Sys Rev Pharm 2019;10 (2):31-33
A multifaceted review journal in the field of pharmacy

EFFECTIVE CONTROL OF SUBTERRANEAN TERMITE Coptotermes curvignathus USING n-HEXANE AND ETHYL ACETATE FROM GAHARU (Aquilaria malaccensis)

Niken Subekti[1], Priyantini Widiyaningrum[1], Isma Nurvaizah[1], and Ro'iyyatul Mar'ah[1]
[1]Biology Department, Universitas Negeri Semarang, Sekaran Gunungpati Semarang, Indonesia

Article History: Submitted: 20.08.2019 Revised: 25.10.2019 Accepted: 21.11.2019

ABSTRACT
Coptotermes curvignathus Holmgren is one of the building pests that have the highest level of damage in Indonesia (Subekti, 2016). This termite control still uses synthetic termiticide. This synthetic termiticide is carcinogenic if exposed to human skin, not on target and causes soil pollution. Therefore, natural termiticide is needed. Alternative natural materials that can be used as natural termiticide is gaharu leaf Aquilaria malaccensi. Research method that used in this study are made extract of gaharu leaf using Soxhlet method, nanoparticle fabricaton of gaharu leaf and eating preferences of subterranean termites Coptotermes curvignathus Holmgren (Ohmura et al. 2000). Result research demonstrate that main compounds nanoparticle from gaharu leaf were n-hexane, methanol and ethyl acetate fractions.
The ANOVA and Tukey's test results showed that termite mortality was significantly higher with the extract treatment compared to the negative control (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%). The conclusion of this research is the extract of gaharu leaf was effective as a natural biotermiticide that is environmentally friendly, right on target and non-polluting.
Keywords: Coptotermes, biotermitiside, mortality, Aquilaria malaccensis,
Correspondance:
Niken Subekti
Biology Department, Universitas Negeri Semarang, Sekaran Gunungpati Semarang, Indonesia
Email:nikensubekti@mail.unnes.ac.id
DOI: 10.5530/srp.2019.2.06

INTRODUCTION

Subterranean termites *Coptotermes curvignathus* Holmgren is an urban pest that can damage buildings and everything in it. Economically, the level of loss caused by the subterranean termites *Coptotermes curvignathus* Holmgren can reach 2 trillion rupiahs a year (Nandika et al. 2015). The problem of *Coptotermes curvignathus* Holmgren as building pests is increasing along with the human need for residential buildings, schools and multi-storey building.

Circulation of termiticide materials in Indonesia is growing very rapidly. The price of synthetic termiticide is getting more expensive. The use of synthetic termiticide is carcinogenic which can cause cancer if exposed to the skin, causing death of non-target organisms and can cause environmental damage. Although termite's mortality is very effective at low doses of this termiticide, the control of termites has not been achieved because the compounds are not selective to target organisms. Further, they contaminate water sources and not readily break down in the soil (Subekti, 2016).

From the description above, termite control technology that environmentally friendly, right on target and does not kill other organisms is needed. Gaharu is an alternative as a termite natural control system. The content of secondary metabolic products of gaharu *Aquilaria malaccensis* has the potential as an environmentally friendly natural biothermalitis. Gaharu (Aquilaria malaccensis) have natural antibacterial and antifungal activities (Dash et al. 2008; Faizal et al. 2017) that could be explained by secondary metabolite compounds that found in leaf extracts (Huda et al. 2009; Khalil et al. 2013). However, prior studies did not yield optimal results, particularly for control of termite attacks in buildings. This study aims to analyze the effectiveness of the use of gaharu leaves as Bio-pest control subterranean termites *Coptotermes curvignathus* Holmgren.

The innovation of this research is implementation of gaharu leaf as a natural termites control using nanoparticle technology. The use of this material has never been done in Indonesia. The advantage of this research is to use agarwood leaves gaharu leaf as a natural material against termites attack.

MATERIALS AND METHODS

Sample Preparation and Nanoparticle Processing.

Five kilograms of Gaharu (*Aquilaria malaccensis*) leaves were extracted and partitioned using the Soxhlet method to obtain methanol, n-hexane, and ethyl acetate fractions. These fractions were qualitatively assessed with phytochemical screening methods. Next, nanoparticles of the n-hexane and ethyl acetate fractions were prepared through the ionic gelation method (Xu *et al.* 2003). Each type of nanoparticle was analyzed morphologically using scanning electron microscopy (Fujita *et al.* 1971) and a particle size analyzer (Burgess *et al.* 2004).

Ionic Gelatin Methode

Chitosan polysaccharide is dissolved in aquenous acidic solution to get the cation of chitosan. This solution is then added drop wise under continuous stirring to polyanionic tripolyphosphate solution. Owing to the complexation between oppositely charged species, chitosan undergoes ionic gelation and precipitates to form spherical particles.

Antitermite Activity Test.

Feed paper was immersed in a nanoparticle solution for 1 hour with 2%, 4%, 6%, 8%, or 10% (v/v) concentration. A positive control was prepared with fipronil at 0.25% concentration (2.5 mL in 1 L of water), and the negative control was solvent only. Termiticidal activity and repellent effect were evaluated using a no-choice feeding test with *C. curvignathus* (Ohmura *et al.* 2000). Four replicates were made for each concentration and control; then termite mortality was measured daily for 7 days.

Data Analysis.

Termite mortality data were analyzed using ANOVA and Tukey's test. The value of LC_{50} was determined using the regression line equation between the concentration log and the probit analysis.

RESULTS AND DISCUSSION

Result research demonstrate that main coumpounds nanoparticle from gaharu *Aquilaria malaccensis* are methanol, n-hexane, and ethyl acetate fractions of gaharu *Aquilaria malaccensis* leaf all contained alkaloids and tannins. Methanol that produced by secondary metabolic products of gaharu (*Aquilaria malaccensis*) leaf extract are toxic, anti-bacterial and antifungal. N-hexane is a poisonous compound which is dangerous if exposed to the skin. Meanwhile ethyl acetate is an organic compound, which is colorless, has a distinctive aroma and volatile. Table 1 shows the secondary metabolic compounds found in gaharu *Aquilaria malaccensis* leaf extracts.

Table 1. Phytochemical Screening Results of Gaharu *Aquilaria malaccensis* Leaf Extracts

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

The result of this study showed that nanoparticles of n-hexane had higher activity against *C. curvignathus* Holmgren than methanol extract and nanoparticles of ethyl acetate (Table 2). This show that a relatively low concentration of n-hexane nanoparticles (4%) was needed to obtain complete mortality. The positive control (0.25% fipronil) caused 100% mortality on the first day. Although fipronil was more effective and efficient, nanoparticles could have several advantages, such as being non-toxic and stable and having a large surface area (Agnihotri *et al.* 2004). The average size of n-hexane and ethyl acetate nanoparticles was less than 300 nm (16.3 and 26.6 nm, respectively), which would allow them to easily penetrate the cells in termite bodies (Mohanraj and Chen 2006).

Table 2. Average Mortality of *C. curvignathus* (%) after 7 Days of Termiticidal Activity Test

Extract	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 ANOVA and Tukey's test results showed that termite mortality was significantly higher with the extract treatment compared to the negative control ($F= 3.116$, $df_{1,2}= 17, 36$; $P< 0.005$). The LC_{50} value of n-hexane nanoparticles against *C. curvignathus* was lower (0.11%) than that of ethyl acetate nanoparticles (0.88%) and methanol extract (5.88%). However, the LC_{50} of both nanoparticles was higher than the LC_{50} of fipronil, 0.00243% (Manzoor *et al.* 2012).

The n-hexane nanoparticles likely had greater bioactivity than ethyl acetate nanoparticles because classes of compounds other than steroids, such as flavonoids, were present. These compounds could increase the anti-termite activity. Various pure flavonoids exhibit antifeedant activity (Ohmura et al. 2000). Foods containing toxins from gaharu *Aquilaria malaccensis* leaf extracts could potentially cause mortality in a termite colony by trophallaxis. Subterranean termites *C. curvignathus* Holmgren have mutualism symbiotic with bacteria, micro fungi and protozoa in the digest (Subekti *et al.* 2017). Microorganism in digest of termites need enzyme for degradation prosess in the body. If toxic compounds include the body of termites, there are toxic compounds that enter the body, the microorganisms will die, so that termites cannot digest their food. This causes mortality of subterranean termites *C. curvignathus*.

Commonly, plants possess biological activity against different insects and other organisms (Hussain *et al.* 2012) . Insects that fed on secondary metabolites would encounter toxic effects which in turn would affect their physiology such as abnormality in the nervous system. The strong termiticidal activity was probably explained by the presence of phytochemicals such as flavonoids, alkaloids and phenolics in that plant. Flavonoids, for instance, have been previously reported to influential with the feeding, reproduction, behavior, and molting process of termite species (Simmonds, 2001). Apart from n-hexane, flavanoids, triterpenoid have also been detected in the methanolic extract of *Aquilaria malaccensis*. Secondary metabolic from plants was reported to have an anti-feedant activity as well as antifungal and antibacterial activities (Abdullah *et al.* 2015). This compound quercetin from secondary metabolic caused 40% mortality against *C. formosanus* through oral application (Boue and Raina 2003), Meanwhile, strong repellency in *Az. indica* might be induced by various active compounds such as azadirachtin, nimbolinin, nimbin, nimbidin, nimbidol, salannin, and quercetin. Azadirachtin is known as a feeding deterrent, insect-growth regulator, repellent, sterilant and inhibits oviposition of insect pests. Other physiological effects that could be caused by azadirachtin are growth reduction, increased mortality or abnormal and delayed molts (Mordue and Nisbet, 2000).

CONCLUSION

n-Hexane and ethyl acetate nanoparticles of gaharu (*Aquilaria malaccensis*) leaf extracts could effectivelly be used to control *C. curvignathus*. n-Hexane nanoparticles at 4% concentration caused higher mortality to *C. curvignathus* than ethyl acetate nanoparticles, and at 0.11% concentration caused 50% termite mortality. The results also showed that *C. curvignathus* Holmgren mortality was concentration and time dependent. A further study should be conducted to understand the made action of chemical compounds plant extracts. Are thus necessary to improve the effectiveness of these naturally-occurring insecticides.

ACKNOWLEDGMENT

This research was supported by Ministry of Research, Technology and Higher Education of the Republic of Indonesia. Under the scheme of National Competency Grant (Contract No. 3.18.3/UN37/PPK.3.1/2019)

REFERENCES

1. Seyburn P. Shinus molle. the minnesota review [Internet]. Duke University Press; 2013 Jan 1;2013(80):59–60. Available from: http://dx.doi.org/10.1215/00265667-2016733
2. Agnihotri SA, Mallikarjuna NN, Aminabhavi TM. Recent advances on chitosan-based micro- and nanoparticles in drug delivery. Journal of Controlled Release [Internet]. Elsevier BV; 2004 Nov;100(1):5–28. Available from: http://dx.doi.org/10.1016/j.jconrel.2004.08.010
3. Burgess DJ, Duffy E, Etzler F, Hickey AJ. Particle size analysis: AAPS workshop report, cosponsored by the Food and Drug Administration and the United States Pharmacopeia. The AAPS Journal [Internet]. Springer Science and Business Media LLC; 2004 Sep;6(3):23–34. Available from: http://dx.doi.org/10.1208/aapsj060320
4. Boué SM, Raina AK. Journal of Chemical Ecology [Internet]. Springer Science and Business Media LLC; 2003;29(11):2575–84. Available from: http://dx.doi.org/10.1023/a:1026318203775
5. Aquilaria agallocha Roxb. SpringerReference [Internet]. Springer-Verlag; Available from: http://dx.doi.org/10.1007/springerreference_68076
6. Faizal A, Esyanti RR, Aulianisa EN, Iriawati, Santoso E, Turjaman M. Formation of agarwood from Aquilaria malaccensis in response to inoculation of local strains of Fusarium solani. Trees [Internet]. Springer Nature; 2016 Oct 8;31(1):189–97. Available from: http://dx.doi.org/10.1007/s00468-016-1471-9
7. Walcott B. Atlas of Scanning Electron Microscopy in Medicine.Tsuneo Fujita , Junichi Tokunaga , Hajime Inoue. The Quarterly Review of Biology [Internet]. University of Chicago Press; 1973 Dec;48(4):659–659. Available from: http://dx.doi.org/10.1086/407893
8. Batubara R, Hanum TI, Risnasari I, Ginting H, Lubis LA. Antioxidant Activity and Preferences Test of Agarwood Leaves Tea (Aquilaria malaccensis Lamk) Based on Leaves Drying Methods. Proceedings of BROMO Conference [Internet]. SCITEPRESS - Science and Technology Publications; 2018; Available from: http://dx.doi.org/10.5220/0008359101590163
9. Hussain M, Javeed A, Ashraf M, Zhao Y, Mukhtar MM, Rehman MU. Aspirin and immune system. International Immunopharmacology [Internet]. Elsevier BV; 2012 Jan;12(1):10–20. Available from: http://dx.doi.org/10.1016/j.intimp.2011.11.021
10. Table 6: Aromatics compounds identified on sugarcane methanolic leaves extracts. PeerJ; Available from: http://dx.doi.org/10.7717/peerj.5445/table-6
11. Qureshi NA. Antitermitic and protozoacidal properties of selected plant extractives against building infesting lower termite Heterotermes indicola (Wasmann) (Isoptera: Rhinotermitidae) and its gut flagellates. 2016 International Congress of Entomology [Internet]. Entomological Society of America; 2016; Available from: http://dx.doi.org/10.1603/ice.2016.114217
12. Mohanraj VJ, Chen Y. Nanoparticles - A review. Tropical Journal of Pharmaceutical Research [Internet]. African Journals Online (AJOL); 2007 Jul 31;5(1). Available from: http://dx.doi.org/10.4314/tjpr.v5i1.14634
13. Mordue(Luntz) AJ, Nisbet AJ. Azadirachtin from the neem tree Azadirachta indica: its action against insects. Anais da Sociedade Entomológica do Brasil [Internet]. FapUNIFESP (SciELO); 2000 Dec;29(4):615–32. Available from: http://dx.doi.org/10.1590/s0301-80592000000400001
14. Simmonds MSJ. Importance of flavonoids in insect–plant interactions: feeding and oviposition. Phytochemistry [Internet]. Elsevier BV; 2001 Feb;56(3):245–52. Available from: http://dx.doi.org/10.1016/s0031-9422(00)00453-2
15. Ohmura W, Doi S, Aoyama M, Ohara S. Antifeedant activity of flavonoids and related compounds against the subterranean termiteCoptotermes formosanus Shiraki. Journal of Wood Science [Internet]. Springer Science and Business Media LLC; 2000 Apr;46(2):149–53. Available from: http://dx.doi.org/10.1007/bf00777362
16. Subekt N, Fibriana F, Widyaningrum P, Adfa M, et al. Determination of the major compounds in the extract of the subterranean termite Macrotermes gilvus Hagen digestive tract by GC-MS method. The Ukrainian Biochemical Journal [Internet]. Co. Ltd. Ukrinformnauka; 2017 Jul 21;89(4):77–82. Available from: http://dx.doi.org/10.15407/ubj89.04.077
17. Xu Y, Du Y. Effect of molecular structure of chitosan on protein delivery properties of chitosan nanoparticles. International Journal of Pharmaceutics [Internet]. Elsevier BV; 2003 Jan;250(1):215–26. Available from: http://dx.doi.org/10.1016/s0378-5173(02)00548-3