

BAMBUSA VULGARIS: CHEMICAL COMPOSITION AND CELL WALL STRUCTURE

**MOHAMAD SAIFUL SULAIMAN¹, SITTI FATIMAH MHD RAMLE^{1*}, BOON JIA GENG¹,
ROKIAH HASHIM², OTHMAN SULAIMAN², NOR IZAIDA IBRAHIM¹ and
NURUL AKMAR CHE ZAUDIN¹**

¹ Faculty of Earth Science, Universiti Malaysia Kelantan, Jeli Campus locked bag 100, 17600 Jeli, Kelantan, Malaysia

² Division of Bioresources, Paper and Coatings Technology, School of Industrial Technology, Universiti Sains Malaysia, 11800 USM, Pulau Pinang, Malaysia

^{1*}Corresponding Author

Email: fatimah.m@umk.edu.my

ABSTRACT

Chemical composition and cell wall structure on Bambusa vulgaris were investigated. The samples were classified in two different age groups which are young and mature. Each bamboo was divided into three portions which are bottom, middle and top. The aim of this study is to investigate the chemical composition which are the percentage of extractive, holocellulose, alpha cellulose, hemicellulose and lignin content and also to visualize the Bambusa vulgaris structure under light microscopy (LM), Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). The methods used to extract all chemical composition were following the TAPPI standard. From this study, it can be highlighted that the higher extractive content and lignin content are 6.51% and 25.97%, respectively which are from top mature of Bambusa vulgaris. Furthermore, the higher content of holocellulose was shown on the top of a young Bambusa vulgaris with 95.07% and followed by the higher content of alpha cellulose on middle mature of Bambusa vulgaris with 55.02%. Lastly, the study has also indicated that higher content of hemicellulose can be found at a bottom of a young Bambusa vulgaris with 48.53%. In this study, the surface of bamboo was visualized by LM to determine their structural figure. It was then followed by a more clear study of cell wall structure that were examined by SEM, and later on illustrated by TEM to most depth anatomically structure of bamboo.

Keywords: Chemical composition, anatomical of bamboo, *Bambusa vulgaris*

INTRODUCTION

Bamboo is one of the most abundant non-timber forest resources in Asia (Hunter, 2003). According to Shanmughavel *et al.*, (2001) there are over 75 genera and 1250 species in the world. On the other hand, the *Bambusa vulgaris*, also known as bamboo (English), and by other tribes in Nigeria as Oparun (Yoruba), Iko (Bini) and Atosi (Igbo) is found in tropical and subtropical areas, especially in the monsoon and wet tropics (Yakubu & Bukoye, 2009), the distinctive part of bamboo is a stem or known as culm, which has upper ground part that contains most of the woody material. It also has a straight, hollow and cylindrical formed of nodes and internodes. The diameter tapers from the bottom to the top with the reduction in culm wall thickness (Biswas *et al.*, 2011). At the internodes, the cells are axially oriented with no radial cell elements such as rays while at the nodes cells provide the transverse interconnections with intensive branching of the vessels occurrences. The outer part of the culm is formed by two epidermal cell layers which consist of the outermost cells are covered by a cutinized layer with a wax coating on the surface and the inner culm that consist of numerous sclerenchyma cells which appears to be thicker and highly lignified (Liese, 1985). In addition, the microstructure of vascular bundles are embedded in parenchyma tissue and distributed across the wall thickness. According Amada *et al.*, (1997) vascular bundles and bundle sheaths are the main parts of this plant, reinforcing the bamboo culm and connecting the nodes to the culm. In this paper, the chemical composition and anatomical structure based on *Bambusa vulgaris* were investigated. Based on previous study, the major constituents of wood or bamboo which are extractive (0-5%), cellulose (45–55%), hemicelluloses (25–35%), and lignin (20–30%) of content (Peng & She, 2014) while the holocellulose which contain hemicellulose and alpha cellulose (70-90%). In addition, according to Satyanarayana *et al.*, (1990) the chemical composition and cell structure of natural fibres were complex because each fibre is essentially a composite in which rigid cellulose microfibrils are embedded in a soft matrix mainly composed of lignin and hemicellulose. Generally, woody plant extractives are diverse substances such as resin acid, fatty acid, turpenoid compounds and alcohols and most of these substances are soluble in water or neutral organic solvent (Ma *et al.*, 2014). On the other hand, reported by Sandu *et al.*, (2003); Chaowana, (2013) the main chemical composition of bamboo is similar to wood over 90% of which consists of cellulose, hemicelluloses and lignin. On the other hand, based on Peng & She, (2014) study the content of hemicelluloses in bamboo was influenced by the conditions of species, age, climate, harvest and *etc.* and hemicelluloses were also perceived as different from cellulose as they are composed of a series of sugar monomers such as xylose, mannose, glucose, galactose and others (Peterson *et al.*, 2008). Moreover, lignin is a phenolic heteropolymer that constitutes the second most abundant organic constituent on earth, after cellulose (Rastogi & Dwivedi, 2008). Nevertheless, as reported by Shi *et al.* (2012), the high lignin content makes bamboo fibre brittle in comparison to other natural plants. It is found that the lignin was present in all vascular plants and the percentage of their presence in an average of 25% from the terrestrial plant biomass (Whetten *et al.*, 1998). The information on relationship in between the chemical composition and anatomical cell wall structure of *Bambusa vulgaris* is rather limited and has not been clearly explained. Therefore further investigation of *Bambusa vulgaris* is necessary for in-depth understanding about this phenomenon. Furthermore, the main objective of this study is to investigate the separation of *Bambusa vulgaris* based on their ages which are young and mature and also in different portion whereby they were identified and evaluated based on their chemical compositions of their anatomical components. The different anatomical structure of cell wall on *Bambusa vulgaris* would probably lead or could be affected to an indication of properties and composition of bamboo. In this study, methods were described by James, (1942); Wise, (1947) and TAPPI standard are used for chemical composition analyses.

MATERIAL AND METHOD

In this study, the chemical composition analysis that are used were benzene (99.5%), sodium chlorite; NaClO_2 (80%), ethanol (99.8%), acetone, sodium hydroxide (NaOH), sulphuric acid (95-97%) and glacial acetic acid (99%). Cell wall structure was analysed by using light microscopy Model Olympus SZX9, Scanning Electron Microscope LEO SUPRA 55 VP, Field Emission SEM and EFTEM with OMEGA spectrometer. *Bambusa vulgaris* with different ages (young and mature) were used in the experiments. The chemical composition such as extractive, holocellulose, alpha cellulose, hemicellulose and lignin content were investigated. Each age of bamboo were classified to three (3) portions which are bottom, middle and top. Portion of bamboo sample were cut using chainsaw and several chipping of cross section were pulverized using grinder. Powder form was screened to collect the homogeneous size of sample for experiments. Then the structure of cell wall was investigated using Light Microscopy (LM), Scanning Electron Microscopic (SEM) and Transmission Electron Microscopic (TEM). The sample of LM and SEM was cut to 20 cm x 20 cm and were dried at 30 ± 3 °C for 24 hours while TEM sample were cut to the small pieces (diameter = 0.5 mm) and preserved into 3% concentration of glutaraldehyde.

Chemical Composition for Extraction Process

The extractive content *Bambusa vulgaris* was determined according to TAPPI T 264 om-97, (1997). The powder form of bamboo was approximately weighed at 10 gram. Then, the samples were put in the extraction thimble which size 125 mm x 30 mm. The solvent that was used in extraction process were ethanol-benzene (2:1). The amount of the solvent was used in this extraction process is 300 ml for 5 – 7 hours. Then, the solvent which contained extractive was evaporated using Rotary Evaporator machine to remove all the solvent. In the next stage, the extractive was dried in an oven at 103 ± 2 °C for 24 hours and the percentage of extractive content was calculated.

Chemical Composition for Holocellulose

Holocellulose content in *Bambusa vulgaris* was determined according to Wise, (1947) method. Approximately 5 gram air-dried free extractive powder form was used as a sample for holocellulose analysis. Then, total of 9 gram Sodium Chloride (NaClO_2), 30ml 10% acetic acid and 100 ml distilled water were mixed with free extractive sample against the period time (within 5 hours). Next procedure, the flask was closed and heated on a hot plate, while being stirred at 70°C. Lastly, the sample in flask was conditioned and a sample was then filtrated before left to dry. After 24 hours dried, the percentage of holocellulose was calculated.

Chemical Composition for Alpha Cellulose

The alpha cellulose in *Bambusa vulgaris* was determined according to TAPPI 203 os-74, (1997) method. Approximately 2 gram holocellulose powder form was used as a sample for alpha cellulose content analysis. A total of 75 ml 17.5% NaOH in stirring condition for 15 minutes were mixed slowly with holocellulose powder. In the stage, 100 ml of cold distilled water was added into mixture and continuously stirred. After 30 minutes, the sample was filtrated and washed with 8.3% NaOH and 2N acetic acid. The sample was left to dry and weighed in order to measure the percentage of alpha cellulose.

Chemical Composition for Lignin Content

Lignin content of *Bambusa vulgaris* was determined according to TAPPI 222 om-88, (2002) standard. The dry air of extractive free sample was used to determine the lignin content the part of bamboo species. Firstly, 1 gram extractive free sample was mixed with 25 ml of 72% sulphuric acid (H_2SO_4) then mixture was

slowly stirred for 2 hours in cold condition. Next stage, 560 ml distilled water was added and it was heated at 180°C for 4 hours. Lastly, the mixture was filtrated and dried before weighed.

Anatomical structure under Light Microscopy (LM)

Each part from *Bambusa vulgaris* was cut into cross section with 20mm x 20mm x thickness (depending to species of bamboo thickness) as a sample dimension. Then, the samples were directly analysed and photographed by using a light microscopy Model Olympus SZX9, Olympus Optical, Japan.

Anatomical structure analysis using Scanning Electron Microscopy (SEM)

Bambusa vulgaris based on different ages (young and mature) was visualised under Scanning electron microscopy (SEM) to characterize their morphology structure such as vascular bundle, parenchyma fibre, vessel and structure of sclerenchyma. The SEM micrographs were taken from the cross section of samples into 5mm x 5mm size of samples block. The samples with dry condition were coated with gold by an ion sputter coater (Polaron SC515, Fisons Instruments, United Kingdom). Then, the samples were visualised by Scanning Electron Microscope LEO SUPRA 55 VP, Field Emission SEM, Carl-Zeiss, Oberkochen, Germany.

Anatomical Structure on Transmission Electron Microscopy (TEM)

Bambusa vulgaris with different ages were cut into 2.0 x 0.5 mm to each sample. According to Spurr's resin method (1969), the samples were fixed in Karnovsky's fixative with 3% Glutaraldehyde and washed with 0.1 M phosphate buffer. Then, the samples were postfixed in 1% Osmium tetroxide and were left in for 2 hours. Besides, the samples were dehydrated with ethanol 50%, 75%, 95%, 100% of concentration and 100% of acetone then mixture of acetone and Spurr's resin (1:1) were used for the embed sample. In the next stage, transverse section of thickness 1 µm was cut using Sorvall Ultra Microtome (MT500) with diamond knife. After that, the section was stained with 2% uranyl acetate and lead citrate. The transverse section was examined with a LEO in column EFTEM with OMEGA spectrometer, Zeiss, zibra 120.

RESULTS AND DISCUSSION

In this study, chemical composition on *Bambusa vulgaris* was determined to relate with their anatomical structure such as vascular bundle, parenchyma, sclerenchyma, vessel and others using light microscopy, scanning electron microscopy and transmission electron microscopy method.

Extractive Content of *Bambusa vulgaris*

In this study, the percentage extractive of *Bambusa vulgaris* at different ages and portion were determined as shown in Figure 1. According to that figure, the top portion to *Bambusa vulgaris* with 4.96% and 6.51% for young and mature respectively has shown a slightly higher extractive content compared to the middle and bottom portion with 4.27% and 2.92% for young and mature to the middle portion then 3.67% and 2.57% for young and mature to the bottom portion, respectively. In different ages, *Bambusa vulgaris* showed the extractive content on young and mature ages with 3.67%, 4.27% and 4.96% for young and 2.57%, 2.96% and 6.51% slightly increased from bottom to the top, respectively.

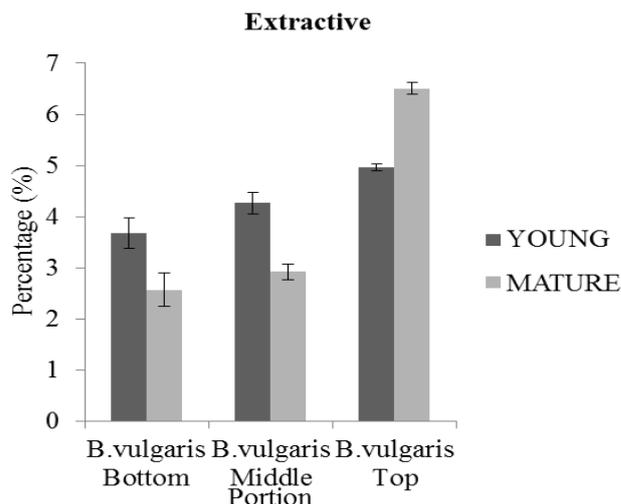


Figure 1: The extractive content on *Bambusa vulgaris* based on their ages and portions.

Figure 1 shows the extractive content which seems mainly depends on the ages and followed by portions separated. Statistical analysis has indicated that there was no significant different ($p \geq 0.05$) between their ages with P equal to 0.39. This may be due to the anatomical structure on mature age of *Bambusa vulgaris* which has a complex and bigger vascular bundle. Therefore, the extractive content was nearly related to density of sample used in experiment. Based on previous study, anatomical was relative with density which is regarded as a function of the ratio of cell wall volume. As such, it is affected by cell wall thickness and structure, cell width, the relative proportions of different types of cells, and the amount of extractives presented (Espiloy, 1985).

Holocellulose Content of *Bambusa vulgaris* on Chemical Base

According to Figure 2, the holocellulose content of *Bambusa vulgaris* in different ages (young and mature) and portion (bottom, middle and top). Figure 2 shows, *Bambusa vulgaris* of young age with 94.96%, 94.24% and 95.07% for bottom, middle and top, respectively was showing a slightly higher value of holocellulose content compared than mature age from *Bambusa vulgaris* with 86.36%, 85.50% and 82.52% for bottom, middle and top, respectively.

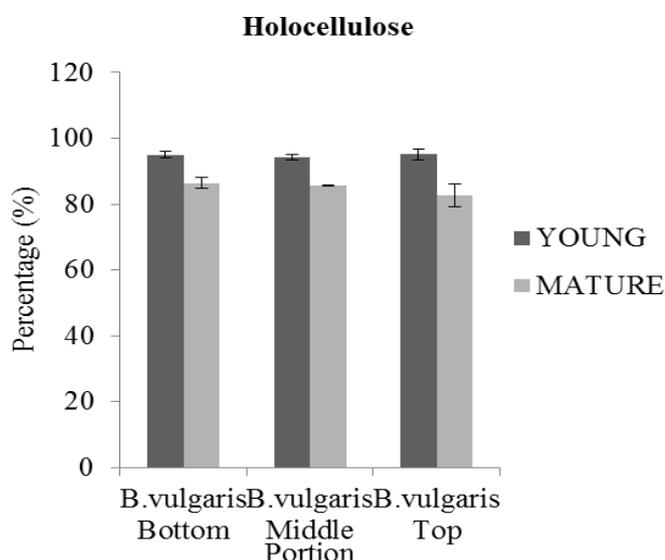


Figure 2: The holocellulose content on *Bambusa vulgaris* based on their ages and portions.

According to Figure 2, the top portion on young *Bambusa vulgaris* has shown that the highest percentage of holocellulose content is 95.07% while the lowest percentage of the holocellulose content showed at top portion on mature *Bambusa vulgaris* is 82.52%. Furthermore, on statistical analysis indicated that there was significant different ($P \leq 0.05$) between their ages with P equal to 0.008. This is due to the anatomical structure of the top portion on young age from *Bambusa vulgaris* may have thicken S_1 layer from secondary wall and rich of sugar content on vascular bundles compared to the other portion which is believed to have contained lots of holocellulose. Supported by Kuhad *et al.*, (2010) report, the holocellulose content was collected using the process of pretreatment to removes lignin and increase surface area of the substrate for saccharification of holocellulose into constituent sugars.

Alpha Cellulose of *Bambusa vulgaris*

Figure 3 shows, the alpha cellulose content of *Bambusa vulgaris* in different ages (young and mature) and portion (bottom, middle and top). The figure indicated that mature *Bambusa vulgaris* with 53.85%, 58.50% and 52.02% for bottom, middle and top, respectively has shown a slightly higher alpha cellulose content compared to the young *Bambusa vulgaris* with 46.43%, 51.25% and 52.93% for bottom, middle and top, respectively. In different portions culm of bamboo such as bottom, middle and top portion, indicated that the middle portion on mature *Bambusa vulgaris* with 58.50% has shown the higher value compare to the other portions.

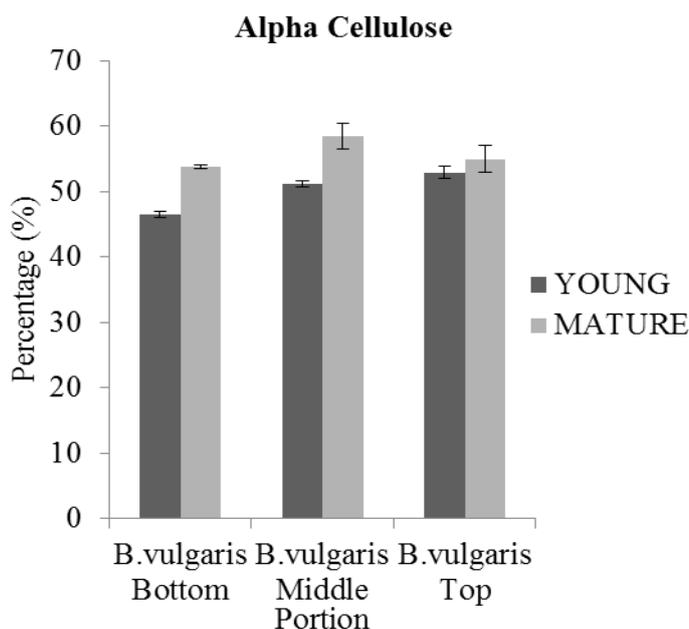


Figure 3: The alpha cellulose content on *Bambusa vulgaris* based on their ages and portion.

Based on statistical analysis, there was a significant different ($P \leq 0.05$) between their ages with P equal to 0.04. It may be due to the anatomical structure, whereby the middle portion on mature age from *Bambusa vulgaris* had a larger fibre size and length in culm of the bamboo species. The study shows that the outside cross section had a complex structure of vascular bundle compared to the inside cross section. According to Widjaja & Risyad, (1985) the fibre size in the middle part of culm is greater than in the bottom and the top, and that the outer part of the culm has longer fibres than the inner part. Based on previous study, from 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 are on the outside the percentage of cellulose is as much as 60% and decreasing to a 20% on the inside (Janssen, 1985).

Hemicellulose in *Bambusa vulgaris*

From Figure 4, shows the hemicellulose content of *Bambusa vulgaris* in different ages (young and mature) and portion (bottom middle and top). Based on the figure, young *Bambusa vulgaris* with 48.53%, 41.71% and 40.48% for bottom, middle and top, respectively showed slightly higher of hemicellulose content compared to the mature *Bambusa vulgaris* with 32.18%, 29.32% and 33.17% for bottom, middle and top, respectively. In different of portion such as bottom, middle and top indicated that the *Bambusa vulgaris* on bottom young age has shown the slightly highest value with 48.53% compared the others.

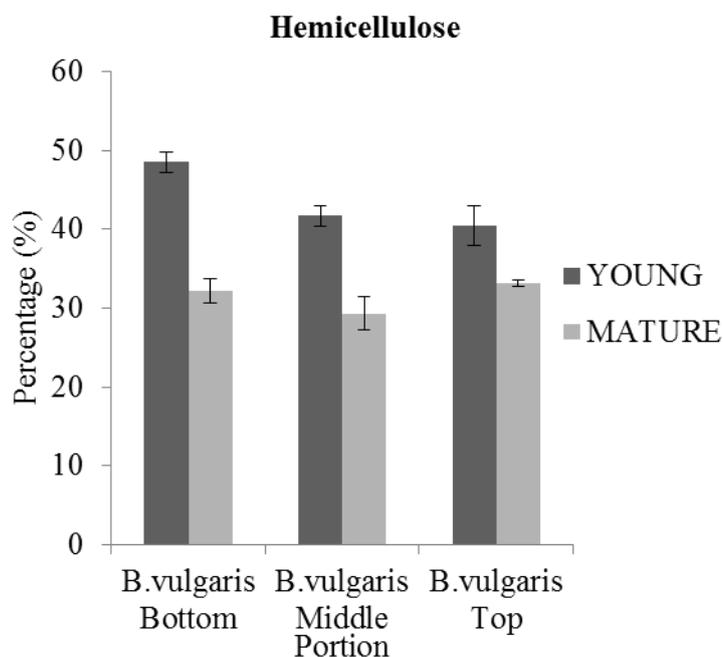


Figure 4: The hemicellulose content on *Bambusa vulgaris* based on their ages and portions.

Based on this study, the statistical analysis shows that there was significant different ($p \leq 0.05$) on their ages with P equal to 0.02. It may be due to the anatomical structure, the bottom portion on young age from *Bambusa vulgaris* which has a thick S_1 layer in vascular bundle of the bamboo species. Based on previous study, after cellulose the hemicelluloses are the next most abundant polysaccharides family in the primary and secondary layers of the plant cell wall. The average degree of polymerization (DP) of hemicelluloses is in a range of 80–200 (Peng & She, 2014).

Lignin Content in *Bambusa vulgaris*

From Figure 5, it can be seen that the lignin content *Bambusa vulgaris* were in different ages (young and mature) and portion (bottom, middle and top). From that figure, *Bambusa vulgaris* on young age with 21.86%, 18.27% and 16.27% were showed in decreasing order value of lignin content from bottom to the top, while mature age with 19.58%, 22.29% and 25.97% has shown increasing order percentage of lignin content from bottom to the top.

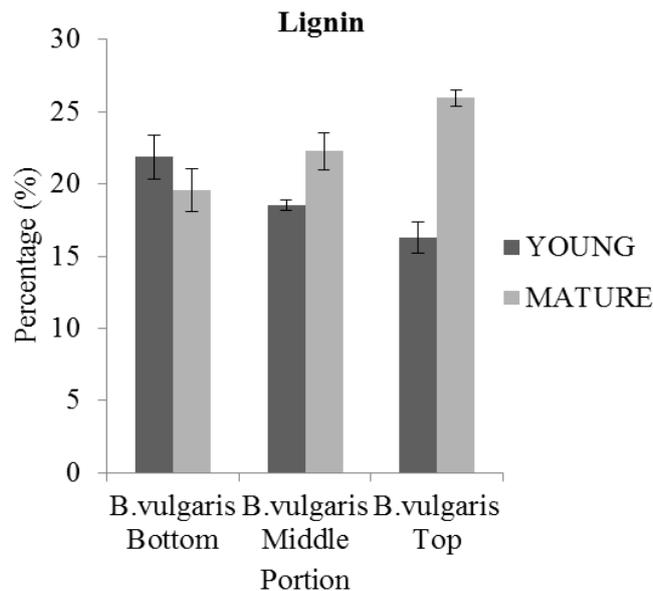


Figure 5: Percentage of lignin content on *Bambusa vulgaris* based on their aged and portions.

Based on this study, different portion such as bottom, middle and top has indicated the top mature *Bambusa vulgaris* with 25.97% which had a slightly highest lignin content compared the others portion. Based on statistical analysis, lignin content between ages was no significant different with P value is 0.20. It may be due to the anatomical structure, the top portion on mature *Bambusa vulgaris* have thicker middle lamella layer and thicker of corner cell in vascular bundle of the bamboo species. Other factors that influence content of lignin composition in elementary fibre are species, the conditions of growth, the age of the bamboo and the part of the culm. According to Liese (1985), the process of bamboo culm tissue to mature within an age were changes from soft and fragile to sprout becomes hard and strong, then proportionally the lignin and carbohydrates were changed during this period time.

Light Microscopy (LM) Analysis under *Bambusa vulgaris*

The anatomical structure *Bambusa vulgaris* of parenchyma cell and vascular bundle were characterized by ages (mature and young) and portion (top, middle and bottom) as shown as in Figure 6. The figure shows that the *Bambusa vulgaris* culm consist parenchyma, vascular bundle, sclerenchyma and vessel. From the Figure 6 the surface area of each image (a; b; c; d; e and f) were standardised to uniform scale. That figure has also shown that bottom portion from young and mature (a and d) were less compact on number of vascular bundle compared than other image which are b, c, e and f. Furthermore, the figure also indicated bottom portion of young and mature was visualized more clearly and completely by the vascular bundle structure. According to Liese (1985), the gross anatomical structure of a transverse section of any culm internode is determined by the shape, size, arrangement and number of the vascular bundles. According to Table 6.1, the morphology structure of *Bambusa vulgaris* from mature age were counted based on number of vascular bundle. Then, based on the surface area calculation, the percentage of vascular bundle was showing the percentage of 12.31 %, 21.54 % and 30.77 % for bottom, middle and top, respectively. According to Liese (1985) the culm tissue is mostly cover by parenchyma and the vascular bundles which are composed of vessels, sieve tubes with companion cells and fibres as shown as in Figure 6. On the other hand, the total culm comprises about 50% parenchyma, 40% fibre, and 10% conducting tissues (vessels and sieve tubes) with some variation according to species. The difference of sclerenchyma and parenchyma were classified based on their contrasting colour which was indicating darker colour as sclerenchymatous tissue and light colour is the parenchymatous ground tissue (Espiloy, 1985). Then based on calculation of number counted

per surface area indicated the percentage of vascular bundle for young *Bambusa vulgaris* which are 27.69 %, 21.54 % and 92.31 for bottom, middle and top, respectively. According to the previous study, at the peripheral zone of bamboo culm the vascular bundles are smaller and more numerous while in the inner parts the vascular bundle becomes larger and fewer as shown in Figure 6. Otherwise within the culm wall, the total number of vascular bundles decreases from bottom towards the top, while their density increases at the same time (Liese, 1985).

Table 1: Percentage of vascular bundle counter.

Portion	Mature <i>Bambusa Vulgaris</i>	Young <i>Bambusa Vulgaris</i>
Bottom	12.31 %	27.69 %
Middle	21.54 %	21.54 %
Top	30.77 %	92.31 %

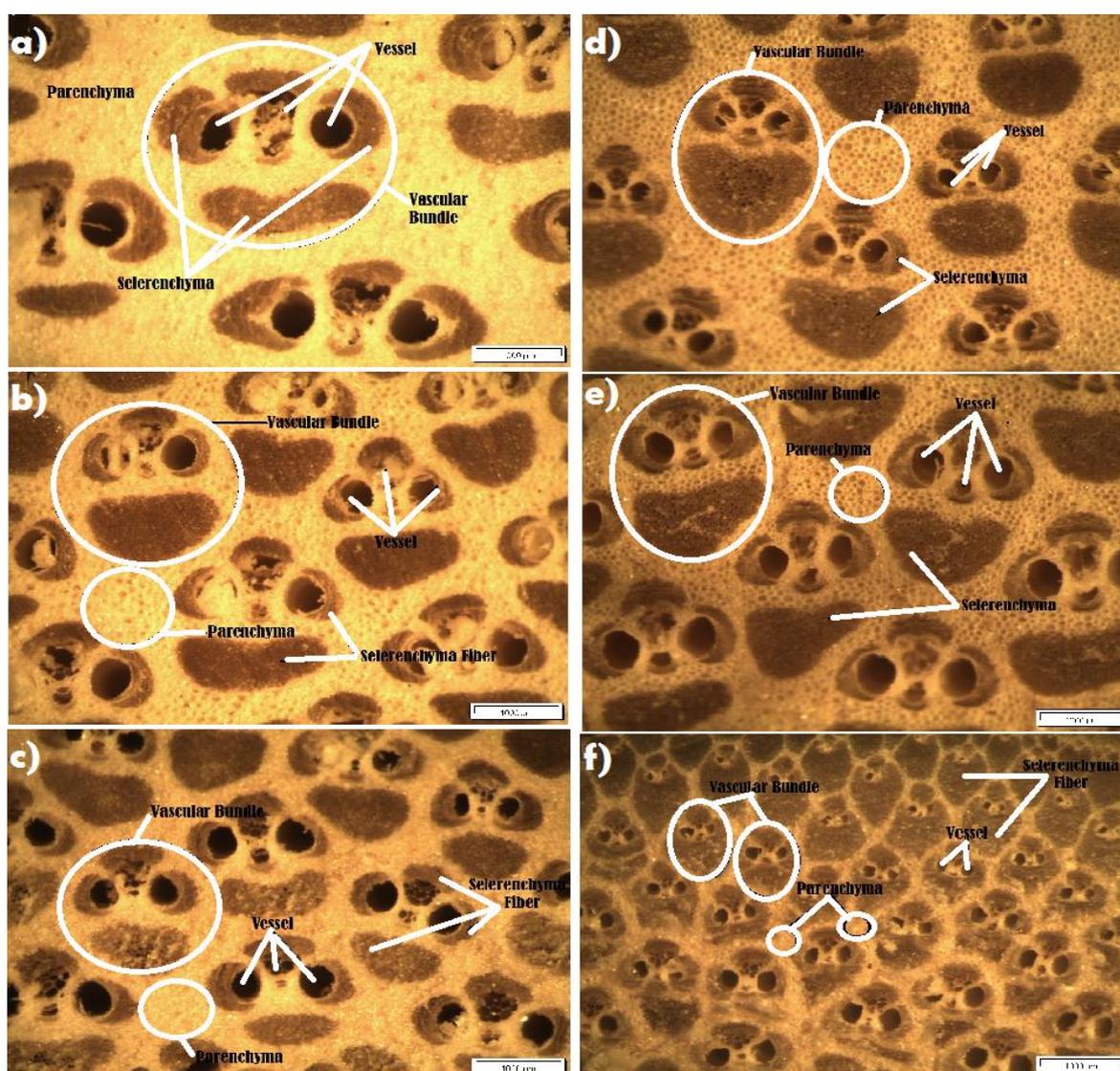


Figure 6: Transverse of mature and young ages for *Bambusa vulgaris* to different portion; a) mature bottom; b) mature middle; c) mature top; d) young bottom; e) young middle and f) young top; viewed by Light Microscopy (LM) showed the vascular bundle, vessel and parenchyma.

Scanning Electron Microscopy (SEM) on *Bambusa vulgaris*

The scanning Electron Microscopy (SEM) has viewed the cell structure clearly of vascular bundle, parenchyma, sclerenchyma and vessel compared than the light microscopy (LM). Nevertheless, SEM was limited to observe in depth cell wall fibre inside individually. Figure 7(a) and Figure 7(b) shows the focus on cell wall structure of bamboo with a different magnification on mature *Bambusa vulgaris*. That figure has shown a clear vascular bundle and sclerenchyma cell on magnification 50x then founded that on magnification 250x the mature *Bambusa vulgaris* which had a starch content on the cell wall structure. Moreover, based on Figure 7(c) and Figure (d) at 50x magnification showed that complete structure of vascular bundle and clear vessel as shown as at magnification 250x with no starch content on the young *Bambusa vulgaris* structure. Based on previous study, the vascular bundles were consisted of xylem, phloem and sclerenchymatous fiber caps (Wang *et al.*, 2012) scattered in the parenchymatous ground tissue while reported by Parameswaran & Liese, (1980); Liese, (1985) a parenchyma wall with the herringbone pattern of fibrillar arrangement whereby the number of layers are mostly restricted to two to four. Based on study also the magnification 50x at both of ages from *Bambusa vulgaris* were illustrated with clearly sclerenchyma structural on vascular bundle part while on parenchyma fibre showed roughly of structural especially on Figure 7(a). As reported Li *et al.*, (2014) on bamboo based the sclerenchyma fibre was characterized by thick cell wall and small cell lumen with an average diameter of 5 mm and the parenchymatous ground cell was characterized by thin cell wall and large cell lumen with an average diameter of 15 mm.

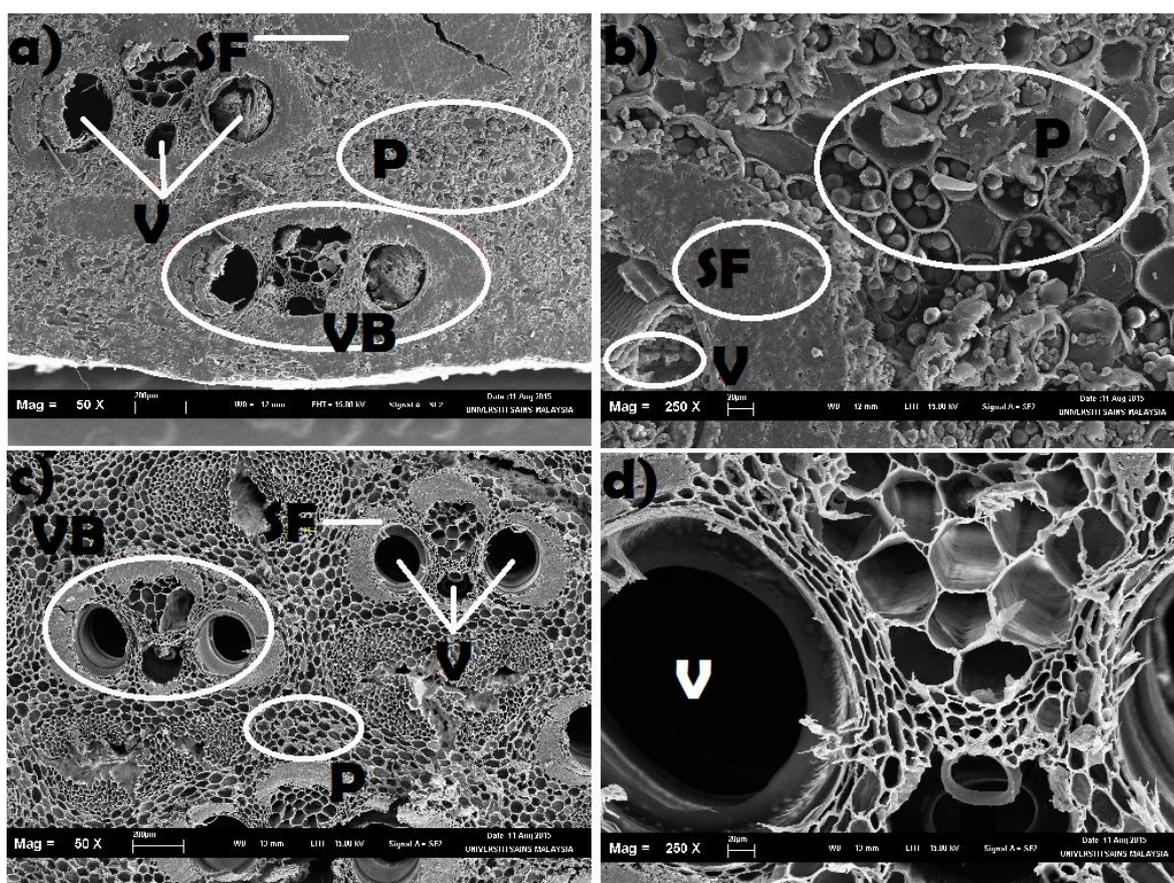


Figure 7: Cross sectional of mature and young ages for *Bambusa vulgaris* to different magnification viewed; a) mature 50x magnification; b) mature 250x magnification; c) young 50x magnification; d) young 250x magnification; using Scanning Electron Microscopy (SEM) showed the vascular bundle (VB), vessel (V), parenchyma (P) and sclerenchyma fibre (SF)

Transmission electron microscopy (TEM) illustrated on *Bambusa vulgaris*

Figure 8(a) and Figure 8(b) shows the mature age and Figure 8(c) and Figure 8(d) shows the young age of *Bambusa Vulgaris* with different magnification. The figure was illustrating the cell wall structure of parenchyma cell with clearly using the Transmission Electron Microscopy (TEM) compared than to SEM and LM. The figure also indicated the primary wall, secondary wall and middle lamella appeared as a thin layer as visualized in Figure 8(b). From the observation, structural of mature *Bambusa vulgaris* is clearly showing all layers (middle lamella layer, secondary layer and primary layer) compared than young age as showed as Figure 8(c) and Figure 8(d). In addition, Figure 8(d) has shown the middle lamella layer which is thicker than what is shown in Figure 8(b). This shows that young age have the most content of lignin compared than mature age of *Bambusa vulgaris*. Reported by Cha *et al.*, (2014) it can be seen from the SEM and TEM images that possibly are the lignin rich at corner middle lamella and it was the most durable. While, young *Bambusa vulgaris* show the secondary and primary layer cannot be visualized with clearly. This is may be the young *Bambusa vulgaris* which has had an immature cell wall structure consisting most spongy fibre and equilibrium in terms of colour tones to each layer.

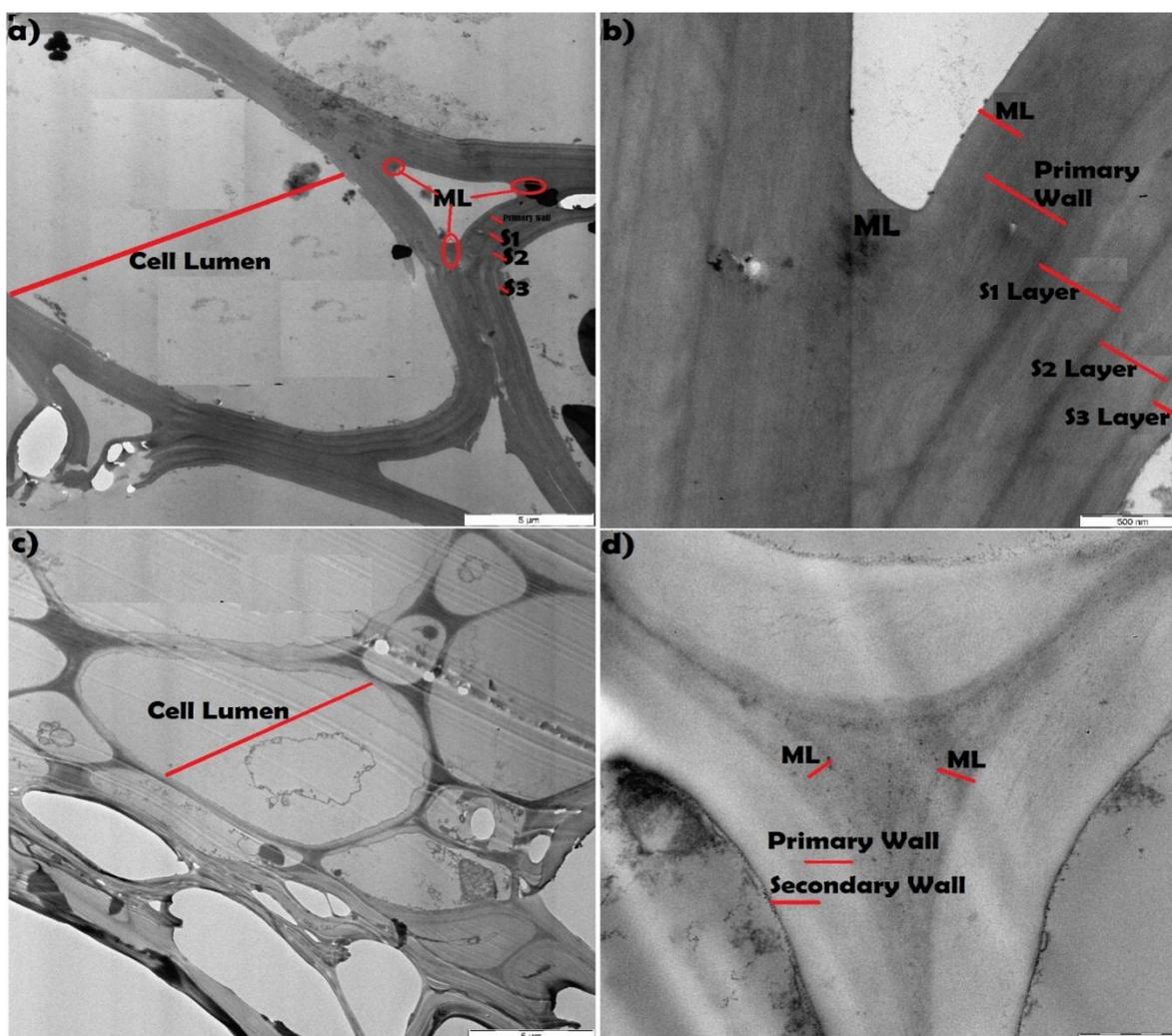


Figure 8: Cross sectional of mature and young age for *Bambusa vulgaris* to different magnification viewed; a) mature 1.3k magnification; b) mature 10k magnification; c) young 1.3k magnification; d) young 10k magnification using Transmission Electron Microscopy (TEM) showed the cell lumen, middle lamella (ML), primary wall and secondary wall.

CONCLUSION

Bambusa vulgaris of the mature age had the higher extractive compared to the other age and portions. The higher extractive was relative to higher density with influence on high cell wall volume such as thick and width cell wall structure. At the same time, from this phenomenon the mature *Bambusa vulgaris* had the high mechanical strength properties and suite to apply for engineered product. While *Bambusa vulgaris* on young age from top portion had the higher percentage of holocellulose yield within over than 90% content. This is because, holocellulose was relatively equipped with lignin content; the part which removed less lignin as shown by the higher content of holocellulose. On the other hand, middle portion on mature *Bambusa vulgaris* has indicated the higher percentage of alpha cellulose. This is because, middle portion had the larger and longer fibre size compared to the bottom and top portion. Consequently there were influences to high content of alpha cellulose on the part. In addition, bottom portion in young *Bambusa vulgaris* had the higher hemicellulose content that were consistence and also had the high content of polysaccharides in the primary and secondary wall. Last but not least, mature age on top portion in *Bambusa vulgaris* was the higher content of lignin. This is because the lignin content was slightly increasing within the age (to mature) and as a binder provided of its most hard and strong fibre on the part. Therefore, due to the results the part had a good potential for mechanical strength by-product and to the excellent properties including biodegradability, biocompatibility, bioactivity, and so on.

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