



Antitermitic Activity of *Cinnamomum parthenoxylon* leaves Against *Coptotermes curvignathus*

MORINA ADFA^{1*}, ANWAR SANUSI¹, SYALFINAF MANAF², IRFAN GUSTIAN¹
and CHARLES BANON¹

¹Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Bengkulu, Jalan W.R. Supratman, Kandang Limun, Bengkulu 38371, Indonesia.

²Department of Biology, Faculty of Mathematics and Natural Sciences, University of Bengkulu, Jalan W.R. Supratman, Kandang Limun, Bengkulu 38371, Indonesia.

*Corresponding author E-mail: morinaadfa@unib.ac.id

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ABSTRACT

The antitermitic activity of *Cinnamomum parthenoxylon* leaves crude methanol extract and its fractions against *Coptotermes curvignathus* was investigated in a laboratory test. A no-choice test was employed for determining antitermitic activity. The results showed that all fractions and crude methanol extract at concentrations tested was reduced the survival of termite and as well as showed feeding deterrent activity compares to the corresponding control. The results of the preliminary phytochemical screening, and refer to previously reported data the secondary metabolites present in the crude MeOH extract and EtOAc fractions are terpenoids, steroids, flavonoids, coumarin, phenolics, benzenoids, chromene, and fatty acid methyl esters. Base on GC-MS data, the chemical components of *n*-hexane fraction showed 13 components which was divided into five groups, fatty acid methyl esters (53.66%), 1,2-benzenedicarboxylic acid, bis (2-ethylhexyl) ester (31.17%), phytol (6.49%), sesquiterpenes (6.47%), and a phenyl propanoid (2.22%). Need further investigation to examine which compounds are responsible for termiticidal and antifeedant activity, either singly or jointly.

Keywords: *Cinnamomum parthenoxylon*, Antitermitic, Termiticidal, Antifeedant, GC-MS and *Coptotermes curvignathus*.

INTRODUCTION

Cinnamomum is one of the large genera of Lauraceae, with more than 250 species worldwide, and is distributed in the tropical and subtropical region of Asia, China, Australia, and America¹. The antioxidant, antimicrobial, anticancer, and insecticidal activities of some *cinnamomum* species has been extensively

investigated²⁻⁵. *Cinnamomum cassia* is the famous one, the main constituents of *C. cassia* barks oil are cinnamaldehyde and used as an antibacterial agent, antifungal, antidiabetic, and to be insecticidal to adults of *Tribolium castaneum* and *Sitophilus zeamais*, and to *T. castaneum* larvae².

Cinnamomum parthenoxylon is widely distributed in South-Eastern Asia. The wood is used

for general construction and furniture materials. It is resistant to insect attack because of its persistent smell. The leaves, stem barks, roots, and stem woods of *C. parthenoxylon* have shown significant antibacterial activity against a wide range of Gram positive and Gram negative bacteria, antioxidant, antifungal, and anticancer activities⁶⁻⁹.

Only a few reported on the effects of *Cinnamomum* spp. on termite control. *C. camphora* exhibited effective repellent performance and feeding deterrent toward *Coptotermes curvignathus*¹⁰, whereas *C. camphora* oil was shown low toxicity against dry wood termite (*Cryptotermes brevis*)¹¹. The essential oils of *C. osmophloeum* and *C. zeylanicum* leaves exhibited great termite resistance¹², and cinnamaldehyde, eugenol, and terpineol in *C. osmophloeum* leaf essential oil exhibited high antitermite activity against *C. formosanus*¹³.

Thus far, to the best of our knowledge, there are no reports describing the antitermite activity of *C. parthenoxylon*. In continuation of our study for natural pesticides in plants¹⁴⁻¹⁶, in this study, the antitermite activity of *C. parthenoxylon* leaves crude methanol extract and its fractions against *C. curvignathus* was investigated, and their chemical components were analyzed.

MATERIALS AND METHODS

General

GC/MS data were measured with a SHIMADZU QP-2010 GCMS, the condition of GC-MS according to previously method¹⁵: a 30 m capillary column DB 1 Agilent (J and W, USA) with id 0.25 mm and 0.1 μ m film thickness was used; column temperature from 70 °C (5 min.) to 260 °C (17 min) at 5 °C/min; injection temperature of 310 °C, and helium as the carrier gas. The percentage of components was calculated by the GC peak area. The mass spectrometer was operated in EI mode at 70 eV. The mass spectra were obtained by ACQ mode scan of the mass range from 20 to 600. The injection temperature and ionization source temperature were 305 and 250 °C, respectively. The compounds were

identified based on the comparison of their retention time (RT) and mass spectra of Wiley, NIST library data of the GC-MS system.

Plant material and termites

The leaves of *C. parthenoxylon* were collected around University of Bengkulu, Bengkulu city, Indonesia. The plant was determined at the Herbarium Bogoriense, Indonesian Institute of Science (LIPI) Bogor, Indonesia. A voucher specimen (MAKG-01) has been deposited at the Laboratory of Organic, Department of Chemistry, University of Bengkulu. The termites for a test are *Coptotermes curvignathus* Holmgren were obtained from the plants that attacked by termites around University of Bengkulu, and termites were fed wet filter papers before the test.

Extraction, fractionation, and phytochemical screening

Fresh leaves of *C. parthenoxylon* (1.4 Kg) were chopped and macerated at room temperature in methanol (5x5.5 L), then the mixture was filtered and concentrated in a rotary evaporator to yield a crude methanol extract (84.5 g). The crude MeOH extract (42.3 g) was dissolved in methanol and distilled water (1:1), and fractionated with *n*-hexane, and ethyl acetate, successively. Removal the solvents in *vacuo* afforded the *n*-hexane (10 g), ethyl acetate (EtOAc) (6.2 g), and methanol-water (MeOH-H₂O) fractions (21.4 g). Phytochemical screening were performed using standard procedures¹⁷.

Antitermite test

A no-choice test was employed for evaluating antitermite activity according to previously described method¹⁵. The concentration of crude MeOH extract, *n*-hexane, EtOAc, and MeOH-H₂O fractions of *C. parthenoxylon* was prepared to 5%, 10%, and 15% (sample mass/filter paper massx100%). The samples were dissolved in 300 μ L *n*-hexane or methanol, the resulting solution was applied to filter papers (3x3 cm, Whatman No. 1). 20 workers and 2 soldiers were used for tested. The number of dead termites was counted daily for 2 weeks, and the mass loss of filter paper was calculated in the end of tested. Three

replications were performed for each sample. The termiticidal activity was evaluated from the mortality (%) average, and the antifeedant activity were evaluated from mass loss (%) of filter paper.

RESULTS AND DISCUSSION

Cinnamomum parthenoxylon leaves methanol extract was separated into three fractions of increasing polarity by fractionation using *n*-hexane, and ethyl acetate. We evaluated the antitermitic activity crude MeOH extract of

C. parthenoxylon leaves and its fractions against *C. curvignathus*, the results were shown in Table 1. No choice forced feeding of *C. curvignathus* on a filter paper impregnated with 5%, 10%, and 15% of the crude MeOH extract, *n*-hexane, EtOAc, and MeOH-H₂O fractions resulted in increased termite mortality with increasing extract and fractions concentrations. All termite dead within 2 weeks for crude MeOH extract, *n*-hexane and EtOAc fractions at a concentration of 15%, while MeOH-H₂O fraction (15%) showed 87.88% mortality after 2 weeks. All fractions and crude methanol extract reduced the survival of termite compares to the control. Filter

Table 1: Antitermite activity of *C. parthenoxylon* leaves crude MeOH extract and its fractions against *Coptotermes curvignathus*

Crude MeOH extract and its fractions	Mortality of termite (%) recorded over time		Mass loss (%) of filter paper 14 days
	7 days	14 days	
Control (MeOH)	3.03	6.06	19.07
Control (n-Hexane)	1.52	4.55	18.53
Crude MeOH extract 5%	37.88	87.88	14.4
Crude MeOH extract 10%	42.42	89.39	12.4
Crude MeOH extract 15%	65.15	100	8.3
<i>n</i> -Hexane fraction 5%	42.42	78.79	12.0
<i>n</i> -Hexane fraction 10%	63.64	95.45	10.3
<i>n</i> -Hexane fraction 15%	81.82	100	8.5
EtOAc fraction 5%	53.03	72.73	12.4
EtOAc fraction 10%	68.18	90.91	11
EtOAc fraction 15%	83.33	100	8.7
MeOH-H ₂ O fraction 5%	15.15	53.03	14.1
MeOH-H ₂ O fraction 10%	31.82	71.21	12.5
MeOH-H ₂ O fraction 15%	48.48	87.88	10.5

paper consumption by the termites after 2 weeks examine to crude MeOH extract of *C. parthenoxylon* leaves and its fractions were compared with that control (Table 1). All fractions and crude methanol extract showed feeding deterrent activity. Average mass loss of filter paper treated with crude MeOH extract and fractions at all concentrations tested was lower than the control, average mass loss of filter paper was decreased as the concentration increased. A similar termiticidal and antifeedant effects were obtained between *n*-hexane and EtOAc fractions, respectively.

The crude methanol extract of *C. parthenoxylon* leaves was subjected to preliminary

phytochemical screening (Table 2). The presence of secondary metabolites such as flavonoids, phenolics, terpenoids/steroids, and coumarins on *C. parthenoxylon* leaves might be responsible for antitermite activity, because some classes of flavonoids and terpenoids have been reported as termiticidal and feeding deterrent to termite¹⁸⁻¹⁹.

The analysis of chemical components of the *n*-hexane fraction of *C. parthenoxylon* leaves was carried out using GC-MS. The *n*-hexane fraction was contain 13 components, and the components are listed in Table 3. The *n*-hexane fraction was characterized by a high content of fatty acid methyl esters 53.66% (hexadecanoic acid,

methyl ester; 10-octadecenoic acid, methyl ester; 9,12,15-octadecatrienoic acid, methyl ester (*Z,Z,Z*); 9,12-octadecadienoic acid, methyl ester (*E,E*); 9-octadecenoic acid,12-hydroxy, methyl ester (9*Z*,12*R*); octadecanoic acid, methyl ester), followed by 1,2-benzenedicarboxylic acid, bis (2-ethylhexyl) ester (31.17%), phytol (6.49%), sesquiterpenes 6.47% (β -caryophyllene, δ -guaiene, caryophyllene oxide, patchouli alcohol), and a phenyl propanoid 2.22% (β -asarone).

Several plants essential oils and its components have been reported to show larvicidal, antitermite or repellent activity. α -Caryophyllene and caryophyllene oxide from *Plectranthus barbatus* and *Zanthoxylum avicennae* essential oils have larvicidal activity against malaria mosquito vectors²⁰, and *Aedes albopictus* Skuse²¹. Zhu *et al.*, (2003) reported that patchouli oil was obtained from *Pogostemon cablin* and its major component patchouli alcohol have toxic and repellent activity against *Coptotermes formosanus*²². The possibility that the toxicity of patchouli oil and patchouli alcohol

against termites have a neurotoxic mode action. This result obtained also agree well with Adfa *et al.*, (2015)¹⁵ that β -asarone at low concentration in *A. calamus* rhizome fractions revealed strong termiticidal activity against *C. curvignathus*. There is a report the presence of 1,2-benzenedicarboxylic acid, bis (2-ethylhexyl) ester in *Sterculia guttata* seeds and its larvicidal activity against *Aedes aegypti* and *Culex quinquefasciatus* larvae²³. Although 1,2-benzenedicarboxylic acid, bis (2-ethylhexyl) ester is the member of the class of phthalates which are used as plasticizers medical devices.

Other studies have confirmed that coumarin (scopoletin), flavonoids (isorhoifolin, rutin, nicotiflorin, epicatechin, kaempferol-3-O-rhamnoside, herbacetin, quercetin-3-O-rhamnoside), benzenoids (4-hydroxybenzoic acid, 4-hydroxybenzaldehyde, 1,2,4-trihydroxybenzene), fatty acid methyl esters (hexadecanoic acid methyl ester, 12-hexadecenoic acid methyl ester), sesquiterpene (blumenol A), chromene dimer (3*R*,4*R*,3'*R*,4'*R*)-6,6'-dimethoxy-

Table. 2: Phytochemical screening of crude MeOH extract of *C. parthenoxylon* leaves

Classes of compounds	Test Applied	Result
Alkaloids	Dragendorff	-
Flavonoids	Shinoda (Mg/HCl)	+
Phenolics	Ferric chloride	+
Terpenoids/ and Steroids	Liebermann-Burchard (H ₂ SO ₄ -Ac ₂ O)	+
Coumarins	Spot test (NaOH)	+

Note: + (detected) and - (not detected)

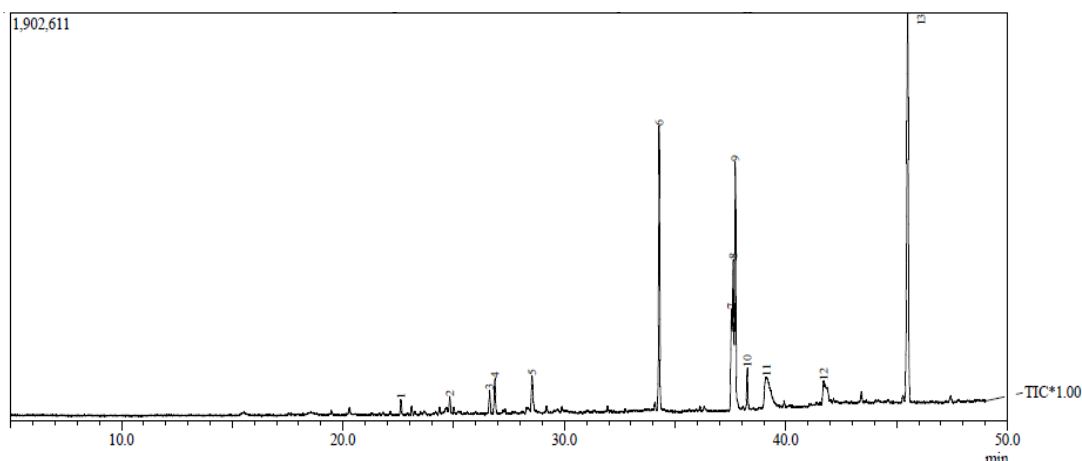


Fig. 1. GC chromatogram of *n*-hexane fraction of *C. parthenoxylon* leaves

Table. 3: Identified chemical components of *n*-hexane fraction of *C. parthenoxylon* leaves

RT (min)	Compounds	Peak area (%)
22.619	β -Caryophyllene	0.95
24.826	δ -Guaiene	0.98
26.622	Caryophyllene oxide	1.53
26.861	β -Asarone	2.22
28.537	Patchouli alcohol	3.01
34.279	Hexadecanoic acid, methyl ester	16.55
37.542	9,12-Octadecadienoic acid, methyl ester (<i>E,E</i>)	6.21
37.622	9,12,15-Octadecatrienoic acid, methyl ester (<i>Z,Z,Z</i>)	9.38
37.717	10-Octadecenoic acid, methyl ester	14.67
38.255	Octadecanoic acid, methyl ester	2.49
39.116	Phytol/ 3,7,11,15-Tetramethyl-2-hexadecen-1-ol	6.49
41.709	9-Octadecenoic acid,12-hydroxy, methyl ester (<i>9Z,12R</i>)	4.36
45.500	1,2-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester	31.17

3,4,3',4'-tetrahydro-2H,2'H-[3,3']bichromenyl-4,4'-diol), and steroid (daucosterol) have been isolated and purified from ethyl acetate fraction of *C. parthenoxylon* leaves²⁴⁻²⁵. Steroid (α -sitosterol) was isolated from petroleum ether fraction²⁵. Scopoletin and 4-hydroxybenzaldehyde have been showed good termiticidal activity, and quercetin-3-O-rhamnoside demonstrated moderate antifeedant activity against *Coptotermes formosanus*^{13,14,26}, whereas 4-hydroxybenzoic acid was claimed as a base material of attractant for termite control agent²⁷.

fractions showed nearly similar activity. These results exhibited that the antitermitic activity of the crude methanol extract of *C. parthenoxylon* leaves and all fractions can be attributed to secondary metabolites present in the extract and fractions. The compounds responsible for antitermitic activity need further investigation, either jointly or independently. *C. parthenoxylon* leaves crude MeOH extract and its fractions might be potential to develop new bio pesticides and wood preservative agent.

CONCLUSION

In conclusion, crude methanol extract of *C. parthenoxylon* leaves and its fractions showed good termiticidal and antifeedant activity against *C. curvignathus*, while *n*-hexane and EtOAc

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