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Anatomical Characteristics of Rhizophora's Leaves as Mangrove Plant Adaptation at Banyuurip Mangrove Center

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ABSTRACT

Rhizophora is a genus of mangrove plants that dominates the Banyuurip Mangrove Center. This plant certainly has an anatomical structure as a form of adaptation to high salinity environments. The purpose of this study was to describe the anatomical characteristics of the leaves of the three Rhizophora species as an adaptation form of mangrove plants in the Banyuurip Mangrove Center and the anatomical variations of the leaves between species. This type of research was descriptive observational in the form of leaf anatomy observations using the whole mount method for longitudinal incisions and the paraffin method for transverse incisions. The results showed that there were anatomical variations among the three Rhizophora species in the Banyuurip Mangrove Center including epidermal cell size, number of epidermal cells, cork warts, number of hypodermis cell layers, hypodermis cell size, stomata size, number of stomata, stomata index, and stomata density. The anatomical characteristics of the leaves of the three Rhizophora species which act as a form of adaptation to mangrove plants are the presence of hypodermis tissue which functions to store water and salt to remove salt content in plants when they abort their leaves, low density of stomata and the presence of cuticles on the adaxial surface of leaves which play a role in reducing the rate of transpiration thus maintaining water to support plant development in saline conditions, cork warts which function as a medium for expelling salt on leaves.

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INTRODUCTION

Mangrove forests usually describe the coastal communities overgrown with shrubs and several typical tree species that can grow well in high-salinity environments (Martuti et al., 2019). In general, the meaning of mangrove forest refers to an ecosystem (Tomlinson, 2016). According to Baderan (2019), the zoning of mangrove forests can be affected by light intensity, sea tides, salinity level, and type of substrate. Physically, mangroves can prevent abrasion and protect the land from sea waves because mangrove trees can slow down the seawater's flow. Thus, the generated energy waves will not reach the mainland. In addition, mangrove plants can stabilize the mud substrate through their roots (Lestari, 2015) and are effective carbon absorbers produced by the atmosphere (Nedhisa and Tjahjaningrum, 2019).

Mangrove forests have a substantial ecological role in supporting the life of marine biota through their role as spawning grounds, nursery grounds, and feeding grounds for various marine biota such as fish and crabs. Mangroves also play a role in producing most of the litter that is a food source for plankton (Lestari, 2015). This ecosystem also supports the economic sector of coastal residents and other ecosystems such as beaches, seagrass beds, and coral reefs (Amin *et al.*, 2019). Mangroves are habitats for several types of plants. Based on research conducted by Puskonser (2014), several plants that generally live in mangrove ecosystems include the genera *Rhizophora*, *Lumnitzera*, *Aegiceras*, *Bruguiera*, *Nypa*, *Avicennia*, *Sonneratia*, *Xylocarpus*, and *Heritiera*.

Banyuurip Mangrove Center is an area with a high enough diversity of mangrove plants, located in Ujungpangkah District, Gresik Regency, East Java (Yona *et al*., 2018). However, this mangrove area has experienced damage in recent years due to changes in the physical environment and community activities (Yunitasari et al., 2020). Thus, mangrove plants that live in this area must be able to adapt to potential environmental changes. Therefore, it is necessary to do research on the adaptation of mangrove plants in this area to formulate strategies for managing mangrove plants in damaged areas, especially for plants that dominate. Hidayat *et al*., (2018) stated that one of the mangrove plant species that can survive and thrive on the coast of Banyurip Village is one of the plants from the genus *Rhizopora* including *Rhizophora apiculata*, *Rhizophora stylosa*, and *Rhizophora mucronata*. The *Rhizophora* is a genus that is widely used by birds as a living habitat in mangrove ecosystems (Mubarrok & Ambarwati, 2019).

The genus *Rhizophora* is a plant that dominates mangrove vegetation and is better known as a mangrove plant. *Rhizophora* is a type of mangrove that is easy to cultivate because its seeds are viviparous. Thus, it can germinate while still on the tree, then become a conservation effort for the Banyuurip Mangrove Center. The research results conducted by Nedhisa and Tjahjaningrum (2019) show that the genus *Rhizophora* has a higher ability to survive and adapt than other mangrove plants. The Rhizophora genus can grow well in habitats with a salinity level of 32-34 ppt (Kolinug *et al*., 2014). Another form of adaptation found in this genus is anatomical adaptation. The *Rhizophora* plant belongs to the non-secreter mangrove species (a type of mangrove that does not have salt glands). Based on Onrizal's research results (2005) on the stems of the *Rhizophora* plant, no salt gland structures were found in the stem's anatomy. In contrast to several other mangrove plants with salt glands to secrete high salt content, such as the genera *Avicennia*, *Aegialitis*, *Aegiceras*, *Limonium*, and *Acanthus* (Onrizal, 2005). Therefore, further research is needed regarding the anatomical adaptations in other parts of this plant, including the leaf organ. By studying the anatomical structure of the leaves, it may provide the insights on the survival of the species in the area,

The anatomical characteristics of the leaves on the *Rhizophora* plant are a form of anatomical adaptation possessed by this plant as a mangrove plant that can grow in mangrove forest habitats (Tihurua et al., 2020). Mangrove forests have several extreme physical factors, such as their habitat with high salinity puddles, high-intensity sunlight, and rivers with muddy soil substrates (Robianto et al., 2020). The anatomical structure of *Rhizophora*'s leaves certainly has unique characteristics compared to terrestrial plants because of its adaptation to high-salinity environments. One of the parts of the leaf that characterizes the form of plant adaptation is the leaf epidermis tissue and its derivatives (Rindyastuti and Hapsari, 2017). Tihurua *et al*., (2020) stated that the cells of the epidermis, stomata, and cuticle are part of the anatomy of *Rhizophora* leaves, which are important to observe regarding their forms of adaptation as mangrove constituents.

Research related to the anatomical characteristics of the leaves in the *Rhizophora* genus at the Banyuurip Mangrove Center is necessary due to the lack of information regarding the anatomy of the leaves in the *Rhizophora* species as their adaptation form in Indonesia. Therefore, this study aims to analyze leaf anatomical variations and anatomical characteristics of three *Rhizophora* species as a form of adaptation to mangrove plants in the Banyuurip Mangrove Center thus providing an insight as to survival of species in the area. The description of leaf anatomical characteristics of the three *Rhizophora* species can be beneficial as an educational facility and a reference for further research. The research results might add information regarding the anatomical structure of *Rhizophora* for several interested parties and the general society. The result of this study can be used as reference for further research because show the mechanism of anatomical adaptation in *Rhizophora* even though this plant that does not have salt glands on its stems.

MATERIALS AND METHODS

This research was a descriptive exploratory study to describe the anatomical characteristics of the leaves in three *Rhizophora* species as a form of adaptation to mangrove plants in the Banyuurip Mangrove Center and the anatomical variations between species. This research was conducted for three months, from December 2022 to February 2023. The sampling was carried out in the Banyuurip Mangrove Center area in Banyuurip

Village, Ujung Pangkah District, Gresik Regency, East Java (Figure 1). Paradermal and transverse sections of the leaves of three *Rhizophora* species were prepared at the Microtechnic Laboratory Department of Biology, Universitas Negeri Surabaya. Meanwhile, observations of the anatomical structure of the leaves were carried out at the Laboratory of Molecular Biology, Department of Biology, Universitas Negeri Surabaya.

Equipments and Materials

Tools and materials are all the tools and materials used in this research, both during sampling, preparation, and anatomical observation. Equipment used in sampling includes stationery, a ruler, a knife, and plastic bags. The materials and equipment used in observing the leaf anatomy are the 5th nodus leaf strands of *Rhizopora mucronata*, *Rhizopora apiculata*, and *Rhizopora stylosa* obtained from the Banyuurip Mangrove Center, FAA (formalin acetic-alcoholic acid), graded alcohol (50 %, 70 %, 95 %, and 100 %), paraffin, solution of alcohol and xylol 3: 1; alcohol and xylol 1: 1; alcohol and xylol 1: 3, absolute alcohol I, absolute alcohol II, xylol I, xylol II, distilled water, 1% safranin, 0.1 %safranin, 0.5% fast green, glycerin, clorox, distilled water, label, tissue, optical microscope, tweezers, pipette, petri dish, razor blade, object glass, rotary microtome, oven, cover glass, and phone camera.

Sampling

Sampling procedure is done by random sampling method of 3 specimens for each species and carried out in the morning. Leaf samples from three *Rhizopora* species (*Rhizopora mucronata*, *Rhizopora apiculata*, and *Rhizopora stylosa*) were obtained from the Banyuurip Mangrove Center area. The sampling was based on a survey and literature review that had been conducted previously regarding the types of *Rhizophora* in the Banyuurip Mangrove Center. Species identification was based on the morphological characters of the genus *Rhizophora* based on the "Flora of Java" Book (Backer and Brink, 1968). Each *Rhizophora* species has its own diagnostic characteristics that distinguish it from other species. *R. apiculata* has branchlets sympodial; leaves are elliptic-oblong-lanceolate, acute, long 7-17,5 cm, wide 3-7,5 cm, midrib mostly tinged with red beneath, petiole 1,5-3 cm with a red flush. *R. mucronata* has sympodial branchlets; leaves broadly elliptic or oblong, cuneate at base, obtuse or acutish, long 11-23 cm, wide 6-13 cm, petiole 2-5,5 cm, green like lower leaf surface. *R. stylosa* has branchlets sympodial; leaves elliptic, acute at base, obtuse, long 9-12,5 cm, wide 4,5-6,5 cm, petiole 2-4 cm, green like lower leaf surface.

Research Procedure

Anatomical examination of *Rhizophora*'s leaves was carried out using two methods, namely the paraffin method for transverse incisions and the whole mount method for paradermal incisions. Observation of leaf transverse anatomical structures using the paraffin method refers to Rahayu and Handayani (2008). *Rhizophora*'s leaves were first fixed in FAA (Acetic acid-alcohol formalin) for 24 hours, then put into a graded alcohol solution with a concentration of 50%, 70%, 95%, and 100%, then followed by alcohol and xylol 3 :1, alcohol and xylol 1:1, alcohol and xylol 1:3, absolute xylol I and II for 3 hours in each solution. Paraffin infiltration was carried out by soaking the leaves in paraffin solution

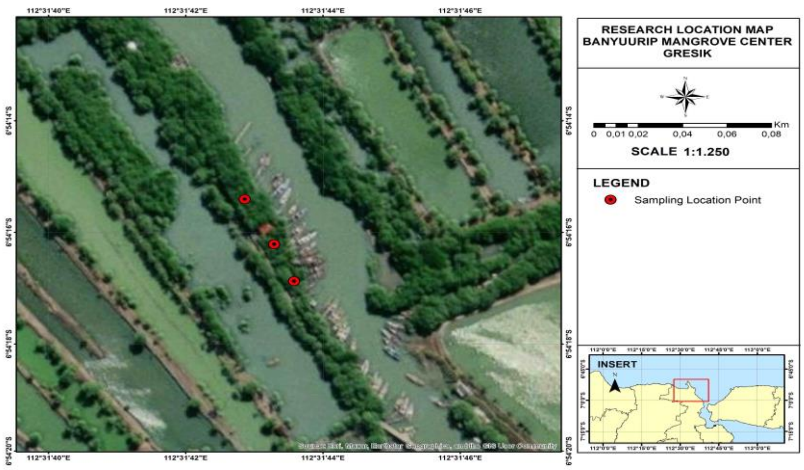

Figure 1. Research location in Banyuurip Mangrove Center. The red point shows the sampling location. Source: https://earth.google.com/

gradually, then forming paraffin blocks which could be sliced with a rotary microtome with a thickness of 15-20 µm. The results of the paraffin slices were placed on the object glass with the help of a brush and water. Furthermore, paraffin-containing object glass was placed in the oven at 35°C for 6 hours and then stained with 1% safranin for 1-2 minutes, followed by dehydration with 70% alcohol, 95% alcohol, and 100% alcohol, then stained with 0.5% fast green in 1-2 minutes followed by absolute alcohol I, absolute alcohol II, xylol I, and xylol II, then a drop of entellan was added and covered with a glass cover.

Observation of the paradermal anatomical structure of the leaves using the whole mount method refers to Marantika (2021). The leaf surfaces at the base, middle, and tips were slashed extremely thin using a razor blade, soaked in Clorox solution until they turned white (about 5 minutes), and then washed in distilled water (aquadest). Furthermore, the incisions were soaked in 0.1% safranin for 2 minutes and washed in distilled water. Then, the incision result was placed on the object glass, given a drop of 30% glycerin, and put on the cover glass. After that, the *Rhizophora*-leaf preparation was observed using an optical microscope with the magnification of 40 x 10. Repeated three times for each sample to obtain the best results, followed by documentation (taking pictures) using a cellphone camera.

Data Analysis

Description of anatomy structure and identification of cells and tissues of the leaves including stomata type, stomata shape, stomata number, stomata length, stomata width, stomatal opening direction, stomata position towards neighboring cells, stomata density, stomata index, cuticle structure, shape and arrangement of epidermal cells, cell layers number (epidermal cell thickness), epidermal cells size, hypodermis tissue, palisade parenchyma, and spongy parenchyma. According to Haryanti (2010), the number of stomata is few if the number of stomata is > 50, quite a lot if there are 51-100, many if 101-200, very many if 201-300, and infinite if > 301. Observations of stomatal density values refer to Juairiah (2014), consisting of low density (< 300/mm2), medium density (300 – 500/mm2), and high density (> 500/mm2). Meanwhile, the calculation of stomata density and index refers to research by Lestari (2006) with the following formula:

$$\text{Stomatal density} = \frac{\text{Number of stomata}}{\text{Field of view area}}$$

Note:
Field of view area = $\frac{1}{4}\pi d^2 = \frac{1}{4} \times 3{,}14 \times 0{,}5^2 = 0{,}19625$ mm^2

$$\text{Stomata index} = \frac{\text{Number of stomata}}{\text{Number of stomata} + \text{Number of epidermal cells}}$$

RESULTS AND DISCUSSION

General Description of Objects and Area

Banyuurip Mangrove Center is a mangrove area that acts as a habitat for several mangrove plants. The results showed 11 species of mangrove plants living in this area, including *Rhizophora mucronata*, *Avicennia marina*, *Avicennia alba*, *Avicennia lanata*, *Sonneratia alba*, *Achantus illicifolius*, *Rhizophora apiculata*, *Aegiceros corniculatu*, *Brugueria gymnoriza*, *Rhizophora stylosa*, *and Lumnitzera racemosa*. *Rhizophora* is one of the dominant genera in this area and is widely propagated for the restoration of the Banyuurip Mangrove Center.

Rhizophora is a mangrove plant that does not have salt glands on its stems. Therefore, this plant has a supportive anatomical structure for its adaptation as a mangrove plant whose habitat is stagnant water with high salinity. The environmental adaptation of mangroves is critical for their continued survival, development, and the intertidal zone because of their distinctive ability to tolerate salt (Vinoth *et al*., 2019). In this study, observations of the anatomical structure of three species of *Rhizophora* at the Banyuurip Mangrove Center, namely *Rhizophora mucronata*, *Rhizophora apiculata*, and *Rhizophora stylosa* were carried out by making transverse and longitudinal sections of leaf preparations based on preliminary tests and references to previous studies (Onrizal, 2005; Tihurua et al., 2020; Nurnida et al., 2012) intended to determine the anatomical variations of the leaves among the *Rhizophora* species studied and the anatomical characteristics of the leaves that act as an anatomical adaptation of *Rhizophora* as a mangrove plant.

Transverse Anatomy of Rhizophora's Leaves

The observations result of transverse anatomy of the leaves of three species of *Rhizophora* showed the presence of tissue constituents in *Rhizophora* leaves. Figure 2 shows some similarities and differences in the characteristics of the three species studied. Characteristics of leaf anatomy in the three species are the presence of epidermal cells, cuticles, hypodermis, sclereid, cork wart, palisade parenchyma tissue, and parenchyma tissue sponge. The anatomical structure of a leaf can be influenced by plant type, plant age, and the environment or habitat of a plant. Based on the results of observations made on the leaves of three *Rhizophor*a species from the Banyuurip Mangrove Center, all three of them have a single layer of leaf epidermal cells on the adaxial and abaxial sides of the leaf, by the results of Nurnida et al. (2012) who stated that the genus *Rhizophora* has a single layer of epidermal cells with a height and width ratio of 1:2, which is also found in the genera *Bruguiera* and *Ceriops*.

All three species showed the presence of a cuticle or waxy coat on the adaxial side of the leaf, whereas on the abaxial side, it was absent. The cuticle is yellow with a flat surface. That is one form of *Rhizophora*'s leaf anatomy adaptation. Cuticles are found in several genera of other mangrove plants, such as *Bruguiera*, *Ceriops*, and *Kandelia* (Nurnida, 2012). Vinoth *et al.* (2019) also revealed that mangrove plants have cuticles on their leaves. The cuticle on *Rhizophora* leaves plays a role in reducing excessive evaporation or transpiration of water by the leaves. That is supported by the statement of Vinoth *et al.* (2019) that the thickness of the cuticle (waxy coat) plays a significant role in retaining water to support plant development in saline conditions. The results showed that the cuticle layer on Rhizophora was relatively thin, in line with the results of a study conducted by Nurnida et al. (2012) that showed the cuticle layer of *Rhizophora* is thinner than that of other mangrove plant genera. The cuticle makes the leaf surface of *Rhizophora* smooth and skinned.

The observations also showed the presence of unstained hypodermis tissue that was densely embedded under the adaxial epidermal cell layer. The hypodermis has a role as a water storage tissue (Cutler *et al.*, 2008). This factor causes the hypodermis cells to be unstained (white). These hypodermis cells are generally found in halophyte plants (which have watery habitats with high salt content) (Tomlinson, 1986). The research results of Vinoth *et al.* (2019) showed that in almost every mangrove species, the water storage tissue originates from the hypodermis tissue except for *Lumnitzera racemosa*. The hypodermis tissue is also used by *Rhizophora* as a salt storage tissue which is absorbed by plants. Salt absorbed by the roots will be collected in these hypodermis cells, which will later be released by the *Rhizophora* plant when the leaves come off. Clough and Attiwill (1982) showed that non-secretory mangrove species lose salt when leaves or other plant parts come off. This statement is reinforced by Balsamo and Thomson (1995) through their research that the cells of the hypodermis of leaves can collect salt and water.

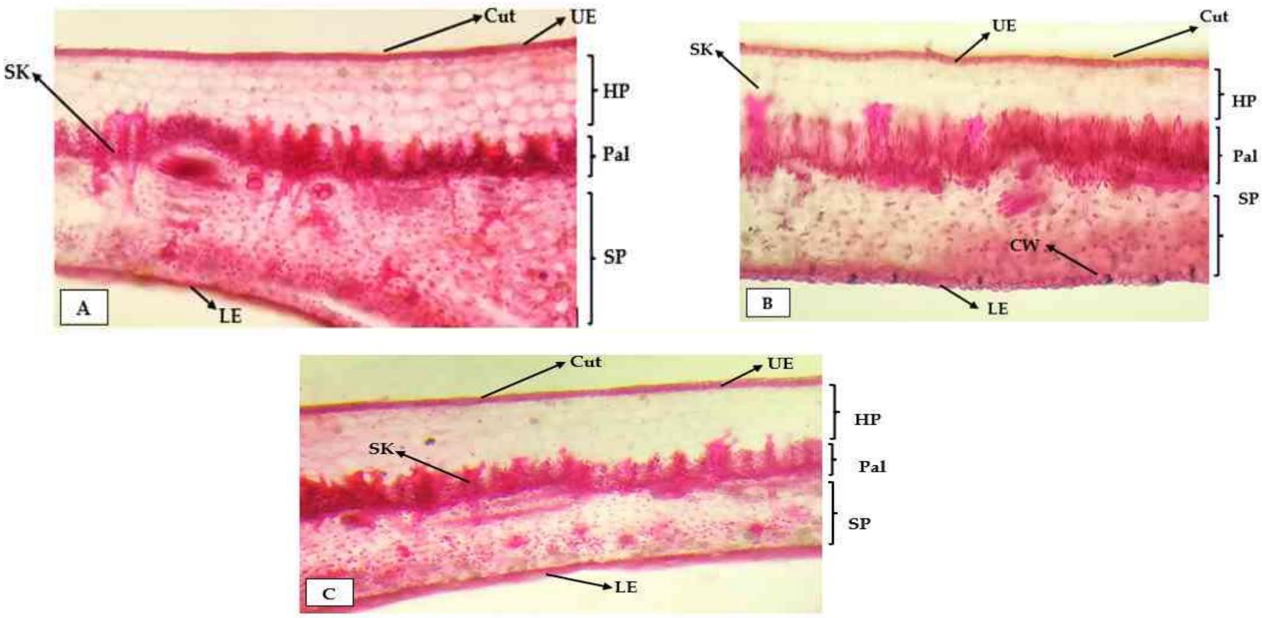

Figure 2. Transverse anatomy structure of three *Rhizophora* species in 10x10 magnification; a) *Rhizophora mucronata*, b) *Rhizophora apiculata*, c) *Rhizophora stylosa*; Upper/adaxial epidermis (UE), lower/abaxial epidermis (LE), cuticle (Cut), hypodermic (HP), Palisade Parenchyma (Pal), Spongy Parenchyma (SP), Sclereid (SK), Cork Warts (CW)

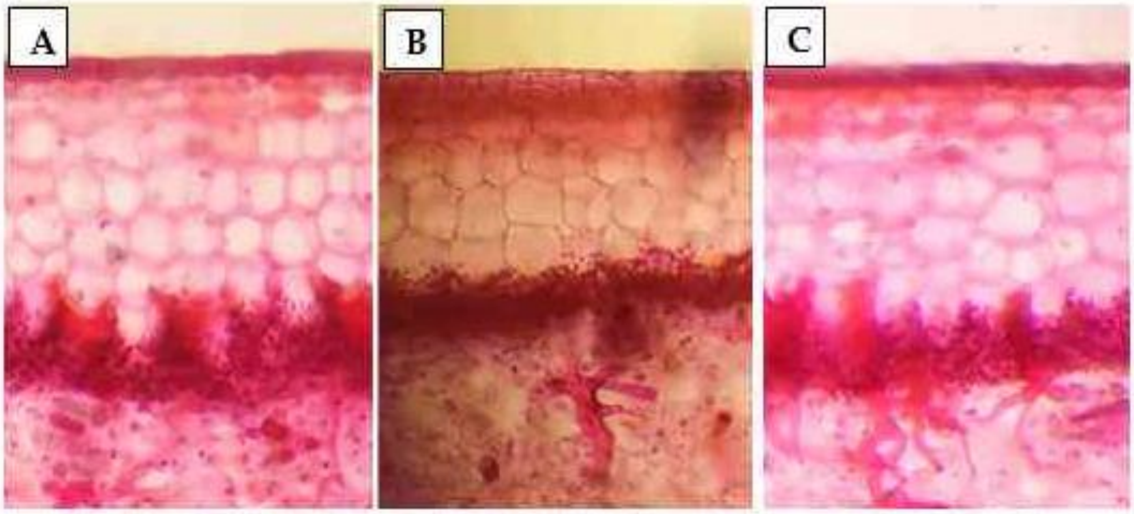

Figure 3. Details of the form of hypodermic cells of three species of *Rhizophora*; a) *Rhizophora mucronata*, B) *Rhizophora apiculata*, c) *Rhizophora stylosa*

Table 1. Observation results of hypodermic tissue in *Rhizophora*'s leaves

Species name	Hypodermic cells length	Hypodermic cells width	Number of hypodermic cell layers
R. mucronata	45-60 µm	36-51 µm	6
R. apiculata	54-69 µm	38-57 µm	4
R. stylosa	49-73 µm	34-46 µm	5

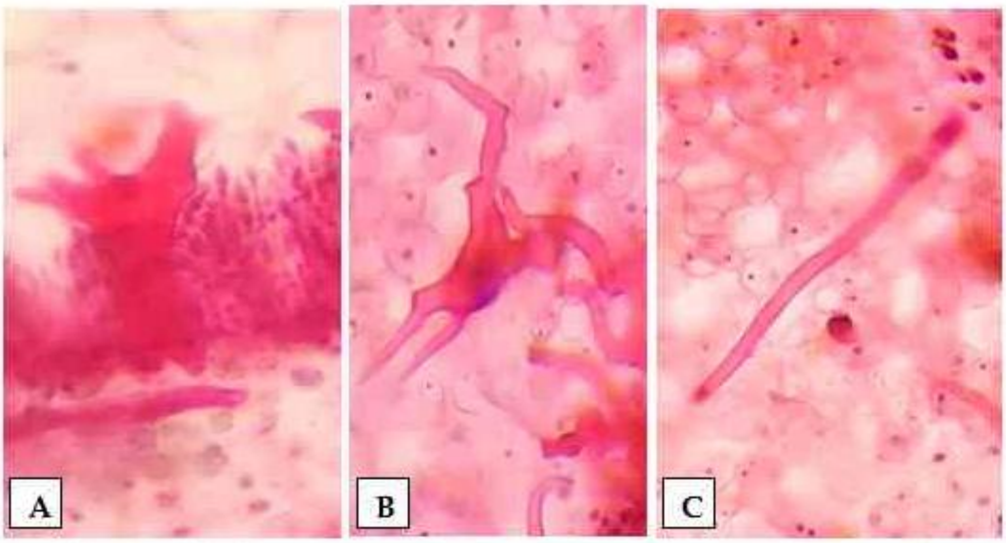

Figure 4. Types of sclereids found in three species of *Rhizophora*; a) osteosklereid, b) astrosklereid, c) trichosklereid

The *Rhizophora* plant carries out a salt-collecting mechanism through hypodermis cells because there are no glands in the stem to secrete salt into the environment. In contrast to several types of mangrove plants, such as the genera *Avicennia*, *Aegialitis*, *Aegiceras*, *Limonium*, and *Acanthus*, which have salt glands on their stems or leaves to secrete a high salt content in these plants (Onrizal, 2005).

The cells in the hypodermic tissue of *Rhizophora* leaves have a rounded hexagon shape. There are variations in the size of the hypodermis cells in the leaves of the three *Rhizophora* species. The hypodermis cells of *Rhizophora mucronata* are 45-60 µm long and 36-51 µm wide, 54-69 µm long and 38-57 µm wide in *Rhizophora*, then 49-73 µm long and 34-46 µm wide in *Rhizophora stylosa.* The size of these hypodermis cells influences the effectiveness of water storage in the leaves of each species. Figure 4 shows that the number of hypodermis cell layers in each species studied also showed variations. *Rhizophora mucronata* has six layers of epidermal cells, *Rhizophora apiculata* has four layers of hypodermis cells, and *Rhizophora stylosa* has five layers of hypodermis cells. The results of this study are reinforced by the results of research conducted by Nurnida et al. (2012) on several species of mangrove plants in Mersing Beach, Johor, which showed that the genus *Rhizophora* has three or more layers of hypodermic cells—two layers in the genus *Ceriops*, one layer of cells in the genera *Kandelia* and *Bruguiera.* Apart from being influenced by the type of plant, the number of cell layers of the hypodermis is also affected by the salt content in the water in this plant habitat, according to its function as a water and salt storage tissue (Waisel, 1972).

The mesophyll tissue in *Rhizophora* plants seen in the transverse section consists of palisade parenchyma and spongy parenchyma, according to the results of a study by Vinoth *et al.* (2019), who showed that the mesophyll tissue in most mangrove plants consisted of palisade and spongy tissue except for *Lumnitzera racemosa.* In the transverse incision of *Rhizophora*'s leaves, sclereids can also be seen between the palisade and spongy parenchyma. This result is reinforced by the research of Vinoth *et al.* (2019), which stated that sclereids are experimental in *Rhizophora apiculata* mesophyll. Sclereids originate from sclerenchyma tissue which functions as a reinforcement network as well as a water storage channel (Tomlinson, 1986). The result of the sclereids found in figure 4 can't be seen clearly due to the thickness of the incision, but the type of sclereids can still be identified from the visible shape.

Based on their shape, the sclereids found are categorized as osteosclereids, asterosclereids, and trichosclereids (Figure 4). Sklereid on the leaves is one of the diagnostic characteristics of the genus *Rhizophora.* Nurnida *et al.* (2012) stated that sclereids in mangrove plants were only found in the *Rhizophora* genus and not in other mangrove plant genera. Osteosclereids are sclereids with a bone-like shape with each end enlarged, lobed, and often branching, usually found on the leaves of certain dicot plants and inside the seed coat (Palennari et al., 2016). Asterosclereids are sclereids with several branches like stars and often leaves (Nugroho, 2021). This is reinforced by the statement of Palennari et al. (2016) that asterosclereids are sclereids that have various types of branches and form star-like patterns, usually found in leaves. Trichosclereids are sclereids with one regular branching, elongated, and hair-like (Palennari et al., 2016).

In addition to the hypodermis and sclereid tissues, there were also found the scattered black spots (cork warts) in the abaxial epidermis of *Rhizophora apiculata* that were absent in the other two species. This is based on research conducted by Tihurua *et al.* (2020) on *Rhizophora apiculata* leaves taken from the Mangrove area in the Banggai Islands, Central Sulawesi, which shows that *Rhizophora apiculata* has cork warts. Black spots or cork warts were also found on the abaxial lamina epidermis of leaves from the genera *Bruguiera* and *Ceriops* (Nurnida, 2012). These cork warts are one of the characteristics of the anatomical adaptation of leaves in plants that live in high-salinity habitats and function as a medium for excessive salt excretion in leaves (Baijnath and Charles, 1980).

Paradermal Anatomy of Rhizophora's Leaves

The results of paradermal anatomy observations on the leaf epidermis of the three *Rhizophora* species showed that all three had the same epidermal-cells-shape in the form of polygonal cells with different cell sizes, the epidermal cell walls were flat, tightly arranged, and irregular. The results of this study are reinforced by previous research conducted by Vinoth et al. (2019) that showed that the epidermal cells in *Rhizophora* and several other mangrove plants are polygonal in shape. There are anatomical variations in the size and number of epidermal cells. These two characteristics are inversely proportional, where the larger the size of the epidermal cells, the fewer the number of epidermal cells that appear in one field on a microscope. *Rhizophora mucronata* has an epidermal cells length of 20-24 μm and a width of 17-21 μm. Epidermal cells in *Rhizophora apiculata* have a length of 19-23 μm and a width of 12-19 μm. *Rhizophora stylosa* has epidermal cells with a length of 29-35 μm and a width of 17-25 μm. The number of epidermal cells in one field of view in *Rhizophora mucronata* is 750 – 780 cells, in *Rhizophora apiculata* is 770 – 220 cells, and in *Rhizophora stylosa* is 730 – 750 cells.

Table 2. Observation results of size and number of epidermal cells

Species name	Epidermal length	Epidermal width	Number of epidermal cells in one field of view
R. mucronata	20-24 µm	17-21 µm	750 – 780
R. apiculata	19-23 µm	12-19 µm	770 – 220
R. stylosa	29-35 µm	17-25 µm	730 – 750

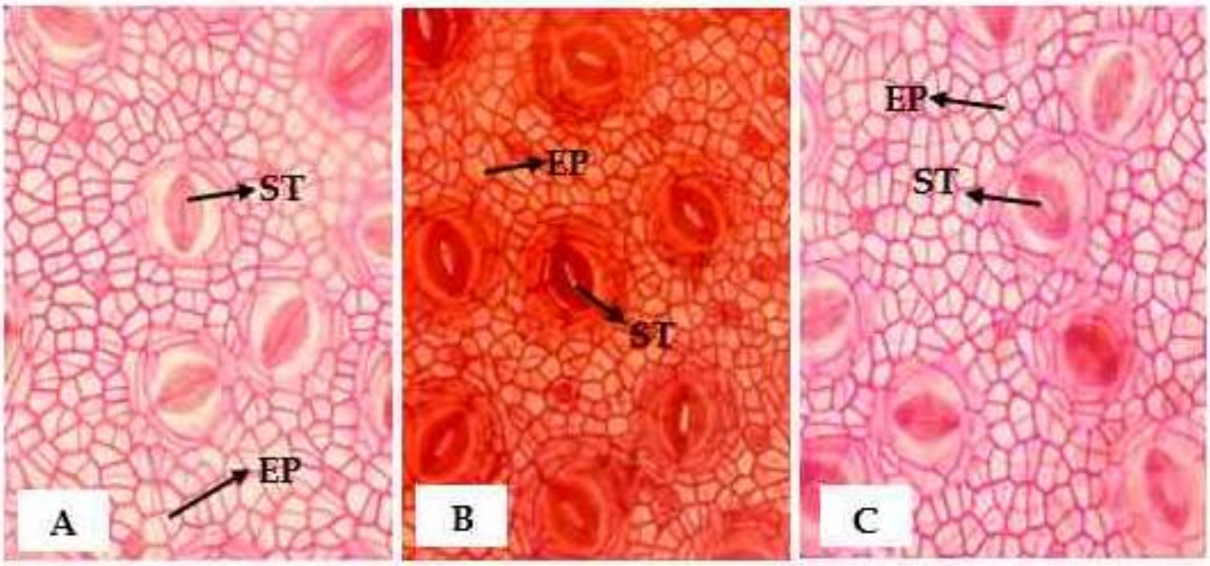

Figure 5. Abaxial paradermal anatomical structures of leaves of three *Rhizophora* species at 40x10 magnification; a) *Rhizophora mucronata*, b) *Rhizophora apiculata*, c) *Rhizophora stylosa*; Stomata (ST), Epidermis (EP)

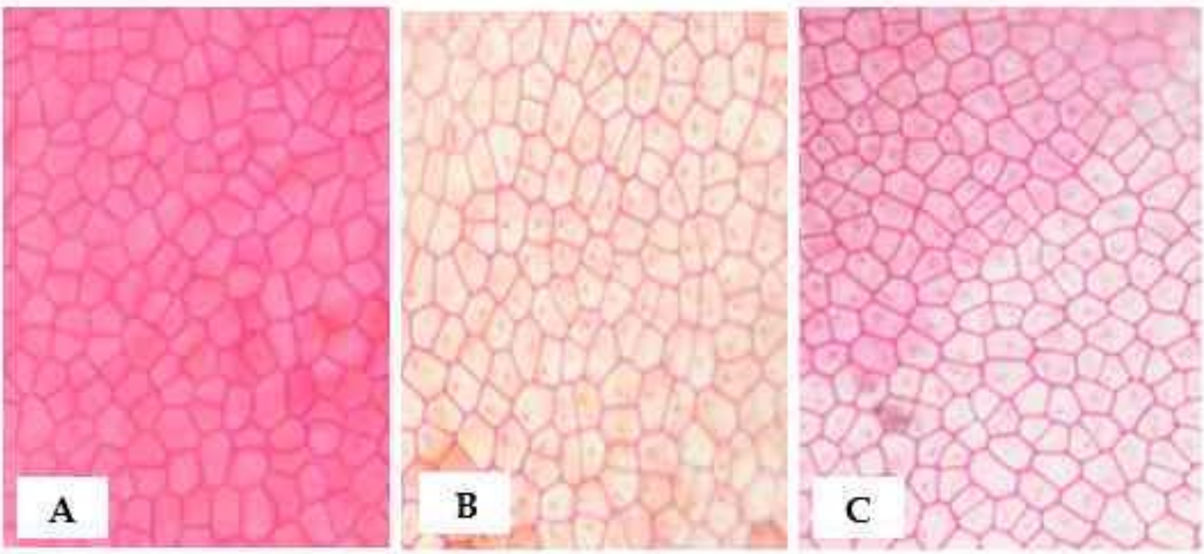

Figure 6. Adaxial paradermal anatomical structure of leaves of three *Rhizophora* species at 40x10 magnification; a) *Rhizophora mucronata*, b) *Rhizophora apiculata*, c) *Rhizophora stylosa*

Apart from showing the anatomic variations in the leaf epidermis, paradermal sections of *Rhizophora* leaves also exhibit the presence of epidermal derivatives in the form of stomata. The research results of Vinoth *et al.* (2019) showed that the leaf epidermis structure in mangrove plants consists of cuticle, epidermal tissue, and stomata, except for *Avicennia marina* plants which have glandular and non-glandular trichomes in the epidermal tissue. Stomata in *Rhizophora* are only found on the abaxial side of the leaf and are absent on the adaxial side. This condition of stomata also occurs in several other mangrove plant genera, such as *Sonneratia*, *Osbornia*, *Lumnitzera*, and *Laguncularia* (Sukardjo, 1996). The results of research conducted by Retno (2015) revealed that stomata located on the abaxial surfaces of leaves are not only found in halophytic plants such as *Rhizophora*, but also in xerophytic plants such as *Euphorbia splendens*, hydrophytes such as *Ipomoea aquatic*, and mesophytes such as *Hibiscus rosa-sinensis*. Figure 5 shows that the three *Rhizophora* species studied had the same type of stomata, which is parasitic. However, the type of parasitic stomata cannot be a distinctive character of the Rhizophoraceae family because this type is also found in plants belonging to the families Magnoliaceae, Rubiaceae, and Mimosaceae (Sutrian, 2011).

The parasitic type of stomata has each guard cell joined by one or more neighboring cells with parallel axes (Palennari et al., 2016). The results of this study are different from those of Tihurua et al. (2020), who stated that the type of stomata in *Rhizophora apiculata* is anomocytic, in which the shape of the epidermal cells that surround the stomata or are around the stomata is the same shape as other epidermal cells but in this study the type of stomata in *Rhizophora* is parasitic. The three *Rhizophora* species studied have kidney-shaped stomata. This shape indicates that the stomata are flanked by two kidney-shaped guard cells (Salisbury and Ross, 1995). The direction of stomata opening in the genus *Rhizophora* is perpendicular to the guard cells. The location or position of the stomata is parallel to the neighboring cells.

Based on the results of measurements of the length and width of the stomata, it was found that the three types of *Rhizophora* studied showed variations in stomata size. *Rhizophora mucronata* has the largest stomata, while *Rhizophora apiculata* is the smallest. *Rhizophora mucronata* has stomata with a length of 51-56 μm and a width of 26-37 μm. *Rhizophora apiculata* has a stomata size of 41-50 μm and a stomata width of 24-34 μm, and *Rhizophora stylosa* has a stomata length of 42-52 μm and a stomata width of 26-37 μm. All three species showed variations in the number of stomata. *Rhizophora mucronata* and *Rhizophora stylosa* have an average number of stomata of 9-12, while *Rhizophora apiculata* have an average number of stomata of 12-15. According to Haryanti (2010), the number of stomata is categorized as few if there are >50, quite a lot (51-100), lots (101-200), very many (201-300), infinite (>301). Therefore, the number of stomata in the three *Rhizophora* species can be classified as low. This is influenced by the size of the stomata and the distance between them.

Based on the calculation of stomata density values, *Rhizophora apiculata* has a higher average value of stomatal density than the other two species, while *Rhizophora mucronata* has the lowest stomata density value. *Rhizophora mucronata* had a stomatal density of 50.955/mm2, Rhizophora apiculata had a stomatal density of 66.242/mm2, and *Rhizophora stylosa* had a stomatal density of 61.146/mm2. The stomatal index of the three *Rhizophora* species at the Banyuurip Mangrove Center is different. The stomatal index is calculated using a formula based on research by Lestari (2006). The calculation results show that the highest stomatal index is owned by *Rhizophora apiculata*, while *Rhizophora mucronata* has the smallest. *Rhizophora mucronata* had a stomatal index of 0.01694, *Rhizophora apiculata* had a stomatal index of 0.01825, and *Rhizophora stylosa* had a stomatal index of 0.01739.

Table 3. Observation results of size and number of stomata

Species name	Stomata length	Stomata width	Number of stomata cells in one field of view (400x magnification)
R. mucronata	51-56 μm	26-37 μm	9-12
R. apiculata	41-50 μm	24-34 μm	12-15
R. stylosa	42-52 μm	26-37 μm	9-12

Table 4. Density and index of stomata of three *Rhizophora* species

Species Name	Stomatal Density Average (/mm^2)	Stomatal Index Average
R. mucronata	50.955	0.01694
R. apiculata	66.242	0.01825
R. stylosa	61.146	0.01739

Stomatal density is grouped based on Juairiah (2014), namely low density (<300mm2), medium density (300-500/mm2), and high density (>500/mm2). Therefore, stomata density in the three *Rhizophora* species is classified as low. The low density of stomata is one of the anatomical adaptations of *Rhizophora*. Mangrove forests generally have intense sunlight, which results in high air temperatures (Onrizal, 2005). The low value of stomatal density can prevent excessive water transpiration so that *Rhizophora* leaves have enough water content considering that this plant lives in an environment with high levels of salt and sunlight. This condition is different from plants that live on land, where the higher the sunlight, the greater the transpiration rate. Mangrove plants have a relatively low transpiration rate due to adaptations to the anatomical structure of their leaves. The results of Tomlinson's research (1986) stated that the transpiration rate of mangrove vegetation ranged from 1.5 to 7.5 mg/dm2/minute. This transpiration rate is lower than that of terrestrial plants, which ranges from 10 – 55 mm/dm2/minute.

The variation in stomata density also has an impact on the survival of each species. Based on the research results, it is known that the lowest stomatal density value is owned by Rhizophora mucronata and the highest stomatal density value is owned by Rhizophora apiculata. In mangrove plants, the lower the density of stomata, the more water reserves are needed to survive. In addition, Rhizophora mucronata is also a species of the genus Rhizophora, which is the easiest to cultivate and can be found in mangrove areas (Nedhisa and Tjahjaningrum, 2019). This species also has better adaptability and is more tolerant of various types of substrates than other species (Rambu et al., 2019). The result of this study can be used as reference for conducting further research on the salt content in each *Rhizophora* leaf nodes, the amount of salt released by *Rhizophora* when it sheds its leaves, and the mechanism of cork warts formation.

CONCLUSION

Based on the results of the research that has been done, it is concluded that there are variations in the anatomical characteristics of the three *Rhizophora* species in the Banyuurip Mangrove Center, including epidermal cell size, number of epidermal cells, cork warts, number of hypodermis cell layers, hypodermis cell size, stomata size, number of stomata, density of stomata, and stomata index. The anatomical characteristics of the leaves of the three *Rhizophora* species that act as a form of adaptation to mangrove plants in the Banyuurip Mangrove Center are the presence of hypodermis tissue which functions to store water and salt to remove salt content in plants when they drop their leaves. This makes leaves of *Rhizophora* thick and watery. cork warts which function as a salt-expelling medium in leaves, and low stomatal density and the presence of cuticles on the adaxial surface of leaves which play a role in reducing the transpiration rate thereby preserving water to support plant development in saline conditions. The cuticle makes the leaf surface of *Rhizophora* smooth and skinned.

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