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# Morphology, anatomy and trichome properties of *Lamium truncatum* Boiss. (Lamiaceae) and their systematic implications

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# Abstract

The anatomy, morphology and trichome distribution on the aerial parts of *Lamium truncatum* Boiss. were studied in order to understand the usefulness of these characteristics for systematic purposes. *L. truncatum* is a Mediterranean element which grows on shady hedge banks, glades, *Quercus coccifera* L. macchie and forest at an altitude of 300-1650 m in the eastern part of the Mediterranean region of Turkey, western Syria, Palaestina and Israel. Some anatomical characters such as the distances of vascular bundles between the corners in the stem, the number of palisade and spongy parenchyma layer of the leaf blade, and the petiole anatomy provide information of taxonomical significance. Three main types of trichomes (peltate, capitate glandular and non-glandular) were observed on the stem, inflorescence axis, leaf and calyx surfaces of *L. truncatum*. Glandular trichomes were present in abundance on the inflorescence axis, calyx, abaxial side of the leaf especially on veins, and apex of the filament, but non-glandular ones were mainly situated on the leaf and stem. Moreover, notes on morphology and nutlet features of the species are given.

Keywords: Anatomy, Glandular and non-glandular trichomes, *Lamium truncatum*.

## Introduction

Lamium L. is composed of nearly 40 species distributed extensively in Europe, eastern Asia, northern Africa, north of the Atlas mountains and Macaronesia. Its diversity centre lies in the Irano-Turanian and the Mediterranean phytogeographic regions (Mennema, 1989). Lamium species are used in official and traditional medicines in Anatolia, Europe and China, possessing antioxidant, anti-inflammatory, blood tonic, uterotonic, antiplasmodic, antiseptic, uterotonic, trauma, hypertension, chronic bronchitis, pharyngitis and other properties. (Bremness, 1995; Baytop, 1999). Moreover, some Lamium species are also grown in parks and gardens as ornamental plants (Rudy, 2004). There are a number studies on taxonomy, morphology, anatomy, trichome micromorphology, palynology and cytology of Lamium (Mill, 1982; Gill, 1983; Mennema, 1989; Abu-Asab and Cantino, 1994; Baran and Özdemir, 2009) and the other genus (Kahraman and Celep, 2010; Kahraman et al., 2010a). The usefulness of the structure of the vascular bundles in petioles for species identification in the family Lamiaceae has been demonstrated (Metcalfe and Chalk, 1972). The taxonomic significance of the structure of trichomes is well known in the Lamiaceae and related families (Metcalfe and Chalk, 1972; Kahraman et al., 2010b). The morphology, distribution and frequency of glandular trichomes are used as discriminative characters at subfamiliar level in the Lamiaceae (Ascensão et al., 1995). Pollen morphology has been pointed out to be useful in systematics of the Lamiaceae (Abu-Asab and Cantino, 1994). However, the anatomical properties, nutlet and trichome structure of most Lamium species have not yet been investigated. Most of the taxonomic problems in the genus arise from its unique biological characteristics. In general, identification of Lamium species is easy, but the nomenclature of the names is exceedingly complicated. Although over 160 names have been described at species and infrapspecific rank, only ca. 40 species are generally recognized (Mennema, 1989). Anatomy and trichome morphology of Lamium truncatum Boiss. have not been studied previously and also such investigations on Lamium species are rather limited. Therefore, the present study aims to investigate morphologic and anatomic features of L. truncatum, to determine the various types of trichomes and their distribution on the aerial parts and to evaluate the usefulness of these characters for systematic purposes.

# Material and methods

Since 2002, the first author has carried out extensive field studies and collected a large number of *Lamium* specimens from their natural habitats. Moreover, population size and phenological properties of the species were observed. During the studies, the author first examined all available specimens of the species in various local (Ankara University, Department of Biology: ANK, Gazi University, Department of Biology: GAZI) and international herbaria (British Museum: B, Edinburgh Herbarium: E, Conservatoire et Jardin Botaniques de la Ville de Geneve: G, Kew: K and Leningrad Herbarium: LE). Plant specimens for anatomical studies were collected during the flowering period from natural populations in Osmaniye (in the vicinity of Düziçi, Düldül Mountain, above Çitli village, 1026 m, 37° 19' 456" N 36° 28' 910" E, June 2009, Celep 1637 & A. Kahraman)

	Lamium truncatum	Flora of Turkey
Stem (cm)	20-60	(28-) 35-45
	Glabrous to sparsely pilose below; inflorescence	Glabrous below, sparingly pilose above;
	sparsely pilose with very short glandular and peltate	inflorescence glandular
Leaf (mm)	15-80 x 8-55	20-60 (-77) x 13-35 (-50)
	eglandular pilose with very short glandular capitate	Eglandular pilose
	on main veins and peltate	
Petiole (mm)	10-45	-
	eglandular pilose with peltate	-
Calyx (mm)	10-19	10-14 (-17)
	the tube is to 9 mm, the teeth is to 10 mm	Teeth c. 1.5 x tube
Corolla (mm)	15-30	(17-) 25-27
	Upper lip is 7-13	-
	Lower lip is 4-7	-
	Lateral lobes 4-7	-
	Tube is 12-20	-
Filament length (mm)	7-18, short glandular capitate at apex	-
Anther length (mm)	1.5-2.8	-
Pistil length (mm)	13-28	-
Nutlet measurement (mm)	2.88-3.24 x 1.56-1.85 mm	3.5-3.6 x 1.2-1.4

Table 1. Morphological characters of Lamium truncatum based on the present study and 'Flora of Turkey' (Mill 1982).

Table 2.	Anatomical characteristics of <i>Lamium truncatum</i> . SD= standart deviation	
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	Width (µm)		Length (µm)	
	Min-Max	Mean $\pm$ SD	Min-Max	Mean $\pm$ SD
The root anatomy				
Peridermis cell	24.99-74.66	42.78±12.15	20.62-45.49	30.12±6.69
Parenchyma cell	15.25-39.35	26.21±7.20	12.09-26.86	19.44±3.73
Vessel	16.58-62.42	36.38±13.55		
The stem anatomy				
Epidermis cell	14.80-32.49	23.26±4.91	15.75-25.11	19.54±2.32
Parenchyma cell	24.78-65.82	41.12±11.18	10.96-59.90	34.30±9.60
Trachea cell	18.27-36.51	28.35±5.30		
Pith cell	29.00-148.10	76.59±29.37		
The leaf anatomy				
Adaxial cuticle	1.45-2.56	2.01±0.27		
Abaxial cuticle	1.03-2.42	$1.68 \pm 0.41$		
Adaxial epidermis	14.2-58.72	28.08±11.67	15.2-41.08	25.11±5.74
Abaxial epidermis	12.35-30.30	$18.81 \pm 4.84$	8.84-25-94	16.24±4.26
Palisade cell	12.1-22.76	17.42±2.60	18.93-35.26	28.16±4.57
Spongy cell	13.02-40.65	22.41±6.89	12.73-31.12	22.37±5.27
Midrip trachea	9.20-19.27	13.69±3.07		
Midrip parenchyma	20.02-59.66	36.42±10.47	18.96-46.97	31.15±8.42
The petiole anatomy				
Adaxial epidermis	14.15-26.72	20.31±3.77	13.60-23.47	18.75±2.81
Abaxial epidermis	13.46-23.80	19.58±2.96	15.02-25.00	19.59±2.95
Parenchyma cell	26.46-62.52	32.92±12.35	23.62-57.06	40.38±9.72
Trachea cell	11.80-23.18	17.19±2.96		

Table 3. Distribution of peltate, capitate glandular and non-glandular trichomes on different parts of Lamium truncatum.

Plant material	Peltate	Capitate Glandular	Non-Glandular
	Type I	(Stalk 1-3 cells)	Type III
		Type II	
Stem	-	-	+
Inflorescence axis	++	++	++
Adaxial leaf surface	-	-	++
Abaxial leaf surface	+++	++	++
Petiole	+	+	+
Calyx	++	++	++

(-) absence of trichomes, (+) a few trichomes, (++, +++) increasing presence of trichomes.

province in the Mediterranean region of Turkey. Voucher samples are stored in the Department of Biological Sciences, Middle East Technical University, Ankara, Turkey. The taxonomical description of the species followed Mill (1982). Anatomical investigations were performed using an average of 30 fresh specimens kept in 70% alcohol. The paraffin wax method was applied for preparing cross-sections of middle parts of mature roots, stems, leaf blades and petioles. The sections were stained with safranin-fast green (Johansen, 1944) with some modifications relating to staining time and amount of additions to the stains, and then they were mounted on slides using entellan. Slides were viewed and photographed with a Leica DM1000 light microscope. Trichomes were obtained from stems, inflorescence axis, leaf blades, petioles and calyces and studied with a stereomicroscope and a light microscope. Sections were made with a Leica RM2125RT rotary microtome using the paraffin wax method and by hand using commercial razor blades. Thirty measurements for each type of trichome were taken. They were studied using a Leica DM1000 light microscope with  $100 \times$  to  $400 \times$  magnifications. The types and distributions of trichomes are described. The general trichome terminology follows Metcalfe and Chalk (1972), Payne (1978), Navarro and El Oualidi (2000) and Kahraman et al. (2010b).

# Results

## Expanded morphological characteristics

L. truncatum (Fig. 1) is a Mediterranean element which grows on shady hedge banks, glades, Quercus coccifera L. macchie and forest at an altitude of 300-1650 m in the eastern part of the Mediterranean region of Turkey, western Syria, Palaestina and Israel (Mill, 1982; Mennema, 1989). It is a perennial herb with a well-developed creeping rootstock. The stem is ascending to erect and clearly quadrangular. The stem is approximately 20-60 cm height, glabrous to sparsely pilose. The leaves are simple, ovate to ovate-oblong, 15-80 x 8-55 mm, eglandular pilose with very short glandular capitate on veins and peltate hairy. Leaves edges are crenate to doubly crenate, and the venation is reticulate-pinnate. The petiole is 10-45 mm, eglandular pilose with short glandular capitate and peltate hairy. The inflorescence is a verticillate cyme and sparsely pilose with very short glandular capitate and peltate. The verticillasters are 1-4, and 6-10-flowered. The bracts, just like the leaves, are petiolate and ovate, 15-65 (-90) x 10-55 mm. The calyx is 10-19 mm, tubular to slightly campanulate and the tube is to 9 mm. The calyx has 5 lanceolate teeth and to 10 mm. The bilabiate corolla is purplish-pink with whitish to cream tube and purple spotted lower lip. The corolla is 15-30 mm. The upper lip is 7-13 mm and truncate with white eglandular pilose and peltate hairy. The lower lip is 4-7 mm and the lateral lobes of the lower lip are narrowly lanceolate and 3-7 mm. The corolla tube is curved and 12-20 mm. The stigma is bifid and the pistil is 13-28 mm. Stamens are didynamous. Anther thecae are divaricate and hairy, 1.5-2.8 mm. The filaments are 7-18 mm and short glandular capitate hairy at the apex (Table 1).

#### Anatomy of the root, stem, leaf and petiole

Root: in transverse section, the periderm layer on the outermost surface is thick and its cells are squashed or breaking up. There is a multi-layered parenchymatic cortex (4-9 layers) under the periderm and its cells are 12.09-26.86 x 15.25-39.35  $\mu$ m (Table 2). The xylem consists of vessel



Fig 1. General appearance of Lamium truncatum.



**Fig 2.** Cross-sections of the root of *Lamium truncatum*. cp: cortex parenchyma, pe: periderm, pr: pith rays, t: trachea, x:xylem. Bar: 100 µm



**Fig 3.** Cross-section of the stem of *Lamium truncatum*. co: collenchyma, cp: cortex parenchyma e: epidermis, t: trachea. Bar: 100 μm.



**Fig 4-5.** Cross-sections of the leaf blades of *Lamium truncatum*. ab: abaxial epidermis of midrip, ad: adaxial epidermis of midrip, gh: glandular hair, le: lower epidermis, pp: palisade parenchyma, p: parenchymatic cells, sp: spongy parenchyma, ue: upper epidermis, vb: vascular bundle. Bars: 100 μm

members and tracheids. Vessel members are circular or hexagonal. The xylem rays are composed of 1-4 rowed rectangular cells. (Fig. 2). Stem: The stem is clearly quadrangular in transverse section. The epidermis is covered by a thin cuticle. The epidermis consists of 1 layer oval, squarish or rectangular cells. Underneath the epidermis, multi-layered plaque collenchyma cells are located at the corners of the stem. The cortex is composed of 4-11 layers of irregular oval or rectangular parenchymatic cells with intercellular spaces. Vascular bundles are next to each other. The size of vascular bundles at the corners is larger than that of the bundles between corners. The cambium are hardly visible and distinguishable. The pith is large and comprised of hexagonal or orbicular parenchymatic cells with intercelular spaces in the centre of stem. (Fig. 3). Leaf: in transverse section the lamina and mid-rib show the upper and the lower epidermides covered with a thin cuticle layer. The thickness of both epidermis cuticles is nearly equal. There are eglandular hairs on both epidermides, but glandular hairs can bee seen only lower epidermis especially on the veins. Both epidermides consist of uniseriate, oval, squarish or rectangular cells. Upper epidermal cells are larger than lower epidermal cells or nearly equal to them. The leaf is of the bifacial type. The mesophyll is composed of elongated rectangular palisade parenchyma and isodiametric spongy parenchyma cells. Measurements of these cells are given in Table 2. The palisade parenchyma is 1 layered. The spongy parenchyma cells are 2-4-layered with large intercellular spaces (Fig. 4). The mid-rib forms a projecting part. Transverse section of the midrip shows that the adaxial surface is flat to concave and the abaxial surface is convex (Fig. 5). Arc-shaped vascular bundles are surrounded by a parenchymatic bundle sheat. There is one large vascular bundle in the center. Petiole: a transverse section of the petioles shows that the adaxial surface is concave and the abaxial surface is convex. There are eglandular and glandular hairs on both surface. The epidermis is composed of 1-2 layers. The epidermal cells of both surfaces are more or less rectangular to oval. Parenchyma cells are hexagonal or orbicular and their dimensions are 23.62-57.06 x 26.46-62.52 µm (Table 2). There are two colleteral vascular bundles in the centre and one small subsidary bundle in the petiolar wings (Fig. 6). Two layered collenchyma can be found in the

abaxial epidermis, which are 3-7 layered in the petiolar wings.

# Trichome morphology

Three different trichome types on the stems, inflorescence axis, leaf blades, petioles and calyces of L. truncatum 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). In the capitate trichome, the length of the stalk should be more than half the height of the head, whereas peltate trichomes are short with a uni- or bicellular stalk and a large secretory head with 4 to 18 cells arranged in one or two concentric circles (Werker et al., 1985). Type I is the typical peltate glandular trichome and consists of a basal epidermal cell, a very short monocellular stalk (8-20 µm) and a broad, round multicellular secretory head (15-60 µm) consisting of four or eight cells in a single shield (Fig. 7a, b, c). Type II is a capitate glandular trichome composed of a basal epidermal cell, unicellular to bicellular stalk of variable length (5-30  $\mu$ m), a neck cell (5–15  $\mu$ m) and a large, cutinized, unicellular or bicellular secretory head (20-60 µm width, 20-50 µm length) (Fig. 7d, e, f). Type III is a non-glandular trichome composed of one or multicellular basal epidermal cell. It is unicellular to tricellular, uniseriate, unbranched with variable length (40-600 µm) (Fig. 7g, h, i).

## Nutlet morphology

Nutlets of *L. truncatum* are ovate in their outline and trigonous in transverse section (Fig. 8). Mature nutlets of *L. truncatum* are 2.88-3.24 mm long and 1.56-1.85 mm wide. The hilum is trigonous and its diameter is 0.40-0.60 mm. The nutlet surface is glabrous and brown with small whitish spots. Hairs can be seen at the hilum and base of the nutlets (Fig. 8).

## Discussion

The present study sought to provide useful information on the anatomy and trichome morphology of *L. truncatum*. This is the first report on the examined characteristics of the species. Stem, leaf and inflorescence characteristics, and nutlet

properties of L. truncatum were found to be more different than reported in 'Flora of Turkey' (Mill, 1982) (Table 1). Measurements and observations of some characters of L. truncatum are presented here for the first time (Table 1). Metcalfe and Chalk (1972) gave some useful information about root anatomy of the family Lamiaceae. They pointed out that the pith rays of roots of the family are composed of 2-12- or more-rowed cells. Our findings are consistent with those of Metcalfe and Chalk (1972) and those of some other investigated members of Lamiaceae (Dinc and Öztürk, 2008; Baran and Özdemir, 2009; Kahraman et al., 2010b, 2010c). The pith rays of Lamium lycium Boiss. are composed of 1-4rowed (Baran and Özdemir, 2009). Salvia chrysophylla Stapf has 1-24 rowed rays (Kahraman et al., 2010b) and Salvia ballsiana has 1-3 (-4) rowed rays (Kahraman et al., 2010c). The present study on transverse sections of the root of L. truncatum showed that the species has 1-4 rows of ray cells. Metcalfe and Chalk (1972) stated that stems of many genera and species of the family Lamiaceae are quadrangular with well-defined collenchyma in the four angles. They also reported that scleranchymatous tissue surrounds the phloem groups of vascular bundles. Like L. lycium (Baran and Özdemir, 2009) and S. chrysophylla (Kahraman et al., 2010b), a well developed multi layered collenchyma was distinguishable at the corners of the quadrangular stem of L. truncatum. Sclerenchyma was not observed in the transverse sections of the L. truncatum like L. lycium (Baran and Özdemir, 2009) in contrast to those described by Kahraman et al. (2010b; 2010c) and Metcalfe and Chalke (1972). The vascular cambium was seen in the cross-sections of L. truncatum like L. lycium (Baran and Özdemir, 2009), Stachys yildirimli Dinç (Dinç and Öztürk, 2008) and stems of some Salvia species (Kahraman et al., 2010b; 2010c). All vascular bundles in the stems of L. truncatum are next to each other. However, bundles between the corners in the stem of L. lycium (Baran and Özdemir, 2009) and S. halophila Hedge (Kaya et al., 2007) are separated from each other by parenchymatous cells. Therefore, the distances of vascular bundles between the corners are useful characters for distinguishing the species in the genus Lamium. The leaf of L. truncatum has 1 layered palisade parenchyma and 2-4 layered spogy parenchyma, however that of L. lycium has 2-3 layered palisade parenchyma and 2-3 layered spongy parenchyma (Baran and Özdemir, 2009). Therefore, the number of palisade and spongy parenchyma layer of the leaf are useful characters for distinguishing the species in the genus. Metcalfe and Chalk (1972) pointed out that the structure of the vascular bundles in the petiole of the species of Lamiaceae could be used as a diagnostic character. In the petiole of L. truncatum, there are two-lobed vascular bundle in the centre and there is a single small subsidiary bundle in petiolar wings like L. lycium. However, the central vascular bundles of L. truncatum are connected to each other via narrow vascular tissues in contrast to L. lycium (Baran and Özdemir, 2009). Therefore, the anatomical properties of the petiole are useful characters for distinguishing the species in the genus. Like other members of the Lamiaceae, L. truncatum carries both peltate and capitate glandular trichomes, as well as nonglandular ones. Whereas the inflorescence axis, calyx, the leaf midrip, petiole and filaments possess glandular trichomes, the stem, leaf blade, bract, petiole and inflorescence axis bear non-glandular trichomes. Peltate trichomes of the Lamiaceae often comprise a broad head of several secretory cells (up to 16), a wide short stalk and a basal epidermal cell (Hallahan, 2000). The present study showed that L. truncatum has the peltate trichomes (Type I, Fig. 7a, b, c), like L. lycium (Baran and



**Fig 6.** Cross-section of the petiole of *Lamium truncatum*. ab: abaxial epidermis, ad: adaxial epidermis, co: collenchyma, p: parenchyma, svb: subsidiary vascular bundles, vb: vascular bundles. Bar: 100 μm



**Fig 7.** Light micrographs of trichomes of *Lamium truncatum*. Type I peltate glandular trichomes on the (a): leaf, (b): petiole, (c): inflorescence, Type II glandular capitate trichomes on the (d): calyx, (e): inflorescence, (f): leaf. Type III non-glandular trichomes on the (g): inflorescence, (h): leaf, (i): petiole. Bars:a-h:  $10 \mu m$ , i:  $100 \mu m$ .



Fig 8. Light micrographs of the nutlet of *Lamium truncatum*. hi: hilum

Özdemir, 2009) and Lamium galeobdolon (L.) L. (Uphof and Hummel, 1962), composed of a four or eight-celled head in a single circle which is agreement with previous studies (Serrato-Valenti et al., 1997; Corsi and Bottega, 1999; Hallahan, 2000; Kamatou et al., 2007). However, in other species of the same family, such as Origanum L. species (Bosabalidis and Tseko, 1984) and Satureja thymbra L. (Bosabalidis, 1990), a higher number of head cells are arranged in two concentric circles. Capitate glandular trichomes have significant taxonomic character and form part of the floral specialized properties for pollination in the Lamiaceae (Navarro and El Oualidi, 2000). They greatly vary in structure and size. Similar capitate glandular trichomes were observed in Lamium lycium, Leonotis leonurus (L.) R.Br., Salvia blepharophylla Brandegee ex Epling and Salvia chrysophylla (Ascensão et al., 1995; Ascensão and Pais, 1998; Bisio et al., 1999; Baran and Özdemir, 2009; Kahraman et al., 2010b). However, distribution and density of the capitate glandular tichomes of L. truncatum are less than them. In the present study, the majority of the capitate glandular trichomes were found on the inflorescence axis, calyx, veins of the leaf and apex of the filament. Non glandular trichomes are very common in the member of the Lamiaceae. The non-glandular trichomes of the L. truncatum are the same with type III-subtype A of Salvia chrysophylla (Kahraman et al., 2010b). However, non-glandular trichomes is shorter in L. truncatum than S. chrysophylla. Types of trichomes observed and their distribution are given in Table 3. Previously, the nutlet size of L. truncatum was reported as 3.5-3.6 x 1.2-1.4 mm (Mill, 1982), however, according to our measurements, the nutlet size of the species is 2.88-3.24 x 1.56-1.85 mm. As well as, trichomes on the nutles are reported for the first time for the genus (Fig. 8). This could be occur different ecological conditions. To sum up, the morphological properties of the species were different to those reported by Mill (1982) in terms of stem, leaf and inflorescence characteristics, and nutlet properties. On the other hand, anatomical features of the species is presented for the first time in this study. According to results, stem, leaf and petiole anatomical features provide useful characters for distinguishing species in the genus.

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