

Research Article

Leaf Anatomical Structure of Five True Mangrove Species in Pemogan Village, Denpasar – Bali

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ABSTRACT

Anatomy is an approach to study physiological processes that occurs in plants. Even though the mangrove community is known as one of the communities with the largest carbon stock, data regarding the anatomical structure of its leaves is still insufficient. Therefore, this research aims to determine the anatomical structure of leaves in several mangrove species and to compare those structures. Samples of mangrove species were obtained from Simbar Segara Batu Lumbang mangrove area in Pemogan Village, Denpasar City, Bali. The species are used i.e. (1) *Lumnitzera racemosa* (Combretaceae) in landward zone; (2) *Xylocarpus granatum* (Meliaceae) in landward zone; (3) *Aegiceras corniculatum* (Primulaceae) in middle zone; (4) *Rhizophora apiculata* (Rhizophoraceae) in middle zone; and (5) *Sonneratia alba* (Lythraceae) in seaward zone. Anatomical data was obtained by the free hand section method through the midrib and a paradermal method. The data was analysed descriptively, then continued with principal component analysis (PCA). Based on the results, *L. racemosa* and *S. alba* are species with amphistomatic (the other species were hypostomatic) and isobilateral leaves (the others were dorsiventral). These two characters are the dominant in PCA. Epidermal tissue, palisade mesophyll, sponge mesophyll, vascular bundles, and calcium oxalate crystals are evenly found in all species. *Rhizophora apiculata*, *A. corniculatum*, and *X. granatum* have hypodermal tissue as a dominant character in PCA. Secretory tissue and salt glands are only observed in *A. corniculatum*. The five species have three different stomata types, namely tetracytic (*L. racemosa*), anomocytic (*X. granatum*, *A. corniculatum*, *R. apiculata*), and staurocytic (*S. alba*).

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INTRODUCTION

Mangroves are plant communities that play an important role in mitigating climate change with their ability to absorb and to store large amounts of carbon. The average amount of carbon stock in the world's mangrove ecosystem is estimated around $115 \pm 7 \text{ Mg C ha}^{-1}$ as above ground carbon stock and $741 \pm 29 \text{ Mg C ha}^{-1}$ as below ground carbon stock (Kauffman et al. 2020). The amount of carbon stock in mangroves involves two main mechanisms, namely sequestrations through photosynthesis and sediment trapping by their root system. Specifically, in photosynthesis, the anatomical structure of mangrove leaves plays an important role in this process. The two main tissues in mangrove leaves that play a role in photosynthesis are palisade and spongy mesophyll which are differentiations of parenchymal tissues to carry out the assimilation process. Despite having these tissues, the assimilation process in mangroves requires additional structures to maintain their physiological conditions within the optimal range (Tatongjai et al. 2021). These additional structures are an essential mechanical adaptation to environmental stresses in the mangrove habitat (Tihurua et al. 2023).

Mangroves have a specific habitat which is in intertidal areas affected by sea tides. This habitat is difficult for the other plant species to grow because it has various environmental stresses, such as rapid wind movement, high water tides, periodic inundation, high light intensity, and fluctuations in water salinity. Related to the photosynthesis process in leaf, adaptation to these stresses can be observed at the anatomical level (Filartiga et al. 2022; Strock et al. 2022). Several anatomical characteristics of leaves related to the adaptation to environmental stresses in mangroves are the presence of thick hypodermal tissue, salt glands, secretory tissue, sclereids, and the arrangement of tissue in the leaves including their shape and thickness (Sánchez et al. 2021; Tatongjai et al. 2021; Tobing et al. 2022; Tihurua et al. 2023).

Not all mangrove species have similar tissue structure, because they do not always have the same niche. Mangrove niches can be simply divided based on zones, such as the landward zone, the middle zone, and the seaward zone. Each zone has different environmental conditions that tend to be gradual from landward to seaward (particularly the sediment from muddy to sandy and from low to high salinity), so that the species composition will change and form a unique ecotone (Wang et al. 2019; Irawan et al. 2021). In Bali, Kitamura et al. (1997) categorised mangrove species based on major distribution in landward, middle, and seaward zone. The landward zone commonly features *Bruguiera gymnorhiza*, *Ceriops tagal*, *Excoecaria agallocha*, *Lumnitzera racemosa*, *Sonneratia caseolaris*, and *Xylocarpus granatum*. In the middle zone, *Aegiceras corniculatum*, *Rhizophora mucronata*, *R. apiculata*, and *R. stylosa* are present. Conversely, *Sonneratia alba*, *Avicennia marina*, and *A. alba* are typically found in the seaward zone. Under certain conditions such as the sea sedimentation that affect the salinity fluctuation, several species can overlap and be found in other zones, such as *R. apiculata*, *S. alba*, *A. marina*, and *A. alba* which can be spread across various zones.

The mangrove area of Simbar Segara Batu Lumbang is located in Pemogan Village, Denpasar City and has all three mangrove zones. The mangrove area is relatively sustained because of the community whose actively conserves the mangroves with various activities and cleans up the trash that floated from the sea. Because this area reflects natural mangrove conditions, it is such an appropriate location for foundational research on mangrove ecosystems, particularly for investigating the anatomy of mangrove leaves. Study on the leaf anatomy of mangrove species in Bali is still insufficient, even though this anatomical structure is closely related to the physiological processes (Filartiga et al. 2022; Strock et al. 2022), especially in carbon absorption and adaptation to the environment that become a distinct ecosystem service in

mangroves. Therefore, as an initial step in compiling the database, this study aims to determine the anatomical structure of mangrove leaves in different zones in the Simbar Segara Batu Lumbang mangrove area of Pemogan Village, Denpasar, Bali.

MATERIALS AND METHODS

Study Site

The study was conducted in January–May 2023 in the Batu Lumbang area located in Pemogan Village, Denpasar. The Batu Lumbang area is utilised as one of the tourist attractions managed by the Simbar Segara Batu Lumbang fisherman group, where around 81 % of the area is mangrove forest (Mursyid et al. 2022). This study used an exploration method (Rugayah et al. 2004) by exploring the mangrove area in the Batu Lumbang area. The research location is shown in Figure 1 with a red line indicating the exploration route for sampling (south towards the sea) with more than 5 km. The exploration started at the coordinate S: -8.719185° , E: 115.201130° and ended at S: -8.733596° , E: 115.197620° . The landward zone of mangrove explored by foot, but for the middle zone to seaward zone we used canoe to reach the representative locations.

Sample Collections

The exploration in the mangrove area of Batu Lumbang obtained ten true mangrove species: *Aegiceras corniculatum*, *Avicennia marina*, *Bruguiera gymnorhiza*, *Ceriops tagal*, *Excoecaria agallocha*, *Lumnitzera racemosa*, *Rhizophora apiculata*, *R. mucronata*, *Sonneratia alba*, and *Xylocarpus granatum*. The identification was using Flora of Java volume 1 (Backer & van den Brink 1963) and mangrove database from Handbook of Mangrove in Indonesia: Bali and Lombok (Kitamura et al. 1997). Among those species, five species that resembled different families were chosen by prioritising their natural distribution zone (Table 1). Landward zone composed by *X. granatum* and *L. racemosa*, middle zone composed by *A. corniculatum* and *R. apiculata*, while the seaward zone represented by *S. alba*. Three leaves from three different individual were collected with the following criteria: (1) the leaves have fully bloomed; (2) the



Figure 1. Mangrove exploration routes in Batu Lumbang, Denpasar.

Table 1. Taxonomical data of sampled mangrove species. The accepted name obtained from POWO (<https://powo.science.kew.org/>), while the vernacular name refers Kitamura et al. (1997).

No	Family	Species	Vernacular Name	Coordinate sample
1.	Combretaceae	<i>Lumnitzera racemosa</i> Willd. Synonym: -	Saman-sigi, kedukduk, truntun, susup, lasi.	-8.722494°, 115.200861°
2.	Meliaceae	<i>Xylocarpus granatum</i> J.Koenig Synonym: <i>Carapa granatum</i> (J.Koenig) Alston; <i>Carapa indica</i> A.Juss.	Banang-banag, siri, nyirih, niri.	-8.720863°, 115.200865°
3.	Primulaceae	<i>Aegiceras corniculatum</i> (L.) Blanco Synonym: <i>Umbraculum corniculatum</i> (L.) Kuntze; <i>Rhizophora corniculata</i> L.	Kacangan, kacang-kacang, gedangan, klungkum.	-8.724433°, 115.200498°
4.	Rhizophoraceae	<i>Rhizophora apiculata</i> Blume Synonym: -	Bakau, bakau putih, mangi-mangi.	-8.727390°, 115.200151°
5.	Lythraceae	<i>Sonneratia alba</i> Sm. Synonym: <i>Blatti alba</i> (Sm.) Kuntze	Prapat, bogem, pupat, prepat.	-8.729688°, 115.200921°

colour were dark green or darker than the young leaves; (3) in the fourth position from the shoot; and (4) there are no pest or disease infections. The collected leaves were cleaned in running water to remove dirt that stuck to the surface of the leaves. The leaves were then dried with tissue, put it into a sample bottle, and poured with 70 % alcohol until all parts of the leaves were completely submerged.

Anatomical Data Collections

Two methods were used for anatomical observation. First is the transversal section method through the midrib to observed the layers of leaf structure, and the second is paradermal section method and paradermal replica to observe the structure of the leaf epidermis. The transversal section used the free-hand section method according to Lux et al. (2015) with some modifications in clearing solution (using 5 % sodium NaClO) and staining solution (using 1 % safranin and 1 % fast green). The leaves were removed from the sample bottle, then dried with tissue. To facilitate cutting, the sample was clamped between carrots, then cut as thinly as possible using a cutter. The cut results were re-immersed in 70 % alcohol for 5 minutes, then the sample transferred to 5 % NaClO solution and soaked for 2-5 minutes. After the sample was transparent, it was then washed in 70 % alcohol for 2 minutes. The sample stained with 1 % safranin for 1 minute, then rinsed with 70 % alcohol for 2 minutes and continued with 1 % fastgreen staining for 1 minute. After staining, the sample were rinsed with 70 % alcohol for 1 minute, then observed using through microscope that equipped with Optilab. Scaling was done using Image Raster software.

Paradermal sections were made using the free hand section method also from samples that had previously been stored in 70 % alcohol, using modified method from Lux et al. (2015). The samples were removed from the bottle, then dried using tissue and then incisions were made on the upper and lower surfaces of the leaves. The incisions (samples) were soaked in 5 % NaClO solution for 5-10 minutes. The NaClO solution was then replaced with 70 % alcohol and soaked for 5 minutes. The samples were then stained with 1 % safranin for 3 minutes and then rinsed again with 70 % alcohol. The samples were then placed on a glass object and added with one drop of aquadest and then covered with a cover glass. Another method, namely a paradermal replica, was made by applying clear nail polish to the surface of the leaves that had previously been cleaned with tissue (without cutting). The nail polish that had been applied was waited for 10 minutes to dry. After that, clear tape was attached to the nail polished part, then the tape was pulled and attached to the glass object. The preparations produced from both methods were then ob-

served on a microscope that equipped with an Optilab device, while the scaling and cell counting were carried out using Image Raster software. The paradermal replica was easier than paradermal sections, but not all species were suitable for this method. Three repetitions were used in every samples. The observed characters consisted of stomatal type and stomatal index calculated using the formula retrieved from Poole and Kurschner (1999):

$$\text{Stomatal Index (SI)} = \frac{\Sigma S}{\Sigma S + \Sigma E} \times 100 \%$$

Annotations:

ΣS = Number of stomata in one field of view

ΣE = Number of epidermal cells in one field of view

Data Analysis

The data obtained were analysed descriptively to determine the anatomical characteristics of the mangrove leaves. The data were presented as leaf anatomy images and comparison tables. The comparison table summarises all observable characters, both synthetic characters and diagnostic characters of the mangrove species. In addition, further analysis was carried out in the form of Principal Component Analysis (PCA) to determine the characters that are unique to the anatomy of mangrove leaves. Character scoring uses a numerical taxonomic approach by considering the Adansonian principle according to Sokal and Sneath (1963). All the numerical analysis conducted in Multi-Variate Statistical Package (MVSP v.3.13.) software by Kovach Computing Services.

RESULTS

Leaf anatomical structure of mangrove in Batu Lumbang

Lumnitzera racemosa

Lumnitzera racemosa leaves are succulent leaves with a not very prominent leaf vein. Based on a transverse section through the midrib (Figure 2a), the observed tissues consist of upper epidermal, upper palisade mesophyll, spongy mesophyll, vascular bundles, lower palisade mesophyll, and lower epidermal, while the non-protoplasmic components observed are drusen-type of calcium oxalate crystals. The presence of upper and lower palisade mesophylls indicates that this leaf is an isobilateral type. Although both palisade mesophyll tissues consist of two layers of cells, the cells in the upper palisade mesophyll are longer than the lower. The vascular bundle was in arc shape with collateral position.

The characteristics of the stomata are shown in Figure 2b1 and 2b2. The stomata presence on the upper (adaxial) and lower (abaxial) sides of the leaf indicate that *L. racemosa* has amphistomatic leaves. The type of stomata is determined by the shape and position of the subsidiary cells that flank the guard cells. The type of stomata in *L. racemosa* is tetracytic, because the guard cells are surrounded by four subsidiary cells (two in lateral and two in poles of guard cells). The SI value in *L. racemosa* is $9.57 \pm 1.38 \%$ on the adaxial surface and $6.42 \pm 0.91 \%$ on the abaxial surface.

Xylocarpus granatum

Transversal section of *X. granatum* (Figure 3a) shows the leaf structure consisting of upper epidermal, 2-3 layers of hypodermal, 2-3 layers of palisade mesophyll, spongy mesophyll, vascular bundles, and lower epidermal, while the non-protoplasmic components observed are drusen-type of calcium oxalate crystals. The vascular bundle has an arc shape, have a small addition vascular bundle above it, and surrounded by lignified cells, the same type as reported by Tihurua et al. (2023). Based on the characteristics of the stomata in Figure 3b1 and 3b2, the type of stomata in *X. granatum* is anomocytic which

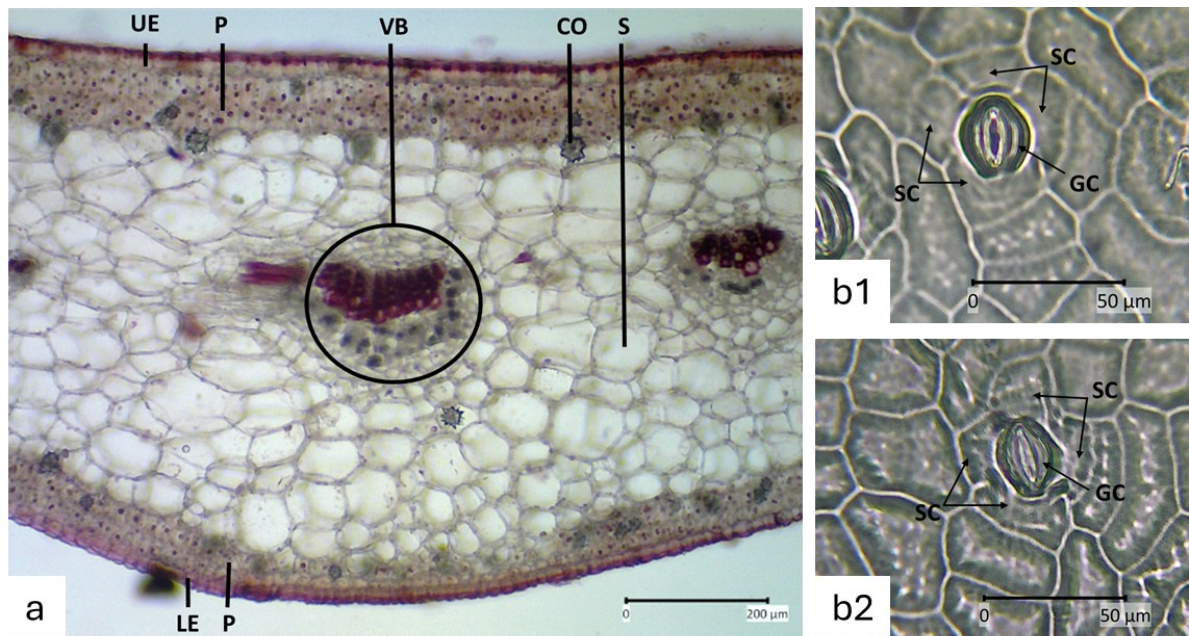


Figure 2. Leaf anatomical structure of *L. racemosa* (a) transversal section through the midrib, (b1) replica paradermal of the adaxial surface, (b2) replica paradermal of the abaxial surface. Annotations: UE: upper epidermal, LE: lower epidermal, P: palisade mesophyll, S: spongy mesophyll, VB: vascular bundle, CO: calcium oxalate crystal (drusen-type), GC: guard cell, SC: subsidiary cell.

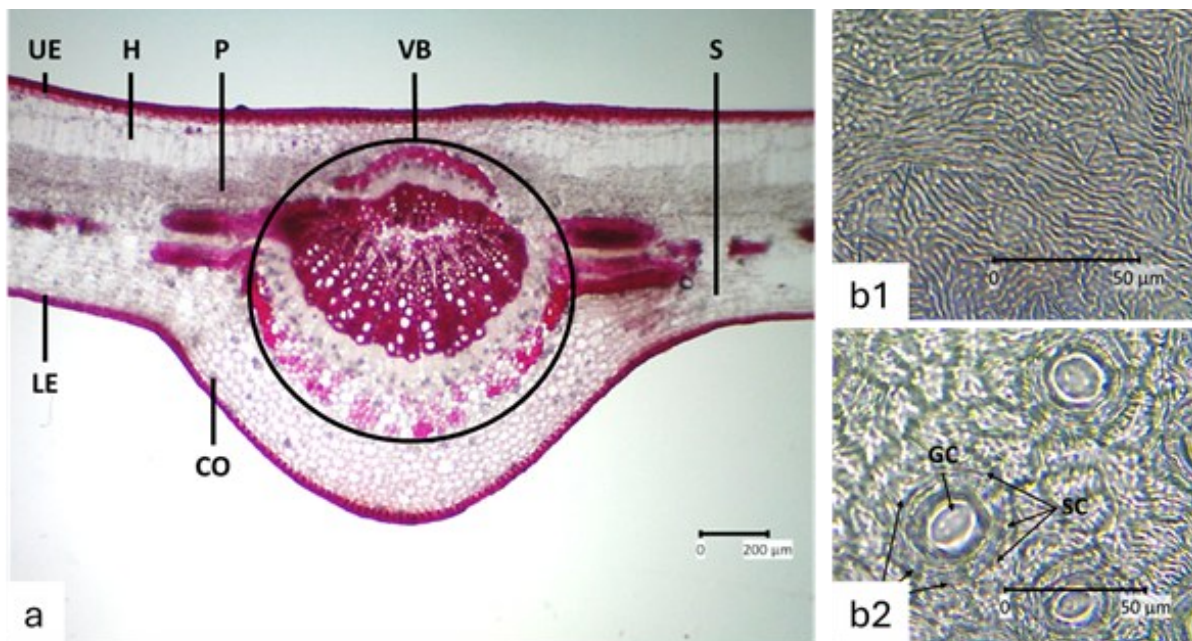


Figure 3. Leaf anatomical structure of *X. granatum* (a) transversal section through the midrib, (b1) replica paradermal of the adaxial surface, (b2) replica paradermal of the abaxial surface. Annotations: UE: upper epidermal, LE: lower epidermal, H: hypodermal, P: palisade mesophyll, S: spongy mesophyll, VB: vascular bundle, CO: calcium oxalate crystal (drusen-type), GC: guard cell, SC: subsidiary cell.

is the guard cells surrounded by subsidiary cells which have the same shape and size with the epidermal cells. Stomata are only found on the lower surface (abaxial) which indicate the leaf of this species is a hypostomatic. The SI value in *X. granatum* is 13.95 ± 2.25 %.

Aegiceras corniculatum

The anatomical structure of *A. corniculatum* (Figure 4a) shows a structure consisting of upper epidermal tissue, 3-4 layers of hypodermal, 2-3 layers of palisade mesophyll, spongy mesophyll, vascular bundles, secretory tissue, salt

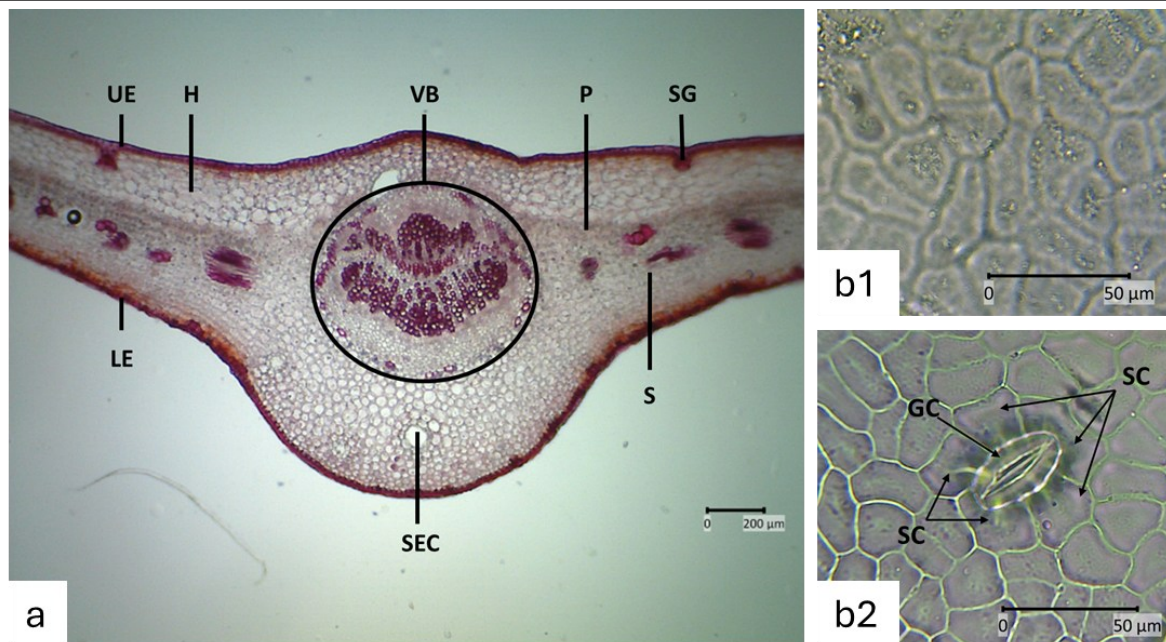


Figure 4. Leaf anatomical structure of *A. corniculatum* (a) transversal section through the midrib, (b1) replica paradermal of the adaxial surface, (b2) replica paradermal of the abaxial surface. Annotations: UE: upper epidermal, LE: lower epidermal, H: hypodermal, P: palisade mesophyll, S: spongy mesophyll, VB: vascular bundle, SEC: secretory tissue, SG: salt gland, GC: guard cell, SC: subsidiary cell.

glands, and lower epidermal. The vascular bundle resembles that of *X. granatum*, forming an arc vascular bundle with an additional vascular bundle on the upper part of the arc, as observed by Tihurua et al. (2023). Based on the characteristics of the stomata in Figure 4b1 and 4b2, the type of stomata in *A. corniculatum* is anomocytic which is the guard cells surrounded by subsidiary cells which similar to the epidermal cells. The SI value in *A. corniculatum* is $8.27 \pm 0.67 \%$.

Rhizophora apiculata

Leaf transversal section through the midrib of *R. apiculata* (Figure 5a) is arranged by upper epidermal tissue, 4-7 layers of hypoderms, 2-3 layers of palisade mesophyll, spongy mesophyll, vascular bundles, sclereids, and lower epidermal. In addition, there is drusen-type of calcium oxalate crystals. The vascular bundle composed by two arc which bend outside, and a small vascular bundle addition in the centre. Sometimes it also resembles separated vascular bundles that form closed ring with additional vascular bundle in the centre (Tihurua et al. 2023). In addition, there are also cork warts in the spongy mesophyll to the lower epidermis. Based on the characteristics of the stomata in Figure 5b1 and 5b2, the type of stomata in *R. apiculata* is anomocytic which is the guard cells surrounded by subsidiary cells which have the same shape and size with the epidermal cells. Stomata are only found on the lower surface (abaxial), so it is called a hypostomatic leaf. The SI value in *R. apiculata* is $4.71 \pm 0.45 \%$.

Sonneratia alba

The leaves of *S. alba* are succulent with fairly clear midrib. This species has leaf with a layer of upper and lower epidermal tissue, 3-4 layers of upper and lower palisade, sponge, vascular bundles, sclereids, and drusen-type of calcium oxalate crystals (Figure 6a). The vascular bundle composed by two flat arcs of xylem and phloem, similar as other *Sonneratia* species reported by Tihurua et al. (2023). The upper arc tends to smaller than the lower arc. The presence of two upper and lower palisade mesophylls indicates that this leaf is

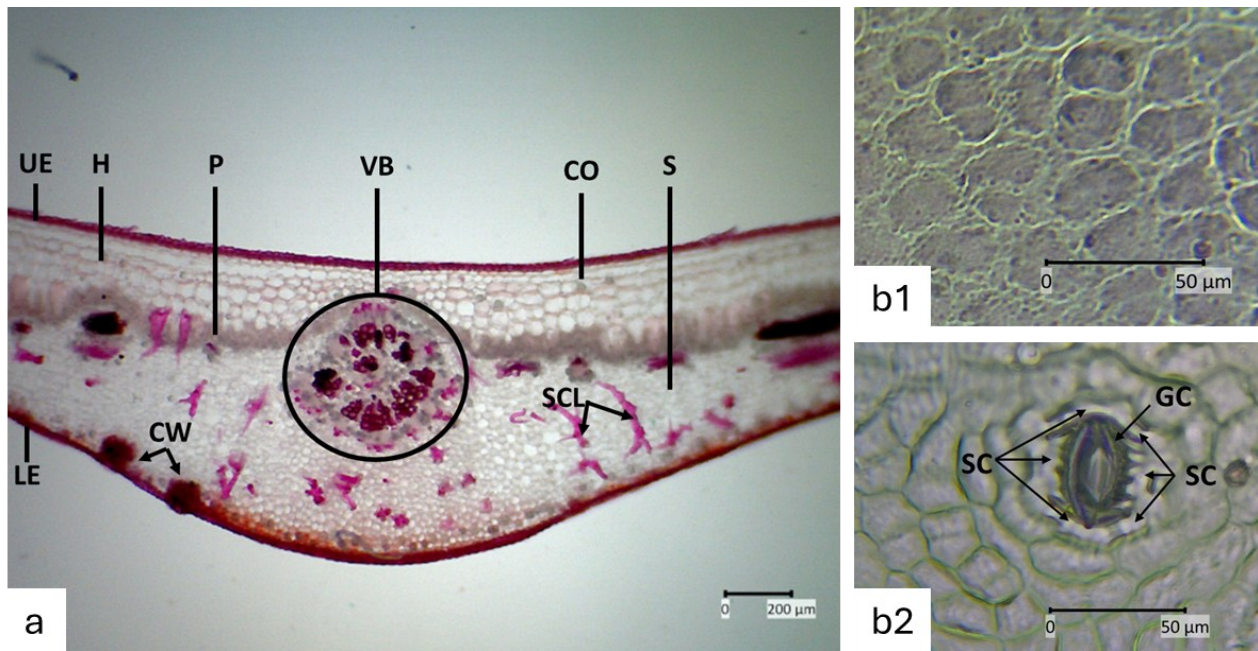


Figure 5. Leaf anatomical structure of *R. apiculata* (a) transversal section through the midrib, (b1) replica paradermal of the adaxial surface, (b2) replica paradermal of the abaxial surface. Annotations: UE: upper epidermal, LE: lower epidermal, H: hypodermal, P: palisade mesophyll, S: spongy mesophyll, VB: vascular bundle, CO: calcium oxalate crystal (drusen-type), SCL: sclereids, CW: cork warts, GC: guard cell, SC: subsidiary cell.

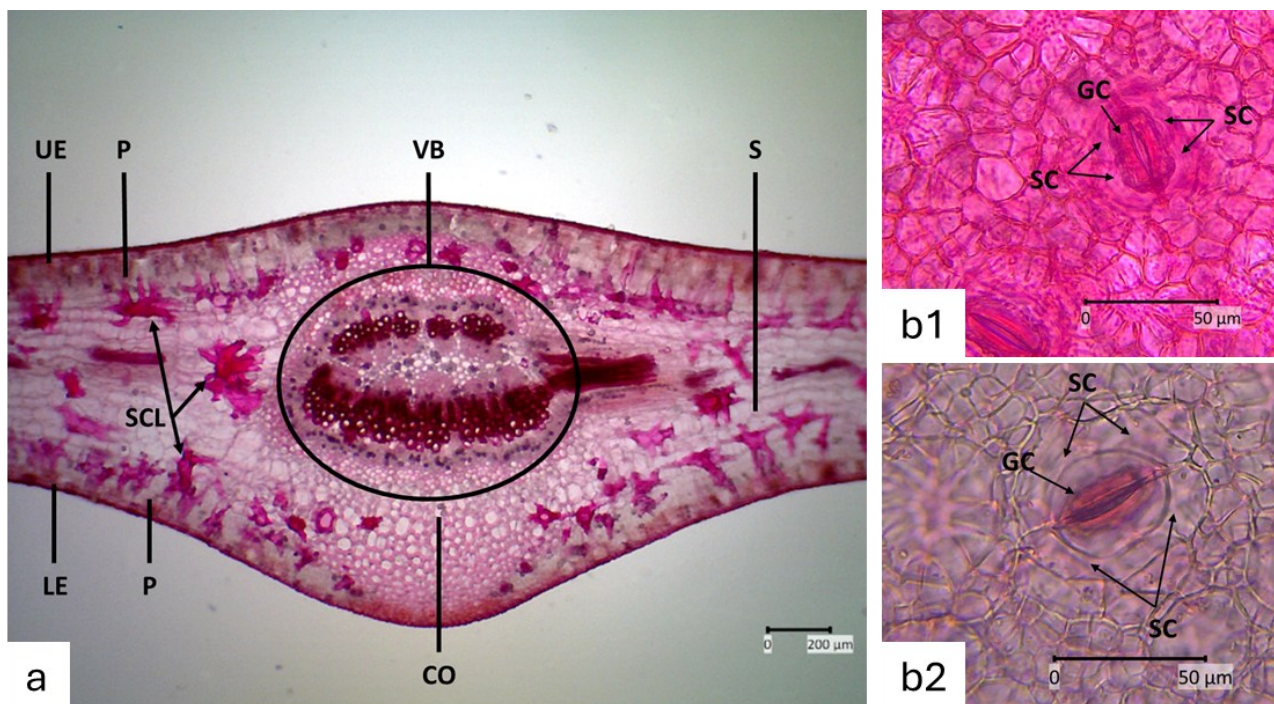


Figure 6. Leaf anatomical structure of *S. alba* (a) transversal section through the midrib, (b1) paradermal of the adaxial surface, (b2) paradermal of the abaxial surface. Annotations: UE: upper epidermal, LE: lower epidermal, P: palisade mesophyll, S: spongy mesophyll, VB: vascular bundle, CO: calcium oxalate crystal (drusen-type), SCL: sclereids, GC: guard cell, SC: subsidiary cell.

an isobilateral. Based on the characteristics of the stomata (Figures 6b1 and 6b2), the type of stomata in *S. alba* is staurocytic because of the guard cells surrounded by 4 subsidiary cells in perpendicular positions, but parallel to the guard cells which giving an X-position of subsidiary cells. This species is grouped in to amphistomatic leaf as stomata are found on the upper (adaxial) and lower (abaxial) surfaces. The SI value in *S. alba* was 2.56 ± 0.52 % on the adaxial surface and 1.90 ± 0.25 % on the abaxial surface.

Structural comparison of leaf anatomical traits in mangrove

Comparison of anatomical structures of mangrove leaves is shown in Table 2. Among the 15 characters, *R. apiculata* and *S. alba* have the most characters (11 characters) while *X. granatum* has the fewest characters (9 characters). In terms of character, salt glands and secretory tissue are the rarest which is only observed in *A. corniculatum*. A principal component analysis (PCA) was carried out to determine the presence of unique anatomical characters in a species. Of the 15 characters, selection was carried out by eliminating characters with the same value, so that 11 characters were remained. After that, scoring was carried out by giving a score or value to the character and was presented in the form of a scoring table as shown in Table 3. The results of this scoring were used to carry out PCA. Based on the PCA results, 4 axes are needed to obtain a cumulative score of 100 %, while the 3 main axes have obtained a cumulative percentage value of the variance of 91.48 % as shown in Table 4. Pankhurst (1991) stated that the accuracy of the PCA results is classified as good or appropriate if the cumulative value on the three main axes is more than 80 %.

Visualisation of PCA results is in the form of a scatter plot with vectors which represent the eigenvalues (Figure 7). The scatter plot is made with axis 1 and axis 2 with a cumulative percentage of 81.75 %, which means that the PCA results accommodate 81.75 % of the variation in the data. The value of axis 1 (57.05 %) is higher than axis 2 (24.70 %), so the priority of cluster separation is more determined by axis 1 or the x-axis than by axis 2 or the y-axis. In general, there are two main clusters formed, namely cluster-I (blue) consisting of *S. alba* and *L. racemosa*, and cluster-II (red) consisting of *R. apiculata*, *A. corniculatum*, and *X. granatum*. The characters that are responsible in grouping the cluster-I are K3 (lower palisade mesophyll), K11 (stomatal type) which overlapping with K3, and K9 (adaxial stomatal index). These two characters are found in *S. alba* and *L. racemosa*, while the other three species do not have this character. The character K9 (adaxial stomatal index) is only found in amphistomatic leaves, while *R. apiculata*, *A. corniculatum*, and *X. granatum* have hypostomatic leaves. The character K3 (lower palisade mesophyll) only found in isobilateral leaves, while the three species in cluster-II have dorsiventral leaves. Another character is K11 (stomatal type) which is tetracytic in *L. racemosa* and staurocytic in *S. alba*, while the three species in cluster-II have anomocytic type. Mangrove species with dorsiventral leaves have thick hypodermal, so K1 (upper hypodermal) is the main vector that groups cluster-II.

DISCUSSION

The anatomical structure of mangrove leaves resembles the anatomical structure of dicotyledonous plants, but with slight additions and modifications to the constituent tissues. In the epidermal tissue, there is a thick cuticle layer on the upper and lower surfaces of the leaves. The cuticle is an exudate in the form of wax produced by the epidermal tissue to prevent excessive evapotranspiration (Sánchez et al. 2021). This is a common adaptation mechanism found in plants with habitats that have high light intensity (Chorchuhirun et al. 2020; Sánchez et al. 2021).

In some species, such as *X. granatum*, *A. corniculatum*, and *R. apiculata*, there is a hypodermal layer just below the upper epidermal tissue. The hypodermal is a derivative of the epidermal tissue which is generally composed of large and elongated cells. The hypodermal plays a role in preventing excessive water loss due to environmental stress such as high light intensity and salinity by preventing osmotic shock, so that it can maintain tissue osmotic pressure (Chorchuhirun et al. 2020; Sánchez et al. 2021).

The tissue below is palisade mesophyll. In dorsiventral leaves, this tis-

Table 2. Leaf anatomical comparison of the observed mangrove species.

No	Character	Species				
		LR	XG	AC	RA	SA
1	Upper epidermal	+	+	+	+	+
2	Upper hypodermal	-	2—3 layers	3—4 layers	4—7 layers	-
3	Upper palisade mesophyll	2 layers	2—3 layers	2—3 layers	2—3 layers	3—4 layers
4	Vascular bundle	+	+	+	+	+
5	Sponge mesophyll	+	+	+	+	+
6	Lower palisade mesophyll	2 layers	-	-	-	3—4 layers
7	Lower epidermal	+	+	+	+	+
8	Salt gland	-	-	+	-	-
9	Secretory tissue	-	-	+	-	-
10	Calcium oxalate crystal	+	+	-	+	+
11	Sclereid	-	-	-	+	+
12	Cork warts	-	-	-	+	-
13	Adaxial stomatal index	9.57 ± 1.38 %	-	-	-	2.56 ± 0.52 %
14	Abaxial stomatal index	6.42 ± 0.91 %	13.95 ± 2.25 %	8.27 ± 0.67 %	4.71 ± 0.45 %	1.90 ± 0.25 %
15	Stomatal type	Tetracytic	Anomocytic	Anomocytic	Anomocytic	Staurocytic

Annotations: (+) indicating present, (-) indicating absent, LR=*Lumnitzera racemosa*, XG=*Xylocarpus granatum*, AC=*Aegiceras corniculatum*, RA=*Rhizophora apiculata*, SA=*Sonneratia alba*.

Table 3. Characters selections and scores of observed leaf anatomical characteristics.

Code	Character	Species				
		LR	XG	AC	RA	SA
K1	Upper hypodermal [0 = absent, 1 = 2—3 layers, 2 = 3—4 layers, 3 = >4 layers]	0	1	2	3	0
K2	Upper palisade mesophyll [0 = 2 layers, 1 = 2—3 layers, 2 = >3 layers]	0	1	1	1	2
K3	Lower palisade mesophyll [0 = absent, 1 = 2 layers, 2 = >2 layers]	1	0	0	0	2
K4	Salt gland [0 = absent, 1 = present]	0	0	1	0	0
K5	Secretory tissue [0 = absent, 1 = present]	0	0	1	0	0
K6	Calcium oxalate crystal [0 = absent, 1 = present]	1	1	0	1	1
K7	Sclereid [0 = absent, 1 = present]	0	0	0	1	1
K8	Cork warts [0 = absent, 1 = present]	0	0	0	1	0
K9	Adaxial stomatal index [0 = absent, 1 = <5 %, 2 = >5.1 %]	2	0	0	0	1
K10	Abaxial stomatal index [0 = 0—5 %, 1 = 5.1—10 %, 2 = >10.1 %]	1	2	1	0	0
K11	Stomatal type [0 = anomocytic, 1 = tetracytic, 2 = staurocytic]	1	0	0	0	2

Table 4. Eigenvalues for each axis in PCA.

Value	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	3.65	1.58	0.69	0.48
Percentage	57.05	24.70	10.72	7.52
Cumulative percentage	57.05	81.75	92.48	100.00

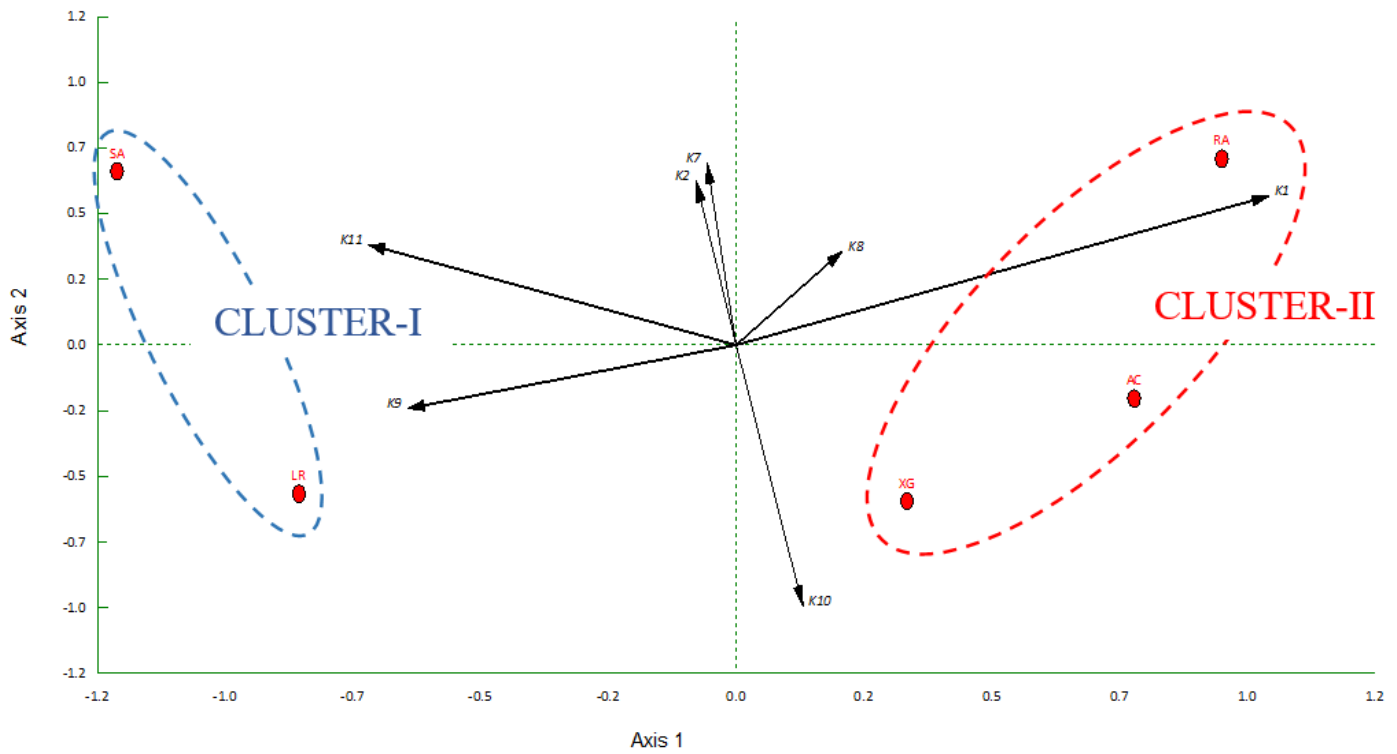


Figure 7. Scatter plot of PCA based on observed leaf anatomical structures in selected mangrove species.

sue is only found in the upper part, while the lower part occupied by spongy mesophyll tissue. Species with dorsiventral leaves are *X. granatum*, *A. corniculatum*, and *R. apiculata*, while others (*L. racemosa* and *S. alba*) are isobilateral. Indirectly, this shows the relationship between mangrove species with and without hypodermal structures. Species *X. granatum*, *A. corniculatum*, and *R. apiculata* have dorsiventral leaves with hypodermal layers, while *L. racemosa* and *S. alba* have isobilateral leaves without hypodermal. This hypodermal character is one of the cluster separation factors in PCA analysis as shown in Figure 7. Since the hypodermal related to the water stress by salinity and light intensity, *X. granatum* as a landward species have lesser hypodermal layers than *A. corniculatum* and *R. apiculata* which adapted in middle zone with higher salinity. Differences in the hypodermal layer were strongly influenced by genetic factors of each species. However, when related to environmental stress, a thicker hypodermal layer frequently found in a species which exposed to high levels of sunlight. Karabourniotis et al. (2021) stated that the presence of the hypodermal layer reduces the energy from potentially destructive sunlight, allowing the mesophyll tissue below to obtain optimal energy for photosynthesis. So, among *A. corniculatum* and *R. apiculata*, the hypodermal in *R. apiculata* were denser as an adaptability in full light intensity than in *A. corniculatum*.

Based on their function, palisade and spongy mesophyll tissues are responsible for the photosynthesis process with slightly different priorities. Palisade mesophyll tends to play a role in processes which involving sun light, so the density of chloroplasts is very high in the column-shaped palisade cells (Sánchez et al. 2021; Tobing et al. 2022). In contrast to spongy mesophyll with polygonal or irregular cells with air space between cells that are responsible in air circulation. Despite their different functional priorities, these two tissues show adaptive responses to environments stressed by high light intensity and salinity. The adaptation is by forming a dense cell arrangement (in palisade mesophyll, the cells are very slender and dense) with few air chambers (air chambers in spongy mesophyll are fewer and smaller than in majority of dicotyledonous species) to increase photosynthetic efficiency and prevent excessive transpiration (Parida et al. 2004; Sánchez et al. 2021; Tatongjai et

al. 2021).

To prevent the leaf structure from being damaged by strong winds, there are additional structures in the form of sclereids that are spread across the hypodermal and mesophyll tissues to provide mechanical support to the leaf structure. Not only as a supporting structure, sclereids are also able to prevent a decrease in turgor pressure while preventing herbivores (Vinoth et al. 2019; Tatongjai et al. 2021). These sclereids were observed in *R. apiculata* and *S. alba* in form of astrosclereid and rhizosclereid. Astrosclereids are star-shaped sclereids with elongated and relatively uniform branches, while rhizosclereids have several branches that are longer than others (Rao & Bhupal 1973; Tatongjai et al. 2021). Additionally, in the hypodermal and mesophyll tissues, drusen-type calcium oxalate crystal within idioblasts can be observed. Calcium oxalate crystals originate from the accumulation of metabolic waste that is generally found around the phloem tissue, then spreads to other tissues (Tatongjai et al. 2021). Physiologically, calcium oxalate crystals play a role in detoxifying heavy metals (Franceschi & Nakata 2005) and also as a source of CO₂ for photosynthesis (Tooulakou et al. 2016). In several taxonomic studies, the characteristics and distribution of calcium oxalate crystals are one of the main diagnostic characters for distinguishing between species (Raeski et al. 2023).

In addition, there are also structures in the form of secretory tissue and salt glands. Of the five species, these two structures are only found in *A. corniculatum*. Secretory tissue is characterised by the presence of epithelial cells around the cavity, while the salt glands are epidermal derivatives that distributed found on the adaxial and abaxial surfaces of the leaves (Naskar et al. 2021). The salt glands in *A. corniculatum* are one of the unique structures, which causes the leaves of this species have fine salt grains on the surface of the leaves. With a habitat that has relatively high salinity compared to fresh water, mangrove species have several salt excretion mechanisms which are classified into salt secretion, salt exclusion, and salt accumulation (Parida & Jha 2010). The mechanism in *A. corniculatum* is called salt secretions because salt excretion is carried out through secretory tissue, including salt glands (Parida & Jha 2010; Naskar et al. 2021). In *Bruguiera*, *Rhizophora*, *Sonneratia*, and *Xylocarpus*, salt excretion is carried out by the salt accumulation method by accumulating salt in cells in leaf tissue which are then shed at certain periods or when mature (Parida & Jha 2010; Tihurua et al. 2023). Another mechanism is salt exclusion by carrying out ultrafiltration on the roots which is commonly found in most mangrove species (Parida & Jha 2010).

In *R. apiculata*, there is a structure in the form of cork warts. This structure develops from the abaxial surface of the leaf and plays an important role in air circulation from the leaves to the roots in anoxic environments (Evans & Bromberg 2010; Naskar et al. 2021). Naturally, this structure can be found in various plant species, including mangroves. The PCA results in Figure 7 show the K8 vector (cork warts) which leads to *R. apiculata*. This indicated that this study agreed to Tihurua et al. (2023) which stated that cork warts is a distinguished anatomical characteristic for *Rhizophora*.

The common epidermal derivative found in leaves is stomata. These five mangrove species have different types of stomata. Our findings for *S. alba* (staurocytic and anomocytic type), *R. apiculata* (anomocytic type), *X. granatum*, and *A. corniculatum* (anomocytic type) align with previous research (Tihurua et al. 2020, 2023; Sabandar et al. 2021). While for the *L. racemosa*, we found different types of stomata according to the previous study. Tihurua et al. (2020) found anomocytic and anisocytic type for *L. racemosa*, while in this research have observed tetracytic type.

Based on their position, leaves with stomata found on the upper (adaxial) and lower (abaxial) surfaces of the leaves, such as in *L. racemosa* and

S. alba. While, species with stomata only on the lower (abaxial) surface are found in *X. granatum*, *A. corniculatum*, and *R. apiculata*. The separation of species clusters based on the presence of stomata was also confirmed through PCA analysis (Figure 7). Based on the stomatal index (SI) value, there is a gradual pattern based on its life zone. This is due to the sensitivity of stomata to environmental changes, so that the SI value and other stomatal characteristics indirectly indicate environmental conditions (Zhu et al. 2018; Khan et al. 2022). In hypostomatic leaves, *X. granatum* living in the landward zone has the highest SI (13.95 ± 2.25 %), followed by *A. corniculatum* (8.27 ± 0.67 %) in the middle zone, and the lowest in *R. apiculata* (4.71 ± 0.45 %) in the middle zone facing the sea. The high SI value (K10 = abaxial stomatal index) in *X. granatum* causes the position of this species in the PCA diagram separated from *R. apiculata* and *A. corniculatum*. Similar things are also found in amphistomatic leaves which is the SI value of *L. racemosa* in the landward zone is always higher than *S. alba* in the seaward zone on the adaxial and abaxial surfaces. Another thing found in this study is the SI value on the adaxial surface which is always higher than the abaxial surface. In *L. racemosa*, the SI value on the adaxial surface was 9.57 ± 1.38 %, while on the abaxial surface it was 6.42 ± 0.91 %. In *S. alba*, the SI value on both surfaces was very small with 2.56 ± 0.52 % on the adaxial surface and 1.90 ± 0.25 % on the abaxial surface.

The SI analysis was popular due to the sensitivity in environmental changes, even in species level. In this study, the SI value related to the water stress due to the salinity and light intensity, similar to the effect in hypodermal layers. The landward species (*X. granatum*) facing lower salinity fluctuation, resulting in higher SI value. On the contrary with *R. apiculata* that adapted in high salinity fluctuation, which developed a lower SI value. The lower SI value generally reflects a strategy to conserve water by reducing transpiration, although this often comes at the cost of limiting CO₂ uptake and photosynthesis, unless compensated by other anatomical or physiological adjustments. The other factors that also related to the water regulation and gas exchange were the stomatal density and stomatal conductance (Royer 2001; Hetherington & Woodward 2003). The SI value also related to the leaf types. The landward species such as *X. granatum* and *L. racemosa* have different preferences. Hypostomatic leaf in *X. granatum* have SI 13.95 ± 2.25 %, while in amphistomatic leaf of *L. racemosa* have lower SI but occur in both side of the leaf surfaces to compensate that (adaxial 9.57 ± 1.38 %, abaxial 6.42 ± 0.91 %). It was the same between *R. apiculata* and *S. alba*.

CONCLUSIONS

The anatomical structure of leaves in five mangrove species belonging to five different families has different compositions between species. Based on the presence of stomata, *L. racemosa* and *S. alba* are species with amphistomatic leaves, while the others are hypostomatic leaves. Based on tissue symmetry (marked by the presence of lower palisade mesophyll), *L. racemosa* and *S. alba* are isobilateral leaves, while the others are dorsiventral leaves. Epidermal tissue, palisade mesophyll, spongy mesophyll, vascular bundles, and calcium oxalate crystals are evenly found in all species. In *R. apiculata*, *A. corniculatum*, and *X. granatum*, there is hypodermal tissue below the upper epidermal tissue. Secretory tissues and salt glands are only observed in *A. corniculatum*. Sclereids as a supporting tissue are found in *S. alba* and *R. apiculata*. The five species have three different stomata types, namely tetracytic (*L. racemosa*), anomocytic (*X. granatum*, *A. corniculatum*, *R. apiculata*), and staurocytic (*S. alba*). Based on the PCA results, the grouping of *L. racemosa* with *S. alba* is caused by leaves that are amphistomatic (there are stomata on the adaxial surface of the leaf/K-9) and isobilateral (have lower palisade mesophyll/K-3), while the grouping in *R. apiculata*, *X. granatum*, and *A. corniculatum* is predom-

inantly caused by the presence of hypodermis (K-1).

AUTHOR CONTRIBUTION

I.M.S.W. designed the research, collected and analysed the data, wrote the manuscript, and supervised all the process; N.K.R.P. collected samples and anatomical data, analysed the data, and wrote the manuscript; N.M.G. designed the research, wrote, and proofread the manuscript.

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CONFLICT OF INTEREST

Please state any conflict of interest regarding the research or the research funding.

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