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Taxonomical, phytochemical, antioxidant and antibacterial study of some medicinal plants of the Myrtaceae family

Shaemaa Muhi Hasson AL-AMERY *[1], Noor Mahmood NAJI [1], Rihab Edan KADHIM [1], Evan Ibrhaim MERHIJ [1], Yazi Abdullah JASSIM [1]

[1] Department of Biology, College of Science, University of Babylon, Iraq.
*E-mail: shaemaamuhi79@gmail.com

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ABSTRACT: This paper addresses a comparative study of three medicinal plants of the myrtle family (*Callistemon macropunctatus* Dum. Cours. Court; *Eucalyptus camaldulensis* Dehnh.; *Myrtus communis* L.). The morphological characteristics of the pollen grains and some anatomical characteristics of the leaves and stems were evaluated, with quantitative estimates of the phenolic and flavonoid compounds of the leaves and determination of the efficacy of the aqueous-alcoholic extract of the leaves as an antioxidant based on the DPPH method; in addition, the efficacy of the extract as an antibacterial for some pathogenic strains, Gram-positive (*Staphylococcus aureus* and *Klebsiella pneumonia*) and Gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) was evaluated. The results showed the taxonomic importance of pollen grain characteristics in separating the Myrtus communis species and the effective role of anatomical characteristics in separating the studied genera. Furthermore, the study's results showed the efficacy of the leaf extract of the studied genera as a strong antioxidant and an effective antibacterial for both types of pathogenic bacterial strains. The greatest efficacy of the extract was for the leaves of the *Myrtus communis* species due to the high content of phenolic compounds and flavonoids.
Keywords: Myrtaceae; *Callistemon macropunctatus*; *Myrtus communis*; *Eucalyptus camaldulensis*; antioxidant.

Estudo taxonômico, fitoquímico, antioxidante e antibacteriano de algumas plantas medicinais da família Myrtaceae

RESUMO: Este trabalho aborda um estudo comparativo de três plantas medicinais da família das murtas (*Callistemon macropunctatus* Dum. Cours. Court; *Eucalyptus camaldulensis* Dehnh.; *Myrtus communis* L.). Foram avaliadas as características morfológicas dos grãos de pólen e algumas características anatômicas das folhas e caules, com estimativas quantitativas dos compostos fenólicos e flavonoides das folhas e determinação da eficácia do extrato aquoso-alcoólico das folhas como antioxidante com base no método DPPH; além disso, avaliou-se a eficácia do extrato como antibacteriano para algumas cepas patogênicas, Gram-positivas (*Staphylococcus aureus* and *Klebsiella pneumonia*) e Gram-negativas (*Pseudomonas aeruginosa* and *Escherichia coli*). Os resultados mostraram a importância taxonômica das características dos grãos de pólen na separação da espécie *Myrtus communis* e o papel efetivo das características anatômicas na separação dos gêneros estudados. Além disso, os resultados do estudo mostraram a eficácia do extrato de folhas dos gêneros estudados como um forte antioxidante e um antibacteriano eficaz para ambos os tipos de cepas bacterianas patogênicas. A maior eficácia do extrato foi para as folhas da espécie *Myrtus communis* devido ao alto teor de compostos fenólicos e flavonoides.
Palavras-chave: Myrtaceae; *Callistemon macropunctatus*; *Myrtus communis*; *Eucalyptus camaldulensis*; antioxidante.

1. INTRODUCTION

Myrtaceae Juss. is one of the widespread plant families of angiosperms, also known as the myrtle family. There are approximately 132-142 genera and 5950 species. It is widely distributed in tropical America and Australia. Most members of the family have a high economic value, especially the genera of the subfamily Myrtoideae, including *Psidium guajava* (guava) and *Syzygium aqueum* (Rose apple), as their fruits are considered to have a high nutritional value. The raw oil material can also be extracted from some species, including Backhousia citriodora and Eucalyptus spp., some used as spices, wood, and ornamental plants. Gum has been extracted from some species of the subfamily Myrtoideae (WILSON, 2011; LAMBERT et al., 2013; MOHAMED et al., 2023). Taxonomically, the family was classified into two subfamilies (Leptospermoideae and Myrtoideae), although some authors contested this classification (WILSON, 2005; WILSON, 2011) classified the family into two subfamilies (Myrtoideae and Psiloxyloideae) based on plastid DNA phylogeny. After that, Lucas (2007) and Stevens (2017) classified the family into 17 tribes, 15 tribes within the subfamily Myrtoideae and 2 within the Heteropyxidoideae (Psiloxyloideae). The Heteropyxidoideae genera are

characterized by having dry capsular fruits, and the leaves are alternate or whorled. As for the genera of the subfamily Myrtoideae, they have fleshy fruits and opposite leaves, and the genera with fleshy fruits are found in two tribes: Myrteae and Syzygieae (THORNHILL et al., 2012).

The genus Callistemon R. Br includes about 37 species of shrubs. This tree is known in folk medicine for its anti-cough and bronchitis properties. Due to its essential oils, it has an insecticidal effect and is an anti-bacterial and anti-fungal agent (GOYAL et al., 2012). Several studies have also indicated the importance of the Callistemon genus in treating digestive system disorders, and it has also been used in traditional Chinese medicine to treat hemorrhoids (RATHORE; RAI, 2022). The species *C. macropunctatus* is known as the scarlet bottle brush. Distinctive tree flowers with red-pink stamens with yellow anthers. The oils extracted from the tree's leaves are important as an antibacterial agent for some bacterial strains (MAURYA et al., 2009).

Eucalyptus L. is one of the important genera in the Myrtaceae family. It is widespread in the world and includes more than 300 species. The leaves of *Eucalyptus camaldulensis* have medicinal and biological importance, including antioxidant, antimicrobial, larvicidal and pesticidal properties, and antifungals. Several studies have indicated that *E. camaldulensis* is a rich source of many biologically active metabolites. The ethyl acetate extract of the leaves of this plant has shown significant antimicrobial and antischistosomal activity and strong effectiveness against *S. mansoni* larvae due to the extract containing several phenolic compounds that could be responsible for this activity (GHAREEB et al., 2018).

Myrtus L. is a small genus of the family Myrtaceae. It is widespread in the Mediterranean region and the Middle East. Myrtus species are rich in volatile oils, phenolic acids, flavonoids, tannins, anthocyanin pigments, and fatty acids (YANGUI et al., 2021). *Myrtus communis* L. is the only species of the genus, widespread in the Northern Hemisphere, known as myrtle. The leaves and fruits of the species *M. communis* have been considered as a medicine and an aromatic plant due to their content of phenolic compounds and essential oils (USAI et al., 2018; YANGUI et al., 2021).

2. MATERIALS AND METHODS

2.1. Morphological study

The method of Al-Amery (2018) was adopted in preparing pollen grains. The flower buds of each species were collected, and the anthers were dissected from the flower of each specimen. Then, the pollen grains were spread to prepare the slides for microscopy. More than 25 pollen grains for each species were measured, and the lengths of the polar and equatorial axes were measured using the ocular lens under 40X and photographed using a camera mounted on a Zeiss compound microscope under power 40X.

2.2. Anatomical study of the leaf and stem

2.2.1. Surface view of leaf epidermis

Al-Amery (2018) method was adopted in preparing the leaves epidermis. The Peeling method was adopted to get the upper and lower epidermis. The prepared epidermis was spread on the middle of the slide containing a drop of glycerin and covered with the slide cover. The models were examined, and the stomata and epidermal cell measurements were taken under a Zeiss compound microscope using an ocular micrometer at 40X. Photographs were taken using a Zeiss camera.

2.2.2. Transverse section of the stem

The manual sectioning method was adopted to prepare the sections. The plant stem was cut into pieces with a length of 1-1.5 cm from approximately the middle of the stem. A sharp blade was used for cutting. The sections were stained with safranin dye and then transferred to clean glass slides containing drops of glycerin. The slides were examined under a compound microscope and photographed using a camera mounted on a Zeiss microscope, and measurements were taken using an ocular micrometer at 4 X and 10 X (AL-AMERY, 2018).

2.3. Collection and identification of plant samples

Fresh plant samples of the studied species were collected from different regions during January 2024. The identification of the plant specimens was verified by a botanist, Shaemaa Muhi Hasson, at the Herbarium of the Biology Department, College of Science, University of Babylon. The samples were washed with filtered water and then placed on paper to dry for (3-5) days in the shade. At room temperature, finely ground using an electric grinder (blender to obtain dry plant powder), then collected in clean glass containers until use (ABDULRAHMAN et al., 2021).

2.3.1. preparation of plant extraction

The aqueous-methanolic solvent was used to prepare the plant extract of the leaves of the studied species, according to the following steps (JENA et al., 2021).

Place 80 gm. of leaf powder in a 1 L volumetric flask in a mixture consisting of (water and methanol (400/400) mL and close the opening of the container tightly with aluminum foil to avoid evaporation of the mixture and air oxidation. The flasks were placed in a water bath at 37 Cº for 2 hours at high speed. The extracts were filtered in two stages, the first using medical gauze and the second using a kit of Layers of Whatman-type (1) filter paper. Place the filtrate in the centrifuge at 2500 rpm for 10 minutes. Place the filter in a clean glass dish and then place it in the oven at 40 Cº for 2-5 days. To be dried well. Scrape the extract after it dries completely, then store it in clean containers until use.

2.3.2. Determination of bioactive compounds in studied genera leaves

TFC were quantified according to the described method by Chang et al. (2002) with minor modifications. Extract 0.5 ml was mixed with 1.5 ml distilled water and 0.2 ml 5% $NaNO_2$, and the resultant solution could stand for 2 min at room temperature (27± 2°C). Subsequently, 0.2 ml of $AlCl_3$ 10% in ethanol and 0.6 ml sodium hydroxide 1N were added successively with vortexing in each step. The samples were incubated in the dark at room temperature for 10 min, and the absorbance was measured at 510 nm using a spectrophotometer.

Total phenolic content was estimated based on the Folin-Ciocalteu reagent assay detailed by Zheng; Wang (2001) and Liu et al. (2002). An aliquot (1 mL) of extracts and a standard solution of gallic acid (100 mg mL^{-1}) were added to a 25 mL volumetric flask containing 8 mL distilled water; 0.5 mL of Folin-Ciocalteu reagent was added to the mixture and shaken.

After 5 min, 10 ml of 7% Na_2CO_3 solution was added to the mixture. The solution was diluted to volume (25 mL) with distilled water and mixed. After incubation for 90 min at room temperature, the absorbance was taken at 765 nm.

2.3.3. Determination of the Antioxidant activity in vitro

The ability of the aqueous-alcoholic extract of the leaves of each species to scavenge the free radical DPPH was measured according to the method described by Beiranvand et al. (2021) with some modifications. The measurement was made by reacting 3 ml of each extract (3.12, 6.25, 12.5,25, 50, 100 and 200 mg mL^{-1}) with 100 ml of methanol with 4 mg (0.1 mM) of DPPH; the mixture was kept in a dark place for 30 minutes at room temperature. After this, the absorbance was read at 517 nm using an absorbance spectrophotometer. The percentage effect of the extract in scavenging DPPH was calculated using the following equation:

DPPH scavenging effect (%) or percent inhibition = [(A0-A1) / A0 × 100]

A0 = the absorbance of the control reaction.
A1 = the absorbance in the presence of a standard sample.

2.3.4. Determination of the in vitro antibacterial activity

Four pathogenic bacteria, two species (gram-positive and gram-negative), were obtained from the Advanced Microbiology Laboratory (for postgraduate studies) at the University of Babylon - College of Science - Department of Biology. The VITEK-2 Compact System confirmed the diagnosis.

To prepare the inoculum, colonies from an overnight culture of bacteria isolates were transferred to a 5 ml tube of normal saline, which was then adjusted to 0.5 McFarland standard to obtain a culture with 1.5×108 CFU mL^{-1} (KRISTANI et al., 2022).

This method was done on Muller Hinton agar, detailed by ROCCHETTI et al. (2019):

- Turbidity of each bacterial isolate compared to McFarland 0.5 standard to get the right concentration;
- 0.1 mL of each bacterial isolate was added to a petri dish containing Muller Hinton agar, spread by a spreader, and left the dishes for 1 hr;
- Wells were made using a cork borer (2 mm diameter), as it was an equal distance between the well and the other;
- The extract was dissolved to obtain various concentrations (1000 mg/ml as stock) and, by dilution, three concentrations (500, 250, 125 mg mL^{-1}).
- 40 microliters of each concentration were added to each well and incubated at 37 ° C for 24 hr. Inhibition zones were measured using a ruler.

3. RESULTS

3.1 Morphological study of pollen grains.

The current light microscope results noted limited variations in the characteristics of the pollen grains of the studied species, including the dimensions and shape of pollen grains in the polar and equatorial views.

Among the studied characteristics, pollen grains are 3-colporate (the style characterized by three pores and three grooves arranged around the equator), which was observed in the pollen grains of the studied species.

Size of pollen grains: Based on this characteristic, the studied species were classified as belonging to the small size group, in which the grain size ranged less than 25 µm, according to Erdtman (1971). In the polar view, the maximum of the average axis length was 20.75 µm in *E. camaldulensis*, while the minimum was 16.5 µm. in M. communis. In the equatorial view, the minimum length of the polar and equatorial axis was in *C. macropunctatus,* and the maximum was in *E. camaldulensis* (Table 1, Figure 1).

The shape of pollen grains. The oblate and oblate spherical shape was observed among the pollen grains of the studied species.

Table 1. The variations in the characteristics of pollen grains of the studied species were measured with micrometers.
Tabela 1. Variações nas características dos grãos de pólen das espécies estudadas medidas com micrômetros.

Species	Polar view	Equatorial view		Pollen shape	
		Polar axis Length	Equatorial axis length	Polar outline	Equatorial Outline
Calistemon macropunctatus	(17.5-22.5) 20	(12.5- 15) 15	(7.5-12.5) 10	Oblate - spherical	Oblate
Eucalyptus camaldulensis	(17.5-23.75) 20.75	(17.5-23.75) 21	(15-16.25) 15		
Myrtus communis	(13.75-17.5) 16.5	(12.5-18.75) 16.25`	(10-15) 12.5		

The values inside the parentheses represent the minimum and maximum, and outside the parentheses represent the average.
Os valores dentro dos parênteses representam o mínimo e o máximo, e fora dos parênteses representam a média.

3.2. Anatomical characteristics

Surface view of the leaf epidermis (Table 2). The epidermal cell walls at the adaxial and abaxial surface of the leaf are characterized by being straight in the *C. macropunctatus* and *E. camaldulensis* and slightly - undulated in the *M. communis*. This characteristic had taxonomic importance in separating *M. communis* from the studied genera Figure (2). As for the stomata and the density of their spread on both surfaces, the species were divided into two groups:

1: Hypostomatic: The spread of stomata on the abaxial surface of the leaf and their absence on the adaxial surface, as in *M. communis* and *C. macropunctatus*.

2: Amphistomatic: the spread of stomata on both leaf surfaces, and it is the dominant type in the epidermis of *E. camaldulensis* leaves.

Regarding stomatal complexes, the predominant pattern is anomocytic, with a small prevalence of anisocytic stomatal pattern in the leaf epidermis of *E. camaldulensis* and *M.*

communis. The tetracytic pattern was observed in the leaf epidermis of *M. communis* and staurocytic in the leaves of *E. camaldulensis*. In contrast, the cyclic pattern was observed in the leaf epidermis of *C. macropunctatus*.

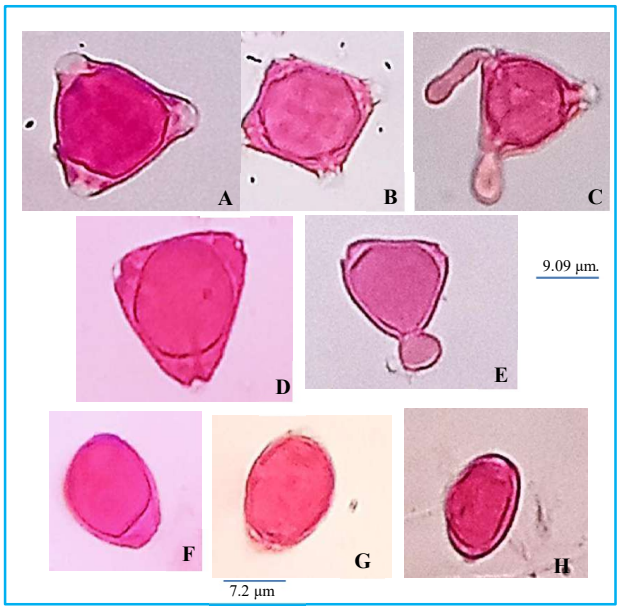

Figure 1. Variations in the shapes of pollen grains. 1: Polar view 4 0 X A-C= *Calistemon viminalis*. D = *Eucalyptus camaldulensis*. E= *Myrtus communis*. 2: Equatorial view 40X F = *Calistemon viminalis* , G= *Eucalyptus camaldulensis* . H= *Myrtus communis*.
Figura 1. Variações nos formatos dos grãos de pólen. 1: Vista polar 40X A-C= *Calistemon viminalis*. D = *Eucalyptus camaldulensis*. E= *Myrtus communis*. 2: Vista equatorial 40X F = *Calistemon viminalis*, G= *Eucalyptus camaldulensis*. H= *Myrtus communis*.

As for the dimensions of the stomata, the study results noted that the upper surface of M. communis leaf epidermis is devoid of stomata at the adaxial surface. The average dimensions of the stoma ranged between (20X21 - 30X26.25) µm in the two species, *C. macropunctatus* and *E. camaldulensis*, respectively.

On the lower surface of the leaf epidermis, the minimum average stomatal dimensions were (20.6X17.5) µm in M. communis and (26.25X21.25) µm in *E. camaldulensis*.

As for the average dimensions of epidermis cells, for the adaxial surface, the minimum limit was 29.75 X 21.87 µm in *C. macropunctatus*, while the maximum limit was 45 X 27.5 µm in *M. communis*. As for the abaxial surface, the average dimensions of the stomata ranged from 20.6 X 17 µm as a minimum in *M. communis* and 26.25 X 21.25 µm as a maximum in *E. camaldulensis*. The stomata index is higher in the *M. communis* leaf epidermis compared to the two species under study.

i) Transverse section of the Stem (Table 3). Regarding the general shape of the section, the semispherical shape was observed in the two species *C. macropunctatus* and *M. communis* and tetragonal in *E. camaldulensis* (Figure 3).

The outer layer of the section is composed of epidermal cells covered by a thin layer of cuticle, represented by a single row of cells for all the species studied. In terms of the type of hairs spread on the surface of the epidermal cells, the presence of non-glandular hairs of different lengths was observed on the stem epidermis of *C. macropunctatus* and, to a lesser extent, on the stem of *M. communis*. It was not observed in the stem epidermis of E. camaldulensis. This is a distinctive characteristic of separating *E. camaldulensis* (Figure 3A).

Under the epidermis, there is a cortex region consisting of lamellar collenchyma in the stem of *C. macropunctatus* of angular collenchyma and then parenchyma in the stem of *E. camaldulensis*, and parenchyma tissue in the stem of *M. communis*. Thus, it was possible to use this characteristic to separate species (Figure 3).

The study of the cross-sections of the stem showed the spread of various types of crystals within the cells of the cortex region. The results recorded the spread of prismatic crystals in the stem of *C. macropunctatus*. At the same time, rosette crystals spread within the cells of the cortex region, the pith region, and the vascular system of the stem of *E. camaldulensis* and *M. communis* (Figure 3D, G, I).

In the vascular system region, the vascular bundles were characterized as collateral in all species. This also had taxonomic importance in separating *M. communis* (Figure 3). The pith region is characterized by parenchymal cells with thickened and pitted walls. It has also been noted to contain storage cells, especially in *C. macropunctatus*.

Table 2. Variations in the quantitative and qualitative characteristics of the leaf epidermis of the species under study. Measured in micrometer µm.
Tabela 2. Variações nas características quantitativas e qualitativas da epiderme foliar das espécies em estudo. Medidas em micrômetro µm.

Species	stomata on the upper surface		stomata on the lower surface		Average (cells length x cells width)		The stomatal pattern	The nature of epidermis cell walls	
	Stoma Length	Stoma width	Stoma length	Stoma width	The upper surface	The lower surface		The upper surface	The lower surface
Calistemon Macropunctatus	(7.5-22.5) 20	(20-30) 21.7	(10-25) 22.5	(17.5-30.5) 17.5	29.75 X 21.87	25 X 22.5	Cyclocytic	Straight	Straight
Eucalyptus Camaldulensis	(27.5-	(22.5-30) 26.25	(22.5-30) 26.25	(17.5-25) 21.25	33.75 X 23.25	33.75 X 20	Anomocytic, Staurocytic	Straight	Straight
Myrtus. Communis	-	-	(17.5-23.75) 20.6	(15-20) 17.5	45 X 27.5	21.25 X 16.25	Anomocytic, Anisocytic, Tetracytic	Slightly Undulate	Slightly Undulate

The values inside the parentheses represent the minimum and maximum, and outside the parentheses represent the average.
Os valores dentro dos parênteses representam o mínimo e o máximo, e fora dos parênteses representam a média.

Table 3. Variations in the qualitative characteristics of the stem epidermis of the species under study.
Tabela 3. Variações nas características qualitativas da epiderme do caule das espécies em estudo.

Species	Section shape	Epidermis	Cortex	Vascular bundle	Pith
Calistemon macropunctatus	Semiovoid – semispherical	One row	Lamellar Collenchyma, Prismatic crystal	Bicollateral	Storage parenchyma , pitted
Eucalyptus camaldulensis	Polygon (quadrilateral)	One row	Angular Collenchyma + Parenchyma, Rosette crystal	Bicollateral	pitted cells
Myrtus. Communis	Semispherical - Polygon	One row	Parenchyma tissue, Rosette crystal	Collateral	Parenchyma tissue

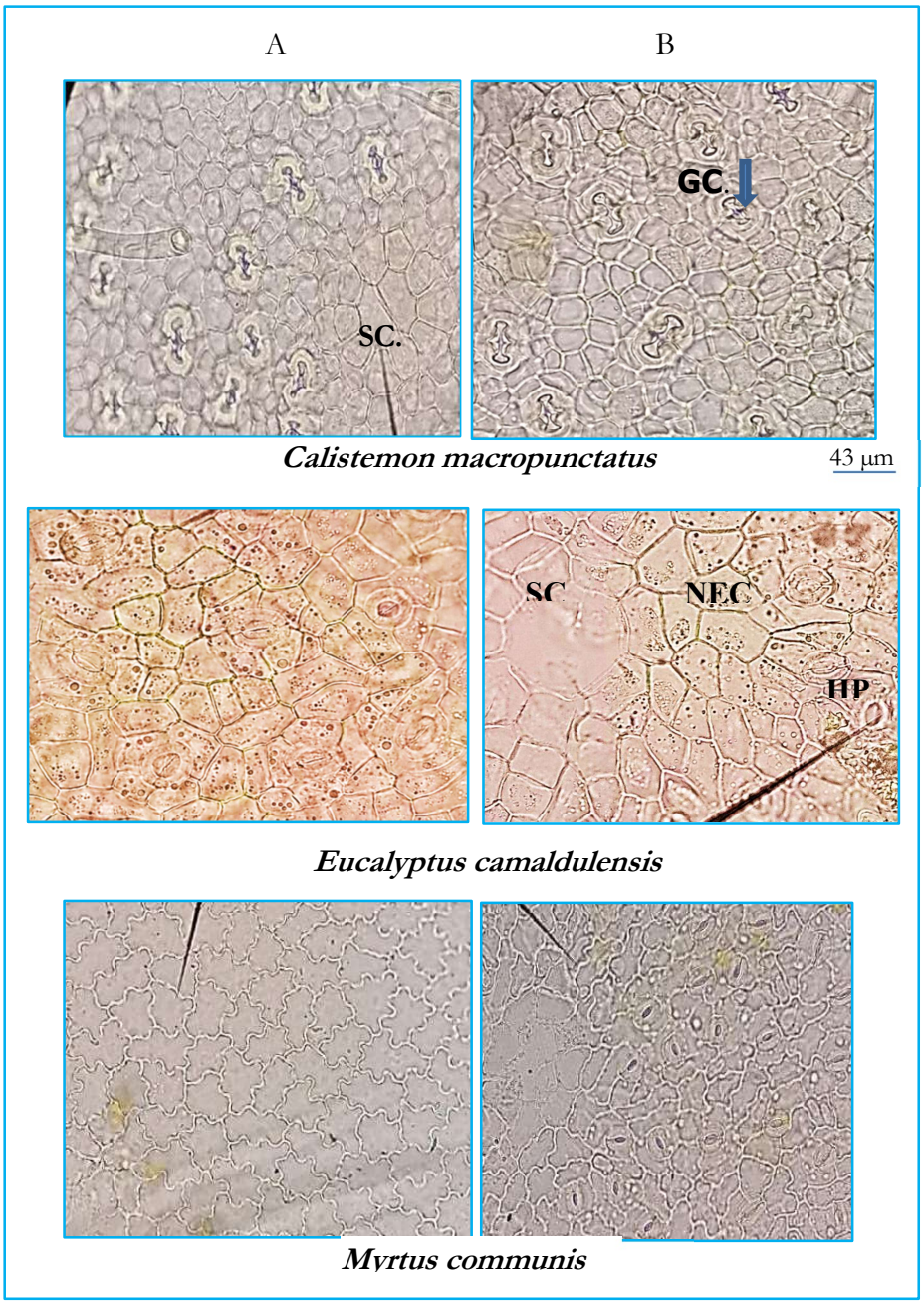

Figure 2. Changes in leaf epiderms characteristics of studied species. A: Upper Epidermis; B: Lower Epidermis. SC=Secretory cavity. HP=hair position. NEC=Normal epiderm cells. GC=Guard cell.
Figura 2. Alterações nas características da epiderme foliar das espécies estudadas. A: Epiderme superior; B: Epiderme inferior. SC=Cavidade secretora. HP=posição do pelo. NEC=Células normais da epiderme. GC=Célula guarda.

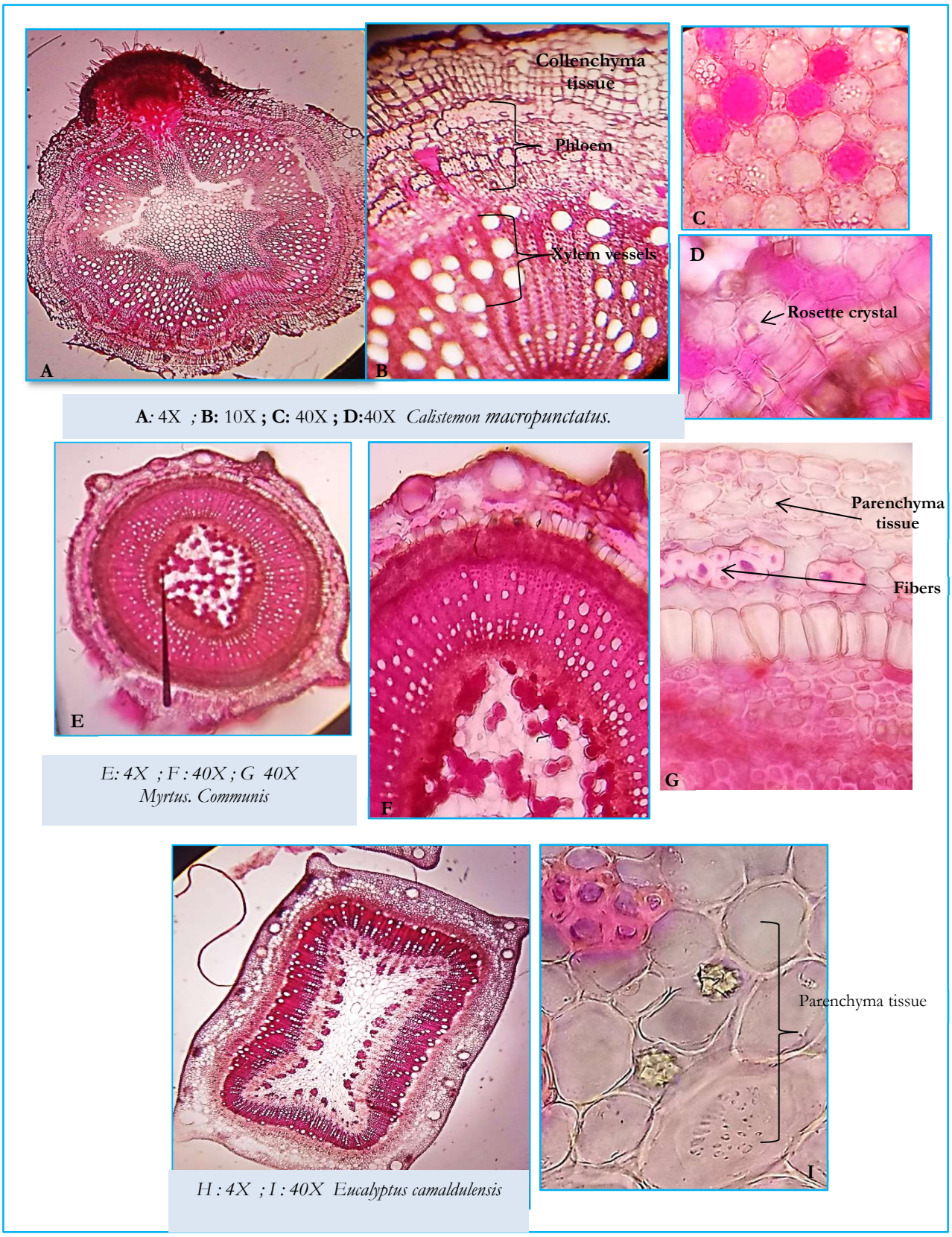

Figure 3. Variations in the cross-section characteristics of the stems of the studied species.
Figura 3. Variações nas características da seção transversal dos caules das espécies estudadas.

3.3. Chemotaxonomy study (Phytochemical)

The current research included a quantitative estimation of phenolic and flavonoid compounds in the leaves of the studied species (Table 4). The analysis noted that the leaves of M. communis have a high content of phenolic and flavonoid compounds, followed by the species *E. camaldulensis* and *C. macropunctatus*.

Table 4. Quantitative estimation of phenols and flavonoids in the studied species.
Tabela 4. Estimativa quantitativa de fenóis e flavonoides nas espécies estudadas.

No.	Components (µg g^{-1})	*Calistemon macropunctatus*	*Eucalyptus camaldulensis*	*Myrtus communis*
1.	Phenolics	12.31	57.53	123.44
2.	Flavonoids	21.06	15.23	35.81

3.4. Estimation of the antioxidant activity of leaf extract

The antioxidant activity of the extracts used in this study was estimated by determining the concentration of the extract that can reduce or inhibit the absorption of the free radical DPPH by half (IC_{50}) (Table 5). The 50% aqueous-methanol solvent was used to make a plant extract of *C. macropunctatus*, *E. camaldulensis* and *M. communis* leaves. Different concentrations were also taken: 200, 100, 50, 25, 12.5, 6.25, and 3.12 µg mL^{-1}.

The study recorded the highest rate of scavenging activity: 95 % in the *M. communis* leaves extract, 93 % in the *E. camaldulensis* leaves extract, and 90 % in the *C. macropunctatus* leaves extract, at 200 µg mL^{-1}. It has been observed that the scavenging activity increases with increasing concentration of the plant extract.

As shown in Table 6, the leaves extract of the studied species has high antioxidant activity properties compared to the effectiveness of Ascorbic acid, where the value of IC_{50} was 11.8 µg mL^{-1} in *M. communis*, 21.97 µg mL^{-1} in *E. camaldulensis* and 26.97 µg mL^{-1} in *C. macropunctatus* (Figures 4,5,6).

Table 5. Percentage of scavenging activity in the leaves extract of the studied species.
Tabela 5. Porcentagem de atividade sequestrante no extrato de folhas das espécies estudadas.

Conc. (µg mL^{-1})	Scavenging activity (%)			
	Calistemon macropunctatus	*Eucalyptus camaldulensis*	*Myrtus. communis*	*Ascorbic acid*
0.0	0	0	0	0.0
200	90.1	93.5	95.03	77.25
100	80.71	82.67	86.26	74.39
50	74.43	72.50	78.46	72.49
25	69.57	67.00	67.69	64.34
12.5	61.48	65.00	66.77	54.50
6.25	50.14	56.50	53.22	50.79
3.12	28.29	30.67	35.44	38.94

Table 6. Antioxidant Activity of plant extracts against Free-Radicals.
Tabela 6. Atividade antioxidante de extratos vegetais contra radicais livres.

Plant	Solvent	IC_{50} (µg mL^{-1})	Mark
Calistemon macropunctatus	Methanol	26.97	Strong
Eucalyptus camaldulensis		21.97	
Myrtus communis		11.08	

3.5. Estimation of the antibacterial activity of leaf extract

The current study showed that the extract of *M. communis* leaves showed stronger inhibitory activity against the bacterial strains under study and that *Klebsiella pneumonia* was more sensitive to the activity of the extract, as the diameter of the inhibition zone reached (19 µg mL^{-1}). While Staphylococcus aureus was more sensitive to the extract of *C. macropunctatus* and *E. camaldulensis* leaves, as the diameter of the inhibition zone reached 18 µg mL^{-1}. The current results also showed that the higher concentration effectively affected the inhibitory effect (Figure 8).

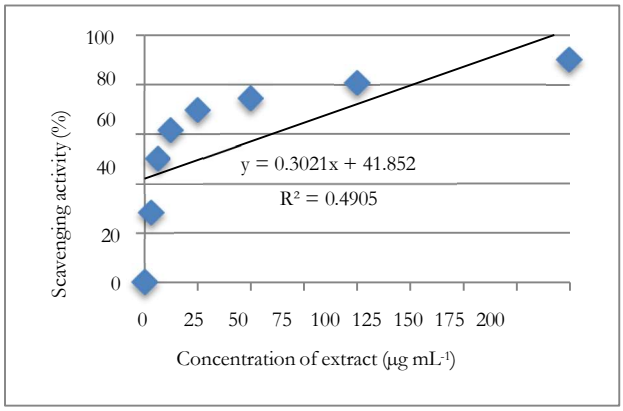

Figure 4. The radical scavenging activity (%) of *Calistemon macropunctatus*.
Figura 4. Atividade de eliminação de radicais (%) de *Calistemon macropunctatus*.

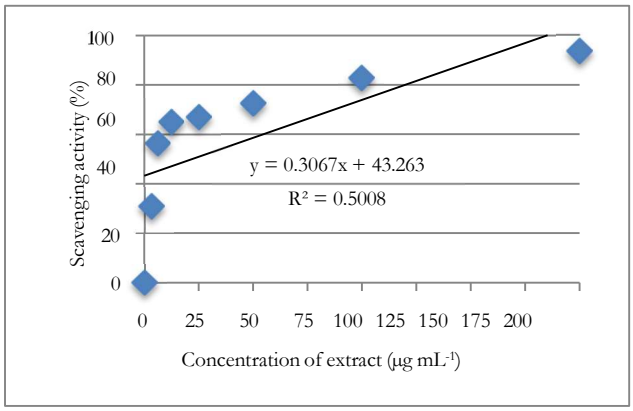

Figure 5. The radical scavenging activity (%) of *Eucalyptus camaldulensis*.
Figura 5. Atividade de eliminação de radicais (%) de *Eucalyptus camaldulensis*.

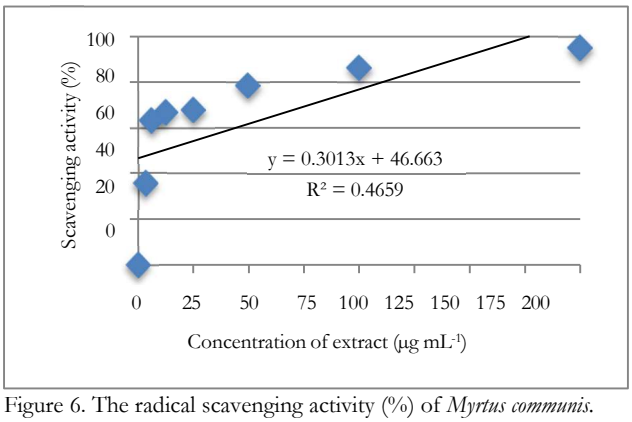

Figure 6. The radical scavenging activity (%) of *Myrtus communis*.
Figura 6. Atividade de eliminação de radicais (%) de *Myrtus communis*.

4. DISCUSSION

It was noted from the current results of the light microscope that there are limited variations in the characteristics of the pollen grains of the studied species in terms of the dimensions, shape of pollen grain in the polar and equatorial views, and other characteristics. It was

observed from the results of the current study that the pollen grains of the studied species were 3-colporate (the style characterized by the presence of three pores and three grooves arranged around the equator); these results are in agreement with a study of (NASCIMENTO; CARVALHO, 2019).

Table 7. Antibacterial activity of leaves extract of the studied species.
Tabela 7. Atividade antibacteriana do extrato das folhas das espécies estudadas.

Mater (A)	Concentration (µg mL^{-1}) (B)	*Klebsiella pneumoniae*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*
		Mean±S.D			
Control	Control	0	0	0	0
C. macropunctatus	1000	17±2.2	18±0.9	16±3.0	15±1.6
	500	12±1.3	13±1.4	12±1.3	11±1.7
	250	0	0	0	0
	125	0	0	0	0
E. camaldulensis	1000	17±2.2	18±0.9	17±2.2	16±3.0
	500	13±1.4	11±1.7	12±1.3	12±1.3
	250	9±1.1	0	0	0
	125	0	0	0	0
M. communis	1000	19±2.8	16±3.0	18±0.9	16±3.0
	500	14±2.0	12±1.3	13±1.4	12±1.3
	250	11±1.7	10±1.5	10±1.5	0
	125	0	0	0	0
LSD (0.05) for (A*B)		3.912	4.007	3.506	3.822

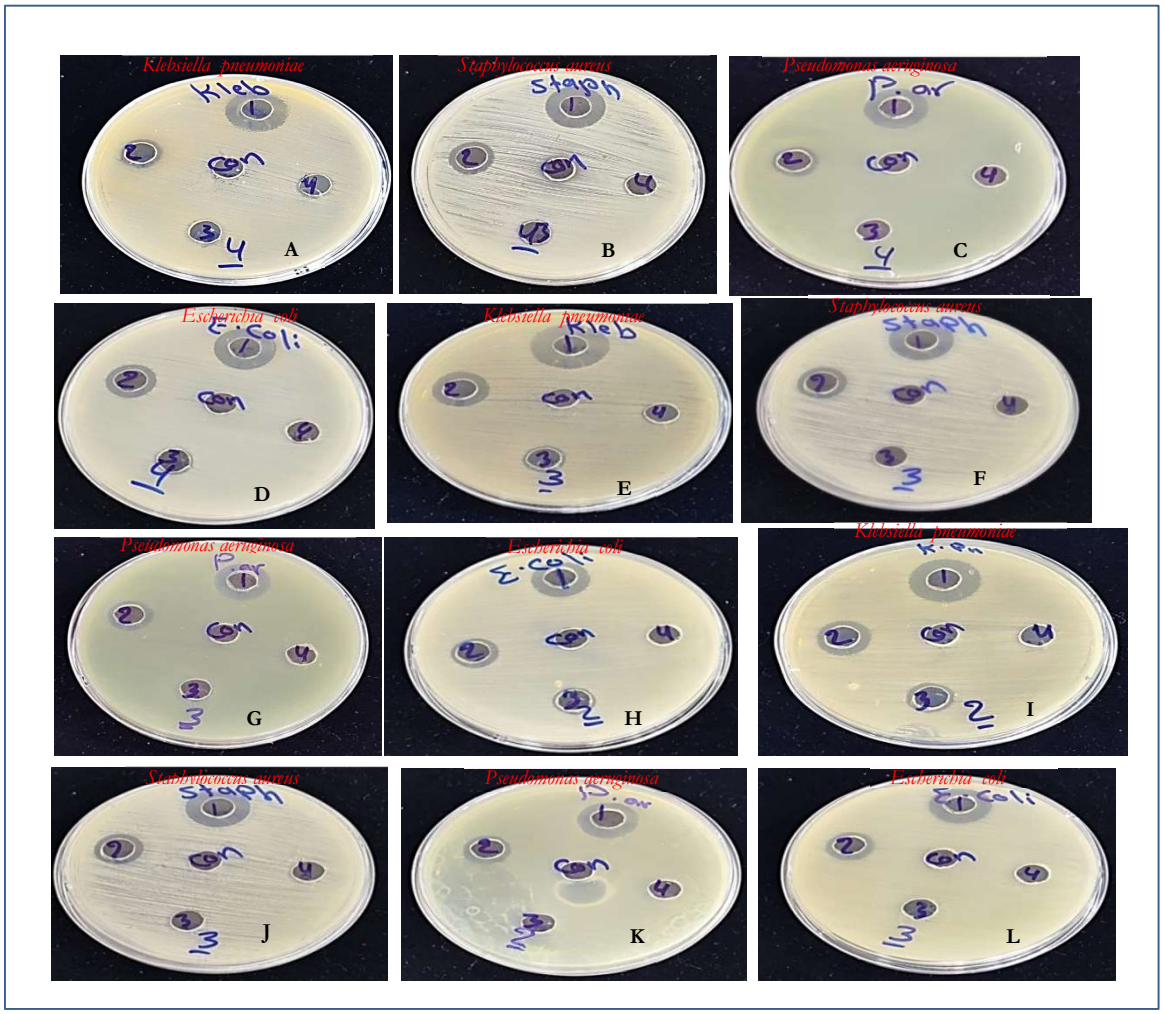

Figure 8. The inhibitory effect of the leaf extract of the studied species against some pathogenic bacteria. A-D= *Calistemon macropunctatus*; E-H = *Eucalyptus camaldulensis*; I-L= *Myrtus communis*.
Figura 8. Efeito inibitório do extrato foliar da espécie estudada contra algumas bactérias patogênicas. A-D= *Calistemon macropunctatus*; E-H = *Eucalyptus camaldulensis*; I-L= *Myrtus communis*.

In terms of pollen grain size, as shown in the results, the pollen grains of the studied species were classified within the small size group, according to Erdtman (1971). These results are consistent with the study of THORNHILL et al. (2012) and Nascimento; Carvalho (2019). The pollen grain size characteristic in the polar and equatorial view was of taxonomic importance in separating *M. communis* from *C. macropunctatus* and *E. camaldulensis*. The variation in pollen size is caused by either anomaly in meiosis or hybridization (MATSUDA, 1928; AYTUG et al., 1971). Also, the size of pollen grains is important after the characteristics of germinating pores and ornamentation of exine in the classification of genera and species (CHANDA; GHOSH, 2013).

Erdtman (1971) mentioned that pollen grains vary in shape. The results recorded the oblate-oblate spherical shape of the pollen grains of the studied species. These results agree with a study by Nascimento; Carvalho (2019).

The results of the current study confirmed the taxonomic importance of the pollen grain's characteristics, in addition to the phenotypic and anatomical study. Studying the phenotypic characteristics of the pollen grains is an effective attempt to find genetic evolutionary relationships between the tribes. It is even more important to separate several taxonomic ranks after using the scanning electron microscope (SEM) (PERVEEN, 1999).

Anatomical characteristics play an important role in interpreting the scientific facts used in plant taxonomy. They have been proven to be more important and useful in determining the highest taxonomic ranks, such as genera or families, as strong taxonomic evidence (METCALFE; CHALK, 1950; STACE, 1965; RADFORD et al., 1974).

Some good taxonomic characteristics were observed from the results of the current study in separating the species under study. As for the cell walls of the leaf epidermis, this characteristic was important in separating the species *M. communis*, as the cell walls were characterized by being slightly – undulate, while they were straight in *C. macropunctatus* and *E. camaldulensis*. These results are consistent with the study of Metcalfe; Chalk (1950). Stace (1965) explained that environmental conditions, including humidity, play an important role in determining the style of the anticlinal cell walls.

As for the stomata, their spread density on the leaf surface and the type of stomatal complexes, the results recorded the Hypostomatic type in the leaf of *M. communis*. The current results also showed types of stomatal complexes that have a role in separating the studied species. Thus, this trait had taxonomic importance in separating the studied species. These results are consistent with what Hosney et al. (2018) mentioned regarding the multiplicity of stomata patterns in the genera of the Myrtaceae family.

As for the dimensions of the stomata, at the adaxial surface, it was noted from the study results that the upper surface of M. communis leaf epidermis is devoid of stomata, which is a good taxonomic characteristic for separating the species. The average dimensions of epidermis cells reveal that the minimum was 21.25 X 16.25 µm in *M. communis,* and the maximum was 33.75 X 20 µm in *E. camaldulensis*. From the above, it was concluded that the characteristics of leaf epidermal cells have good taxonomic importance in separating the studied genera.

As for the cross-section of the stem, the study's results showed clear differences in the characteristics of the internal anatomy of the stems of the studied species. Regarding the general shape of the section, the tetragonal shape of the stem of *E. camaldulensis* was observed, while it was a semispherical shape in the two species, C. *macropunctatus* and *M. communis*.

As for the internal anatomy of the stem, from the outside, the section consists of epidermal cells covered by a thin cuticle layer, which is represented by a single row of the cells in all the species studied. This characteristic agrees with the results of some studies (AL-EDANY; AL-SAADI, 2012). The study also recorded the spread of unicellular non-glandular hairs of different lengths on the stem epidermis. Their spread was dense in *C. macropunctatus*, while they were less widespread on the stem epidermis of *M. communis* was not observed in the stem epidermis of *E. camaldulensis*. This is a distinctive characteristic of separating *E. camaldulensis*.

After the epidermis, there are the cells of the cortex. This region was characterized by types of plant tissues that had taxonomic importance in separating the studied species, as shown in the study results. The pith region is characterized by parenchymal cells with thickened and pitted walls. It has also been noted to contain storage cells, especially in *C. macropunctatus*.

As for the Chemotaxonomy aspect, the current research included a quantitative estimation of phenolic and flavonoid compounds in the leaves of the studied species. *M. communis* is an important medicinal plant because it is rich in biologically active secondary metabolites (YANGUI et al., 2021). Also, *M. communis* leaves are rich in phenolic acids and flavonoids. Several studies have confirmed the importance of *E. camaldulensis* leaves, which have several medicinal and biological activities due to the leaves being rich in active compounds, including eucalyptanoic acid, flavonoids, and acylated pentacyclic triterpenoids (GHAREEB et al., 2018). In addition, the essential oils in the leaves of *C. macropunctatus* are important, and their effective role as antimicrobial and anti-insect (GOYAL, 2012).

Moreover, based on the chemical compounds recorded during the current study, the antioxidant activity of the leaf extract was estimated as good classification evidence for comparison between the studied species. The results of the study showed that the aqueous-methanolic extract of the leaves of the studied species has a strong antioxidant effect compared to the effectiveness of ascorbic acid, where the value of IC_{50} was 11.8, 21.97 and 26.97 µg mL^{-1} in *M. communis*, *E. camaldulensis* and *C. macropunctatus*, respectively. These results may be due to a high percentage of fatty acids and phenolic, flavonoid, and terpene compounds, some of which were estimated during the research. These results are consistent with the study of Yanguia et al. (2021).

In addition, the results of the current study confirmed the effectiveness of the leaf extract of the studied species as an antibacterial against some pathogenic bacterial strains. The strongest effect was for the leaf extract of *M. communis*, and the bacteria *Klebsiella pneumonia* was more sensitive to this extract. Also, the extracts of the other studied species had an effective role in the inhibitory effect of the studied bacterial species. Several studies have praised the importance of the leaf extracts of the studied species and their strong effectiveness as an anti-inflammatory and antischistosomal activity (BESUFEKAD et al., 2017; GHAREEB et al., 2018).

This activity is attributed to several powerful phenolic compounds in the composition of the leaves.

5. CONCLUSIONS

The results of the current study proved the importance of the morphological characteristics of pollen grains and the anatomical characteristics of leaves and stems in classifying the studied species. In addition, the current results confirmed the effectiveness of the leaf extract of the studied species and its medical effectiveness as an antioxidant and an antibacterial against several pathogenic bacteria due to the effective chemical composition of the leaves.

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Data availability: The corresponding author can obtain study data by e-mail.

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