

STEM AND LEAF ANATOMY OF THREE TAXA IN LAMIACEAE

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Abstract

Stem and leaf anatomy of *Origanum rotundifolium* Boiss., *O. vulgare* L. ssp. *viride* (Boiss.) Hayek, *Teucrium hircanum* L., belonging to Lamiaceae were characterized and compared. Some important differences were determined in the taxa. In addition, trichome diversity was investigated by light microscopy and three main types of trichomes (peltate, capitate glandular and non-glandular) were observed in three taxa.

Introduction

The Lamiaceae is a large family which is represented by about 258 genera and 3500 species in the world (Duarte and Lopes 2007). It is the third largest family in Turkey with 45 genera and 574 species of which 256 are endemic (Güner *et al.* 2000) and the country is regarded as a gene center for this family (Başer 1993).

Origanum L. genus is represented by 25 taxa in the Turkish flora (Güner *et al.* 2000). Almost a half of them (12) are endemic to Turkey. Some species contain phenolic substances such as carvacrol and thymol. Thymol is also used as a starter in the synthesis of some pharmacological active ingredients, for tooth filling, in the preparation of antiseptic baths and soaps and in the preservation of some foods (Baytop 1999). *Teucrium* L. is a large and polymorphic genus of the Lamiaceae. The genus *Teucrium* comprises totally 44 taxa belonging to 8 sections in Turkish flora (Yildirimli 2008). Trichomes are among the most useful taxonomic characters in *Teucrium* L. (Navarro and El Oualidi 2000) described infrageneric relationships between sections depending on trichome types.

Many species of Lamiaceae have been used as herbal teas in Turkey and most of them have great importance due to their economic values and many of them are used as raw material in cosmetic industry. Some species are used in official and traditional medicines in Anatolia, Europe and China (Baytop 1999, Cui *et al.* 2003). Some species are cultivated as ornamental plants (Rudy 2004). The morphology and anatomy of several species have been studied so far (Tahir *et al.* 1995). In Lamiaceae, the systematic value of trichome types was demonstrated by Abu-Assab and Cantino (1987) in the subtribe Melittidinae (Dumort.) Endlicher. Glandular trichomes producing essential oils of commercial value are widespread on leaves and flowers of the Lamiaceae and their structures have been examined anatomically and micromorphologically by several authors (Akçin *et al.* 2011, Kaya *et al.* 2007). In recent years, anatomical characters have been used in taxonomy (Ağbagwa and Ndukwu 2004, Kharazian 2007, Eminagaoglu *et al.* 2012, Ozcan *et al.* 2014).

The main object of this study is to investigate detailed description of anatomical structures of the stem and leaf in three Lamiaceae taxa by using light microscopy. The variation in leaf characters is discussed with respect to their potential systematic value and in relation to our previous work.

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Materials and Methods

Plant samples were collected from Artvin Province and dried according to herbarium techniques and stored in Artvin Coruh University Herbarium (ARTH). The localities and collection data are listed in below.

Origanum rotundifolium Boiss.; A8 Artvin: Yeni mahalle (İskebe), stony slope, 500 m, 08.vii.2011, Ö. Emin. 8836.

O. vulgare L. ssp. *viride* (Boiss.) Hayek; A8 Artvin: Hatila Valley National Park, roadside, 570 m, 08.vii.2011, Ö. Emin. 8839.

Teucrium hircanum L.; A8 Artvin: Hatila Valley National Park, roadside, 600 m, 08.vii.2011, Ö. Emin. 8838.

Anatomical investigations were carried out on the cross-sections of the upper and lower stems, the leaves and surface sections of the leaves. All sections were prepared manually and stained with Hematoxyline solution. They are preserved on the permanent slides in glycerin-gelatin solution. The photographs of the sections were taken using an Olympus BX-53 research microscope with digital camera attachment DP-73.

Five cross-sections from at least three different individual plants of each taxon were measured for each sample to assess the consistency of anatomical characters. Ten peripheral slides were prepared for each taxon and 50 stomatal lengths were measured on each slide. The stomatal index was calculated.

Results and Discussion

In this study, three taxa belonging to Lamiaceae were investigated and their anatomical characters are represented here for the first time from Turkey. Two of the investigated taxa belong to the genus *Origanum* and one species belongs to the *Teucrium*. Cross sections in the middle parts of stems and transverse section of the lamina, midrib and both epidermises of leaf were investigated, and representative anatomical characters are summarized in Tables 1 and 2.

Table 1. Stem features of three Lamiaceae taxa.

Characters	<i>O. rotundifolium</i>	<i>O. vulgare</i> ssp. <i>viride</i>	<i>T. hircanum</i>
Epidermis length (µm)	14.47 ± 0.59	13.53 ± 0.84	14.53 ± 0.34
Epidermis width (µm)	25.73 ± 1.12	19.00 ± 0.57	20.87 ± 1.48
Cortex thickness in the corner (µm)	201.93 ± 19.84	143.40 ± 4.85	203.40 ± 5.14
Number of cortex layer	12 - 13	11 - 14	12- 15
Corner collenchyma thickness (µm)	112.73 ± 10.88	71.60 ± 7.67	125.00 ± 4.37
Vascular bundle			
Phloem length (µm)	56.53 ± 4.99	58.87 ± 1.37	67.90 ± 1.21
Xylem length (µm)	294.13 ± 7.72	352.80 ± 15.84	339.20 ± 10.29
Trachea size (µm)	30.31 ± 0.96	23.05 ± 0.81	28.83 ± 0.93
Pith cell size (µm)	79.20 ± 3.59	66.73 ± 2.36	85.65 ± 2.11

The stem anatomical characters on the three taxa of this family showed similar anatomical characteristics, but some important distinctions are also found among the taxa. The stem structure in all the investigated species is more or less uniform. In cross-section, the stem is quadrangular, collenchyma tissue covers a large area at the corner and one-two layers between the corners. These features are previously reported in some other investigated members by several authors

(Metcalf and Chalk 1972, Bosabalitis and Kokkini 1997, Gönüz and Özörgücü 1999, Dinç *et al.* 2008). On the other hand, the cortex size and collenchyma thickenes in the corners, trachea size and pith cell size *O. vulgare* ssp. *viride* has the smallest values among the three taxa. The vascular cambium is visible in two *Origanum* taxa, but not the other. Two investigated *Origanum* taxa have pith cavity at the center of the stems. In previous study Gönüz and Özörgücü (1999) reported starch in *O. onites* L., but two *Origanum* taxa investigated in the present study do not have starch.

Table 2. Leaf anatomical characteristics of three Lamiaceae taxa.

Characters		<i>O. rotundifolium</i>	<i>O. vulgare</i> ssp. <i>viride</i>	<i>T. hircanum</i>
Upper epidermis	length(μm)	20.97 \pm 0.93	22.07 \pm 0.82	17.30 \pm 1.84
	width (μm)	32.17 \pm 2.53	32.07 \pm 2.58	26.00 \pm 3.47
Lower epidermis	length(μm)	19.93 \pm 1.06	14.88 \pm 0.69	9.30 \pm 0.44
	width (μm)	30.63 \pm 2.13	21.40 \pm 1.13	13.40 \pm 0.97
Midrib mesophyll	breadth (μm)	447.20 \pm 35.41	378.40 \pm 10.68	538.40 \pm 27.30
Lamina mesophyll	breadth (μm)	208.93 \pm 7.92	134.27 \pm 3.94	86.90 \pm 3.47
Trachea size	(μm)	14.90 \pm 0.66	12.17 \pm 0.36	16.35 \pm 0.76
Vascular bundle	length (μm)	168.80 \pm 11.18	140.80 \pm 4.07	219.03 \pm 41.41
	breadth (μm)	260.20 \pm 6.30	360.00 \pm 35.54	442.07 \pm 10.43
Number of the palisade cell line		1 - 2	1	2
Number of the spongy cell line		4 - 7	4 - 5	1 - 2
Adaxial cuticle thickness (μm)		4.10 \pm 0.24	4.10 \pm 0.10	2.80 \pm 0.20
surface epidermal cell		Polygonal shape, sinuous wall	Polygonal shape, straight wall	Irregular shape, sinuous wall
	Stomata length(μm)	22.64 \pm 0.99	23.45 \pm 0.68	0
	width (μm)	16.36 \pm 0.66	17.17 \pm 0.39	0
Stomatal index		11.52 \pm 0.98	5.99 \pm 0.57	0
Number of stomata (1 mm ²)		56.25 \pm 5.92	65 \pm 6.11	0
Abaxial cuticle thickness (μm)		3.50 \pm 0.22	3.80 \pm 0.20	2.40 \pm 0.24
	surface epidermal cell	Irregular shape, sinuous wall	Irregular shape, repand wall	Irregular shape, sinuous wall
Stomata	length(μm)	23.08 \pm 0.41	22.81 \pm 0.26	20.62 \pm 0.07
	width (μm)	16.58 \pm 0.64	17.17 \pm 0.39	16.92 \pm 0.12
Stomatal index		15.76 \pm 0.71	21.62 \pm 0.62	18.27 \pm 1.10
Number of stomata (1 mm ²)		121 \pm 5.79	335 \pm 14.97	430 \pm 29.95

\pm : Standard error.

Origanum vulgare has four subspecies in Turkey and *O. vulgare* ssp. *viride* is one of them. Petiole anatomical features of *O. vulgare* ssp. *vulgare* were examined by Akçin *et al.* (2011) and multi-lobed and broadly arc shaped vascular bundle, eglandular and glandular hairs were reported. In addition, leaf anatomy of *O. vulgare* ssp. *viridulum* (Martrin-Donos) Nyman was previously investigated from Greece by Bosabalitis and Kokkini (1997). This taxon is a synonym of *O. vulgare* ssp. *viride*. Bosabalitis and Kokkini (1997) investigated three subspecies of *Origanum vulgare* from Greece and reported that the leaves of *O. vulgare* subsp. *viridulum* have the thinnest lamina (totally 57.53 \pm 04.46) among the four subspecies. Moreover, it was reported that the cells of the palisade parenchyma were not elongate as in the typical dicot leaf and they were short, leaving large intercellular spaces between them.

Our results are generally in accordance to these previous reports. In Turkey, however, the palisade cells of *O. vulgare* ssp. *viride* are elongate and almost a half of the leaf lamina. Furthermore, Bosabalitis and Kokkini (1997) reported that the upper leaf surface possesses more

peltate glandular trichomes than the lower one. Lamina mesophyll thickenes of this taxon is found to be $134.27 \pm 03.94 \mu\text{m}$. According to this result, the plant growing in Turkey has much thicker leaf lamina than that of Greece. Additionally, stomatal frequency per mm^2 in both surfaces in Turkey is many more than that of Greece (Table 2). The vascular bundle is arc shaped and two-lobed in the leaf midrib.

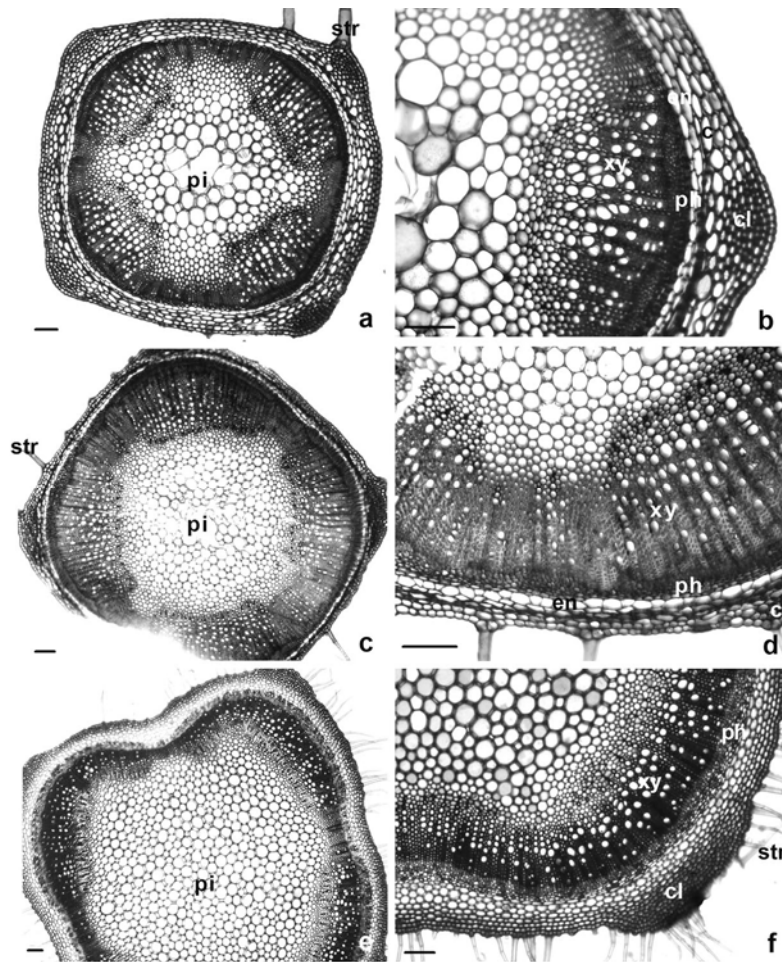


Fig. 1. Cross section of stem. a, b: *Origanum rotundifolium*, c, d: *O. vulgare* ssp. *viride*, e, f: *Teucrium hircanum*. cl: collenchyma, en: endodermis, ph: phloem, pi: pith, str: simple trichome, xy: xylem. Bar = 100 μm .

Trichome types and morphology of the indumentum have taxonomic values in Lamiaceae (Metcalf and Chalk 1972, Cantino 1990, Navarro and El Oualidi 2000). Three different trichome types on the stems of the investigated taxa were observed: peltate, capitate glandular and non-glandular trichomes. Capitate glandular and peltate trichomes can be distinguished by head size and stalk length (Ascensão and Pais 1998). Non-glandular trichomes are sparse in *O. rotundifolium*, while it is densely found in *Teucrium hircanum* (Figs 4, 6). Two examined *Origanum* taxa have peltate glandular trichomes, sunken inside the epidermal layers, while these

are one stalk cell in *T. hircanum*. Mráz (1998) mentioned that glandular trichomes have also a taxonomic value at the specific and a subspecific level in *Teucrium*. In addition to simple non-glandular trichomes, flagelliform hairs are observed in *T. hircanum* (Fig. 5). These type trichomes were previously reported in *T. montanum* by Dinç *et al.* (2011).

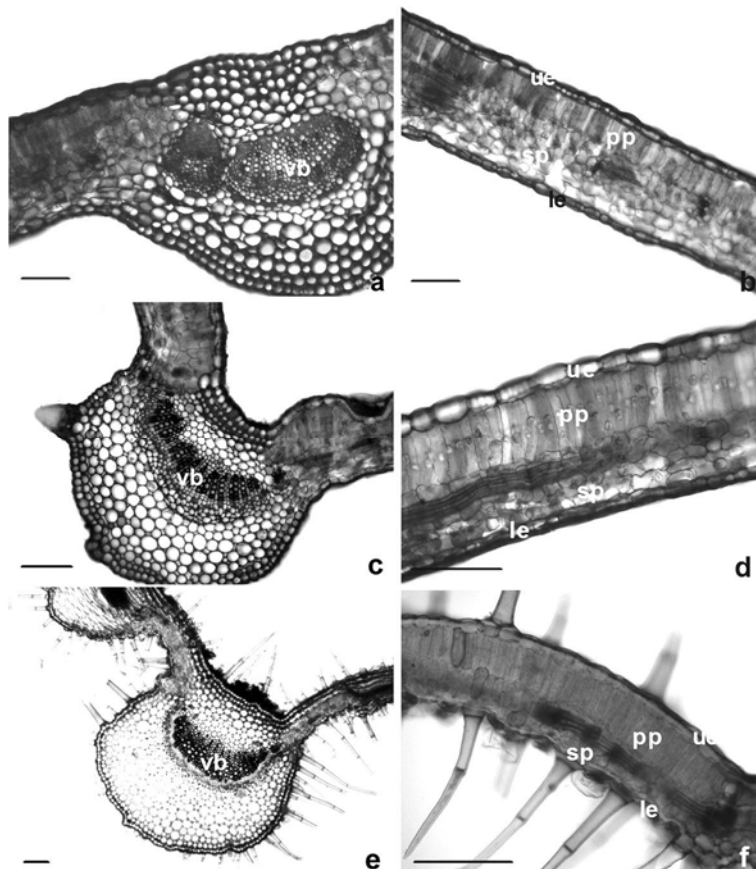


Fig. 2. Cross section of leaf. a, b: *Origanum rotundifolium*, c, d: *O. vulgare* ssp. *viride*, e, f: *Teucrium hircanum*. le: lower epidermis, pp: palisade parenchyma, sp: spongy parenchyma, ue: upper epidermis, vb: vascular bundle. Bar = 100 μ m.

The leaves of the three taxa show dorsiventral mesophyll. However number of palisade layer and spongy layer differ among the taxa. The palisade tissue covers almost a half of the mesophyll in *O. vulgare* ssp. *viride*, while it comprises much of the mesophyll (about 70 - 80%) of *T. hircanum* (Fig. 2f). It is reported that high density of trichomes and thick palisade tissue of mesophyll could be interpreted as xerophytic characters. These features were reported in *T. sandrasicum* (Dinç *et al.* 2008), *T. montanum* and *T. polium* (Dinç *et al.* 2011). The upper epidermal cells are distinctly larger than the lower ones in *T. hircanum*, while it is almost equal to the lower one in *O. rotundifolium*. The spherocrystals were reported in *T. sandrasicum* by Dinç *et al.* (2008), but they are not found in *T. hircanum* or other two taxa. *T. hircanum* has hypostomatic leaf and densely trichomes occurring the epidermis. These characters are also interpreted as xeromorphic type and previously reported in *T. sandrasicum* by Dinç *et al.* (2008).

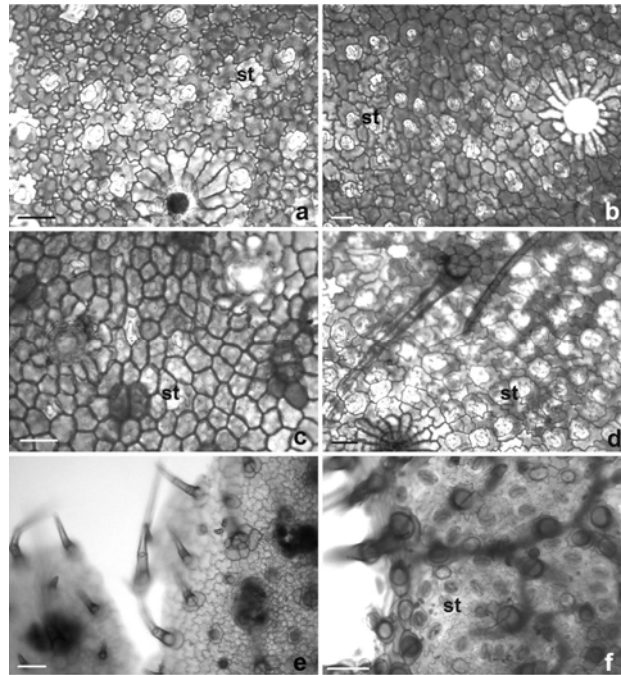


Fig. 3. Peripheral section of leaf. a, b: *Origanum rotundifolium*, c, d: *O. vulgare* ssp. *viride*, e, f: *Teucrium hircanum*. a, c, e: adaxial surface, b, d, f: abaxial surface. st: stomata. Bar = 50 μ m.

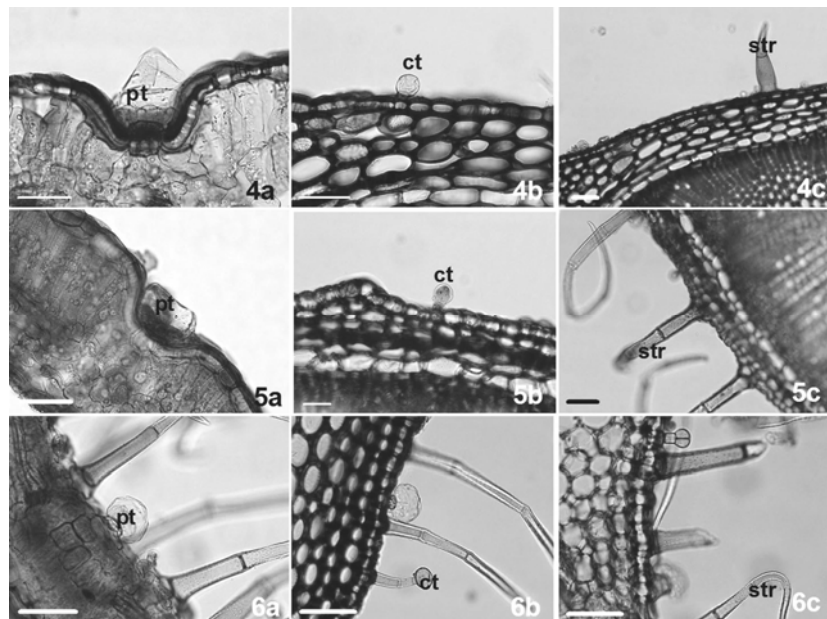


Fig. 4-6. Trichome types. 4: *Origanum rotundifolium*, 5: *O. vulgare* ssp. *viride*, 6: *Teucrium hircanum*. a: peltate trichome, b: capitate trichome, c: simple trichome. pt: peltate trichome, ct: capitate trichome, str: simple trichome. Bar = 50 μ m.

In peripheral sections, it is observed that the two *Origanum* taxa have amphistomatic leaf, while *T. hircanum* has one. Inamdar and Bhatt (1972), who studied 33 species in 17 genera of the Lamiaceae, found that in the majority of species, stomata occurred exclusively on the lower leaf surface. However, Cantino (1990) mentioned that both hypostomatic and amphistomatic leaves are found in the members of the family, the presence of the latter type of leaves being slightly more frequent. Stomata are diacytic or rarely anomocytic type in the two *Origanum* taxa, whereas it is anomocytic type in *T. hircanum*. Stomata densely occur in abaxial surfaces of all the taxa. According to the dimension, *T. hircanum* has many more stomata occurred only abaxial surface. On the other hand, between *Origanum* taxa, the number of stomata in *O. vulgare* ssp. *viride* is three times as many as that of *O. rotundifolium*. Stomatal lengths are almost equal in all taxa.

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