

Anatomical and micromorphological studies on leaves of *Salvia* L. species in NE Iran

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Abstract

This study has examined anatomical and micromorphological characters of leaves of 10 Salvia species growing in the north east of Iran. For anatomical study, the matured dry leaves were selected and fixed in FAA solution. Manual cross sections of the leaves were prepared and stained by differential staining. For leaves micromorphological study, the epidermal cells and trichomes were noticed by SEM. Some differences were perceived in the shape of midribs outline, the number of collenchymous and parenchymous cell layers and the shape of sclerenchymous tissue above the phloem in the midrib. As to the leaves micromorphological findings, the differences between the shape of trichomes on the leaves surface were observed. According to the results, the shape of midribs outline showed significant variation among the studied species.

Keywords: Anatomy, Micromorphology, Iran, *Salvia*, Sclerenchyma, Collenchyma.

• Introduction

Salvia L. belongs to Lamiaceae family has 56 species in Iran which 17 species distributed in NE Iran [1]. Due to essential oils in leaves trichomes, *Salvia* leaves are applied to disinfect, decrease blood sugar and anti spasm. Also, DNA synthesis is decreased by chemical composition of leaves [2- 4]. The previous reports of leaves anatomical and micromorphological studies were related to morphological, leaves anatomical and karyological studies on *S. blepharoclaena* Hedge & Hub.[5], micromorphological, anatomical and pollen ornamentation studies on four desert species of *Salvia* in center of Iran [6] and anatomical research on *S. viridis* L., *S. nemorosa* L., *S. nutans* L., and *S. sobrogensis* [7- 8]. Moreover anatomical characters of *S. sclarea* L. and *S. forskahlei* L. were studied by Ozdemir *et al.* [9- 10]. In the present research, leaves anatomical and micromorphological characters of 10 *Salvia* species in NE Iran were evaluated for the first time. The aim of this study was to observe the variation of internal structure in *Salvia* leaves.

Materials and methods

For anatomical study, the matured dry leaves of *S. nemorosa*, *S. atropatana* Bunge, *S. chorassanica* Bunge, *S. leriifolia* Bent, *S. sclarea*, *S. aethiopis* L., *S. macrosiphom* Boiss, *S. chloroleuca* Rech & Aellen , *S. virgara* L., *S. ceratophylla* L. were selected from specimens kept in FUMH herbarium and fixed in FAA solution. Then, manual cross sections were prepared and stained by green methyl and carmine, after that, photographed

by different magnification of light microscope Labomed model CZ500 and Dino capture camera. Their localities were presented in **Table 1**. Some anatomical characters such as the shape of outline of midribs, the number of collenchymous and parenchymous cell layers and the shape of sclerenchymous tissue above the phloem in the midrib were analyzed. Moreover, leaves surface were observed by SEM to assess the shape of trichomes and epidermal cells.

• **Table 1. The locality of studied *Salvia* species**

Species	Locality
<i>S. atropatana</i>	West north of Quchan, Galil, 2100m, Zangouei, 25824, FUMH; west north of Boujnord, Misi nou mount, 1800m, Joharchi & Zangouei, 20819); west north of Boujnord, Gouinik mount, 1435m, Joharchi & Zangouie, 40134, (FUMH).
<i>S. chorassanica</i>	Between Quchan- Drgaz, northern slope of Allahoakbar mount, 1650m, Joharchi & Zangouie, 16868, (FUMH); North of Masshad, Kalat road, southern mounts of Sandough shekan pass, 1550m, Jopharchi & Zangouie, 16825, (FUMH).
<i>S. sclarea</i>	Boujnord, Badranlou pass, 20 km Boujnord, 1450 m, Jopharchi & Zangouie, 10625, (FUMH); West of Boujnord, base of road, 1485m, Jopharchi & Zangouie, 40436, (FUMH)
<i>S. aethiopsis</i>	west South of Boujnord, Rein, 1700m, Jopharchi & Zangouie, 37949, (FUMH); west north of Boujnord, between Koinic and Baghlogh, Jopharchi, 33706, (FUMH); West of Boujnord, after GharehNowDeh, 1485m, Jopharchi&Zangouie, 40442, (FUMH).
<i>S. ceratophylla</i>	North of torbate Heidarieh, Sfiuj mount, 1450m, Joharchi & Zangouie, 2000G, (FUMH); Mashhad, west of Miamai, Ardolok mount, 1000m, Joharchi & zangouie, 20322, (FUMH).
<i>S. leriifolia</i>	West of Sabzevar, mountains of east Sarough, 1650m; , Joharchi & Zangouie, 42420, (FUMH)Gonabad, Ab Sanou mount, , Joharchi & Zangouie, 12835, (FUMH);
<i>S. macrosiphon</i>	Between Srakhs- Mashhad, Bazangan, Joharchi & Zangouie, 16756, (FUMH); West north of Ghaen, Dashte Baiaz, 1900m, Joharchi, 34480, (FUMH).
<i>S. chloroleuca</i>	East of Quchan, Iadak, 1700m, Joharchi & Zanghouie, 12890, (FUMH); North of Mashhad, Kardeh, 1100m, Joharchi & Zanghouie, 12929, (FUMH).
<i>S. virgata</i>	Kalate naderi, 1100m, Zangouie, 11198, (FUMH); Torbate Heydarieh, 1340m, Rafeie & Zangouie, 23176, (FUMH).
<i>S. nemorosa</i>	East south f Boujnord, Esfidan, 1561m, Joharchi & Zanghouie, 40219, (FUMH); Quchan, Oghaz kohneh, 1800m, Faghihnia & Zangoure, 29451, (FUMH).

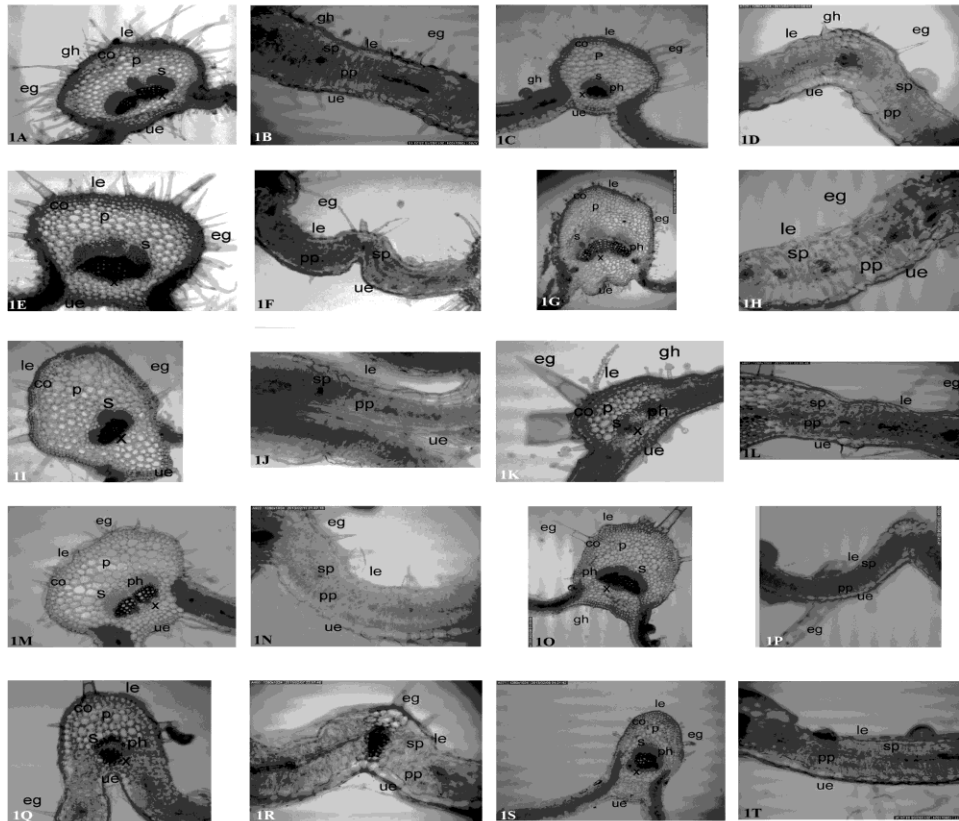
Results

The results of leaves cross sections and electro micrographs of leaf epidermis tissue in *S. nemorosa* displayed: 1) elliptical midrib outline with undulated abaxial side. 2) peltate glandular trichomes. 3) 1- 3 cellular and conical- verrucose non glandular trichomes with

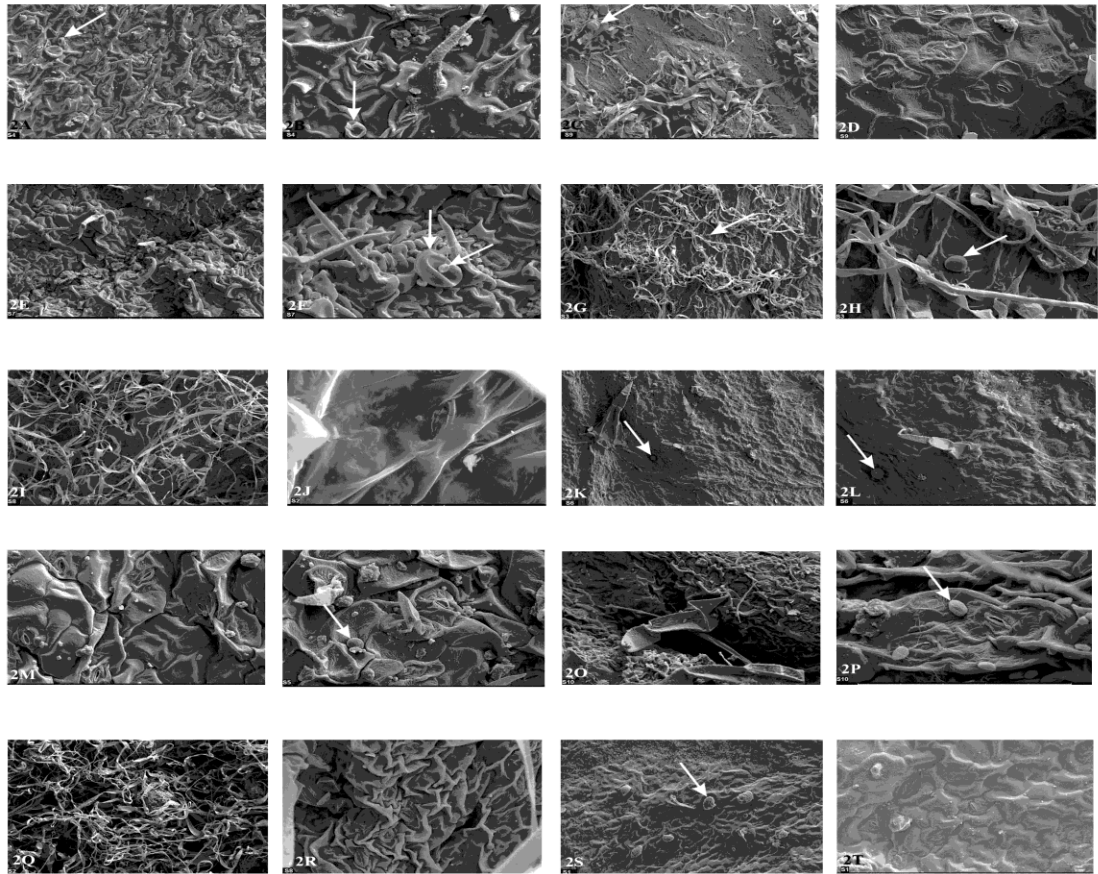
single basal epidermal cell. 4) 3- 4- layered collenchymous and 6- layered polygonal parenchymous tissue after adaxial epidermis layer. 5) three discontinuous sclereid bundles above two vascular bundles. 6) dorsi- ventral mesophyll (Figs. 1A- B, 2A- B). In *S. atropatana* were noticed, 1) circular- elliptical midrib outline with prominent abaxial side. 2) spherical peltate glandular trichomes with reticulate surface. 3) 4- 5 cellular and ribbon shaped non glandular hairs with smooth surface. 4) 2- layered collenchymous and 7- layered polygonal parenchymous tissue after adaxial epidermis layer. 5) crescent shaped sclereid bundles above the single vascular bundle. 6) dorsi- ventral mesophyll (Figs. 1C- D, 2C- D).

S. virgata showed, 1) sac shaped midrib outline with two lobed abaxial side. 2) capitate glandular trichomes with long stalk and verrucose peltate glandular trichomes,. 3) 2- 5 cellular, verrucose subulate (awl shaped) non glandular hairs with curved apex. 4) 2- 3- layered collenchyma and 7- 8- layered polygonal parenchymous tissue after adaxial epidermis layer. 5) discontinuous sclereid bundles above the single vascular bundle. 6) isolateral mesophyll (Figs. 1 E- F, 2 E- F). In *S. leriifolia* were perceived, 1) circular midrib outline with deep two lobed abaxial side. 2) spherical peltate glandular hairs with smooth surface. 3) 2- 3 cellular, so long- thin, ribbon shaped non glandular trichomes with stripped surface. 4) 1- 2- layered collenchymous tissue and 9- 10- layered oblong polygonal parenchymous cells. 5) three discontinuous sclereid bundles above the single vascular bundle. 6) dorsi- ventral mesophyll. (Figs. 1 G- H, 2 G- H). *S. ceratophylla* indicated, 1) ovoid midrib outline with prominent abaxial side. 2) 2- 5 cellular, so long and densely ribbon shaped non glandular trichomes. 3) 1- 2- layered collenchyma and 7- 8- layered oblong polygonal parenchymous cells. 4) three discontinuous sclereid bundles above the vascular bundles. 5) dorsi- ventral mesophyll. (Figs 1 I- J, 2 I- J). In *S. macrosiphon* were observed, 1) semi- circular midrib outline with flat abaxial side. 2) capitate glandular trichomes with two cellular stalk and peltate glandular trichomes. 3) 3- 4 cellular, large, acute and polyhedral- ribbon shaped non glandular trichomes with verrucose apical cell, basal epidermal cell and different cell shapes. 4) single layered collenchyma and 3- 4- layered polygonal parenchymous cells. 5) two sclereid bundles above two vascular bundles. 6) dorsi- ventral mesophyll. (Figs.1K- L, 2 K- L). In cross section of midrib of *S. chorassanica*, 1) sac shaped midrib outline with flat abaxial side. 2) spherical peltate glandular trichomes with smooth surface. 3) 2-3 cellular, short, verrucose, polyhedral non glandular trichomes with basal epidermis cell. 3) 1- 2- layered collenchyma and 6- layered polygonal parenchymous cells. 4) two vascular bundles with two thick cap shaped sclereid above them. 5) isolateral mesophyll were noticed (Figs. 1M- N, 2 M- N). *S. sclarea* showed, 1) sac shaped midrib outline with undulated abaxial side. 2) stripped ovoid peltate trichomes. 3) 2- 3 cellular, verrucose polyhedral ribbon shaped non glandular trichomes. 4) 4- layered collenchymous and 8- 9- layered polygonal parenchymous cells. 5) four sclereid bundles approximately surround the vascular bundles. 6) dorsi- ventral mesophyll (Figs. 1O- P, 2 O- P). In *S. aethiopsis*, 1) semi- circular to campanulate (cup shaped) midrib outline with cavity at abaxial side. 2) peltate glandular trichomes with smooth surface. 3) so long ribbon shaped non glandular trichomes. 4) single layered collenchyma and 4- layered polygonal parenchymous cells. 5) crescent shaped sclereid bundles above the vascular bundles. 6) dorsi- ventral mesophyll were observed (Figs. 1Q- R, 2 Q- R). In *S. chloroleuca* was noticed, 1) oblong elliptical midrib outline with prominent abaxial side. 2) capitate glandular trichomes with

2- 3 cellular stalk and elliptical peltate glandular trichomes with reticulate surface. 3) 2- 3 cellular, polyhedral, subulate (awl shaped) non glandular trichomes. 4) 3- 4- layered collenchymas. 5) 5- 6- layered polygonal parenchymous cells above and below the vascular bundles. 6) sclereid sheath around the vascular bundles. 7) dorsi- ventral mesophyll (Figs. 1S- T, 2 S- T).



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 Fig 1. Cross section of leaf: Cross sections of leaves ($\times 40, 100$). A,B) *S. nemorosa*. C,D) *S. atropatana*. E,F) *S. virgata*. G,H) *S. leriifolia*. I,J) *S. ceratophylla*. K,L) *S. macrosiphon*. M,N) *S. chorassanica*. O,P) *S. sclarea*. Q,R) *S. aethiopis*. S,T) *S. chloroleuca*.



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Fig 2. Electromicrographs of leaf epidermis: Cross sections of leaves ($\times 40, 100$). A,B) *S. nemorosa*. C,D) *S. atropatana*. E,F) *S. virgata*. G,H) *S. leriifolia*. I,J) *S. ceratophylla*. K,L) *S. macrosiphon*. M,N) *S. chorassanica*. O,P) *S. sclarea*. Q,R) *S. aethiopsis*. S,T) *S. chloroleuca*.

- **Discussion**

The results of present research showed the shape of leaf midrib outline in studied species varied elliptical- circular while in *S. sclarea*, *S. virgata* and *S. chloroleuca* were observed sac shaped. The abaxial side changed flat to lobed sometimes prominent. Most of species had peltate glandular trichomes with smooth and verrucose surface although, in *S. sclarea* stripped peltate glandular hairs was noticed. Non glandular trichomes varied

polyhedral to ribbon shaped with smooth or verrucose surface. The minimum and maximum number of collenchymous layers after adaxial epidermis were observed in *S. macrosiphon*, *S. sclarea* and *S. aethiopsis* respectively. Also, *S. macrosiphon* and *S. leriifolia* had minimum and maximum parenchymous layers after adaxial epidermis layer. Moreover, only in *S. atropatana* and *S. chorassanica*, the sclerid bundles were crescent and cap shaped above the phloem and in *S. chloroleuca*, it surrounds the vascular bundles although in the others were discontinuous bundles. Also polygonal parenchymous cells were perceived in all studied specimens. According to the results, *S. macrosiphon* showed significant differences from another ones such as semi- circular midrib outline with flat abaxial side, capitate glandular trichomes with 2 cellular stalk and the minimum parenchyma and collenchyma layers after adaxial epidermis. METCALF and CHALK pointed to polygonal parenchymous cells and 1- 3- layered collenchymous cells around the midrib in *Salvia* leaf [11]. OZDEMIR and SENEL reported continuous or discontinuous sclereid bundles above the phloem in *S. forskahli* [9]. JAFARI and NIKIAN presented dichotomous hairs on the *S. macilenta* Boiss. leaves surface [6]. OZDEMIR & al. reported “adaxial epidermal cells were larger than abaxial epidermal cells and leaf was dors-ventral. Palisade parenchyma was 1- 2- layered. In the median vein of leaf, phloem and sclerenchyma surrounding it were clear and xylem rays were four. Moreover, epidermal cells had sinuous walls in the superficial sections. Stomata were diacytic and present on both surface of leaf” [7]. Furthermore, BERCU & al. reported in *S. nemorosa*, “dors- ventral leaf, numerous glandular and non- glandular trichomes with different in structure, shape and size”. [8]. OZKAN and SOY pointed “*S. blepharoclaena* had single layered epidermis with capitate and peltate glandular and non- glandular trichomes. The upper epidermis cells are bigger than the lower epidermis cells. Parenchyma cells are 2-3- layered and stomata was diacytic” [5].

Conclusion

In conclusion, characters such as the shape of outline of midrib, the shape of sclereid bundles above the phloem, ornamentation of glandular trichoms, the shape of non glandular trichoms were varied among the studied species. It seems the shape of outline of midrib was helpful character to identify specimens.

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