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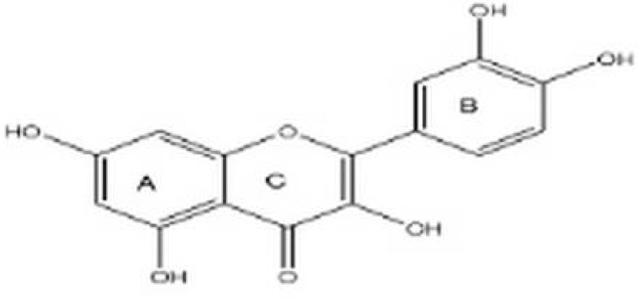

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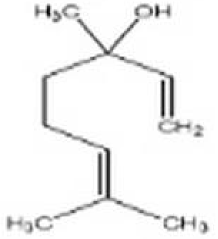

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Subterranean Termite Durability of Three Inferior Wood Species Collected from Indonesian New Guinea Coated with Two Coating Systems

Sasarari Yuliana, Reinardus Liborius Cabuy, and Wahyudi

Abstract

Wood is very susceptible material from termite attacks. A simple, cheapest method to prevent this attack is coating treatment. On this work, three inferior wood species collected from Manokwari – Papua Barat, namely Pulai (*Alstonia scholaris),* Binuang (*Octomeles sumatrana*), and Jambu (*Syzygium* sp.) were coated with two coating systems of oiled and water- based coatings. Coating was applied using paint brush on wood samples with dimensions, 50 cm long x 5 cm wide x 2.5 cm thick. Durability was conducting with grave yard test, where half-length of wood samples, coated and uncoated samples, were buried on the yard for the three consecutive months. Wood durability was evaluated using two variables, wood weight loss, and wood damage, respectively, and analyses into other variables likes wood resistance class, level of resistance, degree of damage, and natural durability classes. The results indicated that both coated systems, oiled and water based, had an average of spreading rates from 0.005-0.015 g/cm^2. Weight loss of three inferior species of wood recorded from the control samples are 38.65%, 12.12%, and 6.14% for Pulai, Binuang and Jambu, respectively. Treatment with coating systems, weight loss could be prevented into 95.1%, 58.2% and 56.0% to control for Pulai, Binuang and Jambu, respectively. Wood resistance class of Pulai is IV for Susceptible (control) and fallen into Moderate – Resistance with coating systems, from Durable into Moderate Durable or shifting from degree of damage 3 into 2. Binuang species initially is Moderate Resistant (III) and improve into Resistant (II), and has natural durability from Non-Durable with degree damage of 3 into Very Durable with degree damage of zero. Jambu is similar to the Binuang, from Moderate resistant to Resistance and natural durability from Moderate Durable to Very Durable after being coated with two coating systems. It is summarized that coating systems available in the local market could protect wood from subterranean termites and these coating regimes could be applied into daily practices in our local environment or houses.

Keywords: wood durability; subterranean termite; inferior wood species; coating systems

Introduction

Wood is biological material available on earth produced by wooden plant that ecologically and environmentally sustainable for any utilizations. This renewable material has been usages from the beginning human civilization across the globe. It has natural characteristics like beauty in color, hardness, convertible and workable to any wood products. Since human civilization, wood has been utilized for construction of bridges, houses, huts, and others. However, one of main obstacle in wood life utilization or wood life service is organism attacks, mainly termite attacks (Subekti *et al*. 2018). Several treatment have been applied to protect wood in service, ranging from wood preservation with chemical, coating with coating system and wood barriers.

Recently, wood supplied from the natural forest is declining in volume, numbers of species, dimension, and quality (Wahyudi and Arifudin 2018). Sawn timber marketed and supplied for local used in Papua and Papua Barat province have been dominated by well-known species, Merbau (*Instia* sp.) and Matoa (*Pometia* sp.), respectively (Wahyudi *et al*. 2017). Two lesser known species which could be an alternative species of sawn timber in Papua and Papua Barat province are Lulu (*Celtis latifolia* Planch) and Jambu (*Syzygium* sp.) (Wahyudi *et al*. 2014).

Two inferior wood species of Pulai and Binuang, are enormous in nature, growing at any secondary forests or opened-lands near the local community gardens across low land areas in Indonesian New Guinea, Papua and Papua Barat Province. They are fast growing species, pioneer and dominant species at any secondary forests. These trees are naturally stand with an average of 60 cm up in diameter breath height (DBH) with 15 m height. Other wood species that have comparable attribute to the superior species are Jambu (*Syzygium* sp.) and Simpur (*Dillenia* sp.), as they have comparable machining properties to Matoa (*Pometia* sp).

In Indonesian New Guinea, these wood species have not been utilized for construction purposes but they are used for pallet boards for cement boxes construction and due to their low density, and susceptible from wood organism, mainly subterranean termites. Today, these three wood species mainly used for packaging material, mainly due to their low density, where Pulai has density of 0.3 g/cm^3, Binuang of 0.4 g/cm^3 and Jambu of 0.63 g/cm^3 (Wahyudi *et al*. 2014).

Wood durability is related to the resistance of timber against any attacks from destroyed organisms, mainly subterranean termites. In order to lengthen life service of wood or timber, wood preservation using chemicals that is toxic to environment was applied, but recently bio-natural agents have been explored and applied for timber preservation, as this is less toxic to surrounding nature (Hadi *et al.* 2016). Another method to improve wood durability is coating treatment, where wood surfaces were layered or coated with substances, either oiled and water-based coating system (Krisdianto *et al*. 2018). Wood coating is aimed to provide a protective layer or barrier from the wood deteriorated agents including termites. Coating is one of several method to improve wood durability by protecting from fungi, termite attacks, and other physical and chemical causes (Kaboorani *et al*. 2017; Pánek *et al.* 2019a; Pavlič *et al.* 2021).

Coating treatments are applicable in various ways in layering or polishing the surfaces of timber with hand sprayer, painted brush or the others (Rowell and Bongers 2015). These methods are technically achievable compared to the preservation ordinary methods, where toxic, corrosive substances and preservation installation are highly required. The simplest and cheapest coating treatment is to apply coating material or systems available on the market.

In daily uses, coating systems are divided into two categories, oiled and water based-coating systems (Pánek *et al*. 2019a). Oiled based coating systems is using oiled, mainly petroleum based for their liquid, while water based is water as the main solvent. Three oiled based coating systems were employed, ranging from paint, melamine, and varnish, respectively. Water based coating is acrylic based material. The main objective of this work, therefore, is to examine effectivity of four coating systems on wood durability against subterranean termite conducted with grave yard test.

Materials and Methods

Wood Sample Preparation

Wood samples of sawn timber were purchased from the local supplier in Manokwari town, each timber has dimension of 5 cm thick x 10 cm width x 400 cm long, and free from bark, split check, knot, and deformation. Sawn timber was cross cut with circular saw (Makita 8" 5008M) into two length, 5 cm thick x 10 cm width x 200 cm length, then further cross cut into 5 cm thick x 10 cm width x 100 cm length and four cross cut-samples were obtained. Each sample was ripped to produce 16 sticks of 5 cm thick x 5 cm width x 100 cm length, then ripped into 2.5 cm thick x 5 cm width x 50 length and 32 sticks were collected and mixed randomly. Hand planner (Makita M1901B) and sand paper (Kinka 60-100) were used for preparing the final samples. Four sticks were selected for moisture content and wood density samples, 2 cm length x 2 cm width x 2 cm thick. 15 sticks were further cut for coating systems with grave-yard test.

Each coating system has 3 replicates, 3 control samples, and 4 treatment of coating, therefore, 15 samples, 2.5 cm thick x 5 cm width x 50 cm length, were used for each wood species.

Coating Treatment

Coating treatments were applied manually using 2 inches paint brush (Palazzo 50, 70 mm), to all surface of wood samples. Glue spreading rate (g/cm^2) was calculated by weighting the sample after and before coated. The coated samples were placed in opened air temperature for drying. Four coating systems were applied, which could be divided into two based characteristics, water and oil-based coating systems. Water based coating was Mowilex WS 503 (Mowilex, Indonesia). Three oil-based coating system are Melamine (Melanic 1200 clear base, PT. Nipsea Paint and Chemical, Jakarta), Varnish (Nipsea Paint and Chemical, Jakarta), and Wood and Metal Paint (Avian, Jakarta).

Grave Yard Test

Graveyard test is similar to in-ground testing, where 20 cm bottom section of the coated samples were buried into ground (30 cm wood samples above ground) and left in place for three months (Hadi et al., 2016). Initial installment of wood sample for conducting graveyard test is illustrated in Figure 1. Grave tests were conducted at the back yard of Laboratory building Faculty of Forestry university of Papua Manokwari, using random design.

Figure 1 Instalment of the samples used for grave yard test.

Research Variables

This work has four variables, ranging from coated spreading rate (SR) expressed in g/cm^2 (Okahisa *et al*. 2019), weight loss (WL) in percent (%) (Hadi *et al*. 2016), level of wood resistance, and class of natural durability (Sornnuwat 1996), and rating system for visual classification (EN 252). These variables were calculated using the following equations: SR = $(W_i - W_o)/WS$ (g/cm^2), where W_i= weight of sample after been coated (g); W_o = weight sample prior being coated (g); WS = total surface of wood sample (cm^2). WL =

($W_1 – W_2$)/W_1 x 100% where W_1 = weight of sample prior the graveyard test (g); W2 = wight of samples after the grave yard test (g).

Wood resistance levels are determined according to percent mass loss, listed in Table 1, whereas classification of natural durability of wood is determined with the visual rating and presented in Table 2.

Table 1. Wood resistance level based on the percent mass loss of wood

Class	Percent weight loss (%)	Level of resistance
I	0	Highly resistant
II	1-3	Resistant
III	4-8	Moderate
IV	9-15	Non-resistant
V	>15	Susceptible

Table 2. Classification of natural durability of wood

Degree of damage	Termite attack on wood	Classified to natural durability
0	No damage	Very durable (VD)
1	Hardly visible damage	Durable (D)
2	Superficial and slightly inner damage	Moderate durable (MD)
3	Moderate inner damage	Non-durable (ND)
4	Heavy inner damage	Perishable (P)

Results and Discussion

The mean values for wood density, coated spreading rate, weight loss, class, level resistant, and classified natural wood durability are displayed in Tabel 3.

As been illustrated by Table 3, the highest spreading rate of coating system was recorded from Pulai (*Alstonia scholaris*), coated with OB_2 and OB_3, where each has 0.014 g/cm^2, and the lowest was recorded from Binuang (*Octomeles* sp), coated with OB_1 of 0,005 gr/cm^2. An average of spreading rates of WB in general (0.01 g/cm^2) is equal to an average of OB1-3 of 0.01 g/cm^2, and this average of the spreading rates recorded from this research is lower than those reported by (Wahyudi and Arifudin 2018) for 0.19 g/cm^2 for gluing a glulam made from Sago bark combined with two less used species applied for both surfaces. Lower in glue spreading rates recorded from this investigation are probably because human error, where the workers are different to those previous experiment.

Tabel 3. The mean values for wood density, coated spreading rate, weight loss, wood resistant class, level resistant, and classified natural wood durability are displayed in Tabel 3.

Wood species	Density (g/cm^3)	Coating system	SR (g/cm^2)	WL (%)	Class	Level of resistant	DD	classified natural durability
Pulai	0.32	Control		38.654	V	Susceptible	3	ND
		Mowilek	0.011	8.468^C	IV	Moderate resistant	2	MD
		Paint	0.014	8.646^C	IV	Moderate resistant	2	MD
		Varnish	0.014	1.892^d	II	Resistant	2	MD
		Melamine	0.012	25.177e	V	Susceptible	2	MD
				11.041^a				
Binuang	0.42	Control		6.141	III	Moderate resistant	3	ND
		Mowilek	0.009	2.742^d	II	Resistant	0	VD
		Paint	0.009	9.242^c	IV	Non-resistant	0	VD
		Varnish	0.007	6.952^c	III	Moderate resistant	0	VD
		Melamine	0.005	2.387^d	II	Resistant	2	MD
				5.331^b				
Jamb	0.63	Control		5.043	III	moderate resistant	2	MD
		Mowilek	0.009	2.940^d	II	resistant	0	VD
		Paint	0.012	1.783^d	II	resistant	0	VD
		Varnish	0.011	1.563^d	II	resistant	0	VD
		Melamine	0.008	3.992^d	III	moderate resistant	0	VD
				2.571^b				

Remarks : SR= Spreading rate, WL= weight loss, DD= degree of damage, ND=non-durable, MD= moderate durable, VD= very durable, S= susceptible, MR = moderate resistant, R=resistant, NR= Non-resistant

Percent WL recorded from the control samples for three species are higher that those recorded from the three coated system used, such as 38.65% recorded from Pulai, followed by 12.12% of Binuang and 6.14% of Jambu. It seems that Pulai coated with OB_2 could prevent WL by 95.1% compared to the control. Similarly, coating applied for other two wood species could prevent WL by 58.2% and 56.0 % for Jambu dan Binuang, respectively. These results summarize that coating systems had an impact to enhance the life services of wood material from subterranean termite tested. Coating systems are usually employed to extent the change of color, protect wood surface from biotic and abiotic factors, and mostly for softwood end products (Gobakken and Westin 2008; Gurleyen *et al*. 2019). It is also supported that coating systems could protect weight lost of wood during service, mainly due to termite attack (Amemiya and Marsuoka 1979).

Analisis of varian (ANOVA) for percent WL amongst three wood species are highly significant (p=0.001), but four coating regimes had a significant impact to prevent WL (p=0.027). Post hoc test using least significance difference (LSD) $_{0.05}$ indicated that the WL recorded from Pulai is different to Binuang and Jambu. Amongst four coating regimes, their means for the WL are not significance different, however, when the coating regimes were applied to different wood species, the WL resulted different percentage (Tabel 3). The WL recorded for Jambu are lesser than the others.

Table 3 indicates that in general wood resistance class and level of resistant for three inferior wood species from Indonesian New Guinea, using percent of wood weight loss (Table 1), could be improved. Pulai without coating treatment or control has wood resistant class V and it could be improved by coating with OB_2 into wood resistant class II. Pulai control has Susceptible (S) for level of resistant and improve to the Resistant (R) after being coated OB_2. Binuang control samples are fallen into wood resistance class IV or Not Resistant (NR) and coated with WB and OB_1 could improve wood class resistance into class II for Resistant (R). Jambu originally (control sample) was classified into moderate wood resistant class III or Moderate (M), after being coated with WB and OB_2 and OB_3 could improve wood resistant class into II or Resistance (R). It could be highlighted that two coating regimes could improve wood resistant class grade and wood resistant level categories. These three inferior wood species had light density and bright color, presumably they had low extractive substances that could be a toxic substances for termite (Amaliyah *et al*. 2020; Nakai and Yoshimura 2000).

With regard to the degree of damages (DD) and classification of natural durability, Pulai without treatment (control) had degree of damage (DD) for scale 3, and after being treated with coating, it is upgraded from into 2 scale or from Non-Durable (ND) into Moderate Durable (MD). Binuang from DD level 3 of control sample into DD 0-2 meaning Very Durable (VD) into MD. Remarkable improvement is achieved for Jambu, four coting regimes could upgrade DD class 2 into DD 0 or from MD into VD. Pulai wood natural durability could be improved from Non-Durable (control) into moderate durable by coating both WB and OB system. Similarly, Binuang treated with two coating regimes can be upgrade from ND into MD and D. Remarkable achievement could be pointed into Jambu, where its natural durability could be improved from MD (2) into VD (0), meaning from moderate durable (control) into very durable (coating systems).

It highlights that coating systems are not only improve the natural durability of wood against subterranean termite ((Hadi *et al*. 2016; Zulfiana *et al*. 2020) but also stabilize wood from biotic and abiotic factors when the wood is exposed outdoor and having direct contacts water, sunlight and other factors (Knapic *et al*. 2018; Okahisa *et al*. 2019; Pánek *et al*. 2019).

Application of wood coating to improve wood life services by layering wood surface and enhance the natural beauty of wood could be conducted using simple method with low cost and the coating material available in local market (Knapic et al. 2018; Ozgenc et al. 2012). This coating treatment could be applied into any wooden product with

Conclusions

It is summarized that three inferior wood species coated with two coating regimes had different durability performance tested against subterranean termite. Coating systems, oil dan water bases, could improve natural durability of three inferior wood species collected from Indonesian New Guinea.

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Bio-Attractant of Termites Bait from Waste Paper and Extract *Ocimum basilicum* Linn against Subterranean Termites *Coptotermes curvignathus* Holmgren

Farah Diba, Palguna Wiranata, Nurhaida, Muhammad Dirhamsyah, and Rudi Hartono

Abstract

Termites were the most destroying wood organism and one of the methods for controlling termites and suppressing their population is the baiting system. One of the conditions of the baiting system method is that the bait must be able to attract termites. Mostly the attractant is made from an extract of the plant. This research aimed to evaluate the attractants of termites bait from waste paper and extract of basil leaf (*Ocimum basilicum*) against subterranean termites *Coptotermes curvignathus* Holmgren. Leaves of *Ocimum basilicum* extracted with maceration process with ethanol. After extraction, the yield was made into five concentrations, consisting of 2%, 4%, 6%, 8%, and 10%. The waste paper was made into termite bait with a square bait with measure 2 cm long x 2 cm wide x 1 cm thick. After that, the extract of *Ocimum basilicum* was poured into the bait in each concentration. The termite's bait is then exposured to termites for 21 days. The number of termites was 50 workers and 5 soldiers. The variable of research was termite mortality and weight loss of termite bait. The result of the research showed that the average termite mortality value was 18.79% - 97.58%. The highest termite mortality was achieved at a 10% concentration of extract *Ocimum basilicum*. Its followed by a concentration of 8% with a mortality value of 91.52%; a concentration of 6% with a mortality value of 78.18%; a concentration of 4% with a mortality value of 61.82%, and a concentration of 2% with a mortality value of 51.52%. Meanwhile, in the control treatment, the mortality value was 18.79%. The average weight loss of termite bait was 3.41% - 8.36%. The highest weight loss was achieved on concentration 8% and this bait was more attractive to termites. The important results of the research was termite's bait from waste paper and extract of *Ocimum basilicum* was attractant to subteranean termites *Coptotermes curvignathus* and the optimum concentration was 8%.

Keywords: attractant, *Coptotermes curvignathus*, *Ocimum basilicum*, subterranean termites, termites bait.

Introduction

Termites are social insects that live in communities and are known as cellulose-eating insects that can damage wood and buildings. Every year the losses caused by termites in Indonesia are around Rp 224 billion – Rp 238 billion (Nandika *et al* 2015). An effort that can be made to reduce losses caused by termite attacks is to carry out controls aimed at suppressing termite populations and termites attacks (Kutana *et al* 2018). The termite baiting system technique is considered more effective than other termite control techniques because other control techniques such as spraying the chemical (termiticide) are considered to endanger human safety and health and the surrounding ecosystem can be contaminated with termiticide (Sucipto 2009). The baiting system technique is unique because this system should attract termites and makes termites eat the bait. Therefore the bait should have properties or aroma to attract termites to consume the bait (Permana dan Husni 2017).

Materials that have a scent that can attract termites to approach and consume the bait are called bio-attractants (Septiana dan Husni 2017). Currently, the attractant used on termites bait was chemicals thought to be not environmentally friendly. Natural attractants or bio-attractants can be obtained from various types of plants, especially plants with distinctive aromas (Permana dan Husni 2017). Basil leaf (*Ocimum basilicum*) hasthe potential a termite bait attractants because it contains aromatic compunds which attract termites to consume the bait (Indrayani *et al* 2017) and in the research of Simbolon *et al* (2015) resulted 41 termites from 55 termites moved to the samples that had been given basil leaf extract.

Basil leaf extract consists of aromatic compounds namely methyl eugenol (2.24%), methyl linoleate (1,49%), and toluene (4,46%), and this compound attract termites to consume the bait (Noviansari *et al* 2013). Basil leaf can be combined with materials that contain high cellulose to strengthen the bait because cellulose is the main food of termites. HVS paper waste is a material that contains high cellulose (Franceschin *et al* 2010). According to Muin *et al* (2015) termite bait formulations from degraded Pine wood, HVS paper waste, paperboard waste and newsprint waste with a mixture of boiled soybean water can be used as feed ingredients to support termites baiting system techniques.However, there have been no reports on the utilization of the HVS paper waste with basil leaf extract as a bio-attractant of termites bait to control termites with a baiting system. The study aimed to evaluate the extract of basil leaf (*Ocimum basilicum)* as a bio-attractant of termites bait, and analyze the optimum concentration of basil leaf as a bio-attractant against *Coptotermes curvignathus* Holmgren. The research outcome is to provide the information on effectiveness of basil leaf extract as bio-attractant of termites bait against subterranean termites and can be used as an environmentally friendly bio-attractant in wood preservation.

Materials and Methods

Sample Preparation

The research was carried out at the Laboratory of Wood Technology, Forestry Faculty Tanjungpura University. The young leaf of basil (*Ocimum basilicum*) around 10 kg was obtained from a plantation in Ahmad Yani Street Pontianak City. Subterranean termites *Coptotermes curvignathus* was from a secondary forest in Sungai Ambawang, Kubu Raya district. The termites were kept in laboratory for one month before being used as a sample for testing.

Basil Leaf Extraction

The process of extraction of basil leaf was referred to Kumalasari and Andiarna (2020) that air-dried basil leaf are crushed into powder and filtered using 40 mesh and 60 mesh screens. The sample used was the powder of basil leaf which passes 40 mesh filter and was retained by the 60-mesh filter. Furthermore, 200 grams of basil powder was dissolved in 800 ml of ethanol and macerated for 48 hours. The result of maceration is filtered and re-extracted until a clear solution is obtained. After that, the liquid extract was filtered on the rotary evaporator at a speed of 50 rpm and temperature 45°C for 1 – 2 hours to remove the ethanol solvent. The yield of the extract was counted as follows (Bihari *et al* 2011):

$$\text{Yield of extract (\%)} = \frac{a}{(1-\mathrm{x})\mathrm{b}} \times 100\%$$

a = weight of basil extract (gram)
b = weight of initial basil powder (gram)
x = water content

Termite Bait Preparation

HVS paper waste which has something print in the paper was crushed using a hammer mill with a size of 20 mesh and made into termite bait refer to Severtson and Majer (2006). The density of the termite bait sample was made into 0.5 g/cm^3. The HVS paper waste was soaked in distilled water for 30 minutes and then air-dried using a filter cloth. After that, 2 grams of material was formed using a molded box with a size length of 2 cm, width of 2 cm, and of thickness 1 cm. The sample bait was then put in an oven with temperature of 40±2 °C for 48 hours. Then the termite bait was dripped with 1.2. ml of basil extract in each concentration (60% of the weight of the material) and drained for 5 minutes. The concentration of basil extract used were 2%, 4%, 6%, 8% and 10%. Five replication was made for termite bait on each concentration. Furthermore, the termite bait sample was put in an oven for 48 hours at a temperature 40±2 °C to protect it from fungi attack (Septiana and Husni 2017). Before testing termites all the termite bait was weight to determine the initial weight of the termite bait sample.

Laboratory Assay on Basil Termite Bait

Laboratory assay to evaluate the attractants of termites bait was conducted refers to Ohmura *et al* (2000). The test tools use a plastic cup with a diameter of 5 cm. Each test plate was filled with 10 grams of sterile sand and moistened with 2 ml of water to maintain moisture. Plastic gauze with a diameter of 3 cm is placed on the sand to prevent direct contact of the sample with the sand. Furthermore, the termites bait was put in front of plastic gauze and termites was put inside the cup (50 workers and 5 soldiers). Observations were made for 21 days, and all the plastic cups were stored in a dark room with a temperature of 26.9 °C – 28.3 °C and humidity of 70% - 82%. The research was conducted in five replications. The variable of research was termites mortality and weight loss of termites bait. Determination of the termites mortality and termite bait weight loss value was calculated by the following formula (Owoyemi *et al.* 2011):

$$\text{Mortality (\%)} = \frac{N_1 - N_2}{N_1} \times 100\%$$

N_1 is the number of initial termites
N_2 is the number of termites at the end of the test

$$\textit{Termites bait weight loss (\%)} = \frac{B_1 - B_2}{B_1} \times 100\%$$

B_1 is the weight of the initial termite bait (gram)
B_2 is the weight of the termite bait at the end of the test (gram)

Data Analysis

Research on termite bait from basil leaf extract (*Ocimum basilicum*) and HVS waste paper against subterranean termite *Coptotermes curvignathus* using analysis of variance (ANOVA) with completely randomized design (CRD) according to Gaspersz (1994). The concentration of basil (*O. basilicum*) leaf extract were 5 concentration, consist of A_0= 0% (control), A_1= 2%, A_2= 4%, A_3= 6%, A_4= 8%, and A_5= 10%. Each concentration has 3 replications. The test results of the analysis of variance with the F test have a significant or very significant effect will continue to evaluation the difference between the treatment with honest significant difference (BNJ) test.

Result and Discussion

Yield of Extract *Ocimum basilicum*

The result of maceration process on basil leaf (*Ocimum basilicum*) was 30.93%. This result was good and higher compare to other research, such as Bilal *et al* (2012) reported the extractive values of basil were 4.0% in ethanol; 6.24% in water and 3.7% in ether. Meanwhile Diba et al (2022) reported the extractive values of basil was 29.38%. The form of extract was gel with a dark green color and smells like the original plant, this is thought to be due to the content of eugenol or its methyl eugenol derivatives contained in the basil leaf extract. According to Islamy and Asngad (2018) the eugenol compound is a clear liquid to pale yellow, volatile and gives a certain aroma with the same distinctive aroma as the original plants. Extraction is a process of withdrawing secondary metabolites using solvents (Purushothaman *et al* 2018). The extraction used ethanol which is a polar solution. According to Ohmura *et al* (2000) ethanol can produce the optimal amount of extract.

Termites Mortality

The average value of termites mortality after feeding the termites bait from waste paper and extract of basil leaf (*Ocimum basilicum*) was 51.52% ~ 97.58%, meanwhile, on control treatment, the mortality value was only 18.79%. The concentration of extracted basil leaf of 6%, 8%, and 10% has a very strong activity to inhibit termites and resulted in mortality from 78.18% ~ 97.58%.

The analysis data of termites mortality with completely randomized design showed that the extract of basil leaf on termites bait has a significant effect on termites *Coptotermes curvignathus*. The mortality of termites happened on five days until the end of the evaluation test. Termite feed the bait then spread out the bait with trophallaxis system Ridhwan and Isharyanto (2016) stated the basil leaf has a potential as bio-insecticide. The highest concentration of extract basil in the bait resulted more termite mortality and the bait has a hig palatability to termites. The average termite mortality after feeding the bait from waste paper and extract of basil leaf value was shown in Figure 1.

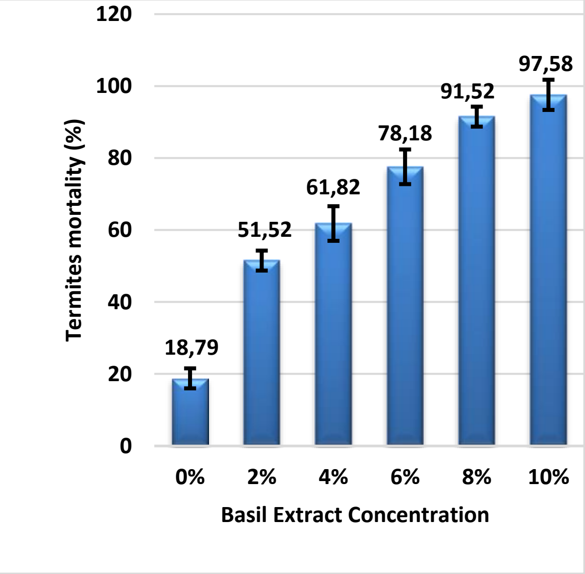

Figure 1. The average value of *Coptotermes curvignathus* termites mortality after feeding the bait from waste paper and extract of basil leaf (*Ocimum basilicum*) with different concentration.

Referring to the research of Suhara (2020), subterranean termite mortality in baits using low concentrations will react more slowly because the compounds contained in baits require time to be absorbed by the subterranean termite. Meanwhile, baits with higher concentrations reacted more quickly and increased subterranean termite mortality. From the experience, we found that the mortality of termites after feed the bait from waste paper and extract basil with concentration 2% was happened after 10 days, meanwhile on concentration 10% the mortality was happened after five days.

Umar and Majid (2020) stated that termites bait methods has the advantage to control termite. Elimination of termite colony population was successfull with the aid of termite behavior of trophallaxis which is the sharing of the bait toxicants among the termite nest mates.The bioatractan from basil leaf made the termites worker share the toxicants among the nest mates then eradicate the colony along the process. The mortality value of *Coptotermes curvignathus* termites after feeding the termite bait from waste paper and extract of basil leaf was from 78.18% ~ 97.58%, almost 100% mortality was achieved at basil concentration of 10%. This results showed that the basil extract has a potential used for bioatractant on bait formulations and to achieved the ellimination process, the bait require additional material, such as from monosccharide and sugar.

Bilal *et al*. (2012) stated the highest concentration of basil extract have the highest bioactive compound which made the highest mortality of termites. The bioactive compound from basil leaf was eugenol, methyl eugenol, and methyl clavical. Kardinan (2007) stated the eugenol and methyl clavical was inhibited and made mortality of flyhouse Musca domestica. Methyl clavical has the effect of anasteticum which works by interfering the nervous system. Meanwhile, eugenol works as a contact poison through the surface of the body. This is because eugenol is easily absorbed through the skin, causes burns and can cause death.

Eugenol can be toxic to termites and has the potential as a termite control bioactive, besides that the eugenol content in plant extracts can also be a bio-attractant for termites, but depending on the type of plant and the concentration used (Indrayani *et al*. 2016). Furthermore, the lowest mortality was at a concentration of 0% with a mortality value of 18.79%. The low mortality at 0% concentration was due to the absence of bioactive components from basil leaf extract, then termite mortality tends to be low. Mortality at a concentration of 0% occurred even though basil leaf extract was not given, presumably due to the inability of termites to adapt to their new environment, and cannibalism between termites was occur (Nandika *et al*. 2015).

Based on the research of Indrayani *et al*. (2017), ethanol extract of basil leaf contains methyl eugenol (2.24%) which has the potential as a bio-attractant and control subterranean termites Coptotermes curvignathus Holmgren. According to Kardinan (2007) methyl eugenol ($C_{12}H_{24}O_8$) is a semi-chemical material that is an attractant that can stimulate the olfactory (sensory device) of insects. Methyl eugenol can affect the behavior of insect animals, such as the behavior of looking for food, laying eggs, sexual relations and others. This statement is supported by Lewis and Forschler (2016) who stated that eugenol was significantly more toxic to subterranean termites *Coptotermes* sp than other chemical compounds such as citral, citronellal, geraniol, and pyrrolidine. The content of eugenol in basil plants is higher than other compounds. Pandey *et al* (2014) stated the main components of essensial oil from basil plant is eugenol with a percentage of 6.6%.

Termite mortality is also influenced by several other factors such as termite conditions, temperature, humidity, and light intensity. In laboratory bioassay, the termites used must be in good condition. Termites are sensitive insects and their activities are easily disturbed, so it is necessary to condition the termite colony before testing. In addition, Oramahi *et al*. (2021) stated subterranean termites Coptotermes curvignathus are termites that require a moist place to live, the ideal temperature and humidity conditions such as their natural habitat. Therefore during the test the temperature should be measured, if it is too high and the humidity is too low, it can trigger an increase in the mortality of subterranean termites. In this study, the average temperature is in the range of 28.5 °C - 30.8 °C, such a temperature is considered to be optimum and ideal for termite life because it is under the living conditions of subterranean termites in nature. This is supported by the statement of Cao and Su (2015) which states that the optimum temperature for the activity of wood-destroying termites is between 24 °C – 32 °C. Meanwhile, the humidity ranges from 70% - 82% and is in accordance with the termites nest. Nandika *et al* (2015) said that the optimum humidity of subterranean termites in living habitat was range between 75% - 90%. In addition to termite conditions, temperature and humidity, during termite laboratory bioassay, it is necessary to store the termites in a dark room, because termites has a cryptobiotic character. Exposure of too much light can interfere termites activity.

Bait Weight Loss

The average value of bait from waste paper and extract of basil leaf (*Ocimum basilicum*) weight loss after feeding by termites was 6.51% ~ 8.36%, meanwhile on control treatment the bait weight loss value was only 3.41%. The concentration of extract basil leaf of 8% has a highest bait weight loss. The average bait weight loss value was shown in Figure 2.

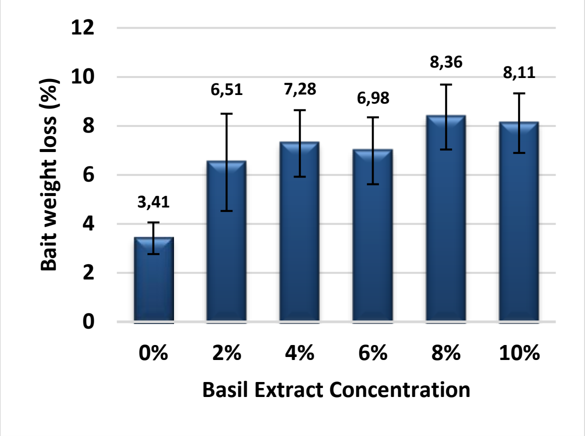

Figure 2. The average value of bait from waste paper and extract of basil leaf (*Ocimum basilicum*) weight loss after feeding by *Coptotermes curvignathus* termites.

Basil leaf extract has a significant effect on termites feeding to the bait. The weight loss of bait without basil leaf extract was 3.41%, meanwhile, the weight loss of bait with basil extract was higher, 6.51% ~ 8.36%. This condition reflect the effect of bio-attractant from basil leaf extract. The rate of termite consumption tends to increase with the higher concentration of basil leaf extract. In line with this result, Carnohan *et al*. (2021) stated the concentration of extract was influence the feeding activity of termites. Some extract with higher concentration tend to increase the preference of termite to feed the bait. According to Diba *et al*. (2017) termite bait active ingredients should be non-repellent and slow acting to ensure the bait will be transport to the nest by worker foraging termites and distribution the active ingredients throughout the colony by trofalaksis system. Bio attractants from basil leaf have a potential in controlling termites. Diba *et al*. (2022) stated the active ingredients in basil leaf extract consist of linalool, methyl chavicol, eugenol, methytl eugenol, flavonoid and saponin. Research of Hikmawanti *et al*. (2019) analysis extract of Ocimum basilicum with GCMS and it had 13 components with the major compound were methyl eugenol (52.60%), caryophyllene (18.75%) and germacrene-D (9.19%). This bio-active compound as an attractant for termites to feed the bait. Nandika *et al*. (2015) states that worker termites use their sense of smell to find food sources because this caste does not have eyes.

The value of bait weight loss is influenced by several other factors, one of which is the material of bait. In this study, the material used was HVS paper waste. HVS waste paper was different from natural food sources for termites. According to Pivnenko *et al* (2015) waste paper contain many chemicals from printing ink, especially polychlorinated biphenyls. The ink remaining on the bait is thought to be toxic to subterranean termites. However, according to Santos *et al*. (2007) subterranean termites genus *Coptotermes* sp and *Macrotermes* sp was able to metabolize and remove lipophilic xenobiotics in the waste paper, and thus the two genera of termites has a potential candidates for the biogenic conversion of waste paper. This research also prove that HVS waste paper was suitable as material for termite bait and not repellent to subterranean termites. The bait after feeding by termite was shown in Figure 3.

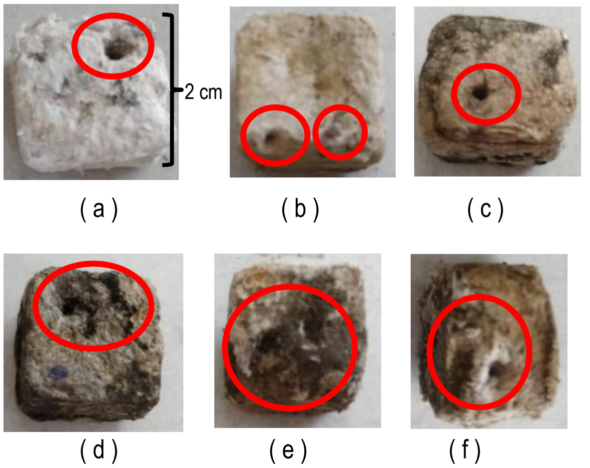

Figure 3. Condition of termite bait from waste paper and extract of basil leaf (*Ocimum basilicum*) after feeding by Coptotermes curvignathus termites
(a) : control; (b) : concentration 2%;
(c) : concentration 4%; (d) : concentration 6%;
(e) : concentration 8%;(f) : concentration 10%;

Figure 3 shows that termites feed on the bait on the sides or bottom of the sample by making holes (red circle in the image). From the results of the study, it can be seen that the feeding activities of termites tend to be in the same hole, so that the change in the shape of the bait is not too significant on the outside, but is dominant on the inside. This is presumably because the termites tend to make hole as part of termite burrow which can be in direct contact between the bait and the sand. In this study there were several components that caused the weight loss of the bait, namely basil leaf extract which have a distinctive aroma that can attract termites and HVS waste paper as a source of cellulose in bait which is very popular as termite food. The mixture of these components can be the main trigger for termites to eat the bait.

According to Kadian and Parle (2012) basil leaf extract contain of methyl eugenol, methyl clavical and linalool. Methyl eugenol has potential as bio-attractant, methyl clavical has an anesthetic effect, which is works by interfering with the work of nervous system. Silalahi (2018) stated linalool is the main constituent of basil leaf extract (52.42%) and has a fragrant aroma and smells good and suspected to be a natural attractant and can attract termites to approach the bait. In addition, according to Sarma *et al* (2011) basil leaf extract has a compound og 1.8 cineol (5.61%) and this compound has a distinctive aroma in plants and can stimulate sensory or olfactory devices, which termites can be attracted to approach the bait and consume the bait.

Basil leaf extract and HVS waste paper are materials favored by termites, and termites are attracted to eat the bait. Simbolon *et al*. (2015) stated that basil leaf extract with a concentration of 10% was the optimum concentration as a bio-attractant against subterranean termites Coptotermes curvignathus. Utilization of HVS waste paper as a basic materials for bait become and additional attractant so that termites are increasingly attracted to consume the bait. This is in line with the research of Muin *et al*. (2015) which stated that HVS waste paper, cardboard waste and news paper waste can be formulated into bait and attractant to termites. In sample testing, the use of HVS waste paper as bait was thought to be good enough to meet the termite feeding needs because all treatments showed a loss of bait weight.

Termite baiting system use small amounts of active ingredients and the main goals was colony elimination. Evans and Iqbal (2015) studied and evaluated about termites baiting used in commercial treatment. They study 15 active ingredients, 23 termite species and 16 countries with mostly the active ingredients was chitin synthesis inhibitor. The result of research showed that baiting system was success to eliminate the colony of termites from genus Coptotermes and Reticulitermes with bait material from paper and 0.5 gram active ingredients. They stated the need of future research to use other active ingredients for termite baiting system. Basil leaf extract could be one promising as active ingredients on termite baiting system.

The baiting system is considered environmentally friendly on termite control method compare to other methods such as spraying with chemical solution or fumigation system. The main goal of termite control is to prevent or remove termites infestations in the area such as plantation or structures. In line with the goal of a termites baiting system, the baiting treatment is to eliminate termites colony and to suppress the populations of termites.

Bait matrix is an important factor of successful on termite baiting system because the bait will be in competition with alternative feeding sites (Iqbal *et al*. 2021). Therefore the bio-attractant compound in termites bait will increase the preference of termites in consume the bait. Result of research showed that the active ingredients of basil leaf extract has a function of bio-attractant compound and acceptance by subterranean termite Coptotermes curvignathus.

Some factor on successful of the termite baiting system is dependent on the knowledge of termite characteristic and behavior. Yii *et al*. (2016) researched the feeding preference test and found that bait size and nutrients supplemented in bait play a role in the food selection of termites. Iqbal *et al*. (2017) stated the size of the bait and the bait station was an important component on termites baiting system. They studied found that termites Macrotermes gilvus and Macrotermes carbonarius was prefer the large bait station. Evans and Iqbal (2015) stated the commercial termite bait was consist of two categories of active ingredients, namely insect growth regulator, and metabolic inhibitor. The metabolic inhibitors such as hydramethalnon, sulfluramid, borates and mirex. The insect growth regulators with chitin synthesis inhibitors such as noviflumuron, hexaflumuron and triflumuron. Extract of plant was a promising active ingredients due to environment consider of the termite control method. The results showed that the treatment with bait from HVS waste paper and concentration of basil leaf extract 8% showed a mortality of termites of 91.52% and the weight loss was 8.36%. Based on these results, it can be stated that termite bait from HVS waste paper and basil leaf extract with concentration 8% was optimal as bio-attractant against subterranean termites. Therefore it can be stated that the mixture of basil leaf extract with HVS waste paper has potential as an attractant to termies to feeding the baiting.

Conclusions

Basil (*Ocimum basilicum*) leaf extract has a function as a bio-attractant to subterranean termites Coptotermes curvignathus. Termite bait using HVS waste paper and basil leaf extract was non-repellent to termites and a promising used for termites bait in control the termites. The optimum concentration of basil leaf extract was 8% with a classification a very strong termite's activity, the average termites mortality value was 91.52% and the weight loss of bait was 8.36%.

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Phenolics Content and DPPH Free Radical Scavenging Activity of *Dalbergia latifolia* Leaf Ethanolic Extract

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Abstract

Dalbergia latifolia or Sonokeling is one of the most well-known tree species that naturally grows in Java Island, Indonesia. It produces purple streaked wood used in luxurious furniture while its leaf can be utilized as forage. The purpose of this study is to determine the bioactivity and phenol contents of *D. latifolia* leaf ethanolic extract. The antioxidant activity and anti-termite activity were measured through the DPPH method and *Neotermes bosei* activity. Furthermore, phenol contents were determined through colorimetric method and ethanolic extract constituents were identified by GC-MS. The DPPH inhibition of ethanol extract of *D. latifolia* leaf exhibited 138.20±2.14 µg/ml. While the measurement of total phenol and total flavonoid content of *D. latifolia* leaf showed a value of 192.67±9.41 mg GAE/g and 55.23±5.11 mg QE/g of dried extract sample. The termiticidal activity of ethanolic extract showed low activity. The GC-MS detection showed fatty acids as dominant compounds. The inhibition of DPPH by *D. latifolia* leaf ethanol extract in this study suggested this leaf is potent as antioxidant agents.

Keywords: Sonokeling, leaf, antifeedant, termiticidal, flavonoid, *Neotermes bosei*.

Introduction

Antioxidant is an important substance that is usually used in the food and beverages industry, animal feed industry as well as the pharmacological industry and serves to control oxidation (free radical scavengers), retard spoilage, and putative health benefits (Finley *et al*. 2011). In food and beverages and animal feed industry, an antioxidant can maintain the food quality/nutrient stability and lengthen the shelf life, meanwhile in pharmacological application, an antioxidant can prevent drug degradation that produces a lower efficacy drugs until a toxic compound generation in drugs so it might lead to some risks for the patient health (Housheh 2017; Gabrič *et al*. 2022). Due to its importance, the global demand for antioxidants is always growing and predicted to grow. For feed antioxidants only, the global consumption has grown from 120.7 million metric ton in 2017 to 131.2 million metric ton in 2020 according to FAO statistics (Anonymous 2022).

Nowadays, synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), 2,6-di-*tert*-butyl-*p*-benzoquinone (BHT-Q), propyl gallate (PG) and tert-butylhydroquinone (TBHQ) are widely used in food industry (Gülçin 2012). It is inexpensive, has higher antioxidant activity (medium to high) than the natural antioxidant, but can cause some harms (such as DNA damage, cancer induction, tumour promotion, etc) if incorrectly use or being used excessively (Gülçin 2012; Xu *et al*. 2021). Even a low concentration of BHT-Q can cause DNA damage (Liu and Mabury 2020). Recent studies focus on the low toxicity, low migration ability and more environmentally friendly synthetic antioxidants or basically pointing to the natural antioxidant (Liu and Mabury 2020). Therefore, massive exploration in finding the natural antioxidants resources that have high antioxidant activity was conducted.

Natural antioxidants such as polyphenols/phenolic compound (flavonoids, tannin, phenolic acids), terpenoids (carotenoids), ascorbic acid (vitamin C), tocopherol (vitamin E), and unsaturated fatty acids (omega 3) were known to have high antioxidant activity (Ali *et al*. 2012; Barbieri *et al*. 2015; Lv *et al*. 2015; Anwar *et al*. 2018; Wang *et al*. 2019). The polyphenol, a well-known hydrophilic antioxidant, together with their hydroxyl groups will act as inhibitors on the 2,2-diphenyl-1-picrylhydrazyl (DPPH). Moreover, it is easily found in plant tissues and its total compound (the total phenolic compound) is known to be highly correlated with antioxidant activity (Kalaycıoğlu and Erim 2016). For flavonoid (Figure 1a) form, especially its B ring, the donated protons (H) interact with the DPPH and convert it into DPPH-H (Pannala *et al*. 2001; Kongpichitchoke *et al*. 2015) in performing the antioxidant activity. Flavonoids are also found as a high anti termite substances against Coptotermes with its C-5 and C-7 hydroxyl groups in A-rings, carbonyl groups at C-4 in pyran rings, and 3-hydroxyflavones and 3-hydroxyflavanones with 3',4'- dihydroxylated B-rings (Ohmura *et al*. 2000). Those phenolic compounds were highly extracted by ethanol, methanol and their mixture (Alara *et al*. 2021). Meanwhile, terpenoid (especially their carotenoid form) and unsaturated fatty acids (see Figure 1b and 1c for chemical structure of monoterpene and fatty acid) that classified as lipophilic antioxidant are depending on their singlet oxygen quenching, hydrogen transfer, or electron transfer and also their long-chain polyunsaturated fatty acids for their antioxidant activity performance (Graßmann 2005; Richard *et al*. 2018). Terpenoid and unsaturated fatty acids were highly extracted by n-hexane and dichloromethane solvents. All those natural antioxidants with high potential development were found in some trees and non tree plants such as *Diospyros abyssinica*, *Acacia auriculiformis*, *Ficus microcarpa*, *Polyalthia cerasoides*, *Uncaria tomentosa* etc in Africa, Algeria, USA, Australia, Brazil, Bulgaria, China, India, Iran, Italy, Japan, Turkey, Poland, Portugal, Thailand, and Malaysia (Krishnaiah *et al*. 2011).

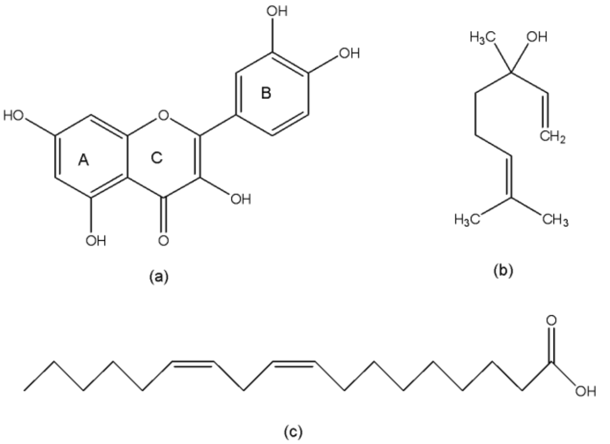

Figure 1. Chemical structure of quercetin (a), linalool (b), and linoleic acid (c).

Each country or each area has its own resource potency of the natural antioxidant that is influenced by the geography, climate, local preference, local wisdom, government policy etc. One of trees that is naturally grown and widely cultivated in Indonesia is *Dalbergia latifolia* Roxb, commonly known as Sonokeling. It is classified as the Fabaceae family and widespread in Indonesia and India natively and in Kenya, Malaysia, Myanmar, Nepal, Nigeria, Philippines, Sri Lanka and Vietnam exoticly (Sukhadiya *et al*. 2020). The wood from this species is utilized as furniture materials and the wood is categorized as fancy wood, meanwhile the leaves are used as forage and in Yogyakarta Indonesia, this species is easily found in community forest in Bantul and Gunungkidul Regency (Santoso *et al*. 2021; Jariyah and Wahyuningrum 2008). This tree might have a high potency of the natural antioxidant resources as other trees in the same genus show the potency. Several *Dalbergia* genus leaf had been studied as natural antioxidant resources and showed a potential results such as *D. katangensis* (Valentin *et al*. 2020), *D. saxatilis* (Koma and Fakunle 2014), *D. odorifera* (Ma *et al*., 2020), *D. sissoo* (Rijhwani and Bharty 2016), *D. paniculata* (Ganga *et al*. 2012), *D. ecastaphyllum* (Lucas *et al*. 2020), and *D. brasiliensis* (Dalarmi *et al*. 2017) in various extractions.

The natural antioxidant of *Dalbergia latifolia* Roxb from its wood and its bark has already been studied in our previous study and shows potential results (Khalid *et al*. 2011; Masendra *et al*. 2020). But the utilization of wood as the natural antioxidant resources is constrained by other wood uses (i.e. furniture and home furnishings). On the other hand, some studies showed that the antioxidant level of leaf is higher than bark (Katekhaye and Kale 2012; Krishna and Nair A 2010). Therefore, the leaf of *Dalbergia latifolia* might be a potential antioxidant resource as the other Dalbergia genus leaf also shows the potency. However, the information of *D. latifolia* leaf extract and its bioactivity is still limited. Due to the natural antioxidant exploration and the demand of its fulfilment, the information of antioxidant activity and phenol contents of *D. latifolia* leaf extract was investigated in this study. The anti-termite activity of the *D. latifolia* leaf extract was also investigated as the leaf might have high phenolic compounds. The ethanolic extracts were used based on the ethanol ability in dissolving the phenolic compounds as well as its renewable and non-poisonous solvent that have a low risk for acute toxicity in pharmaceutical manufacturing processes (Araújo *et al*. 2019).

Materials and Methods

Chemicals and Reagents

Gallic acid, DPPH, and quercetin were purchased from Sigma-Aldrich (Germany), while tectoquinone (2- methyl anthraquinone, 25753-31) was purchased from Kanto Chemical, Japan.

Leaf Maceration

Fresh leaf of *Dalbergia latifolia* (30 g) from a ten-year-old tree (collected in Bantul, Yogyakarta, Indonesia) was macerated with ethanol at room temperature for three days. The solution was then filtered and evaporated through a rotary evaporator. The dried extract was weighed and compared to the fresh initial sample (in percentage). In addition, the leaf maceration was conducted in one replication.

Total Phenolic Content

Total phenolic content was measured with the Folin-Ciocalteu method, referring to Diouf *et al*. (2009). As much as

0.5 ml of *D. latifolia* ethanolic extract (1000 ppm) was reacted with 2.5 ml of Folin-Ciocalteu reagent. The reaction was then left to react for 2 min at room temperature and then 2 ml of sodium carbonate was mixed (7.5% aqueous). Then, the solution was left to react at room temperature for 30 min. 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 Flavonoid Content

The total flavonoid assay was conducted according to Brighente *et al*. (2007). A 2 ml of sample extract (1mg/ml) was mixed with 2% of $AlCl_3.6H_2O$ solution in methanol. The reaction was allowed for 60 min at 20 °C before measuring the absorbance. The absorbance of the mixture was then measured at 415 nm using a spectrophotometer. The results were expressed as quercetin equivalents (mg QE/g) of the sample extract.

Antioxidant Activity

The antioxidant measurement was conducted through literature (Baba and Malik 2015). The DPPH inhibition was measured through the response of 0.1 ml sample with 0.1 mM DPPH. To assess antioxidant activity with IC_{50} value, the sample was measured in different concentrations, and each sample was measured in three replications. In addition, the standard of quercetin is used for comparison as a positive control. The antioxidant activity is calculated through below equation (1):

Antioxidant activity (%) = (Ao-A1)/Ao
(1)

Where Ao is the absorbance of blank and A1 is the sample absorbance.

Anti-termite Activity

In this experiment, a glass with diameter of 7 cm and height of 10 cm, containing sand was used as a place for the termite test. Paper discs with a diameter of 8 mm (Whatman International) containing 5 % (w/w) sample extract (dissolved in methanol) were prepared. The paper discs that contain ethanolic extract of *D. latifolia leaf*, tectoquinone (positive control), and methanol (negative control) were made in three replications. The paper disc was dried in the oven at 60 °C to evaporate the methanol solvent. Twenty worker termites (*Neotermes bosei*) were introduced into the glass with the condition of the termite environment: relative humidity of 70-80% at room temperature. After 21 days the paper discs were taken out, dried and the mass loss was observed. To measure the termiticidal activity, the number of dead termites was also calculated. For negative controls, the starvation sample also was made without feeding.

Gas Chromatography-Mass Spectra (GC-MS)

The 1 µl of ethanolic extract solution with 1000 µg/ml concentration was directly injected into the GC-MS (GCMS-QP 2010, Shimadzu, Japan). The GC column was Rtx-5MS capillary column (30 m x 0.25 mm I.D. and 0.25 µm; GL Sciences, Tokyo, Japan). The detection temperature was 285 °C, the column temperature from 70 °C (2 min) to 290 °C at 5 °C/min, and the injection temperature of 200 °C. The acquisition mass was from 50 to 500 atomic mass units and helium was the gas carrier. The mass spectra from the NIST library were used for comparison with the mass spectra of the sample and the quantification of the compound was calculated with the peak relative method.

Results and Discussions

Yield Extractive and Polyphenols

The extractive content of *D. latifolia* leaf was 2.792 g or 9.27% based on the fresh leaf. The higher extractive content of *D. latifolia* leaf shows that alcohol is one of the good solvents to dissolve the leaf extract. The average total phenols and total flavonoids of *D. latifolia* leaf ethanolic extract are 192.67 and 55.23, respectively (Table 1). This total phenol is 12 times higher than *D. odorifera* leaf ethanolic extract (16± 0.15 mg GAE/g) (Ma *et al*. 2020). Compared to the bark ethanolic extract (210±1.56 mg GAE/g), the total phenol of the leaf from this study is slightly lower. But the total flavonoid of the leaf is slightly higher than the bark (46±3.61 mg GAE/g) (Khalid *et al*. 2011). This suggests that the utilization of *D. latifolia* for pharmacological material might preferably come from leaf due to its faster growth than bark, especially when the Indonesian climate supports the growth all year around.

Table 1. Total phenol, total flavonoid, and antioxidant activity of *D. latifolia* leaf ethanolic extract

Parameter	Value
Total phenol (mg GAE/g)	192.67± 9.41
Total flavonoid (mg QE/g)	55.23± 5.11
DPPH scavenging activity IC50 (µg/ml)	138.20 ± 2

Antioxidant and Anti-termite Activity

The antioxidant activity (IC_{50}) of the *D. latifolia* leaf extract against DPPH radical is shown in Table 1. Compared to the positive control (IC_{50} of quercetin: 47 µg/ml), the *D. latifolia* leaf extract also showed 3 times lower inhibition of DPPH. According to Molyneux (2004), the antioxidant activity of *D. latifolia* leaf ethanolic extract is categorized as moderate activity. In comparison, the resulting activity of antioxidants is stronger than the bark of *D. latifolia* (170 µg/ml) (Khalid *et al*. 2011).

The termiticidal activity of *D. latifolia* leaf ethanolic extract is shown in Figure 2-3. On the no choice antifeedant assay, the paper disc with ethanolic extract was 100% eaten by *N. bosei* which has the same value as the negative control.

The mortality number (Figure 2) of *D. latifolia* leaf ethanolic extract also was similar to that of positive control and lower than that of tectoquinone. The similarity of the mortality rate with the positive control indicates that the toxicity of the samples came from the MeOH solvent instead of the ethanolic extract. These results showed that *D. latifolia* leaf ethanolic extract did not exhibit any antifeedant nor toxicity to *N. bosei* termite. Compared to a similar study, the result differed from *Cibotium barometz* ethanolic leaf extract which was able to exhibit both antioxidant and anti-termite activity (Musman *et al*. 2019). Increasing extract concentration on the paper discs might increase its effectiveness on the termite.

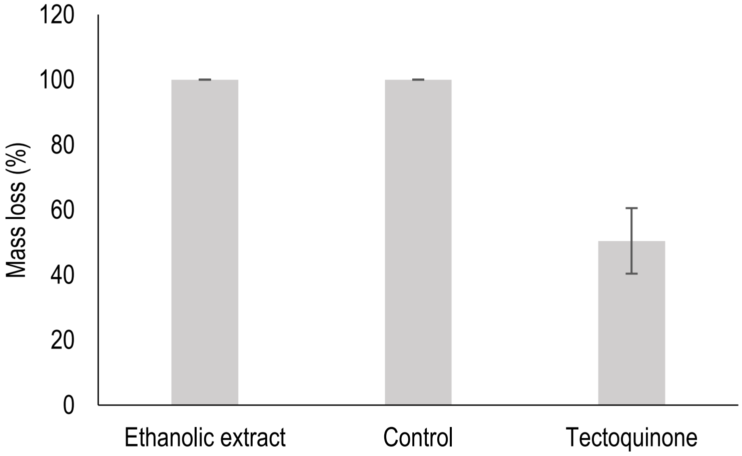

Figure 2. The mass loss of paper disc attacked by *N. bosei.*

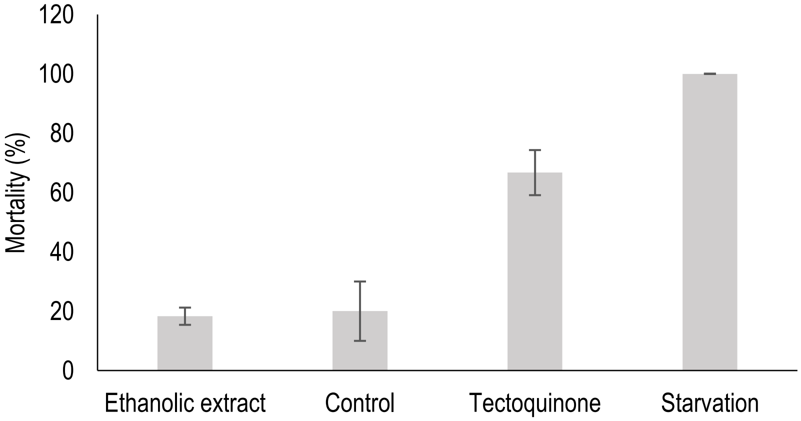

Figure 3. The mortality rate of *N. bosei* against ethanolic extract of *D. latifolia* leaf.

Fatty Acids and Alcohols

Table 2 shows the composition of ethanolic extract of *D. latifolia* leaf. The GC-MS detection by direct injection method exhibited fatty acids and alcohols in the sample. The dominant compound of fatty acid was found as 17-octadecynoic acid, palmitic acid, and linoleic acid, respectively. In the fatty alcohols, the major compound was 14-methyl-8-hexadecyn-1-ol. Other fatty alcohols in the *D. latifolia* leaf extract were phytol and pentadecadien-1-ol. Compared to previous work, these main fatty acids were different from the main types of fatty acid of *D. odorifera* leaves (palmitic acid, linoleic acid, oleic acid) (Ma *et al*. 2020) and *D. ecastaphyllum* leaves (palmitic acid and linolenic acid) (Lucas *et al.* 2020). 17-octadecynoic acid was also detected in other Magnoliopsida plants, i.e. *Momordica cymbalaria* Fenzl and this fatty acid has an anti-hypertensive effects (Gopu *et al*. 2021).

Table 2. GC-MS result of *D. latifollia* leaf ethanolic extract

No	Retention time (min)	Constituents	Percentage	Similarity index (%)
1	27.4	Methyl isohexadecanoate	3.85	95
2	28.4	Palmitic acid	19.17	94
3	30.2	9,12- Octadecenoic acid	3.51	96
4	30.2	Methyl 10-octadecenoate	2.03	96
5	30.5	Phytol	0.53	93
6	30.6	Methyl stearate	1.26	94
7	31.1	17-Octadecynoic acid	20.35	91
8	31.5	Stearic acid	4.63	92
9	31.6	Methyl 9,12-octadecadienoate	0.57	86
10	33.3	Dipalmitin	11.06	85
11	33.4	Arachidic acid	1.52	74
12	33.6	Methyl 18-methylnonadecanoate	0.91	92
13	35.8	Linoleic acid	14.99	89
14	35.9	Pentadecadien-1-ol	1.65	85
15	36.1	Dipalmitin (isomer1)	2.57	84
16	38.7	Dipalmitin (isomer 2)	1.15	84
17	39.6	Diethyl *n*-hexadecylmalonate	0.53	73
18	41.9	Isopropyl linoleate	0.64	84
19	44.8	14-Methyl-8-hexadecyn-1-ol	8.21	89
20	47.1	Palmitone	0.86	90

Attribution of Phenol Contents, Fatty Acids and Alcohols to Antioxidant and Termiticidal Activity

In this study, the free radical scavenging of ethanolic extract of *D. latifolia* leaf was categorized as moderate activity. However, the termiticidal activity was categorized as low activity. It indicates that the phytochemical screening results of *D. latifolia* leaf e.g., phenol contents, fatty acids and fatty alcohols are responsible for DPPH inhibitor rather than for anti-termite activity of *N. bosei*. In previous works, the correlation of phenol contents (total phenol and total flavonoid) and DPPH scavenging activity was observed in leafy vegetables and fruit crops (Chandra *et al*. 2014), *Lantana camara* leaf (Kumar *et al*. 2014), *Ceropegia* species (Chavan *et al*. 2013), and indigenous herbs in Indonesia (Muflihah *et al*. 2021).

In correspondence of fatty acids and alcohols, the compounds that corroborate inhibition of the DPPH is from unsaturated fatty acids and alcohols. In Table 2, the unsaturated fatty acids and alcohols were 9,12- octadecenoic acid, methyl 9,12-octadecadienoate, linoleic acid, pentadecadien-1-ol, and isopropyl linoleate. These unsaturated fatty acids and alcohols, especially the dominant compound of linoleic acid potent to act as antioxidants. This agrees with previous works that linoleic acid and conjugated linoleic acid exhibited as DPPH inhibitor (Ali *et al*. 2012; Lv *et al*. 2015). However, as the phenolic compounds were the most responsible for DPPH inhibitor, the investigation of these compounds through derivative method (GC-MS) is needed in the future works.

Conclusions

The antioxidant and termiticidal activity of *D. latifolia* leaf ethanolic extract were investigated together with their total phenol, total flavonoid, and GC-MS analysis. Observing phenol contents, the *D. latifolia* leaf contained total phenol of 192.67± 9.41 mg GAE/g and total flavonoid of 55.23± 5.11 mg QE/g. Bioactivity of *D. latifolia* leaf ethanolic extract exhibited IC_{50} antioxidant activity of 138.20± 2.14 µg/ml. The GC-MS detection on the ethanolic extract described fatty acids as dominant compounds. The ability of *D. latifolia* leaf extract to inhibit DPPH suggested that this leaf extract is a potent antioxidant. However, due to the activity of *D. latifolia* leaf ethanolic extract to *N. bosei* was in low value, the presence of phenol contents as well as fatty acids and alcohols in the *D. latifolia* leaf did not act for anti-termite activity, but acted as DPPH inhibitor. This shows that local wisdom regarding the use of *D. latifolia* leaves as animal feed has proven to be nutritious and good for livestock health.

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Total Phenolic, Flavonoid, Tannin Content and DPPH Scavenging Activity of *Caesalpinia sappan* Linn. Bark

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Abstract

Caesalpinia sappan is a shrubby Fabaceae tree commonly found in Indonesia, traditionally utilized as natural dye and herbal drink. However, in the making of traditional herbal drink, the bark is often discarded as residues. This research aimed to investigate total phenolic (TPC), flavonoid (TFC), and tannin (TTC) content as well as the antioxidant activity (DPPH scavenging activity) of the bark successive extracted with *n*-hexane, ethyl acetate, methanol, and hot water as well as analyzed it with GC-MS. The result showed the highest amount of TPC (824.16±62.28 mg GAE/g), TFC (185.03±1.91 mg QE/g), and TTC (987.07±30.98 mg TAE/g) in the methanol extract of the bark. GC-MS analysis resulted hydroquinone as a major constituent in the methanol extract. Further, antioxidant activity was found the strongest on methanol extract (IC_{50}=63.48), while correlation between antioxidant activity and TFC was found the highest (R^2=0.93). These findings suggest that bark of *C. sappan* is a suitable source of natural antioxidant with strong activity to DPPH radical.

Keywords: *Biancaea sappan,* bark extract, antioxidant activity, colorimetric, GC-MS.

Introduction

Caesalpinia sappan Linn. is a small-medium sized shrubby tree member of Fabaceae found in South East Asian countries including Indonesia, Vietnam, Myanmar, Philippine, and Malaysia. It is a fast-growing species which able to attain 3.6 m height in its 1st year with proper exposure to sunlight (Mathew *et al.* 2007). Due to its thorny bark, *C. sappan* is often utilized as hedgerow plant to protect farms against vermin such as wild boar (Najiyati *et al.* 2005). Its compact wood can also be utilized for carpentry and musical instrument material (Mathew *et al.* 2007). Moreover, *C. sappan* wood is utilized as source of red dye for textiles and also made into traditional herbal drink by mixing it with other spices, locally called *wedang uwuh* in Yogyakarta, Indonesia (Winarsi *et al.* 2018).

C. sappan is traditionally utilized as herbal drink ingredient in regard to its health benefits. Several bioactivities such as antibacterial, anti-inflammatory, wound healing, and antioxidant has been reported from *C. sappan* wood extract (Zhao *et al.* 2008; Nirmal and Panichayupakaranant 2015; Sucita *et al.* 2019). These bioactivities might be also attributed by several phenolics in the extract which include flavonoids, chalcones, xanthone, and tannin (Sucita *et al.* 2019; Chen *et al.* 2008). Phenolics ability as antioxidant is well reported. Their antioxidant activity is attributed to their ability to donor hydrogen atoms to reactive oxygen and nitrogen species radicals while still being stable due to their structure (Pereira *et al.* 2009). The accumulation of radicals in human body can cause oxidative stress, which is suspected to lead into various diseases including cancer (Adwas *et al.* 2019).

Bark is the outermost part of the tree and acts as a protective layer against environmental and pathogenic threats. One of their defense mechanisms against pathogens and pest are attributed to their accumulation of secondary metabolites (Pásztory *et al.* 2016). Due to this reason, extractives in bark were found in larger quantities compared to wood in general (Sjöström 1993). However, in wood processing, bark is often discarded as residues. In fact, secondary metabolites from bark might be utilized and beneficial for human health. Moreover, extract from the bark of *C. sappan* received less scientific attention compared from its wood and in its traditional utilization for herbal drink in Indonesia, its wood and small part of inner bark often mixed together while the outer bark discarded as a waste. Previous research has reported the antioxidant activity of *C. sappan* wood extract (Setiawan *et al.* 2018). The objectives of this research were to investigate total phenolic, flavonoid, and tannin content, antioxidant activity, as well as compounds identification through GC-MS analysis in *C. sappan* bark.

Materials and Methods

Bark Material and Extraction

The bark sample of *C. sappan* was collected from Forest Research and Education of Wanagama I, Gunung Kidul District, Yogyakarta, Indonesia. Collection was done trees with 9 cm stem diameter. Successive extraction was done to the milled bark sample (100 g). Extraction were done using reflux apparatus with *n*-hexane (6 h), methanol (MeOH) (6 h), ethyl acetate (EtOAc) (6 h), and hot-water (3 h). Each extract was dried with rotary evaporator and stored in a flask in room temperature. The extract yield from each solvents were calculated as percentage of oven-dried weight.

Total Phenolic Content

Briefly, 0.5 ml of extract diluted in dimethyl sulfoxide (DMSO) was added to 2.5 ml of Folin-Ciolcateu reagent (10% v/v) (Merck, Germany) and incubated for 2 mins. As much as 2 ml of 7.5% (w/v) Na_2CO_3 solution was then added to the mixture and sample was incubated for 30 mins under room temperature. Absorbance was measured with UV-Vis spectrophotometer in 765 nm wavelength. Gallic acid in various concentrations were also subjected to the same procedure and calibration curve was made ($y=0.1333x + 0.0042$; $R^2 = 0.99$). The results are expressed as mg gallic acid equivalent (GAE)/g of the sample (Baba and Malik 2015).

Total Flavonoid Content Assay

Extracts were diluted with DMSO and 2 ml of the solutions were added to 2 ml of 2% aluminum chloride ($AlCl_3.H_2O$). The mixture was then shaken and incubated in 22°C temperature for 30 mins. The absorbance of each mixture was then measured using UV-Vis spectrophotometer in 415 nm wavelength. A calibration curve using quercetin was also prepared with the same procedure ($y=0.0388x - 0.0001$; $R^2 = 0.99$) and the results were expressed as mg quercetin equivalent (QE)/g of the sample (Diouf *et al.* 2009).

Total Tannin Content Assay

Preparation; 0.1 ml of extract sample (1000 ppm) was diluted with distilled water (7.5 ml). To the solution, Folin-Denis (0.5 ml) and 1 ml of sodium carbonate (35%) was reacted. The solution was added with distilled water until 10 ml volume. The final reaction was stood at ambient temperature for 30 min and the sample absorbance was read at 760 nm. To calculate total tannin content, the standard of tannic acid was used for calibration ($y=0.694x-0.0079$; $R^2=0.99$), therefore the unit of total tannin content was mg tannic acid equivalent (TAE)/ g dried extract sample (Padmaja 1989).

DPPH Radical Scavenging Activity Assay

DPPH radical scavenging activity was measured by mixing 0.1 ml of diluted extract in four concentration (25, 50, 100, and 200 µg/ml) with 3 ml of 0.1 mM diphenyl-2-picrylhydrazyl (DPPH) (Sigma-Aldrich, USA). The mixture was shaken and kept for 30 mins under 22°C temperature in dark. Blank also prepared with the addition of solvent only. Absorbance was measured at 512 nm wavelength with UV-Vis spectrophotometer and radical scavenging activity was calculated with the following formula:

$$\text{Radical scavenging activity (\%)} = 100 \times (A_0 - A_1)/A_0 \quad (1)$$

Where A_1 is sample absorbance and A_0 is blank absorbance. Antioxidant activity was then expressed as IC_{50} or the concentration needed to inhibit DPPH by 50% in µg/ml.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The MeOH extract of *C. sappan* bark was silylated according to literature (Wijayanto *et al.* 2015). Firstly, 1 mg of sample was dissolved into TMCS (15 µl) and BSA (85 µl). Secondly, the reaction was incubated for 1 hour at room temperature. Then, the sample was diluted with 1 ml of MeOH. For analysis, 1 µl of silylated sample was injected to GC-MS machine. The GC condition: Rtx-5MS capillary column (30 m x 0.25 mm I.D. and 0.25 µm; GL Sciences, Tokyo, Japan); column temperature from 70 °C (1 min) to 290 °C at 5 °C/min; injection temperature of 270 °C; detection temperature of 290 °C; acquisition mass range from of 50-800 amu using helium as the carries gas. GC-mass spectrometry (GC-MS) data were collected with a GCMS-QP 2010 (Shimadzu, Japan). The mass spectrum of sample was compared to NIST library. In this study, peak relative method was applied for calculation of *C. sappan* bark constituents GC-MS analysis.

Statistical Analysis

One-way ANOVA was done using SPSS (IBM, USA) with 95% confidence level. Further, data with significant result was tested with Tukey HSD with 5% significance level. Correlation of colorimetric assay results were correlated with DPPH scavenging activity (100 µg/ml) using linear regression.

Results and Discussion

Colorimetric Assays

The extract yield of each sample were 0.51%, 1.82%, 6.92%, and 7.65% for *n*-hexane, EtOAc, MeOH, and Hot-water soluble extract, respectively. Results of colorimetric assays to measure TPC, TFC, and TTC are showed in Figure 1. In all assays, MeOH extract showed the highest concentration while *n*-hexane showed the lowest. MeOH extract showed the highest value of TPC (824.16±62.28 mg GAE/g), TFC (185.03±1.91 mg QE/g), and TTC (987.07±30.98 mg TAE/g). Further, one-way ANOVA showed significance difference between solvents in all assays ($p<0.01$). Tukey HSD test showed significance difference between all solvents in all assays, where MeOH extract showed significantly the highest amount of TPC, TFC, and TTC.

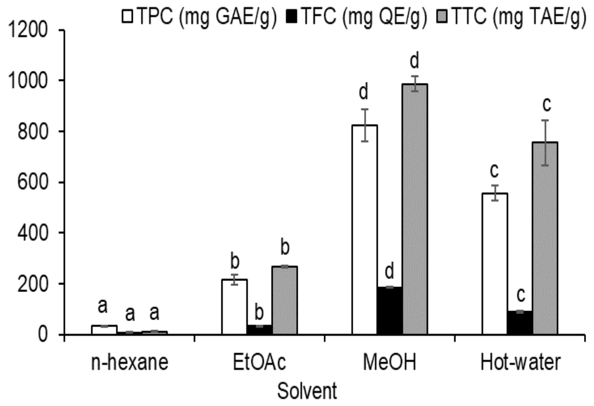

Figure 1. Total phenolic (TPC), flavonoid (TFC), tannin content (TTC) of *C. sappan* extracted with solvents with increasing polarity. Different letters on the histogram (a, b, c, etc.) indicate significant difference with Tukey HSD (5% significance level) between solvents in each individual assay.

Solvent used had significant effect on the results of TPC, TFC, and TTC. Results of TPC indicate that *C. sappan* bark were significantly dominated by phenolic compound in its polar extracts especially in the MeOH soluble extractive, while only a very small amount extracted by *n*-hexane that indicated by some brown colour in the extract. Compared to previous TPC assay on bark of different species, *C. sappan* bark extract can be considered very high in phenolics (Phuyal *et al.* 2020; Wijewardhana *et al.* 2019). Moreover, TPC of *C. sappan* heartwood has been reported in previous research (Febriyenti *et al.* 2018), whereas the amount is slightly lower than the bark from this research. Flavonoid and tannin content were also estimated to be quite high in *C. sappan* bark polar extract by the colorimetric assays. One of flavonoid type compound has been reported in *C. sappan* which is called brazilein, also suspected to give its well-known red colour in the extract (Dapson and Bain 2015). Further, the high amount of TTC might suggest that there are lots of polymeric phenols or flavonoids contained in the extract. Phenolics including flavonoid and tannin is major group of secondary metabolites found in plants and generally bioactive (Miguel-Chávez 2017). The higher amount of TPC in bark indicates its function in bark as a protective measure against pest and disease to its inner tissue. Further, some bioactivities are expected in the polar extract of *C. sappan* bark.

GC-MS Analysis

The detection of MeOH extract of *C. sappan* bark found aromatic compounds as dominant constituents. The compounds can be grouped into phenolic and sugar compounds. The hydroquinone and 2,3-anhydro-d-mannosan were the higher constituents from the phenolic and sugar compounds. The other aromatic compounds were benzoic acid, 1-chloro-2,5-dinitrobenzene, p-diazquinone, 3-methyl-2-nitrophenol, and 2,4-dimethoxyphenol (Table 1 and Figure 2). In comparison, phenolic and sugar compounds such as hydroquinone derivatives were also detected in *Cinamomiun verum* bark (Kankeaw and Masong 2015), benzoic acid in *Terminalia arjuna* bark (Dutta *et al.* 2015), 2,6-dimethoxyphenol and 2,3-anhydro-d-mannosan in *Aesculus chinensis* bark (Li *et al.* 2018).

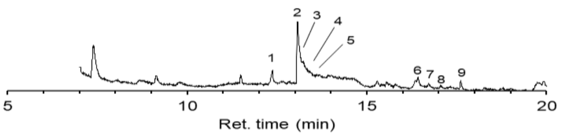

Figure 2. Chromatogram of GC-MS of MeOH extract of *C. sappan* bark; 1. Benzoic acid, 2. hydroquinone, 3. 2,3-anhydro-d-mannosan, 4. 1-chloro-2,5-dinitrobenzene, 5. p-diazoquinone, 6. 3-methyl-2-nitrophenol, 7. 2,6-dimethoxyphenol, 8. 3,4-O-isopropylidene-d-galactose, 9. 2-pentenyl acetate

Table 1. Composition of *C. sappan* bark detected by GC-MS

No.	Ret. time (min)	Constituents	Concentration (% of dried extract)	Similarity index (%)
1	12.4	Benzoic acid	5.4	87
2	13.1	Hydroquinone	51.4	87
3	13.2	2,3-Anhydro-d-mannosan	14.9	60
4	13.3	1-Chloro-2,5-dinitrobenzene	7.1	60
5	13.4	p-Diazoquinone	5.6	60
6	15.4	3-Methyl-2-nitrophenol	2.3	60
7	16.3	2,6-Dimethoxyphenol	2.4	60
8	16.4	3,4-O-Isopropylidene-d-galactose	3.7	65
9	17.6	2-Pentenyl acetate	2.9	80

In this study, some known polyphenolic compounds such as brazilin, brazilein (Dapson and Bain 2015), and sappanol (Uddin *et al.* 2015) were not detected in the GC-MS analysis. Furthermore, the highest detection of hydroquinone in this study indicates hydroquinone as a precursor of polyphenolic in *C. sappan* bark. The high concentration of hydroquinone also suggests that *C. sappan* bark can be utilized as hydroquinone source as well as in pharmaceutical industry.

Antioxidant Activity

Concentration to inhibit DPPH radical by 50% (IC_{50}) value of each extract are shown in Figure 3. Lower IC_{50} value indicates stronger antioxidant activity. The result showed that MeOH extract showed the strongest antioxidant activity, where *n*-hexane extract showed the weakest. Further, gallic acid was used as positive control, where its IC_{50} was slightly lower than MeOH extract.

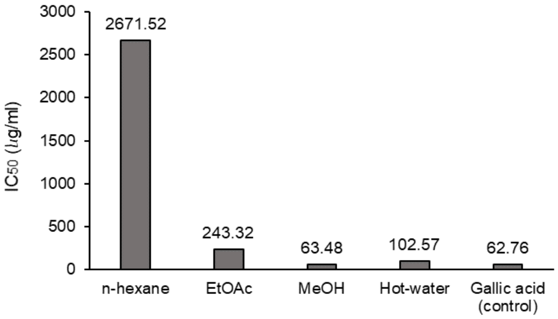

Figure 3. IC_{50} to DPPH radical of each extract.

DPPH is a stable free radical model with dark violet colour that lose its chromophore and turns yellow upon receiving hydrogen atom from antioxidant compounds (Sanchez-Moreno 1999). The result of DPPH scavenging assay suggests that the polar extracts of *C. sappan* bark, especially the MeOH extract, were effectively neutralize DPPH radical with low concentration. This high activity might be attributed by its high concentration of phenolics, flavonoid, and tannin measured in the colorimetric assay. MeOH extract effectiveness to neutralize DPPH radical was comparable to the positive control of gallic acid. By comparing the DPPH IC_{50} of the control, crude MeOH extract of *C. sappan* bark had higher DPPH radical scavenging activity compared to *Albizia adianthifolia,* bark and two well-known antioxidants butyl hydroxytoluene (BHT) and ascorbic acid (Vitamin C) (Brighente *et al.* 2007; Tamokou *et al.* 2012). Further, the detection of aromatic compounds such as hydroquinone and its other derivatives may exhibit antioxidant activity in *C. sappan* bark. Previously, hydroquinone derivative compounds were showed activity against DPPH radical (Kankeaw and Masong 2015). This result indicates that the MeOH extract of *C. sappan* bark is a suitable source of natural antioxidant with strong activity.

Correlation of Total Phenolic, Flavonoid, and Tannin Content to Antioxidant Activity

Correlation between TPC, TFC, and TTC to radical scavenging activity (RSA) are shown in Figure 4. All assays showed positive interaction by linear regression. The highest correlation was showed between TFC and RSA (R^2=0.93), followed by TTC (R^2=0.87), then TPC (R^2=0.84).

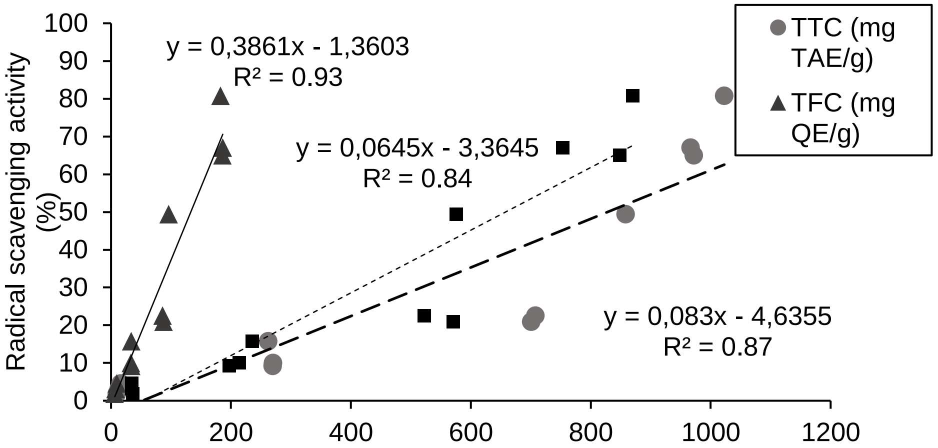

Figure 4. Correlation between colorimetric assays result (TPC, TFC, and TTC) to antioxidant activity (DPPH RSA) in 100 Ɩg/ml concentration.

Phenolic compounds including flavonoid and tannin are well known antioxidant component which is able to neutralize free radicals due to its ability to donate hydrogen atom while still being stable due to its ideal structure characteristic (Amarowicz *et al.* 2004). Linear correlation between phenolic and antioxidant activity has been reported in previous literatures (Shrestha *et al.* 2006; Esmaeili *et al.* 2015). In this research, flavonoid had the highest correlation to antioxidant activity. This result indicates that flavonoid type compounds are more responsible to the antioxidant activity. Similar result of higher correlation between total flavonoid and antioxidant activity was also reported in previous research on several wild vegetables from western Nepal (Aryal *et al.* 2019). Flavonoid is one of the major parts of compound found in *C. sappan* wood and various flavonoid type compounds has been identified from its wood (Namikoshi *et al.* 1987; Shu *et al.* 2008; Zhao *et al.* 2013). Further, the higher correlation of TTC-DPPH might suggest that long chained polyphenols, including that with flavonoids monomer, are more responsible to the antioxidant activity of *C. sappan* bark extract.

Conclusions

Colorimetric assays to measure TPC, TFC, and TTC, as well as antioxidant activity assay by DPPH scavenging activity method, and GC-MS analysis on successively extracted *C. sappan* bark have been conducted. The results showed the highest amount of TPC, TFC, and TTC in the MeOH extract. Further, the strongest antioxidant activity was exhibited in the MeOH extract. The detection of hydroquinone as a major constituent by GC-MS analysis supported the highest result of antioxidant activity. In this study, high correlation between TFC and DPPH RSA indicates that this antioxidant activity is more attributed to flavonoid type compounds. The results of this study indicates that the MeOH extract of *C. sappan* bark is a suitable source of natural antioxidant with strong activity against DPPH radical.

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Fast-Growing Wood-Polymer Nano Composite Characteristics through Nano-SiO$_2$ Impregnation

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Wayan Darmawan, Gilang Dwi Laksono, and Vera Junita Sitanggang

Abstract

Ganitri (*Elaeocarpus sphaericus* (Gaertn.) K. Schum.) and jabon (*Anthocephalus cadamba*) are fast-growing wood species that have low strength and durability class. One of methods for improving the characteristics of ganitri and jabon woods is impregnation. This study objectives were to analyze the effect of impregnation of Melamine Formaldehyde Furfuryl Alcohol (MFFA) copolymer and 0.5% Nano-SiO$_2$ on the physical, mechanical and durability properties of ganitri and jabon woods. The impregnation process was carried out by applying a 0.5 bar vacuum for 1 hour and followed by 2 bar pressure for 2 hours. The results showed that impregnation with MFFA and Nano-SiO$_2$ could improve optimally the physical and mechanical properties and durability of fast-growing woods.

Keywords: ganitri, impregnation, jabon, MFFA, Nano-SiO$_2$

Introduction

Ganitri (*Elaeocarpus sphaericus* (Gaertn.) K. Schum.) and jabon (*Anthocephalus cadamba*) are fast growing species. Ganitri wood has 100% juvenile wood at the age of 6 years (Laksono 2019). Ganitri tree cultivation techniques do not require certain growth requirements and this tree has fast growth (Rahman 2012). Ganitri wood has a soft and light structure with large size, upright, cylindrical stems and light gray to brownish bark. The wood is used for carpentry, and has a useful tree function to protect highways as an urban forest (Prihatini *et al*. 2020b). Based on the research by Prihatini *et al.* (2020b) the mechanical properties (Modulus of Rupture) and physical properties (specific gravity) of ganitri wood are included in the strength class III-IV. The durability of this wood belongs to durable class V (Sani 2015).

According to Darmawan *et al.* (2013) jabon wood that is 7 years old still contains 100% juvenile wood. According to Pandit *et al*. (2011) jabon tree is fast-growing plant, has a relatively high level of cylindrical stems, relatively few knots, and the wood has good mechanical properties for light construction (rafters, battens, window frames, etc). Jabon wood has a yellowish white color and the texture is quite smooth to rough and shiny (Pandit *et al.* 2011). Jabon wood also has low durability, namely durable class V and has a fairly low quality value, namely strong class IV-V (PPKI NI-5 1961).

Jabon and ganitri woods have inferior wood quality, so they need to be modified to improve the quality of the wood (Prihatini 2020a). Wood modification is divided into thermal modification, surface modification and chemical modification. The modification of wood that has been carried out so far is chemical modification, to be precise, the impregnation method. Impregnation is a method that is carried out by inserting impregnant materials or substances into wood with the aim of improving its quality (Hill 2006).

The impregnant material that is often used is Furfuryl Alcohol (FA). FA is derived from the hydrolyzate of agricultural waste which is rich in pentosan, has a strong polarity, a colorless hydrophilic liquid, but forms a hydrophobic dark brown polymer gel when heated in the presence of an acid catalyst (Thygesen 2009). According to Hazarika and Maji (2013) the use of FA impregnation was quite effective in increasing the dimensional stability of wood, but the strength or mechanical properties of wood did not change that much. To overcome this, it is necessary to have a mixture of other materials to improve the mechanical properties of wood, such as Melamine Formaldehyde (MF).

MF resin is one of the very hard and very stiff thermoset polymers that can provide good performance on wood. MF is an amino resin that has various material advantages, such as better hardness, transparency, good boiling resistance, thermal stability, scratch resistance, fire resistance, moisture resistance and makes the wood surface smooth (Bajia *et al.* 2009). According to Yao *et al.* (2017), the mechanical properties of wood that has been treated with MF are higher because MF easily reacts with acidic, alkaline or neutral media. The drawback of using MF is the emission of formaldehyde which can be harmful to health. Mixing Melamine Formaldehyde and Furfuryl Alcohol (MFFA) can reduce the impact of formaldehyde emissions and can improve wood quality, both mechanical properties and durability.

Nanotechnology applications have penetrated into various fields including wood technology. The use of nanotechnology in wood modification can be a good opportunity to help fast growing wood in Indonesia in an effort to improve wood quality. Hazarika and Maji (2013) proved that the addition of Nano-SiO$_2$ to MFFA could increase the durability and resistance of wood to fire. The application of Nano-SiO$_2$ to sengon wood produces density values and dimensional stability that increase effectively (Rahayu *et al.*

2020). Nano-SiO_2 is a white powder consisting of high purity amorphous silica. Nano-SiO_2 particles have advantages such as strong surface adsorption, large specific surface area and surface energy, good dispersion ability (Zhuang and Chen 2019). One of the applications of Nano-SiO_2 is as a composite filter. Nano-SiO_2 can significantly affect the compression, tensile and shear properties of epoxy resin composites (Chira *et al.* 2016). Based above findings, this study aims to analyze the effect of Melamine Formaldehyde Furfuryl Alcohol (MFFA) copolymer and 0.5% Nano-SiO_2 impregnation on the physical and mechanical properties and durability of ganitri and jabon wood.

Materials and Methods

Materials

Ganitri wood (*Elaecocarpus sphaericus* (Gaertn.) K. Schum), jabon wood (*Anthocephalus cadamba*) and dry wood termite (*Cryptotermes cynocephalus*) worker caste were the materials used in this study. Apart from that, there are also other materials used such as melamine, formaldehyde, furfuryl alcohol (Sigma Aldrich Pte. Ltd. China), 70% alcohol, demineralized water, NaOH, Nano-SiO_2 (particle diameter 15 ± 5 nm).

Preparation of Impregnation Solution

The manufacture of MFFA copolymers refers to the research by Prihatini (2020a), Hazarika and Maji (2013) and Yao *et al.* (2017). Melamine, formaldehyde, furfuryl alcohol are placed in a beaker with a mole ratio of 1:3:5. The first step is to mix the melamine and formaldehyde to hydroxymethylated melamine by mechanical stirring. The pre-reaction initiated when the pH of the solution was modified by putting 10% NaOH solution until the pH ranged from 9.4-9.6 and the temperature was slowly incerased to 98 °C. The second step was to react the solution formed with furfuryl alcohol until the solution looked clear which was carried out for 10 minutes. The third step was adding 1.5% anhydrous maleic catalyst which was carried out for 10 minutes. The results of this MFFA copolymer impregnation were made at a concentration of 50%.

After the MFFA is ready, also prepare Nano-SiO_2 which has been soaked in FA for 24 hours by stirring using a magnetic stirrer and sonicated for 30 minutes. After the MFFA is ready, prepare Nano-SiO_2 0.5% which has been soaked in FA for 24 hours by stirring using a magnetic stirrer and sonicated for 30 minutes. Then the MFFA copolymer was poured slowly into the Nano-SiO_2 solution with a magnetic stirrer. The prepared Nano-SiO_2 MFFA mixture was then added to FA with a volume ratio of 1:1. After that, it was continued with the sonication process for 15 minutes.

Impregnation Process

The impregnation method used in this study refers to previous research by Prihatini (2020a) with a MFFA solution and Nano-SiO_2 0.5%. Prior to the impregnation process, the sample was dried in the oven at 103 ± 2 °C until the weight was constant. The wood samples and MFFA solution and MFFA Nano-SiO_2 0.5% are ready to be put into the container. After that, the container containing the wood sample and solution was placed inside the impregnation tube. The sample and solution were given a vacuum of 0.5 bar for 1 hour, followed by a pressure of 2 bar for 2 hours. Then the wood samples were wrapped in aluminum foil and put in the oven at 60 °C for 12 hours. This process is called polymerization process. Then the aluminum foil was opened, the samples were put back into the oven at 103 ± 2 °C for 24 hours.

Weight Percent Gain (WPG) and Density

The analysis were carried out according to the BS 373:1957. The parameters used are WPG and density which can be obtained using the following formula:

$$\text{WPG (\%)}=\frac{(W_2\text{-}W_1)}{W_1}\times 100\%$$

$$\rho\ (\text{g/cm}^3)=\frac{B}{V}$$

Information :

W_1 = Weight of oven dry sample before treament (g)
W_2 = Weight of oven dry sample after treatment (g)
B = Weight after or before treatment (g)
V = Volume after or before treatment (cm^3)

Modulus of Elasticity (MOE), Modulus of Rupture (MOR) and Hardness

The MOE, MOR and hardness samples were made measuring 2.5 cm x 2.5 cm x 41 cm which refers to the ASTM D 143-94. This test used the Instron brand Universal Testing Machine (UTM). The hardness analysis was performed by inserting half a steel ball with a diameter of 1 cm and a cross-sectional area of 1 cm^2 into the wood. The ball is pressed 0.5 cm deep. MOE, MOR and hardness values can be obtained using the formula:

$$\text{MOE}\ \left(\frac{\text{kg}}{\text{cm}^2}\right)=\frac{\Delta PL^3}{4\Delta Ybh^3}$$

$$\text{MOR (kg/cm}^2)=\frac{3P\text{max}L}{2bh^2}$$

$$H\ \left(\frac{\text{kg}}{\text{cm}^2}\right)=\frac{\text{Pmaks}}{A}$$

Information :

MOE = Modulus of Elasticity (kg/cm^2)
MOR = Modulus of Rupture (kg/cm^2)
H = hardness value (kg/cm^2)
Pmax = Maximum load (kg)

ΔP = Load under proportion limit (kg)
ΔY = Deflection at load P (cm)
L = Spacing distance (cm)
b = Sample width (cm)
h = Sample thickness (cm)
A = cross-sectional area (cm²)

Resistance to Dry Wood Termite Attack

Testing for dry wood termites was carried out referring to the Indonesian National Standard (SNI) 7207: (2014). Prepare a sample measuring (2.5 x 2.5 x 5) cm, then trim it using a cutter and code the sample according to the treatment given. The wood sample was then dried in an oven at 103 ± 2 °C until a constant weight was then weighed (W_1). Glass pipes (paralon) are sterilized by wiping a tissue or cotton that has been given 70% alcohol. The pipe is glued to the sample of wood with hot glue. As many as 50 healthy and active worker caste dry wood termites of uniform size were prepared. Termites had to be collected carefully using chicken feathers from the colony container and then put into a petri dish. Fifty dry wood termites of the worker caste were put into a glass tube and then covered with cotton. The wood samples were stored in a dark room at room temperature for 12 weeks. Every 4 weeks, termite activity is observed by removing the cotton and then putting it back on. After 12 weeks, the glass tube was disassembled and the number of live termites on the surface of the sample was counted. After that, clean the wood sample and put it in the oven at 103 ± 2 °C until the weight is constant. then weighed (W_2). Then, calculate the percentage of termite mortality and the percentage of weight loss. The classification of wood resistance to dry wood termites can be seen in Table 1. A sketch of testing the resistance of wood to dry wood termites *Cryptotermes cynocephalus* on a laboratory scale can be seen in Figure 1.

$$\text{Mortality }(\%) = \frac{D}{50} \times 100\%$$

$$WL = \frac{(W_1 - W_2)}{W_1} \times 100\%$$

Where :
D = Number of dead termites after testing
50 = Number of initial termites
WL = Percentage of weight loss (%)
W_1 = Initial oven-dry weight sample (g)
W_2 = Oven-dry weight sample after testing (g)

Graveyard Field Test

Testing for subterranean termites was carried out according to ASTM D 1758-06. Samples measuring (2.5 x 2.5 x 41) cm were sanded until smooth and one end was coated with paint. Sample dimensions were measured with calipers. Samples were oven-dried at 103 ± 2 °C to constant weight and weighed (W_1). Then the samples were planted for 12 weeks in the Arboretum of the Faculty of Forestry and Environment. The distance between the test samples was 30 cm x 60 cm (for the treated samples) (Figure 2). A map of the location of each sample that has been planted in the field is made to facilitate observation and data collection. The condition of the samples was observed every four weeks.

Observations are made to check the condition of the sample which is seen above the soil surface (observations may not remove the sample). After 12 weeks, the sample was removed carefully. The sample was cleaned from the soil and then dried for ± 7 days then put into the oven at 103 ± 2 °C until the weight was constant and weighed (W_2). Then calculate the percentage of weight loss with the formula:

Table 1. Classification of wood durability to dry wood termites *Cryptotermes cynocephalus* (SNI 01-7207-2014)

Class	Durability	Weight Loss (%)
I	Very Durability	< 2.0
II	Hold	2.0 – 4.3
III	Moderate	4.4 – 8.1
IV	Not Durability	8.2 – 28.1
V	Very intolerable	>28.1

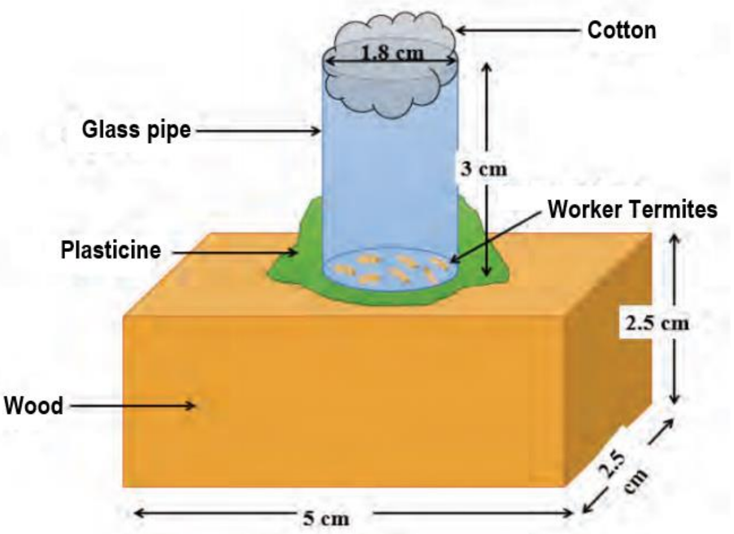


Figure 1. Sketch of testing wood's resistance to dry wood termites.

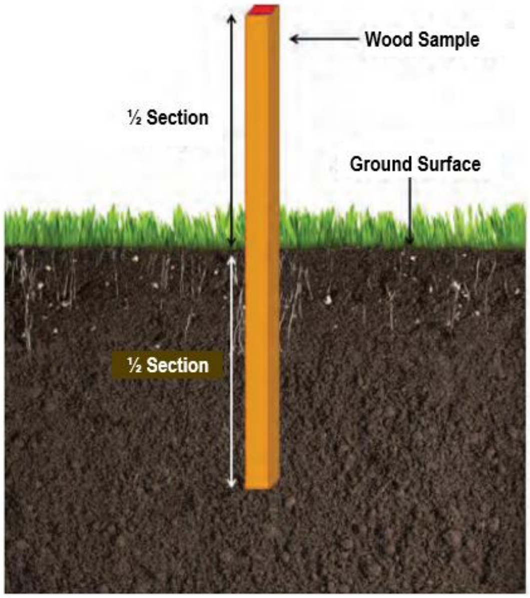


Figure 2. Test sample planting sketch.

$$\text{WL}=\frac{(W_1\text{-}W_2)}{W_1}\times 100\%$$

Information :
WL = Percentage of weight loss (%)
W_1 = Initial oven-dry weight sample (g)
W_2 = Oven-dry weight sample after testing (g)

Data Analysis

The data analysis used in this study was a completely randomized design with two factors; impregnation solution treatment factor (control, MFFA and MFFA Nano-SiO_2 0.5%) and wood species factor (ganitri and jabon).

Results and Discussion

Weight Percent Gain (WPG) and Density

WPG values of ganitri and jabon wood changed when treated with MFFA Nano-SiO_2 0.5% as shown in Figure 3. This shows that the WPG value increased due to the addition of MFFA and MFFA Nano-SiO_2 0.5%. The highest WPG values for ganitri and jabon wood were produced by MFFA Nano-SiO_2 0.5% which had respective values of 59.15% and 75.68%.

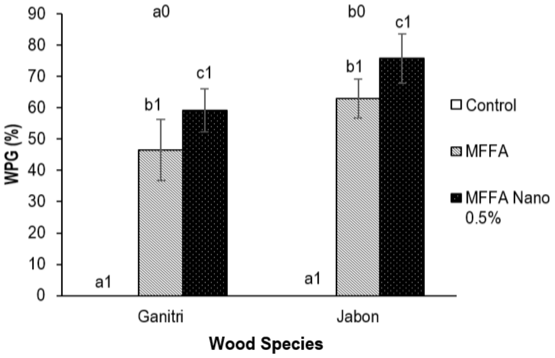


Figure 3. WPG ganitri and jabon woods.

Analysis of variance showed that the interaction between the concentration of the impregnation solution and the wood species factor had a significant effect on WPG. The results of Duncan's further test showed that the WPG in each treatment was not significantly different.

This is in line with Prihatini (2020a) that the addition of MFFA and MFFA Nano-SiO_2 can improve the value of WPG. The higher WPG value for jabon wood is due to the larger pore diameter of jabon wood compared to ganitri wood. According to Martawijaya *et al.* (2005) which states that the pore diameter of jabon is 130-220 µm, and the number of pores is 2-5 per mm^2, while ganitri wood has 84.06-117.94 µm with a number of pores of 5-7 per mm^2.

The density of ganitri and jabon wood obtained from the test showed an increase with the addition of MFFA and MFFA Nano-SiO_2 0.5% solutions. The resulting density value is shown in Figure 4. The highest density values of ganitri and jabon wood were produced by MFFA Nano-SiO_2 0.5%, respectively 0.74 g/cm^3 and 0.59 g/cm^3.

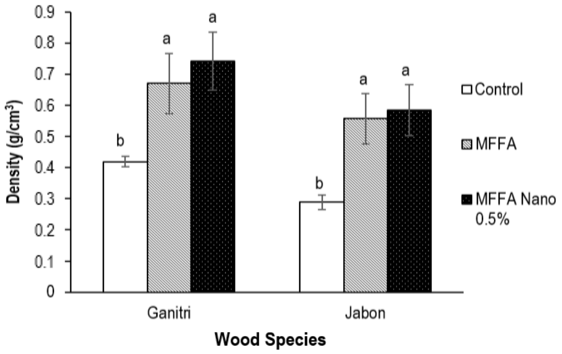
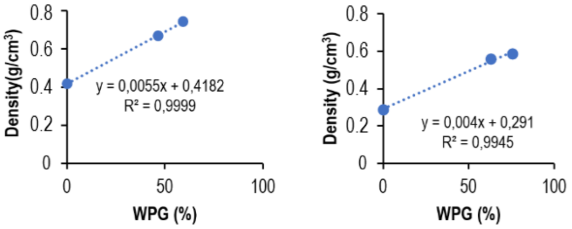


Figure 4. Density ganitri and jabon woods.

The results of the analysis of variance showed that the factors of wood type and impregnant solution had a significant effect on density, but the interaction of the two factors had no significant effect on density. In the further Duncan test results, the control treatment was significantly different from the other two treatments, while MFFA and MFFA Nano-SiO_2 0.5% were not significantly different.



(a) (b)

Figure 5. Density comparison with WPG: (a) ganitri; (b) jabon woods.

Based on Figure 5, it is known that the WPG value is directly proportional to the density value of gantri and jabon woods. This is presumably due to the penetration of the impregnant solution to fill in the voids in the cell walls and wood lumen. This is in line with the research of Hazarika and Maji (2013) which stated that when the MFFA and Nano-SiO_2 were treated, the empty cell walls and lumens were filled. This causes the WPG value and density to increase.

Modulus of Elasticity (MOE)

The elastic modulus shows the ratio between strain and stress below the elastic limit, so that the object will return to its original shape when released (*Mardikanto et al.* 2011). The MOE value generated in this research is presented in Figure 6. Impregnation treatment of MFFA and MFFA Nano-SiO_2 0.5% increased MOE compared to the control shown in Figure 6. The values of ganitri and jabon woods impregnated with MFFA Nano-SiO_2 0.5% were 8214.29 MPa and 7127.27 MPa, respectively.

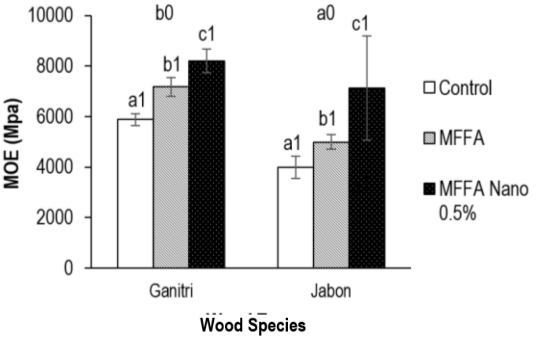
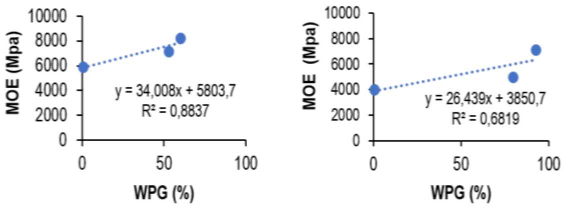


Figure 6. MOE of ganitri and jabon woods.

The results of the analysis of variance showed that the interaction of the impregnation solution concentration factor and the type of wood did not significantly affect the MOE value, but the impregnation solution concentration factor and the wood species factor had a significant effect on the MOE value. Duncan's further test showed that the MOE values of the control, MFFA, and MFFA Nano-SiO_2 0.5% were significantly different.



(a) (b)

Figure 7. Relationship between MOE and WPG values: (a) ganitri; (b) jabon woods.

Based on Figure 7, the increase in MOE value is directly proportional to the increase in WPG. A high WPG value will be followed by an increased MOE value, and vice versa. This is in accordance with research by Riadhi (2017) which stated that the MOE value of sengon wood produced increased in each treatment because Monoethylene Glycol (MEG) and Nano-SiO_2 entered the wood cell walls and filled the empty cell cavities.

Modulus of Rupture (MOR)

MOR in wood shows the resilience of wood in resisting deflection caused by the maximum load received by the wood (Mardikanto *et al.* 2011). The MOR value generated in this study is presented in Figure 8.

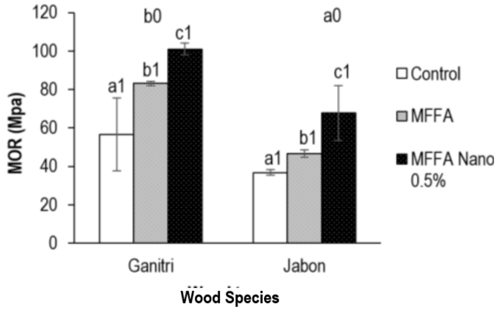


Figure 8. MOR of ganitri wood and jabon woods.

Based on Figure 8 it is known that MFFA and MFFA Nano-SiO_2 0.5% impregnation treatment can increase MOR compared to control. The highest MOR values were obtained in the MFFA Nano-SiO_2 0.5% treatment of ganitri and jabon woods, respectively 101.08 MPa and 67.69 MPa. Nano SiO_2 in the impregnation solution which is already present in the wood and fills the cavities and cell walls of the wood through impregnation, causing the resulting MOR value to be greater than the previous treatment. The wood species factor gives the MOR value of ganitri wood higher than jabon wood. As can be seen from Figure 8, the results of the MOR value with MFFA Nano-SiO_2 0.5% treatment is the optimal treatment. This is in line with Prihatini (2020a) which shows that the MFFA Nano SiO_2 concentration of 0.5% is the optimum concentration for impregnation of ganitri and jabon woods.

Analysis of variance showed that the impregnant solution concentration factor and the wood type factor had a significant effect on the MOR value, but the interaction factor of the impregnant solution concentration and wood species had no significant effect on the MOR value.

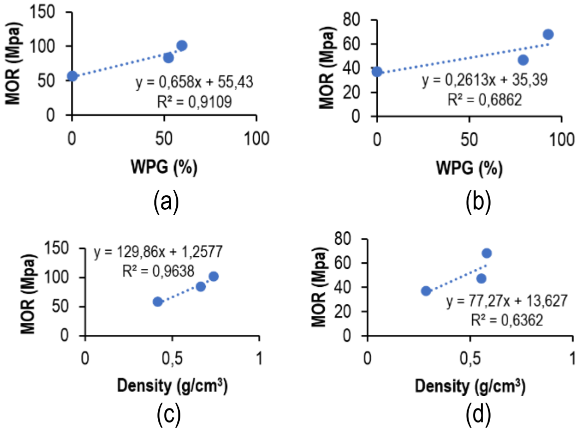


Figure 9. Relationship MOR with WPG of ganitri (a) and jabon woods (b); the relationship between MOR and density of ganitri (c) and jabon woods (d)

Based on Figures 9a and 9b it is known that the WPG value is directly proportional to the MOR value of gantri and jabon woods. Similarly, density is directly proportional to MOR (Figure 9c and 9d). This is thought to be due to polymers penetrating into the wood cell walls and wood cavities. The addition of Nano-SiO_2 concentration can increase the MOR value due to dense wood pores thereby increasing the stiffness of wood (Hoseini *et al.* 2014). The higher the density, the stronger the tested wood is marked by the high MOR value, this is in accordance with the statement of Bowyer *et al.* (2007) that the density of a material is directly proportional to the resulting MOR value.

Hardness

The hardness value of wood can be influenced by several factors, such as density, wood tenacity, adhesion between wood fibers and arrangement of wood fibers (Mardikanto *et al.* 2011). Because wood is anisotropic, it is known that there is radial hardness and tangential hardness. The hardness values produced in this study are presented in Figures 10 and 11.

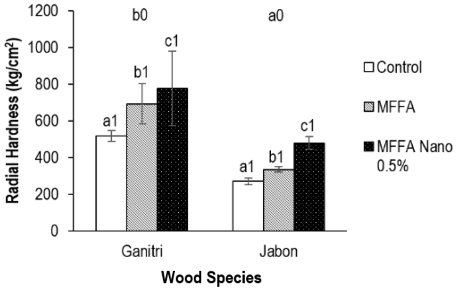


Figure 10. Radial hardness of ganitri and jabon woods.

Based on Figure 10, the radial hardness value obtained has increased in each treatment. Impregnation of MFFA Nano-SiO_2 solution significantly increased the hardness of ganitri and jabon woods, but the increase in hardness of MFFA-impregnated specimens was not significant. Impregnation results in the MFFA Nano-SiO_2 0.5% treatment showed the highest values for the radial hardness of ganitri and jabon wood, respectively 776.73 kg/cm^2 and 479.89 kg/cm^2.

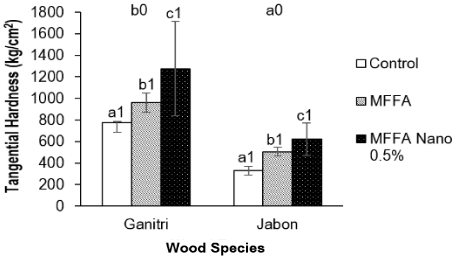
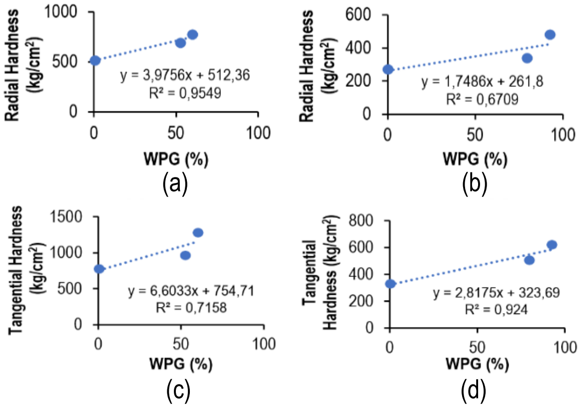


Figure 11. Tangential hardness of ganitri and jabon woods.

The tangential hardness value increased when given the treatment shown in Figure 11. Impregnation treatment with MFFA and MFFA Nano-SiO_2 solution can increase the hardness value obtained. Impregnation results in the MFFA Nano-SiO_2 0.5% treatment showed the highest tangential hardness values of ganitri and jabon woods, 1275.91 kg/cm^2 and 621.74 kg/cm^2 respectively.

Analysis of variance showed that the interaction between the concentration of the impregnant solution and the wood species did not show a significant effect on the radial hardness value, but the interaction had a significant effect on the tangential hardness. The concentration factor of the impregnant solution and the factor of the wood species showed a significant effect on the radial and tangential hardness.

Ganitri wood has higher radial and tangential hardness values than jabon wood. The MFFA Nano-SiO_2 0.5% treatment gave the highest value of all treatments. The results of Duncan's further test showed that the radial and tangential hardness values for each treatment were significantly different.



(a) (b)

(c) (d)

Figure 12. Radial hardness relationship with WPG of ganitri (a) and jabon woods (b); tangential hardness relationship with WPG of ganitri (c) and jabon woods (d).

Based on Figure 12 it shows that the hardness value increases with the addition of the WPG value. Small Nano-SiO_2 particles can spread over a wider surface and have a stronger hardness value on the surface (Zhuang and Chen 2019). The results showed that the tangential hardness was higher than the radial hardness. This is caused by differences in the thickness of the cell wall and the orientation of the constituent cells in each plane. In general, the tangential wall is thicker than the radial wall because there are more holes on the radial wall so that the hardness is lower. According to Bowyer *et al.* (2007) on the radial side, the incoming force will be greeted by the radius cells which are in an open (widened) condition so that the steel ball can penetrate the surface of the wood because it is known that the fingers are the cells that make up wood with thin to very thin walls (Pandit *et al.* 2011).

Resistance to Dry Wood Termite Attack

The value of resistance to attack by drywood termites is indicated by the value of the proportion of mortality and weight loss of wood after being fed drywood termites (*Cryptothermes cynocephalus*). The proportion of wood mortality in treated ganitri and jabon wood increased, this caused the proportion of weight loss in ganitri and jabon wood to be lower. The results of the mortality test produced in the study are shown in Figure 13 and the proportion of weight loss in Figure 14.

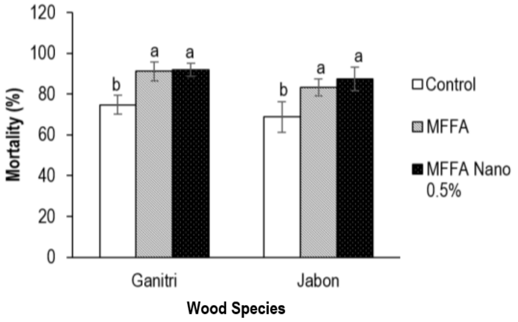


Figure 13. Average mortality percentage of samples after three weeks of feeding *C. Cynocephalus*

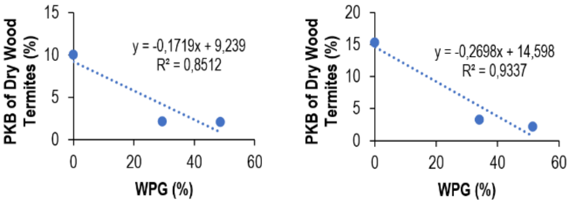
Based on Figure 13, the percentage of mortality increased in each treatment. The presence of Nano-SiO_2 in the MFFA solution can increase the durability of wood. Impregnation results in the MFFA Nano-SiO_2 0.5% treatment showed the highest mortality percentage values for ganitri (92%) and jabon (87.6%) wood. The results of Duncan's further test stated that the control treatment was significantly different from the other treatments, while the MFFA and MFFA Nano-SiO_2 0.5% were not significantly different. The results of the analysis of variance showed that the impregnant solution type concentration factor and the wood species factor had a significant effect on the mortality percentage of dry wood termites, while the interaction factor between the impregnant solution concentration and wood species had no significant effect on the mortality percentage value.

According to Prihatini (2020a) based on the results of SEM (Scanning Electron Microscope) analysis, jabon and ganitri woods before being treated had empty holes, but after being treated with MFFA and MFFA Nano-SiO_2 0.5%, they became evenly covered. It is suspected that this causes the percentage value of mortality to increase which can also increase the toxicity of wood. An indication of the presence of Nano-SiO_2 can be detected by the presence of some white precipitates on the cell walls and gaps between the cells of the jabon and ganitri wood after being impregnated.

The addition of MFFA and MFFA Nano SiO_2 0.5% resulted in a decrease in the percentage value for weight loss as shown in Figure 14. The percentage value for weight loss in the MFFA Nano-SiO_2 0.5% treatment for ganitri and jabon woods had the lowest value of 2.08% and 2.16%, respectively. Ganitri and jabon woods added with Nano SiO_2 can increase resistance to dry wood termite attack. The addition of 0.5% Nano-SiO_2 into the MFFA solution can produce a low value of the proportion of weight loss for each wood. The toxic substances present in wood make it difficult for termites to eat wood. This is supported by research by El-bandary and El-Halaly (2013) which showed that plants treated with Nano-SiO_2 particles could increase their toxicity to insect attacks. Based on research by Zhang *et al.* (2011) Nano-SiO_2 particles are toxic particles apart from ZnO_2, TiO_2 and Al_2O_3.

Figure 14. Percentage of weight loss in dry wood termite testing

The results of Duncan's further test stated that the control treatment was significantly different from the other treatments, while the MFFA and MFFA Nano-SiO_2 0.5% treatments were not significantly different. The results of the analysis of variance showed that the interaction factor between the concentration of the impregnant solution and the wood species did not have a significant effect on the proportion of weight loss after dry wood termite testing, but the concentration of the impregnant solution and the wood species had a significant effect on the proportion of weight loss.



(a) (b)

Figure 15. Relationship between weight loss percentage and WPG: (a) ganitri and (b) jabon woods.

The relationship between the WPG value and the percentage of weight loss is inversely proportional, if the WPG value is high, then the percentage value of weight loss is low. This can be seen in Figure 15. According to Zhang *et al.* (2011), Nano-SiO_2 particles are toxic particles, so that the resistance of wood to attack by wood destroying organisms can be increased which is marked by a low percentage of weight loss.

Graveyard Field Test

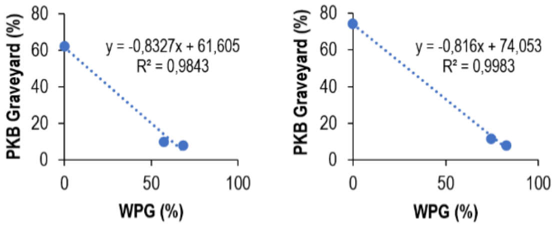
Graveyard Field Tests can be seen through the percentage of wood weight loss caused by wood destroying factors. The lower the weight loss of the wood produced, the higher the level of durability of the wood. The results of the wood grave test produced in the study are shown in Figure 16.

Based on Figure 16, it shows that MFFA and Nano-SiO_2 impregnation treatment can increase the durability of wood. The lowest value was obtained from the MFFA Nano-SiO_2 0.5% treatment for ganitri and jabon woods, respectively 7.82% and 7.76%. The impregnation treatment of MFFA and MFFA Nano-SiO_2 0.5% had a significant effect on the percentage of weight loss. The results of Duncan's further test showed that the ganitri and jabon graveyard test values in the control treatment were significantly different from the other two treatments, while MFFA and MFFA Nano-SiO_2 0.5% were not significantly different.

Figure 16. Percentage of Weight Loss (PKB) graveyard test

Ganitri and Jabon Statistical test results of analysis of variance showed that the concentration of the impregnant solution and the type of wood had a significant effect on the weight loss percentage of the ganitri and jabon wood grave tests, while the interaction factors between the two had no significant effect.

Nano-SiO_2 can increase the durability of wood, this is indicated by the decrease in the percentage of weight loss produced. This is supported by Khoerudin (2021) who shows that silica nanoparticles added to the impregnation solution can increase the resistance of sengon and jabon woods in destroying organisms which is reflected in the low percentage value of weight loss.



(a) (b)

Figure 17. Relationship between Weight Loss Percentage (PKB) and WPG: (a) ganitri; (b) jabon woods.

The relationship between the WPG value and the percentage of weight loss is inversely proportional, if the WPG value is high, then the percentage value of weight loss is low. This can be seen in Figure 17. This is presumably because the Nano-SiO_2 particles can increase the resistance of wood to wood destroying organisms. The factor of the addition of MFFA and Nano-SiO_2 makes the value of wood hardness increase which makes it more difficult for wood termites to eat, so that the resistance of the wood also increases (Tampubolon *et al.* 2015).

Conclusions

Impregnation treatment using a mixture of 0.5% Nano-SiO_2 MFFA was able to increase the values of density, MOE, MOR, hardness and the percentage value of dry wood termite mortality and reduce the percentage of weight loss after being fed to dry wood termites and grave tests.

Ganitri and jabon woods treated with MFFA Nano-SiO_2 with a concentration of 0.5% is the optimum value for now in this study which can increase the strength and durability of the wood. The strong class of ganitri wood has changed, namely from strong class III-IV to III, as well as to jabon wood which initially had a strong class IV-V to IV. In addition, the durability class has also changed in ganitri and jabon woods from durable class IV-V to II.

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4.5. References in text are written as this example: (Palomar *et al.* 1990; Arancon 1997).
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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: Chujo *et al.* 2010.

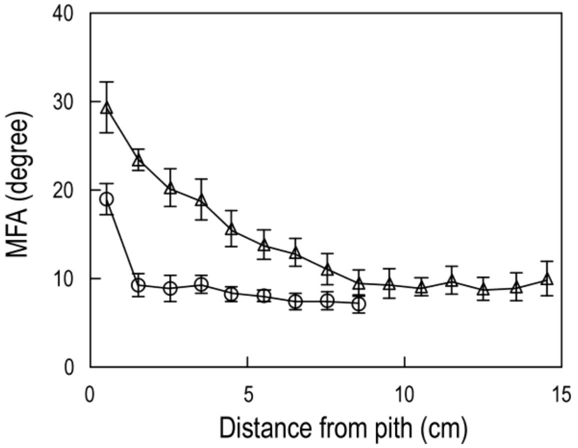

Figure 3. Radial variation of microfibril angle of the S2 layer in tracheid. Open circle, *Agathis* sp.; open triangle, *Pinus insularis*; Bars indicate the standard deviation. (Source: Ishiguri *et al.* 2010)