MICROMORPHOLOGICAL AND ANATOMICAL CHARACTERS OF THE TURKISH ENDEMIC Marrubium trachyticum Boiss. (LAMIACEAE)

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Abstract: In the present study, micromorphological characters of stem, leaf, calyx and mericarp of the Turkish endemic *Marrubium trachyticum* Boiss were investigated using scanning electron microscopy (SEM) and the anatomy of root, stem and leaf using light microscopy (LM). Stellate trichomes with unequal rays and branched hairs were observed on the stem, leaf and calyx. The distributions and densities of glandular trichomes on these vegetative organs were less than the eglandular trichomes. The mature mericarps of the species were ovate in shape and sculpturing pattern was penta-hexagonal colliculate. According to the anatomical results, *M. trachyticum* has secondary root structure. The stem has a distinct collenchyma layer. The stem is surrounded by oval or rectangular epidermal cells with a thick cuticle and is quadrangular in shape. The leaves are bifacial (dorsiventral) with anomocytic stomata in both the upper and the lower surfaces.

Key words: Anatomy, Lamiaceae, Scanning electron microscopy (SEM), Turkey.

Özet: Bu çalışmada, Türkiye için endemik bir tür olan *Marrubium trachyticum* Boiss.'un taramalı elektron mikroskobu (SEM) kullanılarak gövde, yaprak, kaliks ve findıkçığının mikromorfolojik özellikleri ve ışık mikroskobu kullanılarak kök, gövde ve yaprak anatomisi araştırıldı. *M. trachyticum*'un gövde, yaprak ve kaliksi üzerinde eşit olmayan ışınlı stellat tüyler ve dallanmış tüyler gözlendi. Bununla beraber, *M. trachyticum*'un bu vejetatif organları üzerindeki salgı tüylerinin dağılışı ve yoğunluğu, örtü tüylerinden daha azdır. Türün olgun findıkçıkları, ovat şekilliydi ve yüzey süslemesi penta-hexagonal colliculate (beşgenaltıgen şekilli kabartılı)'dir. Anatomik sonuçlara göre, araştırılan tür, sekonder kök yapısına sahiptir. Gövdenin belirgin bir kollenkima tabakasına sahip olduğu gözlendi. Gövde enine kesiti dört köşelidir ve ince bir kutikulaya sahip oval veya dikdörtgen epidermis hücreleri ile çevrilidir. *M. trachyticum*'un yaprakları bifasiyaldir (dorsiventral). Bu tür, yaprağın hem alt hem de üst yüzeyinde anomositik tip stomalara sahiptir.

Introduction

The genus *Marrubium* L. belongs to the Lamiaceae family and includes annual and perennial herbs. The species of the genus have an important distribution in Irano-Turanian and Mediterranean phytogeographic regions. The genus comprises about 40 taxa throughout the world (Hedge 1992) and is represented in Turkey by 21 taxa of which 12 are endemic (Cullen 1982, Davis *et al.* 1988, Ekim *et al.* 2000, Aytaç *et al.* 2012). It is thought that Turkey is the main centre of diversity for the genus *Marrubium* (Akgül & Ketenoğlu 2014). *M. trachyticum* Boiss., growing in steppe, slopes and fields inside the altitude range from 900 to 2500 m a.s.1. (Cullen 1982), is one of the endemic species of the genus in Turkey and has been included in the Red Data Book of Turkish Plants in near threatened (LR-nt) status (Ekim *et al.* 2000).

Marrubium has been used as a traditional medicine for asthma, pulmonary infections, inflammation, and hypotension and also as pain reliever (Meyre-Silva & Cechinel-Filho 2010). Furthermore, there are some reports on *Marrubium* species about their effects on reducing oxidative stress and inflammatory reactions due to their high amount of polyphenol and flavonoid contents (Yousefi *et al.* 2013, 2014). However, studies on *Marrubium* focused mainly on palynological, micromorphological and chemical features of the taxa studied (Akgül *et al.* 2008, Ahvazi *et al.* 2016, Kharazian & Hashemi 2017). Pollen and seed micromorphology generally support identifications of members of some genera such as *Marrubium* and *Stachys* L. (Abu-Assab & Cantino 1994).

Trichomes are the most useful taxonomic characters in the Lamiaceae family and taxonomical significance of trichome structure in this family is well known (Abu-Assab & Cantino 1987, Marin *et al.* 1994, Navarro & El Qualidi 2000, Kahraman *et al.* 2011, Celep *et al.* 2014, Atalay *et al.* 2016, Haratym & Werszko-Chmielewska 2017). Trichomes are widely distributed in the vegetative and reproductive parts of plants of Lamiaceae and distinguished as glandular and non-glandular trichomes (Cantino 1990, Navarro & El- Qualidi 2000). Glandular hairs are widely distributed over the aerial reproductive and vegetative organs of members of the family and their structures have been investigated in a number of studies (Bosabilidis 1990, Ascensao *et al.* 1999, Kaya *et al.* 2003). However, non-glandular trichomes are more common than glandular trichomes within Lamiaceae (Cantino 1990).

Studies on mericarp micromorphology in Lamiaceae have been a useful tool for classification within the family (Navarro & El Qualidi 2000, Dinç & Doğan 2006, Kaya & Dirmenci 2008). The usefulness of nutlet micromorphological characters in Lamiaceae taxonomy were also proved (Jamzad *et al.* 2000, Guerin 2005, Moon & Hong 2006, Kahraman *et al.* 2011). Furthermore, the importance of analysis based on Scanning Electron Microscopy (SEM) has been reported in many genera of the family (Budantsev & Lobova 1997, Jamzad *et al.* 2000, Kahraman *et al.* 2011, Sattl *et al.* 2012).

There exist many anatomical studies concerning Lamiaceae in Turkey (Kaya *et al.* 2000, Uysal 2003, Erken 2005, Celep *et al.* 2011). Although some of these studies are related with *Marrubium* species, detailed investigations and publications on anatomical and micromorphological characters of Turkish endemic *M. trachyticum* are very limited (Akgül 2004, Büyükkartal *et al.* 2016). The present study is the first comprehensive study of anatomy and micromorphology of this endemic species.

The aim of this study is to investigate the detailed characteristics of micromorphological structures of stem, leaf, calyx and mericarp of *M. trachyticum* using SEM and its anatomy of root, stem and leaf using light microscopy.

Materials and Methods

Plant samples were collected from May to July 2014 from natural populations in the vicinity of Bağcılı village in Çorum, Turkey. The specimens were dried according to standard herbarium techniques and stored in the Ondokuz Mayıs University, Faculty of Art and Science Herbarium (OMUB). The taxonomical descriptions of the plants were made according to Flora of Turkey (Cullen 1982).

Dried stem, leaf, calyx and mericarp samples were mounted directly on stubs using double-sided adhesive tape for SEM investigations. The stubs were coated with gold for 5 minutes and then observed and photographed in JEOL-JSM 7001 Scanning Electron Microscope. The terminology of Cantino (1990) and Koul *et al.* (2000) was followed for micromorphological investigations.

Anatomical investigations were performed using an average of 30 fresh specimens which were kept in 70% alcohol. Transverse sections of roots, stems and leaves

and surface sections of leaves were used to make permenant/temporary slides and the slides were viewed and photographed using a Nikon Coolpix 5200 digital camera. All anatomical measurements were done in computer media based on the photographs with the help of the Image J program. Stomatal index was calculated according to the method described by Meidner & Mansfield (1968) for both surfaces of the epidermis.

Results

Micromorphological characteristics

Observation of stem using SEM showed that eglandular and glandular trichomes are present on stems of *M. trachyticum* (Fig. 1A-B). Lanate eglandular trichomes, in particular, are more abundant at the base of the stem. The head of glandular trichomes with short stalk are composed of a spheric cell (Fig. 1B).

Leaf surface is more or less densely covered with eglandular stellate trichomes and eglandular trichomes on the abaxial surface are longer than the adaxial surface (Fig. 1C-D). Glandular trichomes are rare on both side of leaf surfaces. The leaves of the investigated species are amphistomatous and with anomocytic stomata (Fig. 1C). The mean number of stomata per mm² of leaf surface is 15.01 ± 3.01 on adaxial epidermis and 25.64 ± 4.89 on the abaxial epidermis (Table 1). Stomata are more abundant on the abaxial side of the leaf. The upper and lower epidermis consist of cells with strongly sinous walls (Fig. 1C-D).

SEM observation showed that the throat of calyx has more greyish stellate trichomes (Fig. 1E). However, these trichomes are more sparsely on the calyx teeth (Fig. 1F). The distribution and density of the glandular trichomes on calyx are less than eglandular ones (Fig. 1E-F).

The mature mericarps are ovate in outline $(1.01 \times 2.05 \text{ mm})$ and dark-brown in color (Fig. 1G). The sculpturing pattern of mature mericarps are pentahexagonal colliculate (Fig. 1H).

Anatomical characteristics

Root anatomy

The root structure in *M. trachyticum* is more or less uniform. In cross-sections, the periderm layer on the outermost surface is multilayered (Fig. 2A). Cortex consists of 11-12 layers of oval or rectangular parenchymatous cells. Cambium and phloem cells are distinguishable. The thickness of phloem layer is $59.99\pm8.27\mu$ m (Table 2). The xylem consists of vessel members and tracheids. The xylem rays are composed of 7-8 rowed rectangular cells. The pith is completely filled with xylem elements.

 Table 1. Stomata index of leaf of M. trachyticum.

Stomata index	Mean±SD	
Upper leaf surface	15.01±3.01	
Lower leaf surface	25.64±4.89	

SD: Standard deviation

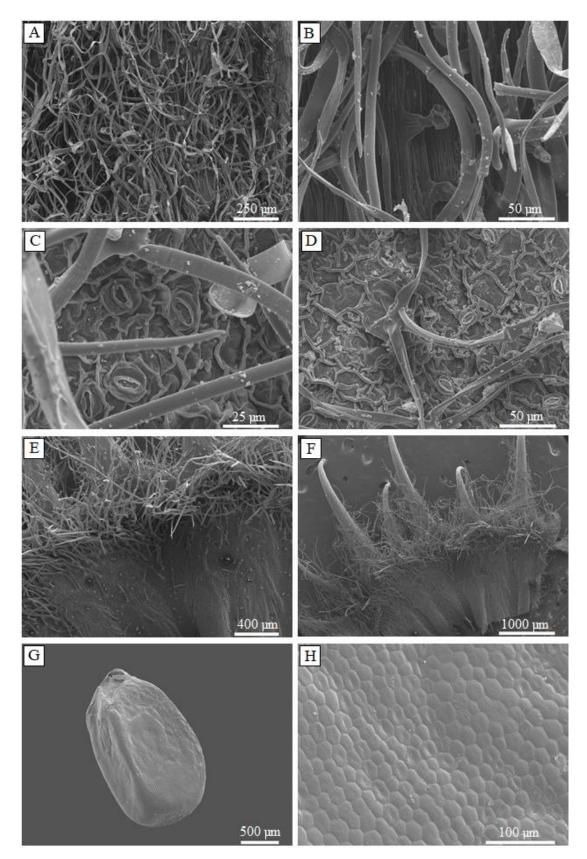


Fig. 1. Scanning electron micrographs (SEM) of *M. trachyticum*. A-Eglandular trichomes on stem. B-Glandular trichomes on stem. C-Upper surface of leaf. D-Lower surface of leaf. E-Trichomes on the calyx throat. F-Trichomes on the calyx teeth. G-General appearance of mericarps. H-Surface details of mericarps.

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Stem anatomy

Cross-section of the stem is clearly quadrangular in shape. The epidermis consists of oval or rectangular cells and is covered by a thick cuticle. There are one-celled or multicellular non-glandular or glandular hairs on the epidermis (Fig. 2B). The collenchyma tissue consisting of 10-12 layers of ovoidal cells is located underneath the epidermis. The cortex tissue is composed of ovoidal and quadrangular cells with thin walls and the thickness of this layer is $130.70\pm23.31\mu$ m (Fig. 2B, Table 2). Beneath the cortex parenchyma, small groups of phloem sclerenchyma cells are located above the phloem and vascular cambium is indistinguishable. Xylem elements are thick-walled. The mean diameter of vessel elements is $20.20\pm4.35\mu$ m (Table 2). The pith consists of large and cylindrical parenchymatic cells and pith cells become smaller towards the central part of the stem (Fig. 2B).

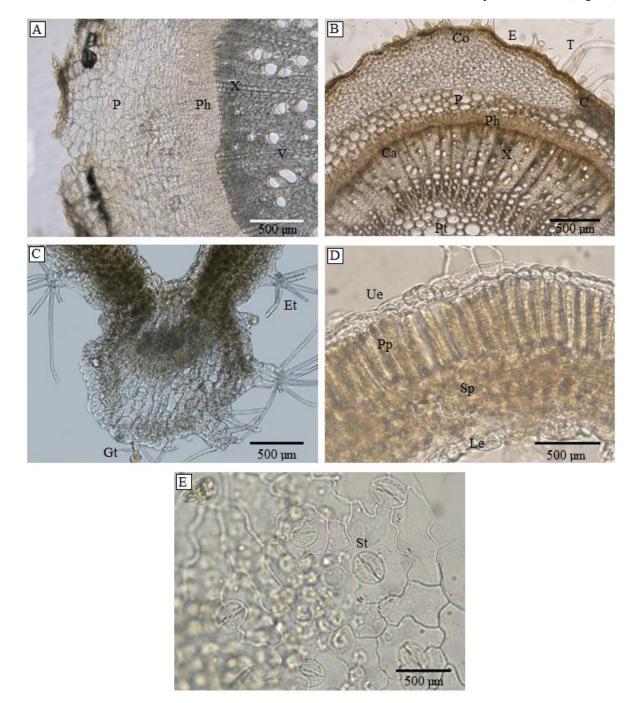


Fig. 2. Root, stem and leaf anatomical structures of *M. trachyticum*. A-Cross-sections of the root (A), the stem (B) and the leaf (C, D). E- Lower surface of the leaf. (C-cortex, Ca-cambium, Co-collenchyma, E-epidermis, Et-eglandular trichome, Gt-glandular trichome, Le-lower epidermis, P-parenchyma, Ph-phloem, Pp-palisade parenchyma, Pt-pith, Sp-spongy parenchyma, St-stomata, T-trichome, Ue-upper epidermis, V-vessel elements).

Table 2. Anatomical characteristics of M. trachyticum.

		Width (μm) (mean±SD)	Length (µm) (mean±SD)
Root	Thickness of periderm	36.46±6.56	-
	Parenchyma cells	23.65±5.97	15.77±3.94
	Thickness of phloem	59.99±8.27	-
	Thickness of xylem	443.34±27.42	-
	Diameter of vessels	24.68±7.76	-
Stem	Thickness of epidermis	71.79±2.91	-
	Parenchyma cells	20.22±3.99	12.60 ± 2.87
	Thickness of cortex	130.70±23.31	-
	Thickness of collenchyma	163.17±33.31	-
	Thickness of phloem	49.11±12.77	-
	Thickness of xylem	114.42 ± 40.50	-
	Diameter of vessels	20.20±4.35	-
	Pith cells	26.66±8.75	20.65 ± 7.80
Leaf	Upper epidermis cells	9.81±2.28	6.93 ± 0.97
	Thickness of palisade parenchyma	41.29±2.47	-
	Thickness of spongy parenchyma	39.71±2.56	-
	Lower epidermis cells	10.38 ± 3.00	10.52 ± 2.89
	Adaxial stomata	22.22±1.22	26.72±0.62
	Abaxial stomata	18.73±1.39	19.46 ± 2.11

SD: Standard deviation

Leaf anatomy

In transverse sections, the upper and the lower epidermises comprise of uniseriate, quadrangular and oval cells. However, lower epidermal cells are larger than upper epidermis cells (Table 2). Both epidermises are covered with non-glandular and glandular hairs (Fig. 2C). 2-3 layers of collenchyma cells are present in the midrib located between the upper and the lower epidermis. The leaves are bifacial (dorsiventral) (Fig. 2D). Palisade tissue is composed of 1-2 layered cylindrical cells with e plenty of chloroplasts. Spongy parenchyma with large intercellular spaces are round or irregular in shape and the thickness of this layer is 39.71±2.56µm (Fig. 2D, Table 2). Vascular bundles are collateral type. In their surface views, the leaves are amphistomatic and epidermal cells have wavy walls (Fig. 2E). Adaxial stomata length and width is higher than the abaxial stomata dimensions (Table 2). The number of stomata per mm^2 in lower surface is higher than in upper surface (Table 1).

Discussion

In this study, micromorphological and anatomical properties of *M. trachyticum* studied by light and scanning electron microscopies were determined to be useful characters.

The taxonomic significance of trichome micromorphology in some members of Lamiaceae has already been evident (Dinç *et al.* 2009, Xiang *et al.* 2010, Kahraman *et al.* 2010, Osman 2012, Celep *et al.* 2014). This study shows that the main hair type in *M. trachyticum* is stellate with unequal rays and branched hairs. Stellate and branched hairs have been previously reported in some other genera of Lamiaceae such as *Stachys L.* (Salmaki *et al.* 2009), *Nepeta L.* (Jamzad 2001), *Lavandula L.* (Upson & Andrews 2004) and *Phlomis L.* (Azizian & Cutler 2008). Our findings are consistent with the results of

Seyedi & Salmaki (2015) and Ahvazi *et al.* (2016) indicating that the stellate and branched hairs were found to be in Lamiaceae family members. However, in the present study, the distributions and densities of glandular trichomes on stem, leaf and calyx of *M. trachyticum* are less than the non-glandular trichomes. It was previously reported that non-glandular trichomes are more common than glandular trichomes in Lamiaceae (Cantino 1990, Osman 2012, Ahvazi *et al.* 2016).

Mericarp surface sculpturing patterns have diagnostic value for species recognition in some members of Lamiaceae (Jamzad *et al.* 2003, Kaya & Dirmenci 2008, Kahraman *et al.* 2010). Furthermore, the importance of the micromorphology of mericarp surfaces has been determined for *Marrubium* (Akgül 2004). Akgül *et al.* (2008) reported that the ornamentation surface in *Marrubium* species is verrucate and the seed shape in the Turkish *Marrubium* species can be related to their habitat. Similar results were also reported by Aytaç *et al.* (2012). According to the findings of our investigations, the mericarp surface in *M. trachyticum* is penta-hexagonal colliculate. Our results confirmed previous studies investigating the mericarp surface in *Marrubium* genus (Akgül *et al.* 2008, Aytaç *et al.* 2012).

Marrubium trachyticum was found to have the same general root anatomy characteristics as in other members of the Lamiaceae family. In root cross sections, the protective tissue was comprised of periderm and the center of the root was composed of tracheary elements. It was previously determined that the pith rays of some species within Lamiaceae, e.g. *Lamium lycium* Boiss. are 1-4 rowed while *Salvia chrysophylla* Stapf. has 1-24 rowed and *S. quezelii* Hedge & Afzal-Rafii has 1-3 (-4) rowed pith rays (Baran & Özdemir 2009, Kahraman *et al.* 2010, Celep *et al.* 2014). Root transverse sections showed that *M. trachyticum* has 7-8 rows of ray cells. Watson & Dalwitz (1978) stated that stems of many members of the Lamiaceae family are quadrangular with well-defined collenchyma on each corners. This character was previously reported in some other *Marrubium* species (Akgül *et al.* 2008, Büyükkartal *et al.* 2016, Tüylü *et al.* 2017). A well developed multilayered collenchyma was distinguishable at the corners of the stem of *M. trachyticum.* Vessel elements were more distinct in stem. The pith cells were parenchymatic becoming smaller towards the central part of the stem.

Anatomical studies showed that the leaves of the *M. trachyticum* was bifacial (dorsiventral). Palisade parenchyma cells were presented in upper surface of the leaf and spongy parenchyma cells had large intercellular spaces in the lower surface. These leaf anatomy

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characteristics coincide with those previously reported by Akgül *et al.* (2008) and Büyükkartal *et al.* (2016) for some other *Marrubium* species. In leaf cross-sections, vascular bundles were collateral type in *M. trachyticum* and the phloem surrounded xylem. Our observation showed that *M. trachyticum* has amphistomatic leaves. Akgül (2004) and Tüylü *et al.* (2017) also reported that the leaves of the some other *Marrubium* species are amphistomatic. In surface sections of leaf, anomocytic type stomata were observed with higher numbers in abaxial surface of the leaf.

In conclusion, some characteristic micromorphological and anatomical features of *M. trachyticum* were reported in detail. The results presented here have the potential to contribute to further taxonomic studies of the genus.

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