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Research Article

CHEMICAL COMPOSITION AND TERMICIDAL ACTIVITY OF *SCORODOCARPUS BORNEENSIS* BECC BARK EXTRACTS

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ABSTRACT

The chemical compositions of the bark extracts of *Scorodocarpus borneensis* plant extracted successively with ethanol, n-hexane and ethyl acetate was analyzed by GC/GCMS. The preliminary phytochemical screening conducted on the crude bark extracts revealed the presence of alkaloids, triterpenoid, phenolic, flavonoids, steroid and saponin which are known to support the bioactive activities of the plant in folk medicine. The anti-termite activity of *S.borneensis* of the two extracts from different solvents against *Coptotermes curvignathus* was evaluated in the present research study. It was found that the ethyl acetate extracts are more toxic than that of n-hexane extract against the *C. curvignathus*. The result showed that ethyl acetate and n-hexane extracts exhibited termite mortality with LC50 values 0.013% and 0.029%. Anti-feedant index of *S.boornensis* extract on *C. curvignathus* was shown that index the n-hexane extract and ethyl acetate *C. curvignathus* were varied from 0.209% to 14.041%. No difference anti-feedant index category for two extract, suggested that the n-hexane and ethyl acetate fraction delivered as toxic to subterranean termites. We hereby report for the first time the major compounds from the n-hexane extracts of *S.boornensis* to be 9-octadecenoic acid (36.50%), hexadecanoic acid (16.68%) methyl 9 octadecenate (15.39%) and octadecanoic acid (4.00%) and the ethyl acetate extract showed the existence of beta-methyl glucocide (23.40%), hexadecanoic (15.56%),1, 2-benzenadicarboxylic acid, bis (2-ethyl (hexyl) ester(12.34%), 9-hexadecenoic acid (12.72%), 3-phenyl-2-propenoicacid (7.53%), bezophenone (6.64%), 5-oxymethyl furfurole (4.78%), 9, 12-octadeca-dienoic acid (3.17%), beta-D-mannofuranoside (2.75%), methyl-m-dioxane (2.23%), hexadedioic acid (2.11%) respectively; all of which contribute individually and or synergistically to the biological activities reported in this study.

Keywords: *Chemical compositions, Scorodocarpus borneensis,termiticidal activity, GC MS*

1. INTRODUCTION

The subterranean termites *Coptotermes curvignathus* (*C.curvignathus*) was widely destructive polyphagus insect pest, which have a wide distribution and has caused the destruction of forestry, agriculture, plantation and wooden buildings. A very large number of tree species has been reported to be attacked by *C.curvignathus*, including conifers, monocotyledonous and dicotyledonous plants [1-3]. In agriculture, it is a serious pest of oil palm (*Elaeis guineensis*) grown on peat soils in Malaysia [4] and coconut grown on peat soils in Indonesia [5]. It is also reported to be a serious pest of rubber (*Hevea brasiliensis*), although on land that has been long planted and replanted with rubber, the incidence of attack appears to lower. Nandika [3] have estimated economic losses due to termite damage by attack wooden buildings in all parts of the Indonesia reaches reached at least US $1 billion in 2015. Subterranian termite control is very important to protect plantations, structure and its components, commonly used antitermite which is a synthetic compounds such as chlorodane, cypermethrin, hydroquinone, and indoxacarb have been used. But all such synthetic pesticides are highly poisonous and kill nontarget organisms. Due to their longer residual persistence in the environment, these have been banned and new alternatives are discovered in form of natural pesticides.

Replacement of synthetic insecticides with bio-pesticides is a universal acceptable and practical approach worldwide [6]. The plants are the reservoir of chemical compounds that are very diverse and has a lot of untapped potential. Higher plants are a rich source of novel natural substances that can be used to develop environmental safe methods for insect control. One of these uses is in agriculture to manage pests with less risk than with synthetic compounds that are toxicologically and environmentally undesirable.

Mechanisms of toxicity of phytochemical compounds or crude extracts of plants against insects is manifested in several ways, such as changing behavior, as repellent substance, inhibiting growth, oviposition deterrence, inhibition of eating, reduction in fecundity and fertility, fumigation activity, mulch barrier [7-11].

Higher plants produce a broad spectrum of secondary metabolites including polyphenols, tannins, quinones, alkaloids, essential oils, sterols, saponins etc. and these phytochemicals show diverse biological properties as insecticides/pesticides. Toxicity to termites depends on the

type of the phytotoxicant, its maximal toxicant transfer, exposure time and toxicant recipient ratios [12].

Many plants have shown as anti-termite [13] or termite repellent such as *Eucalyptus* oil [14]. One of the more interesting plants we had been working with regards to their chemistry and bioactivity is from *S. borneensis* (Olacaceae) [15]. This plant species is family Olacaceae members, who together about 30 genera and 250 species are distributed throughout tropical and subtropical regions. *Olax scandens* Roxb., *Anacolosa griffithii* Mast., *Ochanostachys amentacea* Mast., *Scorodocarpus borneensis* Becc., *Strombosia philippinensis* (Baill) Rolfe and *Ximenia americana* L. are medicinal plants in the Asia-Pacific region. Olacaceae is known as a producer of tannin, glycosida cyanogenetic, polyacetylenic fatty acids, flavonoids and a series of polysulfide compounds [16]. This plant have been reported has content methyl disulfide, some megastigmanes and flavonoids and sulfur-containing compounds from the leaves and fruit [17-19] and some sesquiterpenes and alkaloids from the fruit and bark [20]. To the best of our knowledge, phytochemical profile and efficacy of the plant extract towards wood-degrading termites have not been reported. Therefore, here we reported the potential of *S. borneensis* bark extract as termicidal agent.

2. MATERIALS AND METHODS

2.1. Plant materials

Garlic tree *S.borneensis* was obtained from the Botanical Gardens of Mulawarman University Samarinda, East Borneo. Specimens were identified by applying key plant taxonomy in Biodiversity Laboratory, Department of Biology, Faculty of Science, Mulawarman University. Sample of the collected plant was cut into pieces and dried at room temperature in the laboratory for 2 weeks. Once sample dried, plant parts were separated and smoothed with a uniform texture, measuring 3 mm using an electric mill and then milled. The products were sieved with a sieve sized of 40-60 mesh.

2.2. Extraction of plant materials

The dried powder of stem bark of *S. borneensis* (2.5 kg) was macerated with 95% ethanol in a glass container with a ratio of 1:1. After maceration (7 d), the extracts were filtered using Whatman No. 1 filter paper. The filtrate then was concentrated to dryness by a rotary evaporator and dried in a fume hood until dried crude paste was formed. The yielded extract was brownish (20.50 g, 0.82%) and then tested for phytochemical, followed by fractionation processes with solvents n-hexane and ethyl acetate. The fractionation was diluted with distilled water to be used for a further concentration of the anti-termite material.

2.3. Termites Bioassay

2.3.1. Test Termites

A termite subterranian colony (*C.curvignathus*) was collected from Red Meranti trees, *Shorea leprosula* Miq. attacked by termites in Dipterocarps Forest Arboretum Research Center Samarinda, East Borneo. Termites were identified to species level using the keys and literature provided by Tarumingkeng [21]. Termites were collected and maintained in the aquarium vessel under laboratory conditions with temperature of 25-30°C and humidity of 70-90%. Termites fed with pieces of wet Shorea wood and maintained for 5 days and then ready to be used for bioassay activities.The termites were not feeding for 24 hour prior to the test.

2.3.2. Bioassay Test

Acute toxic and antifeedant responses in termites were evaluated according to Ohmura [22] with some modifications. No-choice bioassay method was used to evaluate the termicide activity of bark garlic tree extractives. Whatman no 1, 8.5 cm in diameter and thickness 1.5 mm filter paper was treated with solution of 0.00, 0.025, 0.025, 0.062, 0.154, 0.387 and 0.972% (w/v) of extractives. This was done by soaking the filter paper overnight in the differentt concentrations of extractive and drying at 80°C to remove the solvent. A piece of filter paper treated with the solvent only was used as the control.

Furthermore, termites removed from logs were maintained, each containing 50 healthy termites consisted of 45 worker and 5 soldier termites were placed onto each filter paper impregnated with the test material in glass bottles 10 cm in diameter with a hard plaster bottom. The test dishes with perforated cover, sterilized sand and filter paper were placed in a moist place to keep the humidity inside the vessel. All the test dishes containing termites was placed in a dark room and kept in a temperature of 25- 27°C and humidity of 75-95%. A few drop of water were periodically added to the bottom edge of each test dishes. Five replications were made for each extract treatment, the percentage of termite mortality was periodecally observed (1, 3, 6 days).

At the end of test period, mortality was calculated for 6 days at each extract concentration variations. The paper discs were taken out from the glass bottles after 6 days and cleaned, oven dried and re-weighed. Termite mortality (percent) and weight loss (percent) of the paper discs were recorded using formula as per the following equations [22].

Termite mortality (%) = No. of dead termites / Total no of test termites x 100% (1)

Correction on rate of mortality = 1 – {(n in T group after treatment/n in group C after treatment) x 100%}; where n = total of termites, T = treatment, C = control (2)

The lost weigth of bait, calculated following equation:

$$\text{Weight loss (\%) of paper disc} = (W1\text{-}W_2/\ W_2) \times 100 \quad (3)$$

Where, W_1 is the weight loss of untreated paper discs (g) and W_2 is the weigth loss of treated paper discs after the termicidal test (g). On the basis of the weight losses of the discs, the indices of the activity of the extracts were calculated.

The absolute coefficient of anti-feedancy (A) was obtained by the following equations [23].

$$A = [(KK - EE) / (KK + EE)] \times 100 \quad (4)$$

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). (29).

Table 1: Indicators to Evaluate the Antifeedant Activity of Extracts

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

2.4. Statistical analyses

Determination of the concentration (% w/v) any extract that could kill as many as 50% of the test termites was done by using probit analysis with log concentration probit mortality [24], in a span of 6 days after treatment. With a 95% confidence interval, values and degrees of freedom of the X^2 values of suitability test (X^2 goodness of fit test) and the regression equation can be determined. If the value of X^2 goodness found significant ($p<0.05$), heterogenity correction factor was used to calculate the confidence limits. Biological data and the death of an effective concentration to kill termites was subjected to analysis of variance and the differences between treatment concentration was determined by Duncan's Multiple Range Test (DMRT) at $p<0.05$. Statistical analysis was performed using SPSS (SPSS Inc., USA) version 21.

2.5. Phytochemical constituent identification using GC-MS

The extracts of stem bark *S.borneensis* (1 g) was loaded on a silica gel column packed with eluted by a mixture of chloroform : methanol : water (7:3:1). The absorbance of the fractions eluted from the column chromatography was measured at a resolution from 254-366 nm using UV-Visible spectrophotometer and the readings were recorded. The fractions that were eluted in column chromatography using chloroform and ethyl acetate (2:8) was subjected to GC-MS equipped with a Shimadzu QP2010 GC/MS apparatus (Shimadzu Corp., Japan) to find out the active principle of the extracts.

The chemical constituent of extracts was analyzed by a GC/MS apparatus (Shimadzu QP2010S, Shimadzu Corp, Japan) equipped with Ri-5MS column Rastex capillary (30 m, 0.25 mm, ionization: EI 70 Ev), heated at temperature of 80°C, pressure of 16.5 kPa, total flow of 20.0 ml/min, column flow of 0.50 ml/min, linear velocity 26.1 cm/sec, purge flow 3.0 ml/min, injector and detector temperature was set at 310°C, using helium as the carrier gas, at a rate of flow 1.0 ml/min.

The spectrum of the unknown component was compared with the spectrum of the known components, showed in the mass spectra and retention indices of GC using reference compounds database from the National Institute of Standards and Technology (NIST) which had more than 62,000 component spectrum patterns. Based on that comparisons,the name of molecular weight and structure of the components of the test material can be identified.

3. RESULTS

3.1. Yield Extracts

The total yields of extractives obtained from parts of garlich tree with different solution extraction are shown in Table 2. On oven-dried basis, the total ethanol extraction obtained from the stem was 0.82%, 0.13 % for hexane extract and 0.30% for ethyl acetate.

Table 2: Content of Extractives of garlic tree, S.borneensis as Obtained by Successive Extraction

Sample Extract	Yield (%, w/v)			
	Crude	n-hexane	ethyl acetate	residual
Bark	0.82	0.13	0.30	0.37

3.2. Characteristic Constituent of Phytochemical Extract

Results of phytochemical screening (Table 3) revealed that flavonoids, steroids and saponins were positive in the crude bark extract of the garlic tree. In the hexane fraction, alkaloids, phenolic and triterpenoids were detected while alkaloids, phenolics and steroidal were also detected in the fraction of ethyl acetate.

Table 3: Some phytochemicals compounds detected in various fractions of the extract of S. borneensis

Phytoconstituents	Material		
	Crude extract	Ethyl acetate fraction	n-hexane fraction
Alkaloid	-	+	+
Phenolic	-	+	+
Flavonoid	+	-	-
Triterpenoid	-	-	+
Steroid	+	+	-
Saponin	+	-	-

+ detected; - not detected

3.3. Antitermitic Activity

The results for antitermite activity are shown in Table 4. The test results on the effectiveness of killing power indicated by the acute toxicity (LC_{50}) on the 6th days at different levels against subterranean termites. From this table, it appears that the toxicity values on termites closely associated with increased concentrations and different types of solvent.

Table 4: Mean mortality rate of *C.curvignathus after treatment* at 6 six days of bark garlic tree extracts

Extract	Concentration (% w/v)	Mean± Std. Error	df	F value	Sig.
Ethyl acetate	Control	8.250±0.85[a]	df_1=5	59.995	0.000
	0.025	21.250±4.028.[b]	df2=18		
	0.062	25.250±1.931[b]			
	0.154	36.750±2.688[c]			
	0.387	50.000[d]			
	0.974	50.000[b]			
N-Hexane	Control	9.00±1.354[a]	df_1=5	227.94	0.000
	0.025	17.25±2.175[b]	df2=18		
	0.062	38.25±1.031[c]			
	0.154	50.00[d]			
	0.387	24.25±0.479[e]			
	0.974	50.00[e]			

3.4. Determination of the lethal concentration (LC_{50}) for the different extract

The lethal concentration (LC_{50}), the concentration required to kill 50% of worker termite due to different extracts at different concentration were showed at Table 5. The lethal concentration (LC_{50}) of ethyl acetate extract of the bark at 0.013% (w/v), followed by n-hexane extract of bark at 0.029% (w/v) for 6 days exposure.

Table 5: Median lethal concentration (LC50) values [%(95% fiducial limits)] at 6 six days of bark garlic tree extracts againts of worker *C. currvignathus*

Extracts	LC_{50}	Chi square	Slope±SE	Intercept
Ethyl acetate	0.013	30.781	0.942±3.810	1.790
n-Hexane	0.029	20.057	3.674±0,556	5.637

3.5. Antifeedant Effect

The results of the effect of garlic tree extract on the activity antifeedant on the subterranean termites, *C.curvignathus* shown in Table 6.

Table 6: Antifeedancy Index (%) of Paper Discs with Addition of Extracts from Bark of the Garlic Tree, *Scorodocarpus borneensis*

Extracts	Concentration (%)	Mean Loses of Bait Paper (gram)	Antifeedant Index A (%)	Degree of antifeedant
Ethyl acetate	0.00	0.095		
	0.025	0.086	4.884	Low
	0.062	0.076	6.451	Low
	0.154	0.070	3.749	Low
	0.387	0.068	1.655	Low
	0.972	0.058	7.891	Low
n-Hexane	0.00	12.04		
	0.025	11.94	0.417	Low
	0.062	11.89	0.209	Low
	0.154	10.52	6.227	Low
	0.387	10.19	7.907	Low
	0.972	9.00	14.041	Low

Degree of antifeedant (A) : 75 ≤ A < 100 : Very Strong; 50 ≤ A < 75 : Strong; 25 ≤ A < 50 : Midle; 0 ≤ A < 25 : Low

From the Table 6 showed that the weight loss of the bait is consumed depends on the fractions and concentration of garlic tree, *S. borneensis*. The antifeedant index in the n-hexane extract and ethyl acetate was between 0.209% and 14.041%. In the bark extract treatment showed weight loss less bait and circumstances indicate that the extract is antifeedant against subterranean termites, *C.curvignathus* in the low category. The mortality trend indicated that termites avoided the filter paper

treated with bark extract. No difference antifeedant index category for two extract, this condition is presumably closely related to the content of bioactive compounds present in the extracts and food additives that are toxic to subterranean termites.

3.6. GC-MS Analyses

The gas chromatography profile of garlic tree bark extract was displayed in Fig. 1 and 2.Compound mass spectrometry identification results are presented in Table 7. From Table 7, are showed of percentage of some main compounds found a lot in different extracts. In the ethyl acetate extract of the bark, the largest contents were beta-methyl glucocide(23.40%), hexadecanoic, methylester(15.56%),1,2-benzena dicarboxylic acid, bis(2-ethyl(hexyl) ester(12.34%), 9-hexadecenoic acid (12.72%), 3-phenyl-2-propenoicacid(7.53%), bezophenone(6.64%), 5-oxymethyl furfurole (4.78%), 9,12-octadecadienoic acid, methyl ester(3.17%), beta-D-mannofuranoside (2.75%), methyl-m-dioxane(2.23%), hexadedioic acid (2.11%).The n-hexane extract of the bark, the main compounds detected were 9-octadecenoic acid (ethyl oleate) (36.50%), hexadecanoic acid, methyl ester (16.68%), methyl 9 octadecenate (Methyl oleate) (15.39%), octadecanoic acid, ethyl stearate (4.00%), progesterone (3.56%), stigmasta-4 ,22-dien-3-one (2.43%) and 1.2 benzenodicarboxylic acid, ester dioctyl (phtalat acid) (1.34%).

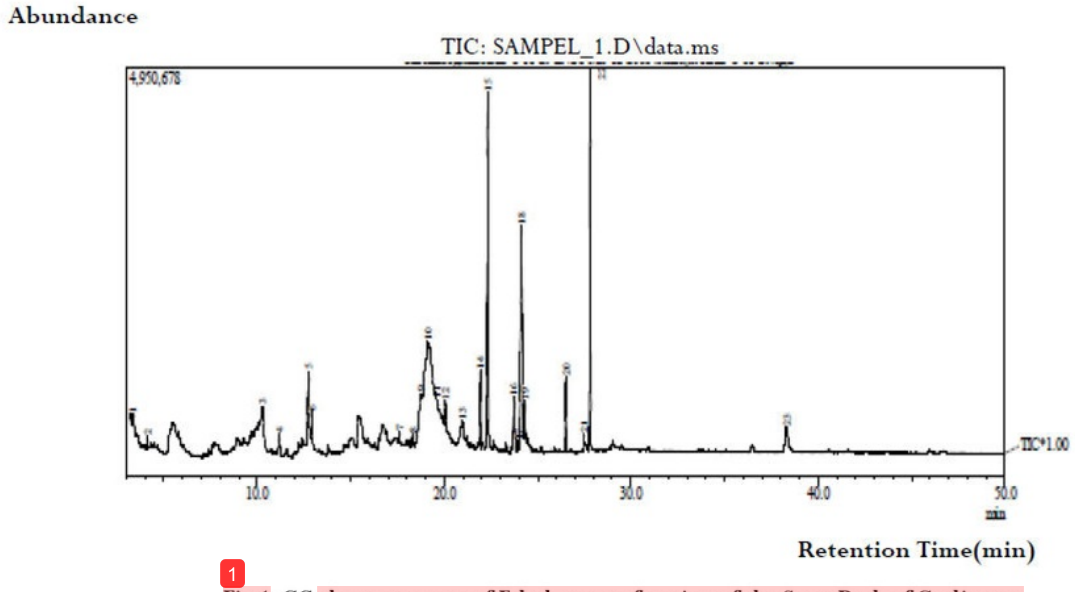

Fig.1: GC chromatogram of Ethyl acetate fraction of the Stem Bark of Garlic tree

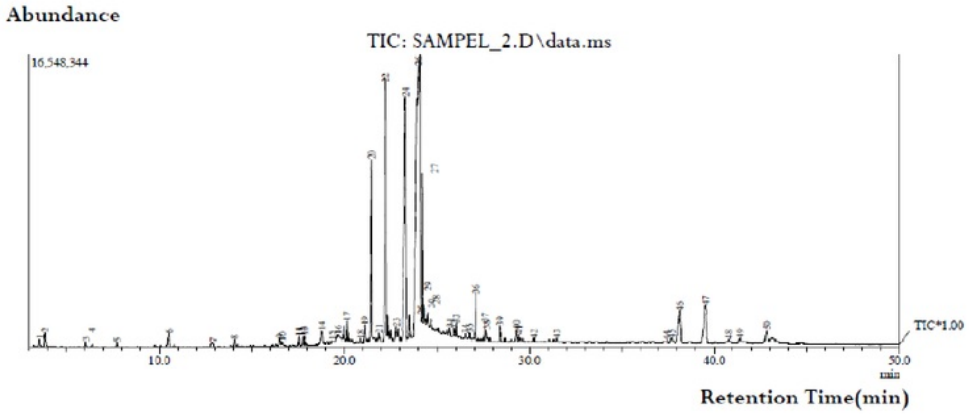

Fig.2: GC chromatogram bark of *Scorodocarpus borneensis* hexane extract

Table 7: Identified compounds and the quantity (% peak area) from the extracts of *S. borneensis* bark

No	Retention time (min)	Compound *)	% Peak area	
			n-hexane	Ethyl acetate
1	3.322	1-Chlorethyl acetate		1.15
2	10.294	Methyl-m-Dioxane		2.23
3	11.184	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one		1.18
4	12.750	5-Oxymethylfurfurole		**4.78**
5	12.923	Ethyl 2,4-hexadienoate		1.67
6	14.387	3-Phenyl-2-propenoic acid		**7.53**
7	18.750	Benzophenone		**6.64**
8	19.125	Beta-methyl-glucoside		**23.48**
9	19.592	6-Methyl-6-(3'-methyl-3'-isopropenylcycloprop-1'-en-1'-yl)-heptan-2-one		1.29
10	20.968	Beta -D-Mannofuranoside, methyl-		2.65
11	21.926	Hexadecanoic acid, methyl ester		2.13
12	22.324	Hexadecanoic acid, methyl ester	**16.68**	**15.56**
13	23.300	Methyl 9 octadecenate	**15.39**	
14	23.700	9,12-Octadecadienoic acid, methyl ester		**3.17**
15	23.858	9-octadecenoic acid	**36.50**	
16	24.127	9-Hexadecenoic acid		**12.72**
17	24.225	Octadecanoic acid, ethyl stearate	**4.00**	
18	24.300	Octadecanoic acid		1.62
19	24.326	1-Nonadecanol	1.08	
20	26.490	Hexanedioic acid, dioctyl ester		2.11
21	27.075	1,2 Benzenodicarboxylic acid,dioctyl ester	1.34	
22	27.810	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester		**12.34**
23	38,140	Stigmasta-4,22-dien-3-one	2.43	
24	39.525	Progesterone	**3.56**	

*)*Abundant component indicated in bold letter (> 3%)*

4. DISCUSSION

Discovery of botanical termicide has been recently emphasized as a potential method for development of ecological safe pesticides. According to Isman *et al.*[25], plants are source rich of organic chemicals on earth. In nature, many plants have unpalatable substances like high content of phenols, alkaloids, flavonoids, terpenes, quinone, coumarin, etc, which play a defensive role againts insect pests. These substances posses wide range of biological activities including antifeedant, oviposition deterrent, insecticidal, ovicidal and Insect Growth Regulators (IGRs). The extracts of *S.borneensis* show the presence of major phytochemical groups responsible for insecticidal activity (Table.7).

In the present study, acute toxicity (LC_{50}) of the bark extracts of garlic tree *S. borneensis* on the termite *C. curvignathus are* 0.013 % for ethyl acetate extract and 0.029% for hexane extract. The results of termite mortality could be the reaction of termites to the toxic, anti-feeding and / or repellent effects (30). In other words, wood extracts are one of the factors that increase the termite mortality.

On the other hand, the results, evidently indicate that *S. borneensis barks* extractives contain biologically active compounds that were potent to *C. curvignathus*. Ethyl acetate extract apparently was a better solvent in extracting the toxic chemical compounds followed by hexane extracts. Ethyl acetate extract completed the mortality of *C. curvignathus* at the concentration of 0.154 % compared to hexane was 0.387% extract at 6 days.

This result demonstrated that toxicity of ethyl acetate extract bark *S.borneensis* termite relatively strong. The different effect of mortality of the extracts could be due to the presence of amounts of secondary metabolites i.e., triterpenoids, alkaloids and phenolics in these extracts which had termicidal effects (Table 7). Extractive content variation obtained was influenced by the kinds of compounds contained in samples and the solubility of these compounds in the used solvents.

This result, supported by Golpayegani et al. [26] in their study on mulberry wood (Morus alba) extractives against *Reticulitermes flavipes* also found that MeOH is the second best solvent besides acetone that give a low termite survival. On the other hand, Syofuna et al. [27] reported that different

compounds obtained from different solvents will show a different effect towards termite resistance. In conclusion of this study, to understand the natural durability of wood against termites, we can't just focus on a single compound alone, but this resistance is a combination of several compounds that are present in the wood.

Many of the identified compounds contribute to different extent to the toxicity of the fraction extract on termite. Decanoid acid is a carboxylic acid that distinctive smell.The smell is thought to stimulate the nerve, so it can attract or repel insects to come. This is in concomintant with the result of Yoshida et al. [28] who reported Japanese commercial termicide against termite *C.formosanus*.

The presence of hexadecanoic acid indicated that the bark extracts might be used as potential pesticide against insects [29, 30]. As far we are aware, this first time an attempt that has been made to describe the toxicity of bark extract from garlic tree bark on the termite. The main compounds found in these extracts are likely to be responsible for toxicity on the termite. In general, all the fraction of garlic tree bark extract showed termicidal activity in comparasion to their respective solven controls. The findings of the present study indicated that the garlic treen bark extracts possessed termicidal properties against *C.curvignathus*.

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