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RESEARCH ARTICLE

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Foliar epidermal micromorphology and its taxonomic implication of some Myrtaceae Juss. taxa

ABSTRACT:

The plants of Myrtaceae family show great economic potential for its medicinal properties, food and ecological importance. So, the leaf micromorphological characters of 26 cultivated species represent 15 genera belonging to family Myrtaceae, collected from Mazhar garden, Al-Bragil, Giza, Egypt, were examined using light and scanning electron microscope to evaluate these characters in taxa identification and delimitation. In this study, nine stomatal types were recognized; anomocytic, anisocytic, cyclocytic, staurocytic, paracytic, brachyparacytic, pericytic, diacytic and actinocytic, while paracytic is the most common. These types occurring were either hypostomatic or amphistomatic exclusively (homostomatic) or in combination (heterostomatic). For each species, the epidermal cell shape, type of anticlinal wall, shape and number of overlying cells associated with the secretory cavities, as well as presence or absence of unicellular trichomes were also recorded. The results showed the presence of abnormal giant stomata in ten taxa only which were considered more diagnostic. Eight sculpturing patterns were scored in these studied taxa; reticulate, ruminate, rugose, sclariform-reticulate, tuberculate, reticulate-foveate, colliculate and pusticulate. Other diagnostic features were recognized *viz.* The shape of periclinal walls of the epidermal cells, epicuticular wax, shape and level of stomatal aperture. An identification key based on the leaf micromorphological characters is provided to distinguish the genera and species.

KEY WORDS:

Myrtaceae, Leaf epidermal micromorphology, SEM.

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INTRODUCTION:

Myrtaceae Juss. (Myrtle family) is considered the ninth largest flowering plant family; it includes trees and shrubs distributed mostly in South America, Australia and Tropical Asia. Myrtaceae include nearly 5950 species belonging to 150 genera (Christenhusz and Byng, 2016). Many taxa are cultivated as ornamentals and for the manufacture of timber, oil, gum, tannin, resin, spices, and fruits. The family is characterized by entire leaves containing oil glands, semi inferior to inferior ovary and numerous stomata.

Several authors (Hickey and king, 1988; Judd *et al.,* 1999; Simpson, 2010) divided Myrtaceae into two subfamilies; Myrtoideae (75 genera) and Leptospermoideae (80 genera). Myrtoideae contained fleshy-fruited genera which are characterized by opposite entire leaves and placed under one tribe Myrteae, while Capsular-fruited genera with spirally arranged or alternate leaves placed in Leptospermoideae which is subdivided into

two tribes Leptospermeae and Chamaelaucieae.

Investigation of micro characters of the leaf surface is considered one of the most powerful taxonomic features that are widely used in the taxonomic studies of many families (Baranova, 1972; Stace, 1984; Jones, 1986; Park, 1994; Hong and Oh 1999; Hong and Son, 2000). Cuticular characters, epidermis, stomata, subsidiary cells and trichomes were found to be valuable in identification of many plants (Ellis, 1976).

Considering Myrtaceae, the leaf morphological investigation including stomatal characters can be used for taxonomic delimitation of the species (Schmid, 1980; Fontenelle *et al*., 1994). Metcalfe and Chalk (1979) documented four essential types of stomata in Myrtaceae; anomocytic, anisocytic, cyclocytic and paracytic. Hussin *et al*. (1992) studied leaf anatomy of *Eugenia* from the Malay Peninsula and stated that the most obvious finding for species identification was the stomatal type. According to several authors (Metcalfe and Chalk, 1979; Van Wyk *et al*., 1982; Fontenelle *et al*., 1994; Soh and Parnell, 2011), the presence of stomata on the abaxial surface only (hypostomatic) is a very common character in Myrtaceae. Also, Baranova (1992) observed the presence of more than one type of stomata in the same species (Heterostomatic) and proposed that, this character is taxonomically valuable. Giant abnormal stomata were recorded in some taxa as *Eugenia* (Van Wyk *et al*., 1982).

Carr *et al.* (1971) and Pole (2012) cleared that the epidermal anticlinal wall characters of Myrtaceae are taxonomically stable and have low interspecific variation. Also, Gifford and Foster (1989) and Retamales and Scharoschkin (2015) stated that, the shape of anticlinal walls is considered as an adaptation to environmental conditions.

According to Wilson (2011) leaves of Myrtaceae are usually glabrous or have trichomes on the leaf blades. Briggs and Johnson (1979) and Metcalfe and Chalk (1979) found that unicellular trichomes are the most common type in Myrtaceae, while in some plants of the family the leaves become glabrous with age. Fontenelle *et al.* (1994) found unicellular trichomes which abscising in adult leaves, leaving a scar in some studied *Eugenia* taxa.

Cuticular studies on the family Myrtaceae have been carried out by Metcalfe and Chalk (1950), Van Wyk *et al*. (1982), and Khatijah *et al.* (1992).

Secretory cavities are observed as oil dots on both leaf surfaces; considered as characteristic feature of Myrtaceae and recorded in many species as *Accara* (Cardoso and Sajo, 2006), *Eugeia* (Defaveri, 2006) and *Eucalyptus* (Santos *et al.,* 2008) which secrete flavonoids. Epidermal cells above secretory cavities are recognized as two overlying cells which are distinct in shape and size. They may be responsible for elimination of secretions accumulated in secretory cavities (List *et al*., 1995). Morphology and number of overlying cells associated with secretory cavities have taxonomic value (Gomes *et al*., 2009). Defaveri *et al*. (2011) recorded secretory cavities and its overlying cells in higher density in adaxial surface than the abaxial one.

The aim of the present study is to describe the foliar epidermal characters of 26 species belonging to family Myrtaceae using light and scanning electron microscope to evaluate these characters in taxa identification and delimitation.

MATERIAL AND METHODS:

In the present study, 26 cultivated taxa represent 15 genera belonging to family Myrtaceae were collected from Mazhar Botanical Garden, Al-Bragil, Egypt during spring season of 2015. The taxa were identified according to Bailey (1949) and Bailey and Bailey (1976). Their synonyms were derived from Tropicos. Voucher specimens were preserved at Botany Department Herbarium, Faculty of Science, Ain Shams University for future studies (Table 1).

Table 1. List of the studied taxa and their synonyms.

Taxa	Synonyms
1. *Acca sellowiana* (O.Berg) Burret	*Feijoa obovata*(O.Berg) O.Berg = *F. schenckiana* Kiaersk.= *F. sellowiana* (O.Berg) O.Berg = *F. sellowiana* f. *elongata* Voronova = *Orthostemon obovatus* O.Berg = *O. sellowianus* O.Berg
2. *Agonis flexuosa* (Willd.) Sweet	*Billottia flexuosa* (Muhl. ex Willd.) R.Br. = *Leptospermum flexuosum* (Muhl. ex Willd.) Spreng. = *L. glomeratum* H.L.Wendl. = *L. resiniferum* Bertol. = *Metrosideros flexuosa* Muhl. ex Willd.
3. *Backhousia citriodora* F. Muell.	
4. *Callistemon rigidus* R. Br.	*Metrosideros aspera* Hoffmanns. = *M. glandulosa* Desf. = *M. linearis* Muhl. ex Willd. = *M. rigida* Dum.Cours. = *M. rigidifolia* Hoffmanns.
5. *Callistemon viminalis* (Sol. ex Gaertn.) G. Don	*Melaleuca viminalis* (Sol. ex Gaertn.) Byrnes = *Metrosideros viminalis* Sol. ex Gaertn.

6. *Corymbia ficifolia* (F.Muell.) K.D.Hill & L.A.S.Johnson	*Eucalyptus ficifolia* F.Muell.
7. *Eucalyptus camaldulensis* Dehnh.	*Eucalyptus acuminata* Hook. = *E. longirostris* F.Muell. ex Miq. = *E. mcintyrensis* Maiden = *E. rostrata* Schltdl
8. *Eugenia supraaxillaris* Spreng.	*Eugenia axillaris* Vell. =*E. cambucarana* Kiaersk.
9. *Eugenia uniflora* L.	*Eugenia brasiliana* (L.) Aubl.= *E. michelii* Lam.≡ *E. myrtifolia* Salisb.= *Myrtus brasiliana* L.
10. *Lophostemon confertus* (R. Br.) Peter G. Wilson & J. T. Waterh.	*Tristania conferta* R. Br.= *Melaleuca conferta* (R. Br.) Steud.
11. *Melaleuca armillaris* (Sol. ex Gaertn.) Sm.	*Metrosideros armillaris* Sol. ex Gaertn.= *Myrtoleucodendron armillare* (Sol. ex Gaertn.) Kuntze
12. *Melaleuca ericifolia* Sm.	*Cajuputi ericifolia* (Sm.) A. Lyons=*Myrtoleucodendron ericifolium* (Sm.) Kuntze
13. *Melaleuca leucadendra* (L.) L.	*Myrtus leucadendra* L. = *Cajuputi leucadendron* (L.) A. Lyons = *Leptospermum leucodendron* (L.) J.R. Forst. & G. Forst. = *Meladendron leucocladum* (L.) St.-Lag.
14. *Melaleuca linariifolia* Sm.	*Melaleuca linariifolia var. typica* (Sm.) Domin=*Myrtoleucodendron linariifolium* (Sm.) Kuntze
15. *Metrosideros excelsa* Gaertn.	*Metrosideros tomentosa* A. Rich.
16. *Myrtus communis* L.	*Myrtus oerstedeana* O. Berg=*M. sparsifolia* O. Berg
17. *Pimenta dioica* (L.) Merr.	*Caryophyllus pimento* Mill. = *Eugenia micrantha* (Kunth) DC. = *E. micrantha* Bertol. = *E. pimenta* (L.) DC. = *Myrtus dioica* L.=*M. pimenta* L.=*M. tabasco* Willd. ex Schltdl. & Cham. = *Pimenta aromatica* Kostel. = *P. officinalis* Lindl. = *P. pimenta* (L.) Cockerell = *P. vulgaris* Lindl.=*Pimentus vera* Raf.
18. *Pimenta racemosa* (Mill.) J. W. Moore	*Amomis acris* (Sw.) O. Berg= *Amomis caryophyllata* (Jacq.) Krug & Urb.= *A. caryophyllata var. grisea* (Kiaersk.) Urb. = *A. grisea* (Kiaersk.) Britton=*A. pimento* O. Berg=*A. pimento var. jamaicensis* O. Berg=*A. pimento var. surinamensis* O. Berg=*A. pimentoides* (DC.) O. Berg= *Caryophyllus racemosus* Mill. = *Myrcia acris* (Sw.) DC. = *M. pimentoides* DC.= *M. acris* Sw.=*Myrtus caryophyllata* Jacq.=*Myrtus pimenta* Ortega=*Pimenta acris* (Sw.) Kostel.=*P. acuminata* Bello = *P. pimento* (O. Berg) Griseb.
19. *Psidium cattleyanum* Sabine	*Eugenia pseudovenosa* H. Perrier= *Guajava cattleyana* (Sabine) Kuntze= *Psidium littorale* Raddi= *P. variabile* O. Berg
20. *Psidium guajava* L.	*Psidium cujavillus* Burm. f. = *P. pomiferum* L. = *P. pumilum* Vahl = *P. pyriferum* L.
21. *Syzygium gratum* (Wight) S.N.Mitra	*Eugenia grata* Wight= *Acmena grata* (Wight) Walp.
22. *Syzygium jambos* (L.) Alston	*Eugenia jambos* L. = *Jambosa jambos* (L.) Millsp. = *J. vulgaris* DC.= *Myrtus jambos* (L.) Kunth
23. *Syzygium malaccense* (L.) Merr. & L. M. Perry	*Eugenia macrophylla* Lam. = *E. malaccensis* L.=*Jambosa domestica* Blume = *J. malaccensis* (L.) DC.
24. *Syzygium paniculatum* Gaertn.	*Eugenia rheedioides* Standl. & Steyerm
25. *Syzygium samarangense* (Blume) Merr. & L. M. Perry	*Eugenia samarangensis* (Blume) O. Berg=*Jambosa samarangensis* (Blume) DC. ≡ *Myrtus samarangensis* Blume
26. *Xanthostemon fruticosus* Peter G.Wilson & Co	

LM investigation:

Lamina epidermal samples were prepared from fresh material by taking fragments of 5 - 10 mm^2 from the median portion of the leaf, soaked in stomatal solution (1 Nitric acid:1 Hydrogen peroxide) from 1 - 2 hours in water bath at 70°C. Then epidermal strips were stained with safranin (Johanson, 1940), mounted on slides, examined using BEL: B103 T-PL light microscope and the photos were taken using Canon Power Shot G12 digital camera.

SEM leaf epidermal study:

Pieces of one-centimeter square were cut from the median portion of the lamina, mounted on stubs and attached with sticky tabs, then coated with gold sputter coater (SPI-Module). The specimens examined both adaxially and abaxially, photographed by scanning electron microscope (JEOL-JSM-5500LV) using high vacuum mode at the Regional Centre of Mycology and Biotechnology, El-Azhar University, Cairo, Egypt. Terminology of epidermal characters using LM and SEM based on Metcalfe and Chalk (1950), Dilcher (1974), LAWG (1999), and Prabhakar (2004).

RESULTS AND DISCUSSION:

Lamina epidermal characters for the 26 studied taxa using light and scanning electron microscope are summarized in tables (2 & 3) and figures (1 - 6).

Table. 2. Lamina epidermal characters for the studied taxa of Myrtaceae

Character / Taxa	Adaxial surface				Abaxial surface				Abnormal giant stomata	Overlying cells	
	Cell shape	Anticlinal walls	Stomata type	Trichomes	Cell shape	Anticlinal walls	Stomate type	Trichomes		Shape	Number
1- Acca sellowiana	Polygonal	Straight	-	Abscising	Irregular	Straight	Actinocytic	coiled	+	Polygonal	2
2- Agonis flexuosa	Polygonal	Rounded	Brachyparacytic & Anisocytic	-	Polygonal	Rounded	Brachyparacytic & Anisocytic	-	_	Polygonal	2
3- Backhousia citriodora	Irregular	Strongly undulate	_	Abscising	Irregular	Strongly undulate	Brachyparacytic & Anomocytic	+	_	Kidney-shaped	2
4- Callistemon rigidus	Polygonal	Straight	Paracytic	Abscising	Polygonal	Straight	Paracytic	+	_	Polygonal	2
5- Callistemon viminalis	Polygonal	Straight	Diacytic	Straight	Polygonal	Straight	Diacytic	+	_	Kidney-shaped	2
6- Corymbia ficifolia	Polygonal	Straight	_	-	Tetragonal	slightly undulate	Anisocytic	_	_	-	-
7- Eucalyptus camaldulensis	Polygonal	Straight	Anomocytic & staurocytic	_	Polygonal	Straight	Anomocytic & staurocytic	_	+	Kidney-shaped	2
8- Eugenia supraaxillaris	Polygonal	Rounded	_	_	Polygonal	Slightly undulate	Paracytic & anisocytic	_	_	Stellate	1
9- Eugenia uniflora	Irregular	Strongly undulate	_	_	Irregular	Strongly undulate	Paracytic & anisocytic	_	_	Kidney-shaped	2
10- Lophostemon confertus	Polygonal	Straight	_	Abscising	Polygonal	Straight	Anomocytic	+	+	Stellate	1
11- Melaleuca armillaris	Polygonal	Straight	Brachyparacytic & Anomocytic & Anisocytic	Abscising	Polygonal	Straight	Brachyparacytic & Anomocytic	Abscising	-	Polygonal	2
12- Melaleuca ericifolia	Polygonal	Straight	Anomocytic	_	Polygonal	Straight	anamocytic	_	+	Polygonal	2
13- Melaleuca leucadendra	Polygonal	Straight	Cyclocytic	Straight	Polygonal	Straight	Cyclocytic	Abscising	+	Stellate	1
14- Melaleuca linariifolia	Polygonal	Rounded	Pericytic	Abscising	Polygonal	Rounded	Pericytic	Abscising	_	_	_
15- Metrosideros excelsa	Polygonal	Slightly undulate	_	Abscising	Polygonal	Slightly undulate	Paracytic	Abscising	_	Stellate	1
16- Myrtus communis	Irregular	slightly undulate	_	Straight	Irregular	slightly undulate	Anomocytic, brachyparacytic, staurocytic	_	_	Kidney-shaped	2
17- Pimenta dioica	Polygonal	Especially undulate knobs	-	_	Irregular	Specially undulate knobs	Paracytic	_	+	Kidney-shaped	2
18- Pimenta racemose	Irregular	Especially undulate knobs	-	_	Irregular	Specially undulate knobs	Anomocytic	_	+	Kidney-shaped	2
19- Psidium cattleyanum	Polygonal	Slightly undulate	_	_	Polygonal	Slightly undulate	Paracytic	_	+	Stellate	1
20- Psidium guajava	Polygonal	Straight	-	Coiled	Polygonal	Straight	Paracytic	coiled	_	Stellate & kidney-shaped	1 & 2
21- Syzygium gratum	Irregular	Strongly undulate	_	_	Irregular	Strongly undulate	Anisocytic	_	_	Kidney-shaped	2
22- Syzygium jambos	Irregular	Undulate Special knobs	_	_	Irregular	Strongly undulate	Paracytic	_	_	Polygonal	2
23- Syzygium malaccense	Polygonal	Straight	_	_	Polygonal	Rounded	Paracytic	_	+	Polygonal	2
24- Syzygium paniculatum	Polygonal	Rounded	_	_	Polygonal	slightly undulate	Paracytic	_	+	Kidney-shaped	2
25- Syzygium samarangense	Irregular	Slightly undulate	_	_	Irregular	slightly undulate	Paracytic	_	_	Kidney-shaped	2
26- Xanthostemon fruticosus	Irregular	Rounded	_	_	Irregular	Rounded	Anisocytic, brachyparacytic	_	_	Stellate	1

(+: present , - : absent)

Table. 3. Lamina epidermal characters using SEM for the studied taxa of Myrtaceae

Characters / Taxa	Adaxial surface						Abaxial surface					
	General sculpturing	Anticlinal walls	Periclinal walls	Epicuticular wax	Stomatal aperture		General sculpturing	Anticlinal walls	Periclinal walls	Epicuticular wax	Stomatal aperture	
					Shape	Level					Shape	Level
1- Acca sellowiana	Reticulate	Raised, smooth	Sunken, smooth	Smooth	--	--	Ill-defined				Semi-circular	Raised
2- Agonis flexuosa	Sclariform-reticulate	Raised, striated	sunken & striated	Striated	Elliptic	Leveled	Rugose	Sunken, smooth	Raised, striated	Striated	Elliptic	Leveled
3- Backhousia citriodora	Ill-defined	Ill-defined	Ill-defined	Granulated	_	--	Ill-defined			Granulated	Elliptic	Raised
4- Callistemon rigidus	Ill-defined	Ill-defined	Ill-defined	Parallel oriented stria	Circular	Raised, stomatal chimney	Ill-defined			Parallel oriented stria	Circular	Raised, stomatal chimney
5- Callistemon viminalis	Ill-defined	Ill-defined	Ill-defined	Parallel oriented stria	Semi-circular	Leveled	Ill-defined			Parallel oriented stria	Semi-circular	Leveled
6- Corymbia ficifolia	Ruminate	Raised, smooth	Sunken with stria	Smooth	_	--	Ruminate	Raised, smooth	Sunken with granules	Granulated	Elliptic	Sunken
7- Eucalyptus camaldulensis	Tuberculate	Sunken with platlets	Raised, smooth	Non-entire platlets	Elliptic	Leveled	Tuberculate	Sunken, granulated	Raised, smooth	Granulated	Elliptic	Leveled
8- Eugenia supraaxillaris	Reticulate-Foveate	Raised, striated	Sunken, striated	Striated	_	-	Ruminate	Sunken, striated	Raised, striated	Parallel grouped platlets	Circular	Leveled
9- Eugenia uniflora	Ill-defined	Ill-defined	Ill-defined	Undulate wrinkled stia	_	-	Ill-defined			Striated undulated	Elliptic	Raised
10- Lophostemon confertus	Colliculate	Sunken, granulated	Raised, granulated	Granulated	_	-	Reticulate	Raised, striated	Sunken, striated	Wrinkled stria	Elliptic	Leveled
11- Melaleuca armillaris	Ill-defined	Ill-defined	Ill-defined	Striated non-entire platelets	Slit-like	Raised, stomatal chimney	Ill-defined			Striated non-entire platelets	Slit-like	Leveled
12- Melaleuca ericifolia	Pusticullate	Sunken, smooth	Raised, smooth	Smooth	Elliptic	Raised	rugose	Sunken, smooth	Raised, smooth	Smooth	Elliptic	Raised
13- Melaleuca leucadendra	Colliculate	Sunken, smooth	Raised, smooth	Smooth	Semi-circular	Raised, stomatal chimney	Colliculate	Sunken, smooth	Raised, smooth	Smooth	Semi-circular	Raised, stomatal chimney
14- Melaleuca linariifolia	Colliculate	Sunken, smooth	Raised, granulated	Granulated	Semi-circular	Leveled	Colliculate	Sunken, smooth	Raised, granulated	Granulated	Semi-circular	Leveled
15- Metrosideros excelsa	Tuberculate	Sunken, undulated stria	Raised, smooth	Undulate straited wrinkles	_	-	Rugose	Sunken smooth	Raised, smooth	Smooth	Circular	Raised
16- Myrtus communis	Ill-defined	Ill-defined	Ill-defined	Undulate straited wrinkles	_	-	Ruminate	Sunken, smooth	Raised, smooth	Smooth	Elliptic	Sunken
17- Pimenta dioica	Pusticulate	Sunken, granulated	Raised, smooth	Granulated	_	-	Pusticulate	Sunken, smooth	Raised, smooth	Smooth	Elliptic	Leveled
18- Pimenta racemose	Ruminate	Sunken, smooth	Raised, smooth	Undulate wrinkled stria	_	-	Tuberculate	Sunken, fine granules	Raised, granulated	Granulated	Slit-like	Leveled
19- Psidium cattleyanum	Pusticulate	Sunken, granulated	Raised, granulated	Granulated	_	-	Pusticulate	Sunken, granulated	Raised, granulated	Granulated	Slit-like	Leveled
20- Psidium guajava	Ill-defined			Undulate wrinkled sria	_	-	Reticulate	Raised with stria	Sunken, smooth	Undulate wrinkled stria	Elliptic	Raised, stomatal chimney
21- Syzygium gratum	Pusticulate	Sunken, granulated	Raised, granulated	Granulated	_	-	Ill-defined	Ill-defined	Ill-defined	Parallel stacked platelets	Elliptic	Raised
22- Syzygium jambos	Ill-defined	Ill-defined	Ill-defined	Striated coiled thread rodlets	_	-	Ruminate	Sunken, granulated	Raised, granulated	Granulated	Elliptic	Raised
23- Syzygium malaccense	Reticulate-foveate	Raised, wide, smooth	sunken with stria	Smooth	_	-	Ruminate	Sunken, smooth	Raised, smooth	Smooth	Elliptic	Raised
24- Syzygium paniculatum	Reticulate	Raised, striated	Sunken, striated	Striated	_	-	Ruminate	Sunken, smooth	Raised, smooth	Smooth	Elliptic	Leveled
25- Syzygium samarangense	Ill-defined	Ill-defined	Ill-defined	Striated coiled thread like	-	-	Ruminate	Sunken, smooth	Raised, striated	Striated	Circular	Raised, stomatal chimney
26- Xanthostemon fruticosus	Reticulate	Raised, smooth	Sunken, smooth	Smooth	-	-	Reticulate	Raised, granulated	Sunken, granulated	Granulated	Slit-like	Sunken

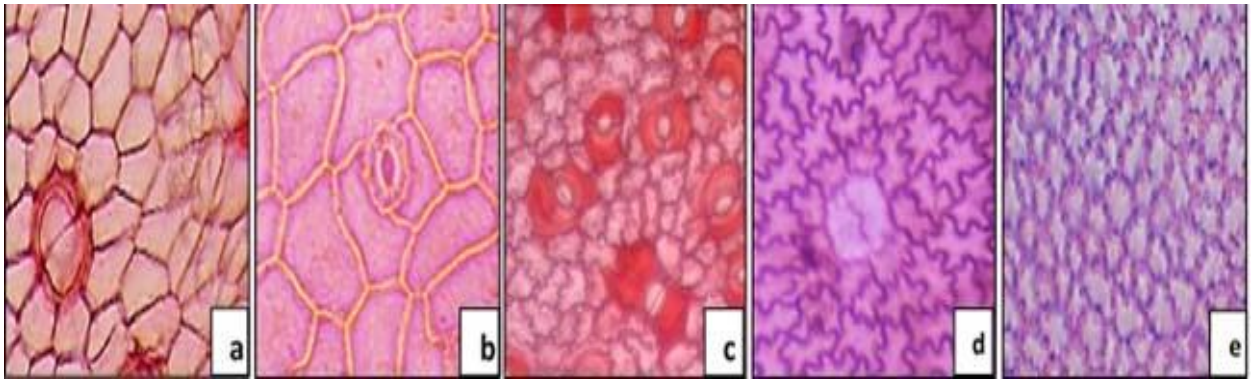

Fig. 1. Light micrographs (LM) of some studied taxa (X = 40) (a-e) showing the shape of epidermal anticlinal walls: a: straight in *Callistemon rigidus*; b. rounded in *Agonis flexuosa*; c. slightly undulates in *Syzygium samarangense*; d. strongly undulates in *Backhousia citriodora*; e. undulates with knobs in *Pimenta dioica*.

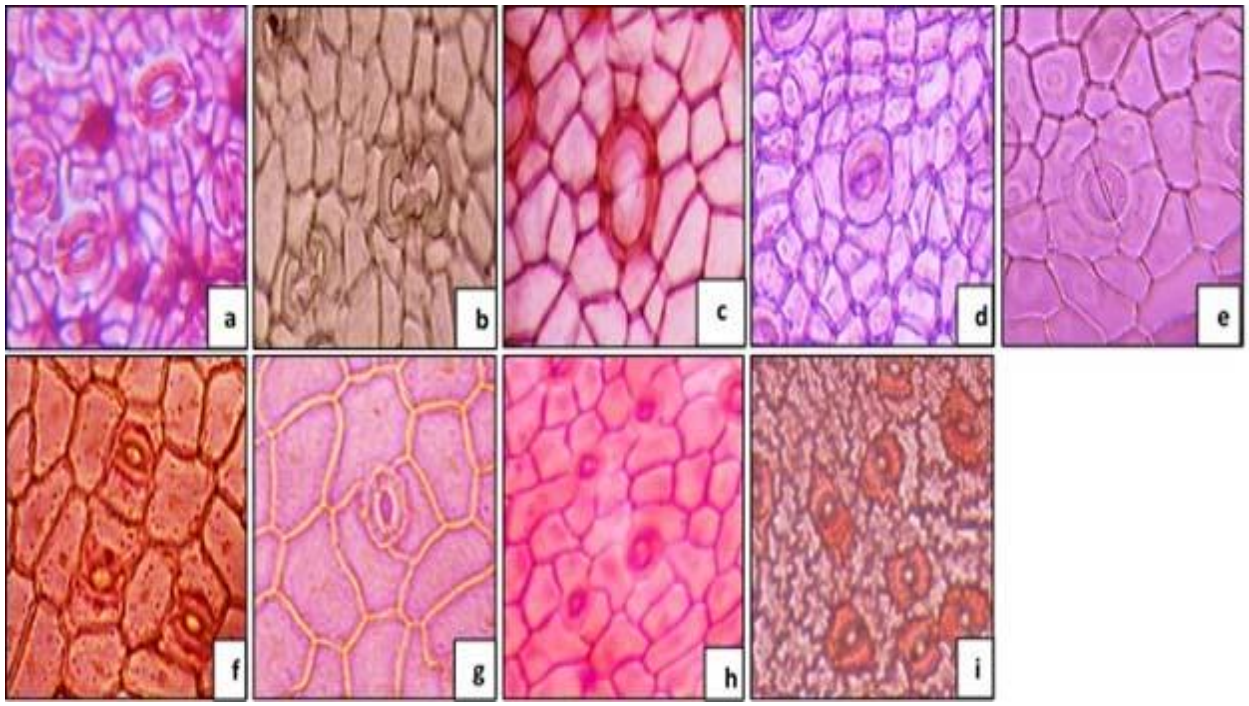

Fig. 2. Light micrographs (LM) of some studied taxa (X = 40) (a-e) showing Stomatal types (X = 40) (a-i): a. Actinocytic in *Acca sellowiana*; b: cyclocytic in *Melaleuca leucadendra*; c: Diacytic in *Callistemon viminalis*; d: pericytic in *Melaleuca linariifolia*; e: Staurocytic in *Eucalyptus camaldulensis*; f: brachyparacytic in *Agonis flexuosa*; g: anisocytic in *Agonis flexuosa*; h: anomocytic in *Melaleuca armillaris*; arrow indicates the abnormal giant stomata, i: paracytic in *Syzygium jambos*.

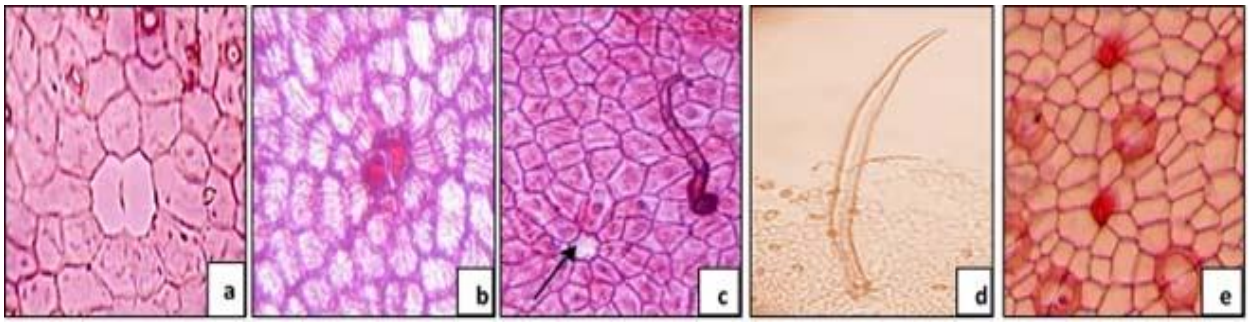

Fig. 3. Light micrographs (LM) of some studied taxa (X = 40) (a-c) showing the shape and number of overlying cells above secretory cavity: a: two polygonal cells in *Agonis flexuosa*; b. two kidney-shaped in *Syzygium samarangense*; c. one stellate cell (arrow) and coiled unicellular eglandular unbranched trichome in *Psidium guajava*. Trichomes (X = 40) (c-e): d. straight unicellular eglandular unbranched trichome in *Backhousia citriodora*; e: base of abscised trichome in *Callistemon rigidus*.

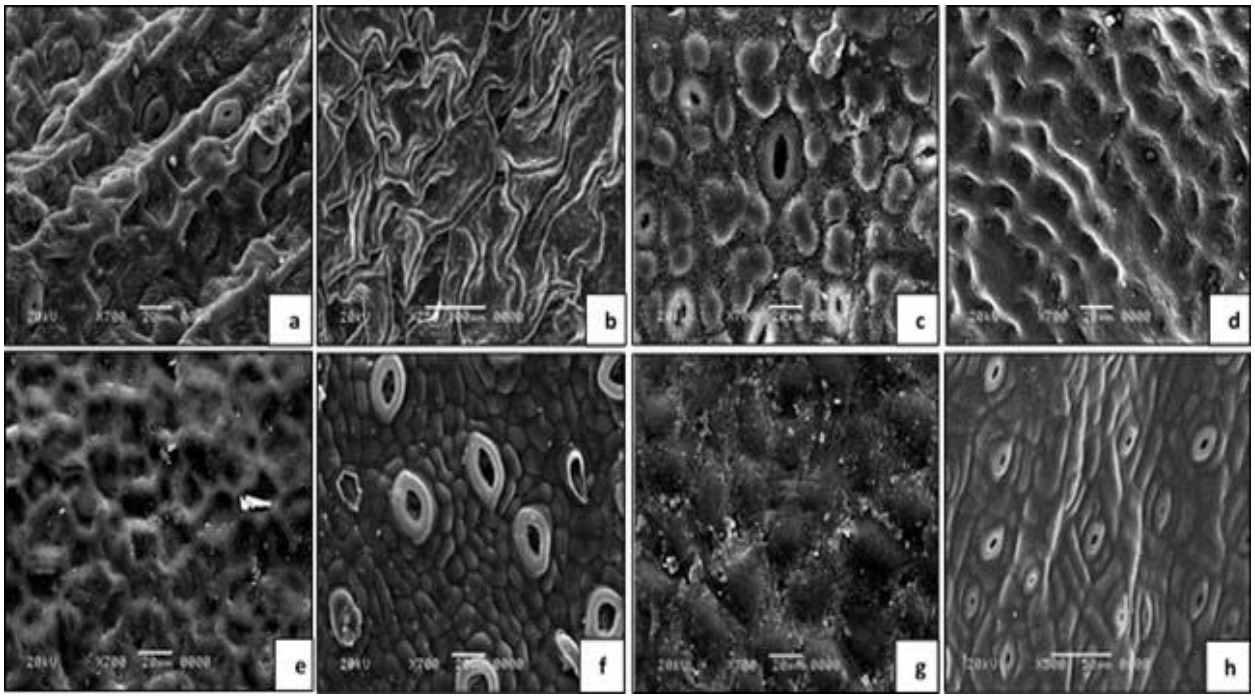

Fig. 4. Major aspects of lamina sculpturing patterns using SEM (a-h) a. sclariform-reticulate on adaxial surface (*Agonis flexuosa*); b. ruminate on adaxial surface (*Corymbia ficifolia*); c. tuberculate on adaxial surface (*Eucalyptus camaldulensis*); d. reticulate-foveate on adaxial surface (*Eugenia supraaxillaris*); e. reticulate on adaxial surface (*Xanthostemon fruticosus*); f. colliculate on adaxial surface (*Melaleuca leucadendra*); g. pusticulate on adaxial surface (*Psidium cattleyanum*); h. rugose on abaxial surface (*Agonis flexuosa*).

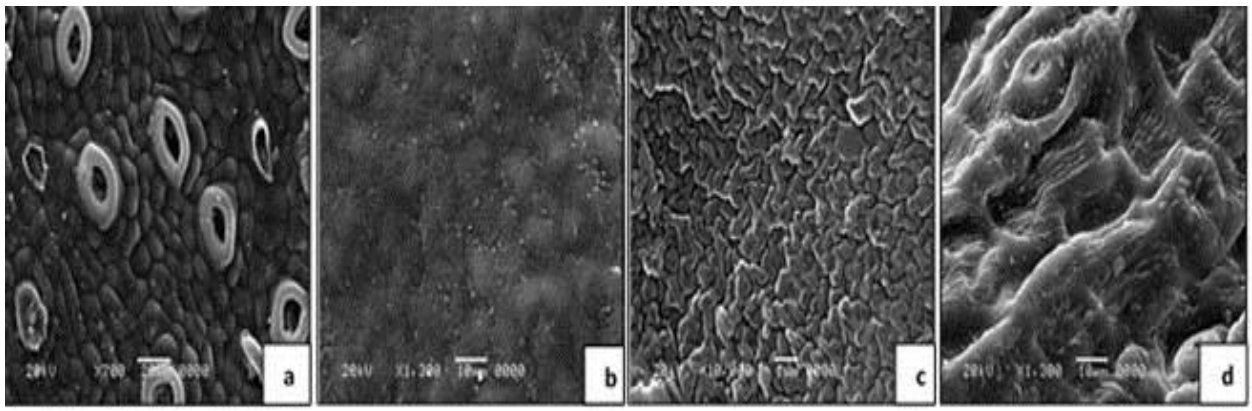

Fig. 5. Scanning electron micrographs showing epicuticular wax patterns (a-d) a. smooth on adaxial surface in *Melaleuca leucadendra*; b. granulated on adaxial surface in *Syzygium gratum*; c. undulate wrinkled stria on adaxial surface in *Pimenta racemosa*; d. striated on adaxial surface in *Agonis flexuosa*.

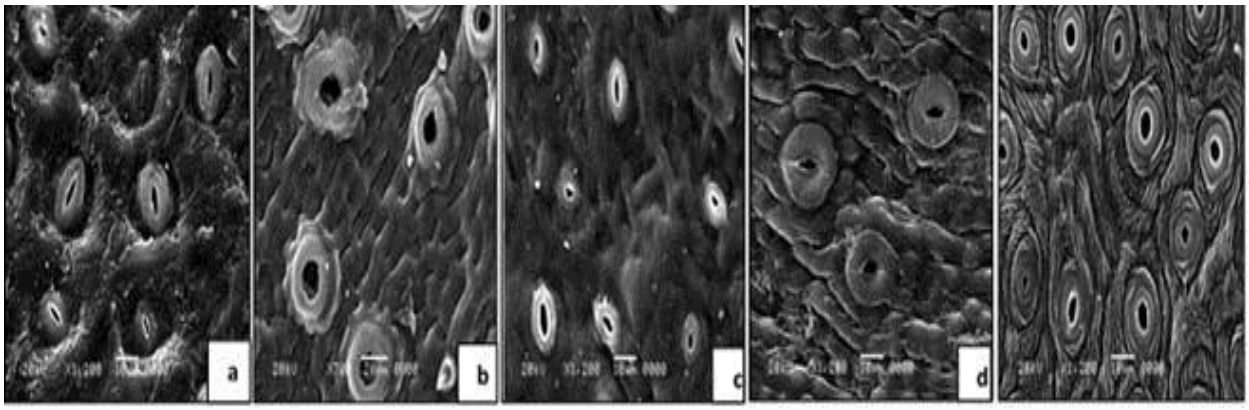

Fig. 6. Scanning electron micrograph showing Stomatal aperture shape and level (a-e) a. slit-like sunken on abaxial surface in *Xanthostemon fruticosus*; b. circular raised with stomatal chimney on abaxial surface in *Callistemon rigidus*; c. elliptic levelled on abaxial surface in *Syzygium paniculatum*; d. elliptic raised on abaxial surface in *Melaleuca ericifolia*; e. circular leveled on abaxial surface in *Eugenia supraaxillaris*.

Surface patterns using light microscope:

Epidermal cell shape:

Acca sellowiana and *Pimenta dioica* revealed polygonal (tetra, penta or hexagonal) shape on adaxial surface and irregular on abaxial surface. Both surfaces showed the same cell shape in 24 studied taxa as they were irregular in *Backhousia citriodora, Eugenia uniflora, Myrtus communis, Pimenta racemosa, Syzygium gratum, S. jambos, S. samarangense* and *Xanthostemon fruticosus* and polygonal in the rest 16 taxa.

The type of anticlinal walls on both ad/abaxial surfaces in most of the studied taxa showed the same pattern, while in some species become different on both surfaces. The anticlinal walls are undulate with knobs in *Pimenta dioica* and *P. racemose* (Fig. 1e), rounded in *Agonis flexuosa* (Fig. 1b)*, Melaleuca linariifolia* and *Xanthostemon fruticosus*, slightly undulate in *Metrosideros excelsa, Myrtus communis, Psidium cattleyanum,* and *Syzygium samarangense* (Fig. 1c), strongly undulate in *Backhousia citriodora* (Fig. 1d), *Eugenia uniflora* and *Syzygium gratum*, straight in the rest nine taxa (Fig. 1a). On the other hand, the remaining five studied taxa showed varied anticlinal shape between ad/abaxial surface. The anticlinal walls of *Corymbia ficifolia* straight on adaxial surface and slightly undulate on abaxial surface. In *Syzygium jambos* it was undulate with knobs on adaxial surface and strongly undulate on abaxial surface. In *Syzygium malaccense* it was straight on adaxial surface and rounded on abaxial surface. While, in *Eugenia supraaxillaris* and *Syzygium paniculatum* it was rounded on the adaxial surface and slightly undulate on the abaxial surface.

Gifford and Foster (1989) and Retamales and Scharoschkin (2015) suggested that the shape of anticlinal walls is considered as an adaptation to environmental conditions as mesophytes mainly have wavy or undulate walls while xerophytes usually have straight ones. Contrary, Fontenelle, *et al.* (1994) supposed that this character is not necessary associated with environmental conditions. Present results agree with this finding, as some of our studied taxa possessed both straight and undulate anticlinal walls. Epidermal anticlinal walls exhibit low intraspecific variation in Myrtaceae (Carr *et al.,* 1971) and regarded as a taxonomically constant character (Pole, 2012).

The leaf type in relation to occurrence of stomata the result of this study revealed that, the hypostomatic leaf type is frequently recorded in 18 studied taxa. This result in accordance with (Metcalfe and Chalk, 1979; Van wyk *et al*., 1982; Fontenelle *et al*., 1994; Soh and Parnell, 2011; Khan *et al*., 2016) who observed the presence of hypostomatic leaves in several Myrtaceae genera. Otherwise, amphistomatic leaf type reported in only eight taxa *(Agonis flexuosa*, *Callistemon rigidus*, *C. viminalis*, *Eucalyptus camaldulensis, Melaleuca armillaris*, *M. ericifolia, M. leucadendra, M. linariifolia*).

The leaf category in relation to stomatal variation, several authors recorded the presence of more than one type of stomata on the same leaf in many Myrtaceae genera and considered heterostomatic leaves common character in that family (Baranova, 1992; Fontenelle *et al*., 1994; Kantachot *et al.,* 2007; Soh and Parnell, 2011; Edany and Al-Saadi, 2012). In this study, heterostomatic category scored in *Agonis flexuosa, Backhousia citriodora*, *Eucalyptus camaldulensis*, *Eugenia supraaxillaris, E. uniflora, Melaleuca armillaris, Myrtus communis* and *Xanthostemon fruticosus*, while homostomatic category (one type of stomata on either adaxial or abaxial surface) observed in the rest 18 taxa.

Stomatal types; actinocytic in *Acca sellowiana* (Fig. 2a), diacytic in *Callistemon viminalis* (Fig. 2c), cyclocytic in *Melaleuca leucadendra* (Fig. 2b), pericytic in *Melaleuca linariifolia* (Fig.2.d.), staurocytic in *Eucalyptus camaldulensis* (Fig. 2e.) and *Myrtus communis*, brachyparacytic in *Agonis flexuosa* (Fig. 2f), *Backhousia citriodora*, *Melaleuca armillaris*, *Myrtus communis, Xanthostemon fruticosus*, anisocytic in *Agonis flexuosa* (Fig. 2g), *Corymbia ficifolia*, *Eugenia supraaxillaris, Eugenia uniflora, Melaleuca armillaris, Syzygium gratum, Xanthostemon fruticosus*, anomocytic in *Backhousia citriodora*, *Eucalyptus camaldulensis, Lophostemon confertus, Melaleuca armillaris*, *M. ericifolia* (Fig. 2f)*, Myrtus communis, Pimenta racemose*, and paracytic in *Callistemon rigidus*, *Eugenia supraaxillaris, E. uniflora, Metrosideros excelsa, Pimenta dioica, Psidium cattleyanum, P. guajava, Syzygium jambos* (Fig. 2i)*, S. malaccense, S. paniculatum, S. samarangense*.

In previous studies (Metcalfe and Chalk, 1979; Fontenelle *et al.*, 1994; Hussin *et al.,* 1992; Kantachot *et al.,* 2007; Defaveri *et al.,* 2011; Soh and Parnell, 2011; Edany and Al-Saadi, 2012; Retamales and Scharaschkin, 2015) recorded the presence anomocytic, anisocytic, cyclocytic, staurocytic and paracytic stomatal types in Myrtaceae. In this study, we recognize brachyparacytic, pericytic, diacytic and actinocytic stomatal types in addition to the previously mentioned types. Paracytic type is the most common as it reported in 11 studied taxa followed by anomocytic type that is recorded in nine taxa. This result in accordance with Haron and Moore (1996)

who also found paracytic and anomocytic very common on family level.

The abnormal giant stomata were found in *Acca sellowiana, Eucalyptus camaldulensis, Lophostemon confertus*, *Melaleuca ericifolia* (Fig. 2h), *M. leucadendra, Pimenta dioica, P. racemose, Psidium cattleyanum, Syzygium jambos* and *S. malaccense*. This result in accordance with Van wyk *et al.* (1982) and Fontenelle *et al.* (1994) who recorded the presence of abnormal giant stomata in some *Eugenia* species.

Trichomes scored for the 15 studied taxa showed a unifying feature of eglandular, unicellular, unbranched type, coiled in *Psidium guajava*, while straight in the remainder 14 taxa. This result is in accordance with Briggs and Johnson (1979), Metcalfe and chalk (1979), and Gomes *et al.* (2009). While, trichomes are absent on both surfaces in 11 studied taxa and abscised in seven taxa when leaves age leaving a scar. Also, this result in accordance with several authors (Fontenelle *et al.,* 1994; Wilson, 2011; Retamales and Scharaschkin, 2015). In *Myrtus communis,* trichomes were present on adaxial surface only. In *Melaleuca leucadendra*, they were present on adaxial surface and abscising leaving a scar as leaf ages on abaxial surface and vice versa in four taxa; *Acca sellowiana, Backhousia citriodora*, *Callistemon rigidus* and *Lophostemon confertus*. However, trichomes were abscising on both surfaces in three taxa; *Melaleuca armillaris, M. linariifolia* and *Metrosideros excelsa*, present on both surfaces in *Callistemon viminalis* and *Psidium guajava*. The existence of trichomes in the plants is regarded as a xerophytic adaptation, particularly when it becomes dense (Evert, 2006).

The overlying cells above secretory cavities were observed in 24 studied taxa and varied in shape and number. *Psidium guajava* (Fig. 3c) had both one stellate and two kidney shaped types of overlying cells. There is one stellate cell in *Eugenia supraaxillaris, Lophostemon confertus*, *Melaleuca leucadendra, Metrosideros excelsa, Psidium cattleyanum,* and *Xanthostemon fruticosus,* two pentagonal cells in *Acca sellowiana, Agonis flexuosa* (Fig. 3a), *Callistemon rigidus, Melaleuca armillaris, M. ericifolia, Syzygium jambos* and *S. malaccense*. The rest 10 taxa showed two kidney-shaped overlying cells (Fig. 3b). This result agrees with Fontenelle *et al.* (1994), Santos *et al*. (2008), Gomes *et al.* (2009), Defaveri *et al.* (2011), Soh and Parnell (2011), Retamales and Scharaschkin (2015), and Khan *et al*. (2016) who recorded the presence of overlying cells in numerous Myrtaceae genera. Gomes *et al.* (2009) found that, the morphology and number of overlying cells associated with the secretory cavities have a taxonomic value.

Surface patterns using scanning electron microscope:

The adaxial surface:

Surface sculpturing patterns Sclariform-reticulate in *Agonis flexuosa* (Fig. 4a), Ruminate in *Corymbia ficifolia* (Fig. 4b), *Pimenta racemose,* Tuberculate in *Eucalyptus camaldulensis* (Fig. 4c), *Metrosideros excelsa,* Reticulate-foveate in *Eugenia supraaxillaris* (Fig. 4d), *Syzygium malaccense,* Reticulate in *Acca sellowiana, Syzygium paniculatum, Xanthostemon fruticosus* (Fig. 4e), Colliculate in *Lophostemon confertus*, *Melaleuca leucadendra* (Fig.4.f.), *M. linariifolia,* Pusticulate in *Melaleuca ericifolia*, *Pimenta dioica, Psidium cattleyanum* (Fig. 4g), *Syzygium gratum* while ill-defined in the remaining nine taxa.

The anticlinal walls had six major categories; raised striated in *Agonis flexuosa*, *Eugenia supraaxillaris, Syzygium paniculatum*, raised smooth in *Acca sellowiana, Corymbia ficifolia*, *Syzygium malaccense, Xanthostemon fruticosus*, sunken with platelets in *Eucalyptus camaldulensis,* sunken striated in *Metrosideros excelsa*, sunken granulated in *Lophostemon confertus, Pimenta dioica, Psidium cattleyanum, Syzygium gratum*, sunken smooth in *Melaleuca ericifolia, M. leucadendra, M. linariifolia, Pimenta racemose* and ill-defined in the rest nine taxa due to presence of epicuticular wax.

The periclinal walls had four categories; sunken smooth in *Acca sellowiana, Xanthostemon fruticosus*, raised granulated in *Lophostemon confertus*, *Melaleuca linariifolia*, *Psidium cattleyanum, Syzygium gratum*, sunken striated in *Agonis flexuosa*, *Corymbia ficifolia*, *Eugenia supraaxillaris, Syzygium malaccense, S. paniculatum*, raised smooth in *Eucalyptus camaldulensis, Melaleuca ericifolia, M. leucadendra, Metrosideros excelsa, Pimenta dioica, P. racemose* and ill-defined in the rest nine taxa.

The epicuticular wax granulated in *Backhousia citriodora, Lophostemon confertus, Melaleuca linariifolia, Pimenta dioica, Psidium cattleyanum* and *Syzygium gratum* (Fig. 5b), smooth in *Acca sellowiana, Corymbia ficifolia, Melaleuca ericifolia, M. leucadendra* (Fig. 5a), *Syzygium malaccense* and *Xanthostemon fruticosus* and striated in the remaining 14 studied taxa.

Stomatal aperture shape circular in *Callistemon rigidus*, slit-like in *Melaleuca armillaris,* elliptic in *Agonis flexuosa*, *Eucalyptus camaldulensis* and *Melaleuca ericifolia* and semi-circular in *Callistemon*

viminalis, *Melaleuca leucadendra* and *M. linariifolia*.

Stomatal aperture level raised in *Melaleuca ericifolia*, raised with stomatal chimney in *Callistemon rigidus*, *Melaleuca armillaris* and *M. leucadendra* and leveled in *Agonis flexuosa*, *Callistemon viminalis*, *Eucalyptus camaldulensis* and *Melaleuca linariifolia*.

The abaxial surface:

Surface sculpturing patterns tuberculate in *Eucalyptus camaldulensis, Pimenta racemose,* Colliculate in *Melaleuca leucadendra, M. linariifolia,* Pusticulate in *Pimenta dioica, Psidium cattleyanum,* Rugose in *Agonis flexuosa* (Fig. 4h), *Melaleuca ericifolia*, *Metrosideros excelsa*, Reticulate in *Lophostemon confertus*, *Psidium guajava, Xanthostemon fruticosus* Ruminate in *Corymbia ficifolia*, *Eugenia supraaxillaris, Myrtus communis, Syzygium jambos, S. malaccense, S. paniculatum*, *S. samarangense* and ill-defined in the remaining seven taxa.

The anticlinal walls categories are raised smooth in *Corymbia ficifolia*, raised striated in *Lophostemon confertus*, *Psidium guajava,* raised granulated in *Xanthostemon fruticosus*, sunken granulated in *Eucalyptus camaldulensis, Pimenta racemose, Psidium cattleyanum, Syzygium jambos*, sunken striated in *Eugenia supraaxillaris*, sunken smooth in *Agonis flexuosa, Melaleuca ericifolia*, *M. leucadendra, M. linariifolia, Metrosideros excelsa*, *Myrtus communis, Syzygium malaccense, S. paniculatum*, *S. samarangense* and ill-defined in the remaining seven taxa.

The periclinal walls categories are sunken smooth in *Psidium guajava*, sunken striated in *Lophostemon confertus*, sunken granulated in *Corymbia ficifolia, Xanthostemon fruticosus*, raised smooth in *Eucalyptus camaldulensis, Melaleuca ericifolia, M. leucadendra, Metrosideros excelsa, Myrtus communis, Pimenta dioica, Syzygium malaccense, S. paniculatum,* raised striated in *Agonis flexuosa, Eugenia supraaxillaris, Syzygium samarangense*, raised granulated in *Melaleuca linariifolia, Pimenta racemosa, Psidium cattleyanum, Syzygium jambos* and ill-defined in the rest seven taxa.

Epicuticular wax smooth in *Melaleuca ericifolia, M. leucadendra, Metrosideros excelsa, Myrtus communis, Pimenta dioica, Syzygium malaccense* and *S. paniculatum,* granulated in *Backhousia citriodora, Corymbia ficifolia, Eucalyptus camaldulensis, Melaleuca linariifolia, Pimenta racemose, Psidium cattleyanum, Syzygium jambos* and *Xanthostemon fruticosus* and striated in the rest ten taxa. Van wyk *et al.* (1982) found that the epicuticular wax differed from specimen to specimen in *Eugenia* and concluded that it has no taxonomic significance. Fontenelle *et al*. (1994) and Haron and Moore (1996) found that the shape and arrangement of epicuticular wax striation taxonomically significant in differentiating certain *Eugenia* species.

Stomatal aperture shape semi-circular in *Acca sellowiana, Callistemon viminalis*, *Melaleuca leucadendra, M. linariifolia*, circular in *Callistemon rigidus*, *Eugenia supraaxillaris, Metrosideros excelsa*, *Syzygium samarangense*, slit-like in *Melaleuca armillaris, Pimenta racemose, Psidium cattleyanum* and *Xanthostemon fruticosus* and elliptic in the remaining 14 studied taxa.

Stomatal aperture level sunken in *Corymbia ficifolia, Myrtus communis*, *Xanthostemon fruticosus*, raised with stomatal chimney in *Callistemon rigidus*, *Melaleuca leucadendra, Psidium guajava*, *Syzygium samarangense*, raised in *Acca sellowiana, Backhousia citriodora, Eugenia uniflora, Melaleuca ericifolia*, *Metrosideros excelsa, Syzygium gratum*, *S. jambos, S. malaccense* and leveled in the rest 11 taxa**.** With respect to *Eugenia.* Present results agree with Van wyk *et al.* (1982) who found that stomatal aperture elliptic to circular and leveled or slightly sunken below epidermis when viewed with SEM in some *Eugenia* species. While, Haron and Moore (1996) found stomatal aperture mostly elliptic to circular in shape and raised above epidermal cells in *Eugenia*.

In conclusion, leaf epidermal characters using light and scanning electron microscope is a suitable source for taxonomic data that can help in taxa delimitation at both generic and specific levels. One of the diagnostic characters of the studied taxa is the presence of overlying cells over oil cavities, abnormal giant stomata, anticlinal and periclinal walls and lamina sculpturing which have a considerable importance and give additional support for species differentiation. An identification key based on leaf type (hypostomatic or amphistomatic), stomatal combination (homostomatic or heterostomatic), trichomes variation, stomatal types, shape of anticlinal walls, number and shape of overlying cells and lamina sculpturing patterns built to distinguish the studied taxa. Some criteria of leaf can obviously delaminate genera while others are unique to some species. Therefore, the current study recommends that for more accurate taxa delimitation, other descriptive criteria are needed to study in addition to leaf epidermal characters.

Identification key:

An identification key based on leaf micromorphological characters for the studied species of family Myrtaceae was constructed as follow:

I. Amphistomatic leaf type

1. Heterostomatic leaf
   - a. Trichomes abscising — *Melaleuca armillaris*
   - b. Trichomes absent
     - i. Straight anticlinal walls on both surfaces — *Eucalyptus camaldulensis*
     - ii. Rounded anticlinal walls on both surfaces — *Agonis flexuosa*

2. Homostomatic leaf
   - a. Trichomes absent on both surfaces — *Melaleuca ericifolia*
   - b. Trichomes present on both surfaces — *Callistemon viminalis*
   - c. Trichomes abscising
     - i. Paracytic stomata — *Callistemon rigidus*
     - ii. Cyclocytic stomata — *Melaleuca leucadendra*
     - iii. Pericytic stomata — *M. linariifolia*

II. Hypostomatic leaf type

1. Heterostomatic leaf
   - i. Rounded anticlinal walls — *Xanthostemon fruticosus*
   - ii. Undulate anticlinal walls
     - a. One stellate overlying cell — *Eugenia supraaxillaris*
     - b. Two kidney-shaped overlying cells
       - Trichomes present on abaxial surface — *Backhousia citriodora*
       - Trichomes absent on both surfaces
         - ❖ Strongly undulate anticlinal walls — *Eugenia uniflora*
         - ❖ Slightly undulate anticlinal walls — *Myrtus communis*

2. Homostomatic leaf
   - i. Anticlinal walls undulate with knobs on both surfaces
     - a. Pusticulate sculpturing with granulated epicuticular was on adaxial surface — *Pimenta dioica*
     - b. Ruminate sculpturing with undulate wrinkled epicuticular wax on adaxial surface — *P. racemose*
   - ii. Anticlinal walls straight on both surfaces
     - a. straight trichomes — *Lophostemon confertus*
     - b. coiled trichomes
       - actinocytic stomata — *Acca sellowiana*
       - paracytic stomata — *Psidium guajava*
   - iii. anticlinal walls not as mentioned above
     - a. anisocytic stomata
       - strongly undulate anticlinal walls on both surfaces — *Syzygium gratum*
       - straight anticlinal walls on adaxial surface only — *Corymbia ficifolia*
     - b. paracytic stomata
       - one stellate overlying cell
         - Trichomes absent — *Psidium cattleyanum*
         - Trichomes present (abscising) *Metrosideros excelsa*
       - two polygonal or kidney-shaped overlying cells
         - raised stomata with stomatal chimney — *Syzygium samarangense*
         - stomata without chimney
           - ❖ irregular cell shape on both surfaces — *Syzygium jambos*
           - ❖ polygonal cell shape on both surfaces
             - Reticulate foveate sculpturing with smooth epicuticular wax adaxially — *Syzygium malaccense*
             - Reticulate sculpturing with striated epicuticular adaxially *S. paniculatum*

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التركيب الدقيق لسطح الورقة وتأثيره على الوضع التصنيفي لبعض الأنواع في الفصيلة الاسية

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تتميز نباتات الفصيلة الاسية بأهميتها الاقتصادية الكبيرة نظرا لخصائصها الطبية والغذائية والبيئية, وقد تناولت الدراسة التركيب الدقيق لسطح الاوراق في ستة وعشرين نوعا منزرعا تنتمي إلى خمسة عشر جنسا والتي تم تجميعها من حديقة مظهر، البراجيل، جيزة، مصر، ثم تم تعريفها وفحصها باستخدام كلا من المجهر الضوئي والإلكتروني الماسح. وقد اوضحت نتائج الدراسة وجود بعض الصفات التشخيصية الهامة في التركيب الدقيق للورقة والتي ساعدت في التمييز بين الأنواع. أوضحت نتائج الدراسة وجود تسعة أنواع من الثغور وهي anomocytic, anisocytic, cyclocytic, staurocytic, paracytic, brachyparacytic, pericytic, diacytic and actinocytic، وقد كان نوع الثغور الاكثر شيوعا هو paracytic. وقد توزعت الثغور إما على السطح السفلى للورقة (في الغالبية, hypostomatic) أو على كلا السطحين (في بعضها، amphistomatic). وقد تميزت بعض الانواع النباتية بوجود أكثر من نوع من الثغور على نفس الورقة (heterostomatic) والبعض الاخر يحتوي على نوع واحد فقط منها (homostomatic). كذلك تم تسجيل أشكال خلايا البشرة والجدر القطرية والمماسية المميزة لها وشكل وعدد الخلايا المصاحبة للتجويف الإفرازي وكذلك وجود أو عدم وجود الشعيرات. وقد ابرزت النتائج وجود صفة تشخيصية هامة وهي ثغور عملاقة غير طبيعية في عشر انواع فقط من النباتات قيد الدراسة. إضافة لذلك تم تسجيل ثمانية أنماط للزركشة الموجودة على سطح الاوراق في النباتات قيد الدراسة هي reticulate، tuberculate، sclariform-reticulate، rugose،ruminate ، colliculate and pusticulate،reticulate-foveate . كذلك تم تسجيل انماط مختلفة لترسيبات المادة الشمعية على سطح الاوراق الى جانب اشكال ومستوى فتحة الثغر فيها، مما أدى الى بناء مفتاح تعريفي للنباتات المدروسة يعتمد على بعض هذه الصفات التشخيصية الهامة لسطح الاوراق للتمييز بين الاجناس والأنواع.