

Taxonomic Significance of Anatomical Characters in Some Species of the Family Myrtaceae

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ABSTRACT

This paper elaborated the epidermis properties, transverse sections of leaves, petioles and stems of five cultivated species belong to five genera of Myrtaceae. They are *Callistemon viminalis* (Sol. ex Gaertn.) G. Don, *Eucalyptus camaldulensis* Dehnh., *Myrtus communis* L., *Psidium guajava* L. and *Syzygium aromaticum* (L.) Merr. It was clear that certain structural characteristics were of significant importance in separation of these taxa, such as the presence of hypodermis in the leaves of *P. guajava*, the absence of stomata from the abaxial surface of leaves of *C. viminalis* and *P. guajava*, the isobilateral mesophyll in *C. viminalis* and *E. camaldulensis* leaves, the wavy transverse section of stem in *C. viminalis*, the presence of more than one vascular strand in *S. aromaticum* petioles as well as the presence of prismatic crystals in addition to the druses in the petioles of *P. guajava*.

Keywords: Myrtaceae; Plant; Anatomy; Stem; Leaves

1. Introduction

Anatomical properties are indices used in taxonomical studies for more than hundred years [1]. Solereder (1908) [2] summarized a lot of anatomical characters of some dicotyledonous families followed by [3] who gave a synthesis of previous works and their own investigations of the family.

The family is of temperate, subtropical and tropical distribution chiefly centered in Australia and tropical America, including about 130 genera and 3000 species of shrubs and trees [4].

Economically the family is of considerable importance throughout the world, like the edible fruit of the guava (*Psidium*), spices such as the cloves (dried flower buds of *Syzygium aromaticum*), or cultivated domestically as ornamentals notably *Eucalyptus*, bottle brushes (*Callistemon*) and the myrtle (*Myrtus communis*).

Former studies of [5-10] indicated the importance of anatomical features on the palaeobotanical and taxonomical studies. Kantachok *et al.* (2007) [11] stated that leaf anatomical data support morphological evidence for separating taxa of Myrtaceae at the generic level.

Ali *et al.* (2009) [12] focused on the anatomical adaptation of leaves in the genus *Eucalyptus* from the Faisalabad region. While longitudinal sections of leaf axiles of *Eucalyptus regnans* studied by [13].

No anatomical study was performed on the family nor any kind of observations stated on it in Iraq except the

morphological study of [14]. The present study is aiming to introduce an internal description of leaves, petioles and stems for five species of family to determine its taxonomic importance and clarity the interrelationship among the present members of Myrtaceae.

2. Materials and Methods

Fresh material of five species of Myrtaceae (**Table 1**) was collected from gardens throughout Basrah province. The cuticles were prepared by macerating the leaves in Jeffrey's solution (equal parts of 10% chromium trioxide solution and concentrated nitric acid), and therefore mounted in safranin-stained glycerine jelly.

For sectioning, fresh material of leaves, petioles and stems was fixed at least 48 hours in formalin acetic acid alcohol solution (FAA) and preserved in 70% alcohol, then dehydrated in ethyl alcohol series, sectioned on a rotary microtome and stained in safranin and fast green and then mounted in canada balsam [15].

The sections were examined with Olympus CH₄ light microscope and photographed with Digital camera type DCE-2. Stomatal index was calculated as mentioned by [16]. Anatomical terms used are cited from [1,16-18].

3. Results and Discussion

3.1. Lamina: Epidermis and Stomata

Measurements of epidermal cells and stomata are sum-

marized in **Table 1**. There are usually differences in cell form and dimensions between the adaxial and abaxial surfaces of the leaf as well as between taxa studied.

The average length of epidermal cells in the adaxial surface ranged between 18.75 - 42.88 μm and the average breadth was 11.66 - 25.00 μm in *Callistemon* and *Syzygium* respectively. In the abaxial surface the average length ranged between 19.09 - 35.55 μm and average

breadth was 12.81 - 23.21 μm in the same previous taxa respectively.

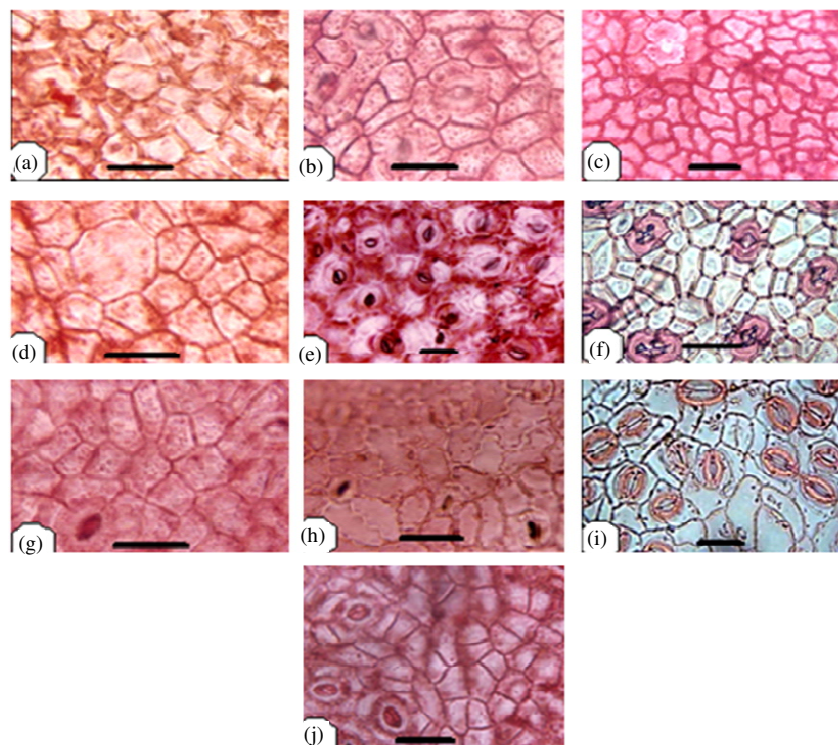
Anticlinal walls of epidermal cells exhibit a little difference between species; they are normally straight, curved sometimes and rarely sinuate (**Figure 1**).

Stomata are rounded or elliptic shaped present on either sides (Amphistomatic leaves) then often more numerous on the abaxial epidermis in the remaining species.

Table 1. Dimensions of epidermal cells and stomata in leaves of Myrtaceae (in micrometer).

Taxa	Epidermal cells				Stomata					Type of stomata
	Upper		Lower		Upper		Lower		Type	
	Length	Width	Length	Width	Length	Width	Length	Width		
<i>Callistemon viminalis</i> (Sol. ex Gaertn.) G. Don	(15 - 25) 18.75	(7.5 - 15) 11.66	(10 - 30) 19.09	(10 - 17.5) 12.81	-	-	A	(10 - 25.5) 22.32	(20 - 35) 21.75	-
<i>Eucalyptus camaldulensis</i> Dehnh.	(12 - 45) 30.00	(10 - 27) 14.88	(12.5 - 50) 25.75	(12.5 - 17.5) 13.75	(25 - 35) 29.25	(20 - 25) 22.50	A	(17.5 - 37.5) 30.62	(30 - 32.5) 30.83	A
<i>Myrtus communis</i> L.	(11 - 32) 21.50	(10 - 14) 12.60	(20 - 35) 27.83	(10 - 15) 13.75	(20 - 25) 21.60	(10 - 15) 13.20	A + H + An	(16 - 20) 18.28	(15 - 20) 18.42	A + H + An
<i>Psidium guajava</i> L.	(25 - 55) 35.75	(12.5 - 37.5) 22.00	(25 - 35.75) 31.33	(16 - 23.21) 20.00	-	-	A + P + H	(15 - 21.6) 20.00	(20 - 25) 19.50	-
<i>Syzygium aromaticum</i> (L.) Merr.	(25 - 67.5) 42.88	(20 - 37.5) 25.00	(25 - 50) 35.55	(15 - 30) 23.21	(32.5 - 42.5) 37.50	(32.5 - 40) 36.25	A + H + P + An	(22.5 - 32.5) 27.18	(17.5 - 25) 21.87	A

A = Anomocytic (Ranunculaceae); H = hemiparacytic; P = paracytic; An = Anisocytic. (The values between arches represent the mean and the values out the arches represent the minimum and maximum values).



(a), (f): *Callistemon viminalis*; (b), (g): *Eucalyptus camaldulensis*; (c), (h): *Myrtus communis*; (d), (i): *Psidium guajava*; (e), (j): *Syzygium aromaticum*; (a)-(e): Upper epidermis; (f)-(j): Lower epidermis, (scale 25 μm).

Figure 1. Surface view of leaf epidermis.

Although ranunculaceous stomata are present in all taxa but *Myrtus*, *Psidium* and *Syzygium* showed another types in addition to the ranunculaceous type (**Table 1**). Guard cells are kidney shaped. Metcalf and Chalk (1950) [3] supported this truth when they pointed that stomata in Myrtaceae are usually ranunculaceous except in a few species. [11,19] found nearly similar types when they observed three types of stomata: anomocytic, paracytic and anisocytic.

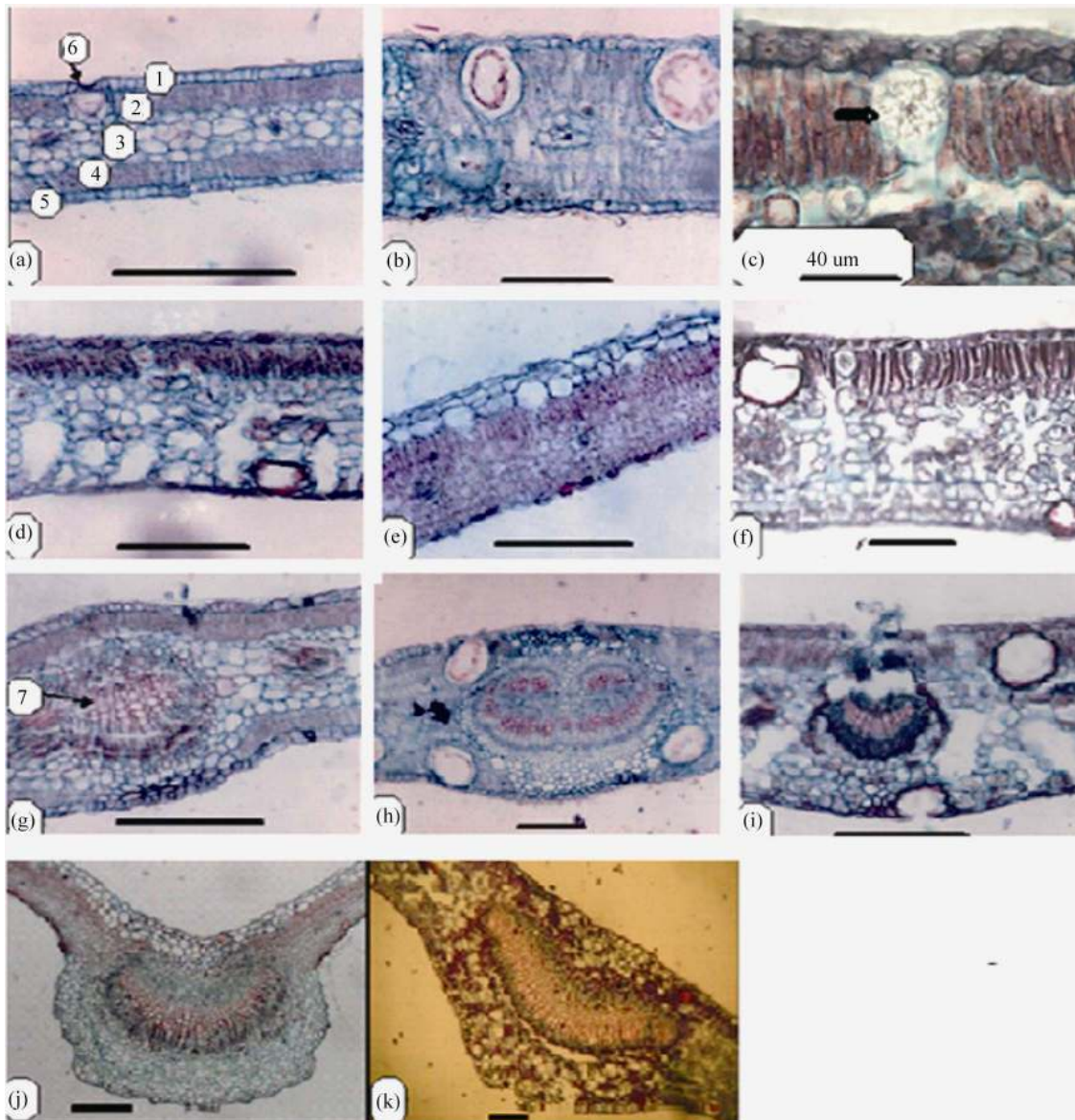
Larger number of epidermal cells is often recorded on the upper epidermis ranging between 127.2 and 420 cells/mm², while it was ranging between 132 and 302 cells/mm² on the lower epidermis in *Callistemon vimi-*

nalis and *Syzygium aromaticum* respectively *i.e.* appositively related with their dimensions. Stomatal complexes can be believed as a significant character in the identification of species.

3.2. Transverse Sections

3.2.1. Transverse Sections of Lamina

Two types of mesophyll has been recognized, isobilateral in *Callistemon viminalis* and *Eucalyptus camaldulensis* or dorsiventral in the other taxa (**Figure 2**) in agreement with the work of [11]. Souza *et al.* (1999) [20] mentioned that mesophyll of *Eucalyptus camaldulensis* is bifacial.



(a), (h): *Eucalyptus camaldulensis*; (b), (g): *Callistemon viminalis*; (c): druses in hypodermis of *Myrtus communis*; (d), (i): *Myrtus communis*; (e), (j): *Psidium guajava*; (f), (k): *Syzygium aromaticum*; 1: upper epidermis; 2: palisad layer; 3: spongy layer; 4: palisad layer; 5: lower epidermis; 6: secretory canal; 7: vascular bundle; (a-f): Lef lamina; g-k: midribs (scale 200 um).

Figure 2. Transverse section of leaf lamina and midribs.

while in *Eugenia pyriformis* the mesophyll was dorsiventral [21], but [3,4] stated that leaves in *Eucalyptus* and *Eugenia* sometimes show the two structures depending on the case of these leaves if are vertical or horizontal.

Thickness of lamina ranged between 180 μm in *Callistemon* and 435 μm in *Syzygium*. Cuticle thickness occurred between 3.95 μm and 10.35 μm in *Callistemon* and *Eucalyptus* respectively (**Table 2**). This increase in cuticle thickness in the latter taxon may be due to the presence of wax in addition to cuticle that [3] mentioned that epidermis coated with wax in some species of *Eucalyptus*.

Epidermal cells are square-shaped or rectangular, uniseriate. Hypodermis present below the upper epidermis in one species (*Psidium guajava*).

Mesophyll of 1 - 2 layers of palisade cells and several rows (up to 15) of compact or loose spongy tissue, or two layers of palisade cells on adaxial and abaxial surfaces enclosing spongy tissue (**Table 2**). Stone cells were recorded in the mesophyll of *Syzygium aromaticum*.

Mid-rib usually grooved from above erect downward, curved or flattened in both directions, supplied with one bicollateral vascular strand variable in shape according to species ranging between ovate, heart-shaped or U-shaped (**Figure 2, Table 3**).

Secretory cavities and cells containing tannin are very common in all unligified tissues in the taxa investigated. Metcalf and Chalk [3] pointed to similar structures. Esau (1977) [17] stated that tanniferous idioblasts and secretory cavities occur in many families including Myrtaceae. Oil cavities located close to both surfaces, glandular, and lined with epithelial-like cells (**Figure 2**). Druses crystals are very common in all species in the mesophyll and cortex of leaves, petioles and stems. Prismatic crystals are seen in the leaf close to the mid-rib in *Callistemon viminalis* and *Psidium guajava*.

There are some anatomical similarities among the leaves, such as striated cuticle, dorsiventral mesophyll, a midrib with a bicollateral vascular strand in an open arc that surrounded by a sclerenchymatic sheath, secretory canal occurred in the sub epidermis, druses and prismatic crystals of calcium oxalate and phenolic compounds [21, 22]. Secretory cavities developed from single epidermal cells, the origin of it was initially schizogenous but ultimately lysigenous at maturity with a breakdown of all the secretory cells within the gland lumen [23], while according to [24] reported that these cavities were located near the epidermis, the origin was from the epidermal meristem without participation of the ground meristem.

3.2.2. Transverse Sections of Petioles

Petioles were variable in length and shape ranging from elliptic to ovoid to semicircular to winged in the trans-

verse section covered with simple hairs in all species. Trichomes of *Psidium guajava* are simple and glandular hairs (**Figure 3**).

Epidermis is one layered, square or rectangular shaped, covered with a layer of cuticle of variable thickness, followed by a cortex of 2 - 3 rows of collenchyma, 2 - 4 rows of chlorenchyma and several rows of parenchyma ranging between 4 - 7. Large secreting cavities and druses are very common in cortical region in *Callistemon viminalis* and *P. guajava* are characterized by the presence of prismatic crystals rectangular (**Table 4**).

According [21] vascular bundle in a centric arrangement in the petiole has open arc, which similar with the open arc observed in the present work.

Vascular system with single, principal arc-shaped vascular strand, widely open, in *Callistemon*, U-Shaped in *Psidium*, *Myrtus* and *Syzygium*, heart-shaped in *Eucalyptus*. *Syzygium* and *Callistemon* were characterized by the presence of additional strands in the wings. Vascular strand is surrounded by sclerenchymatic tissue.

3.2.3. Transverse Sections of Stems

Anatomical characters of stems are represented in **Table 5**. Outline is semi-circular (*Myrtus*), quadrangular (*Syzygium*), quadrangular with wings (*Eucalyptus* and *Psidium*) or wavy (*Callistemon*) (**Figures 4 and 5**).

Epidermis uniseriate covered with a thin or thick layer of cuticle followed by multilayered cortex rich of wide secreting cavities and tanniferous cells. Three types of distinct tissues are recognized in cortex, chlorenchyma, collenchyma and parenchyma in taxa under investigation except the taxa *Eucalyptus*, *Myrtus* and *Syzygium* where chlorenchyma is absent.

Vascular tissue is a continuous bicollateral cylinder in all species. Pith presenting the centre of stem composed of parenchymatous storage cells of isodimetric to polyhedral thin layered cells with more or less large intercellular spaces. Cells are rich of druses crystals. Cell dimensions increases towards the center of stem.

Although the five species investigated belong to five distinct genera, [22] reported that the morphological features of the seed surface sculpture as revealed by SEM show no clear boundaries between the species of the same tribe except minor variation in the anticlinal and periclinal walls, so that the anatomical structures inform additional features for distinction of these species. Some are of high taxonomic significance such as the presence of hypodermis in leaves of *Psidium guajava*, the absence of stomata in the abaxial epidermis of *Callistemon viminalis* and *P. guajava*, the occurrence of isobilateral and dorsiventral leaf type appears to be a good diagnostic characteristic for the generic level, the wavy outline of *C. viminalis* stem and the presence of more than one vascular strand in the petiole of *C. viminalis* and *S. aromatic-*

Table 2. Anatomical characters of lamina in Myrtaceae.

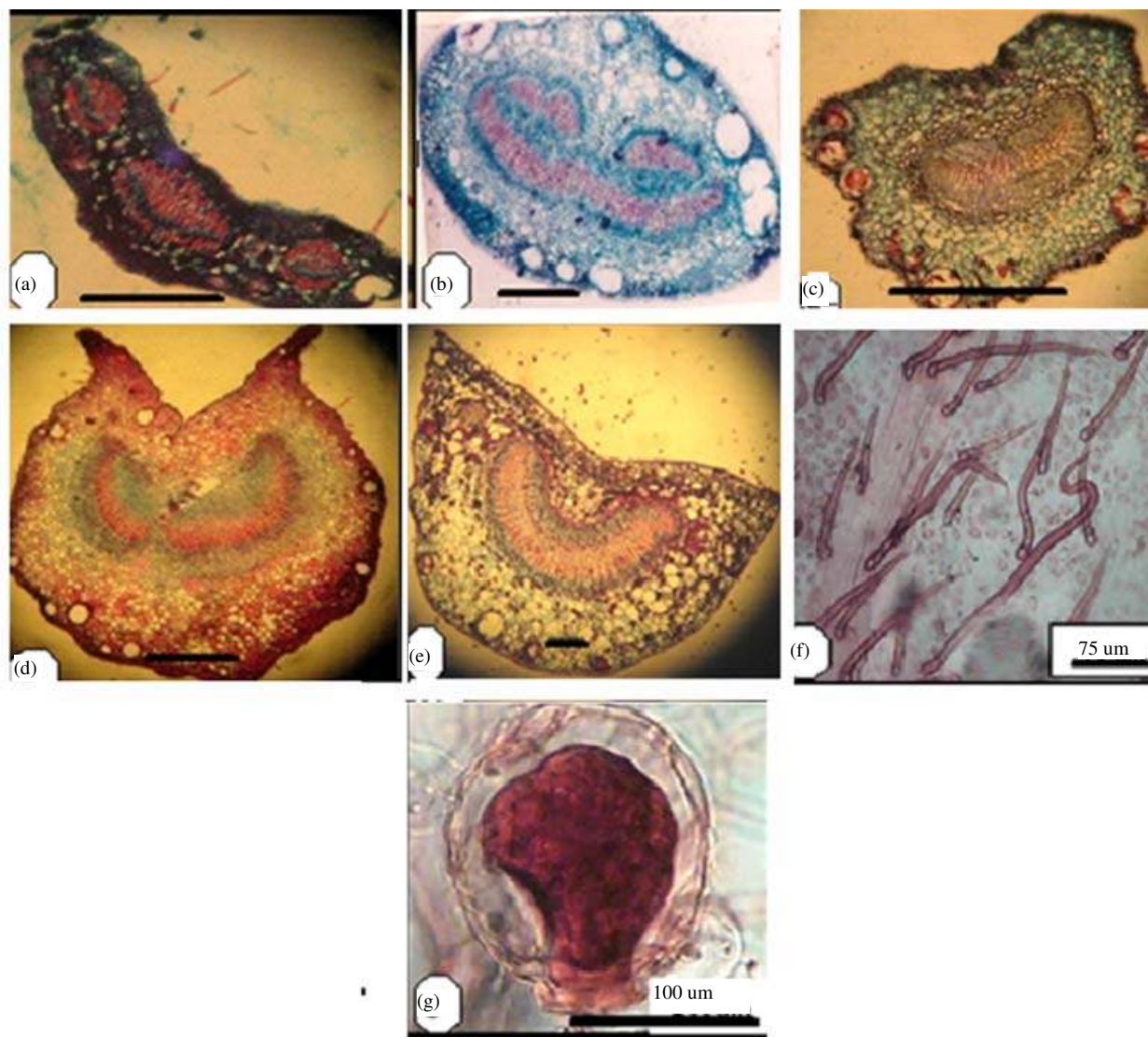
Taxa	Type	Lamina thickness (um)	Cuticle thickness (um)	Epidermis thickness (um)		Number of rows of palisade layer	Thickness of palisade layer (um)	Diameter of palisade layer cells (um)	Number of rows of spongy layer	Thickness of spongy layer (um)	Diameter of spongy layer cells (um)	Number of hypodermis layers
				upper	Lower							
<i>Callistemon viminalis</i> (Sol. ex Gaertn.) G. Don	Iso	(150 - 200) 180,00	(2.5 - 7.5) 3.95	(15 - 27.5) 20,00	(12.5 - 18) 16.25	2	(25 - 50) 41.25	(5 - 10) 7	4 - 6	(75 - 100) 85.62	(15 - 57.5) 33.92	-
<i>Eucalyptus camaldulensis</i> Dehnh.	Iso	(250 - 340) 290,00	(5 - 15) 10.35	(10 - 20) 15,00	(10 - 25) 19.50	2	(20 - 30) 25.40	(7.5 - 17.5) 11.66	1 - 6	(12.5 - 37.5) 25,00	(15 - 27.5) 21.87	-
<i>Myrtus communis</i> L.	Bi	(212.5 - 275) 236.07	(2.5 - 5) 4,00	(10 - 17.5) 13.50	(7.5 - 15) 11.25	1	(30 - 50) 41.42	(5 - 10) 7.25	9 - 11	(162 - 260) 193.33	(7.5 - 62.5) 10.36	-
<i>Psidium guajava</i> L.	Bi	(150 - 220) 190,00	(3.75 - 5) 4.50	(25 - 75) 47.81	(12.5 - 17.5) 15,00	1	(37.5 - 75) 57.08	(7.5 - 17.5) 11.42	7 - 9	(75 - 125) 93.75	(15 - 32.5) 21.66	+2
<i>Syzygium aromaticum</i> (L.) Merr.	Bi	(420 - 450) 435,00	(2.5 - 5) 4.25	(20 - 40) 29,00	(12.5 - 20) 16.42	1 - 2	(12.5 - 37.5) 26.66	(7.5 - 20) 13.50	11 - 15	(70 - 80) 75,00	(15 - 75) 36.94	-

iso = isobilateral; Bi = Bifacial (The values between arches represent the mean and the values out the arches represent the minimum and maximum values).

Table 3. Anatomical characters of mid-rib Myrtaceae (in micrometer).

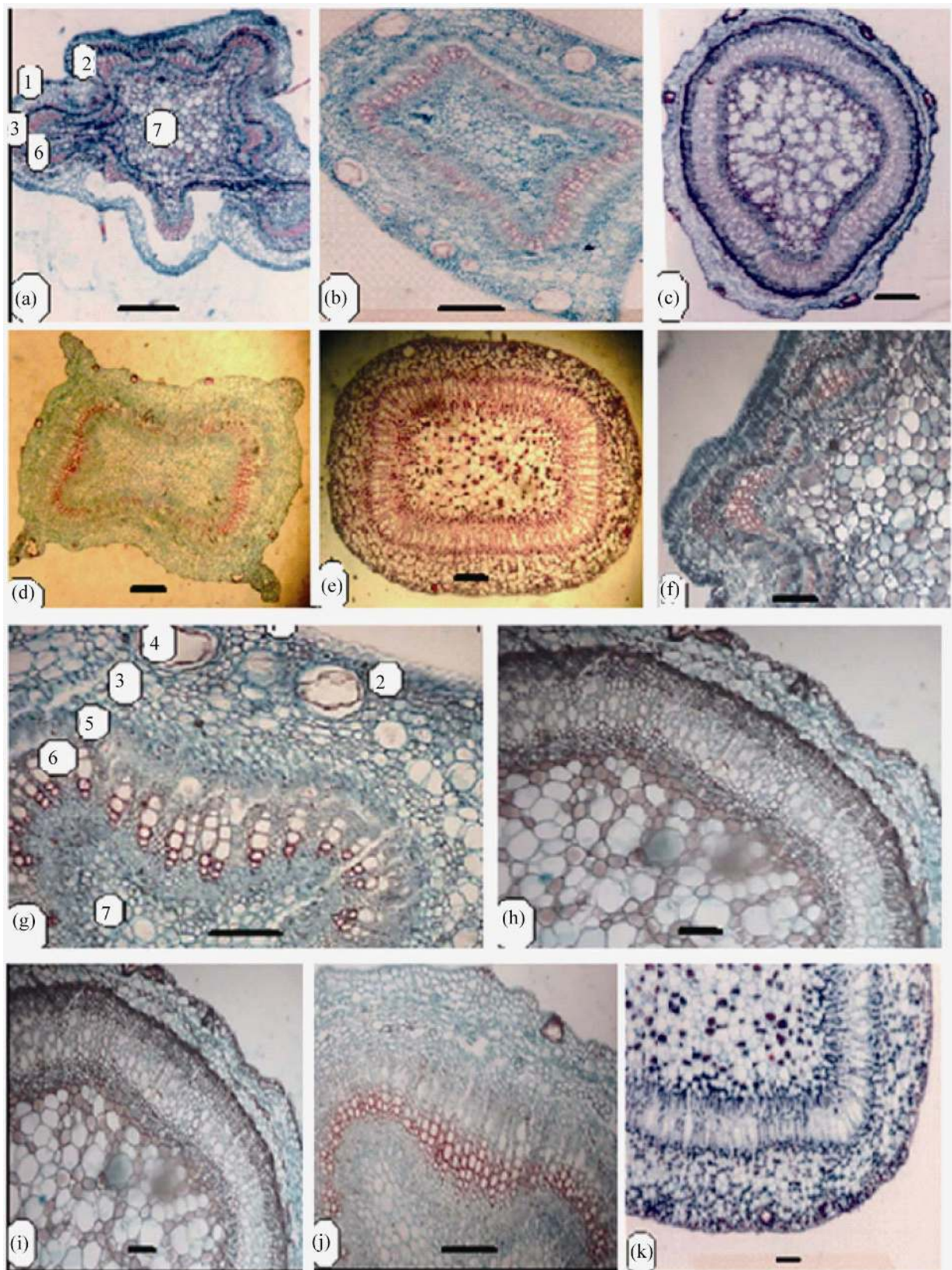
Taxa	Mid-rib shape	Thickness	Length of wood arms	Number of wood arms	Number of vessels in the arm	Mid-rib vascular bundle shape
<i>Callistemon viminalis</i> (Sol. ex Gaertn.) G. Don	Semi flat	(275 - 350) 312.5	(125 - 212.5) 168.75	18 - 24	6 - 12	Ovate
<i>Eucalyptus camaldulensis</i> Dehnh.	Semi flat	(675 - 700) 687.5	(56.3 - 87.3) 70.83	46 - 50	3 - 6	Heart-shaped
<i>Myrtus communis</i> L.	Erect	(257 - 287.5) 270.25	(112.5 - 175) 145.83	15 - 18	3 - 7	U-shaped
<i>Psidium guajava</i> L.	Strongly erect	(780 - 870) 850	(50 - 225) 158.33	51 - 56	4 - 14	U-shaped
<i>Syzygium aromaticum</i> (L.) Merr.	erect	(950 - 1100) 1075	(162.5 - 250) 213.50	60 - 63	11 - 13	U-shaped

The values between arches represent the mean and the values out the arches represent the minimum and maximum.



(a): *Callistemon viminalis*; (b): *Eucalyptus camaldulensis*; (c): *Myrtus communis*; (d): *Psidium guajava*; (e): *Syzygium aromaticum*; (f): simple trichomes; (g): glandular trichomes.

Figure 3. Transverse section of petioles (scale 200 μm).



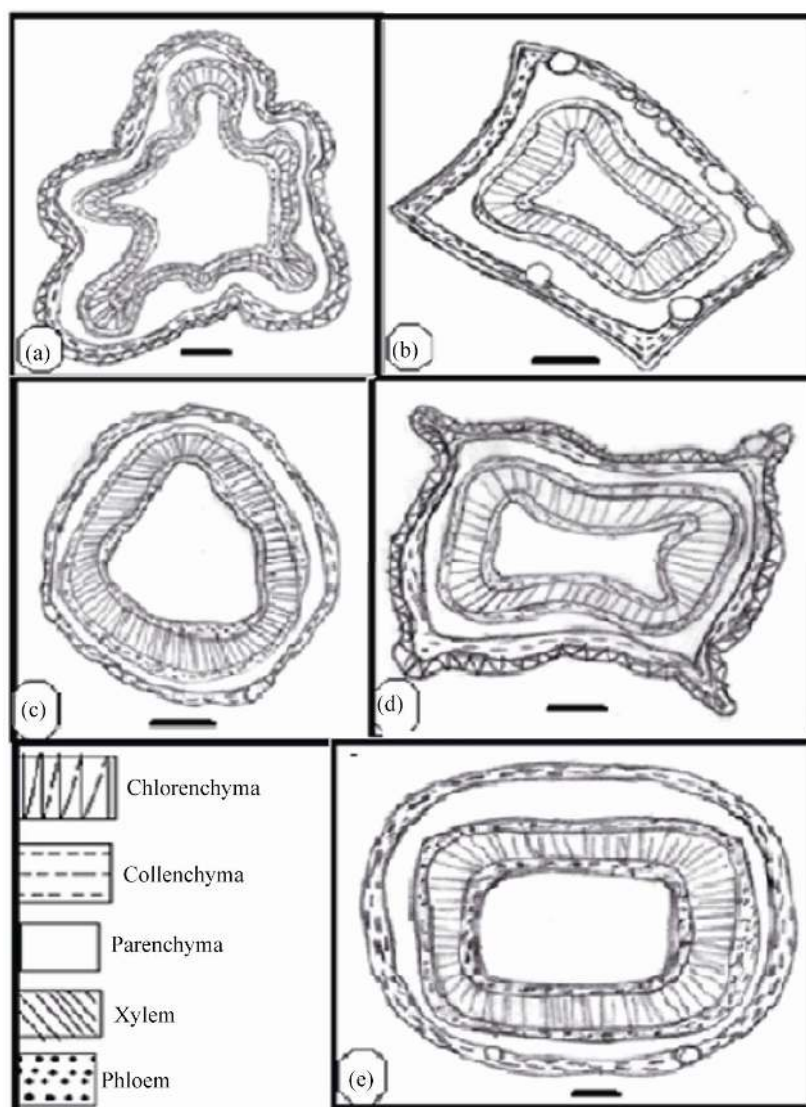
(a), (g): *Callistemon viminalis*; (b), (h): *Eucalyptus camaldulensis*; (c), (i): *Myrtus communis*; (d), (j): *Psidium guajava*; (e), (k): *Syzygium aromaticum*; 1: Epidermis; 2: collenchyma; 3: parenchyma; 4: secretory canal; 5: phloem; 6: xylem; 7: pith. (a-e): scale 200 um, f-k: scale 50 um

Figure 4. Transverse section of stems (scale 50 um).

Table 4. Anatomical characters of petiole in Myrtaceae (in micrometer).

Taxa	Shape	Thickness	Number of vascular bundle	Number of wood arms	Length of wood arms	Petiole vascular bundle shape	Crystal shape
<i>Callistemon viminalis</i> (Sol. ex Gaertn.) G. Don	Elliptic	(307.5 - 317.5) 312.50	1	(15 - 22) 17.00	(172.5 - 180) 175.83	Arc-shaped	D + P
<i>Eucalyptus camaldulensis</i> Dehnh.	Ovoid	(670 - 680) 675.00	1	(44 - 52) 47.00	(250 - 320) 290.00	Heart-shaped	D
<i>Myrtus communis</i> L.	Winged	(292.5 - 305) 300.00	1	(20 - 27) 25.00	(145 - 150) 147.50	U-Shaped	D
<i>Psidium guajava</i> L.	Winged	(720 - 760) 740.00	1	(55 - 62) 57.00	(300 - 350) 325.00	U-Shaped	D + P
<i>Syzygium aromaticum</i> (L.) Merr.	Semicircular	(920 - 960) 943.33	2 - 3	(46 - 50) 47.00	(290 - 320) 303.33	U-Shaped	D

D = Druses, P = Prismatic; The values between arches represent the mean and the values out the arches represent the minimum and maximum.



(a): *Callistemon viminalis*; (b): *Eucalyptus camaldulensis*; (c): *Myrtus communis*; (d): *Psidium guajava*; (e): *Syzygium aromaticum*.

Figure 5. Transverse section of stem (scale 200 um).

Table 5. Anatomical characters of stem in Myrtaceae (in micrometer).

Taxa	Shape	Cuticle thickness	Epidermis thickness	Cortex thickness			Phloem thickness	Length of wood arm	Pith diameter	Stem diameter	Pith/stem diameter
				Chlorenchyma	Collenchyma	Parenchyma					
<i>Callistemon viminalis</i> (Sol. ex Gaertn.) G. Don	Wavy	(4.25 - 7.50) 4.50	(10 - 15) 11.00	(11 - 30) 25.10	(20 - 40) 27.50	(20 - 150) 70.00	(40 - 60) 47.50	(50 - 80) 60.00	(450 - 800) 626.66	(980 - 1110) 1047.50	0.59
<i>Eucalyptus camaldulensis</i> Dehnh.	Quadrangular	(3.75 - 12.5) 8.50	(10 - 30) 22.50	-	(20 - 50) 48.33	(100 - 250) 161.25	(50 - 80) 65.00	(50 - 110) 76.25	(500 - 600) 545.00	(1000 - 1400) 1230.00	0.44
<i>Myrtus communis</i> L.	Semi-circular	(2.5 - 5.0) 3.95	(10 - 15) 10.11	-	(10 - 45) 32.10	(50 - 120) 80.00	(20 - 30) 27.50	(80 - 130) 104.00	(420 - 650) 518.00	(900 - 1100) 994	0.52
<i>Psidium guajava</i> L.	Quadrangular	(2.5 - 5.0) 4.21	(5 - 15) 10.00	(10 - 20) 15.35	(23 - 50) 40.11	(100 - 150) 115.00	(80 - 220) 120.00	(30 - 90) 61.66	(300 - 800) 600.00	(1100 - 1550) 1362.50	0.44
<i>Syzygium aromaticum</i> (L.) Merr.	Quadrangular	(2.5 - 5.0) 3.75	(10 - 15) 11.66	-	(12.07 - 47) 39.80	(170 - 280) 223.75	(50 - 100) 75.00	(120 - 220) 165.71	(700 - 1050) 900.00	(1400 - 2250) 1930.00	0.46

The values between arches represent the mean and the values out the arches represent the minimum and maximum).

cum, as well as the presence of prismatic crystal in the cortex of petiole of *P. guajava* and *C. viminalis*, in addition to the druses and the presence of glandular trichomes in *C. viminalis* and *P. guajava*. This phenomenon has not been reported before.

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