EVALUATION OF ANTITERMITIC ACTIVITY OF DIFFERENT EXTRACTS OBTAINED FROM INDONESIAN TEAKWOOD (*Tectona grandis* L.f)

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The antitermitic activity of different extracts obtained from *Tectona grandis* L.f was investigated against *Coptotermes curvignathus* (Holmgren). The main objective of this work was to determine the mortality rate of termites by the teak wood extracts. Different extracts exhibited different degree of antitermitic activity. A teak wood with age of 39-59 years and 59-79 years were selected from Purwakata and Cepu regions of Indonesia, respectively. As per earlier reports, quinones are considered as toxic to termites, and these quinones are found in abundance in teak wood. Among the extracts of petroleum ether, acetone/water (9:1), and ethanol/water (8:2), the acetone/water (9:1) extracts exhibited strong activity. The surface morphology of extracted wood samples was observed by scanning electron microscopy in order to reveal evidence of change.

Key words: Teak age; Tectona grandis L.f*; Termites; Scaning Electron Microscopy (SEM)*

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INTRODUCTION

Teak (*Tectona grandis* L.f) is one of the major commercial species of Indonesia and has been used for centuries to fulfill the demands for which naturally durable timber is required. Teak is being cultivated in Java, Sulawesi, and many other provinces in Indonesia. It is increasingly used for forest rehabilitation.

Teak wood is also known for its natural resistance against non-native termites (Lukmandaru and Takahashi 2009; Thulasidas and Bhat 2007; Becker 1961). This advantage can be attributed to extractives present in teak wood. There are numerous bioactive compounds isolated from the teak wood (Lukmandaru and Takahashi 2009). Tree age and extractive content have been reported as affecting the natural termite resistances (Lukmandaru and Takahashi 2009; Da Costa et al. 1958, 1961; Rudman et al. 1967). A variety of compounds have been isolated from almost every part of teak, with varied degree of structure, belonging to different classes such as flavonoids, quinones, steroidal compounds, glycosides, and phenolic acids (Ohmura *et al*. 2000). In addition, a plant with such a degree of medical importance is good source to cure a number of human

diseases. Apart from this, other applications such as antitermitic activity are important uses of this plant. Crude extracts are a good source of ingredients for this application.

To evaluate biological activities of extractives of teak wood, various organic solvents have been used by many researchers. Organic solvents such as acetone, *n*-hexane, ethyl ether, ethyl acetate, and ethanol have been used for such studies (Syafii *et. al*. 1987; Syafii and Yoshimoto 1993; Syafii 2002; Ohmura. *et al.* 2000). It has been reported that extracts of ethyl acetate of teakwood have strong bioactivity against termites (Lukmandaru and Ogiyama 2005).

Therefore, in the present investigation, we are reporting the antitermitic efficacy of heartwood extracts of *Tectona grandis* against *C. curvignathus*. Furthermore, insights regarding the relationships between the localities and age of teak and their antitermitic activities are reported.

EXPERIMENTAL

Wood Samples and Extraction

Teak wood was obtained from two places in Indonesia, one group from Purwakarta in West Java and another from Cepu in Central Java. The woods were divided into various groups viz., Group III (30 to 39 years old), Group IV (40 to 49 years old), Group V (50 to 59 years old), Group VI (60 to 69 years old), and Group VII (70 to 79 years old). The sample wood blocks of 2 x 2 x 2 (cm) were cut from teak wood of each age group. The samples were oven dried at 65 ^{o}C for 24 hours and were weighed before treatment. The wood samples were extracted sequentially in soxhlet with petroleum ether, acetone:water (9:1), and ethanol:water (8:2) for 24 hours. The outline scheme of extraction is given in Fig. 1.

Morphological Studies

The microscopic appearances of samples were examined with a scanning electron microscope (Leo Supra, 50 VP, Carl Ziess, SMT, Germany, SEM) using small samples of 1 to 2 mm thickness. Specimens were sputter-coated with gold to a thickness of ca. 10 nm in order to prevent charging during the examination. An accelerating voltage of 15 kV was used to collect the SEM images. The transverse sections of thickness 1 μm were cut using a Sorvall Ultra microtome (MT 500) with a glass and diamond knife.

Termiticidal Test

Whatman No. 41 paper (diameter 25 mm) was dried at 102 $\pm$ 3^{o}C for one hour before it was used as food source for termites. Glass bottles 115 x 10 (mm) were used as experimental containers. The bottles were filled with 30 g of sand sieved through 20 meshes and moistened with 6 mL of distilled water. Whatman No. 41 papers were treated by different extracts by 2%, 4%, 6%, 8%, and 10% (w/w) concentrations, respectively. The untreated paper was used as a control. Termites removed from logs were maintained in glass bottles, each containing 45 workers and 5 soldiers of *Coptotermes curvignathus* (Holmgren). Then each bottle was plugged with a cotton pad and kept in dark conditions at 28 ±2 ^{o}C, 75±5% RH for 21 days. Three replicates were prepared for each test.

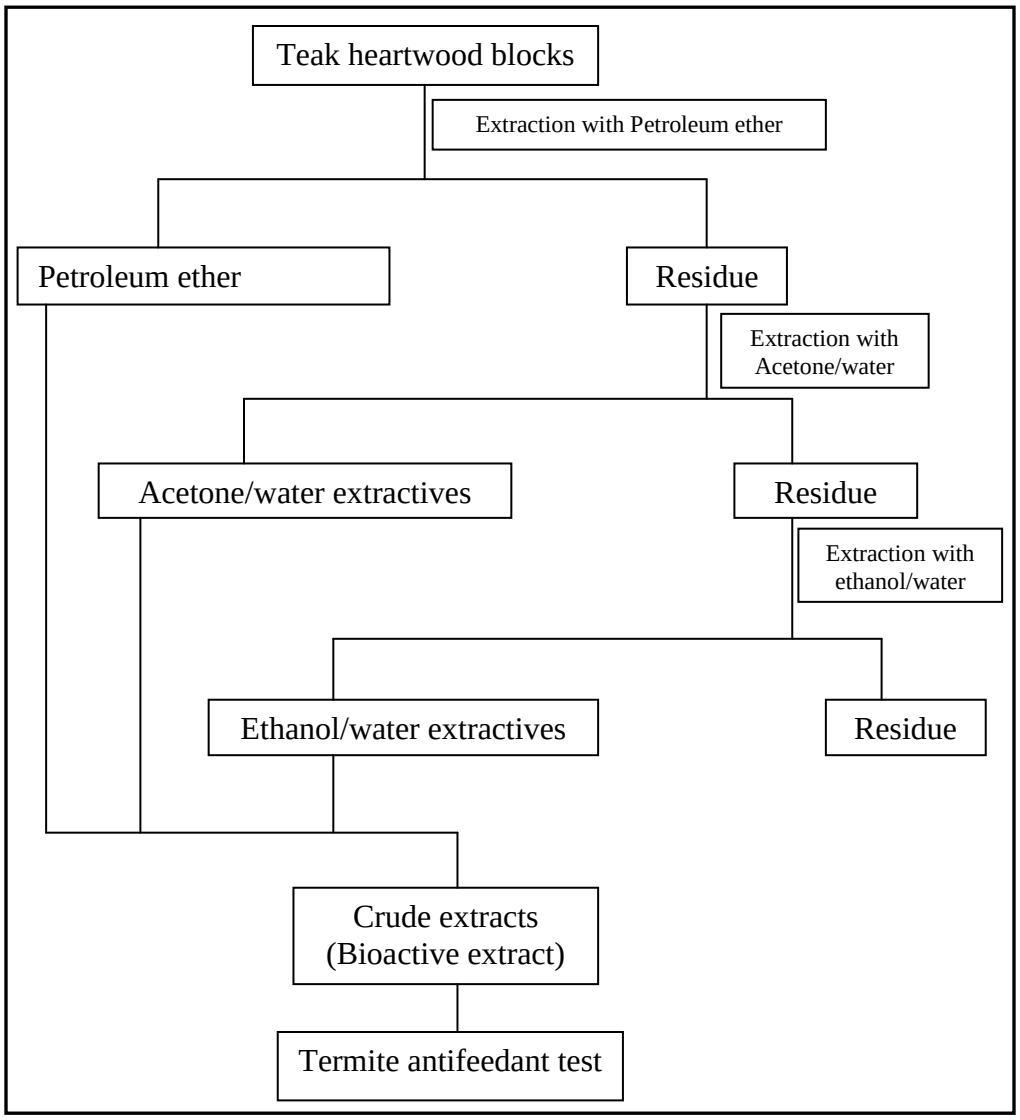

Figure 1. Extraction scheme of teakwood extracts from heartwood extractives teakwood (Haupt *et al.* 2003)

The paper discs were taken out from the glass bottles after 21 days, cleaned, oven-dried, and reweighed. Termite mortality (percent) and weight loss (percent) of the paper discs were recorded as per the following equations (Ohmura *et al.* 2000),

$$\text{Termite mortality (\%)} = \text{No. of dead termites} / \text{Total no of test termites} \times 100 \quad (1)$$

$$\text{Weight loss (\%)} = (W_1\text{-}W_2 / W_1) \times 100 \quad (2)$$

where W_1 is the weight loss of untreated paper discs (gm), and W_2 is the weight loss of treated paper discs after the termicidal test (gm). On the basis of the weight losses of the

discs, the indices of the activity of the extracts were calculated. The absolute coefficient of antifeedancy (*A*) was obtained by the following equation (Ohmura et al. 2000),

$$A = [(KK - EE)/(KK + EE)] \times 100 \quad (3)$$

where *KK* and *EE* are the weight losses of the control and treated discs, respectively. All extracts tested were classified into four classes according to their A values (Table 1). At least 18 replicates were conducted for each parameter. The data was analyzed by analysis of variance (ANOVA) to determine the significance of differences in treatment, using SPPS 11.5 statistical package.

Table 1. Indicators to Evaluate the Antifeedant Activity of extracts

Antifeedancy (%)	Activity level
75 ≤ A < 100	Very strong activity
50 ≤ A < 75	Strong activity
25 ≤ A < 50	Moderate activity
0 ≤ A < 25	Minimal activity

RESULTS AND DISCUSSION

Extraction

The total contents of heartwood extractives obtained from teak woods with different ages and from different localities of teak are shown in Table 2. On oven-dried basis, the total petroleum-ether extraction obtained from the heartwood of teak from Cepu, Centeral Java of about 70-79 years old was 5.68%, which was higher than that from teak tress of Purwakarta.

This finding confirms well the general perception of a positive correlation between the age of a tree and its amount of total extractives. However, there were also variations depending on the geographical site and the age of tree. This finding confirms that the extractives composition on certain woods that are difficult to distinguish anatomically can be used for estimation of the age of the tree.

Table 2. Content of Extractives of Teakwood (%) as Obtained by Successive Extraction

Wood sample of teak (Age)	*Petroleum-ether %*	*Acetone/water 9:1 %*	*Ethanol/water 8:2 %*	*Total %*
30-39 (Purwakarta)	1.19	3.75	0.92	5.86
40-49 (Purwakarta)	4.42	5.34	0.78	10.54
50-59 (Purwakarta)	3.62	5.59	0.83	10.04
50-59 (Cepu)	4.65	5.99	1.11	11.75
60-69 (Cepu)	4.23	5.89	0.87	10.99
70-79 (Cepu)	5.68	6.78	0.81	13.27

(Purwakarta and Cepu are places of origin)

Antitermitic Activity

The results for termite mortality are shown in Table 3. Results revealed that among the extracts, acetone/water (9:1) exhibited a good level of activity. At a concentration of 10%, the acetone/water (9:1) soluble fraction caused termite mortality up to 100%. However, there were also variations related to the age and localities of tree in the toxicity to the termite. It was observed that the greater was the age of a tree, the greater was mortality of termites, which can be attributed to presence of high concentration toxic compounds. Recently, researchers have reported that radial distributions of extractives in the sapwood to heartwood were developed for teak to determine chemical traits related to its natural durability against insects (Niamke et al 2011; Lukmandaru and Takahashi 2009; Bhat et al. 2005). This observation is based on the histochemical investigation, which revealed that metabolic activities throughout the sapwood and within the transition zone lead to the biosynthesis of heartwood extractives from reserve materials (Niamke et al 2011; Datta and Kumar 1987; Nobuchi et al. 1996). This leads to formation of phenolic structures such as anthraquinones and naphthoquinones, which have been identified as important defense compounds against insects and termites. However, there is no trustworthy information to better understand the origin of natural durability through the transformation of non-structural carbohydrates into phenolic compounds accumulated in the heartwood (Niamke et al 2011).

Table 3. Mortality (%) of Termites with Addition of Extracts from Teak after Being Exposed for 21 Days

Wood sample of teak (Age)	Soluble extracts	Concentration (%, w/w)					
		0	2	4	6	8	10
30-39 (Purwakarta)	Petroleum ether	10.0	56.6	84.7	63.3	82.0	100.0
	Acetone/water (9:1)	10.0	85.3	77.3	98.0	94.0	100.0
	Ethanol/water (8:2)	10.0	40.7	65.3	63.3	84.7	99.3
40-49 (Purwakarta)	Petroleum ether	10.0	54.7	75.3	70.7	86.7	100.0
	Acetone/water (9:1)	10.0	73.3	82.0	90.0	100.0	100.0
	Ethanol/water (8:2)	10.0	67.3	74.0	65.3	86.7	100.0
50-59 (Purwakarta)	Petroleum ether	10.0	60.0	66.0	66.7	94.0	100.0
	Acetone/water (9:1)	10.0	70.0	78.7	92.0	100.0	100.0
	Ethanol/water (8:2)	10.0	56.0	64.7	61.3	66.0	96.0
50-59 (Cepu)	Petroleum ether	10.0	44.7	73.3	78.0	92.7	97.3
	Acetone/water (9:1)	10.0	84.0	77.3	96.0	99.3	100.0
	Ethanol/water (8:2)	10.0	61.3	65.3	68.7	93.3	99.3
60-69 (Cepu)	Petroleum ether	10.0	50.7	77.3	70.7	90.7	100.0
	Acetone/water (9:1)	10.0	100.0	72.7	94.7	100.0	100.0
	Ethanol/water (8:2)	10.0	58.0	68.0	53.3	76.7	100.0
70-79 (Cepu)	Petroleum ether	10.0	46.7	82.0	69.3	833	100.0
	Acetone/water (9:1)	10.0	80.7	74.0	100.0	100.0	100.0
	Ethanol/water (8:2)	10.0	52.0	70.0	57.3	90.0	100.0

From Table 3 it is evident that acetone/water (9:1) extracts exhibited a moderate to very strong activity. The extracts of trees with different ages showed different degree of activity. The antifeedancy activity for extractives obtained from teakwood from Cepu and Purwakarta 44.07% to 78.14% and 44.34% to 64.11%, respectively. The summary of statistical analysis results of percent termite mortality is given in Table 4. A significant difference attributable to concentration ($p<0.05$) was observed. Hence, concentration is a vital factor affecting the termite mortality and antifeedancy activity.

Table 4. Summary of Analysis of Variance (ANOVA) for %Termite Mortality *

Source	30-39 (Purwakarta) Petroleum ether					30-39 (Purwakarta) Acetone/water (9:1)				
	SS	DF	MS	*F*	*p*	SS	DF	MS	*F*	*p*
BG	14933.33	5.00	2986.67	53227.72	0.00	17438.46	5.00	3487.69	56557.16	0.00
WG	0.67	12.00	0.06			0.74	12.00	0.06		
Total	14934.01	17.00				17439.20	17.00			
Source	30-39 (Purwakarta) Ethanol/water (8:2)					40-49 (Purwakarta) Petroleum ether				
	SS	DF	MS	*F*	*p*	SS	DF	MS	*F*	*p*
BG	15165.57	5.00	3033.11	245485.83	0.00	14799.94	5.00	2959.99	58710.52	0.00
WG	0.15	12.00	0.01			0.61	12.00	0.05		
Total	15165.72	17.00				14800.55	17.00			
Source	40-49 (Purwakarta) Acetone/water (9:1)					40-49 (Purwakarta) Ethanol/water (8:2)				
	SS	DF	MS	*F*	*p*	SS	DF	MS	*F*	*p*
BG	17266.58	5.00	3453.32	169326.34	0.00	14272.21	5.00	2854.44	139809.41	0.00
WG	0.24	12.00	0.02			0.25	12.00	0.02		
Total	17266.83	17.00				14272.46	17.00			
Source	50-59 (Purwakarta) Petroleum ether					50-59 (Purwakarta) Acetone/water (9:1)				
	SS	DF	MS	*F*	*p*	SS	DF	MS	*F*	*p*
BG	15358.73	5.00	3071.75	98734.70	0.00	17393.16	5.00	3478.63	97836.52	0.00
WG	0.37	12.00	0.03			0.43	12.00	0.04		
Total	15359.11	17.00				17393.59	17.00			
Source	50-59 (Purwakarta) Ethanol/water (8:2)					50-59 (Cepu) Petroleum ether				
	SS	DF	MS	*F*	*p*	SS	DF	MS	*F*	*p*
BG	11585.90	5.00	2317.18	47396.88	0.00	16383.93	5.00	3276.79	141274.63	0.00
WG	0.59	12.00	0.05			0.28	12.00	0.02		
Total	11586.49	17.00				8016.44	17.00			
Source	50-59 (Cepu) /Acetone/water (9:1)					50-59 (Cepu) Ethanol/water (8:2)				
	SS	DF	MS	*F*	*p*	SS	DF	MS	*F*	*p*
BG	15006.41	5.00	3001.28	322525.91	0.00	15006.41	5.00	3001.28	322525.91	0.00
WG	0.11	12.00	0.01			0.11	12.00	0.01		
Total	15006.53	17.00				15006.53	17.00			
Source	60-69 (Cepu) Petroleum ether					60-69 (Cepu) Acetone/water (9:1)				
	SS	DF	MS	*F*	*p*	SS	DF	MS	*F*	*p*
BG	15776.69	5.00	3155.34	316589.16	0.00	18979.60	5.00	3795.92	99023.98	0.00
WG	0.12	12.00	0.01			0.46	12.00	0.04		
Total	15776.81	17.00				18980.06	17.00			
Source	60-69 (Cepu) Ethanol/water (8:2)					70-79 (Cepu) Petroleum ether				
	SS	DF	MS	*F*	*p*	SS	DF	MS	*F*	*p*
BG	13367.79	5.00	2673.56	109372.80	0.00	15634.29	5.00	3126.86	220719.32	0.00
WG	0.29	12.00	0.02			0.17	12.00	0.01		
Total	13368.08	17.00				15634.46	17.00			
Source	70-79 (Cepu) Acetone/water (9:2)					70-79 (Cepu) Ethanol/water (8:2)				
	SS	DF	MS	*F*	*p*	SS	DF	MS	*F*	*p*
BG	18214.21	5.00	1695.34	294701.81	0.00	15280.80	5.00	3056.16	134172.98	0.00
WG	0.15	12.00	0.01			0.27	12.00	0.02		
Total	18214.36	17.00				15281.09	17.00			

* SS: sums of squares; DF: degree of freedom; MS: means of squares; *F*: the ANOVA test static; *p*: probability of the correlation's treatments, BG: between the groups; WG: within the groups were significant at 95% ($p \leq 0.05$)

Table 5. Antifeedancy (%) of Paper Discs with Addition of Extracts from Heartwood Teak after Being Exposed for 21 Days

Wood sample of teak (Age)	Extracts	Concentration (%, w/w)				
		2	4	6	8	10
30-39 (Purwakarta)	Petroleum ether	18.34	24.22	29.03	39.86	42.86
	Acetone/water (9:1)	33.33	43.88	45.98	48.15	50.37
	Ethanol/water (8:2)	17.64	20.48	21.21	19.76	24.22
40-49 (Purwakarta)	Petroleum ether	22.69	29.03	32.45	33.33	36.98
	Acetone/water (9:1)	38.88	49.36	53.84	55.03	66.67
	Ethanol/water (8:2)	18.34	23.45	25.00	28.20	32.45
50-59 (Purwakarta)	Petroleum ether	38.02	40.84	39.86	42.86	43.99
	Acetone/water (9:1)	40.84	42.85	55.04	81.82	100.0
	Ethanol/water (8:2)	21.95	23.53	25.00	31.58	33.33
50-59 (Cepu)	Petroleum ether	25.00	25.78	36.98	42.18	55.04
	Acetone/water (9:1)	36.98	42.86	42.96	47.06	50.49
	Ethanol/water (8:2)	29.03	32.45	33.33	35.96	36.05
60-69 (Cepu)	Petroleum ether	60.00	68.07	68.35	66.81	70.94
	Acetone/water (9:1)	53.85	62.60	66.67	100.0	100.0
	Ethanol/water (8:2)	33.33	34.32	37.93	40.94	41.94
70-79 (Cepu)	Petroleum ether	74.67	75.13	75.44	75.44	75.59
	Acetone/water (9:1)	43.88	66.67	80.18	100.0	100.0
	Ethanol/water (8:2)	36.89	36.98	36.98	39.86	40.84

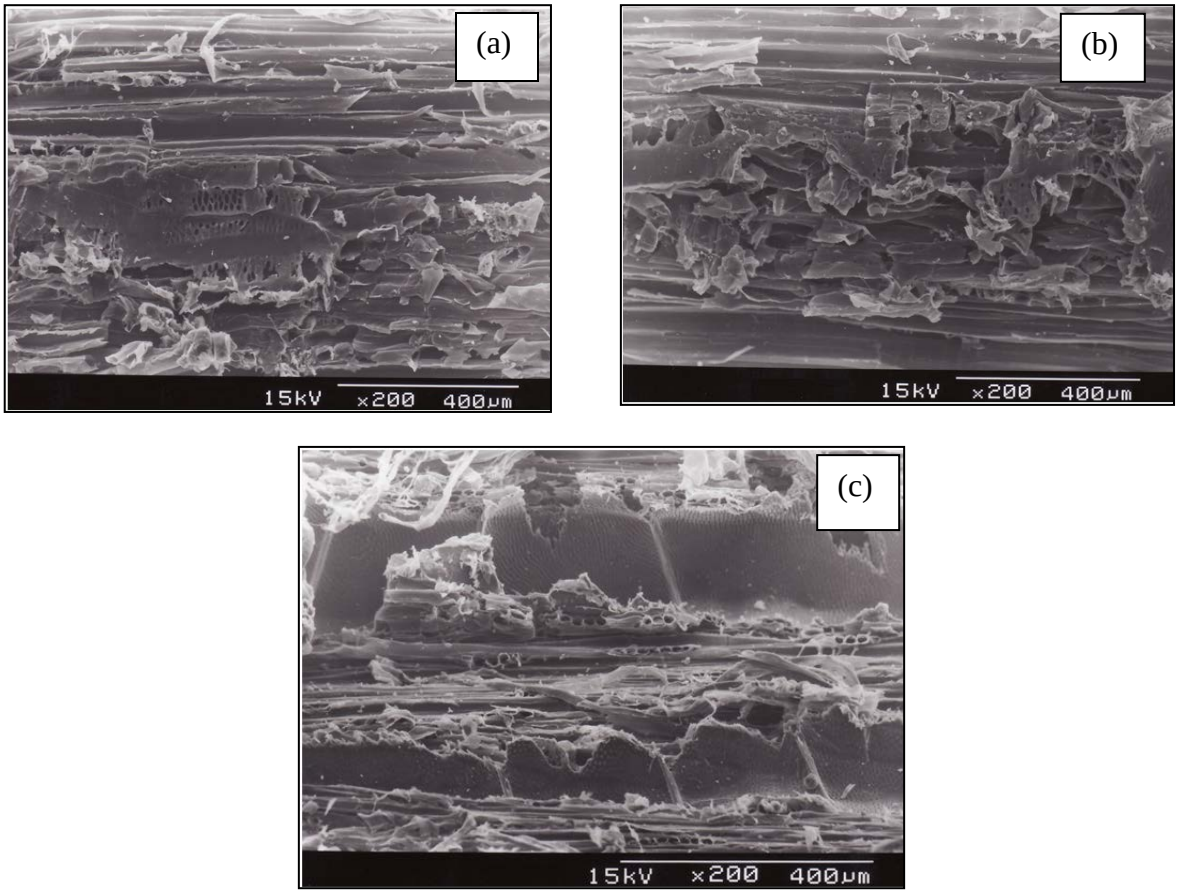

Figure 2. SEM images of (a) petroleum ether, (b) acetone:water (9:1), (c) ethanol:water (8:2) fractionated teak wood samples

Table 6. Summary of Results from Analysis of Variance (ANOVA) for % Antifeedancy

Source	30-39 (Purwakarta) Petroleum ether					30-39 (Purwakarta) Acetone/water (9:1)				
	SS	DF	MS	*F*	*p*	SS	DF	MS	*F*	*p*
BG	1281.95	4.00	320.49	46135.27	0.00	528.08	4.00	10.29	75296.93	0.00
WG	0.07	10.00	0.01			0.18	10.00	60.07		
Total	1282.01	14.00				528.26	14.00			
Source	30-39 (Purwakarta) Ethanol/water (8:2)					40-49 (Purwakarta) Petroleum ether				
	SS	DF	MS	*F*	*p*	SS	DF	MS	*F*	*p*
BG	61.22	4.00	15.30	7063.78	0.00	341.67	4.00	85.42	10748.91	0.00
WG	0.02	10.00	0.00			0.08	10.00	0.01		
Total	61.24	14.00				341.75	14.00			
Source	40-49 (Purwakarta) Acetone/water (9:1)					40-49 (Purwakarta) Ethanol/water (8:2)				
	SS	DF	MS	*F*	*p*	SS	DF	MS	*F*	*p*
BG	1184.15	4.00	296.04	4869.02	0.00	338.18	4.00	84.55	17912.21	0.00
WG	0.61	10.00	0.06			0.05	10.00	0.00		
Total	1184.75	14.00				338.23	14.00			
Source	50-59 (Purwakarta) Petroleum ether					50-59 (Purwakarta) Acetone/water (9:1)				
	SS	DF	MS	*F*	*p*	SS	DF	MS	*F*	*p*
BG	72.83	4.00	18.21	279.93	0.00	8016.42	4.00	2004.11	1015593.82	0.00
WG	0.65	10.00	0.07			0.02	10.00	0.00		
Total	73.48	14.00				8016.44	14.00			
Source	50-59 (Purwakarta) Ethanol/water (8:2)					50-59 (Cepu) Petroleum ether				
	SS	DF	MS	*F*	*p*	SS	DF	MS	*F*	*p*
BG	307.49	4.00	76.87	21118.58	0.00	1868.29	4.00	467.07	88909.95	0.00
WG	0.04	10.00	0.00			0.05	10.00	0.00		
Total	307.52	14.00				8016.44	14.00			
Source	50-59 (Cepu) /Acetone/water (9:1)					50-59 (Cepu) Ethanol/water (8:2)				
	SS	DF	MS	*F*	*p*	SS	DF	MS	*F*	*p*
BG	303.65	4.00	75.91	5274.13	0.00	98.70	4.00	24.68	8336.43	0.00
WG	0.14	10.00	0.01			0.03	10.00	0.00		
Total	303.79	14.00				98.73	14.00			
Source	60-69 (Cepu) Petroleum ether					60-69 (Cepu) Acetone/water (9:1)				
	SS	DF	MS	*F*	*p*	SS	DF	MS	*F*	*p*
BG	199.13	4.00	49.78	206.75	0.00	5675.74	4.00	1418.94	19685.57	0.00
WG	2.41	10.00	0.24			0.72	10.00	0.07		
Total	303.79	14.00				5676.46	14.00			
Source	60-69 (Cepu) Ethanol/water (8:2)					70-79 (Cepu) Petroleum ether				
	SS	DF	MS	*F*	*p*	SS	DF	MS	*F*	*p*
BG	179.83	4.00	44.96	4670.09	0.00	1.39	4.00	0.35	29.60	0.00
WG	0.10	10.00	0.01			0.12	10.00	0.01		
Total	179.93	14.00				1.50	14.00			
Source	70-79 (Cepu) Acetone/water (9:2)					70-79 (Cepu) Ethanol/water (8:2)				
	SS	DF	MS	*F*	*p*	SS	DF	MS	*F*	*p*
BG	6781.36	4.00	1695.34	584599.79	0.00	38.01	4.00	9.50	89.22	0.00
WG	0.03	10.00	0.00			1.07	10.00	0.11		
Total	6781.39	14.00				39.08	14.00			

* SS: sums of squares; DF: degree of freedom; MS: means of squares; *F*: the ANOVA test static; *p*: probability of the correlation's treatments, BG: between the groups; WG: within the groups, were significant at 95% ($p \leq 0.05$)

This finding suggests the more polar eluted extracts have stronger activity against termites. Thus, the strongest activity against termites was exhibited by the acetone/water (9:1) extract. The ANOVA results for % antifeedancy are given in Table 6.

Surface Morphology of Extracted Wood

Scanning electron microscopy was used to observe the transverse sections of dried petroleum ether, acetone:water (9:1), and ethanol:water (8:2) fractionated wood samples. In contrast to petroleum ether extracted samples, the acetone:water and ethanol: water extracts exhibited smooth surfaces (Fig. 2). The apparent difference between the samples can be attributed to dissolution of components from extracted samples.

CONCULSIONS

1. Antifedancy tests were conducted to evaluate the antitermitic activity of extracts from teak wood of different ages and origins against the termite *C. curvignathus.* Results showed that different extracts exhibited different degrees of termite mortality. Among the different extracts, acetone/water extracts possessed the highest mortality rate of termites.
2. The SEM results revealed smoother surface after extraction was carried out.

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