

Anatomical characteristic and fiber morphology of fibrovascular bundle of Indonesian nipa (*Nypa fruticans***) frond**

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1. Introduction

Indonesia is a tropical country that has abundant palm trees. One of the palm trees is known as Nipa (*Nypa fruticans*) which grows in the mangrove forest or wetland area. In Indonesia, Nipa is commonly seen growing densely and forming huge, pure communities along rivers near estuaries to brackish water, and it has an extensive distribution across Indonesia. Nipa provides numerous benefits as a component of the mangrove ecosystem [1-2]. Nipa is also an energy-generating plant, producing alcohol annually as well as producing more alcohol than sugar, sugar cane, and cassava [3]. However, nipa palm is still widely regarded as a plant with no benefits, except for communities living near nipa trees, who have long relied on it for their daily needs [4].

Nipa, a plant containing lignocellulose, has the potential to serve as a substitute material for wood products made from lignocellulose, in addition to its usage as a source of food and energy. Palm fronds have the potential to serve as a natural fiber source for the production of composite boards, reinforcement in polymer composites, and pulp and paper [5-6]. Presently, there is a growing focus on natural fibers, due to their low density, natural fibers exhibit great specific strength. Natural fiber is a readily available, easily recyclable, and cost-effective

Nipa (*Nypa Fruticans*) is a palm tree that grows in wetlands and mangroves. Nipa fronds possess lignocellulose and hold the potential as a viable source for producing composite boards, pulp, and paper. This research aims to identify the anatomical and morphological characteristics of nipah fronds fiber, especially in the fibrovascular bundle. Nipa fronds were observed in the radial and longitudinal directions. There are four zones in the radial direction, consisting of the outer zone (convex and concave), middle zone, and inner zone. The longitudinal positions consist of the bottom, intermediate, and upper. Anatomical characteristics were observed using a light microscope focused on the fibrovascular bundle's characteristics. Fiber morphology was measured at each position with 20 repetitions of each measurement. The results showed that the number of FVB increased from the outer zone, especially convex towards the middle and inner zone. Thus, the outer fronds are denser than the inner zone. Based on observations of fiber morphology, the concave (radial) zone in the intermediate position (longitudinal) has the longest fiber compared to the other parts. Meanwhile, the cell wall thickness is greatest at the bottom of the concave zone. The widest lumen diameter is the convex zone at the bottom position. This research can conclude that the FVB of nipa palm fronds, both longitudinal and radial, are included in quality class III, which is good enough to be used as raw material for pulp and paper.

Keyword: Frond, Fibrovascular Bundle, Fiber Morphology, Nipa

material [7]. The composition of palm leaf fronds includes 35.1% cellulose, 26.4% hemicellulose, 17.8% lignin, 0.3% starch, and 11.7% ash. The leaves and bark of the Nipa plant possess abundant amounts of cellulose, hemicellulose, and lignin, as well as inorganic elements including sodium (Na), potassium (K), chlorine (Cl), magnesium (Mg), calcium (Ca), silicon (Si), phosphorus (P), sulfur (S), and aluminum (Al) [8]. The chemical composition of palm fronds indicates that they can be used as raw materials for lignocellulosic products. The palm frond that has a cellulose content of 42.22%, indicates potential as an acceptable source of raw material for the making of pulp and art paper [9]. Nipa has been extensively studied concerning several themes, including the impact of tapioca and sago adhesives on the quality of Nipa shell charcoal briquettes [10].

Understanding the anatomical and morphological characteristics of palm fronds is crucial for determining their suitability for specific use. Certain palm trees exhibit distinct anatomical features and variations in fiber shape. Anatomical variations will result in corresponding variations in physical and mechanical qualities. Hakim et al. [11] observed that the fibrovascular bundle found in the frond of Arenga longipes exhibits distinct features depending on its longitudinal and radial orientation. The observations indicate that variations in anatomical features along the longitudinal and radial direction of the fibrovascular bundles in sugar palm fronds result in variances in the tensile strength qualities of these bundles. In considering this context, undertaking this research is feasible given the objective of gathering information on the anatomical characteristics of the fibrovascular bundle. The objective of this study was to identify the anatomical and morphological fiber of Nipa fronds (*Nypa fruticans*) as well as analyze the fiber structure of the fibrovascular bundles of nipa fronds.

2. Method

2.1. Sample preparation

The nipa fronds were collected from the mangrove forest located in Sei Nagalawan Village, Serdang Bedagai District, North Sumatra Province, Indonesia. Three nipa fronds were harvested from 10 years old. The frond was collected from the outer part of the main stem, with a diameter of around 8-10 cm. The fronds were severed 30 cm from the primary trunk of the nipa tree. The frond was longitudinally separated into three positions: bottom, intermediate, and upper, each measuring 30 cm. Figure 1 shows that each radial cross-section was separated into four zones: concave, middle, inner, and convex.

Figure 1. The pattern of longitudinal direction and cross section for anatomical and fiber observation. (a1) bottom position; (b1) Intermediate position; (c1) Upper position; (a2) concave zone; (b2) inner zone; (c2) middle zone; and (d2) convex zone

2.2. Anatomical observation

The sample was soaked in 70% ethanol after being sliced to dimensions of approximately 6 cm x 2 cm x 2 cm for sample preservation purposes before being taken in laboratory [11]. The specimen was sectioned using a metal knife with a thickness of around 10-15 µm and placed onto a petri dish. The specimen was immersion in 50 , 70, and 90 % ethanol for dehydration process, respectively. The specimens received an 8-hour safranin (10% of concentration) staining process to enhance the visibility of the surface features of the frond and fibrovascular bundles. The cross-sectional area was determined by measuring various parameters, including the total transverse area of the fibrovascular bundle, the area of vascular tissue, and the area of sclerenchyma fiber tissue (Figure 2). Each measurement parameter was carried out in 30 repetitions. In addition, the proportions of vascular tissue to the whole transverse area and the proportions of sclerenchyma fiber tissue area to the total transverse area were also determined. The anatomical observation was conducted using a light

microscope (Zeiss Primo-Star, Berlin, Germany) that was equipped with a digital camera (iPad 6th, Tokyo, Japan) and imaging analysis software (Labscope-Zeiss-Primo Star, Germany). The anatomical properties were observed using magnifications of 4x and 10x.

Figure 2. An illustration of area occupation measure of fibrovascular bundle. a. Total transverse area; b. non vascular tissue area; c. vascular tissue area

2.3. Fiber morphology observation

The fibrovascular bundle was physically detached from the frond. The frond was incised with a metal knife and the parenchyma tissue was manually extracted. The research process is illustrated in the Figure 3.The fibrovascular bundle was severed at a length of approximately 5 cm (chips). A solution consisting of 60% glacial acetic acid (CH₃COOH) and 30% hydrogen peroxide (H_2O_2) was utilized for the maceration procedure. The ratio of acetic acid glacial and hydrogen peroxide in the solution was 1:1 (v/v). The fibrovascular bundle was immersed in a solution at a temperature of 80 °C, placed in a water bath for a duration of 5 hours, until the individual fibers were detached from the bundle. The fiber was stained with safranin for a duration of 7 hours and subsequently rinsed with distilled water. Ultimately, the fiber was examined using a light microscope (Zeiss Primo-Star, Berlin, Germany) that was equipped with a digital camera (Ipad 6th, Tokyo, Japan) and imaging analysis software (Labscope-Zeiss-Primo Star, Germany).

Figure 3. An illustration of research process. (a). 70% ethanol immersion; (b1). Boiling to soften the frond (b2). Chips of fibrovascular bundle; (c1). Dehydration process; (c2). Maceration; (d1) and (d2). Staining process; and (e). Observation under light microscope

2.4. Measurement of fiber dimensions

Each position and zone were measured for 20 replicant fibers, including their length, diameter, cell wall thickness, and lumen diameter. The cell wall thickness was determined using the provided equations.

$$
cwt = \frac{D-d}{2} \tag{1}
$$

where, cwt is cell wall thickness; D is fiber diameter; d is lumen diameter. The fiber derivate value was calculated by following equation.

$$
rr = \frac{2cwt}{d} \tag{2}
$$

$$
fp = \frac{l}{D} \tag{3}
$$

$$
mr = \frac{D^2 - d^2}{D^2} x \ 100\%
$$
\n⁽⁴⁾

$$
cr = \frac{cwt}{D} \tag{5}
$$

$$
f = \frac{d}{D} \tag{6}
$$

where, rr is Runkel ratio; fp is felting power; mr is Muhlstep ratio; cr is coefficient of rigidity; fr is flexibility ratio; cwt is cell wall thickness; l is fiber length; D is fiber diameter; d is lumen diameter [12-14].

2.5. Statistical analysis

A factorial design with complete randomization has been used to examine the impact of position and zones on the longitudinal and radial aspects of anatomical characteristics and fiber shape of the fibrovascular bundle in Nipa fronds. There were three positions in the longitudinal direction: bottom, intermediate, and upper. Additionally, there were four positions in the radial zones: concave, middle, inner, and convex. The Duncan multiple range test (DMRT) was utilized for determining different treatment levels with a 95% confidence interval.

3. Results and Discussion

3.1. Qualitative Anatomical Characteristic

In general, the fibrovascular bundle of the Nipa frond shows distinct variations in shape and size when compared to the fibrovascular bundle of other palm fronds, such as the salacca frond [15] or the sugar palm frond [11]. The Nipa frond exhibits two locations of sclerenchyma fibers in its periphery FVB form. Moreover, the anatomical structure of the Nipa frond is similar to that of other palm trees. The FVB network is situated within the parenchymal tissues that surrounds it (Figure 4). Various types of tissue were present, including parenchyma, sclerenchyma fiber, metaxylem, protoxylem, and phloem. This study examines the xylem composition in the FVB, which consists of a metaxylem with 1-2 vessels and a protoxylem with 2-6 vessels (Figure 5).

Metaxylem and protoxylem are vascular tissues that contribute to the transportation of water and nutrients in plants [16]. The FVB palm leaf sheaths are distributed throughout the underlying parenchymal tissue. The basic network of parenchyma consists mostly of round cells with thin, soft, and highly hygroscopic walls that function as storage for food, specifically carbohydrates in the form of sugars and starch. Similar to the parenchyma network in the stem of the areca palm, the stem of the nipa palm also possesses parenchyma tissue that exhibits a brighter color than the FVB [15]. The dark color in FVB is caused by the secondary thickening process of the cell walls that make up the sclerenchyma fibers. The thickened secondary cell wall layer of sclerenchyma is composed of cellulose, hemicellulose, and lignin [17].

Overall, according to Figures 6, 7, and 8, the form of the FVB of palm fronds remains consistent in both the longitudinal and radial directions, however there are variations in size. The structure of FVB consists of both a vascular component and a fibrous component. The vascular components in each area of the frond (convex, concave, middle, and inner) are widely spaced and dispersed in a randomly. The FVB of palm fronds exhibits an elliptical morphology, containing vascular tissue at its center. This particular form of FVB has resemblance to the FVB seen in the middle and inner fronds of *Arenga longipes* [15] as well as the fronds of *Salacca zalacca* [18]. The quantity of FVBs in the middle and inner zones surpasses that in the outer regions (both convex and concave).

Figure 4. Cross section photograph of fibrovascular bundle of Nipa frond. Note: a: fibrovascular bundle tissue; b: parenchyma tissue (4x magnification)

Figure 5. Cross section photograph of fibrovascular bundle of Nipa frond. Note: pt: parenchyma tissue; sf: sclerenchyma fiber; mx: metaxylem; ph: phloem; px: protoxylem (10x magnification)

Figure 6. Cross section photograph of fibrovascular bundles of Nipa frond based on bottom position and radial direction with 4x (left side) and 10x (right side). (a) concave, (b) convex, (c) middle, dan (d) inner

Figure 7. Cross section photograph of fibrovascular bundles of Nipa frond based on intermediate position and radial direction with 4x (left side) and 10x (right side) magnification. (a) concave, (b) convex, (c) middle, dan (d) inner

Figure 8. Cross section photograph of fibrovascular bundles of Nipa frond based on upper position and radial direction with 4x (left side) and 10x (right side) magnification. (a) concave, (b) convex, (c) middle, dan (d) inner

3.2. Quantitative anatomical characteristics

Table 1 shows that the total area, vascular tissue area, and non-vascular tissue area of palm frond FVB range from 0.17 to 0.21 mm², 0.06 to 0.09 mm², and 0.10 to 0.12 mm², respectively. The bottom FVB shows a greater surface area at each radial position in comparison to the FVB of the middle and upper of the palm frond. The diameter of the vascular bundles decreases from the bottom to the upper, which is believed to be due to the influence of the vascular bundles' function. The upper serves as the growing point for meristematic tissue. Hence, the FVB area of nipa palm fronds may diminish progressively from the bottom to the upper. The FVB at the bottom, intermediate, and upper positions on the convex side exhibit greater overall FVB area, vascular tissue area, and non-vascular tissue area in comparison to the concave side and the middle and inner zones.

Nevertheless, the non-vascular tissue at the upper position exhibits a discrepancy in area between the outer zone (convex and concave) and the inner zone, with the former having a smaller size. Interestingly, the ratio of non-vascular tissue area to total transverse area (VTA:TTA) has a greater value than the ratio of vascular tissue to total transverse area (nVTA:TTA). Thus, the percentage of the vascular part is greater than the percentage of the sclerenchyma fiber part. This will have an impact on the strength properties of FVB.

The anatomical characteristics of the FVB of the palm frond vary in the bottom, intermediate, and upper. Generally, the outward position (convex) has a larger area compared to the middle and interior regions. When compared to the anatomical characteristics of Arenga longipes and Salacca zalacca, it was found that both species show a significant amount of FVB in the lower region of non-vascular tissue, as opposed to the middle and inner zones [11, 15]. Vascular bundle frond of Salacca Sumatrana fronds exhibited greater size and density at the wood grain (outer zone), whereas it gradually decreased in size and density towards the middle and inner zones [11]. Fibers enhance FVB density, whereas the presence of phloem tissue arteries has a diminishing effect on density. This is similarly comparable to the phenomenon of FVB observed in nipa palm fronds.

3.3. Fiber morphology

Figure 9. depicts the FVB of palm fronds in both longitudinal and radial directions, with various fiber lengths. Fiber lengths range from 1119.31 to 1330.75 µm. According to the International Association of Wood Anatomist (IAWA), the length of palm fiber fronds is classed as medium fiber class [19]. Palm fronds (*Nypa fruticans*) contain the largest fiber length of 1330.75 μ m in the middle of the concave zone, while the shortest fiber is in the bottom of the convex zone with a length of 1119.31 µm. Palm fronds have larger fiber lengths than other non-wood fibers, including cassava stems (650 µm), straw (780 µm), and corn stems (900 µm) [20]. The fibers are shorter than bagasse fibers at 2800 µm [21] and bamboo (*Bambusa vulgaris*) at 2140 µm [22]. Fiber length is an important component in evaluating fiber quality for producing pulp and paper. Fiber length contributes to the paper's tear strength. Long fibers have greater contact with other fibers and have higher structural continuity, resulting in a stronger network than short fibers [23]. Statistically, the longitudinal and radial positions of FVB fibers interact significantly and have a real effect on fiber dimension values. According to the results of additional DMRT tests, the length of FVB fibers in nipa palm fronds at the base of the convex section differs significantly from the concave and middle parts, but not significantly from the inner part. The convex part differs greatly from the entire FVB part in the radial position. Meanwhile, at the upper position, none of the parts differ much.

Figure 9. Histogram of variation fiber length based on longitudinal and radial direction of Nipa frond. CB convex; CK: concave; D1: middle; and D2: Inner

Figure 10. illustrates that the diameter of the FVB fibers of Nipa fronds ranges about 18.29 to 22.85 µm, with the maximum fiber diameter being ingested 22.85 µm at the concave bottom position and the smallest being 18.29 µm at the middle upper position. Palm fronds have a bigger fiber diameter than banana leaf fibers (13.75 µm) and cotton stems (17.2 µm) [20]. Nipa palm fronds have a smaller fiber diameter than hardwood like sengon (*Paraserianthes falcataria*) and acacia (*Acacia mangium*), which have fiber diameters of 43.28 µm and 30.75 µm, respectively [24]. This study found that palm frond fiber had a diameter of 25-26 μm, similar to surian wood (*Toona sureni*) fiber [25]. Fiber diameter influences pulp strength. Fibers with large diameters and thin walls can form strong bonds, resulting in a high strength pulp, whereas fibers with smaller diameters and thick cell walls are more difficult to flatten, reducing the surface of the fibers in making interface bonds [26]. Based on statistical analysis, the fiber diameters at the bottom and intermediate positions indicate that there are substantial differences among all FVB radial positions. The DMRT test reveals a considerable disparity between the outer zones (convex and concave) and the diameters seen in the middle and inner zones. Nevertheless, the upper location indicates a notable disparity between the convex zone and the remaining portion, while the concave and inner sections do not exhibit a major different.

Figure 10. Histogram of variation fiber diameter based on longitudinal and radial direction of Nipa frond. CB convex; CK: concave; D1: middle; and D2: Inner

Figure 11. illustrates that the lumen diameter of nipa fronds' FVB ranges from 6.74 to 10.36 µm. According to the International Association of Wood Anatomist (IAWA), the lumen diameter of nipa palm fronds comes into the small category [19]. The maximum lumen diameter is $10.36 \mu m$ at the base of the concave region, whereas the minimum lumen diameter is $6.74 \mu m$ at the tip of the middle section. The lumen diameter measured in this study exceeds the lumen diameter of yellow bamboo, which is reported as $4.20 \mu m$ [27]. The Duabanga moluccana has a lumen diameter of 39.18 μ m [24]. The mechanical action in the beating process of pulp processing is significantly influenced by the diameter of the lumen. The wider lumen diameter enhances the efficiency of the pulp beating process by allowing liquid to penetrate into the empty space within the fiber.

During the statistical analysis, it was found that the lumen diameter at the base position of the inner part showed a significant difference compared to the entire part. Similarly, the lumen diameter at the middle position of the inner part showed a significant difference compared to the convex and inner parts, but not compared to the concave part. The convex portion at the terminal position shows a notable dissimilarity from the remaining portion.

Figure 11. Histogram of variation lumen diameter based on longitudinal and radial direction of Nipa frond. CB convex; CK: concave; D1: middle; and D2: Inner

According to Figure 12, the FVB fiber cell wall thickness of Nipa fronds have a thickness ranging from 5.56 to 6.24 µm. According to the International Association of Wood Anatomist (IAWA), fiber cell wall thickness is classified as thin to thick walled fiber if its lumen is less than 3 times the cell wall thickness [19]. The maximum cell wall thickness measures 6.24 µm at the bottom of the concave zones, while the minimum cell wall thickness is 5.56 µm at the intermediate position of the concave zone. The cell wall thickness of palm fronds are thicker than those of birch and pine, measuring 2.15 µm and 2.70 µm, respectively [28]. Nevertheless, it has a smaller diameter when compared to the cell wall thickness of flamboyant fibers measuring 6.49 µm [29] and Pterocarpus angolensis fibers at 7.56 µm [30]. An increase in the cell wall thickness results in reduced contact between fibers on the sheet, hence impeding the creation of high-quality paper [29]. However, from a statistical analysis, there is no substantial variation in the cell wall thickness across different positions and zones.

Figure 12. Histogram of variation cell wall thickness based on longitudinal and radial direction of Nipa frond. CB convex; CK: concave; D1: middle; and D2: Inner

3.4. Fiber derivative properties

Table 2 illustrated the range of average Runkle ratio values for FVB fibers derived from nipa palm fronds at different positions, ranging from 1.18 to 1.88. Palm frond FVB fibers have a characteristic of having cell walls with a substantial thickness and a limited diameter of the central cavity. The Runkle ratio value of good grade

fiber for pulp and paper manufacture is less than 1. This characteristic ensures that the fiber is uniformly smooth on the pulp sheet and forms a highly durable connection. The pulp sheets obtained will exhibit significant tear and tensile strength [11]. A high Runkle ratio results in stiff fibers, reduced bond area, and poor bonding ability, leading to diminished mechanical strength of the fiber [31].

The mean felting power of palm leaf FVB fibers at each position falls within the range of 53.74 to 71.90. Fiber orientation is a crucial feature that significantly enhances the strength properties of paper. There is a positive correlation between the felting power value and both tear strength and fiber weave ability [32]. In other words, as the felting power value increases, the tear strength and fiber weave ability also improve. If the obtained felting power value is high, strong bonds will develop between the fibers, leading to increased folding resistance.

The mean Muhlsteph ratio of FVB fibers from nipa palm fronds at each position falls within the range of 78.67- 86.86. During the production of pulp and paper, the utilization of palm frond FVB fibers presents challenges in achieving a flattened structure. This is due to the limited bonding and contact area between fibers, resulting in sheets that have low tear strength and low tensile strength during the generation of pulp. Low Muhlsteph ratio values yield pulp and paper with elevated tear and tensile strength [33].

The mean coefficient rigidity of palm leaf FVB fibers at each site falls within the range of 0.27 to 0.32. The study revealed an inverse correlation between tensile strength and a direct correlation between pulp yield and density. In the paper industry, fibers with a coefficient rigidity value exceeding 0.75 will enhance the interfiber contact area. The observed phenomenon is attributed to a positive correlation between the diameter of the lumen and the diameter of the fiber [11].

The mean flexibility ratio of FVB fibers derived from palm fronds at each site falls within the range of 0.24 to 0.46. Pulping processes favor high FR values. A high flexibility ratio value signifies that the fiber possesses slender walls and exhibits a high degree of malleability. The fiber's capacity for deformation enhances the contact between fiber surfaces, leading to the development of more efficient fiber bonds during the production of pulp sheets [11]. Increasing the flexibility ratio will enhance the bonding area between fibers, resulting in pulp with high tensile strength. This will improve mechanical attributes such as the rupture index and folding resistance, ultimately leading to paper with excellent long-term breaking strength [31].

4. Conclusion

The overall surface area of fibrovascular bundle, vascular tissue, non-vascular tissue, in the Nipa frond was generally larger at the bottom position compared to the intermediate and upper regions. The anatomical characteristic of the FVB of palm fronds gradually decrease from the convex of bottom position to the upper in each zone. The longest fiber is located in the central location of the concave zones, while the biggest fiber diameter, lumen diameter, and cell wall thickness are all found near the base of the concave zone. According to the evaluation of fiber derivatives, Nipa frond FVB exhibits favorable fiber quality for utilization as a raw material in the production of pulp and paper.

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