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Analysis of Lignocellulosic in Various Parts of Nipa Palm (*Nypa fruticans*) Biomass from the Rammang-Rammang Maros Rivers

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The present study aims to characterize the various parts of Nypa palms (*Nypa fruticans*) in order to establish the overall utilization of this biomass as a potential feedstock for chemistry manufacture. *Nypa fruticans* is chemically characterized using The Van Soest Method to identify the content of cellulose, hemicellulose, and lignin molecules. Our findings implied that the total chemical composition of cellulose, hemicellulose, and lignin were obtained to be 36.73-49.20, 2.56-19.11, 11.08-26.72%, respectively. Futhermore, the highest cellulose and hemicellulose contents were found in the frond and shell, respectively. Meanwhile, the highest lignin content was obtained in the husk. The IR spectra examined from 4000 to 500 cm^{-1} of the frond and husk biomass samples clearly showed the typical absorption peaks of cellulose, hemicellulose, and lignin. This indicates the presence of these three components in the *Nypa fruticans* biomass. Overall, each part of nipa palms has superior characteristics and can be used as a source of lignocellulosic for the manufacture of advanced material.

Keywords: *Nypa fruticans*; lignocellulosic; biomass; Van Soest; analysis

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Nypa fruticans, commonly known as Nipa, is a monoecious plant with distinctive characteristics. Unlike its counterparts in the palm family, *Nypa fruticans* exhibits robust growth in river estuaries and brackish water environments. *Nypa fruticans* has a sloping stem with no distinctive stem structure, and its fruits develop at low elevations [1]. The distribution of this plant species is mainly in the equatorial region, extending from Sri Lanka to Southeast Asia to Northern Australia. The plantation area of *Nypa fruticans* in Indonesia is estimated at 700,000 ha, the largest compared to Papua New Guinea (500,000 ha) and the Philippines (8,000 ha) [2]. Nipah trees thrive in Southeast Asia from the north to the Philippines, including Indonesia. In Indonesia, the Rammang-Rammang Geopark is one of the areas that has an abundance of nipah trees. This area is located in South Sulawesi Province, precisely in Maros Regency. Based on data from The Central Bureau of Statistic in South Sulawesi province on 2021, the land area of *Nypa fruticans* in the Rammang-Rammang area ranges from 4000-5000 ha [3]. This shows that *Nypa fruticans* in the area have a lot of potential to be explored.

Nypa fruticans is a mangrove plant that has palm-like tree characteristics and is non-woody. *Nypa fruticans* contains major constituents such as cellulose, hemicellulose, and lignin, making it classified as lignocellulosic biomass [4]. Lignocellulosic biomass plays a very important role as a resource that has abundant carbon content in its chemical structure. Cellulose is widely used as a base material for edible films such as membranes and bioplastics. Hemicellulose is used as a source of producing useful additive compounds such as furfural, 5-hydroxymethylfurfural, and levulinate acid, while lignin can be used as a polymer additive aimed at improving mechanical strength, enhancing antioxidant and antimicrobial activity, and UV protection [5]. Indeed, this indicates that *Nypa fruticans* has great potential as a raw material in various chemical applications such as edible film fabrication, biofuels, a raw material for membrane manufacturing, adsorbents, and various other chemical applications.

Optimising the use of *Nypa fruticans* in several applications is largely determined by an initial understanding of the chemical composition of the biomass. In addition, the main problem of any lignocellulosic biomass processing is the presence of chemical bonds between the carbohydrate and lignin fractions. Therefore, an understanding of the initial

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content of lignocellulose in biomass is essential both for selecting a processing method and for determining a more favourable product. In addition to chemical reasons, the initial identification of the chemical composition of biomass is also important based on the geographical aspects of the plant's habitat. According to study by [6] reported that the different soil chemical characteristics of various biomass growing locations greatly affect its chemical composition. This is supported by the research of [7] who reported the influence of the location of the place of growth on essential oil content in basil leaves based on the height of the location. Although research related to the utilisation of *Nypa fruticans* has been conducted, it does not provide accurate information regarding the chemical composition of nipah in general.

Based on the results of the author's investigation, information related to the analysis of the chemical composition of Nipah trees is very limited. Only two studies were found that analysed and identified the chemical composition of Nipah in a complete and comprehensive manner. In the paper presented by [2] analysed the complete chemical composition of nipah from Balobo Village, Philippines using the TAPPI method. On the other hand, Evelyn and co-workers [8] analysed the chemical composition of nipah from Muntai Village, Bengkalis, Indonesia, using similar method. Therefore, the purpose of this study was to analyse and characterise the chemical composition of lignocellulose in *Nipa fruticans* obtained from the Rammang-rammang River Area qualitatively and quantitatively. A qualitative analysis was conducted using a spectrophotometric method, while a quantitative analysis was conducted using van soest method. Furthermore, the outcomes of the lignocellulose component analysis were contrasted with those of earlier analyses, which can serve as a basis for the use of *Nypa* cellulose from the Rammang-rammang area.

EXPERIMENTAL

Chemicals and Materials

Chemicals used in this work include nipa palm (*Nypa fruticans*) parts obtained from the Rammang-Rammang Water Area, Maros, South Sulawesi, Indonesia, distilled water (Merck), Sulfuric acid 72 % (Merck), acid detergent solution and neutral detergent solution (Sigma-Aldrich), acetone (Merck).

Sample Preparation and Pre-Treatment

The nipa (*Nypa fruticans*) samples used in this study were obtained from the Rammang-Rammang Water Area, Salenrang Village, Maros Regency, South Sulawesi Province, Indonesia. The *Nypa fruticans* parts used consisted of frond, shell, and husk as shown in Figure 1. Prior to analysis, the *Nypa fruticans* were cleaned with water to remove soil and other impurities, then further dried in the sun to remove excess water and moisture. Next, the three nipah parts were cut into small pieces, then ground using a dismill. The milled samples were sieved using a 100 meshsieve. Next, the samples were dried in an oven at 45 ^{o}C for 2 hours and stored in a desiccator before being used in the analysis stage [9].

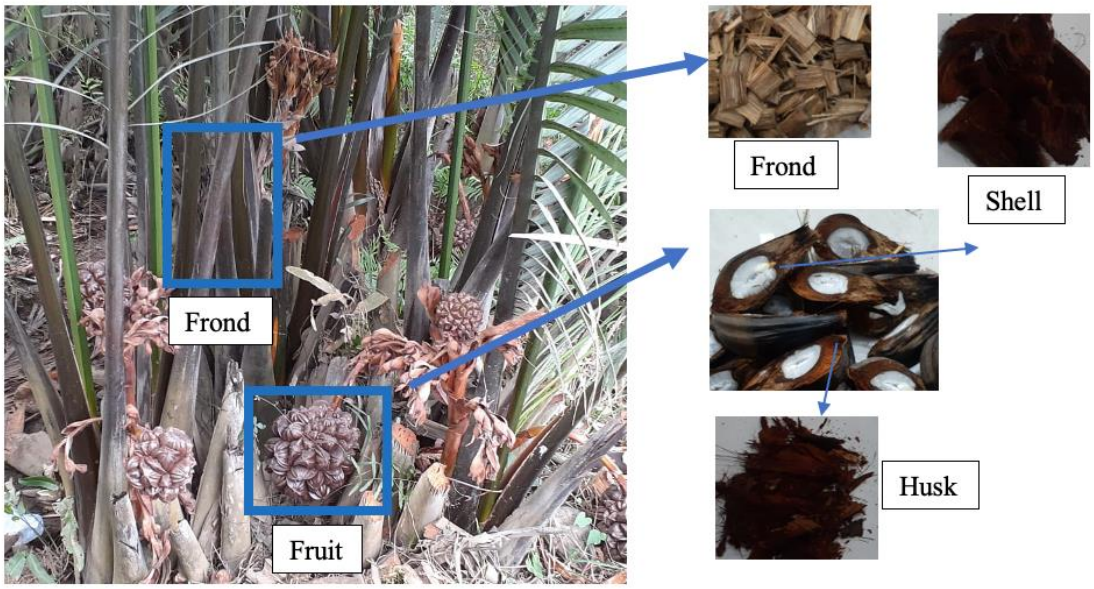


Figure 1. Various parts of nipa palm (*Nipa fruticans*) used in this study.

FTIR Analysis

The analysis was conducted on the dried biomass samples of *Nypa fruticans* using FTIR Spectrophotometer. This analysis was carried out to strengthen the quantitative analysis related to the presence of cellulose, hemicellulose, and lignin components in the biomass sample. Samples of nipah palm fronds and fibre skin were weighed as much as 1 gram. Next, the samples were mixed with KBr pellets and analysed using an FTIR Spectrophotometer to determine the functional groups contained in the biomass samples. FTIR spectra were interpreted using library data of compound functional groups [10].

Determination of Chemical Composition of Nipah Palm (*Nipa fruticans*)

The determination of the chemical composition carried out in this study consisted of the determination of hemicellulose, cellulose, lignin, and ash content in percentage. The method employed was the Van Soest method, a modification derived from the Chesson-Datta method. Lignocellulose analysis using the van soest method consists of three steps, namely the determination of NDF content, the determination of ADF content, and the determination of lignocellulose content [11]. In the first step, 0.3 g (a_1) of *Nypa fruticans* biomass sample was added to 45 mL of neutral detergent solution to determine the NDF content. The solution was closed tightly and placed in boiling water for 1 h then filtered using a sintered glass (b_1) grams with a vacuum pump. The samples were washed using acetone and hot water, then dried in the oven at 60 ^oC for 8 h. The final weighing result was calculated as (c_1) grams. Next step, the determination of the ADF content was carried out using a similar method as NDF content determination, but an acid detergent solution was used instead of neutral detergent solution. In this step, the weight of the initial sample was (a_2) grams, sand glass (b_2) grams, and the final weighing result was calculated as (c_2) grams. The final step, determining the lignocellulose content was carried out by hydrolysis of the remaining fiber from the ADF analysis (c_2) with H_2SO_4 (72%, w/v). The fibers were washed and dried in the oven at 105 ^oC, and the weighing result was calculated as (d) grams. Then, the sample was burned in a furnace at 500 ^oC, cooled in a desiccator, and the weighing result was calculated as (e) grams. Determination of NDF, ADF and lignocellulose concentration (%) is based on the weight difference between the pre-and the post-treatment. The cellulose, hemicellulose, and lignin contents were calculated using the following equations [12].

$$\text{NDF (\%)} = \frac{c_1 - b_1}{a_1} \text{ x } 100\% \quad (1)$$

$$\text{ADF (\%)} = \frac{c_2 - b_2}{a_2} \text{ x } 100\% \quad (2)$$

$$\text{Lignin (\%)} = \frac{e - d}{a_2} \text{ x } 100\% \quad (3)$$

$$\text{Cellulose (\%)} = \text{ADF} - \text{Lignin} - \text{insoluble ash} \quad (4)$$

$$\text{Hemicellulose} = \text{NDF} - \text{ADF} \quad (5)$$

where a_1 and a_2 were the weight of the original sample, b_1 and b_2 means the weight of empty sintered glass, c_1 and c_2 denote the weight of sintered glass plus the remaining filtering after drying, d means the weight of sintered glass plus lignin and insoluble ash, meanwhile denote the weight of sintered glass and insoluble ash after burned in the furnace.

RESULTS AND DISCUSSION

Fourier Transform Infrared Analysis

FTIR analysis was performed to identify the different functional groups in the dried biomass of *Nypa fruticans* with different parts (frond and husk). The spectra obtained are illustrated in Figures 2 and 3. *Nypa fruticans* has the main components of cellulose, hemicellulose, and lignin. The molecular structure of these components can be seen in Figure 4. The three components are composed of alkane groups, esters, alcohol, and aromatic compounds. The FTIR spectra of the two parts are shown in Table 1. The absorption peaks in the range of 3516 - 3111 cm^{-1} in the frond and husk samples show the -OH stretching groups found in both cellulose, hemicellulose, and lignin. The -OH group binds intramolecularly and intermolecularly. Similar results were reported by [10, 13]. Sharp absorption was also shown at 2359 cm^{-1} in the frond sample and 2357 cm^{-1} in the husk sample indicating asymmetric methyl groups and methylene stretching which are functional groups of lignin, cellulose, and hemicellulose [8]. The aliphatic C-H group in FTIR testing is indicated by the presence of peaks in the 2945.40 cm^{-1} and 2947.33 cm^{-1} regions with weak intensity. In the 1703 cm^{-1} and 1701 cm^{-1} regions, there is sharp intensity absorption indicating the presence of C=O stretching in unconjugated ketone groups, carbonyl groups, and aliphatic groups which are typical of hemicellulose [2, 14, 15].

Table 1. Assignment of peaks in FTIR spectra of *Nypa fruticans.*

Peak	Wavenumber (cm^{-1})		Assignment
	Frond	Husk	
1	3518.28-3111.28	3516.35-3020.63	Lignin, cellulose and hemicellulose: -OH intramolecular and intermolecular
2	2945.40	2947.33	Lignin, cellulose and hemicellulose: symmetric methyl and methylene stretching.
3	2752.51- 2359.02	2879.82-2357.09	Lignin and hemicellulose: asymmetric methyl and methylene stretching.
4	1703.20	1701.27	Hemicellulose: C = O stretching in unconjugated ketone, carbonyl, and aliphatic groups. Extractives: C = O stretching in ester carbonyl.
5	1653.05	1651.12	Flavones and calcium oxalate: C = O stretching
6	1585.54-1546.96	1585.54-1546.96	Lignin and extractives: aromatic ring vibration, aromatic skeletal vibrations.
7	1479.45-1437.02	1487.17-1437.02	Cellulose, lignin, and hemicellulose: O-H in-plane bending
8	1346.36	1346.36	C-O deformation
9	1236.41	1234.48	Polysaccharides: C-O stretch and O-H in plane, C=C aromatic ring
10	1190.12	1190.12	Cellulose: C–O–C asymmetric stretching
11	1118.75	1116.82	Cellulose and hemicellulose: C–O–C stretching. Lignin: Aromatic C–H in-plane deformation.
12	1037.74	1039.67	Holocellulose and lignin: C–O stretching.
13	974.08	974.08	Cellulose: C–H deformation

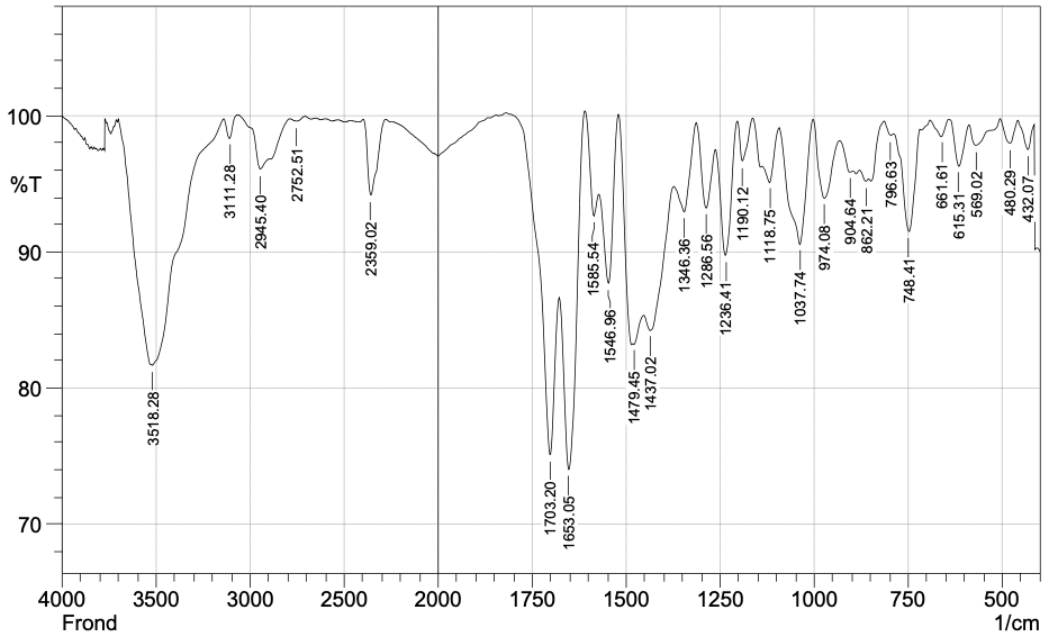


Figure 2. FTIR Spectra of frond part.

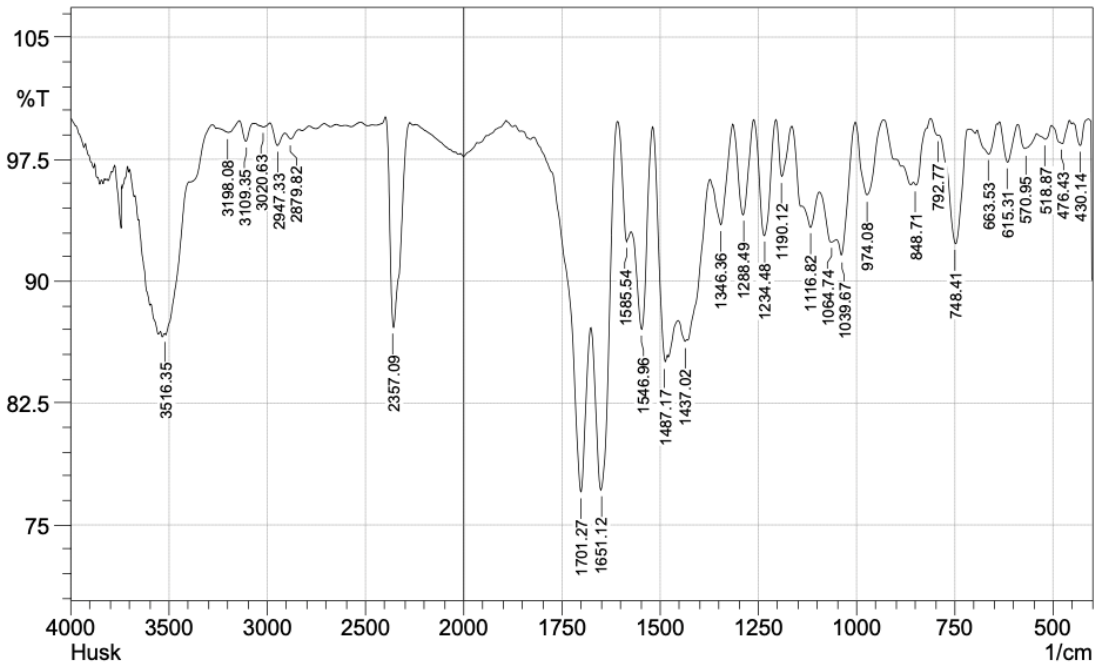
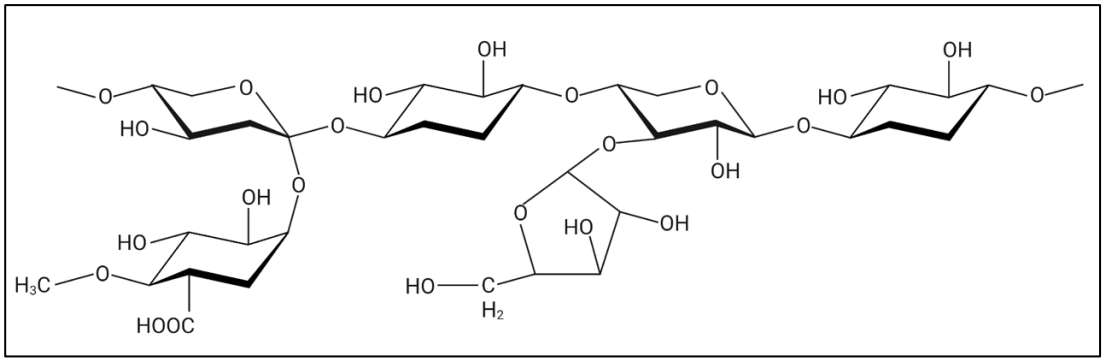
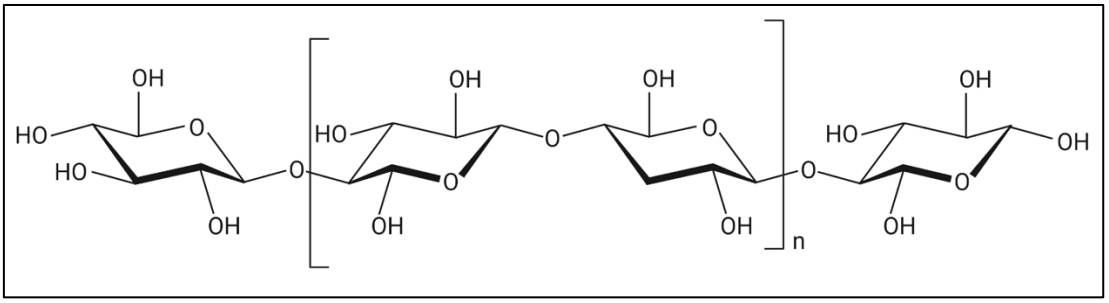


Figure 3. FTIR Spectra of Husk part.



(a)



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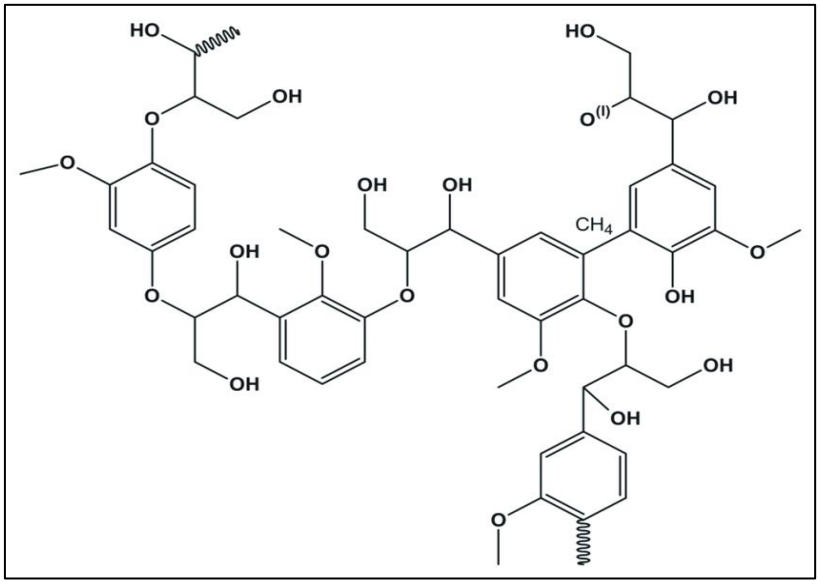


(c)

Figure 4. Molecular Structure of Hemicellulose (a), Cellulose (b), and Lignin (c).

A sharp absorption is shown in the wave number region of 1546 cm^{-1} indicating aromatic ring vibrations of lignin and other extractive components. This absorption was found in both parts of the nipah palm. Similarly, it was reported by [16] that the absorption peak in this region indicates the presence of aromatic ring vibrations of guaiacyl and syringyl fragments. The absorption peak is used in determining the lignin content quantitatively.

The presence of cellulose and hemicellulose in fronds and husks is shown in the wave number range 1190 - 1037 cm^{-1}. In both parts, there is an absorption peak in the 1190 cm^{-1} region which shows the asymmetric stretching of the C-O-C group of cellulose [17, 18, 19]. The absorption peak in the 1118 cm^{-1} region shows the C-O-C stretch in cellulose and hemicellulose [20, 21], the same thing reported by Mohammed and co-workers [22]. A strong absorption peak at 1037.74 cm^{-1} was shown in the spectra of the frond sample indicating the C-O stretch in holocellulose and lignin, which is one of the typical peaks of cellulose compounds in the fingerprint region. This is similar to that reported by Kostryukov and co-workers [16] who used the absorption band in this region to determine the cellulose content quantitatively by deconvolution of IR spectra. However, in the husk sample, the peak for this group is in the region of 1039.67 cm^{-1} and has a peak division with an absorption band in the region of 1064.74 cm^{-1} thus reducing the intensity of the peak and two peaks are seen.

Lignocellulose Analysis

Table 2 shows the percentage of the chemical composition of dry biomass in three parts of *Nypa fruticans* (frond, shell, and husk) consisting of cellulose, hemicellulose, lignin, and insoluble ash content. The cellulose content determined was the total cellulose content in general, which was in the range of 36-49% wt in all three parts of the nipa palm. The highest cellulose content was found in the fronds at 49.20% wt, followed by the shell at 48.55 %wt, and the lowest cellulose content was obtained in the husk at 36.73% wt. In contrast to cellulose, the highest hemicellulose content was obtained in the shell section at 19.11% wt. Of the three parts, it can be seen that the lowest cellulose and hemicellulose content is obtained in the husk, but it has the highest lignin content of 26.72% wt. The high lignin content in the husk was confirmed by the FTIR spectra of the husk at 1546 cm^{-1} (aromatic ring vibrations of lignin) [14, 16, 19], which had a greater absorption intensity than the FTIR spectra of the frond in the same region. In contrast, the low cellulose content of the husk was confirmed by the typical cellulose peak at 1037 cm^{-1} (C-O stretching) [23, 24] which showed peak splitting resulting in low absorption intensity. It was different from the FTIR spectra of the frond in that the region does not show peak cleavage, so the absorption intensity is higher.

The percentages of cellulose, hemicellulose, and lignin in each section of *Nypa fruticans* are summarized in Figure 5, which makes it clear that cellulose components constitute the majority of *Nypa* biomass, while hemicellulose and lignin were present in smaller amounts. These findings seem to be in compliance with earlier research's examination of Nypa chemical components by Tamunaidu and Saka [2] and Evelyn and co-workers [8].

Table 2. Chemical Composition of *Nypa fruticans.*

No	Part of Nipa	Composition (%)					
		ADF	NDF	Cellulose	Hemicellulose	Lignin	Insoluble ash
1.	Shell	60.63	79.74	48.55	19.11	11.08	1.00
2.	Husk	64.47	66.04	36.73	2.56	26.72	0.03
3.	Frond	60.80	78.19	49.20	17.39	11.11	0.49

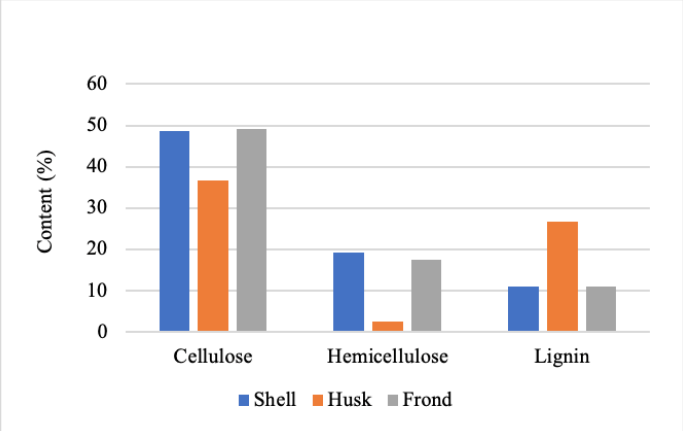
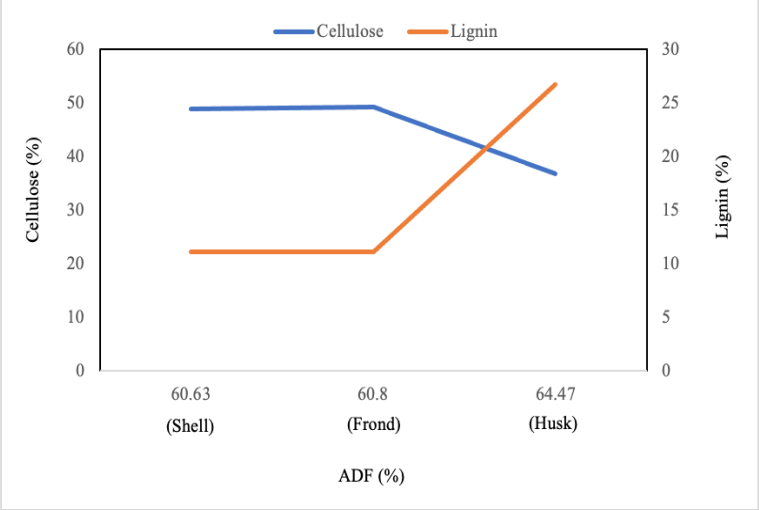


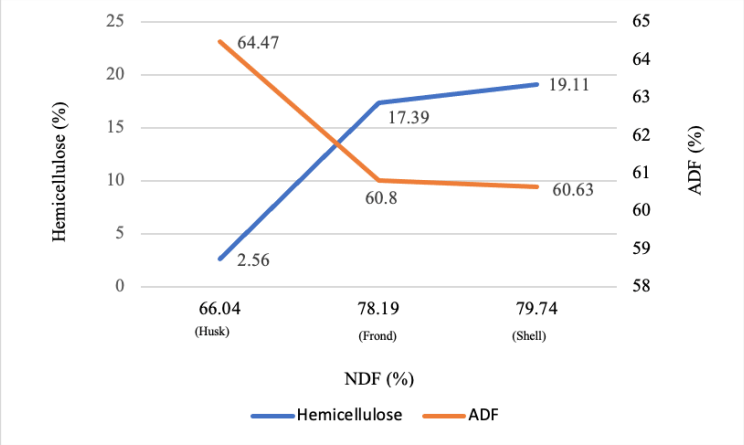
Figure 5. Percentage of cellulose, hemicellulose and lignin content in the three parts of *Nypa fruticans*.

Table 3 shows a comparison of the chemical composition of *Nypa fruticans* from three regions. According to the data, the chemical composition of *Nypa fruticans* analyzed in this work was different from the results of the analysis reported by Tamunaidu and Saka [2] using the TAPPI method, where the highest cellulose content was obtained in the shell at 45.60% wt, and the lowest cellulose content was obtained in the frond at 35.10% wt. Another study by Evelyn and co-workers [8] using the TAPPI method also obtained cellulose content in the frond of 37.30% wt. The TAPPI method was employed for analyzing lignocellulose in pulp and paper, utilizing gravimetric principles and the addition of specific reagents. In contrast, the van soest method employs sequential extraction to produce different fiber fractions (neutral detergent fiber and acid detergent fiber) [25]. Unlike the van soest method, the TAPPI method does not facilitate the separation of cellulose, hemicellulose, and lignin, thereby providing a more generalized estimate of fiber content and limiting the ability to conduct a detailed analysis of lignocellulosic composition in samples.

The Acid Detergent Fiber (ADF) fraction contains cellulose and lignin, whereas the Neutral Detergent Fiber (NDF) fraction is associated with hemicellulose [12]. Thus, the proportion of the ADF fraction and the percentage of lignin substantially determines the cellulose concentration, whereas the percentage of NDF and ADF determines the hemicellulose concentration. In this study, the association between the percentages of lignin, cellulose, and ADF was illustrated in Figure 6a. The higher cellulose concentration identified in the frond and shell was correlated with an increasing proportion of ADF fraction. However, because of the high lignin component, the cellulose concentration drops in the husk portion. An increasing proportion of NDF fraction was correlated with larger concentrations of hemicellulose, as seen in Figure 6b, which exhibits a linear relationship between both of them. Nevertheless, a non-linear relationship was seen between the hemicellulose and ADF fractions.



(a)



(b)

Figure 6. Correlation between ADF, cellulose, and lignin contents (a), correlation between NDF, ADF, and hemicellulose content (b).

Table 3. Comparison of Chemical Composition of *Nypa fruticans.*

Part of *Nypa*	**Cellulose (%)**	**Hemicellulose (%)**	**Lignin (%)**	**Ash (%)**	**Analysis Method**	**Region**	**Ref.**
Frond	35.10	26.40	17.80	11.70	TAPPI Method	Balobo Village, Philippines	Tamunaidu and Saka (2011)
Shell	45.60	23.50	17.30	8.20			
Husk	36.50	21.80	27.30	8.10			
Frond	37.30	24.0	18.30	16.50	TAPPI Method	Muntai village, Bengkalis, Indonesia	Evelyn et al, 2022
Shell	n.d	n.d	n.d	n.d			
Husk	n.d	n.d	n.d	n.d			
Frond	49.20	17.39	11.11	0.49	Van soest Method	Rammang-ramming river, Maros, Indonesia	This work
Shell	48.55	19.11	11.08	1.00			
Husk	36.73	2.56	26.72	0.03			

Noted: n.d (not detected)

Table 3 shows the van soest analysis method used in this study shows analysis results with a higher cellulose content than previous studies. This result aligns with the research conducted by Loelovich [26] utilizing standard samples of whatman filter paper containing 98% α-cellulose, further investigated the efficacy of numerous cellulose determination methods (TAPPI method, NREL method, and van soest method). According to the study, the NREL and van Soest methods yielded 92% and 93% of cellulose, respectively. While, the TAPPI approach obtained only 88%. From this result, the TAPPI method cannot be used to reliably determine the content of polysaccharides (cellulose, hemicellulose, and lignin), which is probably due to a decrease in the output of intermediate products after each subsequent stage as previously described.

The high cellulose content obtained in this study correlates with lower hemicellulose and lignin contents. The highest hemicellulose content of 19.11 wt% was obtained in the shell, while the highest lignin content was obtained in the husk at 26.72 wt%, which had the lowest cellulose and hemicellulose content. The roles and structures of the three parts of *Nypa fruticans* in plant growth are related to the differences in their lignocellulosic composition. It's probably that the frond includes larger amounts of cellulose than the other parts due to the fact it provides the primary structural support for *Nypa fruticans*. Furthermore, the formation and accumulation of plant cell components can be affected by environmental factors such temperature, humidity, light, and soil conditions. Variations in environmental conditions can lead to variations in the chemical composition of particular plant components [12]. Although the cellulose content of the fronds is almost 50% by weight, it is still low compared to wood materials. However, the availability and abundance of *Nypa fruticans* in the ramming-rammang river area of Maros Regency, South Sulawesi, Indonesia, can provide a legitimate basis for exploitation as a raw material in the synthesis of edible film materials, fuels, and other chemical processes. The chemical composition of cellulose, hemicellulose, and lignin is closely related to the Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) components, which have weight percentages in the range of 60-64 and 66-79% in the three parts of *Nypa fruticans*, respectively. ADF includes mostly cellulose and lignin, while NDF includes ADF as well as hemicellulose [8].

A minor fraction in *Nypa fruticans* that represents the non-biodegradable components present in biomass is insoluble ash. *Nypa fruticans* contains a higher ash content than most lignocellulosic materials, such as bagasse, wood, and even other palm tree biomass. In this study, the highest insoluble ash content of 1% wt was obtained in the shell, followed by frond and husk at 0.49 and 0.03% wt, respectively. This result is quite different from the research reported by Tamunaidu and Saka [2], who obtained the highest ash content of 11.7% wt in nipah fronds and the lowest ash content of 5.1% wt in leaves, while in shells and husks it was 8.2 and 8.1% wt, respectively. This confirms that the different analytical methods used greatly affect the chemical composition values obtained for the same type of biomass. In addition, differences in the location of biomass growth also affect the chemical content of biomass because the intake of nutrients into the plant through the roots is highly dependent on soil fertility and nutrient content [27]. According to a study by Manullang and Rezki [6] reported that factors such as soil moisture content, sunlight levels, and temperature can affect photosynthesis, plant growth, and ultimately the chemical composition of biomass. In addition, different soil types can also affect the availability of nutrients and minerals for plants.

The Research by Nur and Baitanu [7] reported the effect of the place of growth on the chemical content profile of basil leaf essential oil. The results showed that basil leaves grown in the highlands (Tinggimoncong District, South Sulawesi, Indonesia) had a higher essential oil content than basil leaves grown in the lowlands (Bontoala District, Makassar, Indonesia). Another study by Diez and co-workers [20] reported that the chemical composition of plants is strongly influenced by the altitude at which they grow. This result aligns with the study by Mega and Beta [28] identifying the effect of the height of the growing place on the content of caffeine compounds in coffee, showing that coffee plants growing in higher locations have a greater content of caffeine compounds. Therefore, based on these reasons, it can be assumed that the difference in chemical content (cellulose, hemicellulose, and lignin) between *Nypa fruticans* identified in this study and previous studies is strongly influenced by the place of growth.

CONCLUSION

Based on the results of the analysis using the Van Soest method, it was found that the cellulose, hemicellulose, lignin, and insoluble ash content of the three parts of *Nypa fruticans* were different. The highest cellulose content was obtained in the frond section at 49.20 wt%. The highest hemicellulose content of 19.11 wt% was found in the shell, while the highest lignin content was found in the husk at 26.72 wt%. The results demonstrate that the chemical composition of *Nypa fruticans* growing in the Rammang-Rammang region, Maros, Indonesia is different from the chemical composition of *Nypa fruticans* from other regions. Apart from regional differences, differences in the analytical methods used also influence the identification results of the chemical composition of *Nypa fruticans*. The high cellulose content in *Nypa fruticans* analyzed in this study provides an illustration that *Nypa fruticans* biomass has great potential to be used as raw material for the fabrication of edible films, fuel, and other applications in the field of materials chemistry.

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