



Anatomy of peduncle and petiole of the medicinal genus *Salvia* L. (Lamiaceae) in Northeast of Iran

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Abstract

The medicinal genus *Salvia* L. belongs to Lamiaceae family with more than 900 species worldwide. It is a very variable and taxonomically difficult genus. This genus has two important centers of diversity viz Asia and Mediterranean area. This investigation used anatomical traits to find distinguishable characters between the *Salvia* species distributed in Northeast of Iran. For anatomy study, cross sections of peduncle and petiole were prepared by hand using razor blade and stained by Safranin and Fast green. In this investigation we studied petiole and stem anatomy of 18 taxa of *Salvia* species from northeastern Iran to determine valuable anatomical characters. Principal Component Analysis and Cluster analysis were used for statistical studies. The result of this study showed that some characters such as collenchyma layer in peduncle, shape of petiole, presence of collenchyma in peduncle and petiole shape of xylem seem to be used as the discriminating characters.

Keywords: Anatomy, Petiole, Peduncle, *Salvia*, Iran



1. Introduction

The genus *Salvia* L. contains over of nearly 1000 species distributed cosmopolitan in the world and largest genus of the family Lamiaceae (Walker and Sytsma 2007). *Salvia* species have medicinal, economical and ornamental value (Ella and Ravid, 1985). This species are used as antibacterial, antioxidant, antidiabetic and antitumor properties (Kamatou et al., 2008). The name of this genus is derived from “Salveo” that means “to save, to recover” in Latin (Estilai et al., 1990). This genus indicates a considerable range of variation in morphology, secondary metabolites and pollination biology (Walker and Sytsma 2007). *Salvia* species indicate high hybridization and therefore diagnose species is difficult. Petiole vascular sheath structure in the family Labiatae has a taxonomic significant. Researchers reported that the variation in leaf structure is correlated with ecological conditions. Nakipoğlu (1993) divided vascular sheaths of petiole on two types: species with and without basal leaves. Vascular bundle of *Salvia* species with basal leaves is divided into fragments whereas without basal leaves is single and large. The aim of the study is to investigate the taxonomical significance of petiole and peduncle anatomy as characters in separating the species of the genus *Salvia* distributed in Northeast of Iran.

2. Materials and Methods

The species (Table 1) were identified according to morphological descriptions in Flora of Iranica (Hedge, 1982a), Flora of Turkey (Hedge, 1982b), Flora of USSR (Pobemidova, 1954) and Flora of Pakistan (Hedge, 1990). For anatomical studies, plant materials were used from FUMH (Ferdowsi Univesity Mashhad Herbarium). To soften dried samples, the specimens preserved in glycerin, alcohol and water for a month and then they were fixed with FAA (formalin 13ml, acid acetic 5ml and alcohol 50% 200ml) (Johansen, 1944) for two weeks. Cross sections of peduncles and petioles were prepared by hand using of razor blade. Sections were discolored by sodium hypochlorite and then they were stained. For the staining, we used safranin and fast green (Johansen, 1944) for 15 minutes and 5 second, respectively. In order to make permanent slides, cross sections mounted with Entellan. Then, measurements and photos were taken using an Olympus light microscope with a C-3AD-4 camera. For 19 anatomical characters, Kruskal-Wallis test was performed to determine which characters significantly differentiated the taxa of *Salvia*. For grouping the similar taxa, cluster analysis was applied using UPGMA implemented in NTSYS (ver2) and then Principle Component Analysis (PCA) was performed using CANOCO ver 4.5 software.

3. Results



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This study was performed with 13 quantitative and 6 qualitative characters. Results of Kruskal-Wallis test indicated that there are not any significant differences between species according to anatomical characters. In other words, the anatomical characters Table (1): list of species, collectors, herbarium numbers and localities of the genus *Salvia* used for the current study

Species	Voucher specimen
<i>S. santolinifolia</i> Boiss.	Tabas to kharv; 700m; Zokaie; 656.
<i>S. eremophila</i> Boiss.	Tabas, between Sange paravarde and Neyestan; 1100m; Rafeie and Zangooei; 35273.
<i>S. tebesana</i> Bunge	SE Birjand, near Near to Nehbandan; 1500m; Rafeie and Zangooei; 28320.
<i>S. macrosiphon</i> Boiss.	SE kalat, between Jalil abad to Ghale no; 1200m; Joharchi; 43000.
<i>S. reuterana</i> Boiss.	N Bojnord, between Naveh and Ghatalmish; 1195m; Memariani & Zangooei; 42522.
<i>S. spinosa</i> L.	Ferdows, abegarm; 1500m; Joharchi; 32280.
<i>S. leriifolia</i> Benth.	NW Gonabad, kamare ziarat; 1159m; Rafei and Zangooei; 26879.
<i>S. shariffi</i> Rech.f.& Esfand.	S Birjand, Band dare; 2000m; Faghihnia and zangooei; 25184.
<i>S. virgata</i> Jacq.	N Farooj, between Ghale safa and Oghaze taze; 1870m; Memariani and Zangooei; 43120.
<i>S. nemorosa</i> L.	Ghoochan, Oghaze kohne; 1800m, Faghihnia and zangooei; 29451.
<i>S. syriaca</i> L.	SE Kalate naderi, between kheshte Naderi and Kalat; 1200m; Faghihnia and zangooei; 42032.
<i>S. aethiopsis</i> L.	NW Bojnord, between Gooing and Baghlogh; Joharchi; 33706.
<i>S. atropatana</i> Bunge	Ghoochan, Gulil; 2100m; Faghihnia and zangooei; 25824.
<i>S. ceratophylla</i> L.	Gonabad, Nakhoo valley; 1600m; Rafei and Zangooei; 28597.
<i>S. sclarea</i> L.	Golmakan, Kalate asheghan, Rashed and Ayatollahi; 13387.
<i>S. chorassanica</i> Bunge	Between Mashhad and Neyshabour, Ghare kohneh; 2290m; Joharchi; 40341.
<i>S. chloroluaca</i> Rech.f.& Aellen	N Farooj, between ghale safa to Oghaze kohne; 1870m; Memariani & Zangooei; 43120.
<i>S. pachystachys</i> Trautv.	W Bojnord, Ghorkhood; 2470; Memariani and Arjmandi; 44001.



could not completely separate species under study because the overlapping variations among the anatomical characters are somewhat high. *Salvia leriifolia* is almost separated from other species because its xylem is rectangle and large, but xylem of other species is Table (2): The measured characters and their coding used in the current study.

Characters	Coding
Number of Collenchyma Layers in Peduncle	Number
Presence of Collenchyma in Peduncle	Present = 0, absent = 1
Shape of Xylem in Peduncle	Arched=0, Quadrangular=1
Thick of Xylem in Petiole	μm
Thick of Phloem in Petiole	μm
Thick of Sclerenchyma in Petiole	μm
Thick of Parenchyma in Petiole	μm
Thick of Collenchyma in Petiole	μm
Thick of Epiderm in Petiole	μm
Thick of Phloem in Peduncle	μm
Thick of sclerenchyma Layers in Peduncle	μm
Thick of Parenchyma Layers in Peduncle	μm
Thick of Collenchyma Layers in Peduncle	μm
Thick of Epiderm in Peduncle	μm
Shape of Xylem in Petiole	Arched=0, Quadrangular=1
Presence of Collenchyma in Petiole	Present = 0, absent = 1
Shape of Petiole	Arched=0, Quadrangular=1
Continuous Vascular Bundle in Peduncle	Continuous=0, Gapped=1
Thick of Xylem in Peduncle	μm

circle and small. The central vascular bundle of petiole in the species including *S. leriifolia*, *S. virgata*, *S. nemorosa*, *S. syriaca*, *S. pachystachys*, *S. macrosiphon*, *S. reuterana*, *S. spinosa*, *S. sharifii*, *S. aethiopsis*, *S. atropatana*, *S. chorassanica*, *S. chloroleuca*, *S. sclarea* and *S. ceratophylla* are divided into pieces, while that of *S. tebesana*, *S. santolinifolia* and *S. eremophila* which belong to group D of Flora Iranica are single and large. Under the epidermis of *S. tebesana*, *S. santolinifolia* and *S. eremophila* there is not collenchyma tissue. In these species parenchyma cells are large and without any typical form. *Salvia virgata* and *S. nemorosa* have been separated from other species (Figs 1, 2, 3), because vascular bundle peduncle of these species was larger than that of other species. In peduncle, the collenchyma tissue is located under the epidermis and it is composed of 2-12 layers. For instance, *S. ceratophylla* has two collenchyma layers and *S. virgata* is distinguished with 12 layers. Parenchyma cells are small and without intercellular spaces. Sclerenchyma groups are located between parenchymatous cells. Vascular bundles are large in corners. The pith of the peduncle is large with parenchymatous cells.

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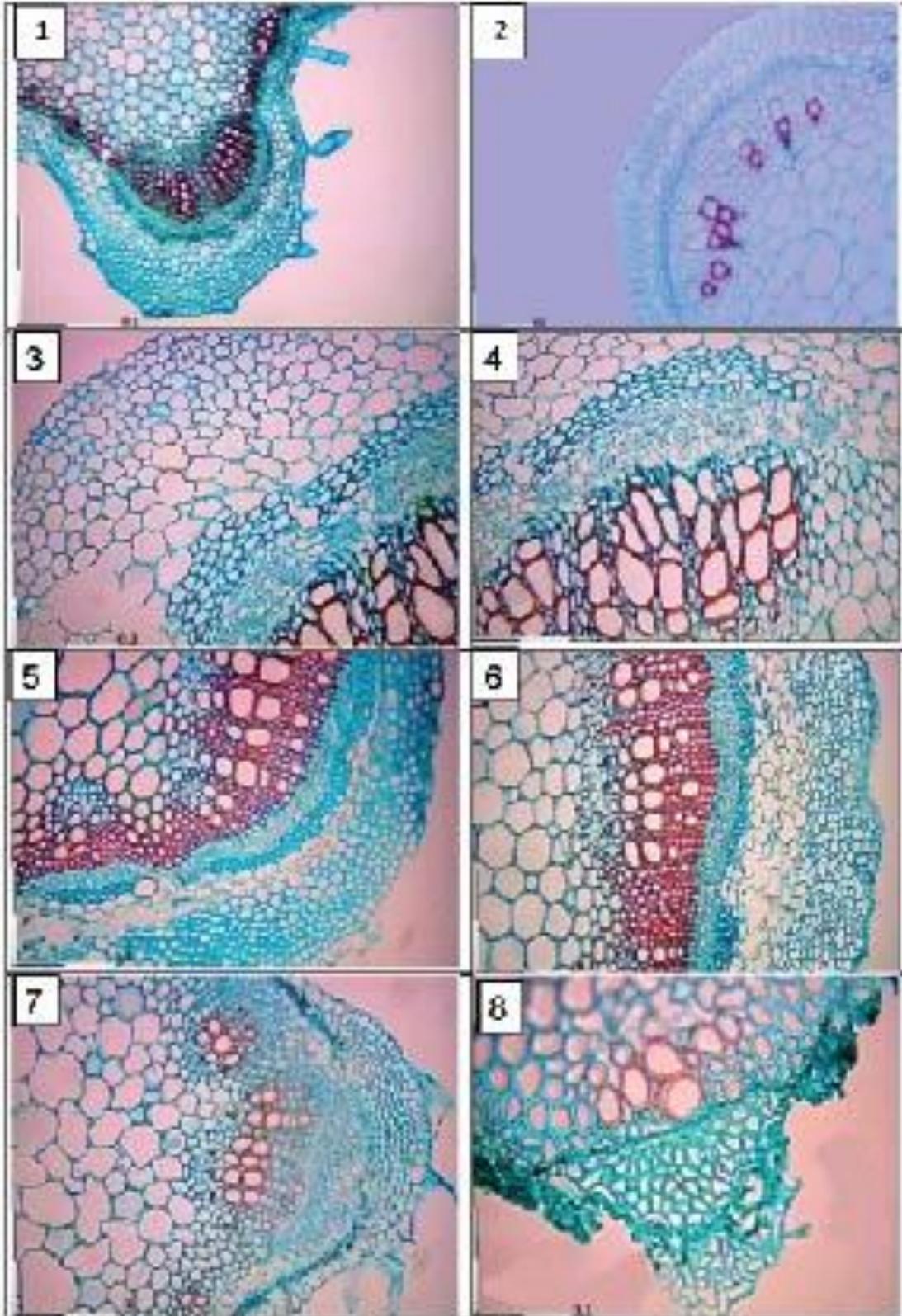
CIVILICA

سازمان جهاد کشاورزی

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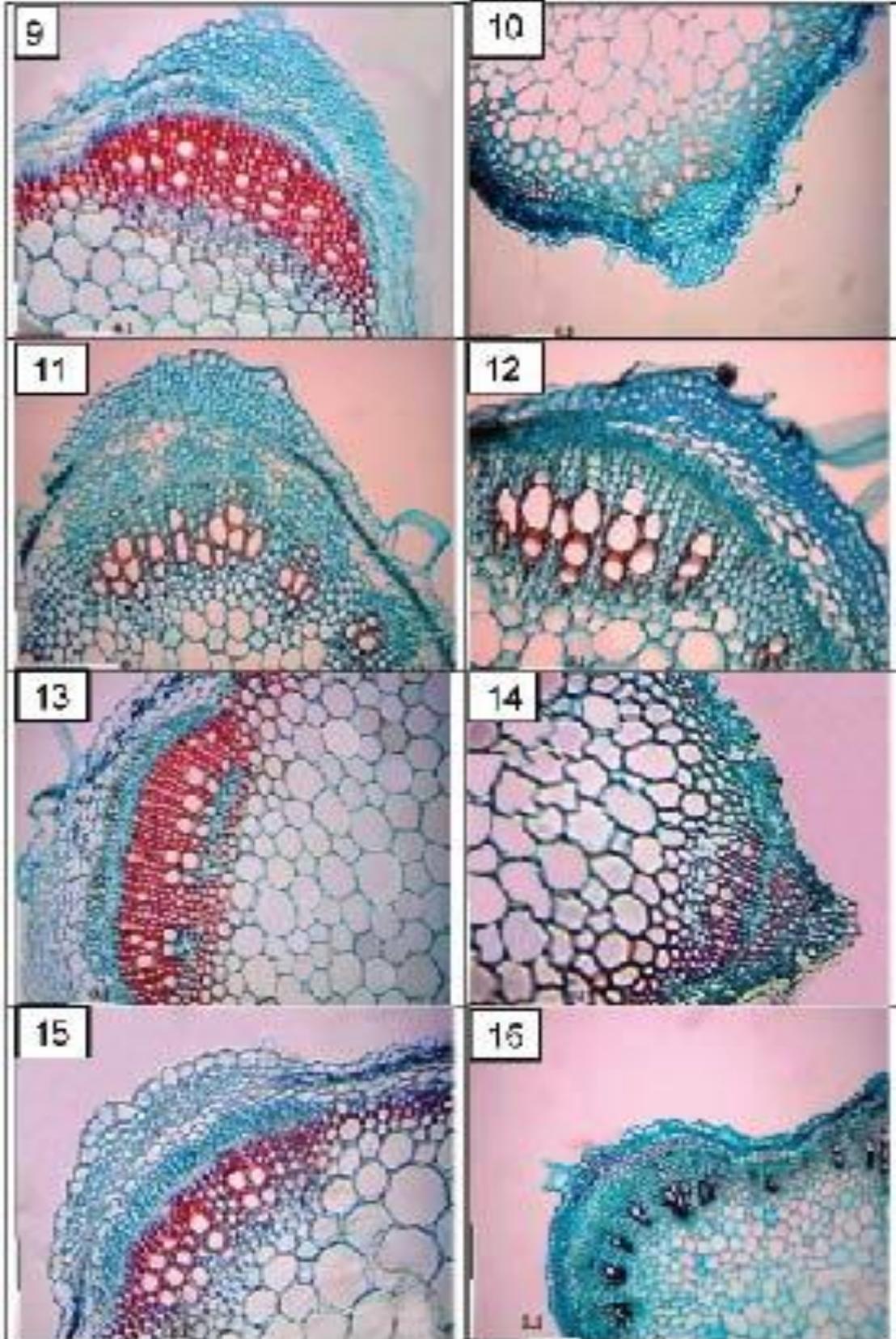
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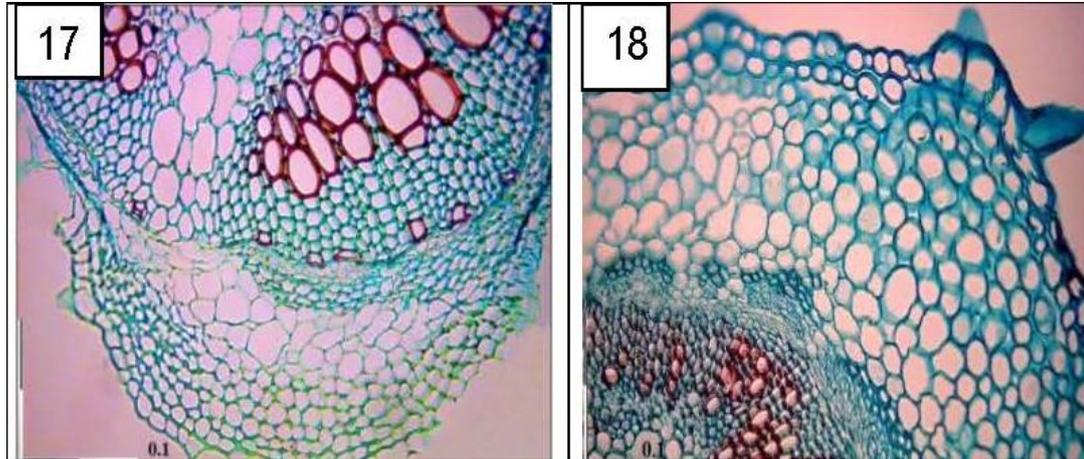
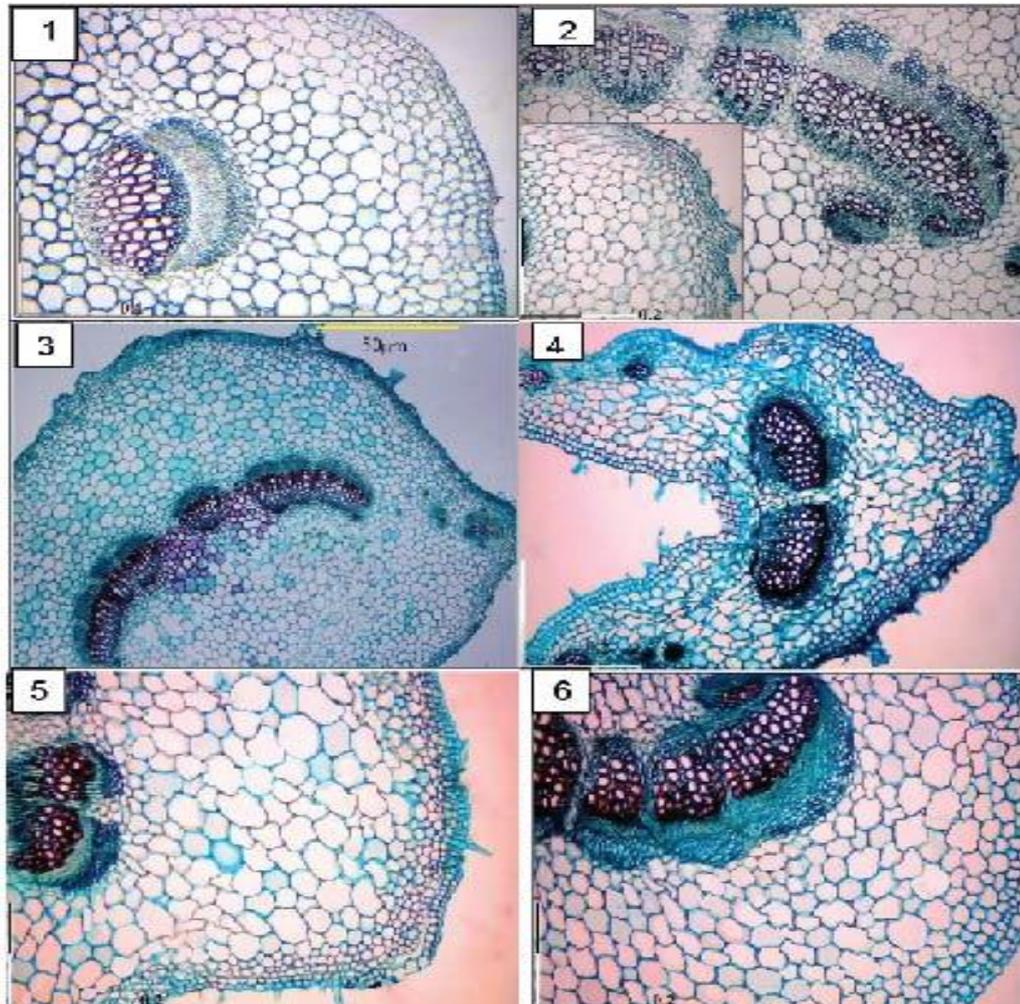


Figure (1): Cross section of the peduncle. 1 *S. aethiopsis*, 2 *S. ceratophylla*, 3 and 4 *S. leriifolia*, 4 and 5 *S. chorassanica*, 6 *S. nemorosa*, 7 *S. reuterana*, 8 *S. tebesana*, 9 *S. sclarea*, 10 *S. santolinifolia*, 11 *S. syriaca*, 12 *S. macrosiphon*, 13 *S. virgata*, 14 *S. eremophila*, 15 *S. pachystachys*, 16 *S. spinosa*, 17 *S. chloroleuca*, 18 *S. shariffi*.



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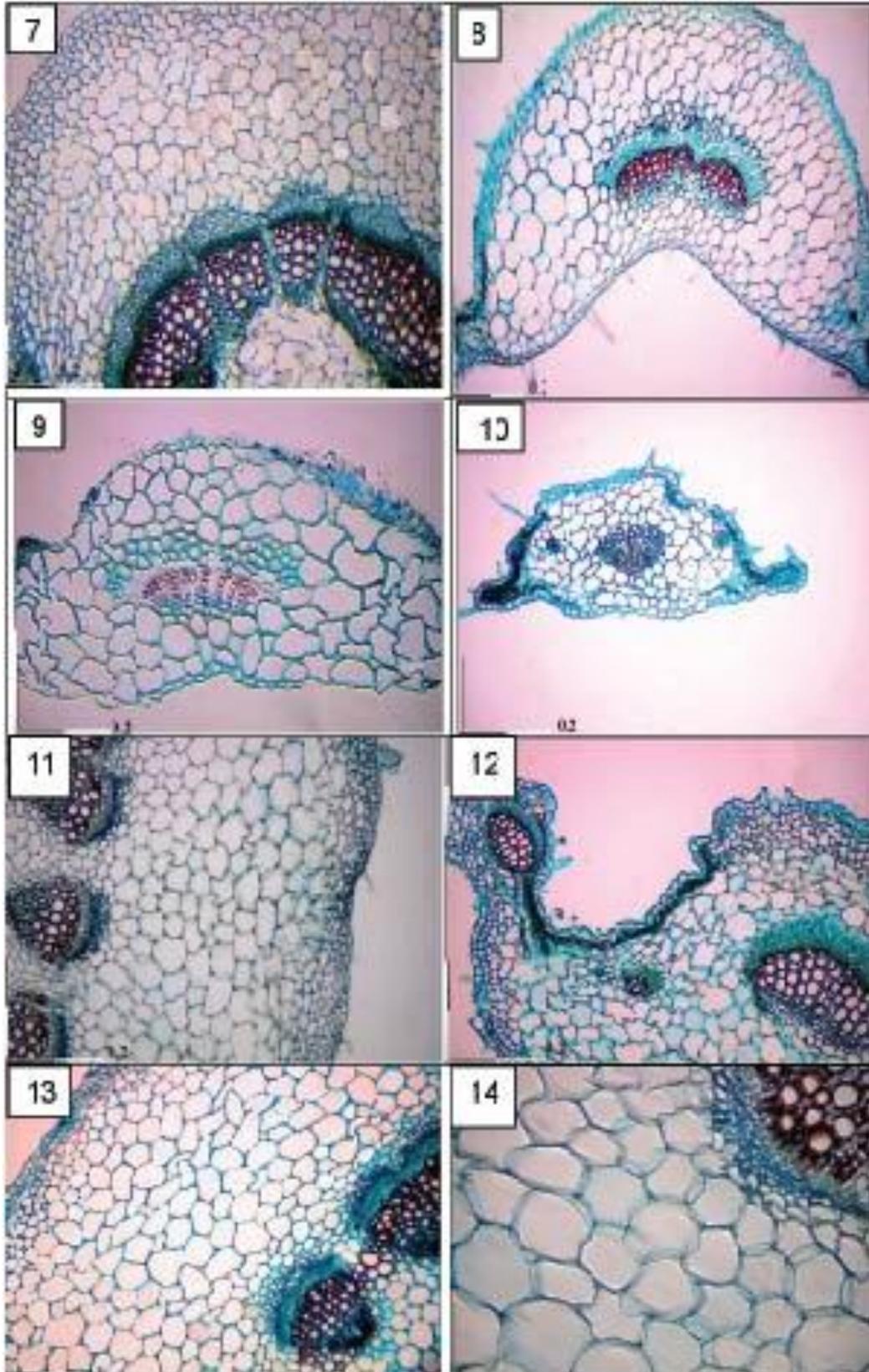
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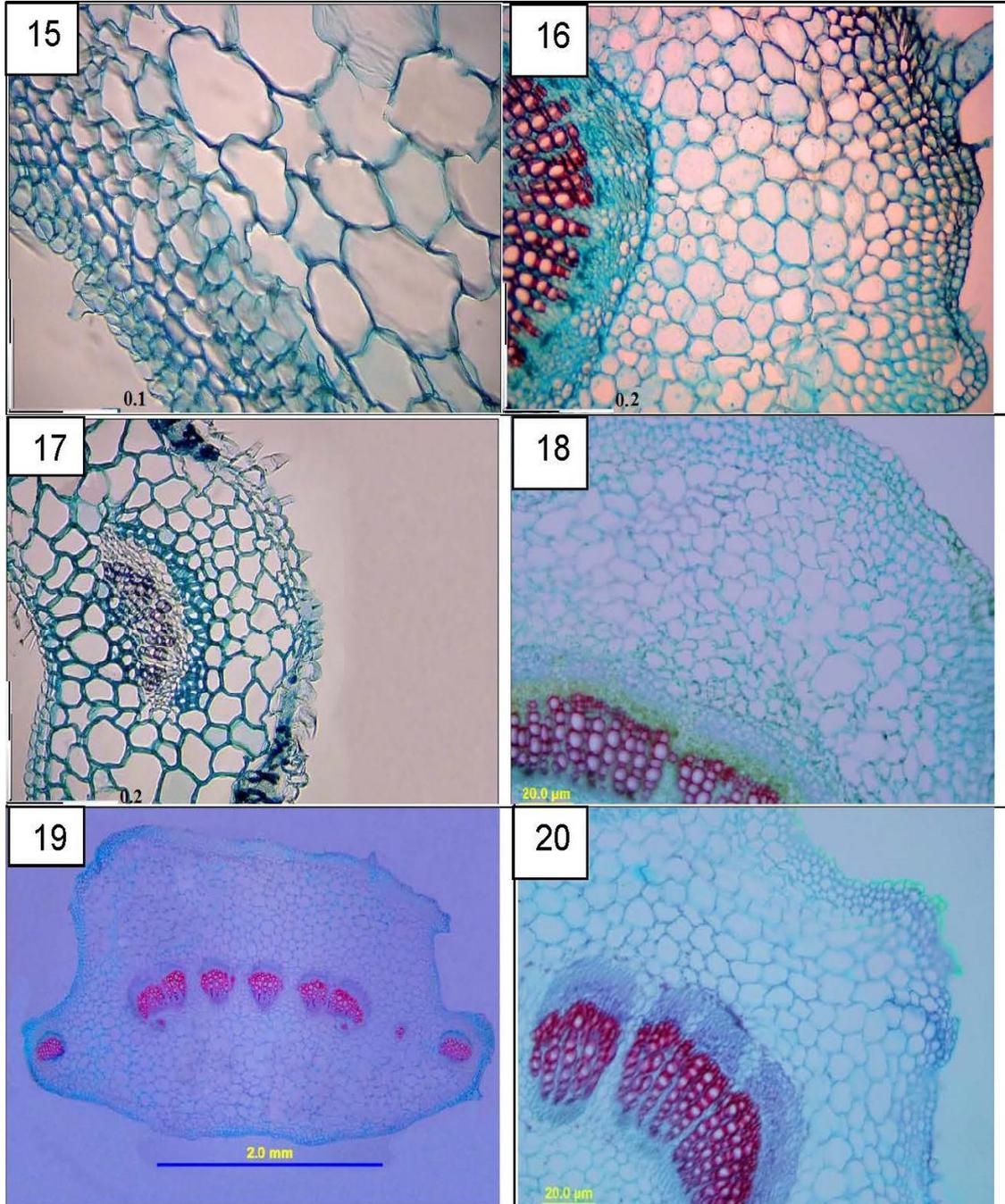


Figure (2): Cross section of the petiole. 1 . *S. leriifolia*, 2 . *S. chloroleuca*, 3 . *S. spinosa*, 4 . *S. chorassanica*, 5 . *S. syriaca*, 6 . *S. nemorosa*, 7 . *S. virgata*, 8 . *S. pachystachys*, 9 . *S. tebesana*, 10 . *S. santolinifolia*, 11 . *S. shariffi*, 12 . *S. reuterana*, 13, 14 and 15 . *S. macrosiphon*, 16 . *S. sclarea*, 17 . *S. eremophila*, 18 . *S. aethiopsis*, 19 . *S. ceratophylla*, 20 . *S. atropatana*.

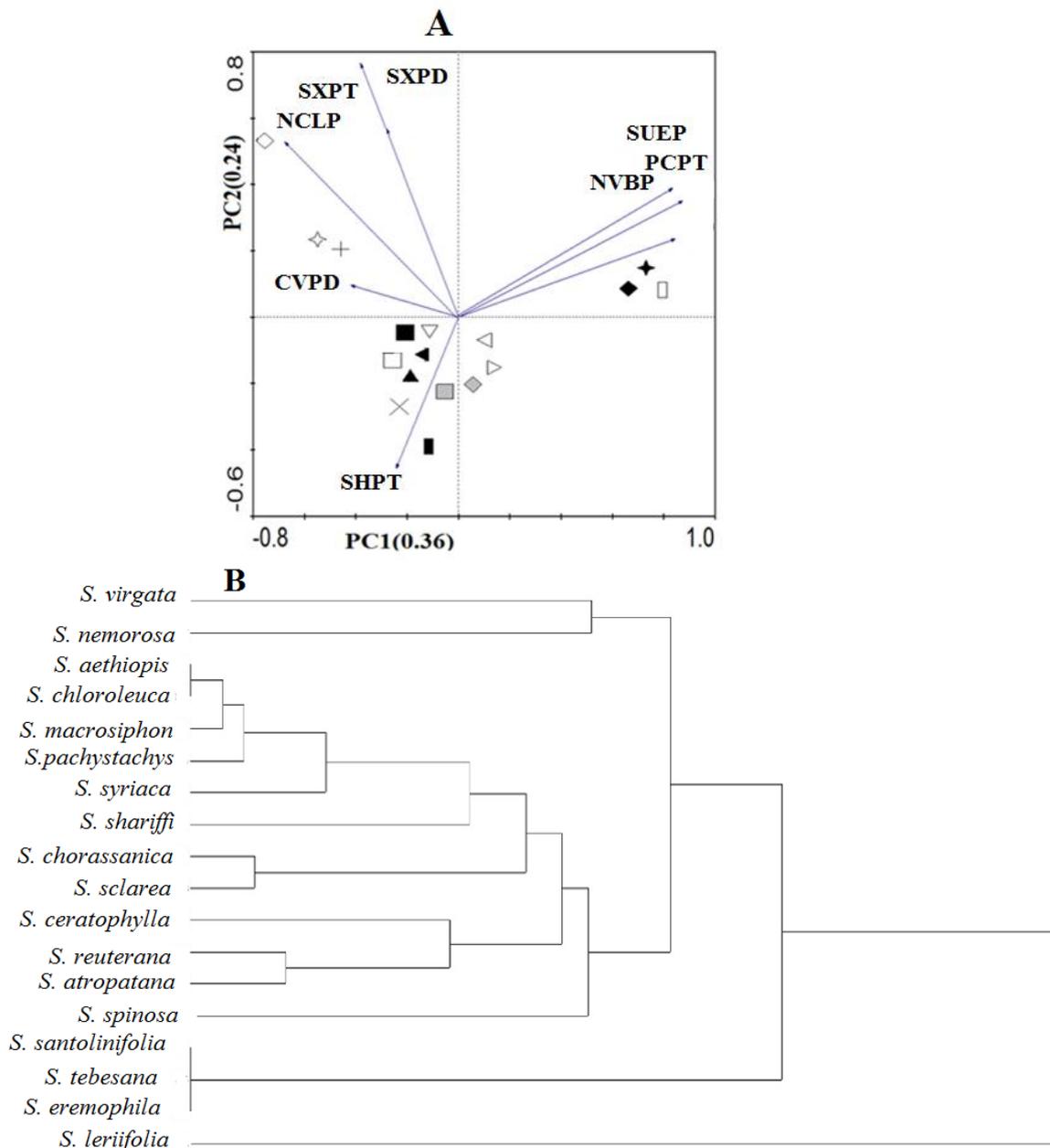


Figure (3): A, Ordination of taxa based on PCA. B, Cluster analysis of taxa by UPGMA. ● *S. eremophila*, ● *S. pachystachys*, ◻ *S. tebesana*, ★ *S. santolinifolia*, ◀ *S. chloroleuca*, ■ *S. chorassanica*, ◊ *S. leriifolia*, × *S. aethiopsis*, S. *atropatana*, ■, ◻ *S. sclarea*, ◁ *S. macrosiphon*, ▽ *S. reuterana*, ▷ *S. spinosa*, ◻ *S. shariffi*, ◊ *S. syriaca*, ▲ *S. ceratophylla*, + *S. virgata*, ◊ *S. nemorosa*.



4. Discussion

The cross-section peduncle of the species of *Salvia* are distinguished with the epidermis of a layer, the number of rows of collenchyma cells 2-12 layers, for example, the number of collenchyma layers in *S. ceratophylla* is 3-2 layers and reaches up to 12 layers in *S. virgata*. *S. virgata* and *S. nemorosa* are composed of many of layers in vascular tissue. The collenchyma layers consecutively have been isolated by the vascular tissue. Collenchyma tissue in *S. tebesana*, *S. eremophila* and *S. santolinifolia* is not existed in the below of epidermis and it is replaced by the Sclerenchyma tissue. This character has been an isolating characteristic for this species in the analysis of the PCA (Fig. 3). Among the investigated species of *Salvia*, a few anatomical differences were observed between species on the qualification of peduncle and species seem to be similar to each other in terms of anatomical characters. For this reason, most species were at the same order in the PCA analysis. The vascular bundles of the peduncle are similar in all species except for the *S. leriifolia* in which the shape of xylem in the vascular sheath of petiole and peduncle is rectangular and it varies within the other species. Regarding this character, *S. leriifolia* is a distinct species in the analysis of PCA and CA. Chlorenchyma tissue has been decorated in the corners of peduncle tissue in all the species. In overall, the anatomical characters could not separate the species of the genus *Salvia* according to the analyses of the PCA and the CA.

5. Conclusion

In this study we investigated the taxonomic significance of petiole and peduncle anatomy of some *Salvia* species distributed in Northeast of Iran. After soften dried samples and staining the sections by safranin and fast green, 19 anatomical characters were measured and then they were analyzed. Results indicated that the number of epidermis layers, the number of rows of collenchymas, the presence and absence of sclerenchyma tissue and the number of vascular bundles are the characters which could separate the species under study.

6. References

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