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## Anatomical Observations on Nutlets of Some *Salvia* Species (Lamiaceae) from West Azarbaijan in Iran

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**Abstract:** Present survey was performed on thirteen species of *Salvia* from West Azarbaijan in Iran with the aim of illustrating species interrelationships. It includes comparative anatomy of the species based on nutlets transverse sections. These sections were examined using light microscope and detailed description of nutlet anatomical feature for all examined taxa is provided. In this study its found that the nutlets showed a considerable uniformity from anatomical point of view. However these species can be differentiated based on thickness of pericarp layer and parenchymatous layers colour.

**Key words:** *Salvia*, Lamiaceae, pericarp anatomy, Iran

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### INTRODUCTION

The family Lamiaceae includes 200 genera and 3000 species. The genus *Salvia* L. with over 900 species is probably the largest member of the family Lamiaceae. The two largest centres of the genus are in America and in South-West Asia (Hedge, 1992). Anatolia is a major centre for *Salvia* L. where 50.6% of the 87 species are endemic in Turkey (Vural and Adigüzel, 1996). Fifty-eight species of the genus *Salvia* (Lamiaceae) are found in Iran of which 17 are endemic. The rate of endemism in the *Salvia* in Iran is ca. 29% (Mozaffarian, 1996). In recent studies on this plant species it has been observed that the compounds decrease DNA synthesis in the cell. This feature is important in the diagnosis and treatment of cancer (Nakiboğlu, 1993). A gelatinous substance is produced from the seed that has mucilage. This substance is used as a good varnish and sweetener in Mexico (Estila and Hashemi, 1990).

Species of the genus *Salvia* L. have high hybridization rate (Haq, 1981).

Since most of the genus of Lamiaceae are important in medical and economic terms the members of the family need to be reviewed 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 (Nakiboğlu, 2002). The structure of the conductive bundles in the cross-section of the leaf petioles of the Lamiaceae members may be useful taxonomically (Metcalf and Chalk, 1950).

Numbers of the conductive bundles in the cross-sectioned petioles of the *Salvia* L. species varied among the species (Nakiboğlu and Oğuz, 1990). Anatomy of the stems have shown that the numbers of rays in every species can be used as a species-distinguishing feature (Osdemir and Senel, 1999).

Studies on nutlets in the Lamiaceae have proved taxonomic hierarchy. Ryding (2001) and Hye-Kyoung and Hong (2006) in a series of papers have made a detailed study of pericarp structure in Lamiaceae. Pericarp anatomical studies of some species of *Salvia* has been examined by some researchers (Wagner, 1914; Wojciechowska, 1961; Hedge, 1970). There are almost no reports on the pericarp anatomy of the species found in Iran. The aims of the present study are to offer a proved detailed description of the pericarp structure of the genus *Salvia* and to indicate species interrelationships.

### MATERIALS AND METHODS

We initiated the anatomical studies in the Laboratory of Anatomy and Morphology in Biology Department of Urmia University with living materials which were collected from their natural habitats in West Azarbaijan in Iran during mid May and end of June 2006.

For pericarp anatomical studies dry nutlets were placed for 10 days in a mixture of distilled water, 96% ethanol and glycerol taken in equal proportions. In the middle part of nutlets 8 µm thick cross-sections were made using a rotary microtome. The paraffin method was used

Table 1: Anatomical measurements on *Salvia* species

Taxons	Mean thickness of endocarp (µm)	Mean thickness of sclerenchymatous cell (µm)	Mean thickness of mesocarp (µm)	Mean thickness of mucilage (µm)	Mean thickness of cuticle (µm)
<i>S. multicaulis</i> VAHI	3.00±0.00	2.12±0.22	0.66±0.16	1.17±0.10	0.10±0.02
<i>S. ceratophylla</i> L.	3.16±0.606	3.09±0.10	3.66±0.33	1.42±0.08	0.24±0.08
<i>S. nemorosa</i> L.	2.00±0.48	1.14±0.05	0.50±0.00	1.54±0.02	0.26±0.09
<i>S. atropatana</i> BUNGE	7.16±0.61	2.18±0.14	4.33±0.66	0.98±0.02	0.33±0.07
<i>S. candidissima</i> VAHI	2.66±0.44	1.98±0.08	0.75±0.00	1.65±0.14	0.25±0.03
<i>S. virgata</i> JACQ	1.83±0.16	1.41±0.11	1.16±0.16	1.17±0.11	0.10±0.04
<i>S. sclarea</i> L.	2.00±0.00	1.54±0.14	0.91±0.08	1.52±0.00	0.20±0.02
<i>S. verticillata</i> L.	1.83±0.16	1.04±0.12	0.83±0.16	1.33±0.22	0.40±0.12
<i>S. syriaca</i> L.	2.00±0.00	1.49±0.02	0.91±0.08	2.24±0.00	0.29±0.09
<i>S. macrochlamys</i> BOISS	3.66±0.33	2.90±0.25	3.00±0.00	3.76±0.40	1.04±0.16
<i>S. aethiopsis</i> L.	2.33±0.33	1.52±0.12	0.75±0.14	2.18±0.07	0.13±0.02
<i>S. hybrangea</i> DC.ex Benth	0.91±0.08	1.54±0.26	0.75±0.00	1.84±0.00	0.21±0.05
<i>S. urmiensis</i> BUNGE	2.00±0.00	1.28±0.04	0.50±0.00	0.85±0.07	0.25±0.08

Table 2: Analysis of variance for parameters in *Salvia* species

Source of variation	df	Mean squares				
		cl	ml	pl	sl	el
Between groups	12	0.172	1.714	5.137	1.277	7.472
Within groups	26	0.020	0.040	0.155	0.068	0.354
Total	38					

cl: cuticular layer, ml: mucilaginous layer, pl: parenchymatous layer, sl: sclerenchymatous layer, en: endocarp layer

Table 3: The localities of plant species

Taxons	The localities of plant species
<i>S. multicaulis</i> Vahl	Naghadeh:Soltan Yaaghub Vallay Oshnavieh:Barden Zard Urmia:Shohada Vallay , Khan Vallay Oshnavieh road Urmia: Nooshinshahr, Keshish Mountain
<i>S. ceratophylla</i> L.	Urmieh: Gharebagh Village Urmieh: between Gharebagh and Agziarar Village
<i>S. nemorosa</i> L.	Urmia :ShohadaVallay Chaldran Salmas:between Koozehrash and Sarichichak Village Maku :Siahcheshmeh
<i>S. atropatana</i> Bunge	Urmia: Ghooshchi Mountains Oshnavieh Road
<i>S. candidissima</i> VAHI	Urmia:Marmisho
<i>S. virgata</i> Jacq	Khoy: Greese Village and road of Ghareziaaddin
<i>S. sclarea</i> L.	Maku: Siahcheshmeh Urmia: Nazloo Village Urmia:Shamlakan Village Khoy: 25 km of road of Ghareziaaddin Sardast:25 km of Piranshahre
<i>S. verticillata</i> L.	Urmia:Ghooshchi Mountains Urmia: Marmisho Maku :Bazargan
<i>S. syriaca</i> L.	Urmia:Ghooshchi Mountains Oshnavieh: Mir Abad Jungle
<i>S. macrochlamys</i> Boiss. and kotschy	Urmia: Marmisho
<i>S. Aethiopsis</i> L.	Khoy: Greece Village
<i>S. hybrangea</i> DC.	Urmia: Kaboodan Island Maku: Siahcheshmeh Ghareziaaddin Road
<i>S. urmiensis</i> Bunge	Nagadeh:Soltan Yaaghub Vallay

for preparing a cross section of nutlets and these cross-sections were stained with Hematoxylin-Eosin (Johnsen, 1940). Suitable sections were taken from these materials for microscopic studies..The thirteen species of *Salvia* growing in West Azarbaijan were selected as study materials (Table 3). The thickness of the layers in the nutlets was measured micrometrically and the results are given in Table 1. All the chemicals were purchased from Merck, Germany.

Values are expressed as means±SE of three replications. For statistical analysis, one-way analysis of variance followed by ANOVA and Tukey's test were used. p<0.05 was considered significant (Table 2).

## RESULTS AND DISCUSSION

The results obtained from the pericarp anatomical studies are presented (Table 1, 2 and Fig. 1).

The basic structure of pericarp layer was found to be uniform in all investigated taxa but there was clear differences in thickness of pericarp layer and

parenchymatous layer's colour . In all the taxa studied the thickness of cuticular layer is 0.08-1.36 µm and that of mucilaginous layer is 0.25-4.24 µm. In the middle of pericarp structure there are layers of yellowish, brown, dark-brown or black parenchymatous cells with thickness from 0.5 to 4 µm. Trackeid groups inside these layers have a sheath of elongated parenchymatous cells around them. The upper cell layers of the mesocarp are broader than the innermost one. In all investigated taxa the sclerenchymatous layer of the endocarp has a smooth sculpture with 0.8-3.2 µm thickness. The cells in the sclerenchymatous region consist of a layer of vertically arranged bone cells. There is a star-like cavity which is enlarged and branched at the centre, this cavity has pit canals towards the apex as well as the base. In all investigated taxa thickness of endocarp layer is between 0.75 and 8 µm.

***S. multicaulis* VAHI:** The thickness of cuticular layer is 0.08-0.16 µm and that of the mucilaginous layer is 0.96-1.28 µm.

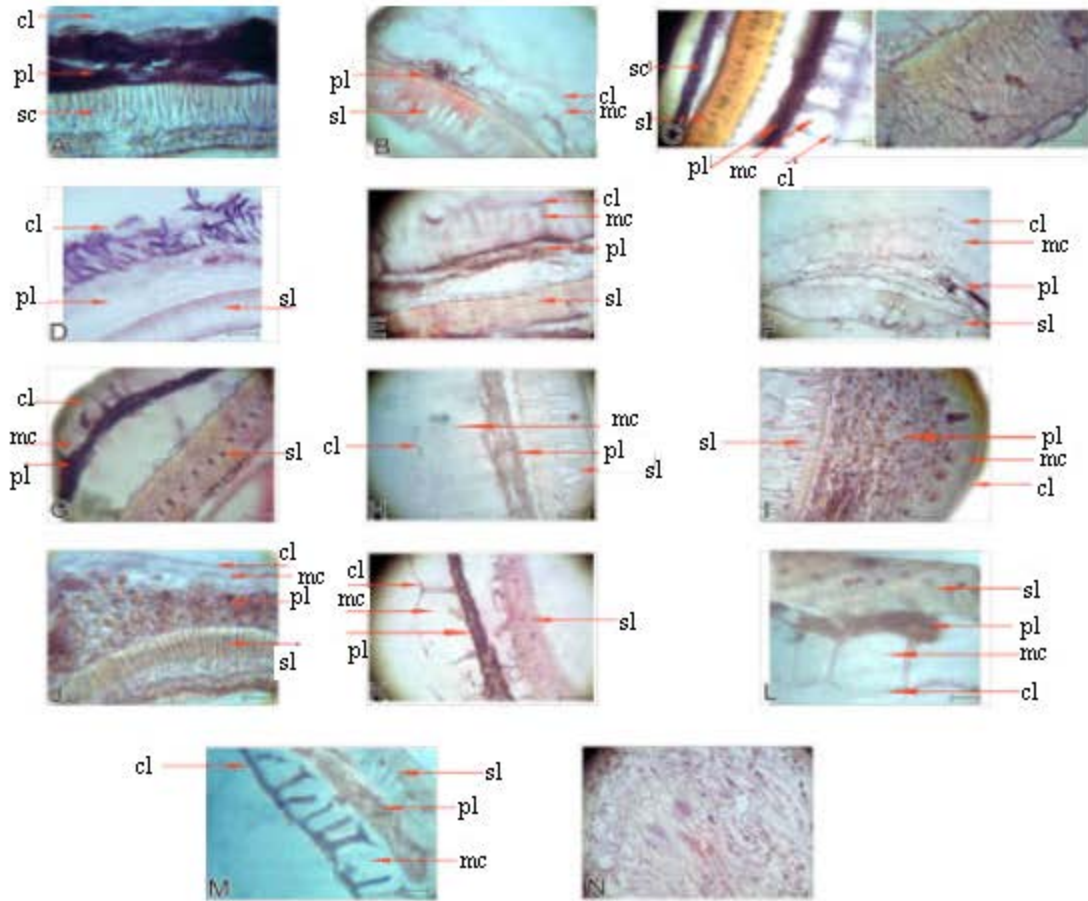


Fig. 1: Percarip transverse sections of *Satvia* species

- A: *S. hydrangea* Scale bar: 25 μm  
 B: *S. atropatana* scale bar: 25 μm  
 C1, C2: *S. multicaulis* scale bar: 25 μm,  
 C2: Details of sclerenchymatous layer scale bar: 10 μm  
 D: *S. ceratophylla* scale bar: 25 μm  
 E: *S. sclarea* scale bar: 25 μm  
 F: *S. vertisilata* scale bar: 25 μm  
 G: *S. candidissima* scale bar: 25 μm  
 H: *S. macrochlamys* scale bar: 25 μm  
 I: *S. eathiopis* scale bar: 25 μm  
 J: *S. urmiensis* scale bar: 25 μm  
 K: *S. nemorosa* scale bar: 25 μm  
 L: *S. syriaca* scale bar: μm  
 M, N: *S. Virgate* scale bar: 25 μm  
 N: Details of paranchymatous layer  
 Cl: Cuticle layer,  
 mc: Mucilaginous cell,  
 pl: Paranchymatous layer,  
 sl: Sclerenchymatous layer  
 Sc = Seed coat

In the centre of the pericarp structure there are 0.5-1  $\mu\text{m}$  thick, dark-brown parenchymatous layer, under the mesocarp layer there are bone cells with 0.9-2.56  $\mu\text{m}$  and endocarp layer is 3  $\mu\text{m}$  thick.

***S. ceratophylla* L.:** The thickness of cuticular layer is 0.08-0.32  $\mu\text{m}$  and that of the mucilaginous layer is 1.32-1.6  $\mu\text{m}$ . In the centre of the pericarp structure, there are 3-4  $\mu\text{m}$  thick, brown parenchymatous layers, under the mesocarp layer there are bone cells with 2.88-3.2  $\mu\text{m}$  and endocarp layer is 3-5  $\mu\text{m}$  thick.

***S. nemorosa* L.:** The thickness of cuticular layer is 0.08-0.32  $\mu\text{m}$  and that of the mucilaginous layer is 1.52-2.24  $\mu\text{m}$ . In the centre of the pericarp structure, there are 0.5  $\mu\text{m}$  thick, dark-brown parenchymatous layers, under the mesocarp layer there are bone cells with 1.04-1.2  $\mu\text{m}$  and endocarp layer is 1-3  $\mu\text{m}$  thick.

***S. atropatana* BUNGE:** The thickness of cuticular layer is 0.24-0.48  $\mu\text{m}$  and that of the mucilaginous layer is 0.96-1.4  $\mu\text{m}$ . In the centre of the pericarp structure, there are 3-5  $\mu\text{m}$  thick, brown parenchymatous layers, under the mesocarp layer there are bone cells with 1.92-2.4  $\mu\text{m}$  and endocarp layer is 6-8  $\mu\text{m}$  thick.

***S. candidissima* VAHI:** The thickness of cuticular layer is 0.2-0.32  $\mu\text{m}$  and that of the mucilaginous layer is 1.44-1.92  $\mu\text{m}$ . In the centre of the pericarp structure, there are 0.75  $\mu\text{m}$  thick, black parenchymatous layers, under the mesocarp layer there are bone cells with 1.88-2.16  $\mu\text{m}$  and endocarp layer is 2-3.5  $\mu\text{m}$  thick.

***S. virgata* JACQ:** The thickness of cuticular layer is 0.08-0.16  $\mu\text{m}$  and that of the mucilaginous layer is 0.96-1.36  $\mu\text{m}$ . In the centre of the pericarp structure, there are 1  $\mu\text{m}$  thick, brown parenchymatous layers, under the mesocarp layer there are bone cells with 1.2-1.6  $\mu\text{m}$  and endocarp layer is 1.5-2  $\mu\text{m}$  thick.

***S. sclarea* L.:** The thickness of cuticular layer is 0.16-0.24  $\mu\text{m}$  and that of the mucilaginous layer is 1.52  $\mu\text{m}$ . In the centre of the pericarp structure, there are 0.75-1  $\mu\text{m}$  thick, brown parenchymatous layers, under the mesocarp layer there are bone cells with 1.36-1.84  $\mu\text{m}$  and endocarp layer is 2  $\mu\text{m}$  thick.

***S. vertisillata* L.:** The thickness of cuticular layer is 0.24-0.64  $\mu\text{m}$  and that of the mucilaginous layer is 0.88-1.6  $\mu\text{m}$ . In the centre of the pericarp structure, there are 0.5-1  $\mu\text{m}$  thick, brown parenchymatous layers, under the mesocarp layer there are bone cells with 0.8-1.2  $\mu\text{m}$  and endocarp layer is 1.5-2  $\mu\text{m}$  thick.

***S. syriaca* L.:** The thickness of cuticular layer is 0.16-0.48  $\mu\text{m}$  and that of the mucilaginous layer is 0.96-1.36  $\mu\text{m}$ . In the centre of the pericarp structure, there are 1  $\mu\text{m}$  thick, brown parenchymatous layers, under the mesocarp layer there are bone cells with 1.44-1.52  $\mu\text{m}$  and endocarp layer is 1.52-2  $\mu\text{m}$  thick.

***S. macrochlamys* BOISS:** The thickness of cuticular layer is 0.9-1.36  $\mu\text{m}$  and that of the mucilaginous layer is 3.53-4.24  $\mu\text{m}$ . In the centre of the pericarp structure, there are 3  $\mu\text{m}$  thick, brown parenchymatous layers, under the mesocarp layer there are bone cells with 2.4-3.2  $\mu\text{m}$  and endocarp layer is 3-4  $\mu\text{m}$  thick.

***S. aethiopsis* L.:** The thickness of cuticular layer is 0.08-0.16  $\mu\text{m}$  and that of the mucilaginous layer is 2.32-2.8  $\mu\text{m}$ . In the centre of the pericarp structure, there are 0.5-1  $\mu\text{m}$  thick, brown parenchymatous layers, under the mesocarp layer there are bone cells with 1.28-1.68  $\mu\text{m}$  and endocarp layer is 2-3  $\mu\text{m}$  thick.

***S. hydrangea* DC. ex Benth:** The thickness of cuticular layer is 0.16-0.32  $\mu\text{m}$  and that of the mucilaginous layer is 0.25  $\mu\text{m}$ . In the centre of the pericarp structure, there are 0.75  $\mu\text{m}$  thick, dark parenchymatous layers, under the mesocarp layer there are bone cells with 0.75-1  $\mu\text{m}$  and endocarp layer is 1.28-2.08  $\mu\text{m}$  thick.

***S. urmiensis* BUNGE:** The thickness of cuticular layer is 0.12-0.4  $\mu\text{m}$  and that of the mucilaginous layer is 0.72-0.96  $\mu\text{m}$ . In the centre of the pericarp structure, there are 0.5  $\mu\text{m}$  thick, yellowish parenchymatous layers, under the mesocarp layer there are bone cells with 1.2-1.36  $\mu\text{m}$  and endocarp layer is 2  $\mu\text{m}$  thick.

**The cuticular layer:** Analysis of species average comparison variance conducted with ANOVA ( $p < 0.05$ ) revealed that there is a significant difference about the cuticular layer thickness among species also Tukey's test showed that species *S. hydrangea*, *S. atropatana*, *S. multicaulis*, *S. ceratophylla*, *S. sclarea*, *S. vertisillata*, *S. candidissima*, *S. aethiopsis*, *S. urmiensis*, *S. nemorosa*, *S. syriaca*, *S. virgata* are placed in a homogenous class and *S. macrochlamys* is placed in a second class. The layer thickness difference among species belonging to the same class is not considerable.

**The mucilaginous layer:** Analysis of species average comparison variance conducted with ANOVA ( $p < 0.05$ ) and Tukey's test results showed that based on the mucilage thickness species are divided into six class: species *S. urmiensis*, *S. atropatana*, *S. virgata*, *S. multicaulis*, *S. vertisillata*, *S. ceratophylla*;

*S. atropatanaa*, *S. multicaulis*, *S. virgata*, *S. vertisillata*, *S. ceratophylla*, *S. sclarea*, *S. nemorosa*; *S. multicaulis*, *S. virgata*, *S. vertisillata*, *S. ceratophylla*, *S. sclarea*, *S. nemorosa*, *S. candidissima*; *S. vertisillata*, *S. ceratophylla*, *S. sclarea*, *S. nemorosa*, *S. candidissima*; *S. candidissima*, *S. hydrangea*, *S. aethiopsis*, *S. syriaca*; *S. macrochlamys* belong to classes 1 to 6, respectively.

**The mesocarp layer:** Analysis of species average comparison variance conducted with ANOVA ( $p < 0.05$ ) and Tukey's test results showed that based on the mesocarp layer thickness, species are classified into 3 classes: species *S. urmiensis*, *S. nemorosa*, *S. virgata*, *S. hydrangea*, *S. candidissima*, *S. aethiopsis*, *S. vertisillata*, *S. sclarea*, *S. syriaca*, *S. virgata*; *S. macrochlamys*, *S. ceratophylla*; *S. atropatanaa*, *S. ceratophylla* belong to the classes 1 to 3, respectively.

**The sclerenchymatous layer:** Analysis of species average comparison variance conducted with ANOVA ( $p < 0.05$ ) and Tukey's test results showed that based on the sclerenchymatous layer thickness species are classified into 6 class: species *S. hydrangea*, *S. sclarea*, *S. vertisillata*, *S. nemorosa*, *S. urmiensis*, *S. virgata*, *S. syriaca*, *S. aethiopsis*; *S. urmiensis*, *S. virgata*, *S. syriaca*, *S. aethiopsis*, *S. sclarea*, *S. hydrangea*, *S. candidissima*; *S. virgata*, *S. syriaca*, *S. aethiopsis*, *S. sclarea*, *S. hydrangea*, *S. candidissima*; *S. multicaulis*; *S. syriaca*, *S. sclarea*, *S. aethiopsis*, *S. hydrangea*, *S. candidissima*, *S. multicaulis*; *S. atropatanaa*; *S. syriaca*, *S. aethiopsis*, *S. sclarea*, *S. hydrangea*, *S. candidissima*, *S. multicaulis*, *S. atropatanaa*, *S. macrochlamys*, *S. ceratophylla* belong to classes 1 to 6, respectively.

**The endocarp layer:** Analysis of species average comparison variance conducted with ANOVA ( $p < 0.05$ ) and Tukey's test results showed that based on the endocarp layer thickness species are classified into five classes: class one consists of species: *S. vertisillata*, *S. virgata*, *S. urmiensis*, *S. sclarea*, *S. nemorosa*, *S. syriaca*, *S. aethiopsis*, *S. candidissima*; class two consists of species: *S. vertisillata*, *S. virgata*, *S. candidissima*, *S. urmiensis*, *S. nemorosa*, *S. syriaca*, *S. aethiopsis*, *S. candidissima*, *S. multicaulis*; class three consists of species: *S. candidissima*, *S. multicaulis*, *S. macrochlamys*, *S. aethiopsis*, *S. syriaca*, *S. nemorosa*, *S. urmiensis*; class four consists of species: *S. candidissima*, *S. multicaulis*, *S. macrochlamys*, *S. ceratophylla* and class 5 consists of species *S. atropatanaa*.

In conclusion our results highly support the results given by other workers on other species of *Salvia* L. (Wagner, 1914, Wojciechowska, 1961; Hedge, 1970).

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