Anatomical feature of royal palm leaf sheath

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Abstract. Royal palm (\textit{Roystonea regia}) is an ornamental plant species which are copiously planted in many landscapes in the urban area. At the end of their life cycle, the palm fall naturally and it becomes waste. The purpose of this study was to evaluate the anatomical characteristics of both macroscopic and microscopic features to explore some potential uses. This study found that the length average of the leaf sheath of royal palm was 161.60 cm with the width of 53.47 cm, and the thickness was about 3.54 mm which was decreasing from the center or core to the edge. The percentage of vascular bundle was increasing from edge to core at the width-radial direction and was followed by the increasing of diameter of vascular bundle toward core of the leaf sheath. The type of vascular bundle was type I with the majority component of the vascular bundle at the positon in the center part in the leaf sheath cell structure. Based on its properties the leaf sheath has the potential to be used as the particle component for composite boards.

Keywords: royal palm, vascular bundle, anatomical structure, macroscopic, microscopic

1. Introduction
Waste is the residue of activities that are closely related to environmental pollution \cite{1,2}. Based on the characteristics, waste is divided into four groups: liquid, solid, gas and B3 waste. The liquid waste consist of 99.9\% of the water component, and 0.1\% the solid component. The solid waste consists of 70\% of organic matter and 30\% of inorganic material. While the gas waste contains CO$_2$, CO, SO$_2$, NO$_x$, and then B3 are hazardous and toxic raw materials \cite{3,4,5}. However, there is also agricultural waste which is residue from the agricultural production process. Agricultural waste can be food crops, farm waste and livestock waste \cite{6}. One of the agricultural waste is the royal palm (\textit{Roystonea regia}) leaf sheath.

Royal palm is an ornamental plant species which are broadly planted in the city park area \cite{7}. With the increasing interest in royal palm species, it can increase the waste of palm leaf sheath. Leaf-sheath is a flaking palm skin that was shredded by the palm. The palm leaf sheath will fall as it turns brownish as they matured. The palm leaf sheath has a thick and hard skin, the older the palm get, the harder and the wider it becomes \cite{8}. The fallen royal palm leaf sheath is a high potential afforestation waste. According to \cite{9} royal palm leaf sheath is one of the silviculture wastes that has potential as a composite reinforcement. However, the utilization of a royal palm leaf sheath is not optimal.

Developing the potential of the royal palm leaf sheath is necessary to observe the characteristics of the royal palm leaf sheath, including its basic features such as the macroscopic and microscopic

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characteristics. Studies on the anatomical structure of the palm have been conducted by several researchers such as [10] in 18 palm species, [11] in pineapple, sansevieria, kenaf, abaca sisal, and coconut. Based on the results of the study, the anatomical properties of the palm leaf sheath material have a great influence on other properties such as mechanical properties, physical properties and potential applications of these materials. However, there is no information regarding the anatomical features of the royal palm leaf sheath. Therefore, this study will portray the anatomical features of the royal palm leaf sheath.

2. Purpose of research
Based on the introduction, the purpose of this study was to determine the anatomical features of the royal palm leaf sheath for more optimum utilization.

3. Research method

3.1. Samples Preparation
The research was conducted from April to July 2019, at Wood Design and Engineering Laboratory and Wood Anatomy and Quality Improvement Laboratory, Faculty of Forestry, IPB University. The material used for the study was the leaf sheath of the royal palm. The leaf sheath was divided into three parts according to the length-longitudinal direction i.e. base, middle, and end (figure 1a). Those three parts were further segmented into others three parts according to their width-radial direction i.e. edge, transition, and core (figure 1b). The test was conducted using five replications with each replication samples came from five different trees.

![Figure 1a](image1.png) ![Figure 1b](image2.png)

**Figures 1.** (a) Illustration of sample making according to the length-longitudinal direction (b) palm leaf segmentation according to their width-radial direction

3.2. Macroscopic observation
The macroscopic observation was done to evaluate the leaf sheath cell structure condition before microscopic observation was carried out. Macroscopic observations were made on regards leaf sheath length-longitudinal direction and width-radial direction. The thickness measurement was made according to the length-longitudinal direction (base, middle, and end) and width-radial direction (edge, transition, and core). The test was conducted using five replications. Macroscopic observations were also made on the cross-section segment at radial direction using a loupe with 100 times magnification. The results of the macroscopic observations were used to determine the percentage or frequency of
vascular bundles/0.25 mm². The percentage or frequency of vascular bundles was calculated using ImageJ version 1.52 software [12].

3.3. Microscopic observation

The paraffin method was used to determine the microscopic cell structure features of the royal palm leaf sheath [13]. As stated in this method, the samples were fixed in FAA solution (formaldehyde solution, acetic acid, 95% ethanol and distilled water) at a ratio of each solution is 10:5:50:35. After fixation for 48 hours, the samples were dehydrated for 1 hour using multilevel ethanol solution (50%, 70%, 80%, 90%, 96%, and 100%) [14,15]. Following the dehydrated samples, they were immersed in xylene I and II solutions for 1 hour each [13]. Then the samples were embedded in flowing paraplast I for 1 hour and continued with paraplast II for 2 hours. The next step was making the paraffin block. The paraffin block was sliced with a thickness of 3 to 4 μm [16]. The best slicing (sample tissue didn’t tear) followed by immersion in a 1% safranin solution for 6-8 hours. Before observing the samples, the colored incisions were washed with multilevel ethanol solution (70%, 80%, 95%) for 5 minutes each. Microscopic observation was employed using a digital microscope equipped 40 times magnification lens. From the results of microscopic observation, it can be used to determine the components of the vascular bundle, the type of vascular bundle and the average diameter of the vascular bundle.

4. Results and discussion

4.1. Macroscopic observation

Royal palm is one of the monocotyledonous plants, not a woody substance [17]. The mean length of the leaf sheath of the palm is 161.60 cm and the mean width is 53.47 cm. Meanwhile, the thickness of the royal palm leaf sheath as shown in Figure 2. The average value of thickness was 0.70 mm in the edge part, 1.90 mm in the transition part, and the average value of 7.94 mm in the center or core part with the average thickness of leaf sheath was 3.54 mm. The average thickness reduction percentage from the transition segment to the edge was 64% and the average thickness reduction from the core to the transition segment was 74%. The highest palm leaf thickness is in the middle segment, followed by the base and the end segment.

![Figure 2](image_url)

**Figure 2.** Three different parts of length (base, middle, end) and radial direction of royal palm (edge, transition, core)

The results of the analysis of variance for the interval 95% showed that the thickness of the leaf sheath was not affected by the segmentation according to the length but according to the width (table 1). According to Duncan's test results, the thickness of the leaf sheath differs greatly from the width in the transition segment and the edge segment, but the thickness of the core was relatively similar. The
closer it was to the edge of the palm leaf, the more it receded. It was due to the different proportions of vascular bundles on the substance. The proportion of vascular bundles and parenchyma increased toward the core. In the bamboo and oil palm, the core is thicker in the center because the percentage of vascular bundles increased towards the center or inner area [18]. Vascular bundles were known as supportive tissue and were associated with structural functions. The parenchyma was a food storage network and had high water holding capacity.

**Table 1.** Recapitulation of analysis variance of the leaf sheath segmentation to the royal palm thickness

| Direction | Significant |
|-----------|-------------|
| Length    | 0.449*      |
| Width     | 0.000**     |
| Length*Width | 0.711*      |

*tn*: not significant difference at 95% confidence intervals, ***: significant difference at 95% confidence intervals

![Macroscopic features of different segments](image)

**Figure 3.** Cross section macroscopic features of three different segments in regards of length-longitudinal direction (base, middle, end) and three different segments in regards of width-radial direction (edge, transition, core) using 100 times magnification

Figure 3 showed the result of macroscopic observation using 100 times magnification louppe/0.25 mm². Based on the features, it can be seen that there was a difference in the shape and quantity of the
vascular bundle within the three segments. The core segment had a more rounded form of vessel bundle compared to the other two segments, while the transition and the edge segment tended to be more oval shape.

The vascular bundle contains a fiber bundle which functions as a reinforcement [19]. The number of vascular bundles per unit area or the percentage of the vascular bundle was dependent on each part of the leaf sheath. Figures 3 and 4 showed the percentage of the vascular bundle that increased from the edge, transition, and core segments. The same results were also reported by [19,20,21,22], the percentage of vascular bundles in the light-colored species tends to increase as it approached the bark.

![Figure 4](image_url)

Figure 4. The ratio of royal palm vascular bundles on three different segments by its length-longitudinal direction (base, middle, end) and width-radial direction (edge, transition, and core)

4.2. Microscopic observation

Figure 5 showed the anatomical elements found in the leaf sheath of the royal palm. The royal palm leaf sheath component consisted of a vascular bundle and parenchyma tissue. Both elements determine the properties of the palm tree. The parenchyma is a tissue with a relatively thin cell wall. The parenchyma tissue was functioned as a palm leaf filler and included a vascular tissue [23]. Vascular bundles consisted of a fiber bundle, metaxylem, phloem, and protoxylem. Royal palm had a metaxylem ranges from 1 to 4 per vascular bundle, and protoxylem ranges from 2 to 8. Protoxylem and metaxylem were the major xylems that have developed since the beginning of growth [24]. Although protoxylem was formed in parts of the palm that have not yet completed their growth stages, according to [25], metaxylem was formed after primary growth was completed.

![Figure 5](image_url)

Figure 5 Vascular bundle of royal palm, Vb: Vascular bundle, Pd: Parenchyma tissue, (a) Fiber bundle, (b) metaxylem (c) phloem, (d) protoxylem
There were three types of shape of the vascular bundle that served as a basis for classifying monocotyledonous plants [27]. Type I had a large vascular bundle with the components of the vascular bundle and tend to be central. Type II had smaller vascular bundles compared to type I, and components of vascular connection tend to approach the bottom. Type III had an eye-shaped vascular bundle. This can be seen in figure 6 (a), (b) and (c).

![Figure 6. The type of vascular bundle (a) Type I (b) Type II (c) Type III](image)

Figure 6 showed a cross-section at radial direction of the leaf sheath royal palm in the transition, edge and core segments. Based on Figure 6, it can be seen that three segments were included in Type I, with components of vascular bundles that tend to be central. Type I was also found in Cocoseae, Borasseae and Phoenicia types [10]. Type I vascular bundles contained relatively small amounts of holocellulose (cellulose, alpha-cellulose) and lignin, therefore the leaf sheath is not suitable to be used as a building material but it has the potential as a composite reinforcing material [26]. On the other hand, type II vascular bundles had relatively high holocellulose content, while the lignin content was low, so this type of material is generally used as a building material. As types, III and IV had relatively high holocellulose and lignin content, that can be used as building materials, pulp industry, and other cellulose derivatives.

![Figure 7. Vascular bundles of royal palm leaf sheath cross section obtained from (a) edge, (b) transition, (c) core segments (40 x magnification cartoon lens) (— —) diameter](image)

Vascular bundle size was a paramount indicator of vascular bundle density. Figure 8 showed the diameter of the vascular bundle at three different segments. The diameter of the vascular bundle at the edge segment was smaller that the transition and the core segments, but the transition and the core were relatively similar. The diameter of royal palm leaf sheath in the edge section was 480.4219 µm, and the transition was 550.6948 µm. While, the core is a bigger size from the other, with the diameter vascular bundle was 608.1847 µm. This phenomenon is displayed in figures 2 and 6 that the vascular bundles towards the core were rounded.
5. Conclusion
The leaf sheath consists of two major components, namely vascular bundles and parenchyma with the type I vascular bundle. The percentage of the vascular bundle decreased from the core to the edge segments. The dimension of the vascular bundle the edge segment was smaller than the transition and the core segments. Because of these properties, the leaf sheath is less suitable for use as a building material but more suitable for use as a composite reinforcement material.

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