

## **Termiticidal activity of an extract of *Brugmansia candida* leaves against a subterranean termite *Coptotermes gestroi* Wasmann and a drywood termite *Cryptotermes cynocephalus* Light**

Didi Tarmadi<sup>1,\*</sup>, S. Khoirul Himmi<sup>1,2</sup>, Sulaeman Yusuf<sup>1</sup>

<sup>1</sup> Research and Development Unit for Biomaterials, Indonesian Institute of Sciences (LIPI), Jl. Raya Bogor KM. 46, Cibinong, Bogor 16911, Indonesia

<sup>2</sup>Research Institute for Sustainable Humanosphere (RISH), Kyoto University, Gokasho, Uji, Kyoto 611-0011, Japan

### **Abstract**

Termiticidal activity of *B. candida* leaves against a subterranean termite *Coptotermes gestroi* and a drywood termite, *Cryptotermes cynocephalus* were conducted. The extraction of *B. candida* leaves resulted in three major fractions, n-hexane, ethyl acetate and aquadest. Bioassay against *C. gestroi* and *C. cynocephalus* were evaluated by a no-choice feeding test, JWPA no 12 1992 and forced-feeding test, respectively. The result of bioassay against *C. gestroi* and *C. cynocephalus* indicated that n-hexane fraction and ethyl fraction delivered higher termite mortality compared to the aquadest fraction. The results of soil treatment against *C. gestroi* also indicated that all fractions of *B. candida* were not able to meet proper efficacy. Weight loss of the samples was not significantly different between the three fractions in bioassay against *C. gestroi*, but the weight-loss results were significantly related to concentration. The weight loss of the samples after bioassay against *C. cynocephalus* suggested that the n-hexane and ethyl acetate fractions delivered moderate protection at the highest concentration tested, while the aquadest fraction delivered low efficacy.

Keywords: termiticidal activity, *Brugmansia candida*, *Coptotermes gestroi*, *Cryptotermes cynocephalus*.

### **Introduction**

Termites are widely considered destructive polyphagous insect pests, which largely damage house-hold materials, finished goods, plants and agricultural crops such as sugarcane, millet, barley and paddy (Elango et al. 2012). The Asian subterranean termite, *Coptotermes gestroi*, is a major pest in the Asia Pacific region, recognized as a destructive and economically important species (Scheffrahn et al. 2000). Another important termite species in the region is the Indo-Malaya drywood termite, *Cryptotermes cynocephalus*. Both termites cause serious damage to buildings and structures, estimated at more than \$400 million per year in Southeast Asia alone (Yeap et al., 2011).

Subterranean termite control is very important to protect structures and its components. The control options include wood treatments, soil barrier treatments, and population control using bait systems (Himmi et al. 2012). However, most termiticides are chemicals identified as Persistent Organic Pollutants (POPs) such as aldrin, coldrane, dieldrin, endrin, etc. (UNEP 2000). The utilization of plant extractives as natural preservatives is the appropriate solution along with the rising concern toward a sustainable green-environment. The effort on developing some suitable natural preservatives aims to reduce the use of chemicals-toxic pesticide (Tarmadi et al. 2010). Plant extracts have been reported to have repellent and toxic effect against termite (Blaske et al. 2001), and can be promising alternatives for pest control in the future (Ohmura et al. 2000).

Crude extracts of *B. candida* leaves has been reported to have insecticidal activity against subterranean termite *C. gestroi* (Tarmadi et al. 2007). The aim of this study was to evaluate termiticidal activity of *B. candida* leaves against a subterranean termite *C. gestroi* and a drywood termite, *C. cynocephalus*.

## Materials and methods

### *Fractionation procedure*

*B. candida* was obtained from Bogor, Indonesia. Leaves of *B. candida* were sun dried and powdered through 40 mesh screen. 2500 g leafpowder was macerated using methanol, and filtrate collected and separated from residue. The filtrate was evaporated by rotary evaporator (RV 10 Digital, IKA Works GmbH & Co., Germany) at 40 °C to obtain dried extract. 200 g dried extract was dissolved on 600 ml aquadest and n-hexane (1:1) solution and extracted by separating funnel. Aquadest fraction was separated and added by 300 ml ethyl acetate (EA) for further extraction.

### *Bioassay test*

Bioassay against *C. gestroi* was referred to no-choice feeding test according to Ohmura et. al.[7]. A test container was made of a glass petri dish (rim diameter 6cm height 5cm) with 3 mm hard plaster of Paris at the bottom and moistened with 2 ml of deionizer water. Paper discs (diameter 13 mm; Whatman International) were permeated with extract fraction of *B. candida* in various concentrations 1%, 2%, 3%, 4% and 5% (w/v). The treatment retention was 1.0% (w/w) per disc. The control discs were untreated. The discs were dried at 60~ for 12h followed by drying in a vacuum desiccator for 1 day. Fifty workers and 5 soldiers, and treated paper disc were entered into a test container. Bioassay was carried out for 14 days. Termite mortality was observed every two days and in the final period observation, the mass loss of paper disc was evaluated.

Soil barrier: extract fractions were mixed with methanol at various concentrations (1%; 2%; 3%; 4% and 5%) for the soil barrier test. Each treatment (2.5 ml) was mixed with 7 g of 20 mesh sandy soil. Soil treatments were made 48 h before each test to allow the solvent to evaporate. Not-treated

sandy soil was used as control for this experiment. “Bottle H” test units, designed according to JWPA Standard 13 (1992), were used to evaluate horizontal tunnelling by termites. Sandy soil was used as supplementary, easy access for termites toward the test substances. One hundred and fifty workers and 15 soldiers were placed in one side of the bottle H glass container, while rubber wood (20 mm x 20 mm x 10 mm) was placed in the opposite side as food. Bottle H units were kept in a dark place with controlled temperature 28 ± 2 C and humidity above 85%. The test was held for 21 days and observations conducted every day by recording the length of tunnel penetration (mm) made by termites. Termite survival rate was observed after the test.

Table 1 Efficacy Parameter of Soil Barrier Test

Penetration (cm)	Score	Efficacy criteria
0	0	Very High
0,1 - 1,0	1	High
1,1 - 2,0	2	Moderate
2,1 - 3,0	3	Low
> 3,0	4	Ineffective

Bioassays against *C. cynocephalus* were carried out by non-choice feeding test. Wood samples with dimension of 2 cm × 2 cm × 1 cm were treated with extract fractions of *B. candida* in various concentrations 1%, 2%, 3%, 4% and 5% (v/v), and then placed into a glass box size 4 × 4 × 4-cm with 50 workers and 5 soldier. Termite mortality was observed once per week for 6 weeks and termite mortality and weight loss of wood sample (rubber wood) was determined at the end of test. Generalization of efficacy level was conducted based on efficacy criteria presented in table 2.

Table 2. Efficacy/durability criteria against drywood termite

Class	Efficacy/Durability	Weight loss (%)
I	Sound	< 2.0
II	High	2.0 – 4.4
III	Moderate	4.4 – 8.2
IV	Low	8.2 – 28.1
V	Susceptible	> 28.1

### Statistical Analysis

The relationship test between concentration and mortality was carried out by linear regression ( $P < 0.05$ ), while comparison of mortality rates and weight lost of the samples between the three fractions (n-Hexane, ethyl acetate, and aquadest) were analyzed by using ANOVA (Tukey’s test,  $P < 0.05$ ) (SPSS PASW 18.0).

### Results and discussion

Fig.1. Shows termite mortality by extract fraction derived from *B. candida* leaves at various concentrations. *n*-Hexane fraction was very toxic against *C. gestroi* as it delivered 100% mortality at concentration 3% and more, and the data indicated mortality was not significantly related with concentration ( $R^2= 72,8\%$ ,  $p = 0.06$ ) ( $P < 0.05$ ). Unlike *n*-hexane, both ethyl acetate and aquadest showed relation between concentration and mortality ( $R^2= 95,0\%$ ,  $p = 0.005$  and  $R^2=98,7$ ,  $p = 0.01$ , respectively) ( $P < 0.05$ ). The higher the concentration, the higher mortality rate.

Fig. 1 Shows that *n*-Hexane fraction delivered higher mortality than either the ethyl acetate or aquadest fractions at the same concentration. The data suggest significantly different mortality between the fractions of *n*-Hexane, ethyl acetate and aquadest ( $p = 0.002$ , Tukey's test:  $P < 0.05$ ). The *n*-Hexane fraction indicated no significant difference with the ethyl acetate fraction ( $p = 0.538$ , Tukey's test:  $P > 0.05$ ), but was significantly different from the aquadest fraction, ( $p = 0.02$ , Tukey's test:  $P < 0.05$ ). Based on this result we conclude, that the *n*-hexane and ethyl acetate fractions have higher termiticidal activity against subterranean *C. gestroi* compared to the aquadest fraction.

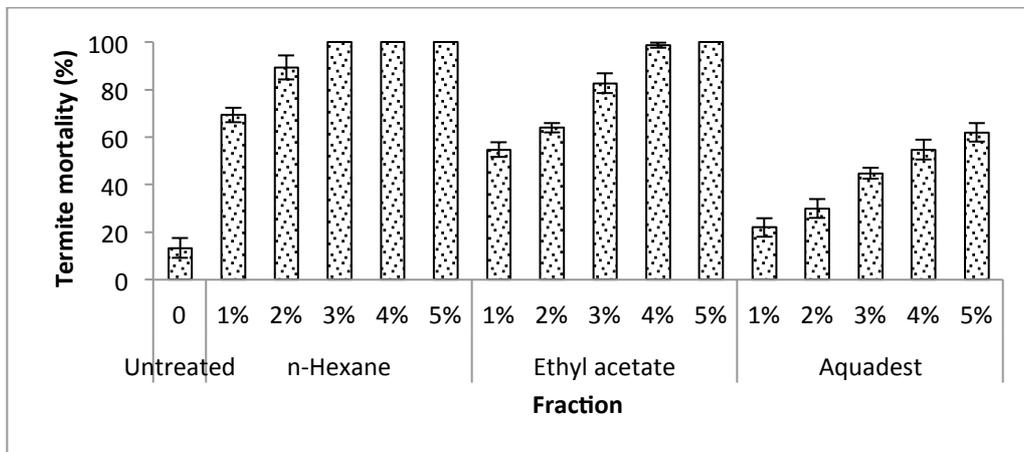


Fig. 1. Daily observation of mortality rate of subterranean termite, *C. gestroi*

Table 3. Efficacy criteria of extract fraction derived from *B. candida* leaves after 3 weeks

Fraction	Concentration (%)	Penetration (cm)	Score	Efficacy criteria	
Untreated	0	5±0	4	Ineffective	
	1	5±0	4	Ineffective	
	2	5±0	4	Ineffective	
	n-Hexane	3	5±0	4	Ineffective
		4	5±0	4	Ineffective
		5	3,77±0,38	4	Ineffective
Ethyl acetate	1	5±0	4	Ineffective	
	2	5±0	4	Ineffective	
	3	5±0	4	Ineffective	
	4	5±0	4	Ineffective	
	5	4,63±0,35	4	Ineffective	
	Aquadest	1	5±0	4	Ineffective
2		5±0	4	Ineffective	
3		5±0	4	Ineffective	
4		5±0	4	Ineffective	
5		5±0	4	Ineffective	

Table 3 Shows the efficacy criteria of the extract fraction derived from *B. candida* leaves after 3 weeks. The results show that the extract fraction from *B. candida* leaves (n-hexane, ethyl acetate and aquadest fraction) at concentration of 1%, 2%, 3%, 4%, 5% were classified as ineffective. It was indicated that the termiticidal activity of the extract fraction derived from *B. candida* leaves is not suitable as a soil barrier.

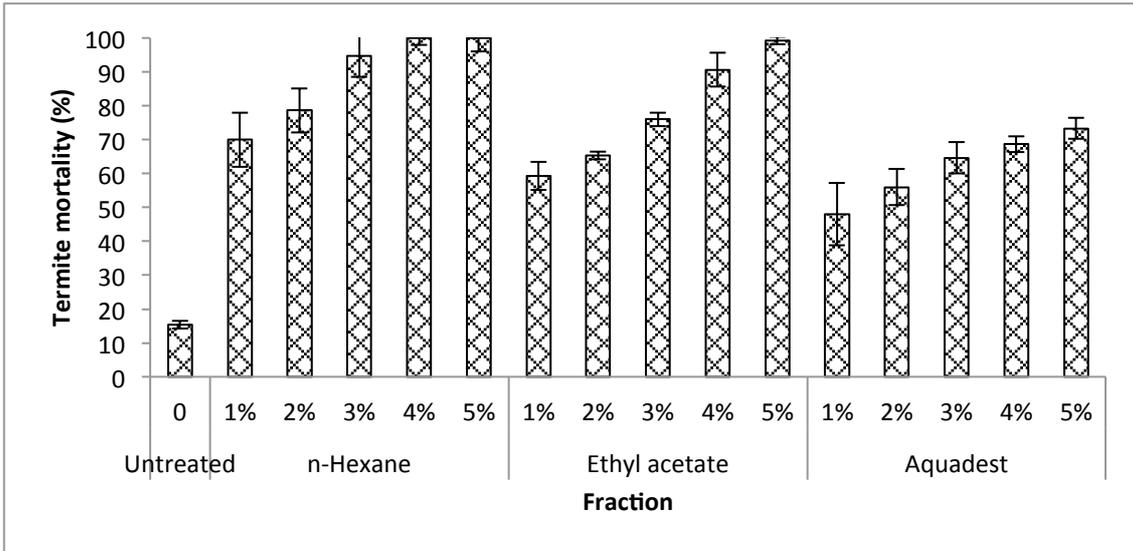


Fig. 2 Mortality of drywood termite, *C. cynocephalus* after 3 weeks in bioassay.

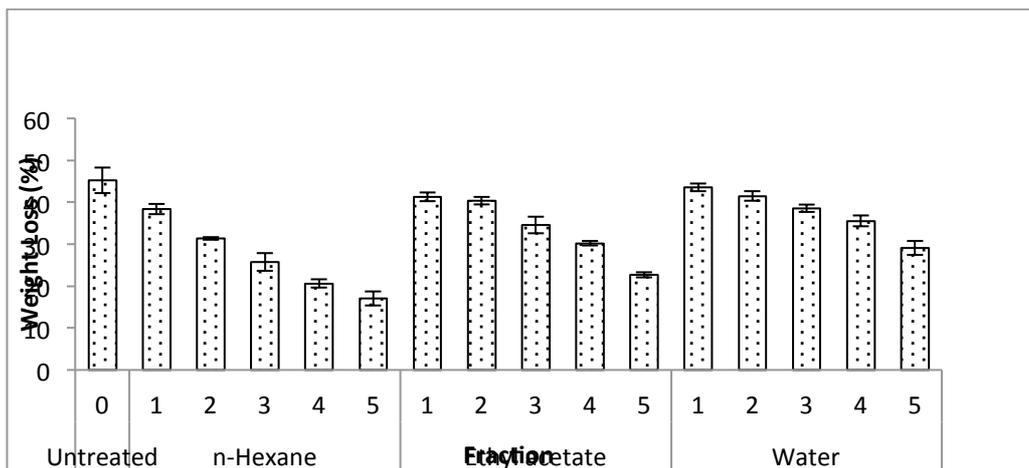


Fig. 3. Weight loss of the samples after 14 days in bioassay with *C. gestroi*

Fig. 2 displays the mortality of *C. cynocephalus* after 3 weeks in bioassay on wood treated with an extract of *B. candida* leaves at various concentrations. The *n*-Hexane fraction delivered 100% termite mortality at 4% and 5% concentration, while the ethyl acetate fraction delivered 90,67% and 99,3% respectively. The aquadest fraction delivered lower mortality than *n*-hexane and ethyl acetate fractions, as it caused 73,33% mortality at 5% concentration. The data suggest that mortality was significantly different between the fractions ( $p = 0.031$ , Tukey's test:  $P < 0.05$ ). Mortality rate of *n*-Hexane fraction was not significantly different from the ethyl acetate fraction ( $p = 0.471$ , Tukey's test:  $P < 0.05$ ) but was significantly different than the aquadest fraction ( $p = 0.026$ , Tukey's test:  $P < 0.05$ ). The result was in accordance with the *C. gestroi* bioassay, suggesting that the *n*-hexane and ethyl acetate fractions have better termiticidal activity than aquadest fraction.

Table 4. Sample weight loss after termite bioassay

Fraction	Concentration (%)	Weight loss (%)*	Efficacy (Class)
Untreated	0	10,21±0,88	Low (IV)
	1	9,89±0,74	Low (IV)
	2	9,44±0,37	Low (IV)
	3	8,82±0,32	Low (IV)
	4	8,06±0,13	Moderate (III)
n-Hexane	5	7,09±0,78	Moderate (III)
	1	10,53±0,31	Low (IV)
	2	10,06±0,93	Low (IV)
	3	9,53±0,42	Low (IV)
	4	9,04±0,28	Low (IV)
Ethyl acetate	5	8,09±0,11	Moderate (III)
	1	10,95±0,72	Low (IV)
	2	10,39±0,37	Low (IV)
	3	9,92±0,25	Low (IV)
	4	9,47±0,13	Low (IV)
Aquadest	5	8,89±0,13	Low (IV)

\* Values are means ± standard deviations from three replications.

Fig. 3 shows the mortality of *C. gestroi* after 14 days in bioassay. The results suggest there were no significant differences between fractions ( $p = 0.095$ , Tukey's test:  $P < 0.05$ ), thus generalization for efficacy performance of the fractions should rely on termite mortality. Weight loss was significantly related with concentration in all fractions (linear regression test,  $P < 0.05$ ), n-Hexane ( $R^2 = 98,6\%$ ,  $p = 0.001$ ), ethyl acetate ( $R^2 = 94,9\%$ ,  $p = 0.005$ ) and aquadest ( $R^2 = 94,9\%$ ,  $p = 0.005$ ). The higher the concentration, the lower the weight-loss and the better protection provided by the fractions.

Table 4 shows weight loss of the samples after the force-feeding test against *C. cynocephalus*. Generalization of efficacy performance was evaluated according to scale on table 1. The data suggested that the n-hexane fraction delivered moderate efficacy at the 4% concentration and more, while the ethyl acetate fraction provided moderate efficacy at the 5% concentration. Other concentrations provided lower efficacy, and all treatments of aquadest fraction did not meet the standard. Linear regression analysis ( $P < 0.05$ ) indicated that weight loss was significantly related with concentration in all fractions, n-Hexane ( $R^2 = 98,0\%$ ,  $p = 0.01$ ), ethyl acetate ( $R^2 = 97,7\%$ ,  $p =$

0.001) and aquadest ( $R^2= 92,3\%$ ,  $p = 0.009$ ). The higher the concentration, the lower the weight-loss and the better protection was provided by the fractions.

### Summary

The extraction of *B. candida* leaves resulted in three major fractions, n-hexane, ethyl acetate and aquadest. Based on the bioassay of the extract of *B. candida* leaves against *C. gestroi* and *C. cynocephalus* the n-hexane and ethyl fraction had better termiticidal activity compared to the aquadest fraction. The results also indicated that all fractions of *B. candida* were not suitable for soil treatment.

### References

- Bläske VU., H. Hertel. 2001. Repellent and Toxic Effects of Plant Extracts on Subterranean Termites (Isoptera: Rhinotermitidae). *Journal of Economic Entomology* **94** (5), 1200-1208.
- Scheffrahn RH, & Su NY. 2000. Asian Subterranean Termite, *Coptotermes gestroi* (=haviandii) (Wasmann) (Insecta: Isoptera: Rhinotermitidae). *University of Florida IFAS Extension (EENY128)*: 1-5.
- G. Elango, G., AA. Rahuman, C. Kamaraj, A. Bagavan, A. Abdul Zahir, T. Santhoshkumar, S. Marimuthu, K. Velayutham, C. Jayaseelan, A. Vishnu Kirithi, G. Rajakumar. 2012. Efficacy of medicinal plant extracts against Formosan subterranean termite, *Coptotermes formosanus*. *Industrial Crops and Products* **36**, 524–530.
- Himmi, SK., D. Tarmadi, M. Ismayati, S. Yusuf. 2012. Bioefficacy performance of neem-based formulation on wood protection and soil barrier against subterranean termite, *Coptotermes gestroi* Wasmann (Isoptera: Rhinotermitidae). *Pro. Env. Sci* **17**, 135–141.
- Ohmura W., D. Shuichi, M. Aoyama, S. Ohara. Antifeedant activity of flavonoids and related compounds against the subterranean termite *Coptotermes formosanus* Shiraki. *J Wood Sci* **46** (2), 149-153.
- Tarmadi, D., AH. Prianto., I. Guswenrivo., T. Kartika., S. Yusuf. 2007. Influence of Bintaro (*Carbera odollam* Gaertn) and Kecubung (*Brugmansia candida* Pers) Extract against Subterranean *Coptotermes* sp. (in Indonesian). *J. wood sci. tech* **5** (1): 38 – 42.
- Tarmadi, D., M. Ismayati, SK. Himmi, S. Yusuf. 2010. Antitermite activity of *Carbera manghas* L seeds extracts. *Proc. the 7<sup>th</sup> Pacific Rim Termite Research Group*. Singapore, 28-31.
- UNEP/FAO/Global IPM Facility Expert Group on Termite and Biology Management. 2000. *Finding Alternatives to Persistent Organic Pollutants (POPs) for Termite Management*. United Nations Environment Programme (UNEP).

Yeap BK, AS. Othman AS, CY. Lee. 2011. Genetic Analysis of Population Structure of *Coptotermes gestroi* (Isoptera: Rhinotermitidae) in Native and Introduced Populations. *Environmental Entomology* **40** (2): 470-476.