	1	0	1	0	1
5	1	0	0	1	0	0	1	0	1
6	1	1	1	1	1	0	1	1	1
7	0	0	0	0	0	0	0	0	1
8	1	0	1	1	0	0	1	0	1
9	0	1	1	1	1	0	1	0	1
10	1	1	1	1	1	0	1	1	1
11	0	1	0	1	0	0	1	1	1
12	0	1	1	1	1	0	1	0	1
13	1	1	1	1	1	0	1	1	1
14	0	0	0	0	0	1	0	1	0
15	1	1	1	1	1	1	1	1	1
16	1	0	1	1	0	0	1	0	1
17	0	1	0	0	1	0	1	1	0
18	0	0	1	1	1	0	1	0	1
19	1	1	1	1	1	0	1	1	1
20	0	1	0	0	1	0	1	1	0
21	0	0	0	0	0	0	0	1	0
22	1	1	1	1	1	0	1	1	1
23	1	1	0	1	0	0	1	1	0
24	0	0	0	0	1	0	0	0	0
25	0	1	1	1	1	0	1	0	1
26	1	1	1	1	1	1	1	1	1
27	1	0	1	1	0	0	1	0	1
28	1	0	0	0	0	0	0	0	0
29	1	1	1	1	1	1	1	1	1

Table 2: Analysis of variance by ANOVA . content of phenolic compounds

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	169.318	8	21.165	42.394	0
Within Groups	8.986	18	0.499		
Total	178.304	26			

analysis, one-way analysis of variance followed by ANOVA and Tukey's test were used ($p<0.05$).

Results and Discussion

TLC studies: All 29 spots and their distribution in species are listed in Fig. 2 and Table 1.

The presence or absence of spot numbers was used in the calculation of the matching coefficient (cm) and coefficient of similarity (cs) (Table 3,4). Cs and cm were calculated with the following formulase:

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 cm and cs, the dendrogram of the species were drawn and the degrees of their relativity established according cm and cs (Fig. 1).

According to the presence or absence of the phenolic spots which were rearranged base upon chemical findings and using numerical analysis, the systematic position of the *Salvia* species was studied chemotaxonomically. The species were categorized as two groups. These groups are: Group A: *S. nemorosa*. *S. hydrangea*, *S. candidissima*, *S. sclarea*, Group B: *S. macrochlamys*, *S. multicaulis*, *S. limbata*, *S. syriaca* and *S. vertissilata*. 15 spots (# 3, 6, 10, 11, 13, 14, 15, 17, 19, 20, 21, 22, 23, 26, 29) are found on the chromatogram of *S. nemorosa*. Eleven of these are considered in common with *S. candidissima*, 10 of them with *S. hydrangea* and 13 with *S. sclarea* and 11 with *S. vertissillata*. 4 spots (# 14, 15, 26, 29) are found on the chromatogram of *S. multicaulis*. Three of these (# 15, 26, 29) are considered in common with *S. macrochlamys*, *S. limbata* and *S. syriaca*. One phenolic spot (# 28) is observed only in *S. syriaca* and one phenolic spot (# 7) is observed only in *S. hydrangea*. Phenolic spot (# 24) is observed only in *S. candidissima*.

Cm and cs values belonging to species clearly show the degrees of species relativity.

Quantification of phenolic compounds: The content of phenolic compounds was determined from the regression equation of a calibration curve ($y = 0.0825x+0.0231, R^2 = 0.98$) and expressed as Tannic Acid Equivalents (TAE). There was a meaningful difference about the level of $\alpha = 0.05$ among the treatments of phenolic compounds (Table 2).

In all the taxa studied the total content of phenolic compounds (mg/g dried weight.) varied between 19.78-27.71 mg/g dried weight. The highest amounts were found in extracts of *S. candidissima* (27.71±0.75 mg/g dried weight.) followed by *S. vertissilata* (26.16±0.12mg/g dried weight), *S. sclarea* (24.04±0.08mg/g dried weight), *S. macrochlamys* (23.79±0.25 mg/g dried weight), *S. hydrangea* (23.09±0.84 mg/g dried weight) *S. limbata* (22.34±0.2 mg/g dried weight), *S. multicaulis* (20.90±0.12 mg/g dried weight), *S. nemorosa* (20.41±0.25 mg/g dried weight). *S. syriaca* had the lowest amount of phenolic compounds (19.78±0.12 mg/g dried weight). Total content of phenolic compounds in *S. slarea* extracts was reported to be 24.0 ±1.1 (mg/g dried weight) (Miliauskas *et al.*, 2003). Analysis of species average comparison variance conducted with ANOVA ($p<0.05$) revealed that there is a significant difference about phenolic content among species. Also Tukey's test results showed that based on total content of phenolic compounds, species are classified as three groups: *S. macrochlamys*, *S. hydrangea*, *S. sclarea*, *S. limbata*, *S. candidissima*, *S. vertisillata*, *S. nemorosa*, *S. multicaulis*, *S. syriaca* belong to groups 1 to 3, respectively.

Table 3: The coefficient of similarity. (Numbers 1,2,3,..and 9 represent species of multicaulis, *S. syriaca*, *S. limbata*,*S. nemorosa*, *S. vertissilata*, *S. macrochlamy*, *S. hydrangea*, *S. sclarea* and *S. candidissima*, respectively).

	Matrix File Input								
Case	9	4	2	1	8	5	6	7	3
9	1.000	0.571	0.765	0.784	0.461	0.141	0.772	0.629	0.754
4	0.571	1.000	0.706	0.800	0.894	0.099	0.855	0.832	0.700
2	0.765	0.706	1.000	0.954	0.830	0.065	0.931	0.438	0.991
1	0.784	0.800	0.954	1.000	0.772	0.036	1.000	0.552	0.996
8	0.461	0.894	0.830	0.772	1.000	0.081	0.828	0.629	0.745
5	0.141	0.099	0.065	0.036	0.081	1.000	0.000	0.288	0.023
6	0.772	0.855	0.931	1.000	0.828	0.000	1.000	0.629	0.972
7	0.629	0.832	0.438	0.552	0.629	0.288	0.629	1.000	0.454

Table 4: The matching coefficient (Numbers 1,2,3,..and 9 represent species of multicaulis, *S. syriaca*, *S. limbata*, *S. nemorosa*, *S. vertissilata*, *S. macrochlamy*, *S. hydrangea*, *S. sclarea* and *S. candidissima*, respectively) .

Case	9	4	2	1	8	5	6	7	3
9	1.000	0.526	0.737	0.737	0.368	0.474	0.684	0.632	0.684
4	0.526	1.000	0.632	0.737	0.895	0.368	0.789	0.842	0.579
2	0.737	0.632	1.000	0.947	0.789	0.263	0.895	0.316	1.000
1	0.737	0.737	0.947	1.000	0.684	0.158	1.00	0.421	1.000
8	0.368	0.895	0.789	0.684	1.000	0.316	0.737	0.579	0.632
5	0.474	0.368	0.263	0.158	0.316	1.000	0.000	0.579	0.105
6	0.684	0.789	0.895	1.000	0.737	0.000	1.000	0.474	0.947
7	0.632	0.842	0.316	0.421	0.579	0.579	0.474	1.000	0.263
3	0.684	0.579	1.000	1.000	0.632	0.105	0.947	0.263	1.000

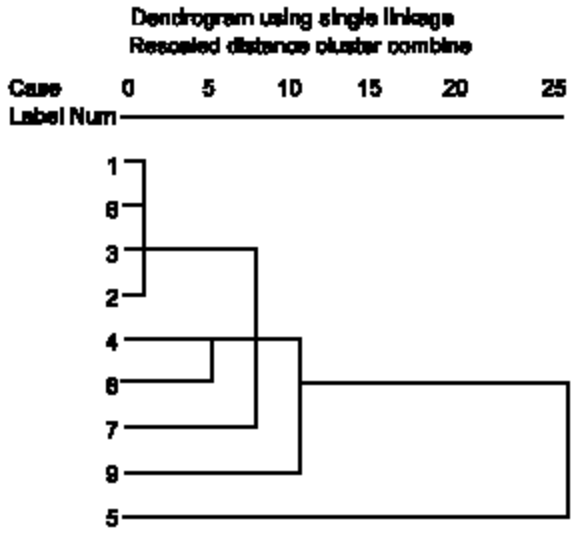

Fig. 1: Dendrogram constructed from coefficient of similarity and matching coefficient

In general, our results agreed with those of published studies conducted on other members of the genus *Salvia* (Nakiboğlu, 2002).

In all the taxa studied, only *S. sclarea* phenolic compounds was reported (24.0±1.1 mg/g dried weight) (Miliauskas *et al.*, 2003).

But, since this was the first study dealling with the content of phenolic compounds, therefore, a comparison with the previously published data has not been possible.

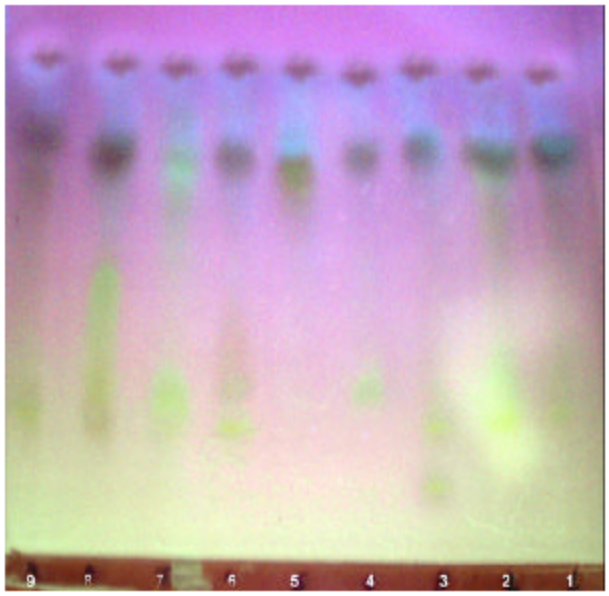

Fig. 2: All 29 spots and their distribution in the species (Numbers 1, 2, 3..... and 9 represent species of multicaulis, *S. syriaca*, *S. limbata*, *S. nemorosa*, *S. vertissilata*, *S. macrochlamys*, *S. hydrangea*, *S. sclarea* and *S. candidissima*, respectively).

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