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Anatomical Branch Wood Properties of *Diospyros* spp.

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ABSTRACT

The distribution of *Diospyros* spp. (Ebenaceae) in Indonesia is greatly wide, but the studies about the botanical aspect are still less attention. The main species of *Diospyros* that already known as high economic value of raw material for furniture industry and also has been studied frequently is *Diospyros celebica* (also known as "the black ebony"). However, the availability of the wood is still constrained by minimum population and limited cultivation. Illegal logging has been a major problem that continuously threatens the population. This study aimed to discover the information of other species of *Diospyros* through the study of anatomical characters. About five species of *Diospyros* used in this study, namely *Diospyros sumatrana, D. ridleyi, D. pendula, D. dictyoneura,* and *D. celebica.* About thirty-three qualitative and six quantitative anatomical characters were observed through maceration and permanent slides. General anatomical characters found in all species of the *Diospyros* were fusiform with reticulate axial parenchymal arrangement, unicellular ray width, and medium fiber cell length. Specific anatomical characters of each species included the differences cell size in quantitative characters and the appearance of special characters such as the vessel perforations, the helical thickening in fibers and vessels, and distribution of black deposits and crystal. The fiber quality of the *Diospyros* used in this study categorized as class III, which can produce paper with moderate tensile strength, breaking, and tearing.

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1. INTRODUCTION

Eboni is a trade name for Sulawesi endemic wood known as *kayu itam, amara, ayu maitong, maeta, sora* and *toètandu* (Martawijaya, *et al*., 2005). According to the Atlas Kayu Indonesia (Indonesian Timber Atlas) Volume I published by the Department of Forestry (Martawijaya, *et al*., 2005), the main species of ebony is *Diospyros celebica* Bakh. However, Sunaryo (2002) mentioned another species which is also considered as ebony, namely *Diospyros rumphii* Bakh. The characterization of *D. celebica* and *D. rumphii* is different. *D. celebica* have brown striped wooden terrace, while *D. rumphii* has a black striped wooden terrace.

Currently, the problem of ebony wood supply is its availability in natural habitats. Most of the ebony grows naturally on their habitat in the districts of Poso, Donggala, Parigi (Central Sulawesi); Gowa, Maros, Barru, Sidrap, Mamuju and Luwu Regencies (South Sulawesi); and Gorontalo Province (Hendromono & Allo, 2008). Ebony grows at an altitude of 0-600 masl (Sunaryo, 2002), on podsolic soil types red yellow, andosols, and podsolic yellowish-brown (Allo, 2002). Ebony populations can be found in small and large numbers or growing solitary (Sunaryo, 2002). In addition, ex-situ conservation is held only at the botanical gardens and collection gardens.

The high economic value of ebony ranged from 80 to 350 million (Kurniawan, 2013), thereby increasing the risk of illegal logging. In order to gather more profits and to fulfill supply demand for the market, illegal logging of ebony often does not consider the maturity level of the wood. Tree harvesting selection becomes crucial to keep the continuity of the natural regeneration process in the habitat. Responding to these problems, since 1972 the government, through the Decree of the Minister of Agriculture No. 54/Kpts/Um/2/1972 has issued a prohibition of cutting down ebony trees with a diameter below 60 cm. This was also supported by the status of ebony species *D. celebica* declared by IUCN as a Vulnerable (VU) category since the last assessment in 1998. Moreover, it has been included in Appendix II CITES, which can only be traded by quota since 12 June 2013.

The distribution of *Diospyros* species is not only found in Sulawesi, but also in other islands such as Java and Kalimantan. The study of *Diospyros* outside of Sulawesi still needs to be done to reveal the potential of *Diospyros* wood as a companion to the true "ebony" species for trading commodities. This study aims to characterize the branch wood anatomy and the fibers dimention of five species *Diospyros* as an initial information of the potential for non-commercial *Diospyros* species.

2. METHODS

The research was conducted in Plant Anatomy Preparation Laboratory, BRIN which previously known as Morphology, Anatomy and Cytology Laboratory - LIPI. The specimens are taken from four *Diospyros* wood xylarium collections from Herbarium Bogoriense*: Diospyros sumatrana* (xm-40)*, Diospyros ridleyi* (xm-249)*, Diospyros pendula* (xm-158)*,* and *Diospyros dictyoneura* (xm-245). As a comparison, we also used fresh wood of *Diospyros celebica* growth around KST Soekarno area.

2.1. Anatomical observation

The woods were prepared in semi-permanent maceration slides and permanent paraffin slides. The maceration slides were prepared through modification of the Schultze method (Sass, 1951), boiled with the HNO_3 1:3 solution until the wood component separated. The permanent paraffin slides were prepared with the double staining method, with modification

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of dehydration solution combination of Tert Butanol-Ethanol-Aquades solution I, II, III, IV, V, and VI and gradually clearing agent combination of Neo Clear and Ethanol (Sass, 1951). Both of the maceration and paraffin slides are using 1% safranin as the coloring solution, and for the paraffin slides also adding the 2% fast green solution for the coloring solution.

Anatomical slides were observed using an optical microscope Nikon Eclipse 80i. Photographs were taken with XCAM Indomicro 1080 PHB camera with magnification 2.4 x 2.4 pixels and Beta View application. The observation of wood components are following the IAWA guidelines for wood microscopic identification (IAWA, 2007), includes 33 qualitative characters and six quantitative characters extracted from vessels, fibers, and parenchyma. For the quantitative characters, the mean value and the deviation standard from the observation of 10 to 25 cells are calculated.

2.2. Phenetic Analysis

Phenetic analysis was conducted using PAST 5.3, with multivariate clustering Neighbour Joining. As much as 20 characters selected are listed in **Table 1** below.

Table 1. Selected anatomical characters used for dendogram analyzing.

Anatomical Characters	Scoring
Qualitative Characters	
Vessel arrangement	1= diagonal or radial pattern only; 2 = diagonal and radial pattern
Vessel groupings	1= solitary dominant; 2= average; 3= multiple or clustering dominant
Vessel frequency / mm^2	1= 5-20/mm2; 2= 20-40/mm2; 3=40-100/mm2
Vessel cells type	1= one type; 2= two type; 3= three type
Perforation plates	1= scalariform perforation; 2= simple perforation
Helical thickenings on vessels	1= absent; 2= present
Vessel length	1= 350-800 µm; 2= ≥ 800 µm
Intervessel pits arrangement	1= opposite, 2= opposite or diagonal with some of them is sclarariform, 3= scalariform to opposite
Intervessel pits size	1= minute; 2= small; 3= medium
Vessel-ray pitting	1= pith rounded (vessel ray pith with reduced borders or apparently simple or distict); 2= pith scalariform (vessel ray pith horizontally scalariform or rounded to scalariform)
Fiber thickness	1= very thin walled; 2= thin to thick walled
Septate fibres	1= absent; 2= present
Helical thickenings on fibres	1= absent; 2= present
Sclereid	1= absent; 2= present
Parenchyma strand length	1= < 5 cells per parenchyma strand; 2= > 5 cells per parenchyma strand
Rays cellular composition	1= procumbent and square; 2= procumbent, square and upright
Black deposit	1= present in rays (parenchyma and/or rays cell); 2= present in vessels and rays (parenchyma and/or rays cell)
Mineral inclusions	1= absent; 2= present
Quantitative Characters	
Fibre cells lumina width	1 = < 10 µm; 2 = ≥ 10 µm
Rays height	1 = 100 – 200 µm; 2 = > 200 – 300 µm; 3 = > 300 – 400 µm; 4 = > 400 µm

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3. RESULTS AND DISCUSSION

3.1. General and specific anatomical characters of *Diospyros* spp. collection from Kalimantan

About 16 general anatomical characters were observed in all samples of *Diospyros*. All species of *Diospyros* spp. have an indistinct growth ring with diffuse-porous. The outline shape of the vessel is circular to oval with non-vestured pits. The parenchyma cells are fusiform with a reticulate arrangement of axial parenchyma. Ray width is exclusively uniseriate, all rays are storied, and the aggregate rays are absent. Disjunctive parenchyma cell walls were found in all of the *Diospyros* specimens. The fiber cell morphology is divided into two types: fiber cells with plain walls (without color) and fiber cells with colored walls. Fibers with brown walls show black or brown patterns (**Figure 1C**). The pits in both of the plain and patterned fibers were absent. The criteria of fiber cell length are medium (length between 900 - 1600 µm). Besides, there were no sheath cells, tile cells, oil cells, and mucilage cells in the radial section of the specimen. Specific anatomical characters of each *Diospyros* species are written on the following **Table 2**.

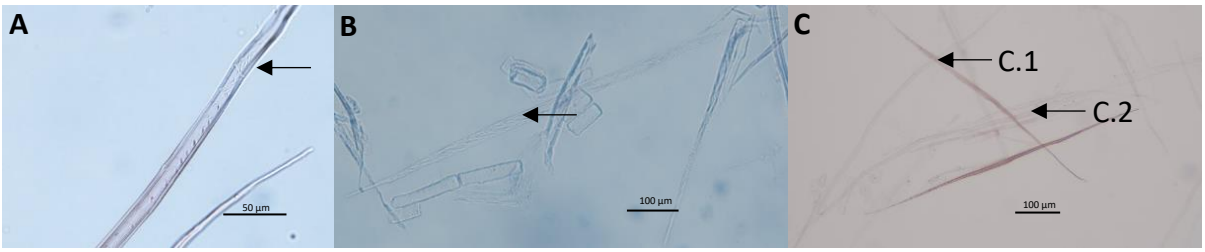

Figure 1. Fibre cells variation: (A) Septate fibres in *D. sumatrana* (scale bar = 50 µm; 200x magnification), (B) Fiber cells with helical thickening in *D. dictyoneura* and *D. ridleyi* (scale bar = 100 µm; 100x magnification), (C) Fiber cells wall variation with (C.1) black or brown pattern or (C.2) without pattern/plain (scale bar = 100 µm; 100x magnification).

The presence of black deposits in *Diospyros* woods can be noted as a unique character for this genus. All specimens in this study present a black deposit in their parenchyma (ray or axial parenchyma), with additional deposits present in the vessels (**Table 2**). Previous research by Hillis and Soenardi (1994) suggested that the appearance of black deposits on *Diospyros* wood was a response to fungal invasion. However, there is no strong evidence to apply in this study because all of the specimens have the black deposit material.

A previous study by Wickremasinghe and Herat (2006) mentioned that the presence of prismatic oxalate crystals can be used as one of the main anatomical characteristics of the *Diospyros*. Between those five species used in this study, two species have crystals present in their ray parenchyma cells, and three species remain absent (**Table 2**). However, the abundance of the crystal among both of the species is different. The crystal is found only in procumbent ray cells in *D. dictyoneura* (**Figure 2A**), but in *D. celebica* it can be found in procumbent and square ray cells (**Figure 2B**).

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Table 2. Specific anatomical characters of *Diospyros* spp. wood collection from Kalimantan.

Anatomical characters	Species				
	D. sumaterana	***D. ridleyi***	***D. pendula***	***D. dictyoneura***	***D. celebica***
Vessel					
Vessel arrangement	Radial pattern	Diagonal pattern	Diagonal pattern	Diagonal and radial pattern	Radial pattern
Vessel groupings	Radial multiples > 4 and clusters, rarely solitary	Solitary, radial multiples 2 to > 4 and clusters	Radial multiples 2 to clusters, rarely solitary	Solitary, rarely radial multiples 2 or more	Radial multiples 2-4 cells, rarely solitary, radial multiples > 4 and clusters
Tangential diameter of vessel lumina (µm)	52.54 ± 7.17	79.6 ± 10.32	67.25 ± 7.99	73.60 ± 8.49	51.72 ± 9.05
Vessel frequency/mm ^{2}	65 vessels	24 vessels	21 vessels	18 vessels	70 vessels
Vessel cells type	Cells with two tail, rarely with one tail or absent	Cells with one or two tail	Cells with one tail, two tail or absent	Dominant with one tail, rarely with two tail	Cells with two tail, rarely with one or absent
Perforation plates	Simple perforation	Scalariform perforation with 4-6 bars	Simple perforation	Simple perforation	Simple perforation
Helical thickenings on vessels	Absent	Absent	Absent	Present	Absent
Vessel length (µm)	417.49 ± 82.1	897.38 ± 137.5	479.42 ± 109.85	436.4 ± 104.00	460.94 ±71.53
Intervessel pits arrangement	Scalariform to opposite	Opposite	Opposite or diagonal, some of them is scalariform	Scalariform to opposite	Scalariform to opposite
Intervessel pits size µm	7.65 ± 4.67 (medium)	6.21 ± 1.21 (small)	7.93 ± 4.41 (medium)	3.87 ±0.35 (minute)	2.78 ± 0.61 (minute)
Vessel-ray pitting	Vessel–ray pits with much reduced borders to apparently simple: pits rounded	Vessel-ray pits simple: pits scalariform horizontal	Vessel–ray pits with much reduced borders to apparently simple: pits rounded	Vessel-ray pits distinct and simple: pits rounded	Vessel-ray pits with much reduced borders, apparently simple: pits rounded to scalariform
Fibers and sclereid					
Fiber length (µm)	1007.98 ± 133.56	1144 ± 169.11	1072.11 ± 191.63	1015.95 ± 99.14	893.75 ± 107.48
Fiber wide (µm)	17.98 ± 3.9	19.41 ± 3.8	14.85 ± 3.18	18.42 ± 2.75	15.28 ± 3.69

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Anatomical characters	**Species**				
	D. sumaterana	***D. ridleyi***	***D. pendula***	***D. dictyoneura***	***D. celebica***
Fiber thickness (µm)	4.00 ± 1.49 (very thin-walled)	4.52 ± 1.42 (very thin-walled)	3.4 ± 1.16 (very thin-walled)	4.88 ± 1.3 (thin to thick walled)	3.69 ± 1.28 (very thin-walled)
Fiber lumina wide (µm)	9.96 ± 3.36	10.36 ± 3.34	8.04 ± 3.21	8.64 ± 2.48	7.91 ± 2.78
Septate fibres	Present	Absent	Absent	Absent	Absent
Helical thickenings on fibres	Present	Present	Absent	Present	Absent
Sclereid	Absent	Absent	Brachysclereid founded in cortex	Absent	Brachysclereid founded in cortex
Axial parenchyma					
Parenchyma strand length	2 – 4 cells per parenchyma strand	3-4 cells per parenchyma strand	3-4 cells per parenchyma strand	>5 cells per parenchyma strand	4-5 cells per parenchyma strand
Ray parenchyma					
Rays height (µm)	358.31 ± 77.25	402.07 ± 78.47	385.87 ± 65.42	420.45 ± 88.15	333.08 ± 59.63
Rays frequency (per µm ^{2})	57 rays	45 rays	65 rays	45 rays	70 rays
Rays cellular composition	Procumbent and square	Procumbent and square	Procumbent and square	Procumbent, upright and square	Procumbent and square
Non-protoplasmic compound and secretory element					
Black deposit	Present on parenchyma axial and rays (1)	Present on vessels and rays (2)	Present on vessels, axial parenchyma, and rays (2)	Present on rays and axial parenchyma (1)	Present on ray cells (1)
Mineral inclusions	Absent	Absent	Absent	Single prismatic crystal present on rays square cells	Single prismatic crystal present on rays procumbent and square cells

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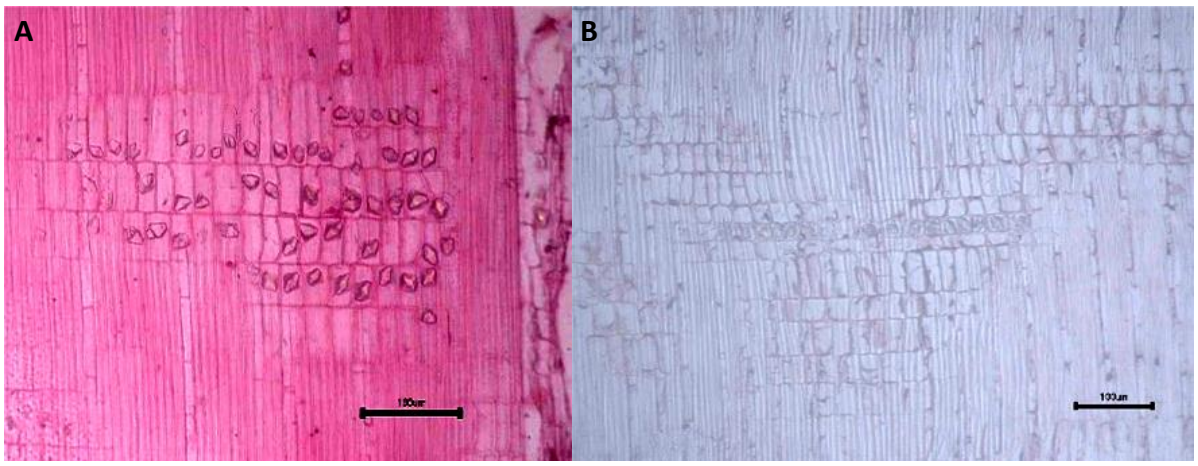

Figure 2. Single prismatic crystal founded in (A) procumbent ray cells of *D. celebica* and (B) square ray cells of *D. dictyoneura* (Radial section; scale bar=100µm; 100x magnification).

Several anatomical characters can be correlated with environmental factors such as climate, rainfall, latitudinal and altitudinal. For example, vessel grouping plays a role in drought or winter stress conditions (Carlquist, 1984) to control embolism in the vessels. Embolism is caused by the air bubbles that entering the vessels due to high evaporation and blocking water transportation (Sperry & Tyree, 1988). Vessels with clustered arrangement acts as a support system to maintain continuous water transportation to plant organs. Plants in dry areas and deserts have a higher percentage of clustered vessels than in tropical areas with high rainfall. In this study, *D. dictyoneura* has a dominant solitary vessel, *D. ridleyi* has a combination of solitary and clustered vessels, while the other species have clustered vessels dominant (see the picture of the vessel arrangement in **Figure 1**). It can be assumed that *D. dictyoneura* was less resistant to drought stress than the other four species. However, further research is needed to compare the physiological responses between those species.

The number of vessels and the diameter were other characters influenced by the climate condition (Wright, 1904) and also by increasing latitudinal and altitudinal (van den Oever, *et al*., 1981). Vessel diameter will increase during the rainy season. Areas with high rainfall also have a wide diameter of the vessel than in dry areas (Wickremasinghe & Herat, 2006). Unfortunately, the samples used in this study were taken at the same location and same period so it can't be used to compare the anatomy characters based on the environmental condition. The woods were taken from Gunung Palong National Park, West Kalimantan, in April-May 2011 with an average rainfall of 6.44 mm (Badan Meteorologi, Klimatologi, dan Geofisika Database Center). However, both characters had very wide variation so it is difficult to apply as a distinguishing feature of taxa (Metcalfe & Chalk, 1950; Rendle & Clarke, 1934). Scientifically, van den Oever *et al*. (1981) revealed a low correlation value between vessel frequency and vessel diameter at the 5% significance limit. Another factor that can cause data bias is the sampling location on the trees such as on the top of the trees, on the diameter of breast height, or near the root. But again, we cannot measure through the sample because all of the samples were taken from the branch with 1-2 cm in diameter. Based on observations, the types of *D. dictyoneura* and *D. ridleyi* which had the least vessels frequency, 5-20 / µm2 and 20-40 / µm2, had the widest vessel diameters, namely 73.6 ± 8.49 µm and 79.6 ± 10.32 µm (**Table 2**).

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3.2 Phenetic analysis based on anatomical characters

The phenetic dendrogram present two main clusters (**Figure 3**). Cluster 1 consists of *Diospyros celebica* and *D. sumatrana,* joining at approximately a distance of 0.05 – 0.1. Cluster 2 consists of *D. ridleyi, D. dictyoneura,* and *D. pendula,* with *D. ridleyi* and *D. dictyoneura* present as a subcluster by joining at a distance of 0.5, and *D. pendula* acts as the sister of this group with a distance of 0.05. The dendrogram shows that *D. celebica* and *D. sumatrana* have the highest phenetic similarity. Both species shared most of similar vessel characters such as vessel arrange in radial, vessel cell type with both long tail present, simple perforation, number of vessels 5-20/µm^2, vessel length 350-800 µm, and vessel with helical thickening present. *D. ridleyi* and *D. dictyoneura* show moderate similarity in the rays and fibers characters. However, *D. dictyoneura* has the longest distance at 3.25 from another species caused by the presence of seven characters that are boldly different from the other species. The characters are vessel arrangement diagonal and radial, vessel grouping in multiple or dominant clustering, number of vessels 40-100/mm^2, vessels with helical thickening present, fiber wall thin to thick, axial parenchyma strand length > 5 cells, and ray cells composition consist of procumbent, square, and upright. The specific characters only perform in the subclade *D. ridleyi* and *D. dictyoneura,* with the ray height > 400 µm. Meanwhile, another clade present mixed characters and can not be used as the marker.

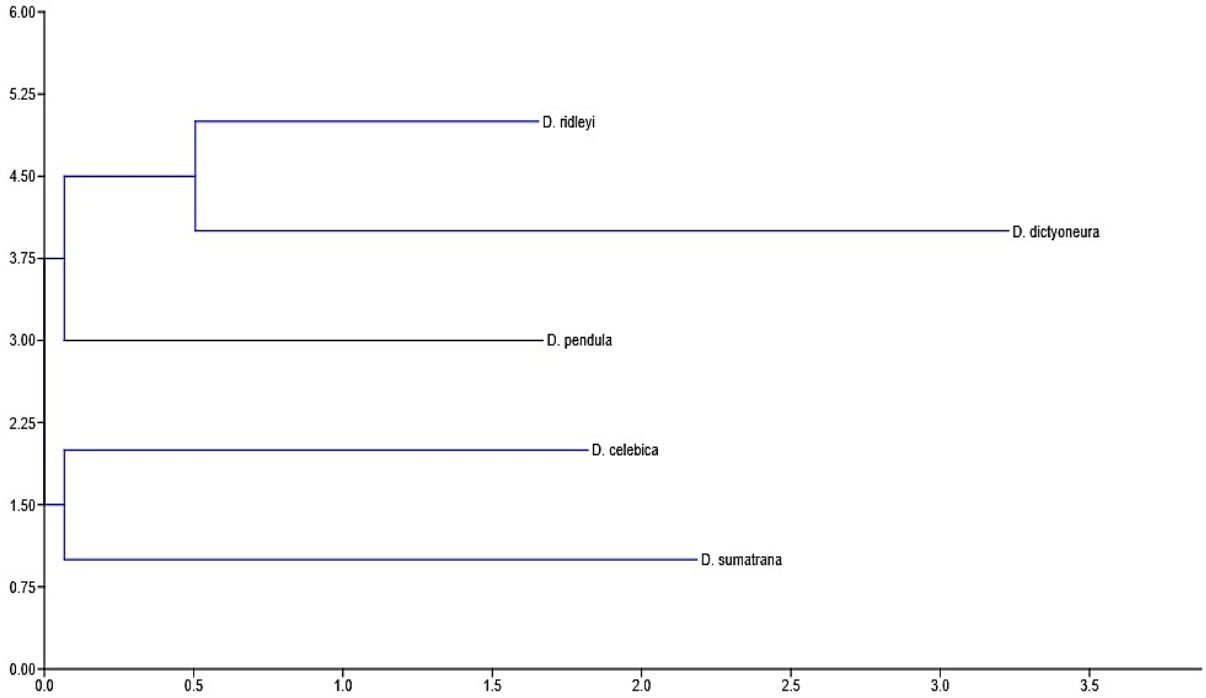

Figure 3. Dendogram of the *Diospyros* based on 20 wood anatomical characters.

3.3 Fiber dimensions derivative values of *Diospyros* spp.

The fiber dimensions derivative values of *Diospyros* wood shows that all of the wood samples are in class III as raw materials for pulp and paper (**Table 3**). The highest total score was found in *D. pendula* with a total score of 300, while the lowest score was found in *D. celebica* and *D. dictyoneura* with a total score of 250.

The ebony wood which is known for its beautiful black pattern terrace has only few species selected as a raw furniture material with high economic value (Bakri, 2008; Martawijaya *et al*., 2005). Hillis and Soenardi (1994) mentioned that the *Diospyros* used as a raw material for

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furniture must have a larger proportion of black wood core, a uniform structure, smooth, and high dry density. In this study, we are not doing the qualitative study to measure the percentage of the black or brown core from the wood samples. It is only observed by the maceration specimen that the fiber cells of the *Diospyros* contain patterned wall fibers and plain wall fibers. Secondary data of field assessment for wood density showed the value of each species are 0,592 g/cc for *D. sumatrana,* 0,604 g/cc for *D. pendula,* 0,520 g/cc for *D. ridleyi*, and 0,541 g/cc for *D. dictyoneura* (Sources: Mr. Cam O Webb, personal communication on 10 June 2020 by email). This value was smaller than the *D. celebica* with 0,823 g/cc wood density (International Centre for Research in Agroforestry Database). Therefore, those four species might be not suggested for furniture commodity.

Table 3. Derivative values five species of *Diospyros* wood.

Species	Fiber length	Runkell Ratio	Felting Power	Flexibility Ratio	Coefficient of Rigidity	Muhlstep Ratio (%)	Total score	Fiber class for Pulp and Paper raw material
D. celebica	893.75	0.93	58.47	0.52	0.24	73.21	250	III
D. ridleyi	1144.00	0.87	58.94	0.53	0.23	71.48	275	III
D. dictyoneura	1015.95	1.13	55.16	0.47	0.27	77.96	250	III
D. pendula	1072.11	0.85	72.17	0.54	0.23	70.68	300	III
D. sumatrana	1007.99	0.80	56.06	0.55	0.22	69.31	275	III

However, the fiber dimensions derivative values of the branch wood specimens shows that all of the wood samples are classified in class III (**Table 3**). The wood in class III criteria are suitable for a raw materials for pulp and paper, but not kind of high-quality paper (Mandang, 1993). Lestari and Hastoeti (2000) mentioned that the wood with class III category will produce the paper with a medium value of tensile, bursting, and tearing strength. Some paper-based product like chipboard or kraft which is not require paper with a smooth surface and translucent are possible to produce with the class III wood fibers.

4. CONCLUSION

Specific anatomical characteristics of the *Diospyros* wood samples are the presence of black deposits in the ray and axial parenchyma cells. Two clades are present from the phenetic analysis using the anatomical characters, namely Cluster 1 consist of *Diospyros celebica* and *D. sumatrana,* and Cluster 2 consist of *D. ridleyi, D. dictyoneura,* and *D. pendula*. There is only a limited characters can be used as the marker of the clades. Sub-clade *Diospyros ridleyi* and *D. dictyoneura* present specific characters of the ray height of more than 400 µm. The four species of *Diospyros* has a lower wood density than *Diospyros celebica,* so this can not be possible to use as a furniture commodity. However, all of the *Diospyros* wood samples have wood quality class III, which can produce paper with a medium value of tensile, bursting, and tearing strength.

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6. AUTHORS' NOTE

The authors declare that there is no conflict of interest regarding the publication of this article. Authors confirmed that the paper was free of plagiarism.

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