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Comparative Anatomical Studies of Some Genera of *Lamiaceae* Family in West Azarbaijan in Iran

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Abstract: The *Lamiaceae* family including 220 genera and 4000 species. This family has an important role as a source of medicinal and aromatic plants of commercial important. The objective of this study, is to provide a detailed description of the leaf, stem and petiole anatomy in 8 genus (*Ballota*, *dracocephalum, lycopus, marrubium, nepeta, ocimum, scutellaria and ziziphora)* of *Lamiaceae* family in West Azarbaijan in Iran. The light microscope images of investigated taxa are presented for comparison.

Key words: Anatomy, *lamiaceae*, west Azarbaijan, leaf, stem, petiole

INTRODUCTION

The *Lamiaceae* family is on of largest and most distinctive families of flowering plants, with about 220 genera and almost 4000 species worldwide. This family has an almost cosmopolitan distribution. Some genera of this family have a great diversity in the Mediterranean and C/SW (Hedge, 1986, 1992; Jamzad *et al*., 2003).

Lamiaceae are best known for the essential oils common to many members of the family. These plants have been surely used by humans since prehistoric times. Evidence from archeological excavations shows that some species of this family, which are now know only as wild plants, has been cultivated at local scales in this past (Rivera and Obon, 1992b). Some species of family are used as food flavorings and vegetables (Naghibi *et al*., 2005). The family *Lamiaceae* has an important role as a source of medicinal and aromatic plants of commercial importance (Kaya *et al*., 2000).

The taxonomic value of the indumentum and its importance in systematic and phylogenetic relationships is well known in *Lamiaceae* and such related families as *Verbenaceae* and *Scrophulariaceae* (Abu-Assab and Cantino, 1987; Cantino, 1992; Metcalfe and Chalk, 1950).

Glandular hairs are widely distributed over the aerial reproductive and vegetative organs of plants of the *Lamiaceae*, a family of great economic importance and their structure has been investigated by many researchers (Bosabalidis, 1990; Maleci and Servettaz, 1991; Servettaz *et al*., 1992; Bourett *et al*., 1994; Serrato-Valenti *et al*., 1997; Kolalite, 1998; Ascensao *et al*., 1999; Rapisarda *et al*., 2001; Kaya *et al*., 2003).

In this study, we report a comparative study on leaf, stem and petiole anatomy of some genera in order to improve our knowledge of their anatomy for systematics and to help separate similar genera.

MATERIALS AND METHODS

The plant material was collected from different localities in West Azarbaijan. Voucher specimens are deposited at the Herbarium of the Science Faculty Urmia University, Iran.

Living material was stored in alcohol: Glycerin in ratio 1:1 for anatomical studies. All sections were taken from leaves, stem and petiole in the middle part of plants. Transverse sections of lamina, stem and petiole and surface preparations of leaves were prepared manually. All sections were embedded in glycerin-gelatin and examined with an light microscope with a camera lucida. The length, width and frequency of the stomata were measured with an ocular micrometer using the surface section from upper and lower parts of the leaf epidermis.

RESULTS AND DISCUSSION

Leaf: Transverse sections of lamina and surface preparations of both epidermises revealed the following elements (Fig. 1).

The adaxial and abaxial epidermises of the leaf consist of uniseriate oval, square and rectangular cells in transverse section. Cells of the adaxial epidermis were larger than those of the abaxial epidermis. Covering trichomes consist unicellular and multicellular.

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Table 1: Overview of leaf anatomical characters of species under investigation

Species	Lamina thinnest (µm)	Leaf type	Stomata frequency(mm²) Adaxial	Abaxial
Ballota nigra subsp. *curdia*	95	Dorsiventral	-	43±1
Dracocephalum moldavica	200	Dorsiventral	54±1	92±1
Lycopus europaeus	140	Dorsiventral	73±1	81±1
Marrubium parviflorum	90	Dorsiventral	46±1	59±1
Nepeta cataria	210	Dorsiventral	38±1	50±1
Ocimum basilicum	237	Dorsiventral	46±1	51±1
Scutellaria pinnatifida subsp. *pichleri*	160	Isobilateral	35±1	51±1
Ziziphora clinopodioides	216	Isobilateral	59±1	68±1

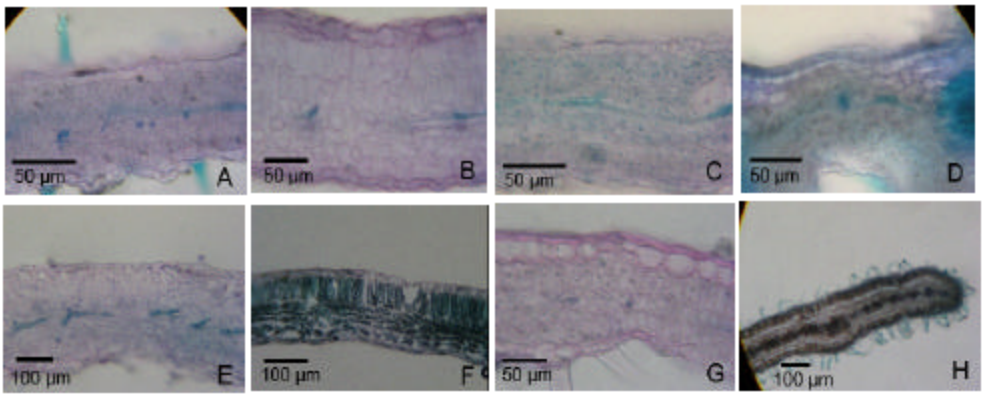

Fig. 1: Transverse sections of lamina: (A) *Ballota nigra* subsp *curdica* (B) *Dracocephalum moldavica* (C) *Lycopus europaeus* (D) *Marrubium parviflorum* (E) *Nepeta cataria* (F) *Ocimum Basilicum* (G) *Scutellaria pinnatifida* subsp *pichleri* (H) *Ziziphora clinopodioides*

Multicellular hairs more abundant. Glandular type was only in *Ballota nigra* subsp. *curdica* and nonglandular existed in all species.

The stomata were diacytic and anomocytic in species, as recorded in other genera of *Lamiaceae* by Inamder and Bhatt (1972). A number of stomata per mm² of leaf on abaxial surface has higher than those of the adaxial surface. A number of stomata range were 92±1 on abaxial epidermis of *Dracocephalum moldavica* and 35±1on adaxal epidermis of *Scutellaria pinnatifida* A.Hamit subsp pichleri per mm². The species exhibit obvious differences in lamina thickness, which ranges from 90-216 µm. The lamina were thinnest in *Marrubium moldavica* (90 µm) and the thickest in *Ocimum basilicum* (237 µm). In all the taxa investigated, leaves were dorsiventral expect *Scutellaria pinnatifida* A. Hamit subsp pichleri and Ziziphora clinopodioides that were isobilateral (Table 1). Type of vascular bundle in all species was collateral.

Stem: Transverse sections taken from the middle part of the stems were observed as follows (Fig. 2).

In all species, the epidermis was composed of a single layer. The collenchyma tissue was located immediately under the epidermis and in corners was thicker than between corners. The shape of collenchyma cell is ovoid. Parenchyma tissue was located under the collenchyma cell. The species exhibit obvious differences in number of layers on collenchyma, parenchyma tissue and also number of vascular bundle (Table 2).

Petiole: Metcalfe and Chalk (1979) pointed out that in many families, especially in *Lamiaceae*, the structure of the petiole was important in terms of taxonomy. Epidermis was single layered. Collenchyma was located under the epidermis. Cortex consist of large parenchyma cell. There was a large vascular bundle in the median region of the petiole and small bundle were present on both sides of this bundle.

A number of layers collenchyma and parenchyma were different in all species. A number of layers collenchyma in *Marrubium parviflorum* (5-6 layers) and *Ziziphora clinopodioides* (1-2 layers) were the highest and the least, respectively. Also, *Nepeta cataria* with 6-8 layers and *Ballota nigra* subsp *curdica* with 1-2 layers have the highest and the least number of layers parenchyma, respectively (Table 3).

The highest number of large and small vascular bundles and the least exist in *Dracocephalum moldavica* (3-6) and *Nepeta cataria* (1+2), respectively (Fig. 3).

Table 2: Overview of stem anatomical characters of species under investigation

Species	A number of parenchyma layers	A number of collenchyma layers		A number of vascular bundle
		Cornera	Between of corners	
Ballota nigra subsp. *curdica*	2-3	12-13	3-4	9-10
Dracocephalum moldavica	5-6	6-7	1-2	9-10
Lycopus europaeus	5-6	4-5	3-4	19-21
Marrubium parviflorum	7-9	8-10	1-2	8
Nepeta cataria	3-4	10-11	3-4	13-14
Ocimum basilicum	6-7	6-7	1-2	8-10
Scutellaria pirnatifida subsp. *pichleri*	5-6	6-7	2-3	4-5
Ziziphora clinopodioides	4-5	6-7	3	8-9

Table 3: Overview of petiole anatomical characters of species under investigation

Species	No. of vascular bundle		No. of layers	
	Median region	Sides	Collenchyma	Parenchyma
Ballota nigra subsp, *curdica*	2	2	4-5	1-2
Dracocephalum moldavica	6	3	3	6-7
Lycopus europaeus	4	3	3-4	4-5
Marrubium parviflorum	4	2	5-6	6-7
Nepeta cataria	2	1-2	3-4	6-8
Ocimum basilicum	4	1	3-4	5-6
Scutellaria pirnatifida subsp. *pichleri*	6	1	3	2-3
Ziziphora clinopodioides	3	1	1-2	2-3

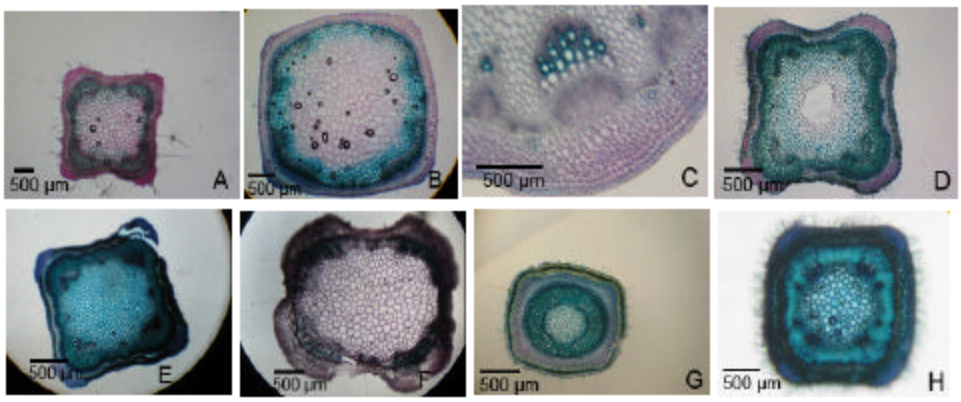

Fig. 2: Transverse sections of stem: (A) *Ballota nigra* subsp *curdica* (B) *Dracocephalum moldavica* (C) *Lycopus europaeus* (D) *Marrubium parviflorum* (E) *Nepeta cataria* (F) *Ocimum basilicum* (G) *Scutellaria pirnatifida* subsp *pichleri* (H) *Ziziphora clinopodioides*

The internal structure of the species under investigation indicate a unique similarity in their anatomical characters in spite of belonging to different genera e.g., in leaf midrib region, all species having a ground tissue consist of lamellar collenchyma, parenchyma cells surrounding the vascular bundle, the vascular tissue was on vascular bundle arched to rounded, the mesophyll at the intercostal regions differentiated into palisade type cells and spongy type cells. In all species studied, stomsta type was amphistomatic except *Ballota nigra* subsp *curdica*.

Lamellar collenchyma located at stem angles and parenchyma cells in between of angles are present. The cortex is characteristic with a bundle sheath followed by outer phloem fibers, mostly as strands well developed at the angles. Pith, is characteristic with a polygonal homogenous parenchymous cells.

In petiole, type of vascular bundle in all species is collateral, collenchyma tissue located under the epidermis and also, parenchyma tissue located under the collenchyma tissue.

In spite of the similarity in most epidermal and internal anatomical characters of these species under investigation the results show that the 8 genus that is growing naturally in the West Azerbaijan can be divided in 3 groups. Group 1: This group includes 5 species:

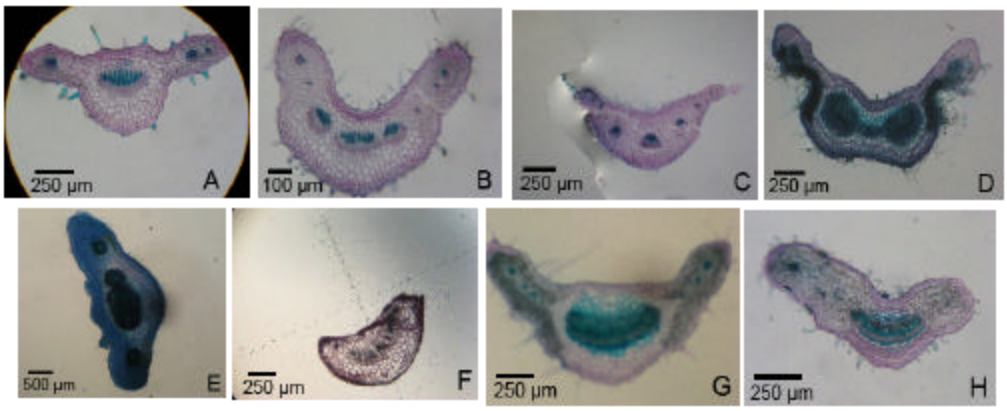

Fig. 3: Transverse sections of petiole: (A) *Ballota nigra* subsp *curdica* (B) *Dracocephalum moldavica* (C) *Lycopus europaeus* (D) *Marrubium parviflorum* (E) *Nepeta cataria* (F) *Ocimum basilicum* (G) *Scutellaria pinnatifida* subsp *pichleri* (H) *Ziziphora clinopodioides*

Nepeta cataria, Dracocephalum moldavica, Lycopus europaeus, Ziziphora clinopodioides and Ocimum Basilicum. These species are characterized by presence of nonglandular, unbranched trichomes, a number of parenchyma and collenchyma layers of petiole and stomata frequency in adaxial and abaxial surface (92±1 -38±1 mm^{-2}).

This group can be divided in 2 subgroup with reference to a number of vascular bundle in stem , length and width of stomata, subgroup 1 including *Nepeta cataria, Dracocephalum moldavica, Lycopus europaeus* and *Ziziphora clinopodioides* and subgroup 2 including *Ocimum basilicum*. Group 2: This group contains the 2 species of *Marrubium parvifloum* and *Ballota nigra* subsp *curdica*, which are characterized by number of palisade parenchyma (1-seriate), lamina thickness and a number of collenchyma layers. Group 3: This group including *Scutellaria pinnatifida A. Hamit subsp pitcheri*, which is characterized by a leaf type (isobilateral), stomata frequency (51±1-35±1 mm^{-2}).

CONCLUSION

All these taxa in group 1, except *Ocimum* have been classified as members of the *Mentheae* by Cantino (1992) and Cantino *et al*. (1992). Within the tribe *Mentheae*, the anatomical studies show that at least 2 subgroups are present (subgroup 1 and 2), which do not correlate with former classification that were based on flower, pollen, embryo or seed morphology.

However, all the taxa in group 1 have been classified as members of the subfamily *Nepetoideae*, the group 2 and 3 have been classified as members of the subfamily *Lamioideae* and *Scutellarioideae*, respectively. However, dividing these taxa in 3 groups agreed with Cantino *et al*. (1992).

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