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Epidermal Structure and Phytochemical Screening of Palm Leaves (*Arecaceae*)

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Abstract

The epidermal structure and phytochemical composition of six palm species (*Arecaceae*) were studied to understand their anatomical characteristics and bioactive compound content. Leaf samples from the phoenix palm (*Phoenix roebelenii*), red palm (*Cyrtostachys renda*), foxtail palm (*Wodyetia bifurcata*), fan palm (*Livistona chinensis*), Christmas palm (*Adonidia merrillii*), and yellow palm (*Chrysalidocarpus lutescens*) were analyzed through microscopic observation and phytochemical screening. The results revealed variations in epidermal cell shape, cell wall structure, and stomatal distribution. Most species exhibited smooth-walled epidermal cells, except for *C. renda*, which had wavy cell walls. Stomata were predominantly tetracytic, with *C. renda* and *A. merrillii* displaying amphistomatic distribution, while the other species were hypostomatic. Phytochemical analysis revealed the presence of flavonoids and saponins in all samples. Triterpenoids were detected only in *P. roebelenii*, *L. chinensis*, and *C. lutescens*. Alkaloids were found in *P. roebelenii* and *C. renda*, while steroids were present in *C. renda*, *W. bifurcata*, and *A. merrillii*. Additionally, tannins were detected in all plant samples except *L. chinensis*. These findings suggest that palm leaves possess significant bioactive compounds that could be explored for pharmaceutical and industrial applications.

Keywords: *epidermal structure, phytochemical screening, palm leaves,* Arecaceae

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Introduction

Palms (*Arecaceae*) are a group of plants widely distributed across tropical regions (Eiserhardt et al., 2011). Species within this family play a crucial role in ecosystems and serve as sources of food, fiber, and raw materials for the pharmaceutical and cosmetic industries (Agostini-Costa, 2018; de Oliveira et al., 2016; Nuryanti et al., 2015). Various palm species are known to produce a broad range of phytochemical compounds, particularly secondary metabolites such as alkaloids, flavonoids, tannins, and saponins. These compounds exhibit significant biological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer properties (Adawiah, 2016; de Oliveira et al., 2016; Syamsurizal et al., 2023).

The presence of bioactive compounds in plants can be detected through phytochemical screening, a preliminary test to identify primary and secondary metabolites in plant extracts. Phytochemical screening can reveal the presence of various secondary metabolites such as alkaloids, flavonoids, tannins, saponins, sterols, terpenes, cardiac glycosides, proteins, carbohydrates, and lipids (Abubakar & Haque, 2020). These secondary metabolites are typically synthesized by secretory cells, including epidermal cells and their derivatives (Fahn, 2000; Ossola & Farmer, 2024; Sabila et al., 2022). As the outermost layer of plant tissue, the epidermis plays a key role in protecting against pathogens, herbivores, and abiotic stress. This protective function is partly attributed to trichomes, epidermal outgrowths that secrete secondary metabolites (Astiti et al., 2021; Zuch et al., 2022).

The epidermal structure of palms includes a waxy layer that helps reduce water evaporation. Additionally, trichomes serve as a defense mechanism against herbivorous insects. Studying the epidermal structures and conducting phytochemical screening of various palm species is essential to uncover their potential applications in health and industry. Moreover, the bioactive compounds produced by palms can serve as raw materials for the development of pharmaceuticals, healthcare products, and other related industries. Research on the epidermal structure and phytochemical composition of palms is expected to support the discovery and development of biologically active compounds for health-promoting products.

Research Methods

Location

The research was conducted from May to September 2024. Palm leaf samples were collected from the area surrounding Universitas Jambi. Observations and phytochemical tests were conducted in the Laboratory of Agroindustry, Medicinal Plants, and Biotechnology, Faculty of Science and Technology, Universitas Jambi.

Equipment and materials

The samples consisted of leaves from six palm species: phoenix palm (*Phoenix roebelenii*), red palm (*Cyrtostachys renda*), foxtail palm (*Wodyetia bifurcata*), fan palm (*Livistona chinensis*), christmas palm (*Adonidia merrillii*), and yellow palm (*Chrysalidocarpus lutescens*).

The materials for epidermal preparation included palm leaves, distilled water, and bleach (Bayclin) as a clarifying agent. Materials for phytochemical testing included palm leaves, methanol p.a., 2N sulfuric acid, concentrated sulfuric acid, Mayer's reagent, concentrated hydrochloric acid (HCl), magnesium powder, 10% $FeCl_3$ solution, distilled water, aluminum foil, and tissue paper.

Equipment used for observing the epidermis and stomata included a razor blade, glass slides, cover glasses, dropper

pipettes, a binocular light microscope, and a camera. Equipment for phytochemical testing included test tubes, beakers, dropper pipettes, spot plates, measuring cylinders, graduated pipettes, a 5 mL volumetric pipette, a rubber bulb, a funnel, a spatula, a hotplate, and an analytical balance.

Epidermal leaf preparation

Epidermal leaf preparation was performed using the fresh leaf sectioning method. Paradermal sections were made from adaxial and abaxial surfaces to observe epidermal structure. Each section was placed on a glass slide, one drop of bleach (Bayclin) was added, and the sample was left for 30 seconds. The bleach was then absorbed with tissue paper, followed by the addition of distilled water, and the section was covered with a cover glass. Observations were made using a binocular light microscope at magnifications ranging from 40× to 400×, and images were documented with a camera.

Plant leaf extraction

Palm leaf extraction was carried out using the maceration method with methanol as the solvent. Freshly collected leaf samples were cleaned and sliced thinly, with a total weight of 10 grams. The slices were soaked in 20 mL of methanol in a closed, dark glass container for 3 days. After the soaking period, the macerate was briefly heated on a hot plate and filtered to obtain the filtrate.

Phytochemical screening

Phytochemical screening of palm leaves was conducted based on the method by Adawiah (2016) with modifications as follows.

Identification of alkaloids

1 mL of methanolic palm leaf extract was placed into a test tube and mixed with 1 mL of 2N sulfuric acid. After shaking, the acid layer was separated. Then, 2–3 drops of Mayer's reagent were added. The formation of a white mist or precipitate indicates the presence of alkaloid compounds.

Identification of steroids and terpenoids

3–4 drops of methanolic palm leaf extract were placed on a spot plate, followed by 2 drops of concentrated sulfuric acid. A blue or green coloration indicated the presence of steroids. A pink, red, or dark red to brown coloration indicated the presence of triterpenoids. The appearance of both colors suggests that the sample contained both steroids and triterpenoids.

Identification of saponins

1 mL of methanolic palm leaf extract was placed into a test tube, followed by the addition of 3 mL of distilled water. The mixture was then boiled in a water bath. After boiling, the tube was cooled and shaken vigorously. The formation of stable foam that persisted for at least 30 seconds indicated the presence of saponins.

Identification of flavonoids

0.5 mL of methanolic palm leaf extract was placed into a test tube. Three drops of concentrated HCl were added, followed by a small amount of magnesium powder. The formation of a red, yellow, orange, or blue color indicated the presence of flavonoid compounds.

Identification of tannins

0.5 mL of methanolic palm leaf extract was placed into a test tube, followed by the addition of three drops of 10% $FeCl_3$ solution. The appearance of a green to blackish-green coloration indicated the presence of tannins.

Research Results and Discussion

Epidermal Structure and Stomata of Palm Leaves

The epidermis is the outermost tissue layer of plant organs, functioning to protect the underlying tissues. The shape,

size, and arrangement of epidermal cells vary among plant species (Rompas et al., 2011). Various epidermal cell structures were observed in six palm species (see **Table 1**). Identical epidermal cell shapes were found on the adaxial and abaxial surfaces of *P. roebelenii*, *C. renda*, and *L. chinensis*, characterized by rectangular and elongated cells. In contrast, *W. bifurcata*, *A. merrillii*, and *C. lutescens* exhibited different epidermal cell shapes between the adaxial and abaxial surfaces. The adaxial epidermis of these species displayed oblique hexangular cells, while the abaxial surface exhibited a combination of oblique hexangular and elongated rectangular cells in *W. bifurcata* and *A. merrillii*. In *C. lutescens*, the adaxial surface displayed both quadrangular and hexagonal cells, whereas the abaxial surface exhibited oblique hexangular and quadrangular cells

The epidermal cell walls of the six palm species were generally smooth, except *C. renda*, which had wavy cell walls. In *P. roebelenii* and *A. merrillii*, the adaxial epidermis ranged from smooth to slightly undulated (see **Figure 1**). Research by Horn et al. (2009) also showed that the shapes and cell wall structures of various palms in the *Arecaceae* family vary, including hexagonal, elongated hexagonal, elongated rectangular, and oblique hexangular forms, with cell walls ranging from smooth to wavy.

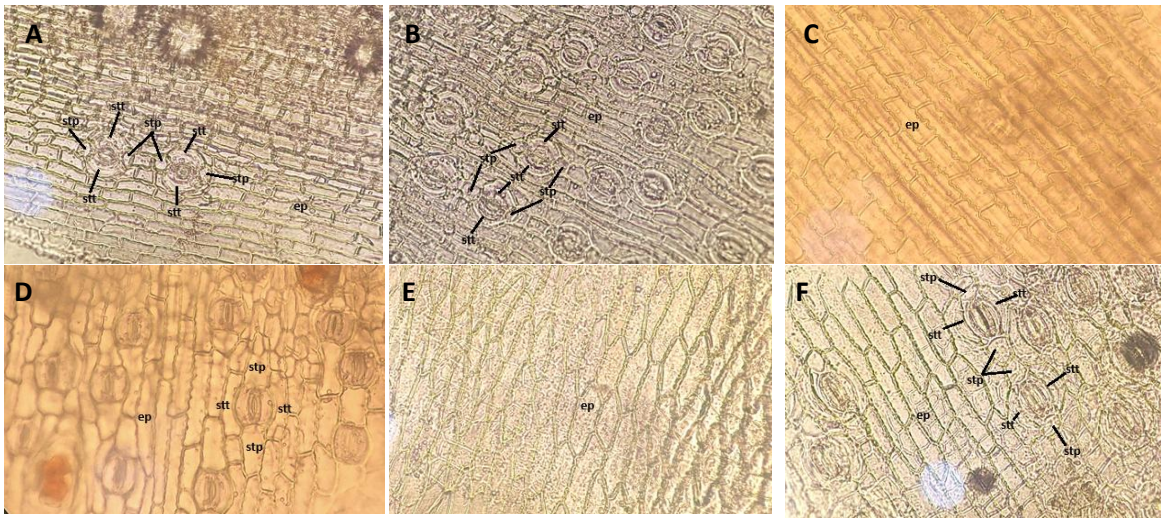
Table 1.
Epidermal structure and stomata of palm leaves

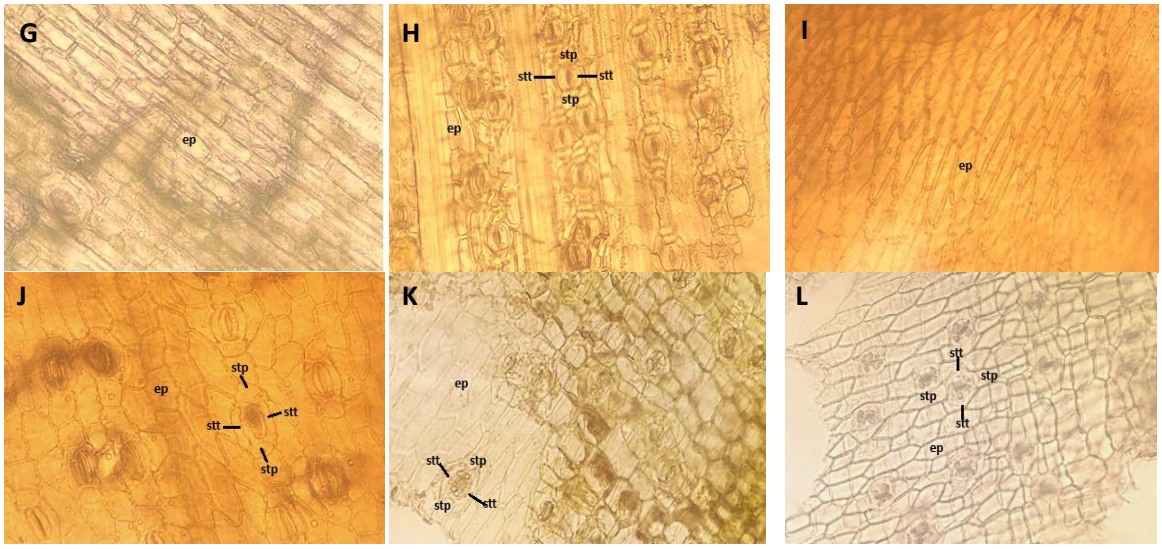
Species	Epidermal Cell Shape		Epidermal Cell Wall		Stomatal Type		Stomatal Density		Stomatal Distribution
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	
P. roebelenii	Rectangular, elongated	Rectangular, elongated	Smooth and undulated	Smooth	Tetracytic	Tetracytic	Sparse	Abundant	Amphistomatic
C. renda	Rectangular, elongated	Rectangular, elongated	Undulated	Undulated	-	Tetracytic	-	Abundant	Hypostomatic
W. bifurcata	Oblique hexangular	Oblique hexangular and rectangular, elongated	Smooth	Smooth	Tetracytic	Tetracytic	Sparse	Abundant	Amphistomatic
L. chinensis	Rectangular, elongated	Rectangular, elongated	Smooth	Smooth	Tetracytic	Tetracytic	Sparse	Abundant	Amphistomatic
A. merrillii	Oblique hexangular	Oblique hexangular and rectangular, elongated	Smooth	Smooth, undulated	-	Tetracytic	-	Abundant	Hypostomatic
C. lutescens	Rectangular and hexagonal	Oblique hexangular and rectangular	Smooth	Smooth	Tetracytic	Tetracytic	Sparse	Abundant	Amphistomatic

The epidermis may develop specialized structures such as trichomes and stomata, which function as adaptations to environmental conditions (Javelle et al., 2011). Stomata are small pores on the leaf surface, flanked by a pair of guard cells, and serve to regulate gas exchange, particularly the exchange of water vapor and CO_2 (Hetherington & Woodward, 2003). While stomata are commonly located on the lower (abaxial) surface of leaves, referred to as hypostomatic, some plant species have stomata on both surfaces, a condition known as amphistomatic (He & Liang, 2018; Rompas et al., 2011).

The distribution of stomata among the six investigated palm species varied. In *C. renda* and *A. merrillii*, stomata were present on both adaxial and abaxial surfaces, categorizing them as amphistomatic. However, stomatal density was lower (sparse) on the adaxial surface and higher on the abaxial surface. Conversely, *P. roebelenii*, *W. bifurcata*, *L. chinensis*, and *C. lutescens* exhibited stomata exclusively on the abaxial surface, indicating a hypostomatic condition. The greater stomatal density on the abaxial side is a common adaptation in terrestrial plants to reduce water loss and respond to environmental stress (Juniza & Chatri, 2021). Additionally, variation in stomatal distribution, arrangement, size, and frequency can occur not only between species but also among genotypes within the same species (He & Liang, 2018).

Figure 1. Upper and lower epidermis of palm leaves at 100x magnification: (A) Upper epidermis of P. roebelenii*; (B) Lower epidermis of* P. roebelenii*; (C) Upper epidermis of* C. renda*; (D) Lower epidermis of* C. renda*; (E) Upper epidermis of* W. bifurcata*; (F) Lower epidermis of* W. bifurcata*; (G) Upper epidermis of* L. chinensis*; (H) Lower epidermis of* L. chinensis*; (I) Upper epidermis of* A. merrillii*; (J) Lower epidermis of* A. merrillii*; (K) Upper epidermis of* C. lutescens*; (L) Lower epidermis of* C. lutescens*.*
Legend: ***ep****: epidermal cell;* ***stp****: polar subsidiary cell;* ***stt****: terminal subsidiary cell*





The type of stomata in plants can be identified based on the arrangement of subsidiary cells surrounding the guard cells. In the six palm species studied, all exhibited tetracytic stomata, which were defined by the presence of four subsidiary cells, two polar and two terminal, arranged perpendicularly and parallel to the stomatal pore, respectively (Megia et al., 2015). The subsidiary cells consist of polar subsidiary cells (stp) and terminal subsidiary cells (stt). Polar subsidiary cells are typically smaller and more compact than terminal subsidiary cells, which tend to be larger and elongated. However, in *C. renda*, the polar subsidiary cells are similar in size to the terminal subsidiary cells, representing a morphological variation within the tetracytic type.

Phytochemical Screening Analysis

Phytochemical screening was conducted on methanolic extracts of various palm species to determine the presence of secondary metabolite compounds in *simplicia* (dried plant material). The screening utilized specific reagents to detect the presence or absence of key classes of secondary metabolites, including steroids, triterpenoids, flavonoids, alkaloids, tannins, and saponins. The results of this screening are presented in **Table 2**.

Table 2

Phytochemical screening results of methanolic extracts from palm species

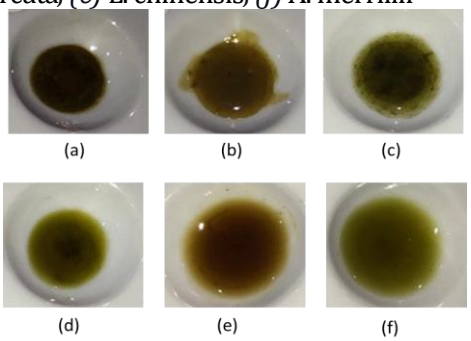
Code	Species	Class of Compounds					
		Flavonoid	**Triterpenoid**	**Alkaloid**	**Saponin**	**Steroid**	**Tannin**
A	*P. roebelenii*	+	+	+	+	-	+
B	*C. lutescens*	+	+	-	+	-	+
C	*C. renda*	+	-	+	+	+	+
D	*W. bifurcata*	+	-	-	+	+	+
E	*L. chinensis*	+	+	-	+	-	-
F	*A. merrillii*	+	-	-	+	+	+

Description: (+) = Presence of compound; (–) = Absence of compound

Steroid and Triterpenoid

The phytochemical tests for steroids and triterpenoids yielded a positive result based on a distinct color change in the test solution: a green or blue coloration indicated the presence of steroids, while a red or pink color signified the presence of triterpenoids. These reactions occured due to the interaction of the test compounds with 98% sulfuric acid and acetic anhydride, leading to the development of characteristic colors (Sangi et al., 2008). Hence, the results of the tests on palm extracts from the *Arecaceae* family indicated positive outcomes for triterpenoid and steroid compounds (see **Figure 2**).

Figure 2. Steroid and triterpenoid test results: (a) P. roebelenii*, (b)* C. lutescens*, (c)* C. renda*, (d)* W. bifurcata*, (e)* L. chinensis*, (f)* A. merrillii

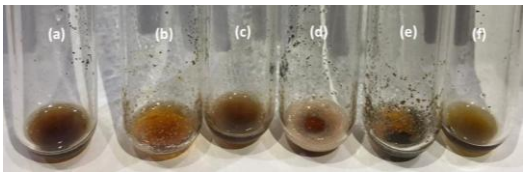


In this study, *C. renda*, *W. bifurcata*, and *A. merrillii* showed positive test results for steroids, indicated by a green coloration of the test solution. Conversely, *P. roebelenii*, *C. lutescens*, and *L. chinensis* tested positive for triterpenoids, as shown by the red color that developed in the solution. These color changes confirm the presence of these bioactive compounds and suggest chemical diversity in secondary metabolites among different palm species.

Flavonoids

Flavonoids are a class of secondary metabolites commonly found in plants, functioning as pigments, insect deterrents, growth regulators, and modulators of physiological processes such as respiration and photosynthesis. Beyond their ecological roles, flavonoids possess significant bioactivity, including antibacterial, antifungal, and antiviral properties (Cushnie & Lamb, 2005).

Figure 3. Flavonoid test results: (a) P. roebelenii*, (b)* C. lutescens*, (c)* C. renda*, (d)* W. bifurcata*, (e)* L. chinensis*, (f)* A. merrillii

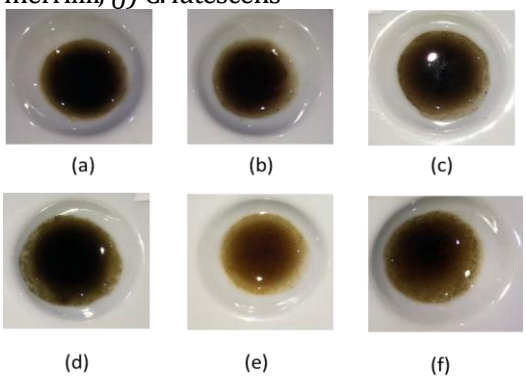


Based on the phytochemical screening results, all six palm species exhibited a positive reaction for flavonoids, indicated by the formation of a red to orange color in the test solution (see **Figure 3**). This color change resulted from the reduction of hydrochloric acid by magnesium metal, which reacted with the flavonoid structure and produced the observed coloration (Sangi et al., 2008).

Tannins

Tannin content was analyzed using a 10% ferric chloride ($FeCl_3$) solution. A positive test for tannins was marked by a color change of the solution to blackish green, which occured due to the interaction between $FeCl_3$ and the hydroxyl groups of phenolic compounds (Sangi et al., 2008). The analysis results showed that tannin compounds were present in five out of six palm species, except L. chinensis, which tested negative (see **Figure 4**). The presence of tannins in most samples suggests potential antioxidant and antimicrobial properties, as tannins are known to play important defensive roles in plants and have been widely studied for their medicinal potential.

Figure 4. Tannin test results: (a) P. roebelenii*, (b)* C. renda*, (c)* W. bifurcata*, (d)* L. chinensis*, (e)* A. merrillii*, (f)* C. lutescens

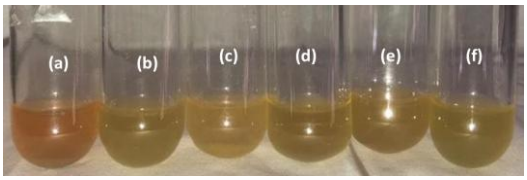


Alkaloid

Alkaloids are nitrogen-containing secondary metabolites commonly found in various parts of plants, including the roots,

stems, flowers, seeds, and leaves. Alkaloids serve multiple ecological functions in plants, such as natural toxins against herbivores and insects, growth regulators, and nitrogen storage compounds (Ningrum et al., 2016).

Figure 5. Alkaloid test results: (a) P. roebelenii*, (b)* C. lutescens*, (c)* C. renda*, (d)* W. bifurcata*, (e)* L. chinensis*, (f)* A. merrillii



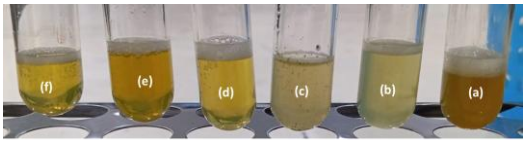
The alkaloid screening using Mayer's reagent on the six palm species showed positive results for *P. roebelenii* and *C. renda*, indicated by the formation of white precipitates (see **Figure 5**). This result is consistent with the findings of Sangi et al. (2008), who explain that the precipitation reaction occurs due to ligand substitution: the free electron pair on the nitrogen atom in the alkaloid molecule displaces iodide ions in Mayer's reagent. Mayer's reagent contains potassium iodide and mercury chloride or potassium tetraiodomercurate(II), which reacts with alkaloids to form an insoluble complex, resulting in visible precipitation.

Saponins

Saponins are glycoside compounds composed of a sugar moiety (glycosyl group), often containing pentose sugars, bonded to a sapogenin aglycone, which may be a steroid or triterpenoid. The glycosyl group is polar, while the sapogenin component is non-polar, making saponins naturally surface-active. When shaken, these molecules form micelles, with the polar groups facing outward and the non-polar groups inward, facilitating the formation of foam (Sangi et al., 2008).

The presence of saponins in plant extracts is indicated by the formation of stable, firm foam after shaking and heating in a water bath. For a positive test, the foam should persist for at least 30 minutes (Junito et al., 2018; Sari et al., 2021). In this study, all six palm species tested positive for saponins, as evidenced by the stable foam formed in each sample (see **Figure 6**). This finding aligns with the results of Adawiah (2016), who also observed foam formation in palm extracts after vigorous shaking.

Figure 6. Saponin test results: (a) P. roebelenii*, (b)* C. lutescens*, (c)* C. renda*, (d)* W. bifurcata*, (e)* L. chinensis*, (f)* A. merrillii



Conclusion

The epidermal tissue of the six palm species (*Phoenix roebelenii*, *Cyrtostachys renda*, *Wodyetia bifurcata*, *Livistona chinensis*, *Adonidia merrillii*, and *Chrysalidocarpus lutescens*) exhibited distinct variations in cell shape, cell wall structure, and stomatal distribution. While some species displayed similar epidermal cell shapes on both adaxial and abaxial leaf surfaces, others showed notable differences. Most species had smooth cell walls, except for *C. renda*, which exhibited wavy cell walls. Stomatal distribution also varied: *C. renda* and *A. merrillii* were amphistomatic, whereas the remaining species were hypostomatic. The dominant stomatal type across all samples was tetracytic, characterized by four subsidiary cells surrounding each pair of guard cells.

Phytochemical analysis revealed the presence of flavonoids and saponins in all species. Triterpenoids were detected exclusively in *P. roebelenii*, *L. chinensis*, and *C. lutescens*. Alkaloids were identified in *P. roebelenii* and *C. renda*, while steroids were present in *C. renda*, *W. bifurcata*, and *A. merrillii*. Additionally, tannins were found in all samples except for *L. chinensis*.

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