Anatomical and Palynological Study of *Myrtus communis* L.

Muazaz A. AL-Hadeethi
Department of biology, college of education of pure sciences Ibn-AL-Haitham, Baghdad University.

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Abstract

This study detailed the properties of epidermis, leaf transverse sections, petioles and stems of the genus *Myrtus communis* L. belong the Myrtaceae family. It was clear that the particular anatomical characteristic were noteworthy and very important of this species, like as the multilayered cortex rich of wide idioblasts and taniniferous cells and the cells are a lot of druses crystals, oil pits situated close to both surfaces and unicellular hairs, likewise druses crystals are extremely regular in this species in the epidermis and cortex of leaf, petiole and stem too. The leaf blade has increase in cuticle thickness due to that epidermis waxen in this species.The pollen morphology of this species was elliptical in equatorial side and triangular in polar side also **the apertures were** tricolporate, mostly syncolpate also the pollen grain narrow colpi.

Key words: *Myrtus communis* , Myrtaceae family, Myrtle, Anatomical study.

دراسة تشريحية وحبوب لقاح لنبات الياس *Myrtus communis* L.

معزز عزيز الحديثي

قسم علوم الحياة - كلية التربية للعلوم الصرفة ابن الهيثم - جامعة بغداد

الخلاصة

تفاصيل هذه الدراسة حول خصائص البشرة والمقاطع العرضية للاوراق، وأعناق الاوراق والسيقان لجنس الآس الشائع *Myrtus communis* L. الذي ينتمي إلى عائلة Myrtaceae. كان من الواضح أن الصفات التشريحية بوجه

خاص كانت ذات أهمية جديرة بالملاحظة ومهة جدا لهذا النوع، مثل القشرة المتعددة الطبقات الغنية بالـ idioblasts الواسعة والخلايا الحاوية على التانينات والخلايا الحاوية على الكثير من البلورات النجمية الشكل ، النقر الزيتية تقع على مقربة من كل من الأسطح والشعيرات وحيدة الخلية، كذلك البلورات النجمية منتظمة للغاية في البشرة والقشرة من الورقة والسويقات والسيقان. نصل الورقة حاوي على طبقة كيوتكل سميكة وواسعة وهذا يعود الى ان طبقة البشرة مغطاة بالشمع في هذا النوع. المظهر الخارجي لحبوب اللقاح في هذا النوع كان بيضاوي الشكل او اهليليجي في المنظر الاستوائي و مثلث الشكل في المنظر القطبي وكذلك الفتحات كانت tricolporate، معظمها syncolpate وايضا حبوب اللقاح ضيقة الفتحات narrow colpi

الكلمات المفتاحية: *Myrtus communis* ، عائلة الـ Myrtaceae، الياس، دراسة تشريحية.

Introduction

Myrtus communis L. is a modest species having a place with the Myrtaceae family which contains between 100 genera and 3000 species developing in tropical, subtropical and temperate locales [1], in Iraq this family contain five genera considered very important medicinal plants [2]. *Myrtus communis* L. was monotypic genus of the species developed in the Northern Hemisphere [3], also considered native in Europe, spread throughout the Mediterranean region and the Middle East where it sowing overland and cultivated including Iraq, Turkey, Iran and Jordan also grows wild in Syria in the mountains of the country's Western-Sham and other countries [4], *Myrtus communis* widespread in the lower mountain valleys, among oak forest also widely cultivated in gardens in lower of Iraq and can see *Myrtus communis* sharp in the Rownduz in the north of Iraq, Baghdad and Abu Ghraib in the middle of Iraq, also cultivate in AL-Amara south of Iraq [2].

To the *Myrtus communis* have several names vary depending on the region in which there is defined, for example, in Syria known as Ace, in Lebanon, Morocco and Tunisia known as basil, in Egypt and Turkey known as Mersin and in Spain known as Ariane also the fruit of Ace is called in the Levant "Hablas or Hmpelas" or Ace grains, in Yemen and southern Saudi known as "Hades" and in some countries of the Maghreb Arab "Hlmos or Hlmos" also called "diving and shlmon", also in the Pharaohs called Ace "Khet Aaos" it means "Basil graves" because placed the Ace on graves when visited also known in Greek as (Amoser) [5], the common name in Iraq is Yas or Habb AL-As it was often eaten by poor people and has used medicinally since very ancient times. also found the name AS in the ancient Assyrian medical

texts, Ibn AL-Baitar since 1240 reproduces short extracts from As and Abu Hanifa and discords who refers to *Myrtus communis* oil or myrtol (Dihn AL-As) [2].The flowers star-like, white in color, have 5 petals, 5 sepals and a groups of tufty stamens, growing from June to September. After the summer, arise the grains it's subglobose to ellipsoid berries and the colors it's black-bluish (or rarely white-yellowish) on maturation, arise around November [6].

The blooms are pollinated by honey bees and have a particular technique of seed spread by warm blooded animals and flying birds, the winter or autumn rains during the climacteric first phases of development can be viewed as altering to augment sexual accomplishment, with seedling settling and survival in Mediterranean situations [3 and 7].

The present review aims to characterize *Myrtus communis* leaves, petioles and stems also scanning of pollen grain as well as identification the important compositions in the cell of the plant parts.

Material and methods

Fresh material of *Myrtus communis* L. was collected from gardens throughout Baghdad district. The epidermis were prepared followed by washing with distilled water and put in 10% KOH, then passed through alcohol series (70, 95, and 100%) for 10-15 minute and then stained in 1% safranin in alcohol for approximately 30-45 minute. Excess stain was washed off with distilled water, dehydrated by Alcohol series (70, 95, and 100%) and cleared with pure xylene 10 minute. Finally, the epidermal samples were put on the slides and mounted by cover slides with Dextrin Plasticizer Xylene (D.P.X) artificial mounting medium according to [8].

For doing sectioning parts, fresh material of leaves and petioles was fixed in (FAA) formalin acetic acid alcohol solution at 48 hours and changed the solution after this time and put in the (70%) alcohol, then sectioned on a rotary microtome and stained in safranin and fast green stain and then mounted with Dextrin Plasticizer Xylene (D.P.X) artificial mounting medium. The prestaining and staining procedure was performed according to [8].

The epidermis using stomatal index [9] as follows:

$$\text{Stomatal index} = \frac{\text{number of stomata}}{\text{number of stomata+number of ordinary epidermal cells}} \times 100$$

The Fresh plant samples of stems were sectioned using hand sectioning method [10] as follows:

stems of selected plant was cut into small pieces of a length between (5-7) cm. Segments were sectioned into thin pieces by razor blade and only stem pieces were treated with 0.5% Sodium Hypochlorite for 5 min. to remove the chlorophyll pigment, then all plant samples were passed through of treatments the following:

1. Stained by 1% safranin in alcohol for (1-2) h.
2. Washing by 70% alcohol to remove the excess pigment.
3. 90% alcohol for 5 mints.
4. 95% alcohol for 2 mints.
5. Absolute alcohol for 2 mints.
6. xylene + absolute alcohol (1:1) for 2 mints.
7. xylene for 2 mints.

Finally the samples were put on the slides and mounted the cover slides by (D.P.X).

All permanent slides were examined by Olympus BH2 light microscope and photographed using Olympus CH3 camera. Pollen samples of *Myrtus communis* were collected. Pollens were prepared by acetolysis method as described by [11]. The observations and measurements carried out by both Light microscope (LM) and Scanning Electron microscope (SEM). For LM analysis, the acetolysed pollens were placed in a small vial and 5-7 drops of silicone oil were added then the samples were mounted on glass slide, sealed with paraffin and investigated under light microscope. The measurements of polar view (P), equatorial view (E) and exine thickness of pollen were done using 10 reading for this species. On the other hand, for the SEM investigation, the acetolysed pollens were suspended in 100% ethanol. Then the suspension was dried on aluminum stub, coated with gold and observed by LEO 1450VP Scanning Electron Microscopy. The terminology and pollen size was depended on [12].

Result and dissection

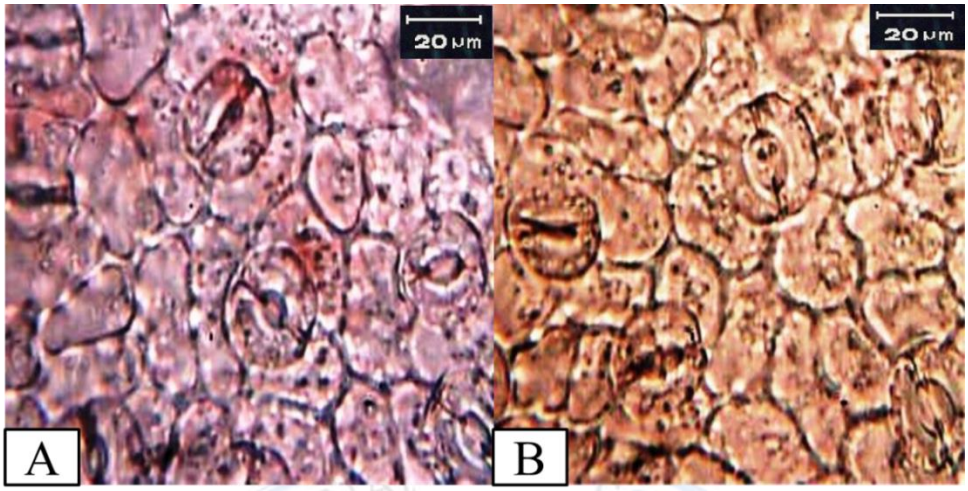
1. Blade : Epidermis and Stomata

Stomata shape in *Myrtus communis was* anisocytic type or ranunculaceous type, guard cells are kidney shaped and the measurements of epidermal cells and stomata are concise in Table 1. There are differences in cell shape between the adaxial and abaxial surfaces of the leaf, the length average of epidermal cells in the adaxial surface ranged 21.50 µm and the average width was 12.60 µm. In the abaxial surface the average length ranged 27.83µm and average width was 13.75µm, also the length average of stomata in the adaxial surface ranged 21.60 µm and the average width was 13.20 µm. In the abaxial surface the average length ranged 18.28 µm and average width was 17.42 µm so the stomatal index was indicate in Table 1. Anticlinal walls of epidermal cells were normally straight, curved sometimes (Figures. 1). [13] referred that although the stomata are ranunculaceous form and appear in all taxa of the family Myrtaceae but showed another shape in addition to the ranunculaceous type, confirming this truth [14 and 15] when they referred that stomata in Myrtaceae family are usually ranunculaceous type exclude some species.

Table 1. Epidermal cells and stomata dimensions in leaves of *Myrtus communis communis communis communis* (in micrometer).

Species	Epidermal cells				Stomata					
	Adixial		Abxial		Adixial		Stomatal index	Abxial		Stomatal index
	Length	Width	Length	Width	Length	Width		Length	Width	
Myrtus communis L.	11-32 (21.50)	10-14 (12.60)	20-35 (27.83)	10-15 (13.75)	20-25 (21.60)	10-15 (13.20)	12.5	16-20 (18.28)	15-19.3 (17.42)	13.6

Note: The numbers between brackets represent average.



Figures 1. Surface view of epidermal cells and stomata in leaves of *Myrtus communis* , A. upper epidermis, B. lower epidermis.

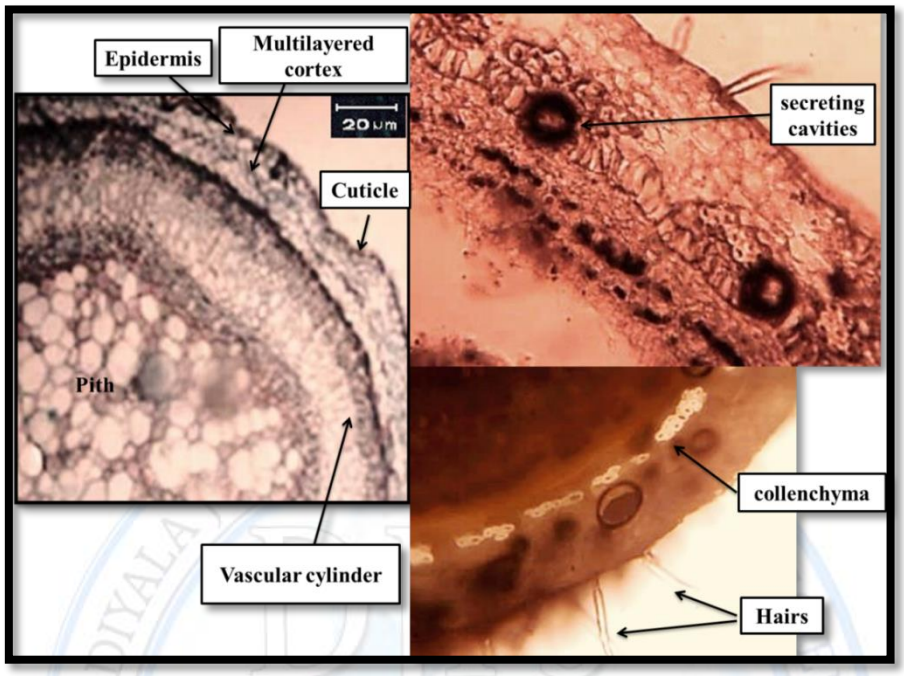
2. **Cross sections**

- **Cross sections of stems**

Anatomical characters of stems are concise in Table 2. Shape of outline stem is semicircular in *Myrtus communis*, (Figures 2). Epidermis is uniserriate covered with cuticle, after epidermis have multilayered cortex have a lot of and wide idioblasts and cells of taniniferous. Cortex consisted collenchyma and parenchyma and the chlorenchyma is absent. Vascular bundles are a continuous bicollateral. Pith appear in the center of stem contain from parenchyma cells of isodiametric to polyhedral layered cells with more large intercellular spaces. Cells are having plentiful of druses crystals. The dimensions of cell growth increase towards the center of stem.

Table 2. Stem anatomical characters in Myrtaceae (in micrometer).

Species	shape	Cuticle thickness (µm)	Epidermis thickness (µm)	Number of wood arms	Pith diameter	Stem diameter
Myrtus communis L.	Semicircular	2-4.5 (3.5)	8.5-14.5 (10.4)	6-9	540-700 (633)	800.5-1201 (1109.4)



Figures 2. cross section of stem in *Myrtus communis* 40X.

- **Cross sections of Leaf blade**

This system represented adaxial and abaxial epidermis. The thickness ranged from 235.3µm. Upper epidermis covered by cuticle, the cuticle thickness ranging 3.5 µm, the lower epidermis have very thin cuticle (Table 3). Thickness of upper epidermis was ranged 13.4 µm, while thickness of lower epidermis ranged 7.5 µm (Table 3). Both adaxial and abaxial epidermis in all studied species showed the presence of trichomes, stomata and ordinary cells.The types of epidermal cells were square-shaped or rectangular, uniserriate. Mesophylls have 2 layers of palisade cells and several rows (up to 3) of compact or loose spongy tissue (Fig. 3).Also the margin of the blade is circular shaped (Fig.4).The mesophyll bifacial mesophyll differentiated into upper palisade parenchyma and lower spongy parenchyma, this result agreement with [14 and16] who referred to that mesophyll of *Eucalyptus camaldulensis* is bifacial.Midrib commonly curved in both directions, supply with one ovate bicollateral vascular (Figure 5, Table 4). Oil lumen located near to both surfaces and unicellular trichomes also druses crystals are very widespread in this species in the epidermis and cortex of leaves, petioles and stems (Fig. 6).Idioblasts or the other

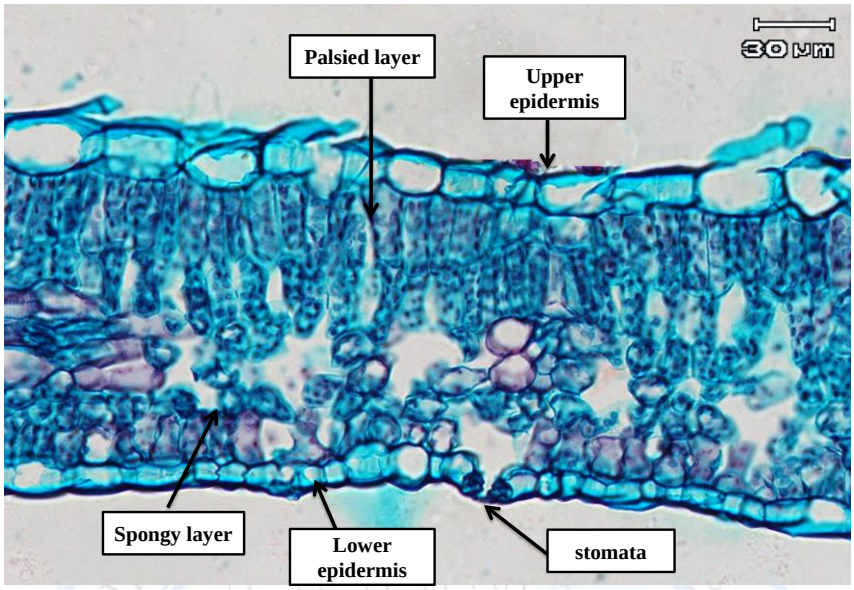
name secretory cavities and cells containing tannin are very common in all un-lignified tissues in this species investigated. [13] refer to similar structures. [17] announced that tanniniferous idioblasts and secretory cavities occur in many families including Myrtaceae. Idioblasts developed from single epidermal cells, the origin of it was initially schizogenous but in the end lysigenous at maturity split-up from all the secretory cells within the gland cavity [18]. While according to [19] pointed that these cavities were located nearby the epidermis, the origin of the idioblasts was from the epidermal meristem without collaboration of the ground meristem.

Table 3. Anatomical characters of lamina in *Myrtus communis* L.

Species	type	Lamina thickness (µm)	Cuticle thickness (µm)	Epidermis thickness (µm)		Number of palsied layer	Thickness of palsied layer (µm)	Number of spongy layer	Thickness of spongy layer (µm)
				Upper	lower				
Myrtus communis L.	bicolateral	235.3	3.5	11-15.5 (13.4)	5.5-10.4 (7.5)	1	20-40 (33.5)	8-10	155-233 (223.5)

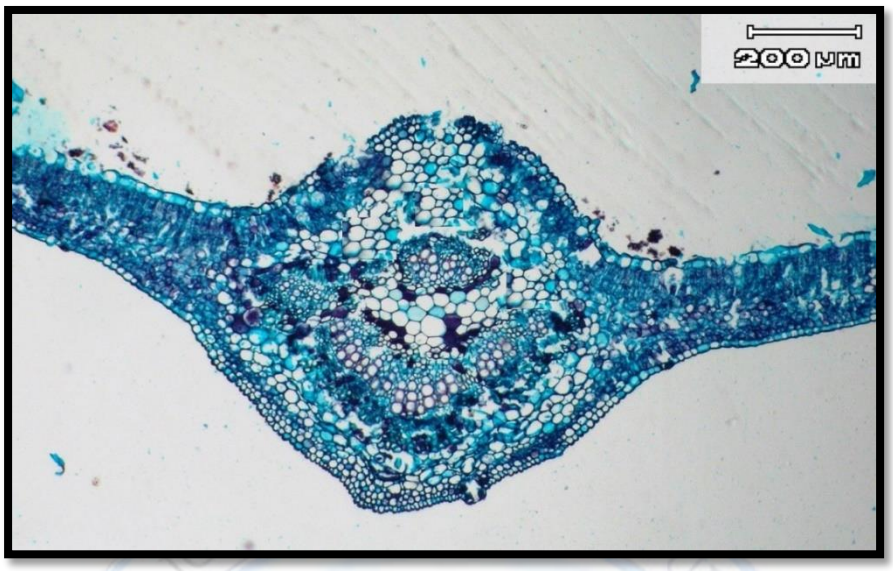
Table 4. Anatomical characters of midrib in *Myrtus communis* L.

Species	Midrib shape	Width (µm)	Length of wood arms (µm)	Number of wood arms	Number of vessels in the arm	Midrib vascular bundle shape
Myrtus communis L.	Erect	(247-267.5) 250.25	(110.5 - 165) 135.53	13 - 17	2 - 8	U-shaped

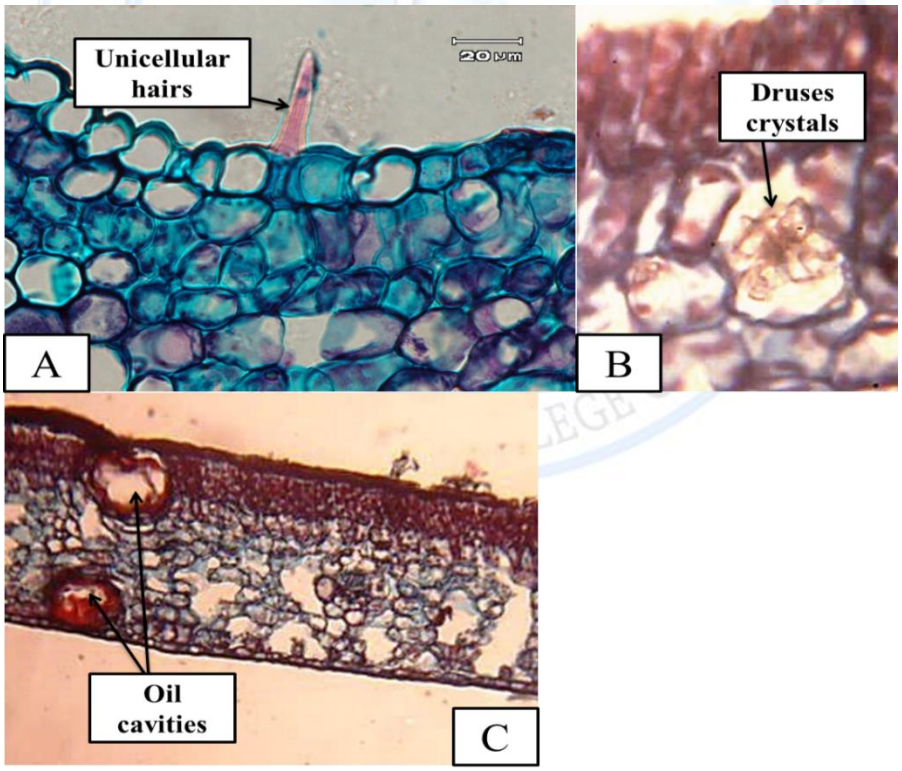


Figures 3. cross section of blade in leaf of *Myrtus communis communis communis communis* 40X.

Figures 4. cross section of margin in leaf of *Myrtus communis communis communis communis* 40X.



Figures 5. cross section of midrib in leaf of *Myrtus communis communis communis communis* 40X.



Figures 6. A. unicellular hairs, B. druses crystals, C. oil cavities in leaf of *Myrtus communis communis communis communis* 40X.

1. **Transverse Sections of Petioles**

Petioles were changeable in shape and length from elliptic to ovoid to semicircular to winged in the transverse section and covered with simple trichomes. Epidermis have one layered, quadrate or rectangular shaped, covered with cuticle of variable thickness, followed by a cortex of 2-3 rows of collenchyma and 2-4 rows of chlorenchyma and several rows of parenchyma ranging between 4–7 (Table 5, Figure 7). According to [20] the vascular bundle in a centric and arrangement in petiole as open arc also the vascular system single, major arc in the center and some diffuse vascular bundle above the major arc, the shaped of vascular system is widely and look like U-Shaped .

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

Species	shape	thickness	Number of vascular bundle	Number of wood arms	Petiole vascular bundle shape	Crystal shape
Myrtus communis L.	Winged	(272.3 - 300) 214.5	1	(23 - 29) 26	U-shaped	Druses

Figures 7. Transverse section of petiole in *Myrtus communis* .

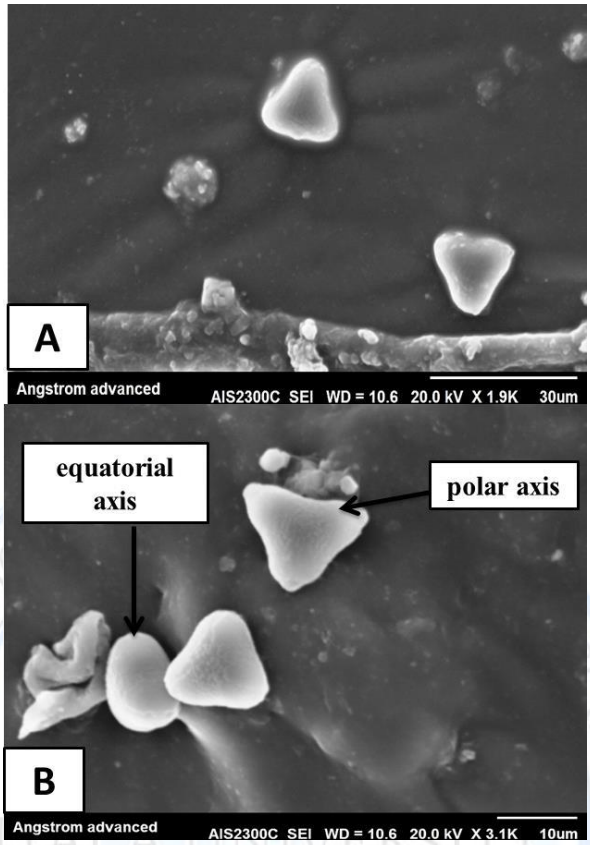
Study of pollen grains

The results of the pollen morphology of ***Myrtus communis*** are summarized in Table 6. It was found that the pollen morphology of this species studied is triangular in polar side, elliptical in equatorial side, the polar side **size** 12.5 (10.9-13.9) µm and the equatorial side 14.6 (13.8-16.8) µm (Figs 8).**The apertures were** tricolporate, also the pollen grain narrow colpi .

The pollen wall very slim psilate or scabrate exine, the thin intine thickens protuberantly underneath the pori. Pollen wall overall about 1.2 µm thick.Pollen description is presented under the tribes recognized by [21] and details of this species follow the reassessment of Myrtaceae by [22].[23] was found that the pollen morphology of ***Myrtus communis*** species is triangular in polar view and elliptical in equatorial view also refer the **size of** polar side is 11.0 µm and the equatorial side is 15.6 µm [24] found that the family Myrtaceae having more than one type of pollen. Also [25] refer that the shape of pollen in ***Myrtus communis*** **similarly** with *Eucalyptus*. The benefit of study pollen grain is considered evidence and index to separate the genus from other [11].

Table 6: Characteristics of pollen grains in *Myrtus communis* (measurement in µm).

Species	Polar axis (µm)	Equatorial (µm axis	E /P Ratio	Shape	Size	Exine thickness (µm	Exine sculpturing
***Myrtus communis* L.**	**(10.9-13.9) 12.5**	**(13.8-16.8) 14.6**	**0.85**	**tricolporate syncolpate**	**Small**	**1.2**	**psilate - scabrate**



Figures 8. Pollen grain of *Myrtus communis* in SEM. Microscope, A: Magnification power = 1.9K, B: Magnification power = 3.1K.

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