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RESEARCH ARTICLE

Foliar epidermal micromorphology: a contribution to the taxonomy of family Oleaceae

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Abstract Oleaceae (olive family), includes 28 genera and about 700 species. Nevertheless, there remains a lot to uncover about the group's historical development, the evolution of various reproductive and dispersal mechanisms, and polyploidization episodes appear to be linked to its diversification. In the current study, foliar epidermal anatomy of 13 plant species and 2 varieties from 7 genera of Oleaceae was examined under a light microscope. The qualitative and quantitative features like stomatal density, size,shape of guard cell, number of epidermal cell, subsidiary cells, and structure and density of trichomes were analysed using a light microscope. This was the first study on the foliar micromorphology of various Oleaceae taxa. Almost all species exhibited hypostomatic type except one in which Amphistomatic type was observed. Most of the stomata were anomocytic while

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some Paracytic and one diacytic stomata were also seen. Maximum stomatal length of (30.80 + 1.44 µm) and lowest of (18.30 + 0.21 µm) was noted. All trichomes observed were unicellular. Both glandular and non-glandular trichomes were observed with highest trichome length (237.75–248.00 = 242.25 + 1.73 µm) and the lowest (100.00–101.75 = 100.75 + 0.32 µm). Plant taxonomists might commence their future research with the micro-morphological aspects of foliar epidermal morphology, which exhibit a number of innovative qualities for accurate taxonomic identification.

Keywords Olive family · Anatomy · Microanatomy · Stomatal complex · Taxonomy

Introduction

Featuring 28 genera more than 700 species, the family Oleaceae commonly referred to as Olive family. Across all continents, this uniformly sized variety of woody plants can be observed growing excluding Antarctica in environments that are both temperate as well as tropical (Dupin et al. 2020). Southeast Asia along with Australia are the two zones with the maximum agglomeration of Oleaceae species (Takhtajan, 2009). Africa as well China and North America have a substantial heterogeneity of species (Green 2004). Mostly species belonging to this family are trees, but some are herbaceous plants as well (*Dimetra craibiana*) a few small bushes, including *Menodora* species, and lianas like *Jasminum* species and *Chionanthus macrobotrys*.Many Oleaceae species are economically valuable since they provide essential oil, fruits (such as olives), timber (such as ash trees), ornamentals, and scents including jasmines and lilacs, etc. Furthermore, members of family Oleaceae are critical ingredients of tropical and temperate ecosystems; for instance, drupes and culinary leaves are abundant in several species and are a major source of food for wild animals. As an illustration, it is known that almost thousands of species (including fungi, insects as well as birds) are associated with the *Fraxinus excelsior* species. Individual species too can sustain a massive number of other organisms (Mitchell et al. 2014).

Oleaceous vegetation are extensively employed in ethnomedicine. The buds of the *Syringa vulgaris* herbage are employed to make wine and alleviate joint discomfort in Belarus. In Southern Italy, the peel of the *Fraxinus. ornus* plant is frequently used to treat diarrhoea and an abnormally low blood cholesterol level. Enhanced liver and kidney operations can be achieved by consuming the fruits of *Ligustrum lucidum* which is native to China. *Olea europaea* leaves are used as a remedy to reduce blood pressure in Greece. In Oman, the essential oil generated from *Olea europaea* is both a laxative and an antiarthritic. Olive oil has also reportedly been traced to carry antioxidant and anti-cancerous properties (De Bruno et al. 2021; El Haouari et al. 2020; Huang et al. 2019). Of the Oleaceae family of flowering plants, jasmines (*Jasminum* species) are traditionally grown. Diverse species belonging to this genus have been demonstrated to feature different biological activities in addition to their pleasing aromas (Mansour et al. 2022).

Consists of 8 generas and roughly 30 species in Pakistan, 22 of which are domesticated. Bushes or trees, very seldom climber. Although the group may be fairly readily classified as afamily, it encompasses a wide range of forms. Some of these forms are stable enough to be quickly identified as species, whereas others, particularly those belonging to the genus Fraxinus, provide challenges for taxonomists (Taylor 1945). Currently, the Oleaceae plant family is classified to form five tribes: Forsythieae, Myxopyreae, Fontanesieae, Jasmineae and Oleeae, which is split up into four further subtribes (Oleinae, Schreberinae, Ligustrinae, and Fraxininae). Although its natural history is still poorly understood, the group's diversification appears to be linked to a few instances of polyploidy (especially a substantial entire genome duplication in the *Oleeae progenitor*) and the development of numerous reproductive and dispersal strategies (Wallander and Albert 2000).Thus, this family exhibits a large assortment of flowers and fruits, notably capsules, samaras, and drupes, associated with various pollination and seed distribution vectors (Dupin et al. 2020). An effective strategy for comprehending plant anatomy is the study of its vegetative organs to solve the related taxonomic issues (Metcalfe and Chalk 1983; Majeed et al. 2022). Research involving multiple families, at the levels of species, genera, or families, reveals how plant anatomy can be used taxonomically with a focus on the morphology of leaves (Kolb et al. 2020; Abbas et al. 2022).

Some taxonomically useful characters perceived to be incorporated into morphological markers. Taxonomic classification and norms depend on the study of morphology (Manzoor et al. 2023). Contemporary taxonomic mechanics have lately been used to tackle several taxonomic issues that are problematic to fully address with conventional morphology (Vogler APMonaghan, 2007). If the taxonomic attributes are merged with both the opposite leaf and scarcity of stipules, Oleaceae seems to be an easily identifiable family (Solereder 1908). The family is eminent by having trichomes with a single celled stem as well as the head splitted into portions by upright walls in a differential cell count with radiated layout and differing sizes; by the existence of acicular as well as prismatic calcium oxalate crystals; by stomata, which are typically utterly bereft of differentiated subsidiary cells; and by the nonattendance of bi-collateral vascular bundles (Kolb et al. 2020).

Through this research, a significant number of micro morphological characters of the epidermal surfaces of several species of the Oleaceae family were revealed. These attributes display fascinating interspecific alterations that are taxonomically relevant. Foliar anatomical features exhibit fascinating interspecific variations that have taxonomically significant value. The arrangement and density of stomata on any leaf surface are largely determined by heredity, although they can also be influenced via environmental conditions, CO_2 availability is one of them (Glover 2000). The three principal cell types that make up the plant epidermis are pavement cells, the subsidiary cells and the guard cells, that encircle the stoma and trichome, generally known as leaf hairs.

In this study, the micromorphological traits of the epidermal surface in slected species of the Oleaceae were identified. The focus of the current study was on foliar epidermal traits of some species of the Oleaceae family. For the first time, microanatomical aspects of some Oleaceae species in context of trichome diversity with potential value for accurate species identification.

Materials and methods

Plant specimen collection and identification

Two varieties and 13 species originating from 7 genera in the family Oleaceae were collected from various regions of Pakistan, such as Khyber Pukhtoonkhwa and Punjab (Fig. 1: Table 1). Pakistan has a rich and diversified floral population. Due to the obvious sites' considerable variation of plant species from various families along with the Oleaceae, these sites were considered for the collection of plant specimens (Sher et al. 2011) during the time period January 2022 to August 2022.

Field visits were arranged to several regions of Pakistan. A field notebook contained all the necessary information that was required for the field. For the purpose of collection and drying, standard herbarium procedures were used. With the aid of the existing literature, along with the flora of Pakistan and the IPNI International Plant Name Index (Croft et al. 1999), the obtained plant specimen were authenticated. Specimen were submitted to Herbarium of Pakistan (ISL).

Application of light microscopy to examine taxonomic traits

The heating technique was followed that was described in the literature for light-microscopy, foliar micro-morphological investigations were carried out by the aid of this method (Nazir et al. 2013). To avoid the collected foliage from dehydrating completely, it was immersed for an hour in water. In a test tube containing 30% nitric acid and 70% lactic acid, an average about 4–5 leaves were examined. The test tube was heated for a couple of minutes to ensure the leaves become translucent. A camel hair brush and needle were used to separate the the adaxial and abaxial leaf surfaces in a Petri dish after pouring it. The epidermis was detached, transferred, and covered with a coverslip after being cleaned with a drop of lactic acid. For each plant specimen, 4–6 samples of each surface were produced. The already made slides were examined using a Meiji microscope, Model MX5200H and an eyepiece with a plan magnification of 40X/0.65.

Qualitative and quantitative traits

Foliar micro-morphological features of leaves of adaxial as well as abaxial epidermis were considered. The morphology of epidermal cells, the existence or lack of stomata and trichomes, and the kinds of them were amongst the qualitative characteristics.

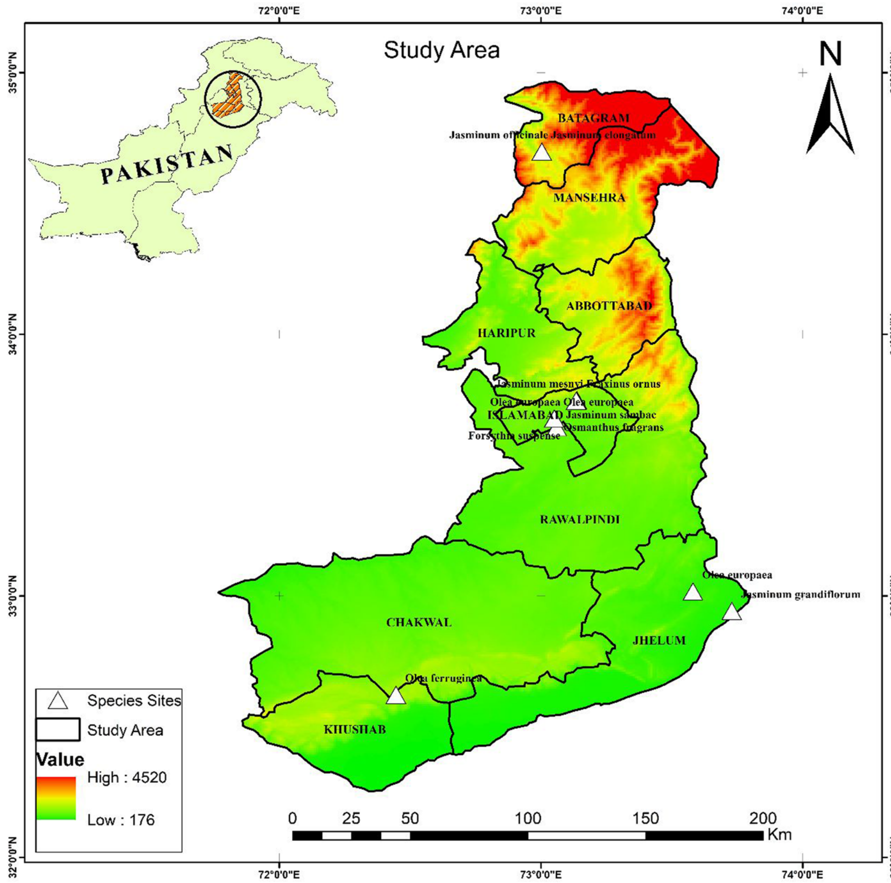


Fig. 1 Map of the study area

Some of the quantitative qualities that were examined included the quantity, length, and thickness of the stomata on the epidermal cells, the stomatal pore, guard cell, subsidiary cells, trichomes, the stomatal index, and the trichome index. The quantitative aspects reported in the previous research (Ashfaq et al. 2019) were evaluated using the SPSS software. (Min–Max = Mean + SE) is the equation that was used to describe quantitative features. For both surfaces, 15–20 values for every character were obtained. On the same ocular the frequency of stomata and the amount of epidermal cells were quantified, and a mean of ten was obtained.

Analysis of statistical data

Stomatal index (SI)

This formula was being used to obtain the stomatal index values.

$$\textit{Stomatal Index} = \textit{Stomata number} / \textit{Epidermal cell number} + \textit{Stomata} \times 100$$

Stomatal index is represented by SI, S represents stomata number per unit area, and the epidermal cell number per unit area (E) are included in the formula as S.I (Sneath and Sokal, 1973).

Trichome index (TI)

With the assistance of the given equation, the trichome index was determined:

$$\textit{Trichome Index} = \textit{Trichome} / \textit{Epidermal cell number} + \textit{Trichome} \times 100$$

Trichome index in this equation,is usually represented by T.I, the trichome number per unit area is represented by T and E represents the epidermal cells number per unit area (Abid et al. 2023).

Table 1 Plant sampling and GPS coardinates along with erbarium vouchering of Oleaceous species

Sr. No	Species name	English name	Collection Site	Altitude(ft)	Collectors	Coordinates	Voucher number	Accession number	Collection Date
1	*F. suspensa* Vahl	Weeping Forsythia	Islamabad	1,770	Tajalla Batool	Lat 33° 44' 16.9620" N Lon 73° 5' 4.1568" E	TB-05	ISL-132063	16-05-2022
2	*F. excelsior* L	Himalayan Ash	Islamabad	1,770	Tajalla Batool	Lat 33° 44' 16.9620" N Lon 73° 5' 4.1568" E	TB-09	ISL-132065	05-06-2022
3	*F. ornus* L	Flowering ash	Islamabad	1,770	Tajalla Batool	Lat 33° 44' 16.9620" N Lon 73° 5' 4.1568" E	TB-04	ISL-132068	02-06-2022
4	*J. grandiflorum* L	Royal jasmine	Jhelum	767.75	Tajalla Batool	Lat 32° 56' 25.9728" N Lon 73° 43' 39.4716" E	TB-15	ISL-132142	05-08-2022
5	*J. mesnyi* Hance	Yellow Jasmine	Rawalpindi	1633.94	Tajalla Batool	Lat 33° 37' 33.8052" N Lon 73° 4' 17.1912" E	TB-03	ISL-132071	13-01-2022
6	*J. elongatum* (P.J.Bergius) Willd	Star Jasmine	Swat	3220	Muhammad Abdullah	Lat 35° 22' 59.99" N Lon 72° 10' 60.00" E	TB-10	ISL-132072	05-06-2022
7	*J. officinale* L	Common jasmine	Swat	3220	Muhammad Abdullah	Lat 35° 22' 59.99" N Lon 72° 10' 60.00" E	TB-14	ISL-132069	21-05-2022
8	*Jasminum sambac* (L.) Aiton	Arabian Jasmine	Rawalpindi	1,667	Tajalla Batool	Lat 33° 37' 33.8052" N Lon 73° 4' 17.1912" E	TB-02	ISL-132067	16-08-2022
9	*L. lucidum* W.T. Aiton	Chinese privet	Islamabad	1,770	Muhammad Zafar	Lat 33° 44' 16.9620" N Lon 73° 5' 4.1568" E	TB-11	ISL-132066	18-05-2022
10	*N. arbortristis* L	Coral Jasmine	Islamabad	1,770	Tajalla Batool	Lat 33° 44' 16.9620" N Lon 73° 5' 4.1568" E	TB-13	ISL-132070	02-04-2022

Table 1 (continued)

Sr. No	Species name	English name	Collection Site	Altitude(ft)	Collectors	Coordinates	Voucher number	Accession number	Collection Date
11	*Olea europaea* L	European Olive	Syed Hussain Village	905	Tajalla Batool	Lat 33° 01' 25.20" N Lon 73° 36' 2.39" E	TB-01	ISL-132140	23-03-2022
12	*Olea europaea L. cv. Arbequina*	European Olive	Islamabad	1,770	Tajalla Batool	Lat 33° 44' 16.9620" N Lon 73° 5' 4.1568" E	TB-07	ISL-132141	16-05-2022
13	*Olea europaea L. cv. Koroneiki*	European Olive	Islamabad	1,770	Tajalla Batool	Lat 33° 44' 16.9620" N Lon 73° 5' 4.1568" E	TB-08	ISL-132062	05-06-2022
14	*Olea ferruginea* Wall. ex Aitch	Indian olive	Soon valley	5,010	Muzammil Aziz	Lat 32° 33'11" N Lon 71° 58'33" E	TB-12	ISL-132061	16-04-2022
15	*O. fragrans* (Thunb.) Lour	Sweet Osmanthus	Islamabad	1,770	Tajalla Batool	Lat 33° 44' 16.9620" N Lon 73° 5' 4.1568" E	TB-06	ISL-132064	05-06-2022

Results

Under a light microscope, leaf epidermal morphological characteristics have been seen. Both of these characteristics, quantitative and qualitative show remarkable variations (Tables 2 and 3).

Taxonomic importance of variations in stomatal patterns

Mostly Anomocytic stomatal types were observed along with a few Paracytic stomata and one diacytic stomata type in Oleaceous flora. Variations of considerable importance in Stomatal cell length width, number and Stomatal index in both upper as well as lower surfaces were observed as mentioned in LM Figs. 2, 3, 4, 5 and 6.

Great diversity in the size of stomata was observed in different species. Stomatal cell length varied from largest (30.80 + 1.44 µm) in *Jasminum grandiflorum* to smallest in *Jasminum elongatum* (18.30 + 0.21 µm) on abaxial surface. Maximum stomatal cell width on abaxial surface was observed in *L. lucidum* (24.50–25.50 = 25.00 + 0.17) µm while minimum stomatal width was reported in *Olea europaea cv. Koroneiki* (10.25–12.25 = 11.25 + 0.35 µm) as shown in Fig. 7. Among the selected species only one species i.e. *J. grandiflorum* showed stomata on the adaxial surface under light microscope. Stomatal index of the selected species showed notable variations from maximum 50% in *J. grandiflorum* to lowest 10.5% in *Olea europaea cv. Arbequina*.

Morphology of epidermal cells and anticlinal wall pattern

Remarkable divergence was percieved in epidermal cell traits like cell length,width, cell shape,size and anticlinal wall pattern within the species. The anticlinal wall showed different patterns in different species and even within a species on the adaxial and abaxial sides.Simple straight to sinuous, Simply undulate, sinuate, Smooth, angular and strightly curved patterns were observed in anticlinal walls. The epidermal cells were mostly irregular to isodiametric in shape but some other shapes like Elongated, V-shaped,

Table 2 Qualitative analysis of adaxial and abaxial surfaces among Oleaceous taxa

Plant species	Leaves Condition	Ad × Ab	ECS	AWP	GCS	St (P/A)	ST	SS	Tri (P/A)	Trichome	DT
F. suspensa Vahl	Hypostomatic	Ad	Elongated V-shaped to rectangular U-shaped	Straight	A	A	A	A	P	Glandular, Unicellular, Capitate	Intercostalzone
		Ab	Irregular and rarely tetragonal	Straight to sinuous	Bean shaped	P	Paracytic	Dumbbell shaped	A	A	A
F. excelsior L	Hypostomatic	Ad	Irregular, isodiametric	Simply undulate, sinuate	A	A	A	A	P	Glandular, Unicellular, Capitate	Intercostal zone
		Ab	Irregular	Smooth, angular	Bean shaped	P	Paracytic	Elongate elliptic	P	Glandular, Unicellular, Capitate	Intercostal zone
F. ornus L	Hypostomatic	Ad	Irregular	Simply undulate	A	A	A	A	A	A	A
		Ab	Irregular	Simply undulate	Bean shaped	P	Anomocytic	Elliptical	P	Non-glandular, Unicellular	Intercostal zone
J. elongatum (P.J.Bergius) Willd	Hypostomatic	Ad	Irregular, Isodiametric	Simply undulate	A	A	A	A	A	A	A
		Ab	Isodiametric, irregular	Slightly undulate	Bean shaped	P	Anomocytic	Elliptical	P	Unicellular Non-glandular	Intercostal zone
J. grandiflorum L	Amphistomatic	Ad	Irregular	Simple undulate	Bean shaped	P	Paracytic	Elliptical	P	Unicellular Non-glandular	Intercostal zone
		Ab	Irregular, isodiametric	Simple undulate	Bean shaped	P	Anomocytic	Elliptical	P	Unicellular Non-glandular	Intercostal zone
J. mesnyi Hance	Hypostomatic	Ad	Irregular	Strightly curved	A	A	A	A	A	A	A
		Ab	Irregular, isodiametric	Simply undulate, sinuate and straight	Bean shaped	P	Anomocytic	Dumbbell shaped	A	A	A

Table 2 (continued)

Plant species	Leaves Condition	Ad × Ab	ECS	AWP	GCS	St (P/A)	ST	SS	Tri (P/A)	Trichome	DT
J. officinale L	Hypostomatic	Ad	Irregular	Sinuate	A	A	A	A	P	Unicellular Non-glandular	Intercostal zone
		Ab	Irregular	Slightly curved	Bean shaped	P	Anomocytic	Elongate elliptic	P	Unicellular Non-glandular	Intercostal zone
Jasminum sambac (L.) Aiton	Hypostomatic	Ad	Irregular, isodiametric	Simply undulate, sinuate and straight	A	A	A	A	A	A	A
		Ab	Mostly polygonal rarely irregular	Straight to little undulate	Bean shaped	P	Anomocytic	Dumbbell shaped	A	A	A
L. lucidum W.T. Aiton	Hypostomatic	Ad	Irregular, Isodiametric	Slightly undulate	A	A	A	A	A	A	A
		Ab	Irregular	Slightly sinuate	Bean shaped	P	Paracytic	Elliptical	P	Unicellular, Glandular, Peltate	Intercostal zone
N. arbor-tristis L	Hypostomatic	Ad	Irregular, Isodiametric	Slightly curved	A	A	A	A	A	A	A
		Ab	Irregular, Isodiametric	Slightly curved	Bean shaped	P	Anomocytic	Dumbbell shaped	P	Unicellular, Glandular, Peltate	Intercostal zone
Olea europaea L	Hypostomatic	Ad	Irregular	Straight	A	A	A	A	P	Unicellular, Glandular, Capitate	Intercostal zone
		Ab	Irregular	Straight	Bean shaped	P	Anomocytic	Elongate elliptic	P	Glandular, Unicellular	Intercostal zone
Olea europaea L. cv. Arbequina	Hypostomatic	Ad	Irregular, isodiametric	Simply undulate	A	A	A	A	A	A	A
		Ab	–	–	Bean shaped	P	Diacytic	Elongate elliptic	P	Unicellular Non-glandular	Intercostal zone

Table 2 (continued)

Plant species	Leaves Condition	Ad × Ab	ECS	AWP	GCS	St (P/A)	ST	SS	Tri (P/A)	Trichome	DT
Olea europaea L. *cv. Koroneiki*	Hypostomatic	Ad	Irregular	Simply undulate	A	A	A	A	P	Unicellular, Glandular, Peltate	Intercostal zone
		Ab	Irregular	Simply undulate	Bean shaped	P	Paracytic	ElongateElliptic	P	Unicellular, Glandular, Peltate	Intercostal zone
Olea ferruginea Wall. ex Aitch	Hypostomatic	Ad	Irregular, Polygonal	Straight to sinuous	A	A	A	A	A	A	A
		Ab	Irregular	Straight to little sinuous	Bean shaped	P	Anomocytic	Elongate elliptic	P	Unicellular, Glandular, Peltate	Intercostal zone
O. fragrans (Thunb.) Lour	Hypostomatic	Ad	Elongated V- shaped to rectangular	Straight	A	A	A	A	P	Unicellular, Non-glandular	Intercostal zone
		Ab	U-shaped Rectangular to rarely pentagonal	Straight	Bean shaped	P	Anomocytic	Elongate eliptic	P	Unicellular, Non-glandular	Intercostal zone

Keywords: Ad, Adaxial; Ab, Abaxial; ECS, Epidermal cell size; AWP, Anticlinal wall pattern; GCS, Guard cell shape; St, Stomata; ST, Stomata type; SS, Stomata shape; Sb, subsidary; Tri, Trichome; DT, Distribution of trichomes; A, Absent; P, Present

Table 3 Quantitative analysis of adaxial and abaxial surfaces of epidermal cells, stomata and trichomes

Taxa	Ad × Ab	L × W	Average No. of Epidermal cell	Epidermal cell (Min–Max = Mean + SE)	No. of Stomata (Avg)	Stomata (Min–Max = Mean + SE)	SI (%)	Trichome Number Per unit area	Trichome (Min–Max = Mean + SE)	TI (%)
F. suspensa Vahl	Ad	L	250	(21.75–23.00 = 22.35 + 0.23)				1	(100.25–105.75 = 103.45 + 1.05)	0.39
		W		(12.75–14.25 = 13.35 + 0.25)					(15.25–21.75 = 17.20 + 1.16)	
	Ab	L	184	(19.75–22.25 = 21.00 + 0.46)	42	(27.75–30.25 = 29.00 + 0.44)	18.58	1	(105.75–133.50 = 125.50 + 5.08)	0.54
		W		(10.25–12.75 = 11.35 + 0.45)		(17.25–19.50 = 18.30 = 0.39)			(21.75–24.75 = 23.00 + 0.55)	
F. excelsior L	Ad	L	188	(23.75–47.75 = 31.25 + 4.34)	–		–	4	(75.25–87.75 = 83.00 + 2.11)	2.08
		W		(13.00–15.75 = 14.75 + 0.48)	–			–	(15.25–18.00 = 16.80 + 0.50)	
	Ab	L	49	(22.00–24.25 = 23.00 + 0.37)	18	(23.75–26.75 = 24.90 + 0.52)	26.86	1	(20.50–22.00 = 21.30 + 0.26)	2.00
		W		(12.75–16.25 = 14.85 + 0.62)		(15.25–17.00 = 16.20 + 0.32)			(12.50–13.50 = 13.05 + 0.18)	
F. ornus L	Ad	L	177	(16.25–40.75 = 24.45 + 4.23)	–	–		–		
		W		(12.75–15.25 = 13.75 + 0.48)						
	Ab	L	97	(11.75–15.75 = 13.2 + 0.70)	38	(19.50–22.75 = 20.90 + 0.54)	28.14	2	(12.00–15.50 = 13.80 + 0.73)	2.02
		W		(7.75–12.75 = 10.65 + 0.88)		(18.00–22.00 = 19.60 + 0.72)			(2.75–4.75 = 3.9 + 0.38)	
J. elongatum (P.J.Bergius) Willd	Ad	L	240	(27.00–28.25 = 27.6 + 0.23)						
		W		(15.25–17.00 = 16.05 + 0.28)						
	Ab	L	87	(20.25–22.00 = 20.95 + 0.31)	42	(17.75–19.00 = 18.30 + 0.21)	32.55	2	(237.75–248.00 = 242.25 + 1.73)	2.24
		W		(12.75–14.00 = 13.40 + 0.23)	–	(12.75–14.00 = 13.30 + 0.21)			(34.50–35.75 = 35.10 + 0.21)	–
J. grandiflorum L	Ad	L	57	(36.00–37.00 = 36.50 + 0.17)	5	(27.75–29.25 = 28.35 + 0.25)	8.06	1	(20.25–21.75 = 21.05 + 0.26)	3.87

Table 3 (continued)

Taxa	Ad×Ab	L×W	Average No. of Epidermal cell	Epidermal cell (Min–Max = Mean + SE)	No. of Stomata (Avg)	Stomata (Min–Max = Mean + SE)	SI (%)	Trichome Number Per unit area	Trichome (Min–Max = Mean + SE)	TI (%)
		W		(20.25–22.00 = 21.20 + 21.20)	–	(22.75–24.75 = 24.00 + 0.35)			(12.50–13.25 = 12.80 + 0.14)	–
	Ab	L	49	(34.25–36.00 = 35.35 + 35.35)	49	(28.25–36.25 = 30.80 + 1.44)	50.00	2	(100.00–104.25 = 101.75 + 0.78)	3.92
		W		(17.75–23.25 = 19.30 + 1.01)	–	(21.75–23.00 = 22.35 + 0.23)		–		
									(15.00–16.75 = 15.85 + 0.32)	
J. mesnyi Hance	Ad	L	147	(30.25–31.75 = 30.85 + 0.25)	–					
		W		(17.50–18.50 = 18.10 + 0.16)						
	Ab	L	105	(27.75–29.75 = 28.90 + 0.38)	60	(22.75–24.25 = 23.35 + 0.25)	36.36			
		W		(16.75–18.50 = 17.60 + 0.38)		(15.75–17.25 = 16.15 + 0.28)				
J. officinale L	Ad	L	77	(10.25–13.75 = 12.75 + 0.63)	–			1	(245.00–246.00 = 245.00 + 0.16)	1.28
		W		(7.75–10.50 = 8.95 + 0.52)					(27.25–29.00 = 28.0 + 0.32)	
	Ab	L	65	(23.25–27.00 = 24.95 + 0.66)	32	(25.25–27.75 = 26.30 + 0.50)	32.98	3	(218.00–227.75 = 223.30 + 1.58)	4.41
		W		(18.75–20.50 = 19.60 + 0.34)		(13.75–15.75 = 14.75 + 0.35			(22.75–24.25 = 23.50 + 0.25)	
Jasminum sambac (L.) Aiton	Ad	L	205	(13.70–14.30 = 14.04 + 0.10)				–		
		W		(7.80–8.70 = 8.20 + 0.16)		–				
	Ab	L	85	(34.50–37.00 = 35.45 + 0.44)	25	(23.25–25.75 = 24.85 + 0.450	22.7			–
		W		(17.25–19.25 = 18.10 + 0.35)		(19.75–21.00 = 20.45 + 0.21)				

Table 3 (continued)

Taxa	Ad×Ab	L×W	Average No. of Epidermal cell	Epidermal cell (Min–Max = Mean + SE)	No. of Stomata (Avg)	Stomata (Min–Max = Mean + SE)	SI (%)	Trichome Number Per unit area	Trichome (Min–Max = Mean + SE)	TI (%)
L. lucidum W.T. Aiton										
	Ad	L		(32.35–34.25 = 32.95 + 0.34)	–					–
		W	144	(17.25–18.75 = 17.85 + 0.29)			–			
	Ab	L	81	(25.50–26.75 = 26.05 + 0.21)	41	(28.00–31.00 = 29.80 + 0.49)	33.60	1	(19.25–20.50 = 19.90 + 0.23)	1.21
		W		(15.00–18.25 = 16.45 + 0.64)		(24.50–25.50 = 25.00 + 0.17)			(12.50–14.00 = 13.20 + 0.28)	
N. arbor-tristis L	Ad	L	37	(37.50–38.75 = 38.15 + 0.23)	–		–	2	(173.25–178.00 = 175.75 + 0.79)	5.12
		W		(20.75–22.25 = 21.40 + 0.26)					(25.00–26.00 = 25.50 + 0.17)	
	Ab	L	28	(18.25–19.50 = 18.95 + 0.21)	14	(23.25–25.50 = 24.65 + 0.39)	33.33	25	(100.25–103.50 = 102.0 + 0.70)	47.16
		W		(7.75–10.00 = 8.85 + 0.45)		(14.50–16.7 = 15.60 0.39)			(15.00–16.50 = 15.65 + 0.26)	
Olea europaea L	Ad	L	102	(20.00–21.75 = 20.80 + 0.28)	–			1	(30.00–31.75 = 30.70 + 0.30)	0.97
		W		(10.50–13.25 = 12.15 + 0.47)		–			(27.50–28.50 = 28.00 + 0.17)	
	Ab	L	76	(22.50–24.25 = 23.30 + 0.32)	39	(22.50–23.50 = 23.00 + 0.17)	33.91	1	(27.50–28.50 = 28.00 + 0.17)	1.29
		W		(15.00–16.75 = 15.65 + 0.30)		(12.5014.25 = 13.25 + 0.30)			(25.75–27.50 = 26.65 + 0.34)	
Olea europea L. *cv. Arbequina*	Ad	L	234	(12.50–13.50 = 13.00 + 0.17)	–		–	2	(50.75–55.75 = 53.10 + 0.96)	0.84
		W		(5.75–10.25 = 8.90 + 0.81)		–			(12.25–13.25 = 12.75 + 0.17)	
	Ab	L	179	(13.25–14.75 = 13.95 + 0.26)	21	(25.00–26.00 = 25.50 + 0.17)	10.5	1	(38.50–48.25 = 41.65 + 1.80)	0.55

Table 3 (continued)

Taxa	Ad×Ab	L×W	Average No. of Epidermal cell	Epidermal cell (Min–Max = Mean + SE)	No. of Stomata (Avg)	Stomata (Min–Max = Mean + SE)	SI (%)	Trichome Number Per unit area	Trichome (Min–Max = Mean + SE)	TI (%)
		W		(8.50–12.25 = 10.15 + 0.77)		(12.00–13.50 = 12.80 + 0.26)			(7.75–11.75 = 9.30 + 0.75)	
Olea europea L. *cv. Koroneiki*	Ad	L	70	(14.50–17.00 = 15.55 + 0.43)	–		–	2	(20.00–21.00 = 20.50 + 0.17)	2.7
		W		(10.00–11.75 = 10.65 + 0.30)		–			(15.00–16.00 = 15.50 + 0.17)	
	Ab	L	57	(21.00–24.50 = 22.80 + 0.56)	19	(25.00–26.75 = 25.70 + 0.31)	25	11	(25.25–26.75 = 25.85 + 0.25)	16.17
		W		(11.75–16.25 = 14.45 + 0.73)		(10.25–12.25 = 11.25 + 0.35)			(20.00–24.50 = 22.00 + 0.97)	
Olea ferruginea Wall. ex Aitch	Ad	L	138	(2.10–10.50 = 8.34 + 1.57)	–					
		W		(10.30–12.10 = 10.92 + 0.32)						
	Ab	L	43	(20.50–23.75 = 21.45 + 0.58)	25	(25.25–30.00 = 27.60 + 0.76)	36.76	5	(20.00–20.75 = 20.30 + 0.14)	10.41
		W		(15.25–28.00 = 19.5 + 2.23)		(14.75–18.00 = 16.10 + 0.60)			(17.50–18.50 = 18.00 + 0.17)	
O. fragrans (Thunb.) Lour	Ad	L	128	(22.00–25.25 = 23.70 + 0.62)				2	(101.25–125.00 = 109.0 + 4.25)	1.53
		W		(18.00–20.50 = 19.50 + 0.48)		–			(24.75–26.25 = 25.4 + 0.26)	
	Ab	L	87	(20.00–21.00 = 20.50 + 0.17)	50	(25.5–26.25 = 25.85 + 0.16)	36.49	2	(100.00–101.75 = 100.0 + 0.32)	2.24
		W		(8.75–10.00 = 9.15 + 0.23)		(19.50–20.50 = 20.00 + 0.17)			(22.75–23.75 = 23.3 + 0.20)	

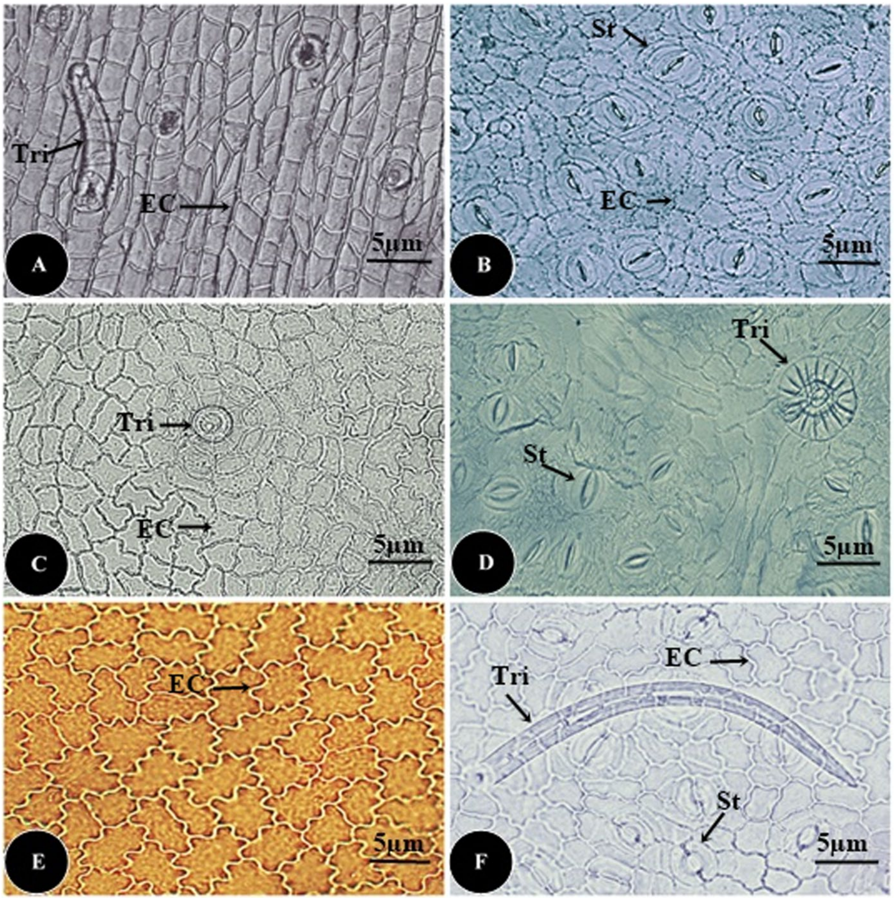


Fig. 2 Plants species of family Oleaceae from Pakistan. Light micrographs (LM) shapes of cells, patterns of walls, stomata and trichomes of Oleaceae. Scale bar = 5 µm (**A**) *F. suspensa* adaxial surface showing Glandular, Unicellular trichomes (**B**) Abaxial surface showing Paracytic stomata (**C**) *F. excelsior* adaxial surface showing Glandular, Unicellular, Capitate trichomes (**D**) Abaxial surface showing Paracytic stomata and Glandular, Unicellular, Capitate trichomes (**E**) *F. ornus* adaxial surface lacking stomata as well as trichomes (**F**) Abaxial surface showing Anomocytic stomata and Non-glandular, Unicellular trichomes. EC, Epidermal cell; St, Stomata; Tri, Trichomes

U-shaped, rectangular and polygonal were also observed. The irregular to isodiametric epidermal cell was most common.It was observed in *F. excelsior* and *L. lucidum* on the adaxial surface while in *J. grandiflorum, Jasminum mesnyi, J. elongatum, Jasminum sambac* and *Nyctanthes arbor-tristis* this type of cells were seen on the abaxial surface. Irregular epidermal cells were observed in *F. excelsior* (Abaxial surface), *Jasminum officinale, Fraxinus ornus, Olea europaea* and *Olea europaea cv. Koroneiki.* Elongated V- shaped to rectangular U-shaped epidermal cells were observed in *Forsythia suspensa* (Adaxial surface) and *Osmanthus fragrans* (Adaxial surface). Irregular polygonal epidermal cells were observed in *Olea ferruginea*.

Length of epidermal cell varied from maximum 38.15 + 0.23 µm in *N. arbor-tristis* to minimum 8.34 + 1.57 µm in *Olea ferruginea* on the adaxial surface and from maximum 35.35 + 35.35 µm in *J. grandiflorum* to minimum13.2 + 0.70 µm in *F. ornus*on the abaxial surface. Width of epidermal cell also revealed great variation in adaxial as well as abaxial surfaces. In the adaxial surface maximum width 21.40 + 0.26 µm was observed in *N. arbor-tristis* and minimum width 8.20 + 0.16 µm was observed in *Jasminum sambac* (Fig. 8). In Abaxial surface maximum width 10.92 + 0.32 µm was observed in *Olea ferruginea* while minimum width 8.85 + 0.45 µm was observed in *N. arbor-tristis* (Fig. 9).

Trichome morphology and variation in trichome characters

The Oleaceae family has a diverse range of trichome patterns. On the lower and upper surfaces of the leaves of the selected species, glandular as well as aglandular trichomes were evident. On both the adaxial together with abaxial leaf surfaces, glandular trichomes of both the capitate and peltate types were irregularly strewn over the entire leaf veins and leaf blade. The essential oil deposition in the subcuticular

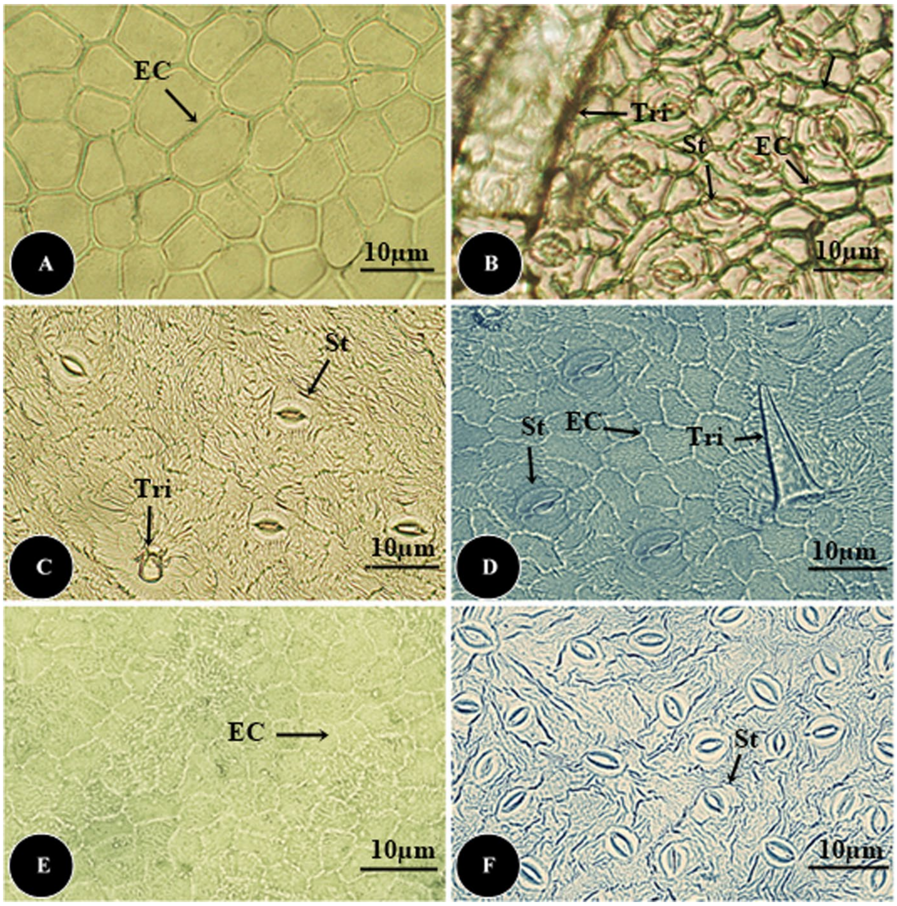


Fig. 3 Plants species of family Oleaceae from Pakistan. Light micrographs (LM) shapes of cells, patterns of walls, stomata and trichomes of Oleaceae. Scale bar = 10 µm (**A**) *J. elongatum* adaxial surface lacking stomata as well as trichomes (**B**) Abaxial surface showing Anomocytic stomata and Unicellular Non-glandular(**C**) *J. grandiflorum* adaxial surface showing Paracytic stomata and Unicellular Non-glandular trichomes (**D**) Abaxial surface showing Anomocytic stomata and Unicellular Non-glandular trichomes (**E**) *J. mesnyi* adaxial surface lacking stomata as well as trichomes (**F**) Abaxial surface showing Anomocytic stomata. EC, Epidermal cell; St, Stomata; Tri, Trichomes

areas is the cause of the spherical head of the Peltate glandular trichomes. Compared to peltate trichomes, capitate trichomes are smaller, with an extending stalk, and a spherical head.Trichomes are completely absent in *J. mesnyi and Jasminum sambac*. On the lower surface (abaxial), trichomes are absent in *F. ornus, L.lucidum* and *Olea ferruginea*. Abaxial surfaces of most species showed trichomes except *Forsythia suspense*, *J. mesnyi, Jasminum sambac*.

Variable trichome index was shown by species and varieties on both adaxial and abaxial surfaces. *N. arbor-tristis* showed the highest trichome index of 5.12 while the lowest 0.39 was seen in *F. suspensa* on the adaxial surface. While on the abaxial surface 47.16 was the peak trichome index that was discerned in *N. arbor-tristis* while the lowest trichome index of 0.54 was observed in *F. suspensa*. Maximum trichome length on the adaxial surface was seen in *J. officinale* (245.00 -246.00 = 245.00 + 0.16) µm and the minimum value was observed in *Olea koroneiki* (20.00–21.00 = 20.50 + 0.17) µm. Similarly maximum trichome width on adaxial surface was seen in *J. officinale* (27.25–29.00 = 28.0 + 0.32) µm while minimum was observed in *Olea europaea cv. Arbequina* (12.25–13.25 = 12.75 + 0.17) µm (Fig. 10). In *J. elongatum* maximum trichome length was observed (237.75–248.00 = 242.25 + 1.73) µm while lowest trichome length was observed in *O. fragrans* (100.00–101.75 = 1.00 + 0.32) µm on abaxial surface. Highest abaxial trichome width was seen in *J. elongatum* (34.50–35.75 = 35.10 + 0.21) µm and lowest was observed in *F. ornus*(2.75–4.75 = 3.9 + 0.38) µm (Fig. 11).

All the trichomes observed are unicellular and were observed on the intercoastal zone. Unicellular non-glandular trichomes are observed in *F. ornus, J. elongatum, J. grandiflorum*, *J. officinale*, *Olea europaea cv. Arbequina and O. fragrans*. Unicellular glandular trichomes are present in *F. suspensa, F. excelsior*, *Ligustrum lucidum*, *N. arbor-tristis*, *Olea europaea*, *Olea europaea cv. Koroneiki and Olea ferruginea*.

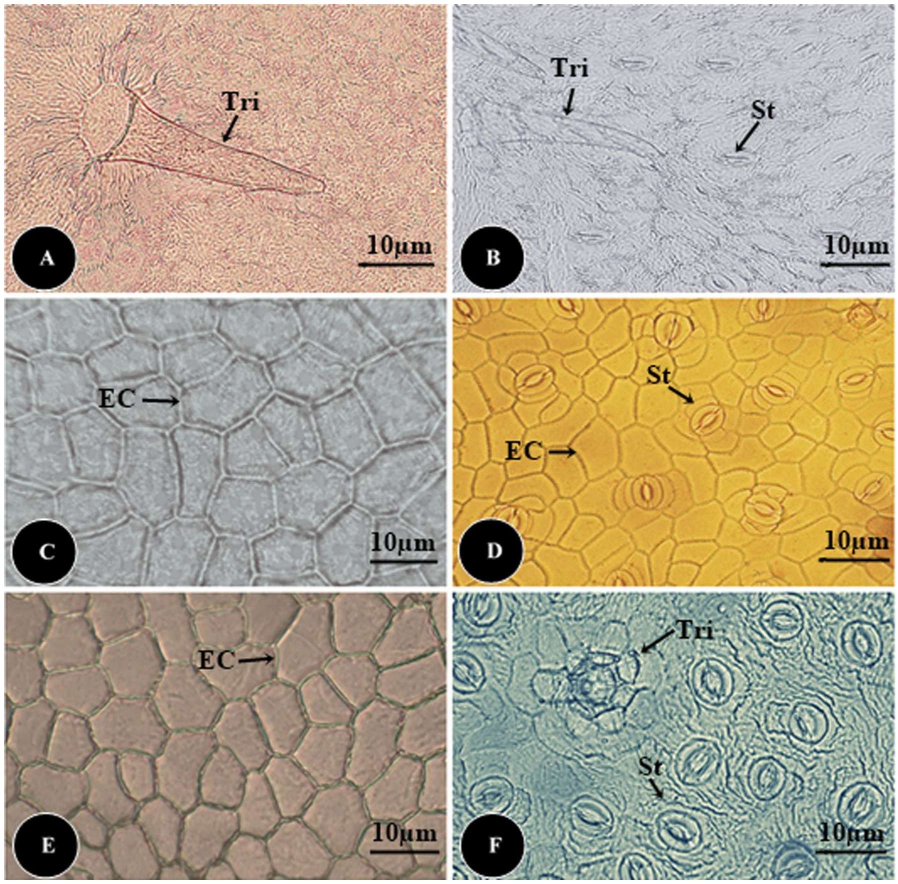


Fig. 4 Plants species of family Oleaceae from Pakistan. Light micrographs (LM) shapes of cells, patterns of walls, stomata and trichomes of Oleaceae. Scale bar = 10 µm (**A**) *J. officinale* adaxial surface showing Unicellular Non-glandular trichomes (**B**) Abaxial surface showing Anomocytic stomata and Unicellular Non-glandular trichomes (**C**) *Jasminum sambac* adaxial surface lacking stomata as well as trichomes (**D**) Abaxial surface showing Anomocytic stomata (**E**) *L. lucidum* adaxial surface with no stomata and no trichomes (**F**) Abaxial surface showing Anomocytic stomata. EC, Epidermal cell; St, Stomata; Tri, Trichomes

Peltate trichomes are observed in *Ligustrum lucidum, N. arbor-tristis*, *Olea europaea cv. Koroneiki and Olea ferruginea*. Capitate trichomes are observed in *Forsythia suspense, F. excelsior and Olea europaea*.

Cluster analysis through dendrogram

15 taxa of Oleaceae fall into two major clusters based the on the difference in qualitative features. Similarity relationships among different species were explored using UPGMA clustering using foliar anatomical characters. The UPGMA phenogram shows two main clusters C1 and C2. The second principle cluster C2 represents section *J. elongatum* and *J. officinale* and *J.sambac*. The first cluster C1 is further divided into two sub-clusters comprising C1A1 of 3 species comprising of *N. arbor-tristis,O. fragrans and f. suspensa* were found to be closely related based on Euclidean distance mapping. The second sub-cluster C1A2 represents 9 species *Olea europaea, O.europaea* L. *cv. Koroneiki, O. ferruginea, F. ornus, F. excelsior*, *J. mesnyi, Ligustrum lucidum*, *Olea europaea cv. Arbequina and J. grandiflorum* (Fig. 12).

Considering the outcomes of this study, a taxonomic key was created in order to describe the Oleaceae flora.

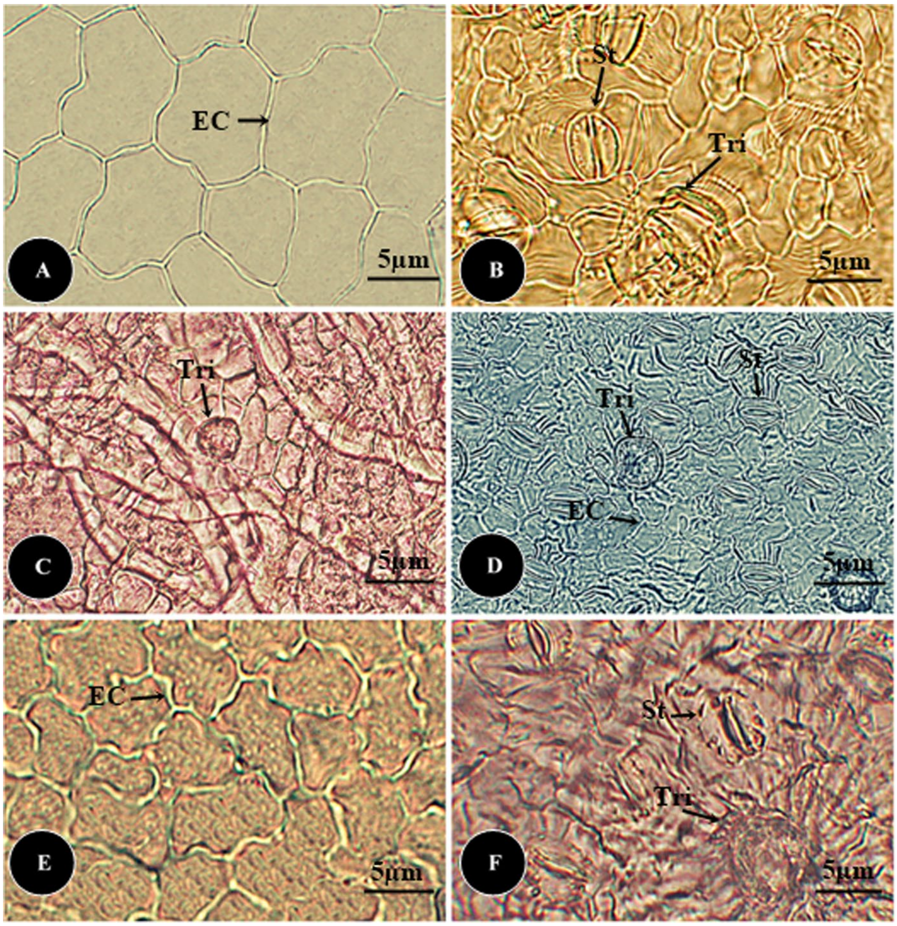


Fig. 5 Plants species of family Oleaceae from Pakistan. Light micrographs (LM) shapes of cells, patterns of walls, stomata and trichomes of Oleaceae. Scale bar = 5 µm (**A**) *N. arbor-tristis* adaxial surface lacking stomata as well as trichomes (**B**) Abaxial surface showing Anomocytic stomata and Unicellular, Glandular trichomes (**C**) *Olea europaea* adaxial surface showing Unicellular, Glandular trichomes (**D**) Abaxial surface showing Anomocytic stomata and Glandular, Unicellular trichomes (**B**) *Olea europaea L. cv. Arbequina* adaxial surface with no stomata and no trichomes (**F**) Abaxial surface showing Anomocytic stomata and Unicellular Non-glandular. EC, Epidermal cell; St, Stomata; Tri, Trichomes

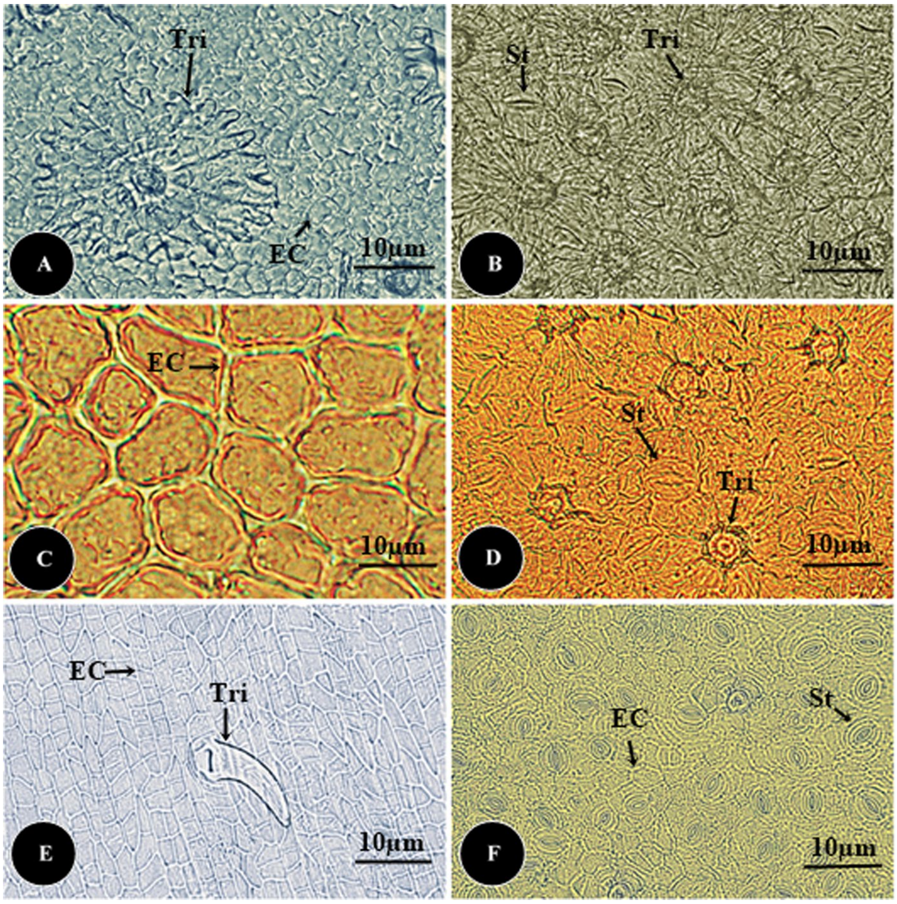


Fig. 6 Plants species of family Oleaceae from Pakistan. Light micrographs (LM) shapes of cells, patterns of walls, stomata and trichomes of Oleaceae. Scale bar = 10 µm (**A**) *Olea europaea* L. *cv. Koroneiki* adaxial surface with Unicellular, Glandular trichomes (**B**) Abaxial surface showing Paracytic stomata and Unicellular, Glandular trichomes (**C**) *Olea ferruginea* adaxial surface (**D**) Abaxial surface showing Anomocytic stomata and Glandular, Unicellular trichomes (**E**) *O. fragrans* adaxial surface with no stomata and Unicellular, Non-glandular trichomes (**F**) Abaxial surface showing Anomocytic stomata and Unicellular Non-glandular trichomes. EC, Epidermal cell; St, Stomata; Tri, Trichomes

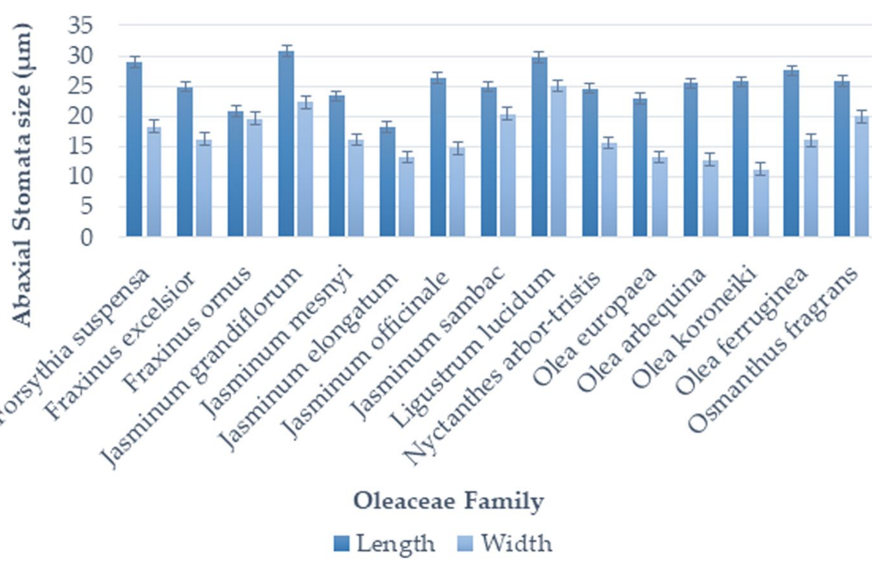


Fig. 7 Pattern of variation in stomata length on abaxial surface

Fig. 8 Showing variation of foliar epidermal length and width on adaxial surface

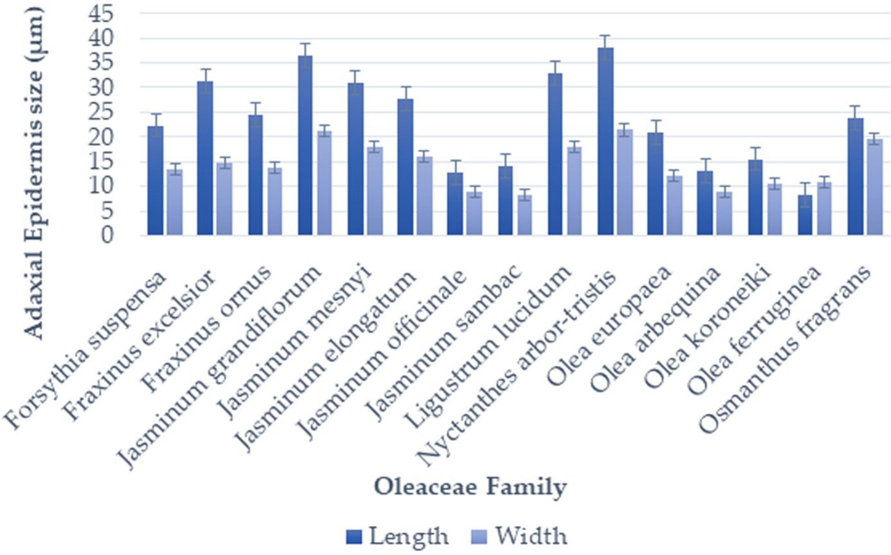


Fig. 9 Showing variation of foliar epidermal length and width on abaxial surface

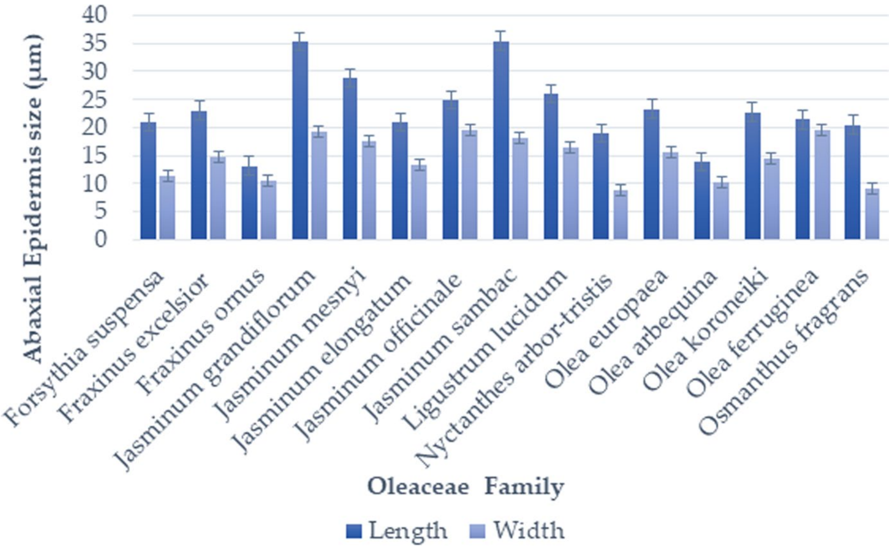


Fig. 10 Showing variation of trichome length and width on adaxial surface

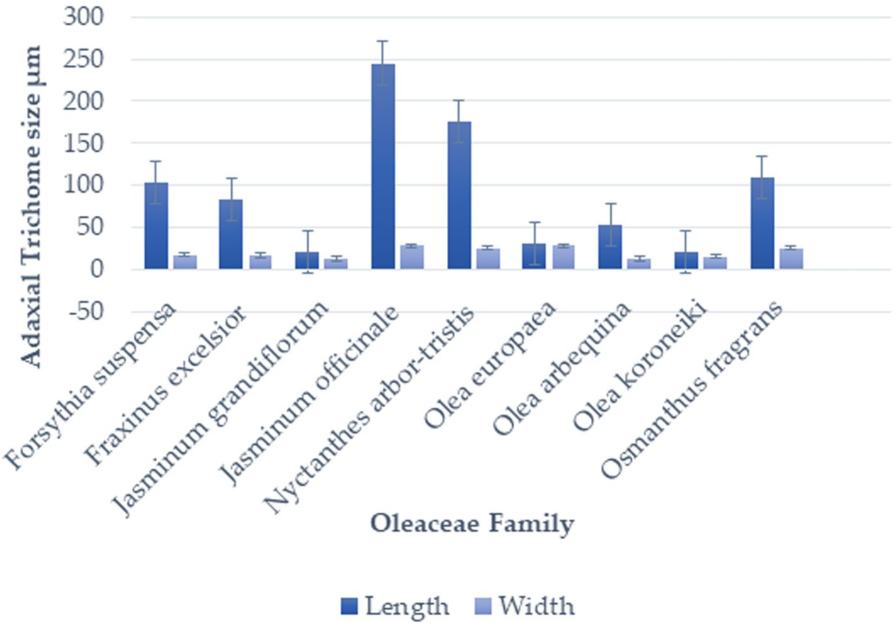
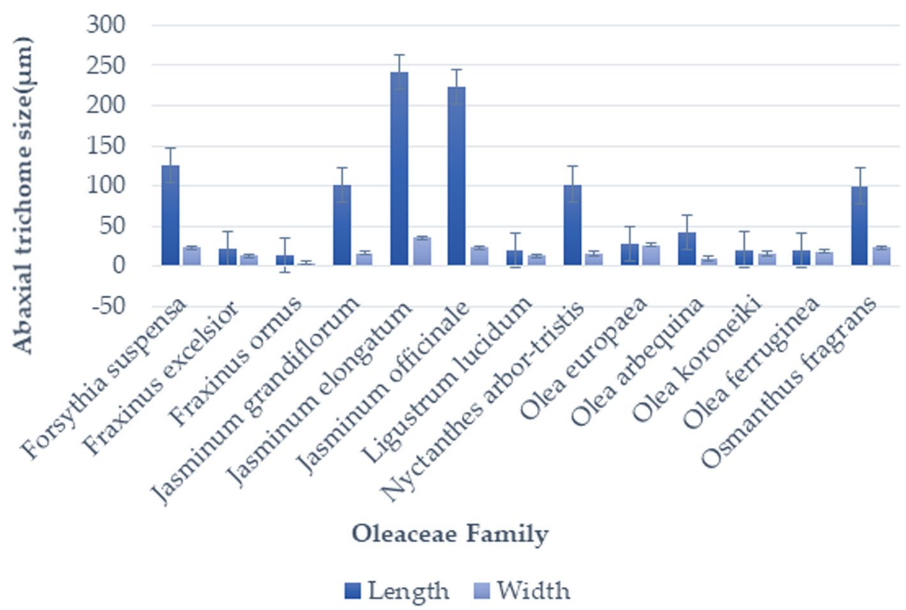


Fig. 11 Showing variation of trichome length and width on abaxial surface



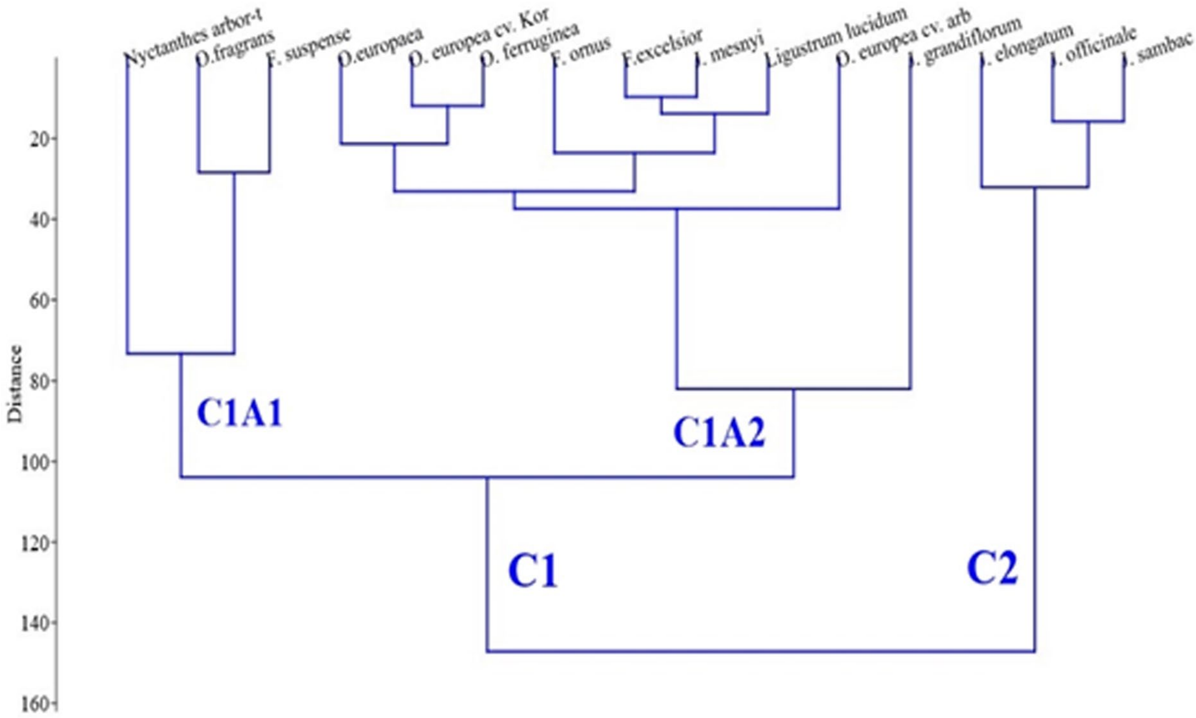


Fig. 12 Cluster groupings via dendrogram of Oleaceae family based on foliar anatomical features

Taxonomic key based on foliar epidermal anatomical characters

1	Dumbbell shaped stomata	*F. suspensa*
	Elongate elliptic shaped stomata	2
2	Anticlinal wall pattern, smooth, angular	*F. excelsior*
	Anticlinal wall pattern, simply undulate	3
3	Epidermal cell shape Irregular	*F. ornus*
	Epidermal cell shape Isodiametric, irregular	4
4	Leaves condition hypostomatic	*J. elongatum*
	Leaves condition amphistomatic	5
5	Stomata shape elliptical	*J. grandiflorum*
	Stomata shape dumbbell	6
6	Trichome absent	*J. mesnyi*
	Trichome present	7
7	Epidermal cell shape irregular	*J. officinale*
	Epidermal cell shape mostly polygonal	8
8	Stomata type anomocytic	*Jasminum sambac*
	Stomata type paracytic	9
9	Stomata shape elliptical	*Ligustrum lucidum*
	Stomata shape dumbbell	10
10	Trichome unicellular, glandular, peltate	*N. arbor-tristis*
	Trichome unicellular, glandular	11
11	Anticlinal wall pattern, straight	*Olea europaea*
	Anticlinal wall pattern, simply undulate	12
12	Stomata type, diacytic	*Olea europaea cv. Arbequina*
	Stomata type, paracytic	13
13	Epidermal cell shape irregular	*Olea europaea cv. Koroneiki*
	Epidermal cell shape, irregular, polygonal	14
14	Trichome unicellular, glandular, peltate	*Olea ferruginea*
	Trichome unicellular, non-glandular	15
15	Epidermal cell shape rectangular to rarely pentagonal	*O. fragrans*

In the surroundings where they are prevalent, plants have favorable impacts, such as minimizing various kinds of pollution. They also serve a variety of other purposes, including as lowering wind speed, serving as a resource economically, and sustaining wildlife (Çetİn 2016; Cetin and Sevik 2016; Guney et al. 2016; Kahraman et al. 2009; Sevik et al. 2019). Plants are now recognized as a measure of the quality and live ability of urban life, used and grown for a variety of purposes (Cetin 2015).

Plants must be grown in environments with the right climatic and edaphic conditions in order to perform their biological tasks. Plants cannot successfully perform the tasks that are expected of them if they are not protected against stress circumstances. The main determinant of the potential a plant possesses for colonization may be its aptitude to adhere to various environmental conditions (Gratani 2014). The degree of individualized adaptation to environmental variables can be observed in the enormous range of foliar morphology (Kaveh et al. 2014). Determining no matter if plants are subject to stress elements in the growing environment where they survive, is nevertheless indispensable. In research work on landscaping, stress factors are frequently applied to plants relying on whether the plants are used beyond their naturalistic environments. It is recognized that these stressful situations alter the physical traits characteristic of that plant. Numerous stressors have been found in research to have an influence on the morphological attributes of plants such as photoperiod stress (Çetİn 2016; Guney et al. 2016), salinity stress (Reddy et al. 2003) and abiotic stresses (Ferris et al. 1996). Furthermore, it is almost certain that stomatal parameters are influenced by environmental factors, peculiarly biotic and abiotic stress (Bañon et al. 2004).

The most intra- and interspecific variable organs among higher plants are concretely the leaves, which has stupendous taxonomic utility. The most crucial organelles for leaf action are stomata. The leaf loses water, and the stomata regulate the input of CO_2 (Xu and Zhou 2008). Environmental conditions have a substantial impact on the density as well as size of stomata (Galmés et al. 2007; Pearce et al. 2006; Xu and Zhou 2008). Numerous researchers have highlighted how environmental factors and stressors, in particular, have an impact on stoma density (Bosabalidis and Kofidis 2002; Dunlap and Stettler 2001; Guerfel et al. 2009).

The most critical step for each and every taxonomist is the taxonomic description of any plant species. It is reliant upon the fundamental instinct, competence and expertise of the taxonomist. For accurate identification of species, the most commonly used methods in the past were the morphological methods. Contemporary taxonomy, however, also encompasses a broad range of strategies, such as anatomy, palynology, phytochemical constituents, genomics, molecular genetics, developmental biology, serological tests, ecology, etc. with the progression of science (Jalal et al. 2021). Blair and Turner, (1972) defined micromorphology emerged as one of the foremost tools for taxonomic identifications of flora. Except for extreme environmental conditions in Antarctica, the members of the family are cosmopolitan in distribution represented by mostly shrubs, trees, rarely herbs and woody climbers. The Olive family is economically important as a consequence of essential oil extraction from various species like *Olea spp*., *Jasminum spp*., fragrances obtained from species of *Jasminum* are appreciated worldwide, Moreover, plants belonging to this family are used as ornamental plants as well. Olives are used for oil extraction and as a source of food as well. (Simon et al. 1995) stated that the attributes that discriminate between elementary and advanced categories and assistance in anatomical and ontogenetically categorization incorporate epidermal architecture and stomatal ontogeny.

The study by Wu-Jan et al. (2009) highlights the distinct micromorphological features of leaf epidermal cells and stomatal apparatuses among 14 Oleeae species, suggesting these characteristics are useful for species identification within the Oleaceous tribe. The variations observed in stomatal shape, epidermal cell arrangement, and glandular spot distribution underscore the taxonomic significance of these traits. Moreover, the commonalities in leaf epidermal micromorphology support the classification of Oleeae as a distinct natural group, providing insights into their systematic evolutionary relationships.

The intention of this study was to foregather useful correlative particulars on foliar anatomical and stomatal complexes, stomatal and trichome index, epidermis traits and foliar anatomical characteristics that can be employed as a useful taxonomic tool for the categorization and delineation of Oleaceae species. Over and above that, it will serve as a foundation for future research and the exploration of morphologically concomitant species via comparative foliar attributes and anatomical characteristics. In view of the fact that leaf parameters are more fitted for systematized categorization compared to other anatomical features, it will be effective in the classification and establishment of intraspecific phylogenetic relationships.

Variations in stomatal patterns and epidermal cells

A stomata is an epidermal orifice that permits gaseous exchange to take place between the intercellular spaces of sub epidermal cells and the surroundings (Eames and MacDaniels 1947). Initially, the stomata were examined based on the existence and placement of accessory cells combined with their process of development, identified four main kinds of stomata (Gole et al. 2013). Current study revealed 3 different stomatal types in the taxa under study under LM.

With 200 species, *Jasminum* is the biggest genus in the olive family Oleaceae (Green and Miller 2009; Kadereit 2004). The genus is thought to be indigenous to tropical as well as warm temperate zones of the world, with a range that stretches from Portugal to the Canary Islands to southern Europe and the entirety of Africa to Formosa, Tahiti, and Australia (Green 1969).

Based on leaf architecture and floral colour, the Jasminum species have been classified into various groups. (Green 1997, 2001). In the current study, 5 *Jasminum* species were analysed, all species were seen to be hypostomatic except for *J. grandiflorum* which was amphistomatic. Similarly all species contained anomocytic stomata except *J. grandiflorum* in which paracytic stomata were observed. Epidermal cells in all these species were irregular to isodiametric. These results are in accordance with the results by (Upadhyay et al. 1989) except for the amphistomatous *Jasminum sambac* and epidermal cell shapes observed by them.

Study of (AL-Hadeethi et al. 2020) showed *F. ornus*L., a genus of blooming medium to big trees, ranges in height from 7 to 10.5 m and can reach 22 m. It has a cylindrical stem and opposite, three-whorled leaves that are bright green in colour. On the top and bottom, the blade's epidermal cells undulate. The adaxial surface is devoid of stomata and only has them on the abaxial surface, which is an anomocytic kind of stomata. The stomatal index observed was 3.11, the stomatal length reached up to 23- 29 (26.5) µm and the width observed was 22–25 (23.5) µm. Present study agrees with the results except for the stomatal index, stomatal length and width which were observed to be 28.14, 16.25–40.75 (24.45) µm and 12.75–15.25 (13.75) µm respectively.

The secretory canals and druses were evident in the *O. europaea* anatomical analysis. The walls of the anticlinal were straight. The stomatal apparatus structure was of anomocytic type seen only on the abaxial epidermis (Najmaddin 2016). Same parameters were confirmed in the present study. In *N. arbor-tristis* stoma was positioned in an insignificant indentation. The orifice is elliptical in configuration and has a delineation of waxy deposition. The stomatal shape was observed to be oval. Guard cells were somewhat protruding, and their peripheral walls had an irregular waxy coating with flakes all over them (Biswas and Mukherjee 2011) On both surfaces in *N. arbor-tristis*, the epidermis was predominantly made up of cells with undulating anticlinal walls; however, the cells that surround the lower portion of the hairs were hexagonal. Stomata were anomocytic, limited to the abaxial surface (Stant 1952). In the current study, irregular, isodiametric, it was reported that epidermal cells had anomocytic stomata and anticlinal walls that were somewhat anticlinal (Fig 12).

L. lucidum is a member of the family Oleaceae. It is an evergreen tree that is native to China. Its height is around 15 m, and its canopy is dense. Uni-strata epidermis, spongy palisade and parenchyma, and vascular bundles are characteristics of leaves (Honaine et al. 2019). In the current research work this plant was found to only have stomata on its lower surface, making it hypostomatic and these stomata were paracytic stomata. No such observations on this species was made in the previous research. Leaf of *O. fragrans* is a typical bifacial leaf. The epidermis was made up of a series of irregular epidermic cells that were stacked together, had thick sticky layers and stomata were only present on the lower epidermis as observed by (Fude et al. 2003).

These traits can be regarded as having taxonomic validity at species or subspecific levels as they enable perceptible demarcation between solitary taxa as well as groups of taxa, potentially deviating from earlier classifications. The results obtained in the current study are in relevance with the previous research proving the accuracy of the literature and thus providing significance traits and basis for solving the problems related to the taxonomic identification of Oleaceous species.

Trichomes

The results of the present study demonstrated that the foliage of family Oleaceae is composed of various trichome shapes on this basis of which species may be divided into two groups according to the types of trichomes present in them. Group I contains species that have non-glandular trichomes.This group includes *F. ornus, J. elongatum, J. grandiflorum*, *J. officinale*, *Olea europaea* L. *cv. Arbequina* and *O. fragrans*. Group II includes all other species in which the trichomes were observed to be glandular. *Forsythia suspense* in contrast to the remarkable multicellular and uniseriate patterns found in *J. elongatum*, glandular trichomes in general had thin walls and sharp peaks. In the species *J. elongatum*, glandular hairs were dispersed across the underlying tissue surfaces of the leaves and varied in length and makeup from glandular head to papillary, as well as in terms of their shape, number of cells, and neck type. Study outcomes are in line with (Ali and Sosa 2015), they focused on the anatomical assemblage of traditional medicines in their research. In their study (Ali and Sosa 2015) observed aglandular trichomes in *J. mesnyi and J. officinales* whereas in the present study *J.mesnyi* did not showed any trichomes under LM.

All the 5 studied species of the largest genus of the family, *Jasminum,*contain unicellular and aglandular trichomes except *J. sambac* in which trichomes were absent as observed under LM. In previous study by (Upadhyay et al. 1989) short, cone shaped and broad trichomes were observed in *J. grandiflorum,* glandular trichomes were observed in *J.mesnyi and J. officinale* while in *J. sambac,* long trichomes with sharp edges were seen. (Kaveh et al. 2014) in their study on two species of *Fraxinus*, *F. angustifolia* and *F. excelsior* from Iran, observed acicular trichomes on different parts of plants like petioles and stems in addition to the leaf surface. On the petioles of the specimens under investigation, there were varieties of capitate as well as acicular trichomes. Upon each specimen of the two species, capitate trichomes were visible. The present study also found capitate tricomes in *F. excelsior* which is in accordance with the study of (Kaveh et al. 2014).

In *F. ornus* trichomes are uniseriate and unicellular, have conical shape in the study of (AL-Hadeethi et al. 2020) which agrees with the current study. (Najmaddin 2016) Observed trichomes in *O. europaea*, no description in given by them. In the current study the trichomes observed in *O. europaea* were Unicellular and glandular.The present study showed Unicellular, glandular trichomes in *Ligustrum lucidum.* These findings were also observed by (Honaine et al. 2019) where trichomes were glandular, containing a unicellular stalk and a head which is multicellular. In a study by (Biswas and Mukherjee 2011) on the micromorphology of leaf of *N. arbor-tristis*, they observed emerging trichomes of all types all over the leaf's surface. Upon the lower surface compared to the upper, greater number of trichomes were observed. The solid trichomes that are aglandular often have a huge base and a verrucose surface. The adaxial surface of the leaf ordinarily has these trichomes standing upright. The veins that link the trichome base have striations that mimic divariaxial adventitious roots. There were also several lens shaped contiguous pits and an additional type of trichome with an upright body and smooth texture. The third category consisted of unsystematically dispersed, simple, fistular hair with terminally compressed lumen that had their pilings on the bottom part. The fourth category revealed the ubiquitous peltate glandular trichomes that cover the entire leaf. The chiasmatic multilateral depression on the peltate trichomes' shield is covered by a single celled stalk. Unicellular,Glandular and peltate trichomes were observed in the current study on the abaxial surface of *Nyctanthes* whereas, no trichomes were observed on the adaxial surface.

Epidermis was lined by trichomes in *O. fragrans,*that create flower-ring structures with the surrounding epidermic cells neatly aligned (Fude et al. 2003). Unicellular,aglandular trichomes were evident on adaxial as well as abaxial surfaces as observed in the present study. This experimentation focuses on the richness of trichomes as well as qualitative and quantitative micromorphological traits in the investigated species. The motif under study manifested that both adaxial together with abaxial surfaces featured trichomes. For species and genus rank identification, the epidermal characteristics, variety, and dispersion of trichomes were key taxonomic traits.

The literature has few descriptions of the anatomy of Oleaceae species, and no such comparative compilation amidst these taxa that applies taxonomic principles to the arrangement of leaves of the species is available. Kiew and Ibrahim, (1982) released a thorough article on the micro-morphology of the 15 Oleaceae species from Malaysia, nevertheless it omits any mention of how the observations should be applied taxonomically. The only type of stomata present on Oleaceae species were anomocytic The only type of stomata present on Oleaceae species were anomocytic (Solereder 1908). The current research work found other kinds of stomata like paracytic and diacytic stomata as well. However, notwithstanding the fact that every single Oleaceae species under study have them, the feature of cuticle crest in stomata is not discussed in the literature.

All of the studied species featured stomata of variable sizes, and on some leaves, it was even feasible to observe two or more sizes on an individual leaf. It has been reported that in some Jasminium species having sinuose anticlinal epidermal walls are present (Solereder 1908) but straightly oriented walls have really been recorded in nearly half of these species, and also in numerous species from Malaysia (Kiew and Ibrahim 1982). Sun and shade grown leaves may differ in their anticlinal epidermal wall undulations, with the latter being more conspicuous. This happens because the cuticle thickens and clenches more readily under the sun, making the walls immovable. On the abaxial surface, there is a propensity for increased undulation. It's probable that the environment has an impact on this quality, with xeric areas having less undulation. (Metcalfe and Chalk 1979). The information gained through the present study will be useful for the taxonomy of the group.

Despite being always present and frequently more numerous on the abaxial surface, glandular trichomes were present on both foliar surfaces. The features witnessed in the Malaysian species were also seen in this examination (Kiew and Ibrahim 1982). Only the long-headed glandular trichomes were observed on *Olea ferruginea*, *Olea europaea*, and *J. officinale*, usually in very small numbers, but the kind of glandular trichomes observed, consisting of a unicellular stem and a multicellular head, is indicative of the Oleaceae (Solereder 1908). Various members of the family also have single celled or one layered tector trichomes together with glandular trichomes (Solereder 1908). There have already been reports of tapered-end unicellular unseriate trichomes in Malaysian Oleaceae species (Kiew and Ibrahim 1982). Multicellular trichomes, another type found in this study, are found on the leaf blades of *Jasminium* species and constitute the domatia in all taxa.

The presence of embellished cuticle on the tector trichomes was one characteristic with taxonomic significance of *N. arbor-tristis* and *Ligustrum lucidum*. Trichomes are well known for their importance in the taxonomy of the cuticle and in the identification of angiosperms, with their ornamentation being seen as the key to accurate identification (Metcalfe and Chalk 1979). Certain species, including *Olea* species, which found substantial sinuosities on the midrib's cuticle, on the abaxial surface, and in some areas on the Leaf, were evident under a light microscope to have multiple kinds of cuticle ornamentation above the epidermis.

The midrib, the petiole, and the anterior surface of the leaf blade of trichomes, all revealed distinct striations on the cuticle, along with prominent striations emphasised on the guard cells encompassing stomata and typical epidermal cells on the abaxial surface, *Jasminium* species is occasionally observed on the guard cells but is more frequently seen around large stomata. Additionally, striations are connected to the Asian species *C. porcatus*, which have undulated striations on their adaxial surfaces, and *C. laxiflorus*, which have linear striations on their abaxial surfaces (Kiew and Ibrahim 1982).

The comparative study by Song and Hong (2013) on Forsythieae leaf epidermal microstructure reveals that, unlike the Oleeae, Forsythieae leaves are mostly hypostomatic, with anomocytic stomatal complexes being predominant. Both tribes show variability in stomatal and epidermal cell structures, but Forsythieae uniquely exhibit a mix of simple unicellular, multicellular non-glandular, and subsessile glandular trichomes. These micromorphological differences underline the importance of leaf epidermal characteristics in the taxonomic identification and classification within and between the tribes.

Conclusion

The results of the current study, supported by earlier literature, demonstrated the importance of studying foliar epidermal anatomy using light microscopy. The foliar epidermal characteristics have enormous potential and are pivotal for accurate species identification of the family Oleaceae. For plant taxonomists to identify a species, micro-morphological features of epidermal cells, stomata and trichomes were analyzed qualitatively as well as quantitatively. For the purpose of precise identification and demarcation of closely related taxa, taxonomic keys for the researched species were constructed based on qualitative characteristics. It is suggested to do additional anatomical and molecular phylogenetic research using sizeable specimens to determine the relationships between the various genera and species within the family.

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Declarations

Conflict of interest The authors declare no conflicts of interest.

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