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Numerical taxonomy of eight *Salvia* L. species using anatomical properties

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ABSTRACT

The anatomical characters of eight Salvia species of the Lamiaceae family have been investigated. The stem is regular or irregular quadrangular, with collenchymatous hypodermis, sclerenchyma group above vascular bundles with different number layers in species. Stems and petioles have glandular and eglandular hairs. Also, a cluster analysis was performed with nineteen anatomical characters. Based on our results, two main clusters separate two groups of species and the taxonomical results have been illustrated.

Keywords: Stem, Petiole, Lamiaceae, Iran.

INTRODUCTION

The genus *Salvia* L. with about 1000 species is probably the largest member of the Lamiaceae family. This genus is distributed extensively in three main regions of the world: central and south America (500 spp.); Western Asia (200 spp.) and Eastern Asia (100 spp.) (Walker & Sytsma, 2007). In Iran, it is represented by 58 species which 17 are endemic (Mozafarian, 1996; Rechinger, 1987). *Salvia* species are used in traditional medicines all around the world, possessing antibacterial, antidiabetic and antitumor properties (Ulubelen, 2003). In addition, many *Salvia* species used as herbal tea and for food flavoring, as well as in cosmetics, perfumery and the pharmaceutical industry (Demirci *et al*., 2003). Most of *Salvia* species have been investigated in point of chemical composition (Bigdeli *et al*., 2005; Gulluce *et al*., 2006; Habibi *et al*., 2004; Lari Yazdi *et al*. 2005 and Rustaiyan *et al*., 2000). And little data is found in anatomical characters, except a few species (Aktas et al., 2009; Baran & Özdemir, 2006; Ceja *et al*., 2005; Cobanoglu, 1988; Kahraman *et al*., 2009; Ozdemir & Senel, 1999; Koyuncu *et al*., 2009). On the other hand, there is general information about anatomical structure of Lamiaceae (Metcalf & Chalk, 1957). Therefore, the present study was undertaken in order to give a detailed account of the anatomical characters in eight species of *Salvia* growing in Iran.

MATERIALS AND METHODS

The materials are collected and procured from different places. The list of species is presented in Table 1. Five samples from each species were used. In order to study histofoliar and stem characters, materials fixed in FAA (Johansen, 1940) and kept in 50% ethanol solution (Berlyn & Miksche, 1976). Cross sections of stem and petiole were prepared by common cytohisto technique. Sections were cleaned with sodium hypochlorite, dehydrated and stained with methyl green 0/1% and carmine 1% for 30 seconds and 15 minutes respectively, then mounted in gelatin. Observations were carried out with Olympus light microscope (Figure1). Qualitative characters of stems and petioles consist of shape and hair type of stem, continuity of fibrous layer, epidermis and cortex cell shape; petiole shapes, epidermis cell shape, hair type of petiole were evaluated (Table 2). Also the stomata type and type of trichomes on an abaxial leaf surface were assayed using SEM (Figure 2). The experimental data of qualitative characters were analyzed by ANOVA for analysis of variance and the differences were compared at alpha 0.05 (Table 3). For phenetic study, 10 qualitative and nine quantitative characters were examined (Table 4). After coding of qualitative characters, Hierachial clustering analysis were performed using SPSS software version 9 with Average linkage method (Norusis, 1999) and the resulting dendrogram was illustrated (Figure 3).

Table 1- The species of *Salvia* studied and their collecting sites

Species	**Locality and voucher specimen no**.
S. atropatana Bunge.	Tehran: Firoozkoh road, 2210 m, Ebrahymiyan 14038
S. macrosiphon Boiss.	Semnan: Semnan road, 1350 m, Ebrahymiyan 14067
S. nemorosa L.	Tehran: Damavand county, 1300 m, Ebrahymiyan 14029
S. sclarea L.	Tehran: Damavand county, 1700 m, Ebrahymiyan 14072
S. splendens Ker. Gawl.	Tehran: Tehran, Jamshidie, 1600m, Ebrahymiyan 14046
S. syriaca L.	Tehran: Varamin, 1400 m, Ebrahymiyan 14011
S. virgata Jacq.	Qazvin: Alamout, 2100 m, Ebrahymiyan 14019
S. xanthocheila Boiss.	Mazandaran: Gachsar, 2900 m, Ebrahymiyan 14062

Table 2- Stem, petiole and leaf characters in eight *Salvia* species

	Stem					Petiole			Leaf	
Species	**shape**	**Hair type**	**Continuity of fibrous layer**	**Epiderm shape**	**Cortex shape**	**Petiole shape**	**Epiderm shape**	**Hair type**	**Stomata type**	**Trichome type**
S. atropatana	regular quadrangular	eglandular	Not- Continuity	elliptic	elliptic	suborbicular	elliptic	eglandular	diacytic	eglandular
S. macrosiphon	irregular quadrangular	glandular eglandular	Continuity	oblong	oblong	oblong	oblong	glandular	paracytic	eglandular
S. nemorosa	rectangular quadrangular	eglandular	Not- Continuity	qudrate-oblong	hexagonal	crescent	elliptic	eglandular	paracytic	glandular+eglandular
S. sclarea	regular quadrangular	glandular	Continuity	circular-ovate	hexagonal	suborbicular	elliptic	eglandular	diacytic	glandular+eglandular
S. splendens	rectangular quadrangular	eglandular	Continuity	circular	Irregular polygonal	reniform	elliptic	eglandular	diacytic	glandular
S. syriaca	rectangular quadrangular	glandular eglandular	Continuity	circular	Irregular polygonal	quadrate	ovate	eglandular	paracytic	glandular+eglandular
S. virgata	regular quadrangular	glandular eglandular	Continuity	elliptic	circular	oblong	elliptic	glandular+ eglandular	diacytic	glandular+eglandular
S. xanthocheila	regular quadrangular	glandular eglandular	Not- Continuity	ovate	hexagonal	suborbicular	elliptic	glandular	paracytic	glandular+eglandular

Table3- Quantitative characters of eight *Salvia* species

Species	**Stem**				**Petiole**						
	Co	Cp	Pr	Sg	Ae	Ac	Pl	Tc	Te	Rc	Pp
S. atropatana	10.333±0.333	6±0	11.333±0.333	6±0	2.666±0.333	1.666±0.333	6.333±0.333	2.666±0.333	1.333±0.333	31.666±0.333	7.666±0.333
S. macrociphon	3.333±0.333	3.666±0.333	18±0.577	5.333±0.333	2.666±0.333	2.666±0.333	13.333±0.333	4±0	2.333±0.333	45±1.154	13.333±0.333
S. nemorosa	8.666±0.333	3.666±0.333	10.333±0.881	3.333±0.333	2.333±0.333	2.666±0.333	6.333±0.333	1±0	3.333±0.333	38.333±0.333	11.666±0.333
S. sclarea	4.333±0.333	3.333±0.333	7.666±0.333	1.666±0.333	2.666±0.333	1.333±0.333	22.666±0.333	1±0	2.333±0.333	57.333±1.763	9.666±0.33
S. splendens	4.333±0.333	3.333±0.333	7.666±0.333	4.666±0.333	1.333±0.333	1.333±0.333	11.333±0.666	4.333±0.666	0±0	18.666±0.333	7.333±0.333
S. syriaca	9.333±0.666	4.666±0.333	9.333±0.333	4±1.154	2.666±0.333	1.333±0.333	6.333±0.333	2.333±0.333	0±0	27.333±0.333	6.333±0.333
S. virgata	5.666±0.333	3±0	14±0.577	3±0	1.333±0.333	2.333±0.333	4.333±0.333	2.333±0.333	2±0	51.666±0.881	4.333±0.333
S. xanthocheila	6.666±0.333	5.333±0.666	8±0.577	4.666±0.333	4.333±0.333	4.333±0.333	19±0.577	7.333±0.333	3.666±0.333	54±2.645	10.333±0.881

Abbreviations: Stem characters: Co= Collenchyma layer; Cp= Cortex parenchyma; Pr= Pith ray; Sg= Schloranchyma group
Petiole characters: Ae= Adaxial epidermis cell; Ac= Abaxial epidermis cell; Pl= Paranchyma layer upper adaxial epidermis; Tc= Trachia in centre of petiole; Te= Trachia in the end of petiole; Rc= Number of xylem rays in the central vascular bundles; Pp= Petiole paranchyma layer

Table 4- Distribution of anatomical qualitative character states among the studied *Salvia* species. Character states are as follows: Stem shape (regular0 / irregular1); hair type of stem (glandular0/ eglandular1/ both of them2); Continuity of fibrous (continuity0/ not-continuty1); epiderm shape of stem (circular or ovate0/ elliptic1/ other state2); cortex shape (circular or elliptic0/ polygonal1); petiole shape (suborbicular0/ oblong1/ other state2); epiderm shape of petiole (elliptic0/ ovate1); hair type of petiole (glandular0/ eglandular1/ both of them2); stomata type (diacytic0/ paracytic1); trichomes type of leaf (glandular0/ eglandular1/ both of them2).

	characters									
species	1	2	3	4	5	6	7	8	9	10
S. atropatana	0	1	1	1	0	0	0	1	0	1
S. macrociphon	1	2	0	2	0	1	1	0	1	1
S. nemorosa	1	1	1	2	1	2	0	1	1	2
S. sclarea	0	0	0	0	1	0	0	1	0	2
S. splendens	1	1	0	0	1	2	0	1	0	0
S. syriaca	1	2	0	0	1	2	1	1	1	2
S. virgata	0	2	0	1	0	1	0	2	0	2
S. xanthocheila	0	2	1	0	1	0	0	0	0	2

RESULTS AND DISCUSSION

The results of the anatomical studies demonstrated that the stem has following tissues:
The cross section of stem in all species showed regular or irregular quadrangular. Epidermis was single layer with ellipsoid, oblong, ovoid or circular cells. Collenchyma was made of three to ten layered under epidermis, projecting at the corners of stem, becoming thin towards edges. Cortex parenchyma under epidermis was three to six layered with oblong, hexagonal or ellipsoid cells. Thick sclerenchyma groups with three to six layers were presented above vascular bundles at the corners. Phloem was located under sclerenchyma. Vascular bundles at the corners were large and sometimes with lobed and at the edge were very small. Pith rays were seven to nineteen rowed. Two hair types of stem were glandular or eglandular. In some species such as *S. atropatana*, *S. macrosiphon*, *S. syriaca* and *S. xanthocheila* both of them were shown. Also large pith always was presented at the center of stem (Figure 1).

In cross section of petiole these characters were shown: The shape of petiole was different in species such as oblong, suborbicular and reniform. In *S. nemorosa*, the petiole shape was cresent and in *S. syriaca* was quadrate (Figure 1). Epidermis, the outermost layer, was formed by cells, which were ovoid, ellipsoid or nearly oblong. Paranchyma with circular cells and inter cellular space had four to nineteen layers under abaxial epidermis. Types of vascular bundles were collateral. At the cross sections of petiole, it has been observed that the number of vascular bundle vascular in the center were different. There were one to eight vascular bundles in the center and 0ne to four small bundles at the ends. The central vascular bundles were sometimes single and lobed or divided into two or three pieces. . Sclerenchyma upon the central vascular bundles was thick. The central vascular bundles had 19 to 60 xylem rays. Hair types of petioles were glandular or eglandular. In *S. xanthocheila* both of them were shown.

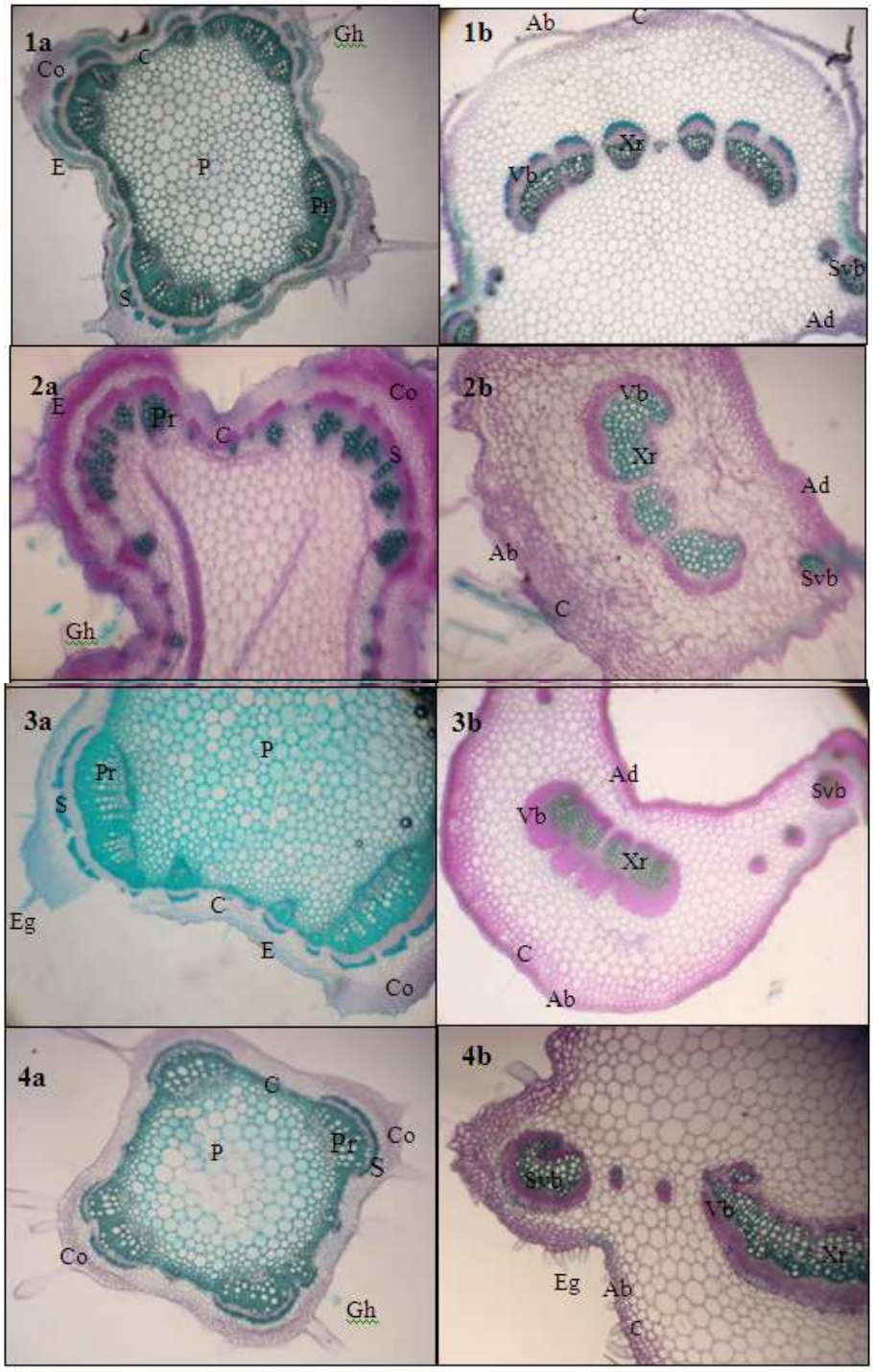

Figure 1- Cross section of stem and petiole in eight *Salvia* species: *S. atropatana* 1a, 1b – *S. macrociphon* 2a, 2b – *S. nemorosa* 3a, 3b – *S. sclarea* 4a, 4b

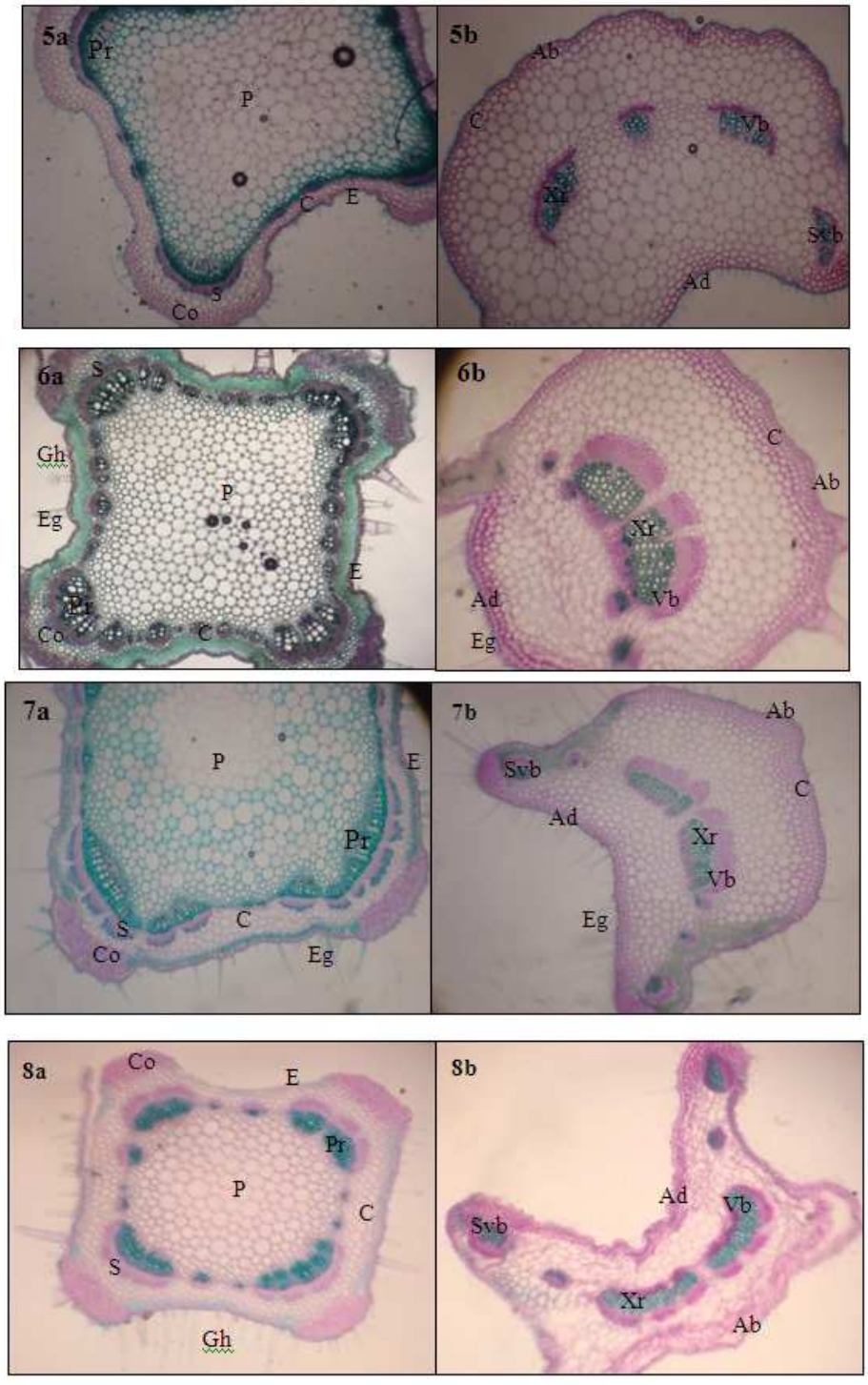

Figure1 continued: *S. splendense* 5a, 5b – *S. syriaca* 6a, 6b– *S. virgata* 7a, 7b – *S. xanthocheila* 8a, 8b

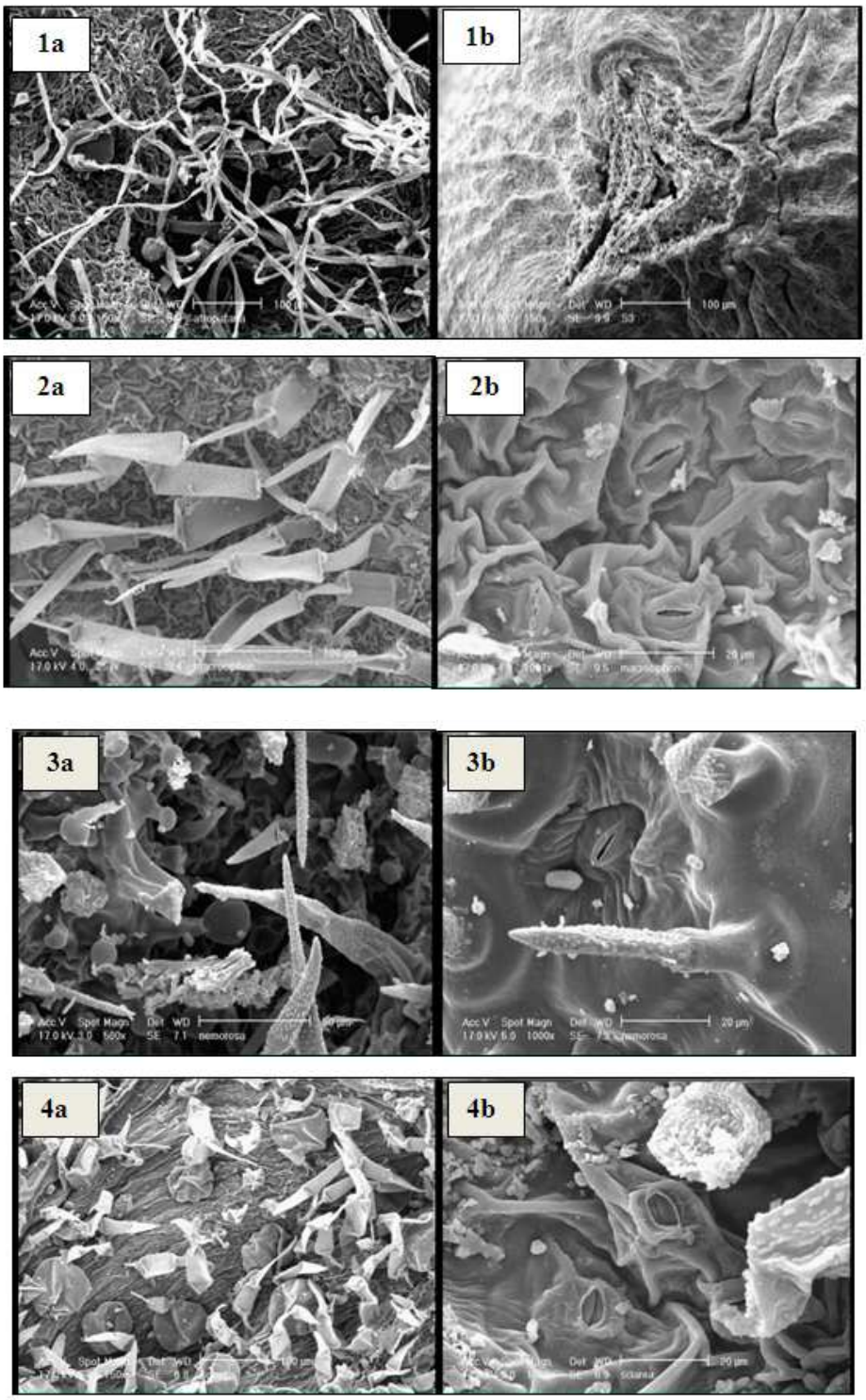

Figure2: Sem photographs of trichomes and stomata in eight *Salvia* species: *S. atropatana* 1a, 1b – *S. macrociphon* 2a, 2b– *S. nemorosa* 3a, 3b – *S. sclarea* 4a, 4b

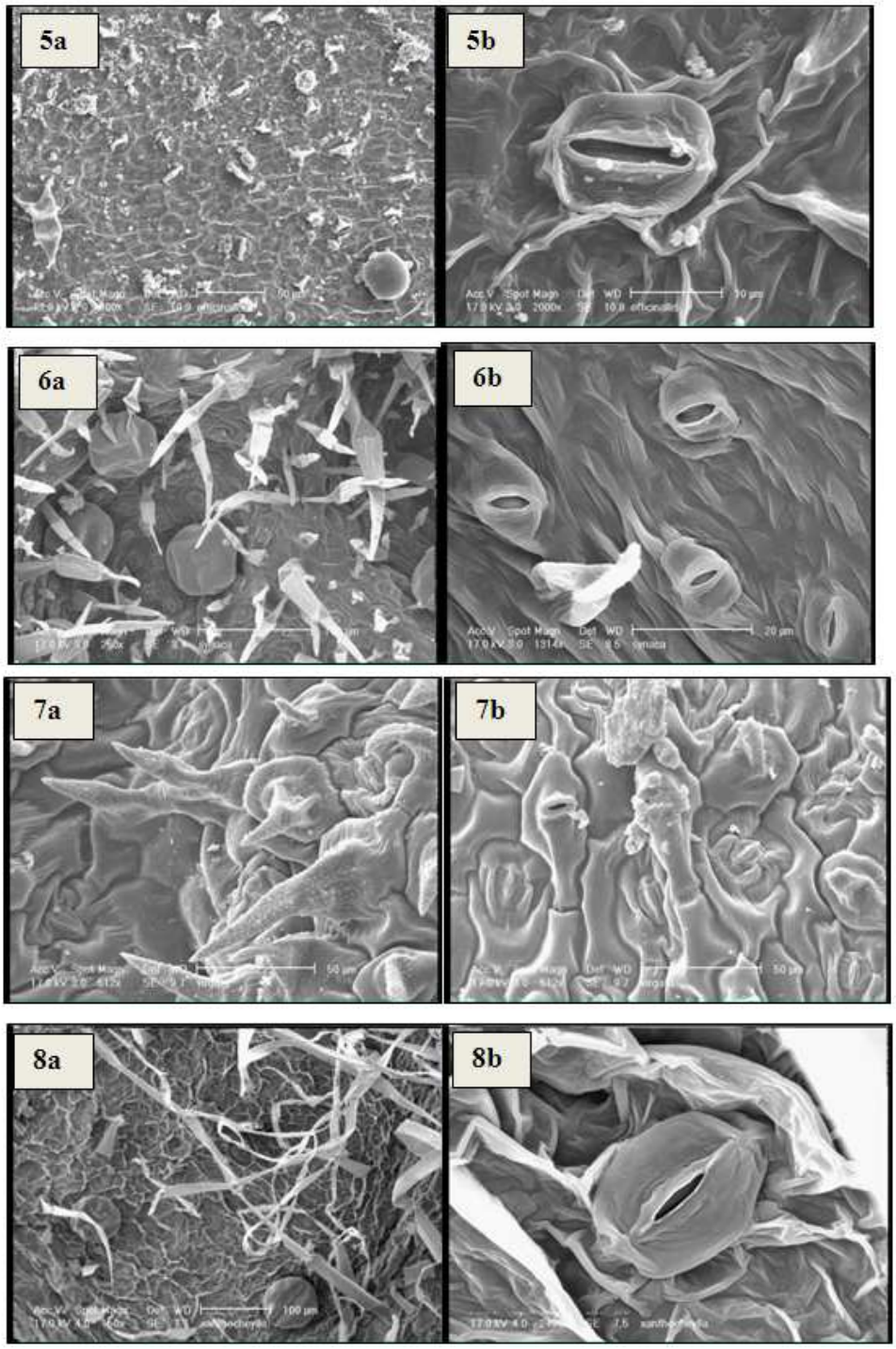

Figure2 continued: *S. splendense* 5a, 5b– *S. syriaca* 6a, 6b – *S. virgata* 7a, 7b – *S. xanthocheila* 8a, 8b

Hairs on upper surface of leaves are eglandular or glandular and in some species both of them was shown. Glandular hairs were in different shapes such as head, capitates or peltate (Figure 2). Also, stomata types were diacytic or paracytic (Figure 4). The variability among species caused that a cluster analysis was performed based on 19 qualitative and quantitative characters. As demonstrated in Figure 3, species divided into two clusters at the linkage distance 20. First cluster comprises three species consist of *S. atropatana, S. xanthocheila* and *S. sclarea*. Five other species were placed in second cluster. This cluster divided into two subclusters. *S. nemorosa* and *S. splendense* were placed in subcluster one at the linkage distance 11 and subcluster two comprised three other species consist of *S. syriaca*, *S. virgata* and *S. macrosiphon.*

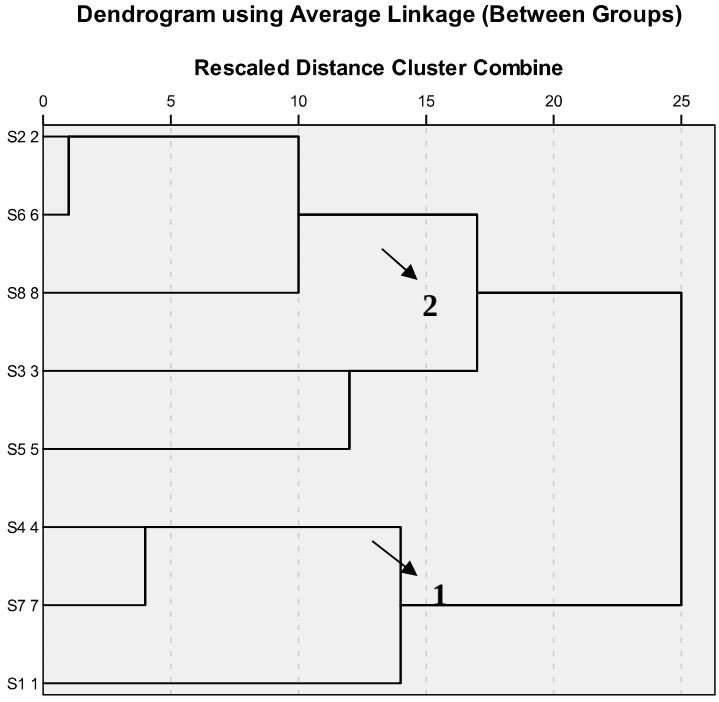

Figure 3- Cluster analysis of eight *Salvia* species using Average linkage with Ward method.
S1- *S. xanthocheila*; S7- *S. athropatana*; S4- *S. sclarea*; S5- *S. splendens*; S3- *S. nemorosa*; S8- *S. virgata*; S6- *S. syriaca*; S2- *S. macrosiphon*

Tribe Menthaea consists of 65 genera and over 2000 species with many economically important herbs and belongs to subfamily Nepetoideae (Harley *et al*., 2004). According to Harley *et al*. (2004), Mentheae can be divided into three subtribes, Salviinae, Nepetinae and Mentheae. Subtribe Salviinae is recognized as a monophyletic lineage, but its largest genus *Salvia* proved to be para or polyphyletic (Walker *et al*., 2004; Walker & Sytsma, 2007). *Salvia* is distinguished from other 72 genera in the tribe Mentheae by having the two posterior stamens aborted and the connective separating the thecae of the two expressed stamens significantly elongated. In the Flora Iranica, *Salvia* is divided into five groups (Grex A-E). The selected species in this present paper belong to two different groups. For instance, *S. nemorosa, S. virgata, S. syriaca* and *S. macrosiphon* are placed in Grex D and *S. sclarea, S. atropatana* and *S. xanthocheilla* in Grex E (Hedge, 1982b). Also, *S. splendens* is an exotic species in Iran placed in grex D. We found that

anatomical characters correlated with classification of these species in Flora Iranica. As demonstrated in Figure 3, two main clusters separate two groups of species. In first cluster the species of Grex E and in second one, all of species belong to Grex D were located. In first cluster, *S. sclarea* and *S. atropatana* are very close to each other. These similarities were shown in qualitative characters such as stem and petiole shape, hair type of petiole and also stomata type. Cluster analysis based on chemical composition is shown that these two species placed in one cluster (Salimpour *et al*., 2011). *S. xanthocheila* separate from these two species in this cluster by having paracytic stomata and glandular hairs of petiole. In second main cluster, species separated into two sublusters. *S. nemorosa* and *S. virgata* have similarities in morphological characters such as leaf shape, leaf indumentum, color of corolla and stamen type, but our data suggests that they have different anatomical characters and positioned into two different subclusters. Also, morphological characters of two other perennial species consist of *S. macrosiphon* and *S. syriac* are similar such as shape of leaf and calyx, color of corolla, shape of nutlet and abscission scar. However their anatomical characters are similar too. On the other hand, we agreed with data of other researchers about the arrangement of vascular bundles in central and edge petiole, number of pith rays of Lamiaceae species is important from the taxonomic point of view (Metcalf & Chalk, 1972; Baran & Özdemir, 2006; Nakipoglu & Oguz, 1990). As shown in Figure1, the number of central vascular bundle of petiole and number of pith rays showed variation between species.

CONCLUSION

In conclusion, we aimed to introduce anatomical properties of eight *Salvia* species growing in Iran. We found some differences beside the similarities between these species in anatomical characters. Types of hairs in stem, petiole and leaf, trachia in center of petiole and some quantitative characters are an important for distinguishing the species. Based on these characters, phenetic relationships between these species were discussed.

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