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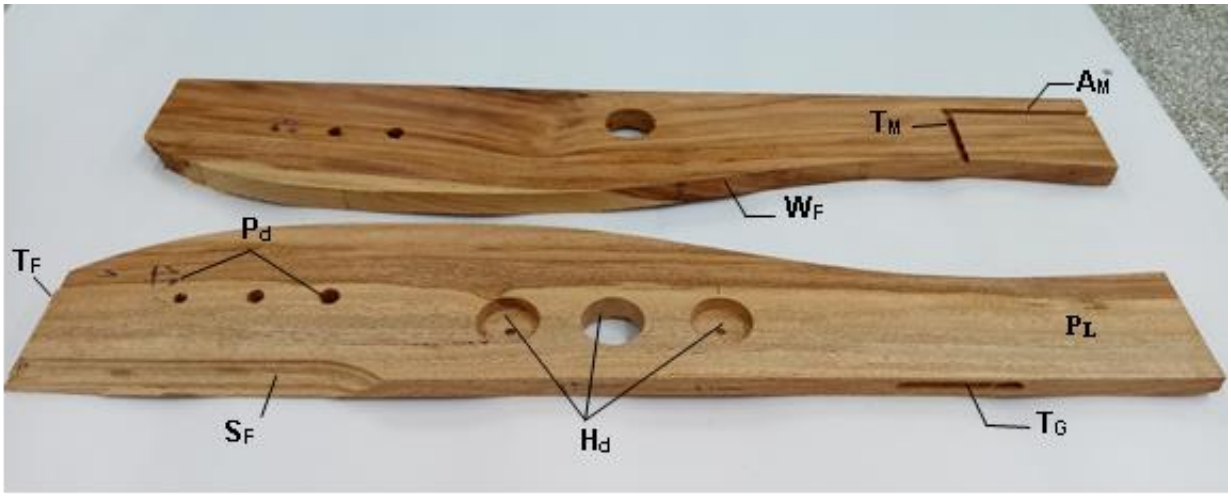

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Variation of Heartwood Proportion and Wood Colour from Fast Grown 5-Year-Old Teak

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Abstract

The heartwood percentage and wood colour of fast plantation grown teak destined for harvest at 5 years of age were characterized using automatic image processing 'ImageJ' routines and CieLab's colour system with the following coefficients: L for lightness, a* for redness, and b* for yellowness. Analyses were conducted on material from different dry and wet sites. Comparison with 6-year-old plantation from a dry site was conducted to study differences arising in older trees. Analyses of variation of those properties between and within different tree diameter classes were also conducted. The results showed that brightness, redness, and yellowness values of 5-year-old teak trees were 60.7, 10.7 and 23.1, respectively. Tree clone had a more dominant effect on wood colour and heartwood proportion than site, thus if specific colour preferences are needed of plantation trees, clone selection is important. The drier site produced larger proportions of heartwood in trees, as well as a more attractive figure. The trees produced heartwood proportions of 20% and 14% from the dry and wet sites respectively. On average, these 5-year-old teak trees already produced 18% heartwood. Faster tree growth (larger diameter) appeared to have produced significantly larger heartwood proportions. Radially, the palest colour (the highest L but the lowest a*b* parameters) occurred in an area between heartwood and sapwood indicating the presence of a transition zone in all the tree samples.

Keywords: extractives, super teak, clone, young teak, wet and dry sites, Indonesia.

Introduction

In Indonesia, there has been an effort to cultivate fast grown plantation teak with the aim to harvest it within 5 years-'super teak'. However Saranpaa (2003) and Thulasidas *et al*. (2006) indicated that timber from shorter rotations brings a lower price on timber markets as it is generally believed to exhibit a lower quality. While intensive timber production may induce changes to the anatomical and technical properties of the wood colour, density, durability, and mechanical properties, potentially this may affect the suitability of using such timber for high quality products (Saranpaa 2003). To test this assertion, an investigation was undertaken of the wood properties to determine the possible utilization potential of the very fast grown teak.

One of the wood properties highly regarded for panelling and furniture is the wood figure (Nocetti *et al*. 2011) which is characterized by the presence of a rich wood-colour and the presence of a coloured heartwood. Teak is highly regarded for its wood colour and this is considered as an important aspect for its marketing (Nocetti *et al*. 2011). Consequently, it is the attractive figure that is one of the major reasons why this species is widely planted commercially (Moya and Marin 2011).

In addition to the effect on figure, wood colour might be useful as an indirect indicator of the level of resistance to insect and fungi attack (Gierlinger *et al*. 2004; Lukmandaru and Takahashi 2008). Wood colour also tends to reflect the chemical makeup of the heartwood (Lukmandaru *et al*. 2009) but to a lesser extent the sapwood. Similarly a study on larch heartwood by Gierlinger *et al*. (2004) revealed that the amount of polyphenols turned out to be strongly correlated with the reddishness (a*) of larch heartwood and this provided an indirect correlation between the red hue and brown rot decay resistance. Furthermore, they proposed that colour characterization to predict decay resistance of the wood might be beneficial in tree breeding programs for optimization of timber utilization.

Nocetti *et al*. (2011) reported that wood colour was possibly affected by environmental influences. Combination of dry climate and fertile site tended to produce more yellowish-brown colour (higher yellowness (b*) parameter which corresponds to the blue yellow axis) of the heartwood. In their study, climate was considered as the first-order contributor for explaining wood colour variation.

Furthermore, according to Trockenbrodt and Jouse (1999), proportion of heartwood become an important criterion influencing the wood quality and potential utilization of young plantation teak. Moya and Marin (2011) mentioned that this property is a very important characteristic of teakwood. According to Pinto *et al*. (2004), heartwood proportion varies between and within species. It is influenced by growth rate, stand type, individual tree characteristics, site conditions and genotype (Pinto *et al*. 2004), as well as by tree clone (Moya and Marin 2011). However, according to Bouslimi *et al*. (2013) only limited tree growth information is available on within-tree heartwood proportion, and site effects.

Teak in this study has been planted in Indonesia since 2007 (first harvesting started in 2012) in many areas which can be classified into wet and dry sites. The objectives of the study were to investigate heartwood proportion and wood colour of the 5-year-old teak planted on different sites at radial and axial variations. The effect of tree clone was also considered to see to what extent those properties are influenced by the genetic sources and the environment. As the variation in the stem diameter of the trees at the same age is quite large, the effect of growth rate (tree growth) on wood colour and heartwood proportion was also considered.

Materials and Methods

Materials Preparation

Eighteen trees ranging from small, medium and large diameter stems from 5-year-old fast grown Jati Utama Nasional (JUN) teak plantation forests in Bogor, West Java, Indonesia, and Magetan, East Java, Indonesia (Table 1), were selected and felled for wood colour and heartwood percentage measurements. For comparison, 6-yearold trees from dry site (Magetan) were also cut down. Three discs at five centimetres thickness were taken from bottom, middle and at the top (stem diameter minimum 7 cm) of each tree. In addition, specimens were taken from sawn boards between the bottom and middle sections to observe the colour variation in the longitudinal direction (along the length of boards). Seven boards were selected as replicates from each of the sites and for each tree diameter class. In total 60 discs and 42 boards were prepared for measurement.

Site Information

Climate data were collected from the Meteorology, Climatology and Geophysics Agency at Darmaga Station, Bogor, West Java (representing Site 1-wet site) and Karangploso Station, Malang, East Java (representing Site 2-dry site) from 2007 to 2013 (during the course of tree growth at the plantations). The climate data covered average temperature, average humidity, and rain fall, number of rain days, air pressure, and wind velocity.

Physical and chemical soil properties were obtained from the primary data collection at both sites. Three soil sub samples were gathered from 0-30 cm depth and quartered to obtain single 0.5 kg samples for soil testing at the Soil Research Centre, Ministry of Agriculture, Bogor, and Lab. of Soil Science and Natural Resource, Faculty of Agriculture, Bogor Agricultural University, Bogor. A summary of climate and soil properties data is presented in Table 2.

Table 1. Selected tree samples***

No	Tree code	Diameter [cm]	Height* [m]	Clone**	Tree code	Diameter [cm]	Height* [m]	Clone**
	Site 1 (West Java)				Site 2 (East Java)			
Class Diameter 1: large (>26 cm)								
1	SA22	30.25	16.00	A	K198	29.3	10.5	B
2	AC18	31.4	18.20	B	S12	29.3	12	C
3	AC23	32.2	13.60	A	S101`	28.7	9.2	C
Class Diameter 2: medium (17-25 cm)								
1	S 50	20.7	11.50	-	S108	21.5	8.5	-
2	S 74	21.3	9.10	-	S153	24.2	6.5	-
3	S 105	22.9	11.40	-	S78	24.5	8.65	-
Class Diameter 3: small (10-16 cm)								
1	S 89	14.3	9.65	-	S27	14.3	6.4	-
2	S 53	11.9	8.90	-	S2	16.6	6.7	-
3	S 120	14.6	10.10	-	S10	16.2	5.8	-
6-year old trees								
1					M73	27.7	10	
2					Y56	27.4	12	

*Economic height: height of stem at the top with minimum diameter of 7 cm
**In this study, DNA testing to check the tree clone was conducted only on wood from the large diameter classes
***Damayanti *et al.* (2019)

Colour and Heartwood Measurements

Colour Measurement. CR10 Tristimulus colourimeter (Konika Minolta®) with 8 mm aperture at the point measurement was used for measuring the colour coefficients of the sapwood, transition and heartwood zones from cross sections of disc samples, and also from the sapwood and heartwood zones of the sawn boards.

Three replicate measurements were conducted at different locations from each zone. All colour measurements were made using standard illumination conditions: D65 and 10 degrees observer (HunterLab 2012). Small circular areas were marked on the faces of test specimens to ensure that

repeatable measurements were performed at the same location. The colour coefficients L a*b* were measured and used to represent wood colour of each sample. All measurement points were treated as target (absolute measurements).

According to HunterLab (2012) procedure, the CIELab colour system estimates the wood colour coefficients using three coordinates: L for lightness represents the position on the black–white axis (L = 0 for black, L = 100 for white), a* for the chroma value, this defines the position on the red–green axis (+100 indicates red shades, -100 indicates green shades), and b* for chroma value and defines the position of the yellow–blue axis (+100 indicates yellow shades, -100 indicates blue shades). Measurements were conducted at room temperature.

Table 2. Summary of climate data and soil properties of plantation sites from 2007 to 2013 at Bogor (Site 1) and Magetan (Site 2)

Climate/Soil Properties	Unit	Site 1 (Wet)	Site 2 (Dry)
Location		Bogor (West Java)	Magetan (East Java)
Elevation	m.a.s.l	157	110
East longitude		106°51'	111°24'
South latitude		6°35'	7°44'
Average temperature	°C	25.8 (25.5-26)	26.9 (26.6-27.1)
Average humidity	%	82.8 (81-85)	76.5 (74-81)
Average annual rainfall	mm	3667 (2845-4051)	2330 (1739-3749)
Number of dry months	Months	0	3
Average number of rain days	Days	23.3	15.6
Wind velocity	Km/Hour	3.3	11-18
Average air pressure	Millibar	989.3	1011.6
Mean tree diameter	cm	22	23
Mean tree height	m	12	8
Ca	Me/100g	13.78	39.63
Mg	Me/100g	2.23	4.76
K	Me/100g	0.54	3.30
ECEC	Me/100g	17.17	19.25
Fe	%	3.38	3.29
Al	%	6.3	5.1
Mn	ppm	1621	950
Cu	ppm	29	50
Zn	ppm	66	156
P (Bray 1*)	ppm	14.80	13.20
Rough Silicate	%	74.68	59.94
pH of H_2O		5.90	7
Soil texture		14% sand, 20% dust, 65% loam	46% sand, 32% dust, 22% loam

Ca calcium, Mg magnesium, K potassium, ECEC Effective Cation Exchange Capacity, Fe iron, Al aluminium, Mn manganese, Cu copper, Zn zinc, P phosphorus

Me/100g=meg+/100g=meg/100g= Mili-equivalent of Hydrogen per 100 g of dry soil (SI cmol+/kg)

*Bray method: Used for the determination of available Phosphorus content in the soil (Sawyer and Mallarino 1999).

Heartwood Measurement. To minimize the human decision making, automatic image processing was used to determine heartwood percentage using ImageJ® freeware routines (Mekhtiev and Torgovnikov 2004). A digital camera was employed to capture images from each sanded disc. An outline of the steps for calculating the heartwood percentage is presented in Figure 1. Total disc area was determined by thresholding B/W images of disks. The heartwood area was conveniently further thresholded due to grey level differences from the background wood to provide proportions of heartwood from each disk.

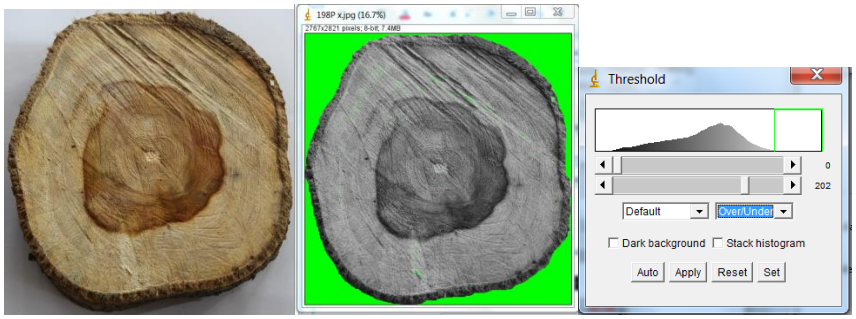

Figure 1. Automatic image processing procedure using ImageJ® to determine heartwood percentage from digital images of disk sections

Data Analysis

Tests to determine if the data were normally distributed were conducted using Saphiro-Wilk W test (Statistica®). Differences between 5- and 6-year-old teak (age differences) and between sites (different teak plantations) were carried out by using Mann-Whitney U-test (Statistica®). Furthermore, variation among different clones, different tree diameter classes and different axial and radial positions were analysed using Kruskal-Wallis ANOVA test (Statistica®). These non-parametric tests were used since the data were not normally distributed.

Results and Discussion

Wood Colour and Heartwood Proportion of 5-year-old Fast Grown Teak

In general, the brightness (L) of 5-year-old teak trees from both plantations was 60.7, and the redness (a*) and yellowness (b*) values were 10.7 and 23.1, respectively. Tests were further conducted to analyse the difference between 5- and 6-year-old JUN teak trees (Table 3). As the 6-year-old teak was only available from the dry site, the comparison was carried out between 5- and 6-year-old teak trees at this site. The older plantation produced significantly lower L and b* and higher a* values.

Table 3. Average colour parameters of 5- and 6-year-old JUN trees (Dry site)

Colour Parameter	Mean and Standard Error				P value
	5 years (N=251)		6 years (N=33)		
L	60.4	0.5	55.5	0.8	0.00**
a*	11.1	0.2	13.1	1.3	0.00**
b*	23.8	0.2	23.2	0.4	0.014*

*= Significant at α =< 0.05; **= Significant at α =< 0.01 according to Mann-Whitney U test

Table 4. Colour of teak planted in Indonesia and India

Age (years)	Annual Rainfall (mm)	Colour Parameter			Authors
		L	a*	b*	
5	1739-3749	60.4	11.1	23.8	This study
5	2845-4051	61.0	10.11	22.15	This study
6	1739-3749	55.5	13.1	23.2	This study
8	1400-1800	65.2	4.3	23.8	Lukmandaru and Takahashi (2008)
30	1700-2500	65.7	4.8	25.48	Lukmandaru and Takahashi (2008)
35	2500-3500	52.3	6.35	21.1	Thulasidas *et al*. (2006)
35	1500-2300	54.0	6.37	23.4	Thulasidas *et al*. (2006)
35	2500-3000	56.4	6.8	23.4	Thulasidas *et al*. (2006)
51	1300-2000	60	5.5	24.25	Lukmandaru and Takahashi (2008)

Table 5. Heartwood proportion of plantation teak grown in Indonesia and Malaysia

Ages (years)	Teak		Authors	Location
	Fast Grown	Teak grown from seed		
3	29.81% (bottom)	25% (bottom)	Wahyudi and Arifien (2005)	Central Java, Indonesia
	16% (stem 2.5-4.8 cm)		Trockenbrodt and Jouse (1999)	Malaysia
4	16.5-63% (stem diameter 5.8-10.9 cm)		Trockenbrodt and Jouse (1999)	
5	22.61 % (average from the base, middle and top part of trees)	20.31%	Sumarni *et al*. (2008)	South Sumatera, Indonesia
	25-65% (stem diameter 6.9-11.4 cm)		Trockenbrodt and Jouse (1999)	Malaysia
	18%		Wahyudi *et al*. (2014)	West Java, Indonesia
	21%		Damayanti (2010)	Tegal, Central Java, Indonesia
	20% (average stem diameter 23 cm)		This study	Magetan, East Java, Indonesia
	14% (average stem diameter 22 cm)		This study	Bogor, West Java, Indonesia
6	44.31%		Anisah and Siswamartana (2005)	Ciamis, West Java, Indonesia
	20.12%			Ngawi, East Java, Indonesia
	23% (average stem diameter 27.5 cm)		This study	Magetan, East Java, Indonesia
7	39.6%	20.3%	Krisdianto and Sumarni (2006); Krisdianto (2008)	Penajam, East Kalimantan, Indonesia
8		58.23% (bottom) 46.30% (middle)	Wahyudi and Arifien (2005)	Central Java, Indonesia

The brightness indices of the 5- and 6-year-old teak from this study were lower than that for the 8-year-old trees planted in Gunungkidul, Jogjakarta. The annual rainfall from the Gunung Kidul area is much lower than the dry site's annual amount. The average L value for 8-year old teak was 65.2 (Lukmandaru and Takahashi 2008). It showed that even in older wood, the very dry environment potentially produced lighter coloured wood. Older teak trees from Kerala, India, aged 35 years planted under rather similar climate conditions to this study produced darker coloured timber with a similar yellowness index (Thulasidas *et al*. 2006). Summary of wood colour of 5- and 6-year-old JUN teak and comparison with older age teak is presented in Table 4.

Gierlinger *et al*. (2004) stated that old trees from natural stands had higher a* values than young trees from the same provenance grown in plantations. However, in this current study, the redness value was almost twice as high as that from older teak. Possibly this was because there are differences between genetic or environmental factors such as soil fertility and climatic conditions (Moya and Marin 2011). It is proposed that measurements of colour from different aged plantations grown at the same location should be conducted to study trends in wood colour due to age differences.

As shown in Table 5, average heartwood proportion from both sites measured from the base to the top part of 5-year-old JUN teak in this study was 18% (average diameter of the discs was 17 cm). This result was similar to the heartwood proportion of 17% for the same teak planted in West Java, Indonesia (Wahyudi *et al*. 2014). Six-year-old JUN teak in this study produced a larger proportion of heartwood (23%) even though it was not statistically significant compared to 5-year-old JUN teak ($p = 0.22$). These values were far lower than the 76-year old teak whose heartwood proportion reached 84% (Wahyudi 2000; Wahyudi and Arifien 2005). An outline of these heartwood proportions of plantation teak of different ages and sites is presented in Table 5 and the appearance of the heartwood of 5-year-old fast grown teak is presented in Figure 2.

Figure 2. Heartwood of 5-year-old fast grown teak from dry site (left) and wet site (right)

Wood Colour and Heartwood Proportion of Wood from Wet and Dry Sites

Differences were observed between wet and dry sites as reflected by different a* and b* coefficients. Even though the L value was higher (the timber was lighter) from the wetter site, there was little influence upon L. In contrast to the wetter site, more distinct figure was observed from the wood from the drier site due to higher redness (a*) and yellowness (b*). This result is in agreement with Nocetti *et al*. (2011) who noted that a dry and fertile site produced more yellow heartwood colour. Besides having drier climate, according to the ECEC (Effective Cation Exchange Capacity) value, Site 2 also has a higher ECEC value and more fertile soil than Site 1. Colour of the teak heartwood from the wet and dry sites is presented in Figure 3.

Figure 3. Appearance of the wood colour of teak wood grown from a wet site (left) and a dry site (right)

Furthermore, the result from this study is also in line with Thulasidas *et al*. (2006) findings for 35 year old teak planted on different wet and dry sites. Their studies revealed that while no significant differences were observed in brightness and reddishness, yellowness of wood from wet sites was less than that from the dry sites; this was proposed to act as a limiting factor for timber prices.

Bhat (1999) in Moya and Calvo-Alvarado (2012) identified two broad wood colour groups from contrasting geographic regions in Asia. They suggested that a uniform golden yellow to brown colour is typical of one group wood from tropical wet climates along the coast, and the second group is represented by a darker colour of the wood from tropical dry climates from central areas. While the L coefficient was not statistically significant in this study, the results are in agreement with the proposed groupings.

It is still unclear which environmental factor is responsible for elucidating wood colour variation, but according to Moya and Calvo-Alvarado (2012), wood from drier climates produces darker wood colour. Slower growth due to dry seasons will create a larger stem diameter (Thulasidas and Bhat 2007), which is in line with the current study (Table 2) although in this study this difference was not significant ($p = 0.89$). The length of dry season and deficit in

soil water reduce the tree growth (shown by significantly shorter trees from the dry site, Table 2) during this period, and induces the heartwood formation (Kokutse *et al*. 2010). Gierlinger *et al*. (2004) and Moya and Calvo-Alvarado (2012) suggested that extractives will be formed at different rates in the heartwood of trees from drier climates than those from humid climates. These are possibly the reasons why heartwood proportion of wood from wet and dry sites were statistically significant in this study. The humidity at the wet site was also higher than that from the dry site (Table 2). In keeping with this, the wood produced from the drier site in this study had a larger proportion of heartwood (20%) than wetter site (14%) as seen in Figure 2.

Gierlinger *et al*. 2004; Kokutse *et al*. 2010; Lukmandaru and Takahashi 2008 suggested that the higher dynamics of heartwood's formation in trees from drier sites produce higher extractives content leading to the formation of a darker and redder wood colour. On the other hand Moya and Calvo-Alvarado (2012) indicated that less extractive material will be produced during the growth of trees in growth-favourable environmental conditions (wet sites) because reserve material is used for other needs of the tree rather than for the formation of extractives and heartwood, so the timber will less dark.

When termite resistance is considered, Lukmandaru and Takahashi (2008) claimed that the wood is more resistant when it is darker and redder. This was based on the moderate correlation between wood brightness and redness and the survival rate in the first week after exposure. However, these authors believe that the relationship between the termite resistance and colour is complex because it is known that the colour of wood tends to be related to the quantity and types of wood extractives. Other studies have attempted to relate wood durability to wood colour (Gierlinger *et al*. 2004), but they may or may not be appropriate for modelling the durability of fast grown teak. Based on studies of old teak (Lukmandaru and Takahashi 2008), the durability of fast grown material has yet to be assessed and potentially it is worthy of further study.

Wood Colour and Heartwood Proportion between and within Clones

Clone factor significantly affected heartwood colour (Moya and Marin 2011). In this study, trees from the same clone planted on the same site produced the same colour coefficients. Furthermore, the same clone if planted on different sites consistently produced the same a* and b* coefficients. Gierlinger *et al*. (2004) stated that the wood colour of the same provenance grown on different sites is similar. However in this study, the L coefficient was different as darker coloured wood was produced from the drier site. The extreme difference in annual rainfall between both sites had an influence on the L coefficients even if the trees originated from the same clone.

Moreover, in this study, it was shown that trees from different clones planted at the same location resulted in different L and b* values. This is supported by Gierlinger *et al*. (2004) who observed that in Larch (*Larix* spp.) trees of different provenances planted on the same site showed different colour characteristics. Consequently, the different clones of teak in this study may have originated from different provenances.

From this section it can be summarized that the colour characteristics of wood from different clones may still appear even if they are planted on a same site. This study showed that clone had a greater influence on the wood colour than site. Thus, it is suggested that if specific colour preferences are needed of plantation trees, clone selection is important (Damayanti *et al*. 2019).

Analysis of variance of heartwood proportion in clones could not be applied as there were only three to six discs from each clone. Table 6 shows the heartwood proportions of the same clone planted at the same site (green rows), different clone planted at the same sites (yellow rows), and the same clone planted on different sites (blue rows). Generally, variation among the clones showed that same clone planted at the same site produced a similar amount of heartwood; also different clone planted at the same site produced different amounts of heartwood, while the same clone planted on different sites exhibited similar heartwood proportions (and consistently, drier sites produced larger heartwood proportions). Similar to that of wood colour, the result showed that the heartwood proportion from young teak was more influenced by clone than site. The result is in good agreement with Moya and Marin (2011).

Table 6. Proportion of heartwood from 5-year-old fast grown teak clones

Clones	Sites	Heartwood Proportion (%)
A	Wet	12
A	Wet	25
C	Dry	19
C	Dry	21
A	Wet	18
B	Wet	28
B	Dry	32
C	Dry	20
B	Wet	28
B	Dry	32

Effect of Tree Size on Wood Colour and Heartwood Proportion

Additional observations were conducted to assess the wood colour variation among different tree diameter classes. The analysis was conducted because a large variation was observed between tree diameters of trees of the same age.

In this study, wood colour characteristics were different among different tree diameter classes. In contrast with the small diameter class, the largest diameter class exhibited the lowest L and the highest a*b* coefficients.

Wood colour affecting the figure of wood in teak exhibited an inverse relationship between L and a*b* coordinates, lightness, redness, and yellowness, respectively. Hence, faster grown trees produced more attractive wood colour, showing a nicer figure.

Because there is a site effect on heartwood proportion, an analysis was also conducted for each of the sites. On the wet site, the faster tree growth produced larger diameter trees which had significantly larger heartwood proportions ($p = 0.02$), *viz* 13%, 9% and 21% for small, medium and the large diameter classes respectively. While, faster growth resulted consistently in the production of a larger proportion of heartwood from the drier site, this was not significant statistically ($p = 0.33$). The proportion of heartwood produced from small, medium and large trees was 16%, 20% and 24% respectively, and this is in line with the results for radiata pine obtained by Harris (1954) in Cown *et al*. (1991) who stated that dominant trees with deep root systems and an adequate water supply would tend to form greater amounts of heartwood.

Wood Colour Variations within Trees

Strong patterns of colour were evident from wood along the tree radius from trees from both sites. Even though the trees are still young, they exhibited clear colour variations from the sapwood, transition zone and the heartwood. Colour coefficients along the axial and radial positions of 5-year-old teak are shown in Table 7.

In the wet site, redness and yellowness index significantly decreased at different axial positions along the tree. The highest a* and b* coefficient was observed at the bottom of the trees, and became paler with increasing tree height. In the dry site, a more uniform colour was exhibited axially. This means that between the bottom, middle and top part of the trees, the L, a* and b* values were similar. According to Moya and Calvo-Alvarado (2012), this more uniform colour within trees produced from the dry site has met one of desired industrial goals for improving the uniformity of wood colour. However, for teak, colour variation of the wood is more visually appealing, but if uniformity is required it can be rendered by staining. Studies on colour variability in the axial direction have not previously been reported.

In the radial direction, the transition zone showed higher L values than the sapwood. On the other hand, redness (a*) was least in the transition zone, and highest in heartwood. The transition zone also had the lowest yellowness (b*), while sapwood had the highest. Redness dominated the heartwood, and yellowness dominated the sapwood. Gierlinger *et al*. (2004) proposed that different colour characteristics between the heartwood and sapwood are probably due to the oxidation and polymerization of phenolic components during and after heartwood formation.

Table 7. Average Axial and radial colour coefficients of 5-year-old teak trees

Colour Parameters	Axial (mean)			P value	Radial (mean)			P value
	Bottom	Middle	Top		Sapwood	Transition	Heartwood	
				Wet Site				
L	60.1	61.5	61.8	0.61	65.1	67.1	52.4	0.00**
a*	10.9	9.6	9.6	0.04*	9.0	8.1	12.7	0.00**
b*	23.2	21.1	21.9	0.01**	23.4	20.5	21.9	0.00**
				Dry Site				
L	61.0	59.2	61.1	0.61	64.0	66.5	53.1	0.00**
a*	10.8	11.6	11.0	0.33	10.1	8.9	13.6	0.00**
b*	23.9	23.7	24.0	0.59	24.4	22.7	24.0	0.00**

*=Significant at α=<0.05; **= Significant at α=<0.01 according to Mann-Whitney U test

Consistently, transition zones had the palest colour (the highest L and the lowest a* and b*). This result is consistent with the transition zone definition proposed by Hillis (1987) as the narrow and pale-coloured zone surrounding heartwood. This relatively white zone devoid of colour from of the transition zone is also due, in part, to that wood having a lower moisture content as can be seen in Figure 4.

The phenomenon of transition zone in teak wood was also observed by Barnacle and Ampong (1974) in teak planted in Ejura, Ghana. They experienced preservative penetration problems associated with poor ingress of treatment chemicals in relatively wide zones of transition zone in 15 cm diameter fence posts from 12-15-year-old un-pruned plantation grown teak. Some posts also showed alternating penetrated and non-penetrated bands in the heartwood. Furthermore, Norton (2012) observed full outer sapwood penetration and virtually no penetration in the heartwood with vacuum pressure impregnation using copper based preservative (CCA and Copper Naphthenate) in six specimens of six and a half year old teak trees planted in tropical north Queensland, Australia. In this case, the transition zone which was apparent in three of six samples was also not penetrated.

In the light of the report from Thulasidas *et al*. (2006), there appears to be a need for further study if improved durability of young plantation grown teak is required since timber from young plantations generally tends to have lower natural durability. The presence of refractory transition zones appears to present additional difficulties for adequate penetration of preservatives similar to that of the heartwood.

Figure 4. Transition zone (arrows) as it appears in cross sections of all logs cut from 5-year old fast grown teak trees

Conclusions

Colour characteristics of the same clone planted at the same or different sites, appeared to produce a uniform colour. Only the lightness intensity (L value) was influenced in wood from very different wet and dry sites. Type of clone had the most dominant effect on wood colour rather than site. Similar to colour, clone had a more dominant effect on the proportion of heartwood than site, and the drier site produced a larger proportion of heartwood. The transition zone was observed along the radius and was shown by measurement to have the palest colour area surrounding the heartwood. This zone also had the highest L and the lowest a* and b* values. More attractive figure was observed from trees planted on drier sites than wetter ones as measured by higher redness (a*), yellowness (b*) and lower lightness values (L).

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Machining Operations on Messassa Wood

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Abstract

This study aimed to evaluate the effect of machining operations on the surface quality of the messassa wood (*Brachystegia spiciformis* and *Julbernadia globiflora*) for use in the furniture industry. The wood cames from Mozambican Miombo Woodland. The following machining operations were performed: planing, shaping, milling, tearing and boring based on technical standard. The wood had a surface quality approval rating above 70% in all tested machining operations. A perfect surface quality was obtained with a feed speed of 6 $m.min^{-1}$ in planing. *Brachystegia spiciformis* had easy workability and extremely well performance compared to *Julbernadia globiflora*. Nevertheless, both wood species have great potential for use in higher value-added products such as furniture and frame production.

Keywords: *Brachystegia spiciformis*,*Julbernadia globiflora,* surface quality, higher value-added products, planing, shaping, milling, tearing, boring.

Introduction

The Mozambican furniture industry needs modernization and expansion as well as demand for new and lesser-used woodspecies in the domestic and international market. Therefore, knowledge of the possible applications of wood from the native species is essential, considering the high biodiversity existing in the biomes of Mozambique and the different technological characteristics of timber species. In recent years, there has been a tendency for research in Mozambique to indicate other potential species for wood production by determining their anatomical characteristics (Uetimane *et al.* 2009 and 2018; Bila *et al.* 2018), chemistry (Lhate *et al*. 2010), physical and mechanical properties (Ali *et al.* 2008; Cristovão *et al*. 2011), and cutting forces (Lhate and Cristovão 2007). Thus, as in the technological characterization of the species, knowledge about machining evaluation and surface quality of wood is extremely important for their use.

The species *Brachystegia spiciformis*. Benth and *Julbernadia globiflora* (Benth.) Troupin commonly known as common messassa and red messassa, respectively, represent the largest availability of commercial timber in the country, with approximately 46% of the total volume (Magalhães 2018). Such species have the potential to be inserted in different sectors of the timber segment for higher value-added produtcs (DNTF 2017). However, due to the lack of knowledge about the behavior of these species under different machining processes, they are relegated to less noble uses in civil construction. It is noteworthy that the optimization in the use of wood involves the knowledge of its characteristics, as well as it is interaction with different machining operations (Silva *et al.* 2005; Aguilera and Muñoz 2011).

According to Brown (1998) and Silva (2002), wood machining is the mechanical process that aims to give the desired shape regarding the dimensions and quality of wood products. In addition, Lucas (2004) affirm that machining and it's cause and effect relationships with the variables involved in the process are paramount for positioning against the competition and for the development of a more efficient manufacturing process. Taylor *et al*. (1999) and Gupta *et al*. (2019) state that during machining process, different species tend to exhibit distinct behavior due to their inherent wood characteristics, such as density, moisture content, anisotropy, presence of silica and minerals, wood grain and texture, juvenile wood, age, reaction wood, presence of knots, among others.

Wood machining includes various operations such as cutting, planing, sanding, milling, boring and turning, requiring specific machines, which can be manual or automatic, aiming a better appearance on their surfaces facilitating a better application of finishing products (Palermo *et al*. 2010; Zamarian, *et al*. 2012). Machining quality can be assessed by calculating normative values for tooth feed (fz) or the presence of surface failures, which are generated as a function of the intrinsic structure of the wood or by the roughness parameters Ra, Rz provided by the roughness meter (Silva 2002; Braga 2014). However, it is common to infer the quality of the machined wood surface through the use of ASTM D1666-11 (2011) which consists of assigning grades after visual inspection of some defects such as raised grain, fuzzy grain, torn grain, crushing, chip marks, etc. In this assessment, lower grade numbers describe surfaces that would be acceptable in a manufacturing environment, while higher grades require additional rework, resulting in additional production costs (Hernández *et al*. 2011).

Thus, the present study aimed to evaluate the behavior of two messassa wood species under machining processes such as planing, shaping (top, side, and winding axial), axial and transverse milling, tearing and boring (pin

and hinge) in order to contribute to the country's timber sector by disseminating scientific and technological information with a view to adapting these species to furniture production, allied to the issues of better use of tropical wood.

Materials and Methods

Materials

Wood from two Mozambican native lesser-used species, *Brachystegia spiciformis.* Benth (red messassa) and *Jubernardia globiflora*. Benth (common messassa) whose basic density are (0.670 and 0.680) g.cm^{-3} respectively, were used. Tangential planks where obtained from15 logs of *B. spiciformis* and 12 logs of *J. globiflora*, of 1500 mm in length, and width variation based on the diameter of the trees, which ranged from (260 to 550) mm. Subsequently, the planks were ripping in a multiple circular saw, into board of size 25 x 150 x 650 mm^3 and conditioned at temperatures of 20 ± 2°C and 65 ± 5°C, with a relative humidity to the moisture content of 12 ± 2%. Then, 20 samples of each species were randomly selected for machining tests. These samples were selected representative of the intrinsic characteristics of the species with smaller heartwood region, when compared with the sapwood, because generally, the heartwood tends to rot in the core and some samples have heartwood and sapwood, in different proportions. This sampling allowed to test the wood as it is marketed in the country.

Methods

Machining Operations Tests. The machining operations tests followed ASTM D 1666-11 (2011). The machining parameters used in the operations followed the methodology of Zamarian *et al.* (2012). It is noteworthy that to avoid the influence of cutting edge wear on the machined surface quality, the sequence of the samples in each operation were randomly selected. With the exception of planing, the average feed speeds presented for the remaining operations were manual and obtained by the ratio of machining offset to the programmed time required to perform. All tests were carried out on the same sample using the following technical machining parameters (Table 1).

Table 1. Machining parameters used for *Brachystegia spiciformis* and *Julbernadia globiflora* wood

Machining operation	Nr.	n (min^{-1})	D (mm)	V_S (m.s^{-1})	V_F (m.min^{-1})
Planing	3	3445	105	18.93	6.0;15.0
Top frame	2	8000	135	56.52	0.65
Side frame	2	8000	135	56.52	0.78
Winding axial frame	4	8000	90	37.68	0.50
Tearing	2	3380	8	1.42	2.58
Axial milling	1	27000	5	7.07	0.78
Transversal milling	1	27000	5	7.07	1.38
Pin drilling	2	1735	8;10;12	0.73;0.91;1.09	0.50
Hinge drilling	2	1735	35	3.18	0.17

Where: Nr. = number of knives; n = tool holder spindle rotation speed; D = tool diameter; Vs – Cutting speed; V_F = Feed speed.

Planing was performed on an *Omil* model DES 400 planer, with frequency of 60 Hz. The samples were planed on opposite faces parallel to the grain with each face having distinct feed speed and constant cutting speed (Table 1). Two passes were made on each side with a depth of cut of 0.5 mm.

Shaping (top and side frame) were performed on a *Invicta* model TI-14 router, crosswise and along the grain respectively using a standard cutter shaping LEN-Profile 24. For winding axial frame, a flat forming knife was used and consisted of performing a winding curve parallel to the grain and along the length of the sample as shown in Figure 1.

The tearing test was performed in the same corner on the side where the winding axial frame was made, using a hand-feed *Invicta* FIC – 15 horizontal drill. Only one rip was made in each sample, whose geometry were 5 x 8 x 70 mm^3 corresponding to the depth, width and length, respectively.

Axial and transverse milling had dimensions of 7 x 50 x 100 mm^3, depth, width and length, respectively, forming an "L" shape. Manual column spindle *Makita* 3709 machine having 1200W, 127V, 60 Hz and engine speeds ranging from 8000 to 27000 rpm were used.

Boring tests were carried out in a single hand-feed type *Motomil* boring machine having 1.735 rpm. For pin hole, three twist profile carbon steel drills having diameters (8, 10 and 12) mm were used. Three holes for hinge (one through hole and two non-through hole) were also drilled using a 35 mm diameter drill as shown in Figure 1.

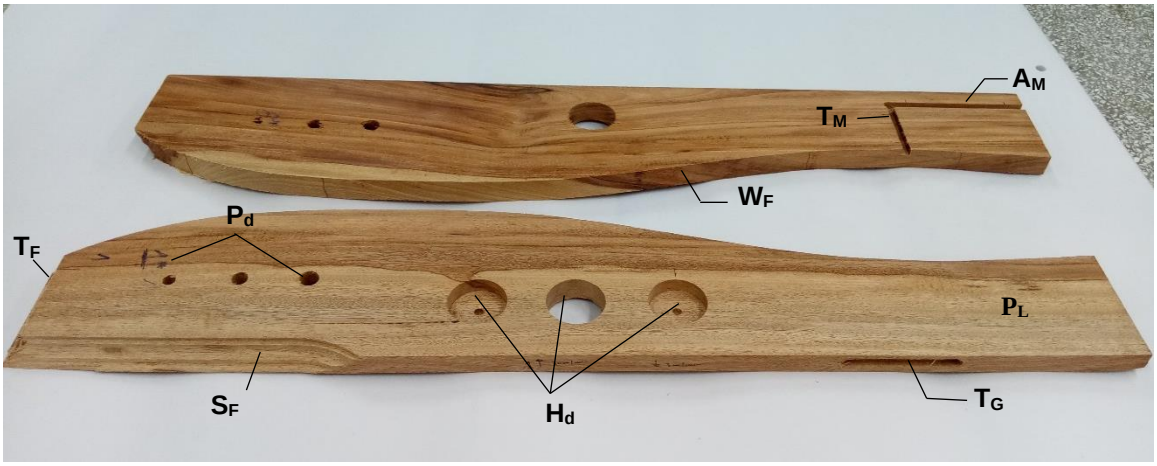

Figure 1. Messassa wood sample in machinig test. P_L – Planing; A_M, T_M – Axial and transversal milling; T_F, S_F – Top and side frame; W_F - Winding axial frame; T_G – Tearing; P_d, H_d – Pin and Hinge drilling.

Assessment of Machining Operations.The machined wood surface in each operation were visually examined according to ASTM D 1666-11 (2011) on post-machining defects. The examination was made by a team of three evaluators who gave (separately) scores ranging from 1 to 5 in defects according to their intensity and frequency. Defect values were compiled for each operation as a percentage for a given grade. Table 2 summarizes the assignment of defect values used in this study.

Table 2. Quality grades used in determining overall performance for each machining operation

Grade	Surface quality criteria*	Performance
1	Surface free of any defects	Excelent
2	Mild to medium raised, torn grain, crushing	Good
3	Strong raised and slight chipped, torn grain, tearouts	Fair
4	Strong raised and mild to medium chipped, torn grain, crushing	Poor
5	Strong raised, chipped, rough-end grain, crushing, tearouts	Very Poor

*Adapted from ASTM 1666-11 (2011)

The assigned value allowed grouping the samples into five surface quality grades: Grade 1 (excellent) - no defects in the sample; Grade 2 (good) - presence of less than 50% of defects; Grade 3 (Fair) - presence of 50% of defects; Grade 4 (Poor) - presence of more than 50% of defects and Grade 5 (very poor) - presence of 100% of defects. Figure 2 illustrates the surface quality grades in planing.

Analysis

As the purpose of this study was to test the messassa wood for use in products whose surface quality is paramount, such as furniture, frames, doors, windows, among others, the Hotelling T^2 test was applied to infer the existence of differences between the grades awarded by the evaluators. Then, a single value per surface was determined when at least two of the three evaluators rated a given sample in the same class or by the arithmetic mean when the three assigned different grade without consensus. The correlation between machining operations by Spearman's test was also verified. The results in machining operations were compared based on percentages of samples as grades 1 and 2.

Results and Discussion

Machining Test

Table 3 shows the percentage of surface quality grade in different machining operations for *B. spiciformis and J. globiflora* under the specified test conditions. It can be seen that most samples of both species had an approval percentage above 70% and graded between 1 and 2 corresponding respectively to excellent and good surface quality. However, it is noteworthy that, only in the tearing operation for *J. globiflora* wood the surface quality was grade 4. In turn, there was no samples graded 5 for both species throughout the study.

The larger observed number of samples graded 1 and 2 in the different machining operations demonstrates a satisfactory performance of both species, therefore, suitable for use in higher value-added products, such as furniture and frame production. The indication of these species for furniture production, for example, is justified by the good performance in axial, transverse and hinge drilling operations (Table 3). In turn, the indication for frame

production is supported by the good results in the top, side and winding frame tests.

In Table 3, it can also be seen that for the planing with feed speed of 6 m.min^{-1} most grades were between 1 and 2. In contrast, the planing with feed speed of 15 m.min^{-1} led to the appearance of defects such as raised, torn and chipped grains, as evidenced by the higher percentage of the samples in grade 3 (fair). Vančo *et al.* (2017) and Kaplan *et al*. (2018) also reported for *Pinus Sylvestris* L. and Oak wood (*Quercus cerris*) respectively, a decrease in surface quality (increased roughness) as a function of increased feed speed. Thus, these results (table 3) demonstrate the importance of planing this species with low feed speed to ensure a good surface quality, as well as to avoid higher wood consumption during sanding that aims to eliminate waviness and smooth the machined surface of the wood (Silva 2002; Laina *et al*. 2017). Burdurlu *et al.* (2005), Hernandez *et al*. (2001), Ratnasingam and Scholz (2007), and Gupta *et al.* (2019) state that parameters such as cutting angles and number of knives also have different effects on the quality of the machined wood surface, but were not analysed in the present study.

Table 3. Percentage of samples in each surface quality grade for machined wood of *B. spiciformis* and *J. globiflora*

Machining operation	Parameter	Grade (%)									
		1		2		3		4		5	
		Bs	*Jg*	*Bs*	*Jg*	*Bs*	*Jg*	*Bs*	*Jg*	*Bs*	*Jg*
Planing	6 m.min^{-1}	60	45	25	35	15	20	0	0	0	0
	15 m.min^{-1}	30	15	25	30	45	55	0	0	0	0
Top frame	-	10	5	85	80	5	15	0	0	0	0
Side frame	-	85	80	15	15	0	5	0	0	0	0
Axial winging frame	-	90	80	10	20	0	0	0	0	0	0
Tearing	-	10	10	60	35	30	30	0	25	0	0
Axial milling	-	85	70	15	25	0	5	0	0	0	0
Transversal milling	-	15	0	85	90	0	10	0	0	0	0
Pin drilling	8 mm	0	20	70	65	30	15	0	0	0	0
	10 mm	15	15	45	60	40	20	0	0	0	0
	12 mm	10	20	55	70	35	10	0	0	0	0
Hinge drilling	Through	35	25	65	60	0	15	0	0	0	0
	Non-through	40	15	60	65	0	20	0	0	0	0

Where: *Bs - B. spiciformis and Jg - J. globiflora*.

Comparison between wood species in planing reveals better performance of *B. spiciformis*. Possibly, this result was due to the species *J. globiflora* having intercrossed grain, which hinders the planing at high feed speed. The surface quality grades obtained in planing are illustrated in Figure 2.

Figure 2. Surface quality grades in planing of *B. spiciformis* (A) and *J. globiflora* wood (B)

The molding operation had a percentage of approved sample above 85 % and rated excellent (grade 1) and good surface quality (grade 2) on the top and side frame (Table 3). In turn, the axial winding frame showed 100 % approval in both species. These operations were performed with different cutting and feeding speeds, all of which were lower in the axial winding frame due to the operation requirement (Table 1). Unlike the other moldings, the winding axial frame is undoubtedly the least delicate due to the orientation of the cutting efforts in relation to the fibers, which favored the observed results. It is noteworthy that, besides the possible effects of wood anatomy, cutting and feeding speeds on the good results obtained in machining operations (Silva *et al*. 2005; Bustos *et al.* 2010), the use of tool without wear and good condition of the machines used contributed to the better quality of the machined wood surface.

It should be noted that the wood needs some care for tearing and boring operation. In these operations both species had a similar performance in which the samples were mainly graded 2, followed by grade 3. Exceptionally for the species *J. globiflora*, in the tearing operation, there were samples graded 4 (Table 3). This grade occurred in samples whose tearing coincided in the sapwood region and was characterized by the chilling and strong pull-out of the fibers (Figure 4). Despite that, this defect little compromises the acceptability of the species for this operation because it can be eliminated with sanding. In addition, further studies on cutting parameters such as cutting speed and feed speed or changes in tool edge angles may improve this operation.

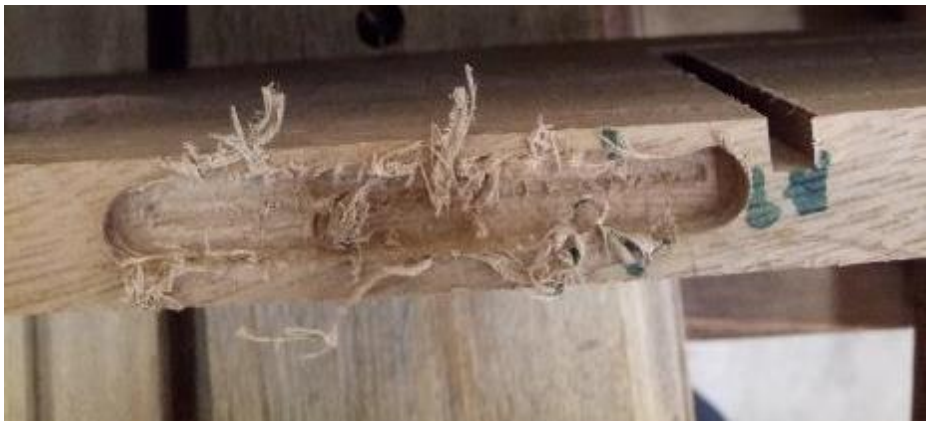

Figure 3. Tear with presence of chills and pullout of the fibers in *J. globiflora* wood

Raised and chipped grains were the common defects observed during boring. In the through holes the evaluation was made in the exit of the drill. For non-through holes (hinge) were assessed at the bottom of the hole. Although some samples were graded 3 in the pin drilling mainly for the *B. spiciformis* species, these do not afect the final surface quality as they will be eliminated during sanding. Overall, the species performed well against the boring operation.

Axial milling also performed satisfactorily with most samples approved in grade 1, equivalent to the high quality wood surface. Particular emphasis should be given to the cross-milling operation which, despite being performed with orientation of cutting effort perpendicular to the fiber arrangement, the wood of both species performed well, with most of samples lying in the graded 2 (good quality) with a percentage of (85 and 90) % for *B. spiciformis* and *J. globiflora* respectively. According to Silva (2002), the wood profiling, especially against the fibers, allows to really evaluate the machinability of the wood, since it is machined under drastic conditions, and can demonstrate its true potential. In addition, according to the same author for intercrossed grain wood species, such as *J. globiflora*, it is recommended concurrent milling, ie the workpiece moves in the same direction of rotation of the machine to minimize the machining defects.

The results obtained for machining operations (except the tearing operation) of studied species are similar to those of wood with good machining, such as the african mahogany species (*Khaya ivorensis*) reported by Carvalho *et al*. (2010). On the other hand, Table 4 presents the comparison of the average grades results found in the present study for the different machining operations in relation to some species also commonly used in the manufacture of furniture such as *Swietenia macrophylla* (Mahogany) basic density of 0.530 g.cm^{-3}, *Ocotea porosa* (Imbuia) of 0.540 g.cm^{-3} and *Eucalyptus grandis* of 0.510 g.cm^{-3} obtained by Silva (2002).

Although the results presented in Table 4 were not obtained under the same test conditions, the comparison with the species commonly used in furniture manufacture illustrates better performance of *B. spiciformis* and *J. globiflora* species in most of machining operations, which shows the suitability of the species under study for more noble uses such as furniture and frame production. Barcík *et al.* (2014), Gaff *et al*. (2015) and Gupta *et al*. (2017) state that parameters such as density, cutting speed, among others, have a direct proportionality with the roughness of the wood, resulting in the improvement of the machining

surface quality as they increase. Hernández *et al.* (2001) and Belleville *et al.* (2016) reported good machining performance as the density of *Picea glauca* and *Eucalyptus* wood increases, respectively. From this point of view, considering the average density of the messassas wood, it can be inferred that it favors the workability and, therefore, the good performance demonstrated in machining operations.

Table 4. Assigned average grades by machining operation for *B. spiciformis* and *J. globiflora,* along with *Ocotea porosa*, *Swietenia macrophylla* and *Eucalyptus grandis*.

Machining operation	*B. spiciformis*	*J. globiflora*	Imbuia*	Mahogany *	*E. grandis**
Planing	2.3 (20.2)	1.6 (33.9)	3.1	1.5	2.1
Top frame	2.0 (15.5)	1.9 (19.1)	3.8	1.7	3.2
Side frame	1.2 (25.0)	1.2 (30.5)	2.3	4.5	5.0
Winding axial frame	1.2 (16.4)	1.3 (18.8)	1.3	-	3.6
Tearing	2.4 (25.4)	2.8 (22.9)	2.4	3.4	2.9
Axial milling	1.2 (22.0)	1.3 (31.2)	2.9	2.9	3.9
Transverse milling	1.8 (16.4)	2.1 (16.4)			
Pin hole	2.4 (14.7)	2.6 (12.9)	2.2	2.4	4.3
Hinge hole	2.3 (11.9)	2.3 (15.4)	-	-	-

*Silva, (2002); Numbers in parenthesis represent the coefficient of variation; - absent

Correlations between Machining Operations

The Spearman's correlation between wood machining operations on *B. spiciformis* and *J. globiflora* wood did not show significant correlations in most cases at 1 and 5 % probability. Exceptionally, it was observed that there was a positive correlation between the transverse milling and the top frame operations, besides the side frame and winging axial frame in both species (Figure 4). However, it is important to state that, in general, the correlations found were weak witnessed by the R^2 coefficient well below 30 %.

The absence or weak correlation between the operations presupposes the non-influence of one operation on the results of the other and may be performed differently from the sequential order adopted in the present research. However, planing is known to be one of the first operations in wood machining.

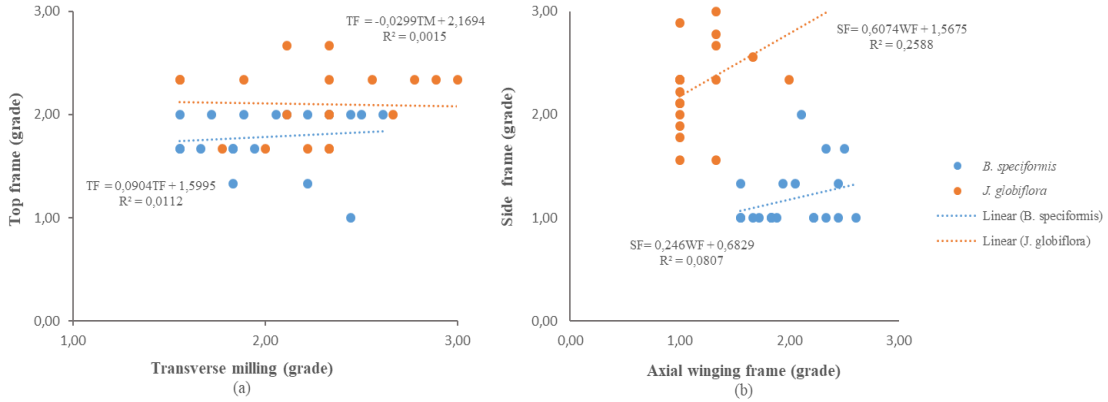

Figure 4. Correlations between top frame (T_F) and transverse milling (T_M) operations (a); and Correlations between side frame (S_F) and axial winging frame (W_F) operations (b) for species *B. spiciformis* and *J. globiflora* wood.

The observed correlations between the operations (Figure 4) may occur due to the possibility of these operators occurring in the same direction relative to the grain direction (Figure 1). There were no relevant literature reports on correlations between these operations. However, studies that aim to verify this trend by varying if the machining parameters in these operations for the same or another target group of species, as well as correlating them with the anatomical properties, are pertinent and needs continued reasearch.

Conclusions

The wood of species *B. spiciformis* and *J. globiflora* presents ease of machining in planing, side frame, top frame, axial and transverse milling, winding axial profiling, tearing, pin and hinge drilling. The surface quality of the machined wood under the test conditions was considered satisfactory and superior for *B. spiciformis* compared to *J. globiflora* specie. Both *B. spiciformis* and *J. globiflora* are suitable for use in the Mozambican furniture industry, especially for furniture and frame production. Thus,

considering the good performance of the species under study in the different machining operations, there is a possibility to promote forest management activities aimed at improving their health, especially in younger stands, in order to guarantee the good quality raw material for the wood industries.

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Extractives Contributing to the Color of *Swietenia macrophylla*'s Bark

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Abstract

The dark red color of *Swietenia machrophylla* King bark is correlated with the extractive constituents such as phenolic compounds. This study, therefore, aimed to investigate extractives from the inner and outer bark of *S. machrophylla* and their effects to color properties. The results showed that the extractive content in the inner bark was higher than the outer except for hot water soluble. In addition, the polyphenols and sugar levels from inner to the outer bark were increased, except in the soluble-sugar of hot water extractive. The highest correlation between the absorbance of methanol, hot water-soluble extracts, and total polyphenols were observed using the visual spectrophotometer. The extractives that contributed to the bark's color were indicated from flavonoids with a precursor such as monophenol of catechol and resorcinol.

Keywords: polyphenols, *S. machrophylla*, polysaccharides, coloration.

Introduction

Swietenia macrophylla King, also known as mahogany, is a species of wood that commonly found in Indonesia, which originated from Central and South America (Brown *et al*. 2003). In Jepara of Central Java, home furnitures such as chairs, bed frames, and other products are manufactured from this wood The utilization of *S. macrophylla* discards the bark as residues. The bark contains more polyphenol and lignin, which produces important medicinal extractives. Previous studies reported that *S. macrophylla* bark has been investigated as an astringent for the wound (Falah *et al*. 2008). The leaf was used for leishmaniasis, abortion medicine (Bourdy *et al*. 2000), cancer, amoebiasis, diabetic, and malaria as a folk medicine in Indonesia (Kadota *et al*. 1990).

Beside its potency for medicinal, it is also used for dye materials. According to Adeel *et al*. (2009), the increasing utilization of plant materials for dyeing is due to the growing awareness of the environment, healthcare and natural colorants that consists of properties such as antimicrobial activity (Yusuf *et al*. 2015). Furthermore, its use has been reported in the species of *Albizia coriaria*, *Morinda lucida*, *Syzgium cordatum*, *Vitellaria paradoxa*, and *Juglans regia* (Wanyama *et al*. 2014; Bukhari *et al*. 2017). Haque *et al*. (2013) reported that the potential use of an anthraquinone compound, known as rubiadin, isolated from *S. mahagoni* bark was for silk fabric dyeing. Emiliana and Widhiati (2002) also stated that a satisfactory result of using *S. macrophylla* bark extract is dying the red snapper fish skin.

The presence of color in the wood of plants is usually related to the extractives content such as flavonoids (Yazaki 2014). Three flavonoid type compounds, namely catechin, epicatechin, and a pale reddish swietemacrophyllanin were isolated from *S. macrophylla* bark and its antioxidant activity was assessed (Falah *et al*. 2008). The chemical composition of its inner and the outer bark lipophilic extractive was also investigated (Arisandi *et al*. 2019). However, there are no present study on the coloring compounds of *S. macrophylla*, especially in its separated inner and outer bark, which are known to be differed in their chemical composition (Masendra *et al*. 2018; Masendra *et al*. 2019; Seki *et al*. 2012). This study aimed to observe the total amount of phenols, flavanols, flavonoids, and polysaccharides contained in the inner and outer bark of *S. macrophylla* and their correlation with the color.

Materials and Methods

Bark Collection and Extraction

The bark was collected from Srikandiratu, furniture industry in Jepara, Central Java, Indonesia, and the leaves was identified in Faculty of Forestry Universitas Gadjah Mada as *S. macrophylla*. The characteristic of inner bark with light red color with thickness of 0.5-1.0 mm was easily peeled and separated from outer bark with dark red color and 1.5-3.0 mm thickness. It was grounded to powder with the inner and outer barks (500 g) successively refluxed for 6 h using *n*-hexane, methanol, and water. The solution was evaporated and the resulting crude extract was weighed.

Phytochemical Tests of the Plants

The bark extracts were subjected to phytochemical screening to identify the main classes of secondary metabolites. The tests were Mollisch for carbohydrates (Browning 1967), frothing for saponins (Kokate 1999), Mayer for alkaloids (Mir *et al*. 2016), ferric chloride from tannins (Trease and Evans 2002), and sodium hydroxide for flavonoids (Browning 1967).

Total Phenols

Total phenols were investigated by the Folin-Ciocalteu method with modification (Diouf *et al*. 2009). Approximately 0.5 ml of an ethanol solution of the sample (0.25 mg/ml) was mixed and incubated for 2 minutes with 2.5 ml of the Folin-

Ciocalteu reagent (10 times dilution). Furthermore, 2 ml of 7.5% aqueous sodium carbonate (Na_2CO_3) was added to the solution, and the mixture was allowed to stand for 30 min at room temperature. The absorbance of the sample was read at 765 nm and the results were expressed as gallic acid equivalents (mg GAE/g based on dry extract weight).

Total Flavanols

Total flavanols were observed by vanillin-HCl assay as described by Diouf *et al*. (2009). In addition, 0.5 ml (0.25mg/ml) of ethanol solution was mixed with 3 ml of vanillin reagent (4% vanillin in methanol) and 1.5 ml of HCl. After 15 minutes incubation of a sample, the absorbance was read at 500 nm, with the results expressed in (+)-catechin equivalents (mg CE/g based on dry weight).

Total Flavonoids

The $AlCl_3$ method is used to determine the total flavonoids (Brighente *et al*. 2007). First, 2 ml of the sample at 1 mg/ml concentration was added to 2% $AlCl_3.6H_2O$ solution and stood after 1 h incubation at 20°C. After that, the absorbance was read at 415 nm, and the results expressed in quercetin equivalents (m QE/ extract).

Total Soluble Polysaccharides

The polysaccharides contents were determined by using the DuBois method (DuBois *et al*. 1956), with 1 ml of hot-water extract mixed with 1 ml of phenol (5%) and 5 ml of concentrated sulfuric acid (98%). The mixture was maintained for 20 min at 25°C, with the absorbance of the sample was read at 490 nm and calculated in glucose equivalents (mg GE/ g sample).

Color Measurements (λ= 300-700 nm)

The absorbance of n-hexane, methanol, and hot water extracts (each 1 mg/ml) was read at a wavelength of 300-700 nm for color measurement.

Gas Chromatography-mass Spectrometry (GC-MS)

The GC-MS data were collected using a GCMS-QP 2010 (Shimadzu, Japan), with 1 µl of silylated sample injected to the GC-MS machine. The GC condition are as follows: Rtx- 5MS capillary column (30 m x 0.25 mm I.D. and 0.25 µm), column temperature from 70°C (2 min) to 290°C at 5°C/min, injection temperature of 200°C, detection temperature of 285°C, and acquisition mass ranging from 50-800 amu using helium as the carrier gas. The mass spectra of samples were compared to the NIST11 library.

Results and Discussion

Extractive Content

The extractive content of outer and inner bark using three different solvents is shown in Figure 1. Previous research by Arisandi *et al.* (2019) found that the *n*-hexane soluble extracts of the inner bark was higher than the outer part. In addition, the methanol extractive content in the inner bark also had a higher value, while the hot water extractive content showed the opposite result. The higher extractive content in the inner bark indicated that this part contains more constituents such as lipophilic or phenolic. Previous studies also reported that the inner bark of six *Pinus* species contained more lipophilics, phenolics, and sugar compounds than the outer bark (Masendra *et al*. 2018; Masendra *et al*. 2019).

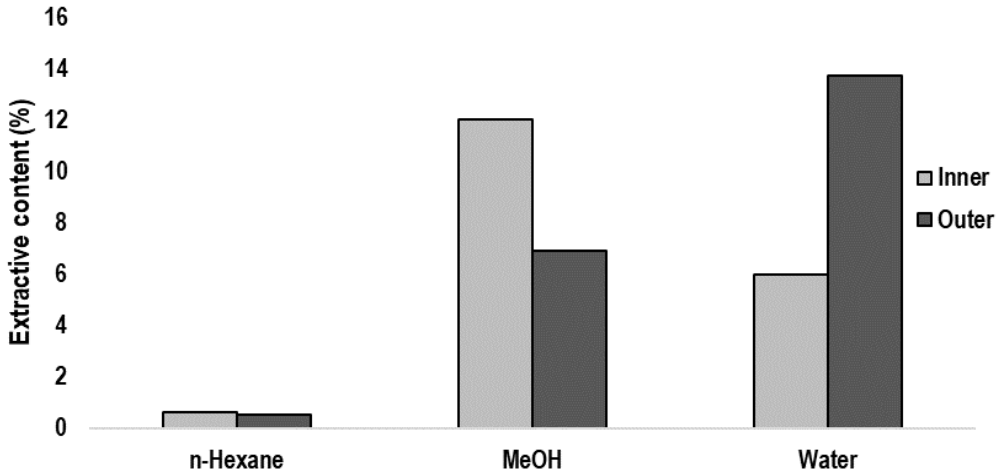

Figure 1. *n*- Hexane, methanol (MeOH), and hot water extractive content of the inner and outer bark of *Shorea macrophylla*

Polyphenols, Sugar, Saponin, and Alkaloid

In the first screening using the qualitative method, the bark of *S. macrophylla* was found to contain alkaloid, saponin, tannin, flavonoid and carbohydrate. However, the alkaloids were only detected in the inner and outer bark of *n*-hexane extracts (Table 1). The presence of saponin was easily detected in the methanol extract of the inner bark than other fractions, while tannin detected on inner bark hot water extracts led to a higher concentration compared to outer bark. Furthermore, the carbohydrate test showed that methanol and hot water extract of inner bark were in lower concentration and undetected, compared to the outer bark. Due to this result, the total polyphenol and soluble polysaccharide content were quantitatively analysed.

Table 1. Qualitative measurement of alkaloid, flavonoid, saponin, and carbohydrate test of *Swietenia macrophylla*

Fraction	Alkaloid		Flavonoid		Saponin		Tannin		Carbohydrate	
	IB	OB	IB	OB	IB	OB	IB	OB	IB	OB
Hexane	+	+	-	-	-	-	-	-	-	-
Methanol	-	-	+++	+++	++	+	+++	+++	-	+++
Water	-	-	++	+++	+	+	+++	+++	+	++

(-): not detected, (+): low detected, (++): moderately detected, (+++): highly detected.

Table 2. Total phenols, total flavanols, total flavonoids, and total polysaccharides from the bark of *Swietenia macrophylla*

Fraction	Total phenols (mg GAE/g extract)		Total flavanols (mg CE/g extract)		Total flavonoid (mg QE/g extract)		Total polysaccharides (mg GE/g extract)	
	IB	OB	IB	OB	IB	OB	IB	OB
Methanol	451.6 ± 15.5	601.3 ± 10.5	112.3 ± 11.8	174.9 ± 6.8	29.8 ± 5.4	66.2 ± 3.3	97.3 ± 38.3	148.2 ± 29.2
Water	143.8 ± 5.4	295.8 ± 2.4	27.3 ± 2.9	51.6 ± 0.5	0.5 ± 0.5	7.1 ± 2.6	91.5 ± 5.8	78.7 ± 3.4

IB: inner bark, OB: outer bark.

The polyphenol measurement showed that the total phenols dominated the composition of extractive in the bark samples compared to total flavanols, flavonoids, and polysaccharides (Table 2). In addition, their concentrations in methanol and hot water content were lower in the inner bark. However, the levels of total polysaccharides in hot water extract from inner bark were higher than the outer.

Phenolic is a main class of secondary metabolites found in plants with broad compounds and known for its bioactivity (Valette *et al*. 2017; Kadir 2017; Al-Huqail *et al*. 2019) with the ability to remove the color of a material (Burtin *et al*. 1998; Kelebek *et al*. 2010). Therefore, the outer bark is often associated with a high amount of phenolic compounds, including flavonoid and flavanol with protective function against pathogens (Popa 2015). Similar patterns between phenolics were found in a study conducted by Masendra *et al*. (2019) in six species of *pinus*, with a high concentration of polysaccharides measured in hot water-soluble fraction of the inner bark. This result was, however, inconsistent with the phytochemical screening carried out by the Mollisch test as the carbohydrate was detected in low concentration in the inner bark. Theoretically, the presence of carbohydrate can be found in both inner and outer bark. However, the present study showed carbohydrate reaction in the inner bark was lower than outer bark. Further, the presence of polysaccharide in hot water-soluble extract of inner bark was expected due to its function in the distribution and storage of nutrients from the root to other parts of the tree (Sjöstrom 1993).

Extractives for Color

The outer and inner colors of the bark extractive were dark brown. The wavelength measurement from 300-700 nm by spectrophotometer showed that a higher solubility was presented by methanol and hot water extract of outer bark followed by the inner bark, respectively (Figure 2). The highest shoulder was observed at 475 nm where the absorbance of methanol and hot water extracts in both barks were 1.684, 1.33, 0.77, and 0.73, respectively.

The presence of more intense shoulder at 475 nm matched with the color properties in the methanol and hot water extracts. The correlations between absorbance at 475 nm, polyphenols measurements (total phenols, flavanols, and flavonoids), and sugar content are linear as shown in Figure 3. However, the correlation between absorbance at 475 nm and polyphenols content was stronger than total polysaccharides. Therefore, methanol and hot water extract color are affected by polyphenols ($R^2 > 0.9$) (Figure 3b).

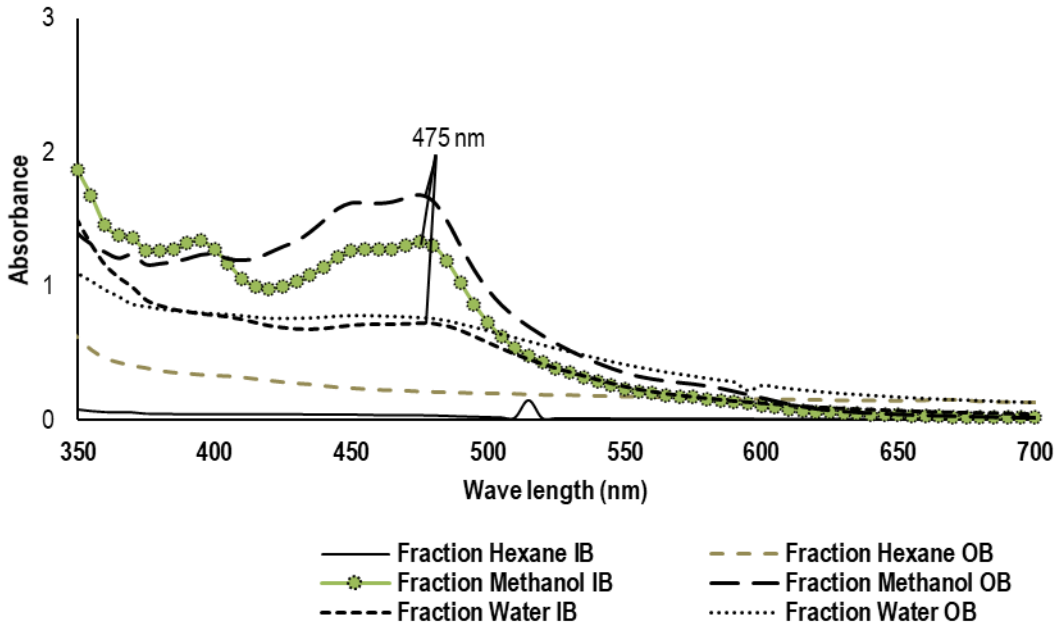

Figure 2. Absorbance of the *n*-hexane, methanol, and hot water extractives from inner bark (IB) and outer bark (OB) of *Swietenia macrophylla*

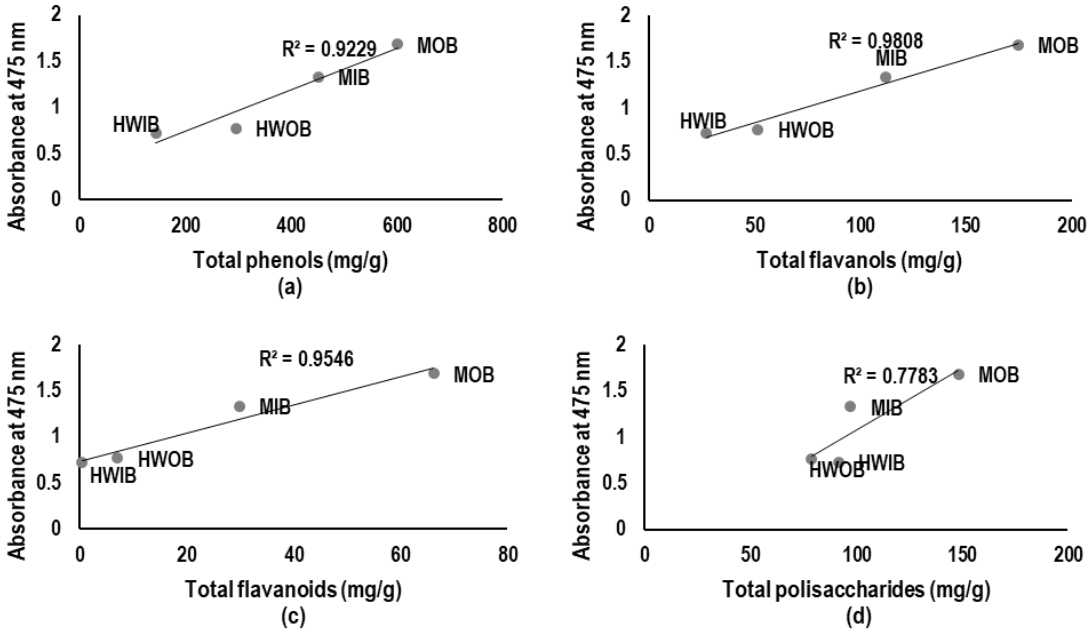

Figure 3. Correlation between absorbance at 475 nm and total phenols (a), total flavanols (b), total flavonoids (c), and total sugars (d). MIB (methanol extract inner bark), MOB (methanol extract outer bark), HWOB (hot water extract outer bark), HWIB (hot water extract inner bark).

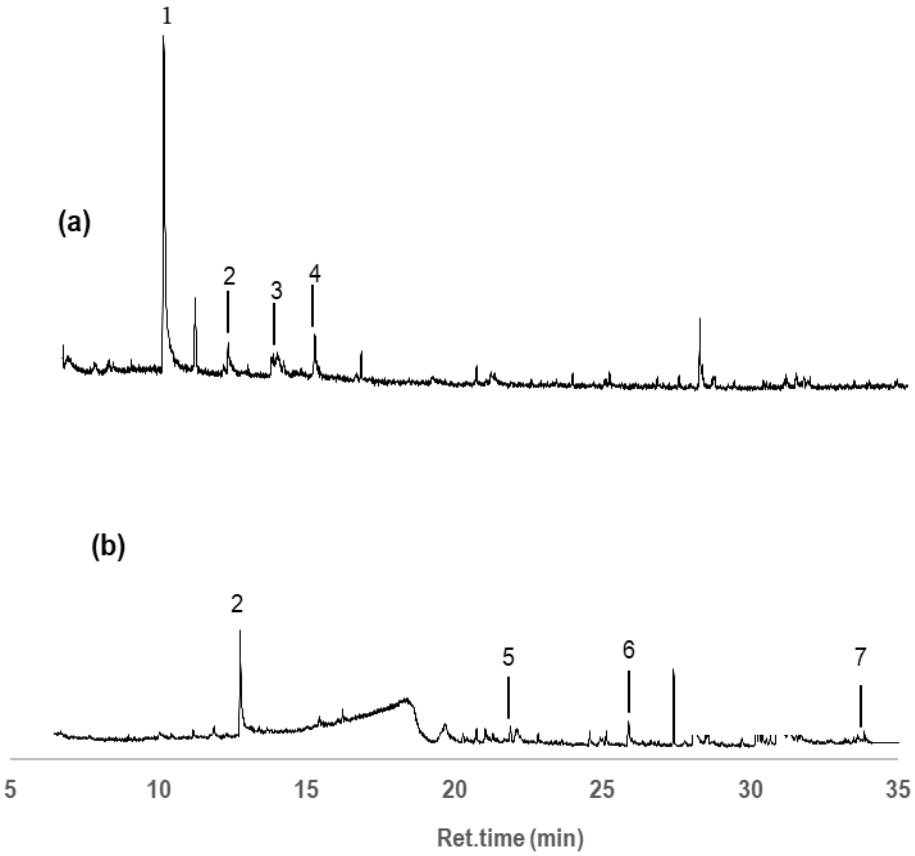

Figure 4. GC-MS chromatogram of outer bark (a) and inner bark (b) methanol extract of *Swietenia macrophylla*; 1. Catechol, 2. Resorcinol, 3. 4-Methylcatechol, 4. Syringol, 5. Antiarol, 6. Syringic acid, 7. Trimethoxycinnamic acid

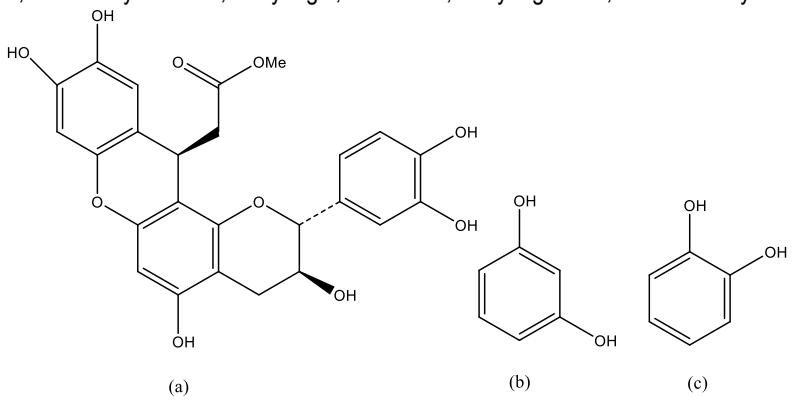

Figure 5. Chemical structure of swieteniemacrophyllanin (a), resorcinol (b), and catechol (c).

The methanol extract absorbance was analysed by GC-MS for phenolic compounds detection. To determine the GC-MS analyses, the methanol was extracted with ethyl acetate, which was in-turn trimethylsilylated (Wijayanto *et al*. 2015). In Figure 4, the chromatogram of methanol extract, along with the detected monophenols in the inner and outer barks of *S. macrophylla* is shown. Catechol and resorcinol were dominant monophenols detected in the outer and inner bark methanol extract, respectively, and responsible for their coloration.

Research carried out by Fallah *et al*. (2008) successfully isolated a reddish pale compound known as swieteniemacrophyllanin (Figure 5a) and two other flavonoids from soluble extracts. The presence of swieteniemacrophyllanin, isolated from the bark of *S. macrophylla* by Falah *et al*. (2008), has the ability to affect the coloration. Therefore, the extractive responsible for coloration in the inner and outer barks are flavonoid with resorcinol structure (Figure 5b), and flavonoids that contain catechol (Figure 5c). Further studies are needed to identify

the polyphenol compounds or flavonoids that contain catechol and resorcinol by HPLC or NMR analyses.

Conclusions

In conclusion, preliminary phytochemical screening was used to detect alkaloids, saponins, tannins, flavonoids and carbohydrates in the inner and outer bark extractives of *S. machrophylla*. The total values of phenols, flavonoids, and flavanols were higher in the outer bark. In addition, the absorbance of methanol and hot water-soluble extract was highest at 475 nm and linearly correlated by polyphenols. The stronger absorbance and the detection of phenolic compounds by GC-MS was in the methanol extract for inner and outer bark of *S. Machrophylla*. Therefore, we suggested that extractives contributing to color in the bark were flavonoids with monophenols structure such as catechol and resorcinol.

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Phenol Contents and Antioxidant Activity of Sonokeling (*Dalbergia latifolia* Roxb) Wood

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Abstract

Dalbergia latifolia or sonokeling is a native species of Java, Indonesia, used as an important wood for furniture and building materials, due to the high of durability and beautiful color. This study, therefore, was aimed to investigate the phenol composition, represented by total phenolic, flavonoid, and flavanol content, as well as antioxidant activity, conducted by DPPH (1, 1-diphenyl-2-picrylhydrazyl) method on *D. latifolia* wood. The sample was extracted using ethanol-toluene solvent in a Soxhlet apparatus, and subsequently subjected to column chromatography. This treatment yielded 12 fractions, which were then evaluated for phenol contents and antioxidant activity. The results showed a high antioxidant activity and total phenolic content in Fr.1- Fr.3, while latifolin was detected and characterized by GC-MS and a literature comparison. Therefore, it was established that the antioxidant activity of *D. latifolia* wood extractives properly correlated with the total phenolic, but not with the total flavonoid and flavanol contents.

Keywords: Sonokeling, DPPH activity, phytomedicine, neoflavonoid, extractives

Introduction

Dalbergia latifolia called sonokeling (Javanese) or Java palisander (English) is a native species from Indonesia, known to possess beautiful wood, with a brown to dark brown color (Orwa *et al.* 2009). In addition, they are classified as highly resistant, naturally durable (Kalynasundaran and Ganti 1975), placed in strength class II (Dwianto *et al*. 2019), and also deliver good acoustical properties (Karlinasari *et al*. 2012). Hence, the wood is commonly used in the manufacture of furniture and building materials.

Based on the chemical properties, Sekine *et al*. (2009) isolated some neoflavonoids compounds from the heartwood, *D. latifolia*, characterized as latifolin and its derivatives, which were then tested for antitermite and antifungi activities (Sekine *et al*. 2009). The wood, bark, and leaves extracts were also reported to confer anticancer and antioxidant effect (Khalid *et al*. 2011; Niraimathi and Sundaraganapathy 2014; Liu *et al*. 2018; Tripathi 2018). Other investigations performed on the genus *Dalbergia* demonstrated the propensity for the leaf extract of *D. saxatilis* to increase kidney toxicity (Ismail *et al*. 2015), *D. sisoo* to function as a photoprotective and DNA protective agents (Yasmeen and Gupta 2016), while *D. parviflora* contained antioxidant isoflavonoids (Castellano and Torrens 2015).

This antioxidant activity is affiliated with the protection of cell body from free radicals continuously which is produced internally, where the excess quantities are responsible for various disease manifestations (Young and Woodside 2001). Numerous radicals are known to be highly reactive with other molecules, e.g., DPPH (1, 1-diphenyl-2-picrylhydrazyl), which is unstable in dark purple color. Meanwhile, phenolic compounds as antioxidants play the role of donating proton to reduce DPPH-H to the nonradical form of DPPH, which is an activity of polyphenols from plants (Ku *et al*. 2007; Gan *et al*. 2010). This study, therefore, investigated the wood extractives obtained from *D. latifolia* wood, in order to determine the phenol contents and antioxidant activity.

Materials and Methods

Sample Collection and Extraction

The sample of *D. latifolia* wood was purchased and collected from a wooden industry in Bantul, Yogyakarta, Indonesia. The 10 g of the heartwood and sapwood were mixed and milled to powder, followed by drying at oven temperature 40°C for a week, and then extraction was conducted using ethanol-toluene (2/1, v/v) in soxhlet apparatus for 6 h.

Column Chromatography and Gas Chromatography Mass Spectrometry (GC-MS) Analysis

Si-gel 60 with size of 63-210 µm (Kanto Chemical Co., Inc., Japan) was used for column chromatography, where *n*-hexane, ethyl acetate (EtOAc), acetone, and methanol (MeOH) were loaded as eluent. Conversely, a GC-MS-QP 2010 (Shimadzu, Japan) machine was implemented to detect the compound, as 1 µl of the sample (1 mg/ml) was directly injected with column temperature from 100° C (1 min) to 320°C at 5°C/min; while that for injection and detection were 250°C and 320°C, respectively. In addition, DB-1 capillary column (30 m x 0.25 mm I.D. and 0.25 µm; GL Sciences, Tokyo, Japan) was used in the machine, using helium as the carrier gas, and the acquisition mass were set from 50-800 amu. Subsequently, the mass

spectrum obtained for each sample was compared with data from the NIST library and the literature (Sekine *et al*. 2009).

Total Phenolic Content (TPC)

The Folin-Ciocalteu method by Diouf *et al*. (2009) was used as a reference during the investigation of TPC. Approximately 2.5 ml of Folin-Ciocalteu phenol reagent (10 times dilution) was mixed with 0.5 ml of the sample (0.25 mg/ml) and incubated for 2 min, then 2 ml of 7.5% aqueous sodium carbonate was added and incubated again for 30 min. Finally, the mixture was placed in the equipment, followed by the sample absorbance reading at 765 nm, and the results of TPC were expressed as (+)- gallic acid equivalents (mg GAE/g extract).

Total Flavonoid Content (TFC)

TFC evaluation involved the $AlCl_3$ method (Brighente *et al*. 2007), where 2 ml of the sample prepared at 1 mg/ml concentration was reacted with 2% $AlCl_3.6H_2O$ solution (2 ml). This mixture was then incubated for 1 h at 20°C, followed by the absorbance reading at 415 nm, and the results expressed in quercetin equivalents (mg QE/g extract).

Total Flavanol Content (TVC)

The TVC was determined using the vanillin-HCl method (Richard *et al*. 1978), where 0.5 ml of the sample (1 mg/ml concentration) was mixed with 3 ml of 4% vanillin reagent and 1.5 ml of HCl. This reaction was performed for 15 min at ambient temperature, followed by the absorbance reading at 500 nm, and standard calibration used was of (+)-catechin (mg CE/g extract).

Determination of DPPH Radicals Scavenging Activity

The determination of DPPH radicals scavenging or antioxidant activity was conducted according to Gao *et al*. (2006), where each 0.1 ml methanolic extract at different concentrations were mixed with 5 ml of 0.004% DPPH in methanol and incubated for 30 minutes. Therefore, the sample absorbance was read at 517 nm, using UV-Vis spectrophotometer, and the antioxidant activity was calculated using the following equation:

$$\text{DPPH scavenged (\%)} = 100 \times (Ao-A1)/ Ao$$

Where Ao is the absorbance of blank and A1 is absorbance of sample. The antioxidant activity also was represented as IC_{50}, which is an expression for the concentration responsible for inhibiting 50% activity.

Chemicals

(+)- Gallic acid (97.5%), (+)- catechin (≥95%), quercetin (≥95%), and 1,1-diphenyl-2-picrylhydrazyl were purchased from Sigma Aldrich (Germany), while heneicosane (≥95%) was obtained from Kanto Chemical Co., Inc. (Tokyo, Japan).

Results and Discussion

Extraction and Isolation

The yield of *D. latifolia* ethanol-toluene extract was not mentioned in this report, although Table 1 shows the result of sample fractionation. Conversely, the isolation process involved the use of column chromatography with *n*-hexane as solvent, whose polarity was increased with EtOAc, acetone, MeOH, and water. At the inception, Fr. 1 and Fr. 2 had the highest yield of 0.74 g and 0.34 g (Table 1), which were collected in the eluent of *n*-hexane 100% and *n*-hexane/EtOAc 80%, respectively. Therefore, it is established that *D. latifolia* wood extractives is dominated by apolar compounds, although Fr. 12 yielded 0.59 g in the MeOH-water soluble fraction (polar compounds). This fraction is observed to possess a comparably higher content, and predicted to comprise of more polar components, including tannins.

Characterization of Fr.1- Fr.12

The 12 fractions were analyzed using GC-MS by direct injection, where only Fr. 1, Fr. 2, and Fr. 3 demonstrated a compound with a higher peak at a similar retention time of 41.5 mins (Figure 1), suggesting the tendency of similar components. Meanwhile, none was detected from Fr. 4- Fr. 12, as the presence of polar compounds possibly requires further processing by silylation or methylation.

Further discussions were performed to characterize Fr. 1- Fr. 3, through a comparison with the mass spectra of latifolin, as demonstrated in Table 2. Furthermore, the molecular weight obtained for Fr. 1- Fr. 3 was at m/z 286, alongside a base relative intensity at m/z 154. Therefore, a similarity was established between the fragmentations and latifolin, as reported by Sekine *et al*. (2009), making it the main compound, based on literature comparisons. This was also demonstrated in previous investigations performed on the genus *Dalbergia*, as an isolate from *D. parviflora* (Muangnoicharoen and Frahm 1982), with the molecular structure displayed in Figure 2.

Table 1. Yield of fractionation of *D. latifolia* wood

Column chromatography of ethanol toluene fraction (3.39 g)		
Eluting solvents	Fraction number	Weight (g)
n-Hexane	1	0.74
n-Hexane-EtOAc (8/2)	2	0.34
n-Hexane- EtOAc (7/3)	3	0.08
n-Hexane- EtOAc (5/5)	4	0.30
n-Hexane- EtOAc (3/7)	5	0.20
n-Hexane- EtOAc (2/8)	6	0.18
n-Hexane- EtOAc (1/9)	7	0.08
EtOAc	8	0.09
EtOAc -acetone (5/5)	9	0.19
Acetone	10	0.06
MeOH	11	0.43
MeOH- water (1/1)	12	0.59
Total		3.28

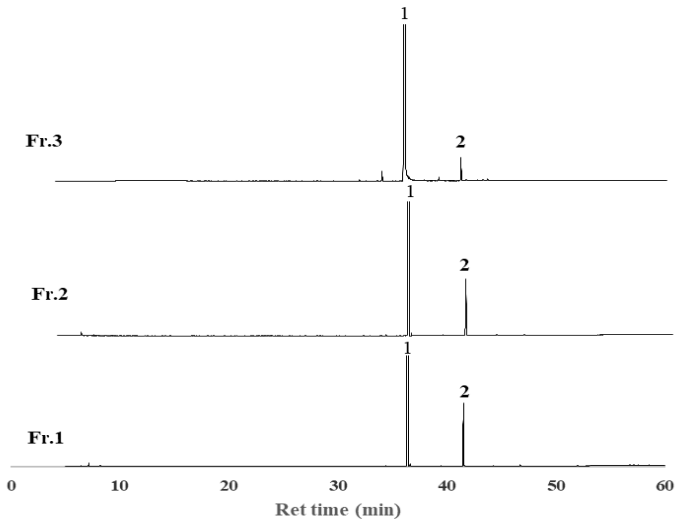

Figure 1. Chromatograms of GC- MS from Fr. 1- Fr. 3; 1. Heneicosane (Internal Standard (retention time: 36.4 min)), 2. Targeted compound (ret. time: 41.5 min) of *Dalbergia latifolia* wood

Table. 2. Comparison mass spectra of latifolin with Fr.1- Fr.3 of *Dalbergia latifolia* wood

Samples	Mass spectra fragmentations
R- (–) - latifolin[a]	286 $[M]^+$ (47), 269 (4), 255 (25), 240 (3), 227 (3), 211 (4), 193 (2), 180 (9), 167 (12), 154 (100), 139 (16), 133 (13), 131 (13), 115 (10), 107 (12), 105 (6), 91 (7), 77 (13), 69 (15), 65 (7), 51 (8)
Fr. 1	286 $[M]^+$ (83), 269 (7), 255 (42), 240 (5), 227 (4), 211 (6), 193 (3), 180 (9), 167 (11), 154 (100), 139 (19), 133 (18), 131 (18), 115 (14), 107 (19), 105 (10), 91 (14), 77 (22), 69 (21), 65 (10), 51 (10)
Fr. 2	286 $[M]^+$ (87), 269 (8), 255 (42), 240 (5), 227 (4), 211 (6), 193 (3), 180 (10), 167 (12), 154 (100), 139 (19), 133 (18), 131 (18), 115 (14), 107 (20), 105 (10), 91 (13), 77 (22), 69 (19), 65 (10), 51 (9)
Fr. 3	286 $[M]^+$ (86), 269 (7), 255 (40), 240 (7), 227 (7), 211 (7), 193 (3), 180 (9), 167 (10), 154 (100), 139 (17), 133 (15), 131 (19), 115 (20), 107 (23), 105 (12), 91 (15), 77 (22), 69 (19), 65 (10), 51 (10)

(a): Sekine *et al*. 2009

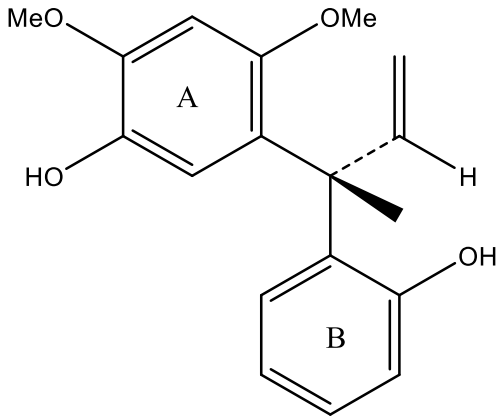

Figure 2. Chemical structure of *R- (–)- latifolin* from *D. latifolia*

Phenol Contents and Antioxidant Activity

The phenol content and antioxidant activity of Fr. 1- Fr. 12 were displayed in Table 3, as the highest TPC concentration was observed in Fr. 1 and Fr. 2, while Fr. 1 and Fr. 4 demonstrated the most significant level of TFC, and high TVC values were identified in Fr. 7 and Fr. 9. A comparison with previous works showed a markedly lower amount of TPC, especially in the Fr. 1 (469.8 mg/g) and Fr. 2 (415.5 mg/g), than in the bark of *D. latifolia* at 641.8 mg/g (Khalid *et al*. 2011), although higher than reported by Tripathi (2018) in the leaves (29.1 mg/g). Conversely, the TFC value for Fr. 1 (171.6 mg/g), Fr. 4 (173.2 mg/g), and Fr. 9 (170.5 mg/g) were higher than previous reports on the bark of *D. latifolia*, at 46 µg/ml (Khalid *et al*. 2011).

The test conducted with DPPH demonstrated a higher level of antioxidant activity in Fr. 1- Fr. 3 (Table 3), which was affiliated with the presence of latifolin as the main compound. Therefore, the high values in these fractions were assumed to have been affected by the neoflavonoids, despite the comparably higher level of the positive control, encompassing cathecin, quercetin, and gallic acid. This study also demonstrated lower antioxidant activity in Fr. 1- Fr. 3 when compared to the bark of *D. latifolia* (Khalid *et al*. 2011). However, the values recorded were higher than *D. saxatilis* woody roots (Isyaka *et al*. 2015).

Table 3. Total phenolic content, total flavanoid, and antioxidant activity of fractions of *D. latifolia* wood

Fraction	TPC[a]	TFC[b]	TVC[c]	DPPH scavenging activity (%)		IC50 (µg/ml)
				500 µg/ml	250 µg/ml	
Fr. 1	469.8	171.6	28.3	72.2	37.6	340.4
Fr. 2	415.5	128.4	28.8	82.0	41.7	303.3
Fr. 3	191.3	144.4	52.8	59.6	26.8	393.1
Fr. 4	187.4	173.2	48.4	40.8	23.2	663.8
Fr. 5	82.2	101.1	31.8	24.5	13.8	1179.2
Fr. 6	109.0	166.4	54.0	31.0	15.8	7848.0
Fr. 7	113.3	162.4	61.6	28.0	15.6	1041.4
Fr. 8	128.8	158.8	52.1	28.1	12.1	944.9
Fr.9	142.8	170.5	56.8	37.3	21.9	592.0
Fr. 10	152.1	97.1	44.9	41.9	26.4	604.6
Fr. 11	69.0	96.6	8.3	26.0	6.7	1110.5
Fr. 12	51.6	104.7	31.4	20.4	11.3	1328.4
Catechin	-	-	-	93.8	92.0	83.3
Quercetin	-	-	-	96.1	93.0	28.7
Gallic acid	-	-	-	94.6	94.5	88.5

(-): not determined, DPPH: 1,1-diphenyl-2-picrylhydrazyl
[a]Determined by Folin-Ciocalteu assay, in units of milligrams (+)-gallic acid equivalent per gram sample
[b]Determined by $AlCl_3$ assay, in units of milligrams quercetin equivalent per gram sample
[c]Determined by vanillin-HCl assay, in units of milligrams (+)-catechin equivalent per gram sample

Correlation between Phenol Contents and Antioxidant Activity

Figure 3 shows the plots between antioxidant activity and phenol contents, which displayed a good pattern against TPC, suggesting the dependence of *D. latifolia* on TPC for effectiveness (3a), while Figure 3b and 3c were resulted in random plots. This relationship is in agreement with several prior studies (Eddebbagh *et al*. 2016; Guedes *et al*. 2017; Amamra *et al*. 2018; Hossain *et al*. 2019).

The measurement of TPC indicates the presence of phenols, hence a better understanding of the particular compound responsible in the antioxidant activity requires the conduction of specific phenol evaluation, through TFC and TVC. Figure 3b showed a low correlation with TFC, although fractions demonstrating high effectivity were generally observed to possess high concentrations. Furthermore, GC-MS data in the current study and a previous work (Sekine *et al*. 2009) reported on the presence of neoflavonoids on the extracts of *D. latifolia*, as the common fractions with more significant activity also possessed higher total flavonoids. The neoflavonoids identified in this research, including latifolin probably does not refer to the total flavonoids measured by the unit of standard, quercetin (Figure 4a). Based on the analysis of regression, the direct correlation against TFC was also weak, which is inconsistent with the previous reports by Eddebbagh *et al*. (2016) and Amamra *et al*. (2018), although in agreement with the study by Ghasemi *et al*. (2009) on peels and tissues of 13 citrus species.

Further determination on more specific phenols was also conducted for TVCs, and the values obtained correlated properly with antioxidant activities (Figure 3c). Similar with TFC, it was impossible to establish a good correlation against TVC, due on the generally opposing values recorded, as shown in Table 3. This outcome suggests the weak dependence of antioxidant activity on TVC, which was expressed in catechin unit, possessing latifolin as the main compound and a different type flavonoid. Conversely, flavanols or catechins are flavanones 3-hydroxy derivatives, also referred to as flavan-3-ols, due to the bound of the hydroxyl group with the C ring at position 3 (Figure 4b). This compound is classified as a neoflavonoid, possessing the 4-phenylchromone skeleton, which is different from the 2-phenylchromen-4-one backbone (Phance *et al*. 2016). The varying concentration of catechins and latifolin as the main compounds in *D. latifolia* wood possibly lead to the reduced value of TVC in Fr. 1- Fr. 12, and is also associated with the absence of a good correlation against antioxidant activity. Meanwhile, the flavanols responsible for the donation of proton in the sample were not detected, which is different from the study of Henning *et al*. (2003) conducted on green tea extract, but in agreement with the research on tea extracts by Gao *et al*. (2013).

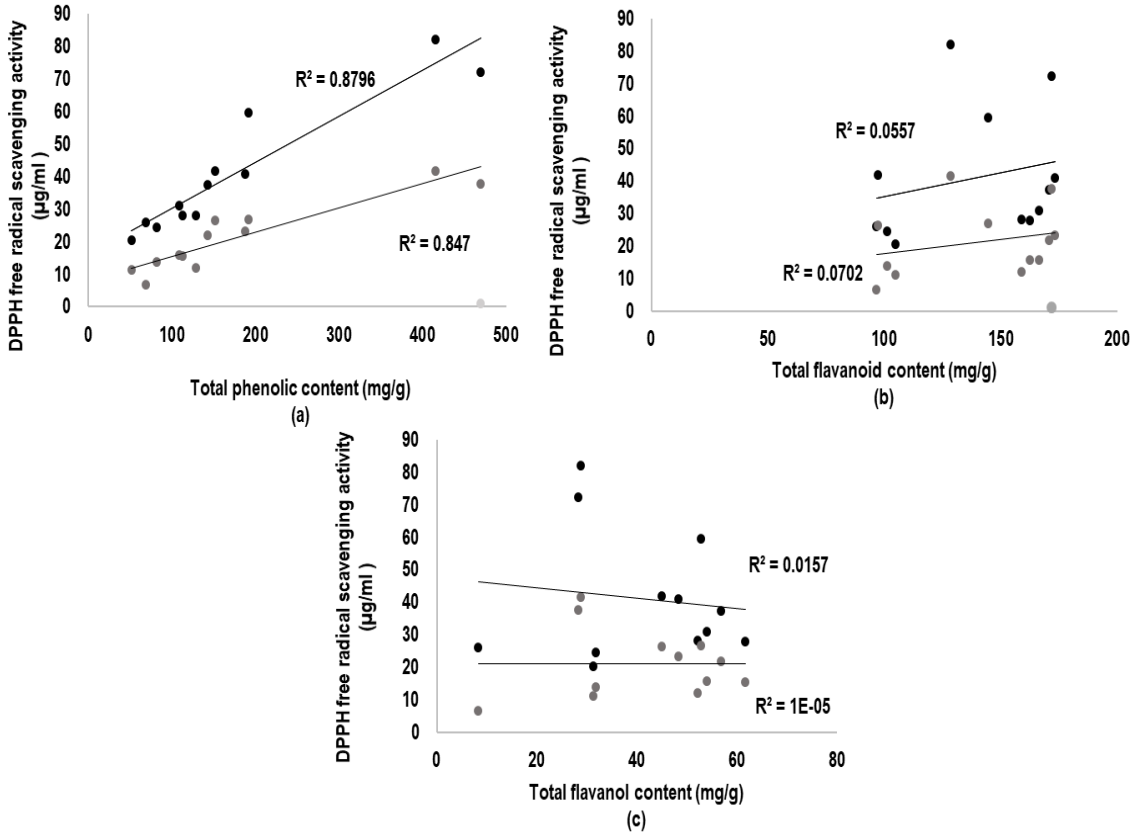

Figure 3. Correlation between antioxidant activity and total phenolic content (a), total flavonoid content (b), and total flavanol content (c). Black circles, 500 µg/ml; gray circles, 250 µg/ml of *Dalbergia latifolia*.

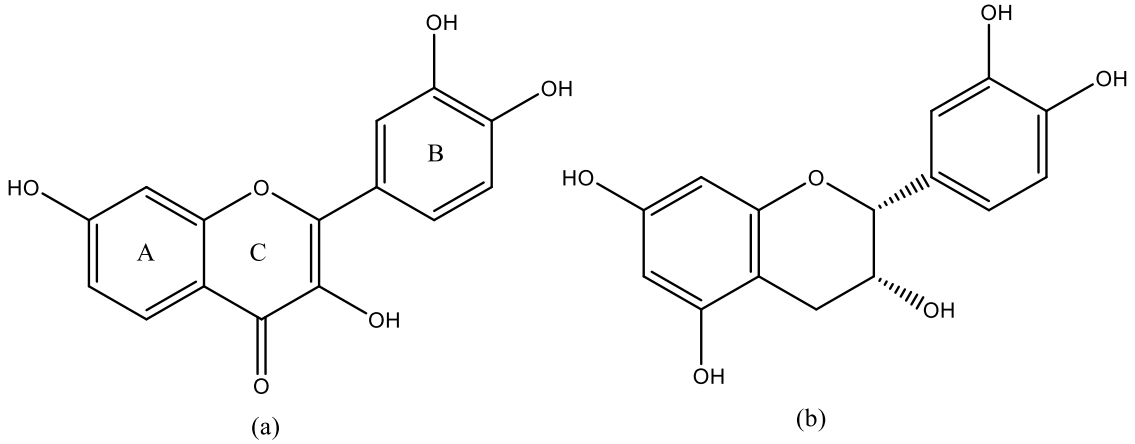

Figure 4. Common chemical structure of quercetin (a) and catechin (b).

Conclusions

Based on the results and discussion, it was established that Fr. 1- Fr. 3 showed comparably higher TPC and antioxidant activity than other fractions. Conversely, more significant levels of TFC were observed in Fr. 1 and Fr. 4, while Fr. 7 and Fr. 9 demonstrated relatively better TVC concentrations. The GC-MS analysis detected latifolin in Fr. 1- Fr. 3, which was assumed to be responsible for antioxidant activity. Furthermore, the differences observed in the results of correlation against TPC, TFC, and TVC suggests the dependence of *D. latifolia* wood on TPC for effectiveness.

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Chemical Compounds, Physicochemical Properties, and Antioxidant Activity of *A. cardamomum* Leaves and Rhizomes Oils on Different Distillation Time

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Abstract

Amomum cardamomum is local cardamom that grows widely and recently developed as an agroforestry crop in Indonesia. Its seeds, leaves and rhizomes are sources of essential oil. Essential oils from cardamom have many benefits for health and flavouring agent. The objectives of this study were to elucidate the yield, chemical composition, physico-chemical properties, and antioxidant activities of leaves and rhizomes oils of cardamom distillated using water-steam distillation for 4, 6, and 8 hours. The chemical composition were analyzed by GC-MS, physicochemical properties were analyzed using ISO standard and antioxidant activity were analyzed by DPPH method. The results showed that *A. cardamomum* oils yield between 0.06-0.33%. The main compound in the oils is 1,8-cineole with the highest percentage was obtained from cardamom rhizomes oil distillated for 6 hours (60.63%). The results of each sample almost have the same quality with specific gravity between 0.899 – 0.909; refractive index between 1.476-1.478; optical rotation between (+)2.05º-(+)2.38º; miscibility in 70% alcohol between 1:7-1:9; and acid number between 0.49-0.69. The leaves and rhizomes oils of *A. cardamomum* showed potent antioxidant activity with the highest antioxidant were obtained from cardamom rhizomes oil distillated for 8 hours with IC_{50}: 0.039 g/ml.

Keywords: distillation time, cardamom oils, leaf, rhizome, chemical composition, physico-chemical properties, antioxidant

Introduction

There are two types of cardamom that are grown in Indonesia namely *Amomum cardamomum* (local cardamom) and *Elettaria cardamomum* (cardamom sabrang) (Suryadinata 2008). *A. cardamomum* is native to Indonesia, endemic to the mountainous areas in western Java. This species is commonly cultivated in Western Java, Southern Sumatra, and Moluccas whereas Java and Sumatra are the major growing areas (Lim 2013).

A. cardamomum is a crop that widely cultivated in Indonesia, because of high economic value, suitable growing site, and can grow well under forest stands. Agroforestry between forest plants and cardamom began to be widely developed in Indonesia such in Ciamis, West Java. Cardamom agroforestry is profiting the farmers by 5.7 times more compared to the rainfed agriculture. In the mountainous area, agroforestry is also a favorite land management system. The implementation of cardamom agroforestry in large scale land use is quite promising for economic and ecological sustainability (Sharma *et al.* 2007). Part of cardamom plant that is generally used to produce essential oil is seed, while the leaves and rhizome cardamom has not been widely utilized. In fact, leaves and rhizome of cardamom can also produce essential oils (Winarsi 2014).

Essential oils may be extracted from different parts of the plant, such as leaves, fruit peels, seeds, bark, wood wicks, and flowers, and are usually obtained from steam or hydrodistillation (Ahn *et al.* 2018). Essential oil of cardamom can obtained by hydrodistillation with distillation time of about 6-8 hours. Cardamom plant produces an optimal seed up to the age of 10-15 years, after that the plants need to be replaced with the new plants. This condition causes the leaves and the rhizomes become waste. Previous study on cardamom provide a high yield (2.43%) from leaves oil of *Elletaria cardamomum* compared to other plants of the Zingiberaceae family (Batubara *et al.* 2016a). While research on the influence of distilation time on yield of cardamom seed oil showed that distillation times give different yield and quality (Rosjidi 1993).

Essential oils have been used in traditional medicine. The availabilities of essential oils seem to have a great potential as anti-inflammatory, anti-bacterial, anti-cancer therapeutic agents, and aromatherapies. In recent years, the essential oils and herbal extracts have attracted a great deal of scientific interest due to their potential as a source of natural antioxidants and biologically active compounds (Bozin *et al.* 2006; Tepe *et al.* 2005; Shaaban *et al.* 2011; Perricone *et al.* 2015).

Seed of cardamom known as queen of spices (Charles and Denys 2013) while cardamom essential oils have some bioactivities such as fever medicine, gout drug, and heartburn medicine. On the other hand, seeds, leaves, and cardamom rhizome also contain essential oils and compounds such as saponins, flavonoids, and polyphenols that have a potential as antioxidants (Winarsi 2014). Antioxidant activity also can be found in essential oils from *Ellettaria cardamomum* leaves (Batubara *et al.* 2016b). Antioxidant has been widely discussed for medical purposes, because its compounds can prevent the reaction caused by the presence of free radicals. To reduce the damage caused by reactive compounds, additive substances with antioxidant activity such as butylated

hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) are widely used as drug compounds. However, It potentially toxic because they are made from chemicals. Therefore, natural antioxidants are in demand because they use natural ingredients, making it safer than synthetic materials. Natural antioxidant can be obtained from essential oils (Pujiarti *et al.* 2015), such as from cardamom oil (Wang *et al.* 2017).

Previous studies have analyzed some cardamom essential oils from seed. However, study of leaves and rhizomes *A. cardamomum* essential oils and hydrodistillation time are still limited. This study were conducted to elucidate the yield, chemical composition, physico-chemical properties, and antioxidant of leaves and rhizomes of *A. cardamomum* oils that were extracted from different times hydrodistillation of 4, 6, and 8 hours.

Materials and Methods

Plant Material and Extraction

Fresh leaves and rhizomes of *Amomum cardamomum* (age 11 years old) were collected from Prangkokan village in Kulonprogo district, Yogyakarta, Indonesia. The cardamom leaves and rhizomes were chopped with a length of 2 cm. For each sample, 5 kg of fresh cardamom leaves and rhizomes were extracted by hydrodistillation (water-steam distillation) for 4, 6, and 8 hours. Essential oil extraction was carried out by a hydrodistillation using 5 kg capacity. The obtained oils were kept in labeled bottles and were stored in fridge before analyzed.

Essential Oil Yield

The oil yields were determined based on dried weight of leaves or rhizomes. The 2 g fresh cardamom leaves and rhizomes were oven-dried each at 103 ± 2°C until its weight constant and water content were calculated.

GC-MS Analysis

The chemical compositions of cardamom oils were analyzed by GC-MS (Gas Chromatography-Mass Spectrometry) QP2010S with Agilent HP 5 column with length 30 m and film thickness 0.25 nm. The carrier gas was Helium and ionizing EI 70 eV. The oven column temperature was set at 70°C and the injection temperature was set to 310°C. The chemical analysis was performed on each sample. GC-MS analysis was performed with retention time 50 minutes. Quantification compounds of cardamom oils were calculated based on the relative peak area (percent area) from chromatogram and chemical components were confirmed by comparing retention time with NIST 147 data base library.

Physico-chemical Properties

Physico-chemical properties of cardamom oils was analyzed based on ISO 4733: 1981 including specific gravity, refractive index, optical rotation, miscibility in 70% alcohol, and acid numbers on each sample. The specific gravity was analyzed using pycnometer with certain temperature of 20ºC. The refractive index was analyzed using hand-refractometer. The optical rotation was analyzed using polarimeter for samples and a control (distilled water). The acid number was analyzed by NaOH 0.1 N titration.

Antioxidant Activity

Antioxidant activity was analyzed by DPPH method (1,1diphenyl-2-picrilhidrazil) based on Molyneux (2004) method with slight modification. Antioxidant activity test used 4 oil concentrations of 0.05 g/ml, 0.1 g/ml, 0.15 g/ml, and 0.2 g/ml. Tests were performed on each sample. The antioxidant percentages were analyzed by spectrometer (WPA brand) number at 515 nm wavelength. The inhibitory concentration 50% (IC_{50}) antioxidant of cardamom oils were determined by probit regression.

Statistical Analysis

All tests and analyses had been done in three replications on each sample. The results were tested by *Completely Randomized Design*/CRD. Significant differences between means were determined by Tukey HSD analysis. $P < 0.05$ was considered statistically significant.

Results and Discussion

The *A. cardamomum* leaves oils in this study have yield of between 0.26-0.33% and cardamom rhizomes oils had yield 0.06%. The yield of *A. cardamomum* oil were obtained from distillation time of 4, 6,and 8 hours were different, however the yield of leaves oils had tendency increasing by the increasing of distillation time. The highest yield of leaves oils were obtained from distillation time of 8 hours. On the other hand, distillation times 4,6, dan 8 hours gave no different yield of rhizomes oils. The yield of *A. cardamomum* leaves oils in this study lower than *Elettaria cardamomum* leaves oils (3.15%) (Batubara *et al.* 2016b), this is probably due to the different species of cardamom leaves were used. Parts of the plant also have effect on the oil produced, rhizome tissue is thicker than leaf tissue of cardamom. The hydrofusion process that occurs becomes more difficult on the rhizomes, resulting in fewer oil yields. This result accordanced with the Jaafar *et al.* (2007) study about the essential oils of the leaf and rhizome of *Etlingera elatior*, where the leaf oil has higher yield (0.0735%) than rhizome oil (0.0021%).

Table 1. Chemical composition of *A. cardamomum* essential oils

No.	Components*	Molecular Formula	Compound Group	Percentage [%]					
				Leaves			Rhizome		
				4 H	6 H	8 H	4 H	6 H	8 H
1	Alpha-Thujene	$C_{10}H_{16}$	Monoterpenes	-	-	-	5.66	12.1	13.3
2	4-Carene	$C_{10}H_{16}$	Monoterpenes	0.6	-	-	-	-	-
3	M-Cymene	$C_{10}H_{14}$	Monoterpenes	28.5	32.5	30.2	13.3	16.5	16.7
4	1,8-Cineole	$C_{10}H_{18}O$	Oxygenated Monoterpenes	55.1	52.2	50.3	51	61	51
5	Alpha-Terpinene	$C_{10}H_{16}$	Monoterpenes	-	-	-	-	-	0.56
6	Beta-Linalool	$C_{10}H_{18}O$	Oxygenated Monoterpenes	-	-	-	-	-	-
7	Linalool	$C_{10}H_{18}O$	Oxygenated Monoterpenes	-	-	-	-	3.3	3.38
8	Cyclohexane-l-ol	$C_{10}H_{16}O$	Oxygenated Monoterpenes	0.26	-	-	-	-	-
9	Alpha-Terpineol	$C_{10}H_{18}O$	Oxygenated Monoterpenes	-	-	-	-	0.57	2.56
10	Limonene oxide	$C_{10}H_{16}O$	Oxygenated Monoterpenes	-	-	-	-	-	0.52
11	Terpineol	$C_{10}H_{18}O$	Oxygenated Monoterpenes	0.18	-	-	-	-	-
12	Sabinenehydrate	$C_{10}H_{18}O$	Oxygenated Monoterpenes	-	-	-	0.9	0.96	0.81
13	Alpha-Terpineol acetate	$C_{12}H_{20}O_2$	Hydrocarbon	-	-	-	1.37	-	-
14	Cyclohexene	$C_{10}H_{18}O$	Oxygenated Monoterpenes	-	-	0.34	-	-	-
15	P-menth-1-en8-ol	$C_{10}H_{18}O$	Oxygenated Monoterpenes	0.31	-	-	-	-	-
16	Alpha-Thujenal	$C_{10}H_{14}O$	Oxygenated Monoterpenes	-	-	-	-	0.47	-
17	Azulenemethanol	$C_{15}H_{26}O$	Oxygenated Sesquiterpenes	-	-	-	-	-	0.37
18	Sabinyl acetate	$C_{12}H_{18}O_2$	Hydrocarbon	-	-	-	-	0.48	-
19	Isosafrole	$C_{10}H_{10}O_2$	Oxygenated Monoterpenes	-	-	-	1.38	0.57	-
20	Thymol	$C_{10}H_{14}O$	Oxygenated Monoterpenes	-	-	-	-	-	0.72
21	Isothujol	$C_{10}H_{18}O$	Oxygenated Monoterpenes	-	-	0.21	0.76	-	-
22	Limonene epoxide	$C_{10}H_{16}O$	Oxygenated Monoterpenes	-	-	0.16	-	-	-
23	Acetamide, N-prophyl	$C_{15}H_{11}NO$	Carboxamide	-	0.41	-	-	-	-
24	Patchoulene	$C_{15}H_{24}$	Sesquiterpenes	10.9	11.1	0.12	1.69	1.36	0.61
25	Chamigrene	$C_{15}H_{24}$	Sesquiterpenes	0.36	-	-	14	-	-
26	Octahydronapthalene	$C_{15}H_{24}$	Sesquiterpenes	-	-	-	1.05	-	-
27	Thujopsene	$C_{15}H_{24}$	Sesquiterpenes	-	0.63	0.37	-	-	0.75
28	Beta-Humulene	$C_{15}H_{24}$	Sesquiterpenes	0.17	-	-	-	-	-
29	Napthalene	$C_{15}H_{24}$	Sesquiterpenes	-	-	0.12	-	-	3.74
30	Beta-Vatirenene	$C_{15}H_{22}$	Sesquiterpenes	0.57	-	0.67	1.71	-	-
31	Cycloisolongifolene,8,9-dehydro	$C_{15}H_{22}$	Sesquiterpenes	0.29	0.84	-	-	-	-
32	Beta-Chamigrene	$C_{15}H_{24}$	Sesquiterpenes	-	-	12.8	-	-	-
33	Alpha-Bisabolene	$C_{15}H_{24}$	Sesquiterpenes	-	-	0.36	1.25	0.46	0.77
34	Cyclohexane	$C_{15}H_{24}$	Sesquiterpenes	0.23	-	-	-	-	-
35	Alpha-Panasinsen	$C_{15}H_{24}$	Sesquiterpenes	1.73	1.68	1.9	-	-	-
36	Germacrene	$C_{15}H_{24}$	Sesquiterpenes	-	-	-	-	-	2.85
37	Methanoazulen	$C_{15}H_{24}O$	Oxygenated Sesquiterpenes	-	-	0.22	-	-	-
38	Gamma-Gurjunepoxide	$C_{15}H_{24}O$	Oxygenated Sesquiterpenes	-	-	-	0.87	-	-
39	Cedrene	$C_{15}H_{24}$	Sesquiterpenes	-	-	0.21	-	0.55	-
40	Aromadendrene	$C_{15}H_{22}$	Sesquiterpenes	-	-	0.36	-	-	-

41	Lanceol	$C_{15}H_{24}O$	Oxygenated Monoterpenes	-	-	0.39	0.96	-	-
42	Spathulenol	$C_{15}H_{24}O$	Oxygenated Sesquiterpenes	0.27	-	-	-	-	-
43	Cycloprop[e]azulene	$C_{15}H_{24}$	Sesquiterpenes	-	-	1.08	-	-	-
44	Azulene	$C_{15}H_{24}$	Sesquiterpenes	0.63	-	-	-	-	-
45	Globulol	$C_{15}H_{26}O$	Oxygenated Sesquiterpenes	-	0.62	-	-	0.48	-
46	Alpha-Bisabolene epoxide	$C_{15}H_{24}O$	Oxygenated Sesquiterpenes	-	-	0.17	-	-	-
47	Ledol	$C_{15}H_{26}O$	Oxygenated Monoterpenes	-	-	-	-	0.63	-
48	Bergamotol	$C_{15}H_{24}O$	Oxygenated Sesquiterpenes	-	-	-	0.91	-	-
49	Dibutyl phthalate	$C_{16}H_{22}O_4$	Hydrocarbon	-	-	-	0.98	0.63	1.12
50	Dimethyl ester	$C_{16}H_{24}O_6$	Hydrocarbon	-	-	-	-	-	0.31
			Total	100	100	100	100	100	100

Note: * : Identification by Nist 147 library, - : not detected, H: distillation hour

GC-MS analysis showed that *A. cardamomum* oils consists of several compounds with different percentages in each sample. Chemical compounds of *A. cardamomum* essential oils showed in Table 1. The main compounds in *A. cardamomum* oils were obtained in this study are 1,8-cineole (50.30–60.95%) and m-cymene (13.29-32.54%). Previous studies also found that 1,8 cineole is the main compound of *A. cardamomum* leaf oil (Riendyani 2014). Ketaren (1985) also stated that the cardamom oil has the main compound 1,8 cineole. In this study, cardamom leaves oils were distillated for 4 hours has the highest 1,8-cineole with percentage 55.11%, while on the cardamom rhizomes oils were distillated for 6 hours oil has the highest 1,8-cineole with percentage 60.95%. Chemical compounds contained in leaves and rhizome of cardamom in this study are mostly included in the monoterpenes, oxygenated monoterpenes, and sesquiterpenes groups (Table 1).

Table 2. Physico-Chemical Properties of *A. cardamomum* Oils

Sample Oils *A. cardamomum*	Specific Gravity (20°C)	Refractive Index (20°C)	Miscibility in Alcohol 70%	Optical Rotation (°)	Acid Number
Leaves oil - distillated for 4 hours	0.903 ± 0.03a	1.476 ± 0.002a	1 : 7.33 ± 0.58a	(+) 2.37 ± 0.09a	0.50 ± 0.08a
Leaves oil -distillated for 6 hours	0.906 ± 0.02a	1.478 ± 0.001a	1 : 7.67 ± 0.58a	(+) 2.14 ± 0.13a	0.56 ± 0.08a
Leaves oil -distillated for 8 hours	0.909 ± 0.03a	1.478 ± 0.001a	1 : 8.00 ± 0.00a	(+) 2.05 ± 0.05a	0.57 ± 0.12a
Rhizomes oil- distillated for 4 hours	0.899 ± 0.00a	1.476 ± 0.002a	1 : 7.67 ± 0.58a	(+) 2.38 ± 0.02a	0.49 ± 0.09a
Rhizomes oil- distillated for 6 hours	0.901 ± 0.01a	1.477 ± 0.002a	1 : 8.00 ± 0.00a	(+) 2.28 ± 0.13a	0.59 ± 0.07a
Rhizomes oil- distillated for 8 hours	0.902 ± 0.01a	1.478 ± 0.001a	1 : 8.33 ± 0.58a	(+) 2.24 ± 0.17a	0.69 ± 0.02a
ISO 4733:1981*	0.191 – 0.938	1.462 – 1.468	1:2 – 1:5	22 - 41	Max. 6

(*Source : ISO 4733:1981)

Note : identical letters (a, b, etc.) mean no significant difference between mean in same column at P<0.05

Physicochemical properties of each sample were analyzed in this study almost had the same qualities and values. Statistical analysis showed no significant difference for each sample. The physicochemical properties of *A. Cardamomum* essential oils from this study are showed in Table 2. The results showed that the *A. Cardamomum* leaves oil had specific gravity value of between 0.903-0.909 while the *A. Cardamomum* rhizomes oils had specific gravity value of between 0.899-0.902. The highest specific gravity were *A.cardamomum* oils distillated for 8 hours, both cardamom leaves oils and cardamom rhizomes oils. The value of specific gravity of *A. cardamomum* oils in this study probably influenced by the present of 1,8-cineol and m-cymene compounds with specific gravity 0.922 and 0.861, respectively (Haynes 2014). Refractive index of *A. cardamomum* leaves and rhizomes olis had value of 1.476-1.478. Cardamom leaves oil which had the highest refractive index value is cardamom oil distilled for 6 hours, while the cardamom rhizomes oil having the highest refractive index is cardamom oil obtained for 8 hours distillation. *A. cardamomum* leaves oils had optical rotation value in average between (+)2.05º-(+)2.37º. While the cardamom rhizomes oils had optical rotation value in average between (+)2.24º-(+)2.38º. The optical rotation value of cardamom oils obtained in this study is lower than the standard by ISO 4733:1981. It probably due to this study used *A. cardamomum* while ISO 4733: 1981 is the standard for cardamom essential oil of *Ellettaria cardamomum*. This study used ISO standard because there is no standard for local cardamom of *A. cardamomum*. The results of the miscibility in 70% alcohol showed that the miscibility of *A. cardamomum* oils in alcohol were generally similar to the

ratio of 1: 7 to 1: 9 with an average miscibility of 1: 8. Miscibility of oil is influenced by the rapidity of oil solubility and the oils quality. If the oil mostly contained by oxygenated components, it will easily to be dissolved in alcohol (Guenther 1987). According to Ketaren (1985), the longer the amount of carbon chain, the more difficult the oil to be dissolved. The *A. cardamomum* leaves oils had an average acid number of 0.50-0.57 while *A. cardamomum* rhizomes oils had an average acid number of 0.49-0.69. The acid number in this study increased with the length of distillation time. The longer distillation time, water and oil contact is also longer and heat causes ester hydrolysis process to increase. Alcohol compounds with high molecules will be oxidized to aldehydes, carboxylic acids, and ketones.

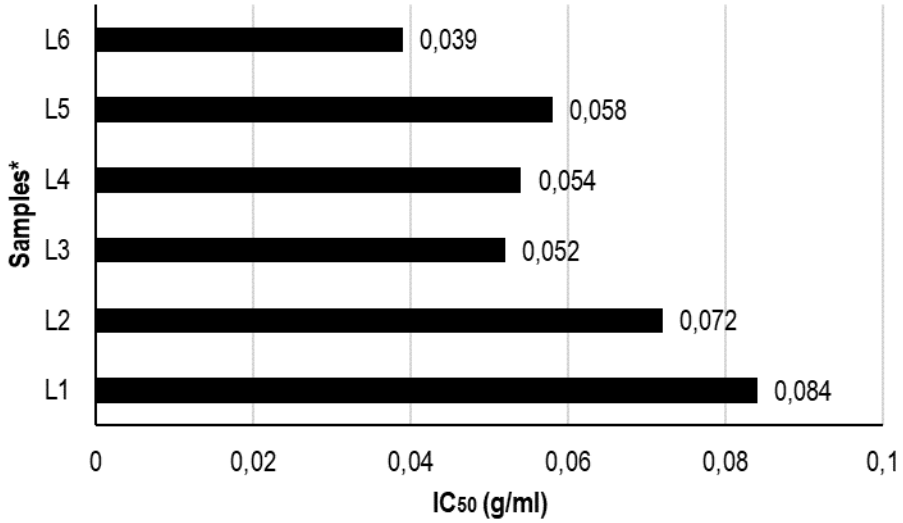

Figure 1. IC_{50} of *A. cardamomum* Leaves and Rhizomes Essential Oils (L1: Leaves oil-distillated for 4 hours, L2: Leaves oil-distillated for 6 hours, L3: Leaves oil-distillated for 8 hours, L4: Rhizomes oil-distillated for 4 hours, L5: Rhizomes oil-distillated for 6 hours, L6: Rhizomes oil-distillated for 6 hours)

This study also tested the antioxidant activity of essential oils which was analyzed by DPPH method. The result showed that the antioxidant activities of *A. cardamomum* oils have tendency increase by increasing the concentration. The concentration of 0.05 g/ml, 0.1 g/ml, 0.15 g/ml, 0.2 g/ml of *A. cardamomum* oils in this study have inhibitory concentration between 40.53 - 57.34%, 52.10 - 65.90%, 59.86 - 78.64%, 71.26 - 86.5%, respectively. Previous study also gave value of antioxidant activity increase by increasing concentration of essential oils (Pujiarti *et al.* 2015). Overall, *A. cardamomum* oils in this study have mild antioxidant activity. IC_{50} values in each of cardamom oil are presented in Figure 1. The lower IC_{50} value had better antioxidant activity, because in small percentages. The antioxidant compound can prevent about 50% radical activity. The highest IC_{50} value of antioxidant activity was found in cardamom leaves oils distillated for 4 hours, while the lowest IC_{50} value of antioxidant activity was found in cardamom rhizomes distillated for 8 hours. The results of chemical analysis of cardamom oil obtained in this study showed that most of the chemical components in *A. cardamomum* oils consists of terpenoid groups from the monoterpenes group, oxygenated monoterpenes, sesquiterpenes and oxygenated sesquiterpenes with the largest percentage is the oxygenated monoterpenes. Alkaloids and terpenoids from medicinal plants extraction have antioxidant activity when are tested with DPPH (Awouafack *et al.* 2013). Antioxidant activity of leaves and rhizomes oils in this study probably caused by the terpenoid contained in these oils such as m-cymene, patchoulene, and others terpenoid compounds. This study also found that average of antioxidant activity of cardamom leaves oils smaller than cardamom rhizomes oils. This is probably due to rhizomes oils have more terpenoid compound such as alpha thujene, linalool, and alpha-terpineol where are not found in leaves oils.

Conclusions

Leaves oils of *A. cardamomum* have higher yield than rhizomes oils. Distillation time had an effect on chemical compound of leaves and rhizomes oils of *A. cardamomum*, in which the chemical components of the oils were varied with the main compound was 1,8 cineole. However, distillation times had no effect on oils physico-chemical properties. *A. cardamomum* leaves and rhizomes oils obtained from hydrodistillation 4,6, and 8 hours had same qulities. Leaves and rhizomes oils of *A. cardamomum* posses mild antioxidant, while the highest antioxidant

activity obtained from rhizomes oils were distillated for 8 hours.

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WOOD RESEARCH Journal

Journal of Indonesian Wood Research Society

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4.2. Values between are written using this symbol (~), e.g. 3.75 ~ 8.92%.
4.3. Editors could modify Figures without changing their substantial meaning.
4.4. References are arranged from A to Z.
4.5. References in text are written as this example: (Palomar *et al.* 1990; Arancon 1997).
4.6. Examples of writing of References: Altschul, S.F.; T.L. Madden; A.A. Schäffer; J. Zhang; Z. Zhang; W. Miller; D.J. Lipman. 1997. Gapped BLAST and PSI-BLAST: A New Generation of Protein Database Search Programs. Nucleic Acids Res. 25: 3389-3402.

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Example of Table and Figure

Table 1. Effects of temperature on *in vitro* growth of seedlings.

Temp. (°C)	Shoot length (mm)	Number of leaf	Fresh weight (g)
25	59.2 ± 10.6[c]	4.5 ± 0.8[a]	0.29 ± 0.13[a]
27	88.5 ± 9.3[a]	4.8 ± 0.9[a]	0.40 ± 0.12[a]
29	75.0 ± 11.1[b]	3.8 ± 0.6[a]	0.30 ± 0.07[a]

Note: Values (average ± standard deviation) with different letters are statistically significant according to Tukey's multiple comparison test. Data were recorded after 4 weeks of culture. MS medium was used as a basal medium without any PGRs. Number of sample = 10.

Source: Chijo *et al.* 2010.

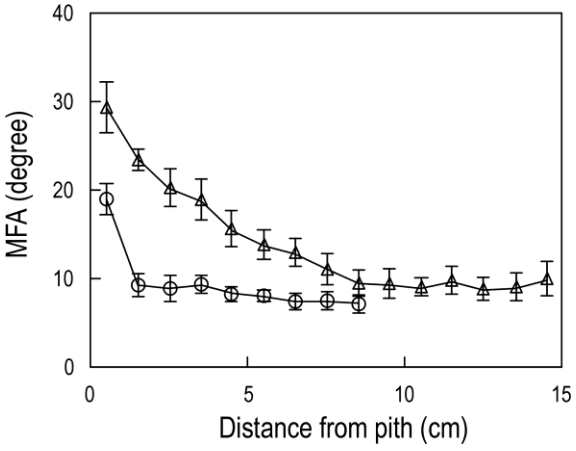

Figure 3. Radial variation of microfibril angle of the S2 layer in tracheid. Open circle, *Agathis* sp.; open triangle, *Pinus insularis*; Bars indicate the standard deviation. (Source: Ishiguri *et al.* 2010)