**POTENTIAL n-HEXANE AND ETHYL ACETATE FROM GAHARU (*Aquilaria malaccensis*) FOR CONTROLLING SUBTERRANEAN TERMITE *Coptotermes curvignathus***

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**INTRODUCTION**

The problem of controlling termites has not been solved until now due to the fact that current termiticides are very effective at low doses, but are not selective to target organisms, contaminate water and water sources and are difficult to decompose in the soil (Nandika et al. 2015). Gaharu leaves (*Aquilaria malaccensis*) are potentially as natural antitermite (Dash et al. 2008). This ability is supported by the content of secondary metabolite compounds in the extract (Huda et al. 2009; Khalil et al. 2013). However, prior studies had not yet reported the optimal results, especially for controlling the termites attack in buliding. This study aimed to compare and analyze the effectiveness of two active compounds of gaharu leaves extracts (n-hexane and ethyl acetate) conducted using nanoparticle technology for controlling *Coptotermes curvignathus.* Therefore, this is expected that the results of this study can be used as an environment-friendly termite controlling solution and have better capability than synthetic chemicals.

**MATERIALS AND METHODS**

**Sample preparation and nanoparticle processing**. Five kg of gaharu leaves (*A. malaccensis*) were extracted and partitioned using soxhlet method to obtain methanol, n-hexane, and ethyl acetate fraction. These fractions were tested qualitatively by phytochemical screening methods. Then, Nanoparticles of both n-hexane and ethyl acetate were prepared through ionic gelation method (Xu et al. 2003). Each nanoparticle was analyzed morphologically using Scanning Electron Microscopy (Fujita et al. 1971)and Particle Size Analyzer (Burgess et al. 2004).

**Anti-termite Activity Test.** The feed paper was immersed in a nanoparticles solution for 1 hour with 0% (negative control), 2%, 4%, 6%, 8%, and 10% (v/v) concentration. Positive control used was fipronil with concentration of 0,25% (2,5 mL in 1 L of water), whereas negative control was solvent only. Termiticidal activity and repellent effect of these two nanoparticles were evaluated by no-choice feeding test with *C. curvignathus* (Ohmura et al. 2000). Four replicates were made for each concentration, and the termite mortalities were measured daily for 7 days.

**Data Analysis**. The termite mortality data were analyzed using ANOVA and continued with tukey test. The value of LC50 was determined using the regression line equation between the concentration log and the probit analysis.

**RESULTS AND DISCUSSION**

Methanol extract, n-hexane and ethyl acetate fraction of gaharu leaves had the same contain: alkaloids and tannins. Table 1 Showed the secondary metabolic compounds found in gaharu leaves extracts.

Table 1. The Phytochemical Screening Result of Gaharu Leaves Fraction

|  |  |  |  |
| --- | --- | --- | --- |
| Test | Pure methanol extract | Fraction | |
| n-hexane | Ethyl acetate |
| Alkaloid | + | + | + |
| Polyphenol/Tannin | + | + | + |
| Flavonoid | + | + | - |
| Saponin | - | + | + |
| Steroid | - | + | + |
| Triterpenoid | + | - | - |

Nanoparticles of n-hexane had the higher activity against *C. curvignathus* than nanoparticles of ethyl acetate and methanol extract (Table 2). It needed relatively low concentration to obtain the perfect mortality. n-hexane nanoparticles of 4% concentration was needed to achieve 100% mortality. Positive control (0.25% fipronil) caused 100% mortality on the first day. Fipronil was more effective and efficient, but nanoparticles had a lot of advantages such is a not toxic, stable during use and wide surface area (Agnihotri et al. 2004). The average size of n-hexane and ethyl acetate nanoparticles was lower than 300 nm (16.3 nm and 26.6 nm respectively), so it can penetrate into termite body cells easily (Mohanraj & Chen 2006).

Table 2. Mortality of *C. curvignathus* After 7 Days of Termiticidal Activity Test

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Extract | Average Mortality of Termites (%) at Concentration (%) | | | | | |
| 0 | 2 | 4 | 6 | 8 | 10 |
| Methanol | 10±3 | 30±3 | 48±1.73 | 54.33±2.3 | 60±3 | 80±3 |
| N. Ethyl acetate | 9±7.2 | 76.67±3.51 | 86.67±3.51 | 92±1.73 | 96±0 | 100±0 |
| N. n-hexane | 12.33±5.03 | 89±1.73 | 100±0 | 100±0 | 100±0 | 100±0 |

The result of ANOVA and tukey test showed that the termite mortality at control (0%) was significantly different with the extract treatment (F= 3.116, df1,2= 17, 36; P< 0.005). The LC50 value of n-hexane nanoparticles against *C. curvignathus* was lower (0.11%) than ethyl acetate nanoparticles (0.88%) and methanol extract (5.88%). However, the LC50 of both nanoparticles was higher than the LC50 of fipronil, 24.3 × 10-4% (Manzoor et al. 2012). The bioactivity of n-hexane nanoparticles was higher than ethyl acetate nanoparticles because it contained classes of other compounds other than steroids that reinforce its activity as anti-termite, such as flavonoids. Various pure compounds of flavonoids in which all compounds exhibit anti-feedant activity (Ohmura et al. 2000). Foods containing toxins from gaharu leaves extractive substances will be spread through trophalaxis so that it can cause mortality in a termite colony.

**Conclusion**

n-hexane and ethyl acetate nanoparticles of gaharu leaves extracts have potential to control *C. curvignathus*. Concentration of 4% n-hexane nanoparticles caused the higher mortality to *C. curvignathus* than ethyl acetate nanoparticles and at 0.11% of that caused 50% termite mortality.

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