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Comparative anatomy on the vegetative organs of genus *Ziziphora* L. (Lamiaceae) from Turkey

Selami Selvi¹ 💿 | Fatih Satil²

¹Department of Medicinal and Aromatic Plants, Vocational School, Balıkesir University, Altınoluk-Balıkesir, Turkey

²Department of Biology, Faculty of Science & Art, Balıkesir University, Balıkesir, Turkey

Correspondence

Selami Selvi, Department of Medicinal and Aromatic Plants, Vocational School, Balıkesir University, 10870 Altınoluk-Balıkesir, Turkey. Email: sselvi2000@yahoo.com

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Abstract

The genus *Ziziphora* L. (Lamiaceae) is represented by five species (nine taxa) in the Turkish Flora. These taxa are *Z. clinopodioides* Lam. (subsp. *elbursensis*, subsp. *filicaulis*, subsp. *kurdica*, subsp. *rigida*), *Z. capitata* L., *Z. persica* Bunge, *Z. tenuior* L., *Z. taurica* Bieb. subsp. *taurica*, and *Z. taurica* Bieb. subsp. *cleonioides* (Boiss.) Davis which to be an endemic taxon for Turkey. They are strongly aromatic herbs which contain rich pulegone and used as herbal teas and spices and for this reason. In this study, comparative anatomy of the genus *Ziziphora* growing in Turkey is presented for the first time. In anatomical studies, cross sections of vegetative organs such as the root, stem, and leaf (lamina and petiole) were examined. In addition, to exhibit stomatal distribution and anatomy on adaxial and abaxial leaves were taken surface sections of the lamina and calculated stomatal index. Lamina and petiole anatomy were shown to be of great importance in the taxonomy of the *Ziziphora* taxa. The presence or absence of sclerenchyma in midrib of lamina and petiole, cortex parenchyma layer, mesophyll structure, and epidermal surface were found to be important characters for identification of *Ziziphora* taxa.

KEYWORDS

anatomy, Lamiaceae, Turkey, vegetative organs, Ziziphora

1 | INTRODUCTION

Ziziphora L. (Lamiaceae) is one of the most important genera containing pulegone of high rates and belonging to in the subfamily of Nepetoideae (tribe Mentheae subtribe Menthinae). They are distributed in East and West Asia, Central Europe, North Africa, and Mediterranean region. The genus *Ziziphora* represents with about 17 species (28 taxa) in the world (Anzalone et al., 1982; Boissier, 1879; Borisova et al., 1954; Dothan, 1978; Edmondson, 1982; Harley et al., 2004; Jalas & Rechinger, 1982; Meikle, 1985; Rechinger, 1964; Strid & Tan, 1991; Tutin et al., 1976; Zhang & D'Arcy, 1994).

It is represented five species (nine taxa) in Turkey as Z. *clinopodioides* Lam. subp. *elbursensis*, subsp. *filicaulis*, subsp. *kurdica*, subsp. *rigida*, Z. *capitata* L., Z. *persica* Bunge, Z. *tenuior* L., Z. *taurica* Bieb. subsp. *cleonioides* (Boiss.) Davis and Z. *taurica* Bieb. subsp. *taurica*. In these taxa, only *Z. taurica* subsp. *cleonioides* is endemic for Turkey and endemism ration is about 11% (Edmondson, 1982; Guner, Aslan, Ekim, Vural, & Babac, 2012).

Ziziphora species have been used as sedative, stomachic, aphrodisiac and carminative in Iranian and Turkish folk medicine (Aghajani et al., 2008; Sezik & Tümen, 1984). In Turkey, members of this genus are called "Dağ Reyhanı," "Filiskin out," or "Nane ruhu" and dried herbal parts are used as herbal tea, condiments and folk medicine (Baytop, 1999; Sezik & Tümen, 1984). They are also used to treat various ailments such as antiseptic and wound healing (Baytop, 1999; Kaya, Satıl, & Dirmenci, 2013; Selvi, Satıl, Martin, Çelenk, & Dirmenci, 2015).

Anatomical studies of Lamiaceae have been carried out by many authors; however, anatomical studies carried out on genus *Ziziphora* were limited with several authors, and these studies were fulfilled by Koca, Erken, Tümen, and Başer (1995), Koca and Tümen (1996), Keshavarzi et al. (2008), and Hatamneia (2008), respectively.

While Z. clinopodioides was studied by Koca et al. (1995), Z. tenuior was studied by Koca and Tümen (1996). In both studies, cross sections were taken from stem, leaf, and calyx of species were examined and discussed their anatomical different. Anatomical studies on Z. capitata, Z. taurica subsp. cleonioides, and Z. taurica subsp. taurica were carried out by Sezik and Tümen (1984, 1988, 1989). In anatomical studies, cross sections taken from stems and leaves were investigated and supported by anatomical illustrations. Keshavarzi et al. (2008) conducted morphological and anatomical studies, they investigated only leaf anatomy. As a result of anatomical studies, they emphasized leaf micro-characters of epidermis showed to be of great importance in the taxonomy of the Z. clinopodioides subsp. Hatamneia et al. (2008) examined cross sections taken from stem, leaves, and petiole of Z. clinopodioides and Z. tenuior growing in Iran.

The objectives of this study were to provide a detailed account of the vegetative anatomy of *Ziziphora* in general by light microscopy and to determine to what extent these anatomical data can be used as a taxonomic character in the genus.

2 | MATERIALS AND METHODS

Plant specimens to needed for anatomical studies were collected from different localities in Turkey and presented taxa used and localities of specimen collection in Table 1. In addition, morphological drawings of the taxa used in this study are shown in Figure 1.

Anatomical studies were carried out on specimens kept in 70% alcohol. Cross sections of stem and leaves were stained with Phloroglucinol-HCL solutions and chlorophyll in leaves was removed with chloral hydrate (Baytop, 1972; Coşkun et al., 2010; Karaismailoğlu and Güner, 2019).

Stomatal density on abaxial and adaxial surfaces of the leaves were counted under a light microscope. Stomatal index was calculated according to the method of Meidner and Mansifield (1968). Stomatal terminology and the leaf epidermal terminology were based on the classification proposed by Wilkinson (1979) respectively. Measurements and photographs were taken using Olympus BX 53 and Nikon Eclipse E200 binocular light microscopes.

3 | RESULT AND DISCUSSION

3.1 | Root anatomy of genus

There is periderm which comprise from phellogen, phellem, and phelloderm on the outermost surface of the root. Phellogen and phelloderm are unclearly. The cortex lies just beneath the periderm. The cortex cells are parenchymatic and cubic, polygonal, or ovale in shape. These cells may be shown as crushed or shredded in some taxa. Endodermis which to be the innermost layer of cortex was cubic or rectangular cells in shape and single-layered. The pericycle is located **TABLE 1** Taxa used for anatomical studies and localities of specimen collection

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Studied taxa	Collection areas and collector's number
Z. clinopodioides	A4 Kastamonu: Ilgaz Mount, behind the Television tower, 2000 m, 20.viii.2009, S. Selvi, (SV 1345)
Z. capitata	A4 Çankırı: Ankara-Karabük path, Kuzören village, 1 km away from İsmetpaşa village, 40° 52′ 0261″N, 032° 36′ 3,536″ E, 1021 m, 09. vii.2009, E. Erdoğan (EE 1016) & S. Selvi.; B1 Balıkesir: Çağış campus, 150 m, 27.vi.2008, S. Selvi (SV 1159)
Z. persica	B4 Isparta: Gelendost, Yenice village, Akyokuş around, 1,000–1,600 m, 12.vii.2009, Q. coccifera scrubs, open field, S. Selvi (SV 1320)
Z. tenuior	A5 Kastamonu: Tosya, 600 m, 01.viii.2009, S. Selvi (SV 1332); B1 Balıkesır: Edremit, Kazdağı, Güvertepe region, 900 m, 05.vii.1989, G. Tümen.; Edremit, Kazdağı, Gürlek locality, 06. vii.1992, G. Tümen
Z. taurica subsp. taurica	B2 Denizli: Acıpayam, Aşağıkatlık district, the old Mine, 09.vii.2009, S. Selvi (SV 1302)
Z. taurica subsp. cleonioides	B2 Manisa: between Sarıgöl and Kiraz, 47 km, 750 m, 21.vi.2006, T. Dirmenci (TD 3154); C2 Denizli: Honaz Dağı, 1800 m, 20.vi.2006, T. Dirmenci (TD 3152)

between the endodermis and phloem. It is composed of parenchyma cells. Xylem composed of vessels and tracheids. Pith rays comprise 1–2 rowed ovale or rectangular cells. The pith completely includes xylem elements (Table 2; Figure 2).

3.2 | Stem anatomy of genus

Cross sections taken from the stem of Ziziphora taxa have exhibited a monolayered epidermis which is composed of oval, cubic, or rectangular cells. The upper surface of epidermis is covered with a thin cuticle and contains glandular and eglandular trc. Eglandular trichomes are acicular or curved, simple, made up from one or more cells (up to 6), mostly having one to three cells, arranged in a single row and having a cuticle with micropapillae (or without) (Figure 3). Two types of glandular trichomes are encountered (e.g., Figure 8): (1) Peltate trichomes are composed of 1 basal epidermal cell, one neck cell, and a broad head of 12-16 secretory cells, 4 or 6 central cells and 8 or 12 peripheral cells (Figure 8). (2) Capitate trichomes are small in size and either consists of (a) a short unicellular stalk and a globose or pear-shaped head cell, or (b) two-cellular stalks and a globose or pear-shaped head cell (Figure 8). Epidermis also includes rarely diacytic stomata type. Underneath the epidermis, multilayered collenchyma cells (60-90 µm) are located at the corners and there are 1-3 rows of chlorenchyma cells between them. The parenchymatic cortex tissue (250-400 µm) consists of 7-10 layered of oval, ovate, or orbicular parenchymatous cells. The single-layered endodermis consists of generally ovale or rectangular cells. Underneath the endodermis is located the pericycle

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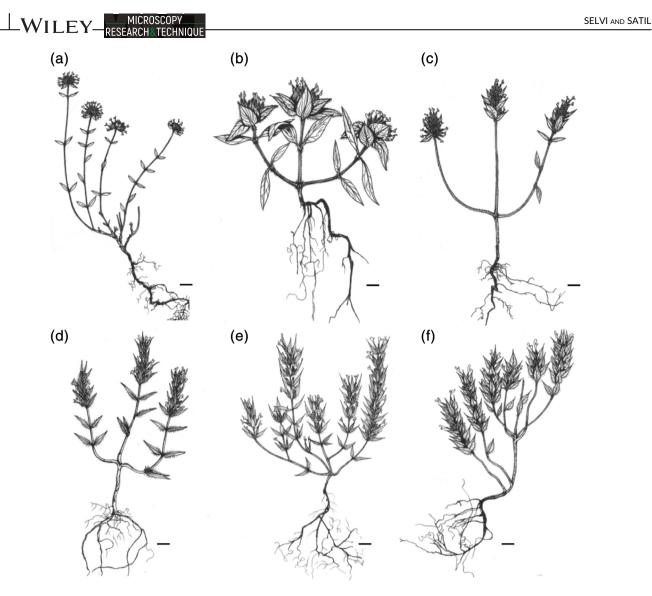


FIGURE 1 General showing of Ziziphora taxa (drawing). (a) Z. clinopodioides, (b) Z. capitata, (c) Z. persica, (d) Z. tenuior, (e) Z. taurica subsp. taurica, (f) Z. taurica subsp. cleonioides. (scale: 1 cm)

TABLE 2	Comparatively root anatomical characters of Ziziphora taxa
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12

	Root anatomical characters									
Таха	Periderm (phellem)	Cortex layer	Phloem layer	Pith ray	Xylem/root ratio (%)					
Z. clinopodioides	1-3	4-6	4-10	1-2	68					
Z. capitata	2-5	1-3	3-5	1-2	82					
Z. persica	1-3	1-3	1-4	1-2	87					
Z. tenuior	2-5	1-3	2-5	1-2	81					
Z. taurica taurica	2-5	2-4	2-4	1-2	80					
Z. taurica cleonioides	2-4	2-4	1-4	1-2	72					

which comprise in groups of 1–2 elongated ovals forming a ring. The phloem (80–120 μm) is surrounded by more or less sclerenchymatous fibers. Cambium is distinguishable. The xylem considerably bulges at ridges. The phloem is 1–4 layered between corners and 2–5-layered at the corners. It consists of irregular or rectangular cells. The cambium is

not distinguishable. The xylem $(500-1,150 \ \mu\text{m})$ comprises trachea and tracheids. The tracheae are orbicular or ovoid while the tracheids are polyhedral. The rays are usually uniseriate or biseriate rarely triseriate. The pith comprises hexagonal or orbicular parenchymatous cells with intercellular spaces (Figure 3; Table 3).

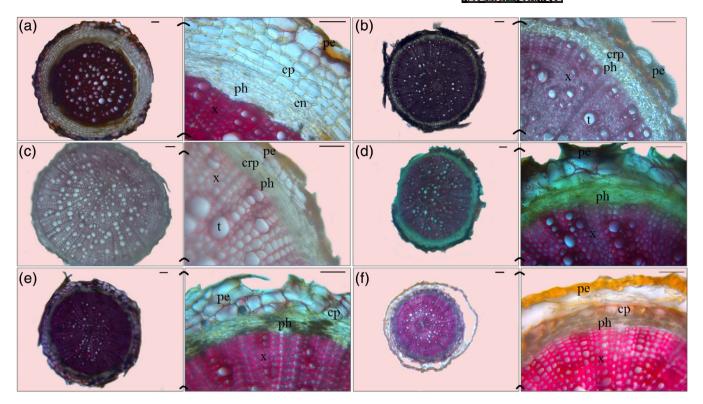


FIGURE 2 Root anatomy of *Ziziphora* taxa. (a) *Z. clinopodioides*, (b) *Z. capitata*, (c) *Z. persica*, (d) *Z. tenuior*, (e) *Z. taurica* subsp. *taurica*, (f) *Z. taurica* subsp. *cleonioides*. pe: periderma, cp: cortex parenchyma, crp: crushed parenchyma tissue, en: endodermis, ph: phloem, x: xylem, t: trachea (scale 50 µm) [Color figure can be viewed at wileyonlinelibrary.com]

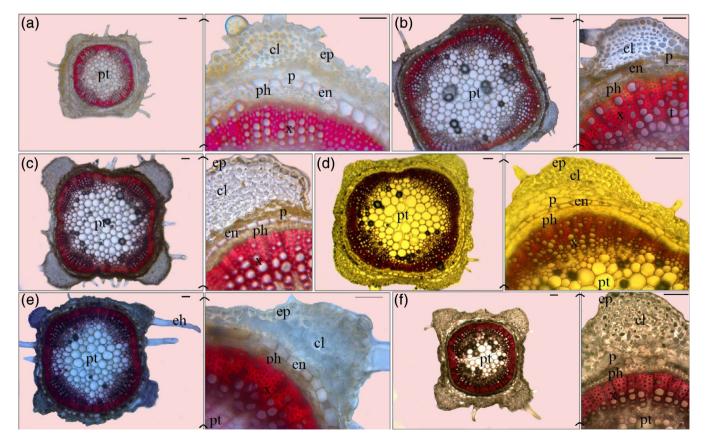


FIGURE 3 Stem anatomy of *Ziziphora* taxa. (a) *Z. clinopodioides*, (b) *Z. capitata*, (c) *Z. persica*, (d) *Z. tenuior*, (e) *Z. taurica* subsp. *taurica*, (f) *Z. taurica* subsp. *cleonioides*. ep: epidermis, eh: egladular hair, p: parenchyma, cl: collenchyma, en: endodermis, ph: phloem, x: xylem, t: trachea, pt: pith region (scale 50 µm) [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 3	Comparatively stem anatomical characters of Ziziphora taxa
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	Stem anatomical characters									
Collenchyma layer			Phloem la	yer						
Таха	Corner	Between corner	Cortex layer	Corner	Between corner	Pith ray	Pith/stem (%)			
Z. clinopodioides	5-8	1-3	2-4	1-2	1-3	1-3	42			
Z. capitata	5-9	1-3	1-3	1-2	2-4	1-2	48			
Z. persica	6-10	1-3	2-5	1-2	1-3	1-2	46			
Z. tenuior	3-7	1-3	2-4	1-2	2-6	1-2	41			
Z. taurica taurica	4-7	1-3	1-4	1-2	1-3	1-2	48			
Z. taurica cleonioides	6-9	1-3	2-6	3-5	2-4	1-2	45			

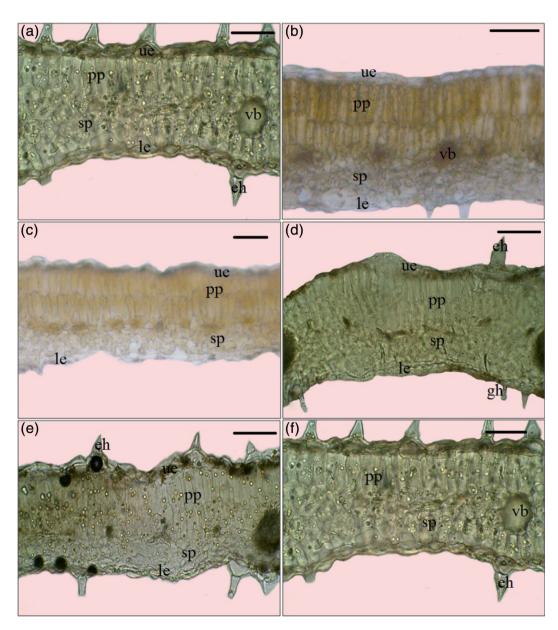


FIGURE 4 Leaf anatomy of Ziziphora taxa. (a) Z. clinopodioides, (b) Z. capitata, (c). Z. persica, (d) Z. tenuior (e) Z. taurica subsp. taurica, (f) Z. taurica subsp. cleonioides. ue: upper epidermis, eh: eglandular trichome, pp: palisade parenchyma, sp: spongy parenchyma, vb: vascular bundle, le: lower epidermis (scale 50 μm) [Color figure can be viewed at wileyonlinelibrary.com]

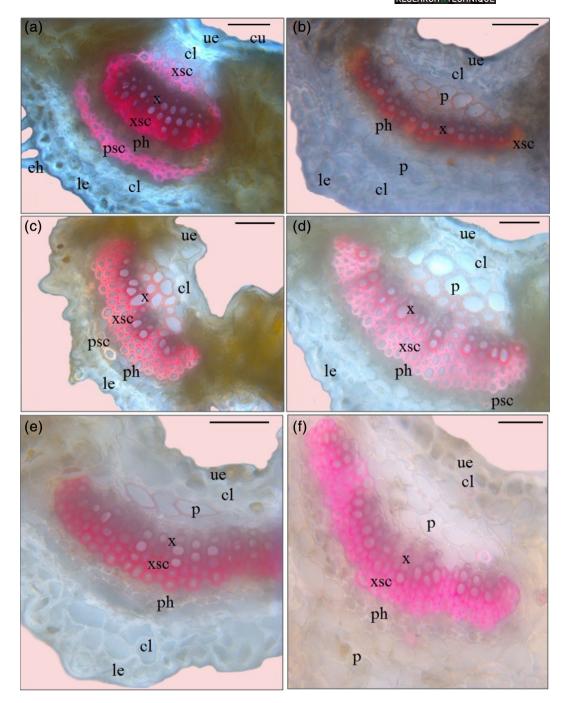


FIGURE 5 Leaf middle vein anatomy of *Ziziphora* taxa. (a) *Z. clinopodioides*, (b) *Z. capitata*, (c) *Z. persica*, (d) *Z. tenuior*, (e) *Z. taurica* subsp. *taurica*, (f) *Z. taurica* subsp. *cleonioides*. cu: cuticle, ue: upper epidermis, eh: egladular trichome, p: parenchyma, cl: collenchyma, xsc: xylem sclerenchyma, psc: phloem sclerenchyma, ph: phloem, x: xylem, le: lower epidermis (scale 50 µm) [Color figure can be viewed at wileyonlinelibrary.com]

3.3 | Lamina anatomy of genus

In the cross section of the lamina, there is a thin cuticle on the upper and lower epidermis (Figure 4). Both epidermal cells are monolayered, isodiametric, and rectangular, oval, or cubic in shape. Surface of epidermis are covered with eglandular (1–4 cells) and glandular (uniseriate and biseriate) trichomes (Figure 4). Eglandular trichomes are acicular or curved, simple, made up from one or more cells (up to 6), mostly having one to three cells, arranged in a single row and having a cuticle with micropapillae (or without) (Figure 5). Two types of glandular trichomes are encountered (e.g., Figure 5): (1) Peltate trichomes are composed of one basal epidermal cell, one neck cell, and a broad head of 12–16 secretory cells, four or six central cells and eight or twelve peripheral cells (Figure 8). (2) Capitate trichomes are small in size and either consists of (a) a short unicellular stalk and a globose or pear-shaped head cell, or of (b) two-cellular stalks and a globose or pear-shaped head cell (Figure 8). Stomata are present on both surfaces of the lamina (amphistomatic type).

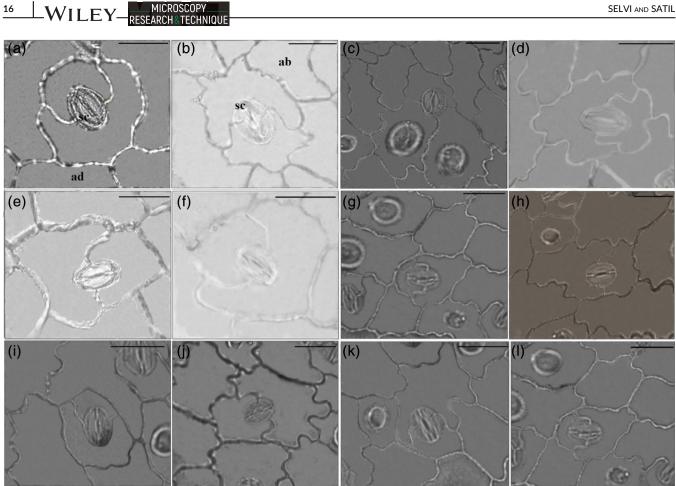


FIGURE 6 Lamina epidermal surface and stomata types of Ziziphora taxa. Adaxial surface: (a, c, e, g, i, k); abaxial surface (b, d, f, h, j, l). Z. clinopodioides (a, b), Z. capitata (c, d), Z. persica (e, f), Z. tenuior (g, h), Z. taurica subsp. taurica (i, j), Z. taurica subsp. cleonioides (k, l). ad: adaxial epidermis, ab: abaxial epidermis, sc: stoma cell (scale 25 μm) [Color figure can be viewed at wileyonlinelibrary.com]

	Lamina ana	Lamina anatomical characters											
			Midrib region										
	Mesophyll	layer		Parenchyma	Parenchyma layer		Sclerenchyma layer						
Таха	Palisade	Spongy	Collenchyma layer	Upper epidermis	Lower epidermis	Xylem (lower)	Xylem (upper)	Phloem (lower)	Phloem layer				
Z. clinopodioides	2	4-8	1-4	1-3	2-4	1-4	1-4	1-3	4-7				
Z. capitata	2	2-6	1-3	1-3	3-5	1-2	-	-	2-4				
Z. persica	2	2-6	1-3	1-3	2-4	2-6	-	1	1-4				
Z. tenuior	2	3-6	1-3	2-3	2-4	2-6	-	1	2-5				
Z. taurica taurica	2	3-6	1-3	2-4	1-3	2-5	-	-	2-4				
Z. taurica cleonioides	2	3-6	1-3	2-4	2-3	2-4	-	-	2-4				

TABLE 4 Comparatively lamina anatomical characters of Ziziphora taxa

Stomata type is diacytic or rarely ranunculaceous (anomocytic) type (Figure 6). In surface section, while anticlinal walls of the upper epidermal cell are straight or mild undulate, anticlinal walls of the lower undulate or mild undulate. The stomatal index is 31-47 (upper surface) and 29-47 (lower surface), while stomatal index ratio is between 1.01 and 1.5

(Table 6). Mesophyll consists of palisade and spongy parenchyma cells. Palisade parenchyma cells are 1-2 layered, cylindrical whereas spongy parenchyma cells are 2-6 layered and round or oval in shape. Mesophyll are bifacial and equifacial types (Figure 4). The midrib region, which forms a projecting part, comprises 1-3 layers of collenchyma adjacent

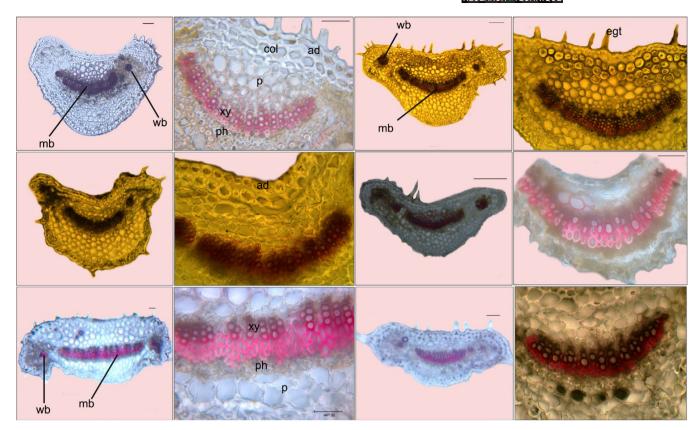


FIGURE 7 Petiole anatomy of *Ziziphora* taxa. (a) *Z. clinopodioides*, (b) *Z. capitata*, (c) *Z. persica*, (d) *Z. tenuior*, (e) *Z. taurica* subsp. *taurica*, (f) *Z. taurica* subsp. *cleonioides*. ad: adaxial epidermis, ab: abaxial epidermis, egt: eglandular trichome, col: collenchyma, p: parenchyma, xy: xylem, ph: phloem, mb: middle bundle, wb: wing bundle (scale 50 μm) [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 5	Comparatively petiole anatomical characters of Ziziphora taxa
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	Petiole anatomical characters									
	Number of v	Numbe	er of layer	ed	Sclerenchyma l					
			Collenchyma							
Таха	Middle	Wings	Ad.	Ab.	Parenchyma	Xylem (lower)	Phloem (lower)	Phloem layer		
Z. clinopodioides	1	1 + 1	1-3	1-3	2-5	1-3	1-3	3-6		
Z. capitata	1	1 + 1	1-3	1-5	2-5	1-3	-	2-5		
Z. persica	1	1 + 1	1-2	1-3	1-4	1-3	-	3-5		
Z. tenuior	1	1 + 1	1-3	1-5	1-3	1-3	-	2-4		
Z. taurica subsp. taurica	1	1 + 1	1-3	1-3	3-6	2-5	-	3-6		
Z. taurica subsp. cleonioides	1	1 + 1	1-2	1-3	2-4	1-3	-	3-5		

the epidermal cells. A single large vascular bundle which surrounded by parenchymatic cells is located in the center (Figure 5; Table 4). The xylem faces the upper surface while phloem faces the lower epidermis. Sclerenchymatic tissue on the xylem and phloem is present or absent (Figures 4 and 5; Table 4).

3.4 | Petiole anatomy of genus

In cross sections taken from the petiole of *Ziziphora taxa* it has been observed that the epidermal cells of both surfaces are oval or rectangular in shape and covered a thinner cuticle.

Surface of epidermis are covered with eglandular (1–4 cells) and glandular (uniseriate and biseriate) trichomes (Figure 8). Eglandular trichomes are acicular or curved, simple, made up from one or more cells (up to 6), mostly having one to three cells, arranged in a single row and having a cuticle with micropapillae (or without) (Figure 7). Two types of glandular trichomes are encountered (e.g., Figure 8): (1) Peltate trichomes are composed of one basal epidermal cell, one neck cell, and a broad head of 12–16 secretory cells, four or six central cells and eight or twelve peripheral cells (Figure 8). (2) Capitate trichomes are small in size and either consist of (a) a short unicellular stalk and a globose or pear-shaped

TABLE 6 Stomata properties of Ziziphora taxa

			Adax	Adaxial epidermis				ial epide	rmis		
Таха		Stoma type	sts	ec	sti	stm	sts	ec	sti	stm	IR
Z. clinopodio	oides	Diacytic	52	184	22.03	$18-29 \times 14-17$	68	212	24.2	$19-31 \times 14-19$	0.91
Z. capitata		Diacytic	40	172	18.9	$18-22 \times 12-17$	68	248	21.5	13-22 × 8-18	0.87
Z. persica		Diacytic	56	240	18.9	$21 - 31 \times 11 - 26$	64	252	20.2	$24 - 31 \times 11 - 25$	0.93
Z. tenuior		Diacytic	48	168	22.2	16-25 × 11-19	60	224	21.1	17-23 × 11-18	1.05
Z. taurica	Subsp. taurica	Diacytic	64	192	25	16-24 × 12-21	84	236	26.2	$17-24 \times 12-17$	0.95
	Subsp. cleonioides	Diacytic	56	192	24.5	15-25 imes 11-19	72	208	25.7	$16-26 \times 11-18$	0.8

Abbreviations: sts, stoma number; ec, epidermis cell number; sti, stomatal index; stm, measurements of stomata; Ir, index ratio.

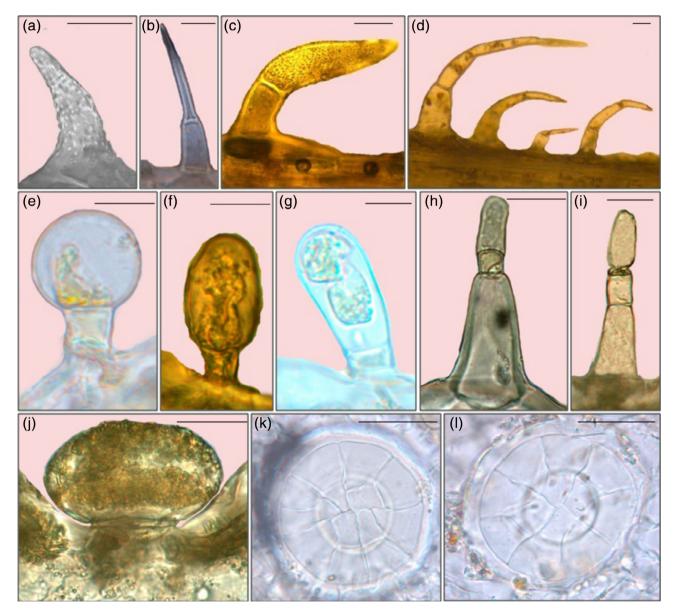


FIGURE 8 Different trichome types observed in vegetative organs of *Ziziphora* taxa. Eglandular trichomes (a–d); glandular trichomes: capitate trichomes (e–i), peltate trichomes (j–l). Stem trichomes (a, c, d, e); lamina trichomes (g, h, i, j, k, l); petiole trichomes (b, f) [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 7 Trichomes observed in Ziziphora taxa

19

	Stem			Leaf			Petiole			
	Glandular				Glandular			Glandular		
	Eglandular	Capitate	Peltate	Eglandular	Capitate	Peltate	Eglandular	Capitate	Peltate	
Z. clinopodioides	1–4 cells Less dense Micropapillae	Dense	12-16 cells Rare	1–3 cells Dense Micropapillae	Rare	12–16 cells Rare	1–3 cells Less dense micropapillae	Less dense	-	
Z. capitata	1–5 cells Less dense Micropapillae	Less dense	12-16 cells Very rare	1–3 cells Rare Micropapillae	Rare	12-16 cells Rare	1–3 cells Rare Micropapillae	Rare	-	
Z. persica	1–6 cells Densely Micropapillae	Rare	12-16 cells Very rare	1–3 cells Less dense Not micropapillae	Less dense	12–16 cells Rare	1–3 cells Rare Micropapillae	Rare	-	
Z. tenuior	1–4 cells Less densely Micropapillae	Rare	12-16 cells Very rare	1–4 cells Rare Micropapillae	Less dense	12–16 cells Rare	1–3 cells Rare Micropapillae	Rare	-	
Z. taurica taurica	1–4 cells Rare Cuticular micropapillae	Rare	12–16 cells Rare	1–4 cells Rare Micropapillae	Dense	12-26 cells Rare	1–2 cells Rare Micropapillae	Rare	12–16 cells Rare	
Z. taurica cleonioides	1–4 cells Rare Micropapillae	Rare	12-16 cells Rare	1–4 cells Rare Micropapillae	Dense	12-16 cells Rare	1–2 cells Less dense Micropapillae	Rare	-	

head cell, or (b) two-cellular stalks and a globose or pear-shaped head cell (Figure 8). There are several layers of collenchyma cells under the epidermis. A single large vascular bundle, which is to be crescent-shaped are located in the middle region. Also, there is one small vascular bundle in each of the petiolar wings. Vascular bundles are of collateral type. The sclerenchyma tissue is well developed outside of the phloem and the xylem (Figure 7a,b; Table 5). The xylem faces the adaxial surface while phloem faces the abaxial surface. Sclerenchymatic tissue on the xylem and phloem is present or absent (Figure 7).

Comparison of root anatomical characters is shown in Table 2. As seen in the table, it is observed that the root structure of *Z*. *clinopodioides* differs according to other taxa. The root cortex of *Z*. *clinopodioides* was 4–6 layered, while the other taxa were found to be 1–4 layered. Again, the phloem layer was 4–10 layered and the other taxa ranged from 1 to 5 layered. While the endodermis and pericycle layer were clearly seen in *Z*. *clinopodioides*, other taxa were not significantly seen. Root anatomy of the family Lamiaceae according to Metcalfe and Chalk (1950) is characterized by pith rays of roots composed of 2–12 or more rowed cells. Our study shows that pith rays of *the genus Ziziphora* consist of 1–2 rowed cells (Table 2).

When the anatomical differences related to the stem were examined, there were no significant differences between the taxa, only changes in the number of layers (collenchyma, cortex, phloem) were detected. Comparison of stem anatomical characters is shown in Table 3.

El-Gazzar and Watson (1970) found that the largest trachea diameter observed in stem of Z. *tenuior* was 15 μ m. In our studies were seen that the smallest trachea diameter is 6.5 μ m and the largest

trachea diameter is 24.5 µm. Significant differences in cross sections and superficial sections taken from leaves between taxa were mostly found in the middle vein region (Figure 5). The adaxial surface of the middle vein in all taxa is flat and slightly curved (concave); Abaxial surface was observed to be outwardly (convex). Whether or not the xylem and the phloem contain sclerenchyma is an important taxonomic character that separates anatomically the leaves from each other. In the Z. clinopodioides, upper and lower side of xylem were surrounded by a sclerenchymatic sheath, but in other taxa, sclerenchyma were found only lower side of xylem. While the xylem sclerenchyma in lamina middle vein of Z. capitata was very few (1-2), all other taxa were found to be more than 2 sclerenchymatic layers. Again, in the middle vein area, the presence or absence of sclerenchyma on the underside of the phloem cells is an important character. Stomata of examined all taxa are surrounded by a pair of subsidiary cells (diacytic type). Stomata are seen on both sides of the lamina and are more intense at the bottom. They are at a higher level than the epiderma cells in the cross section (epistomatic stoma). Stoma index ratio of taxa was observed between 0.8 and 1.05. In addition, it was observed that the number of stomata on the lower surfaces of all taxa examined was higher than the upper surface (Table 6). The usefulness of the structure of the vascular bundles in petioles for species identification in the family Lamiaceae has been demonstrated (Metcalfe and Chalk, 1950). The taxonomic significance of the structure of trichomes is well known in the Lamiaceae and related families (Metcalfe and Chalk, 1950; Selvi et al., 2015). When Table 7 is examined; all taxa have eglanduar and glandular (capitate and peltate) trichomes. But; peltate trichomes were found only in petiole of Z. taurica subsp. taurica taxa. Capitate trichomes are most intense in the leaves of Z. taurica subsp.

taurica and *Z. taurica* subsp. *cleonioides* taxa. Peltate trichomes are 12 or 16 cells and they are rare or not seen in all taxa (especially petiole). Eglandular trichomes; 1–6 cells in stem; 1–4 cells in the leaf and 1–3 cells in the petiole and outer surface of eglandular trichomes are generally covered with micropapillae (Table 7; Figure 8).

Significant differences between taxa in transverse and superficial sections taken from the petiole were found in vascular bundles in the middle region. In *Z. clinopodioides*, there are sclerenchyma layers (1–3 rows), while none of the other taxa were found with sclerenchymatic elements on the phloem elements (Figure 7; Table 5).

4 | CONCLUSIONS

When the root and stem anatomy of *Ziziphora* taxa has been examined, important characters separating taxa each other was not observed. However, in lamina anatomy; characters such as the presence or absence of sclerenchyma in midrib of lamina, mesophyll structure and in petiole anatomy characters such as the presence or absence of sclerenchyma in middle region were shown to be important characters for identification of *Ziziphora* taxa. As a result, it is observed that characters obtained from root and stem anatomy are insufficient on its own to differentiate *Ziziphora* taxa and that lamina and petiole anatomy have important characters separating taxa each other. Usage of anatomical characters together with morphological and micromorphological (trichomes and epidermal surface) characters will certainly remove the problems in the infrageneric classification of the species and will provide important contribution to the systematic of the species.

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ORCID

Selami Selvi 🕩 https://orcid.org/0000-0002-9959-6945

REFERENCES

- Aghajani, Z., Assadian, F., Masoudi, S., Chalabian, F., Esmaeili, A., Anaraki, M. T., & Rustaiyan, A. (2008). Chemical composition and in vitro antibacterial activities of the oil of *Ziziphora clinopodioides* and *Z. capitata* subsp. *capitata* from Iran. *Chemistry of Natural Compounds*, 44, 387–389.
- Anzalone, B., Becherer, A., Ehrendorfer, F., Merxmiller, H., Metlesics, H., Montelucci, G., ... Segelberg, I. (1982). *Flora D'Italia* (Vol. 2, p. 476). Edagricole: Sandro Pigatti.
- Baytop, A. (1972). Anatomical structure of plant drugs. Pharmacy Faculty, Publication 829, Istanbul.
- Baytop, T. (1999). Türkiye'de Bitkiler ile Tedavi. İstanbul: İstanbul Üniversitesi Yayınları.

- Boissier, E. (1879). *Flora orientalis* (Vol. 4, pp. 537–822). Genevae and Basileae.
- Borisova, A. G., Volkova, E. V., Gorshkova, S. G., Klokov, M. V., Knorring, O. E., Kupriyanova, L. A., ... Juzepczuk, S. V. (1954). Labiatae. In B. K. Shishkin (Ed.), *Flora of the U.S.S.R* (Vol. XXI, pp. 273–293). Moskova-Leningrad: Izdatel'stvo Akademii Nauk SSR.
- Coşkun, F., Selvi, S., & Satıl, F. (2010). Phylogenetic relationships of some Turkish Crocus (Iridaceae) taxa based on morphological and anatomical characters. Turkish Journal of Botany, 34(2010), 171–178.
- Dothan, N. F. (1978). Flora Palaestina, Part Three text, Ericaceae to Compositae (pp. 145–146). Jerusalem: Israel Academy of Sciences and Humanities.
- Edmondson, J. R. (1982). Ziziphora L. In P. H. Davis (Ed.), Flora of Turkey and the East Aegean Islands (Vol. 7, pp. 395–399). Edinburgh: Edinburgh University Press.
- El-Gazzar, A., Watson, L., A (1970). Taxonomic study of Labiatae and related genera, *New Phytologist*, *69*: 451–486.
- Guner, A., Aslan, S., Ekim, T., Vural, M., & Babac, M. T. (2012). Türkiye bitkileri listesi (Damarlı bitkiler). İstanbul: Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını.
- Harley, R. M., Atkins, S., Budantsev, A. L., Cantino, P. D., Conn, B. J., Grayer, R., ... Upson, T. (2004). Labiatae. In J. W. Kadereit (Ed.), Flowering plants Dicotyledons:Lamiales (except Acanthaceae including Avicenniaceae) (Vol. 7, pp. 167–191). Germany: Springer.
- Hatamneia, A. A., Khayami, M., Mahmudzadeh, A., Sarghein, S. H., & Heidarih, M. (2008). Comparative anatomical studies of some genera of Lamiaceae family in West Azarbaijan in Iran. *Botany Research Journal*, 1 (3), 63–67.
- Jalas, J., & Rechinger, K. H. (1982). Flora Iranica (pp. 532–551). Graz: Akademische Drück – und Verlagsanstalt.
- Karaismailoğlu, M. C., & Güner, Ö. (2019). Nutlet structures of subsection Fragiles of the genus Stachys (Lamiaceae) from Turkey and their systematic applications. *Turkish Journal of Botany*, 43(5), 659–672.
- Kaya, A., Satil, F., Dirmenci, T., & Selvi, S. (2013). Trichome micromorphology in Turkish species of *Ziziphora* (Lamiaceae). Nordic Journal of Botany, 31, 270–277.
- Keshavarzi, M., Jahandideh, R., & Bokaee, Z. N. (2008). Morphological and anatomical studies on Ziziphora clinopodioides Lam. (Labiatae). Pakistan Journal of Biological Sciences, 11(23), 599–605.
- Koca, F., Erken, S., Tümen, G., & Başer, K. H. C. (1995). Ziziphora clinopodioides Lam., Üzerinde Morfolojik ve Anatomik Araştırmalar, Tr. Journal of Botany, 19, 135–144.
- Koca, F., & Tümen, G. (1996). Türkiye'de Aromatik bir bitki: Ziziphora tenuior L.' nin morfolojik ve anatomik özellikleri, XI. Bitkisel İlaç Hammaddeleri Toplantısı, 22–24 Mayıs 1996 (pp. 203–214). Ankara: Ankara Üniversitesi Eczacılık Fakültesi Yayınları.
- Meidner, H., & Mansifield, T. A. (1968). *Physiology of stomata* (pp. 67–68). London: McGraw Hill.
- Meikle, R. D. (1985). *Flora of Cyprus* (Vol. 2, pp. 1279–1280). Kew: Bentham-Moxon Trust, Royal Botanic Gardens.
- Metcalfe, C. R., & Chalk, L. (1950). Anatomy of the Dicotyledons (Vol. 2, pp. 1041–1051). London: Oxford Univ. Press.
- Rechinger, K. H. (1964). Flora of lowland Iraq (pp. 528-529). Austria: Weinheim.
- Selvi, S., Satıl, F., Martin, E., Çelenk, S., & Dirmenci, T. (2015). Some evidence for infrageneric classification in *Ziziphora* (Lamiaceae:Mentheae). *Plant Biosystems*, 149(2), 415–423.
- Sezik, E., & Tümen, G. (1984). Morphological and anatomical studies on plant used as folk medicine and plant tea in Turkey-II, Ziziphora taurica Bieb subsp. taurica. Doğa Bilim Dergisi, 8(1), 98–103.
- Sezik, E., & Tümen, G. (1988). Türkiye'de Halk İlacı ve Çay Olarak Kullanılan Bitkiler Üzerinde Morfolojik ve Anatomik Araştırmalar VI. Ziziphora taurica Bieb. subsp. cleonioides (Boiss.) Davis. Uludağ Üniversitesi Eğitim Fak. Dergisi (Vol. 3, pp. 65-73).

- Sezik, E., & Tümen, G. (1989). Ziziphora capitata L. Üzerinde Morfolojik ve Anatomik Araştırmalar. Uludağ Üniversitesi Eğitim Fakülteleri Dergisi, 4(2), 13-20.
- Strid, A., & Tan, K. (1991). *Mountain flora of Greece* (Vol. 2). Edinburgh: Edinburgh University Press.
- Tutin, T. G., Heywood, V. H., Burgers, N. A., Moore, D. M., Valentine, D. H., Walters, S. M., & Webb, D. A. (1976). Flora Europaea. Cambridge: Cambridge University Press.
- Wilkinson, H. P. (1979). The plant surface (mainly leaf). In C. R. Metcalfe & L. Chalk (Eds.), Anatomy of the dicotyledons (Vol. 1, pp. 97–165). Oxford, UK: Clarendon Press.
- Zhang, Z. L., & D'Arcy, W. G. (1994). Lamiaceae. In Z. Y. Wu & P. H. Raven (Eds.), *Flora of China*, (*Verbanaceae and Solanaceae*) (pp. 224–225). China: Science Press.

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