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Analytical Chemistry of *Bambusa vulgaris* and *Schizostachyum brachycladum* on 2- and 4-Years Old with Transmission Electron Microscopy.

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ABSTRACT

The changes of the primary chemical compositions in 2- and 4-year-old *Bambusa vulgaris* and *Schizostachyum brachycladum* were investigated. The bamboo culms of known age 2- and 4-year-old harvested from the forest reserve area in Jeli, Kelantan. The bamboo culms segregated into three portions namely the bottom, middle and top. They were then processed and analysed in their primary chemical compositions such as extractive, holocellulose, α-cellulose, hemicellulose, and lignin content. Standard outlined by the Technical Association of the Pulp and Paper Industry (TAPPI) followed. The bottom portion of 4-year-old *B. vulgaris* species possesses higher extractive content to compare the other portions, ages, and species. *B. vulgaris* also possessed a higher percentage of holocellulose yield with over than 90% on 2-year age compared to the *S. brachycladum* species. *S. brachycladum* possessed a higher percentage in α-cellulose and lignin content in the two-year-old culms while hemicellulose showed slightly higher of yield in the 4-year-old. *B. vulgaris* and exhibited an increment in lignin and α-cellulose from 4 to 6% from 2 to 4-year-olds culms, while *S. brachycladum* showed an increase of 1-15% in the holocellulose, α-cellulose and lignin. The high chemical content was related to the high density that gives influence to their hardness, strength, wall thickness and complex cell wall structure.

Keywords: chemical composition, extractive, alpha cellulose, holocellulose, hemicellulose, lignin

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INTRODUCTION

Bamboo is among the most popular and abundant non-timber forest resources in the world [1,2,3]. It is the most significant member of the grass family (*Gramineae*) consisting of more than 1250 species with 75 genera recorded in the worldwide [4,5,33]. About 70 species of bamboo are found growing in Malaysia which comprises about 7% of the lowland and highland forests areas. These bamboos consisted of 45 indigenous and 25 cultivated species [6, 7]. Bamboo is also a fast-growing woody plant and can mature within 3 - 4 years after cultivating [2,8]. It possesses high mechanical properties and currently being considered as an alternative to wood [9]. The distinctive part of bamboo called as culm. It contains most of the woody material with straight, hollow and cylindrical formed of nodes and internodes. Also characterize with diameter tapers from the bottom to the top with the reduction in culm wall thickness [35].

The primary chemical composition in the bamboo culms consisting of cellulose, hemicelluloses, lignin, and extractive. According to [10,11], the chemical contents comprised of cellulose, hemicellulose, and lignin, with the composition of 90%-98% located mostly in the cell wall structure, while the extractive content at 2-10%. Lignin and hemicelluloses (each amounted to about 20-30%), pentosans (20-25%), and holocellulose (60-70%) [12]. While the waxes, tannins, resins and inorganic salts considered as the minor constituents. The cell structure of natural fibers is complicated because each fiber is essentially a composite in which rigid cellulose microfibrils embedded in a soft matrix that mainly composed of lignin and hemicellulose [13]. The woody plant extractives consist of substances such as resin acid, fatty acid, terpenoid compounds and alcohols and most of these substances are soluble in water or neutral organic solvent [14]. The primary chemical composition of bamboo is similar to wood, over 90% consists of cellulose, hemicelluloses, and lignin [1,15,16]. On other hands, hemicelluloses defined as differing from cellulose comprising a series of sugar monomers such as xylose, mannose, glucose, galactose, and others [17]. Lignin is a phenolic heteropolymer constitutes the second most abundant organic constituent on earth after cellulose [18]. The high lignin content makes bamboo fiber more brittle in comparison to other natural plants [19]. Lignin also presents in all vascular plants, and they represent about 25% of the plant biomass [1,20]. The main chemical constituents in bamboo vary depending on the species, age, positions in the culms length and habitat where they grow. The thick bamboo culms chemical constituents so far undertook by researchers, but no previous studies have been recorded on the thin wall of a bamboo culm.

Thus, this study overcomes to compare the chemical constituents between thick and thin species of bamboo due to the difference of age and location of bamboo culms.

MATERIAL AND METHODS

The 2 and 4 year-old culms of natural grown *B. vulgaris* and *S. brachycladum* species randomly selected and harvested from the forest reserve areas in Jeli, Kelantan (the study area composed of tropical rainforest with temperatures ranging from 21° to 32°C, and average annual rain fall from 3000 to 4000 mm per year). The age of selected bamboo culms was identified by a bamboo expert through their morphological characteristics. The bamboo culms selected having diameters 3 to 6 cm, culm length 8 to 15 m and culm thickness 4 to 5 mm for *S. brachycladum*. The diameter, culm length and culm thickness for *B. vulgaris* were 12 to 14 cm, 10 to 20 m and 4 to 10 cm, respectively [21]. They were segregated into portions bottom, middle and top. The culms were cut approximately 30 cm from ground level. Each culm of selected bamboo was cut to the length of 12 meters leaving out the top parts with branches and later subdivided into three equal lengths at about 4 meters per each. The portions were later grounded into powdered form and screened to collect the comparable size of the sample for subsequent studies. The chemical composition analysis used on the bamboo fiber. The chemical properties on *B. vulgaris* and *S. brachycladum* studied to determine the amount of extractive, holocellulose, hemicellulose, α-cellulose, and lignin content present. In this paper, the chemical composition in the cell wall structure investigated. The most widely used methods are variants of the method described by TAPPI standards [22,23].

This study involves the material preparation, determination of extractive, holocellulose, cellulose and lignin content.

Fiber Extraction Procedure

The extractive contents of the two (2) selected bamboo determined according to the method suggested TAPPI standard [24]. The grounded powder form of this bamboo at 10 gm placed in the extraction thimble size 125 mm x 30 mm. The solvent used in the extraction process were ethanol and benzene (at ratio 2:1). About 300 ml of the solution (ethanol and benzene) used in the 5 - 7 hrs extraction process. The solvent contained extractive evaporated using a Rotary Evaporator machine to remove all the solution used. The extractive obtained dried in an oven at 103 ± 2ºC for 24 hrs, and the percentage of extractive content calculated.

Holocellulose Procedure

The holocellulose content determined by studies conducted by [7,21]. Five (5) gram air-dried free extractive powder used as a sample for holocellulose analysis. The total of 9 grams of Sodium Chloride ($NaClO_2$), 30 ml of 10% acetic acid and 100 ml of distilled water mixed with a free extractive sample for a period of up to 5 hrs. The residue heated and stirred at 70ºC before the percentage of holocellulose calculated.

Alpha Cellulose Procedure

The α-cellulose determined according to TAPPI standard [25]. Two (2) gm of the holocellulose powder form used as a sample for α-cellulose content analysis. Altogether 75 ml of 17.5% NaOH in stirring condition for 15 minutes were mixed slowly with holocellulose powder. Distilled water about 100 ml added to the mixture and continuously stirred. After 30 minutes, the sample filtrated and washed with 8.3% NaOH and 2N acetic acid. The sample left to dry and weighed to measure the percentage of α-cellulose.

Lignin Content Analyses

Lignin content determined according to TAPPI standard [26]. The dry air of extractive-free sample used to determine the lignin content on the part of bamboo species. One (1) gm extractive free sample mixed with 25 ml of 72% sulphuric acid (H_2SO_4) then slowly stirred for 2 hrs in cold condition. 560 ml of distilled water added and heated at 180ºC for 4 hrs. The mixture then filtrated and dried before weighed.

RESULTS AND DISCUSSION

In this study, analytical chemistry properties on *Bambusa vulgaris* and *Schizostachyum brachycladum* was determined to analyses their percentages of extractive, holocellulose, hemicellulose, alpha cellulose, and lignin content.

Extractive Content of Two Selected Bamboo Species Using Solvent

Overall the results (see Figure 1) highlighting the extractive contents in the two bamboo species which ranged between 2.6% to 6.5% of the overall chemical contents. Comparison between the two species shows *B. vulgaris* contained 4.15% and *S. brachycladum* 4.04% of extractive. *B. vulgaris* has the high average extractive content in the 2-year-old culms of 4.30%, and *S. brachycladum contain* 4.07% extractive in the 4-year-old culms. Comparison at a different position for both species shows the average extractive content at top portion possess 3.20%, followed by middle at 3.76%, and bottom at 4.00%. The bottom of the two-year-old *S. brachycladum* possesses higher extractive content with 5.28% compared to the top portion with 2.96% while the 4-year-old *S. brachycladum* at bottom portion possess higher extractive content against the top portion with 4.54% and 3.59%, respectively (see Figure 1). The *B. vulgaris* shows overall slightly fewer percentages in the extractive contents. The bottom of the two-year-old *B. vulgaris* with 4.96% indicates marginally higher extractive content compared to the top with 3.67%. While the four-year-old *B. vulgaris* shows, the bottom portion had higher extractive content against the top portion *B. vulgaris* with value 6.51% and 2.57%, respectively.

At different ages, *B. vulgaris* possess the extractive content of 4.96%, 4.27% and 3.67% for the 2-year-old and 6.51%, 2.92%, and 2.57% for the 4-year-old which slightly increases from top to the bottom, respectively. *S. brachycladum* possess 5.28%, 3.76% and 2.96% for 2-year-old and 4.54%, 4.08% and 3.59% for

4-year-old culms which decreases from the bottom to the top, respectively. The extractive content in the *B. vulgaris* decreases between 0.30 % from 2-years to 4-year-old culms. While in the *S. brachycladum,* the extractive increases between 0.07 % from 2-years to 4-year-old culms.

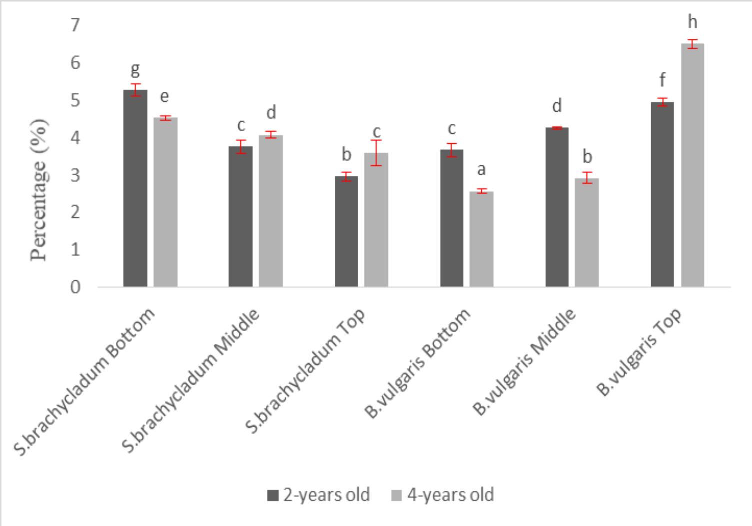

Figure 1: The extractive content on two selected bamboo species based on their ages and portions.

Figure 1 shows the extractive content seems were mainly depends on the species of bamboo and followed by ages and the portions separated. The highest extractive content found at the bottom of 4-year-old *B. vulgaris*. A significant difference exists between the bamboo ages and species at 95% probability. These might be due to the anatomical structure of 4-years *B. vulgaris* having a complex vascular bundle. The extractive content indicated with the apparent relationship between the numbers of vascular bundles [2]. As the number of vascular bundle increases, the number of extractive content increases in bamboo. The extractive content is affected by cell wall thickness and structure, cell width, the relative proportions of different types of cells, and the kind amount of extractives presented [27].

Holocellulose of *Bambusa vulgaris* and *Schizostachyum brachycladum* on Chemical Base

Figure 2 highlighted the 2-years *B. vulgaris* with 95.07%, 94.96%, and 94.24% for the bottom, middle and top, respectively. These holocellulose content are slightly higher compared to the 2-years *S. brachycladum* which possesses 91.04% at the bottom, 90.64% at middle and 90.21% top portions respectively. The 4-years *S. brachycladum* with 89.69%, 87.82% and 85.30% for the bottom, middle and top, exhibited slightly higher in holocellulose content compared to the 4-years *B. vulgaris* with 86.36%, 85.50%, and 82.52% for the bottom, middle and top, respectively. The content of holocellulose in *B. vulgaris* and *S. brachycladum* decreases between 9.97% and 3.03%, respectively from 2-years to 4-years culms.

The holocellulose contents in both bamboos are related to species, ages and portion heights. The bottom 2-years *B. vulgaris* shown that the highest of holocellulose content which is 95.07%, while the lowest of the holocellulose content manifested at top 4-years *B. vulgaris* with 82.52%. The statistical analysis (see Table 1) clarifies that there was significantly different ($p \leq 0.05$) between their ages and species. The holocellulose in the woody material is known to have both the α-cellulose and hemicellulose together [28]. The holocellulose content in bamboo fiber indicated high compared to the holocellulose content in both the softwood and hardwood fiber at only 60-80% and 75%, respectively [29]. According to [10,29], the outer position possesses higher holocellulose content due to the dense distribution of vascular bundle compared to the inner positions. The holocellulose content collected used the process of pre-treatment to removes lignin and increase the surface area of the substrate for saccharification of holocellulose into constituent sugars [30].

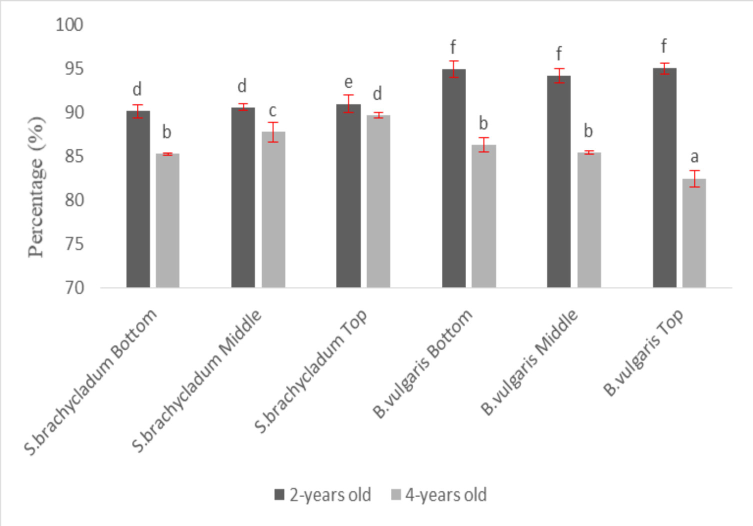

Figure 2: The holocellulose content on two selected bamboo species based on their ages and portions.

Alpha Cellulose of *Bambusa vulgaris* and *Schizostachyum brachycladum*

Figure 3 shows, the α-cellulose content of two selected bamboo species at different ages (2-years and 4-years) and a portion (bottom, middle and top). The result shows that the overall α-cellulose content in both selected bamboo species ranged from 38.76 % to 64.22 %. The highest average α-cellulose content found in *S. brachycladum* (53.30 %) and followed by *B. vulgaris* (53.00 %). Comparison of different age indicated *B. vulgaris* had a high average of α-cellulose content in 4-years (55.79 %) and *S. brachycladum* highlighted higher extractive content in 2-years (60.88 %). At different portion (bottom, middle and top) *B. vulgaris* and *S. brachycladum* in both age show the higher average of α-cellulose content was on the bottom (57.35 %) and followed by middle (53.66 %), and top (48.44 %).

The 2-year-old *S. brachycladum* with 64.22%, 63.72% and 54.70% for the bottom, middle and top, respectively had slightly higher α-cellulose content compared than the 4-year-old *S. brachycladum* with 53.75%, 44.63% and 38.76% for the bottom, middle and top, respectively. The 4-year-old *B. vulgaris* with 58.50%, 55.02% and 53.85% for the bottom, middle and top, respectively was shown higher α-cellulose content compared to the 2-year-old *B. vulgaris* with 52.93%, 51.25%, and 46.43% for the bottom, middle and top, respectively. At the different portions, culm of bamboo at the bottom portion on 2-years *S. brachycladum* with 64.22% had the highest value compared to the others. The α-cellulose in *B. vulgaris* increased between 5.59% from 2-years to 4-years while *S. brachycladum* decreased between 15.17% from the 2-years to the 4-year-old culms.

Based on Table 1, the statistical results show there were significantly different ($P \leq 0.05$) on their ages and species. The differences might be due to the bottom *S. brachycladum* had a thick S2 layer in the vascular bundle of the bamboo species. The TEM is used to view rare specimens which are tissue sections, molecules, and others through which electrons can pass generating a projection image. It was clearly show cell walls of the fibers with possess more than two sections which were S1, S2, S3, and Sn [36]. Furthermore, as known as the bamboo cross section highlighted that the outside position had a complex structure of vascular bundle compared to the inside cross section. From the mechanical side, the distribution of the cellulose in the cross-section of the wall of a culm contributes to the quality of strength of the material which is on the outside the percentage of cellulose is as much as 60% and decreasing to a 20% on the inside [31].

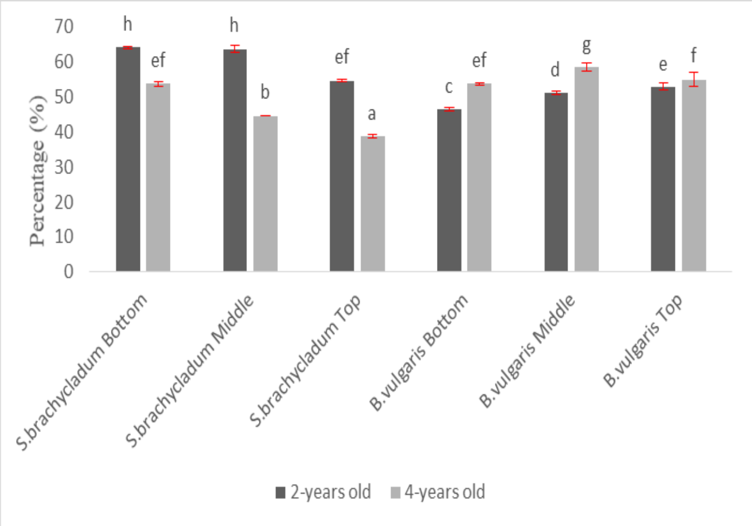

Figure 3: The α-cellulose content on two selected bamboo species based on their ages and portion.

Hemicellulose of *Bambusa vulgaris* and *Schizostachyum brachycladum*

Figure 4 showed the hemicellulose content of the selected bamboo species at different ages (2-years and 4-years) and a portion (bottom, middle and top) investigated. The result shows the overall hemicellulose content in two selected bamboo species ranged from 24.66% to 50.93%. The highest average of hemicellulose content was found in *B. vulgaris* (37.54%) and followed by *S. brachycladum* (35.32%). The values at different age indicated *B. vulgaris* had a high average of hemicellulose content in the 2-year-old culms (43.57%) and *S. brachycladum* highlighted higher average of hemicellulose content in the 4-year-old (41.94%). The average value at a different portion on *B. vulgaris* and *S. brachycladum* shows decreases in value from the top to bottom which is 41.40%, 36.42% and 31.47%.

The 4-year-old *S. brachycladum* with 31.56%, 43.33% and 50.93% for the bottom, middle and top, respectively highlighted the slightly higher of hemicellulose content compared than 2-year-old *S. brachycladum* with 24.66%, 28.45% and 33.00% for the bottom, middle and top, respectively. While, 2-year-old *B. vulgaris* with 40.48%, 41.71% and 48.53% for the bottom, middle and top, respectively highlighted slightly higher of hemicellulose content compared to the 4-year-old *B. vulgaris* with 29.18%, 32.18%, and 33.17% for the bottom, middle and top, respectively. At different of the portion such as the bottom, middle and top shows that the top 4-year-old *S. brachycladum* shows the slightly highest value with 50.93% compared the others. The average of hemicellulose content on *B. vulgaris* decreases between 11.39% from the 2- to 4-year-old, while *S. brachycladum* increased between 13.24%.

The statistical analysis (see Table 1) shows the significantly different (P≤0.05) between ages and species variable. The top of 4-year-old *S. brachycladum* had the higher value as they possessed 4-year-old secondary and primary layer on cell wall structure compared to the others. The previous study shows that the content and chemical composition of hemicelluloses in bamboo influenced by the conditions of species, age, climate, harvest, and others. The hemicellulose content increases from green to yellow colour (young to mature bamboo culms) and from bottom to the top (by height) [32].

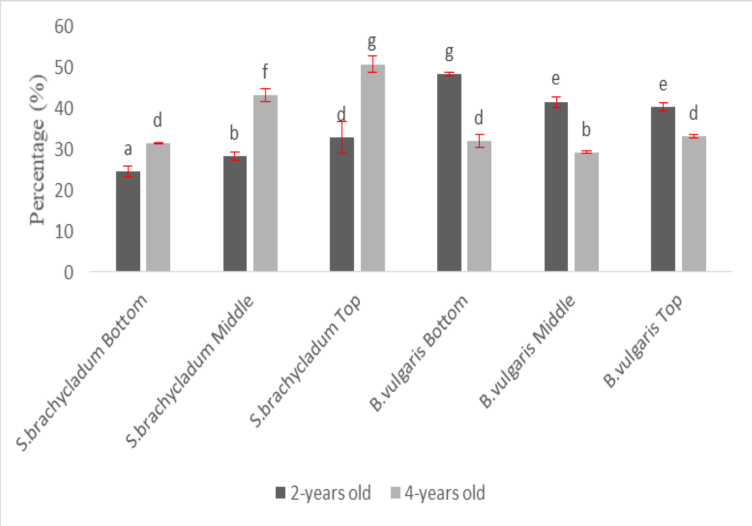

Figure 4: The hemicellulose content of two selected bamboo species based on their ages and portions.

Lignin Content of *Bambusa vulgaris* and *Schizostachyum brachycladum*

Figure 5 indicated that the lignin content of the two selected bamboo species at different ages (2- and 4-year-old) and height positions (bottom, middle and top) investigated. The result shows the overall lignin content in both bamboo species ranged from 16.27 % to 29.81 %. The highest lignin content found in bottom portion *S. brachycladum* (29.81%) and followed by bottom portion *B. vulgaris* (25.97%). The values at different age indicated *B. vulgaris* had high lignin content in the 4-year-old (25.97%) and *S. brachycladum* highlighted higher extractive content in 2-year-old (29.81%). While the average value at a different height positions on *B. vulgaris* and *S. brachycladum* shows decreases in value from the bottom to top which is 24.50%, 20.49% and 18.28%.

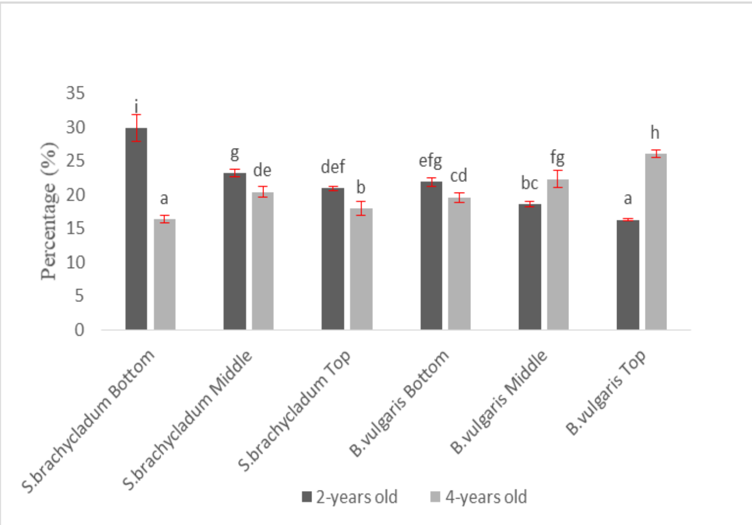

Figure 5: Percentage of lignin content on two selected bamboo species based on their ages and portions.

The 2-year-old *S. brachycladum* with lignin contents of 29.81%, 23.21% and 20.88% for the bottom, middle and top portions, were relatively higher compared than the 2-year-old *B. vulgaris* with 21.86%, 18.54% and 16.27% for the bottom, middle and top, respectively. The 4-year-old *B. vulgaris* which are 25.97%, 22.29% and 19.58% for the bottom, middle and top, respectively was shown slightly higher of lignin content compared to the 4-year-old *S. brachycladum* species with 20.36%, 17.93% and 16.39% for the bottom, middle and top, respectively. The lignin content on *B. vulgaris* increases between 11.17% from 2- to 4-year-old while *S. brachycladum* decreased between 19.22% from 2- to 4-year-old.

At different portion such as the bottom, middle and top indicated the bottom 2-year-old *S. brachycladum* species with 29.81% which is the highest lignin content compared to the others portion. Table 1 highlighted there was significantly different between their ages and species with P≤0.05. It may be due to the anatomical structure at the bottom of the 2-year-old *S. brachycladum* have the thicker middle lamella layer and corner cell in the vascular bundle of the bamboo species as shown in Figure 1. Supported by [34] seen from the TEM images that possibly be the lignin-rich at corner middle lamella and it was the most durable in physically. However, the composition of lignin depended on species, the conditions of growth, the age of the bamboo and the part of the culm. According to [33] bamboo culm tissue matured within the generation and changes to the soft and also fragile sprout becomes hard and durable, then the proportion of lignin and carbohydrates were changed during this period (from young to mature bamboo culms).

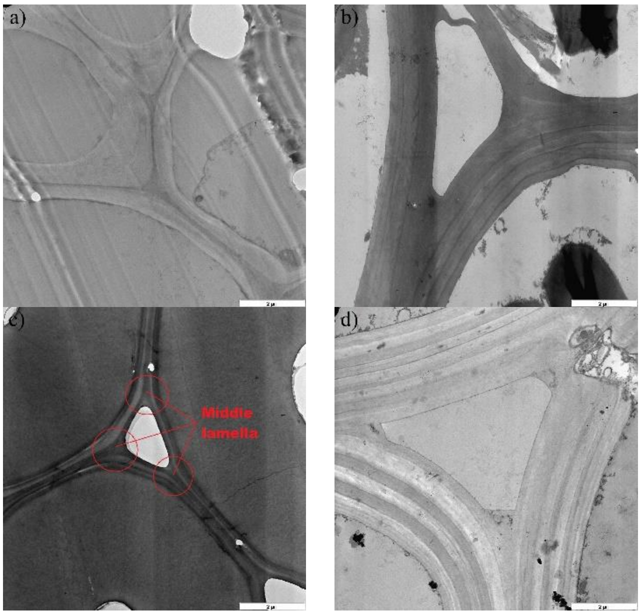

Figure 1. Cross-sectional of the 2- and 4-year-old *Bambusa vulgaris* and *Schizostachyum brachycladum* to 3.2k magnification viewed; a) 2-year-old *B. vulgaris* b) 4-year-old *B. vulgaris*; c) 2-year-old *S. brachycladum*; d) 4-year-old *S. brachycladum* using Transmission Electron Microscopy (TEM).

Table 1: ANOVA on chemical composition on *B. vulgaris* and *S. brachycladum*

Chemical Composition	Sum of Squares		F-Ratio	P-Value
Extractive	Between-Group	41.315	255.453	*
	Within Group	0.353		
Holocellulose	Between-Group	547.442	95.222	*
	Within Group	12.544		
α-cellulose	Between-Group	1794.601	170.067	*
	Within Group	23.023		
Hemicellulose	Between-Group	2297.072	85.699	*
	Within Group	58.481		
Lignin	Between-Group	513.125	59.271	*
	Within Group	18.889		

***** = significant at P≤0.05

CONCLUSIONS

The extractive content in both of selected bamboo species are 4 – 4.3%, 85 – 95% for holocellulose, 46 – 61 for α-cellulose, 29 – 44% for hemicellulose and 18 – 25% for lignin content. *B. vulgaris* shows declined from the 2 to 4 year-old culms in extractive, hemicellulose and holocellulose content with 0.3 – 12 %. The α-cellulose and lignin content increases 4 – 6%. The *S. brachycladum* between 2- to 4-years-old indicated the declining in the contents in holocellulose, α-cellulose and lignin between 3 – 5.2%. The extractive and hemicellulose content increases in values between 1 – 13% in the 2- to 4-years-old which is 1 – 13%.

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