International Journal of Agricultural Technology 2022Vol. 18(1):1-12
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

The efficacy of *Cinnamomum parthenoxylon* roots as a biopesticide towards termite and wood-rotting fungi

Adfa, M.[*], Utomo, B., Oktavia, L., Yudha, S. S., Banon, C., Gustian, I. and Maryanti, E.

Department of Chemistry, Faculty of Mathematics and Natural Sciences, Bengkulu University, Jalan WR. Supratman, Kandang Limun Kota Bengkulu 38371, Indonesia.

Adfa, M., Utomo, B., Oktavia, L., Yudha, S. S., Banon, C., Gustian, I. and Maryanti, E. (2022). The efficacy of *Cinnamomum parthenoxylon* roots as a biopesticide towards termite and wood-rotting fungi. International Journal of Agricultural Technology 18(1):1-12.

Abstract The antitermite and antifungal activity of *C. parthenoxylon* roots constituents, namely *n*-hexane and methanol fractions against termite (*Coptotermes curvignathus*) and wood-rotting fungi (*Trametes versicolor*) was determined. The results showed that both fractions at 15% concentrations were reduced the survival of termite to 100% after 7 and 6 days of treatment. In addition, both fractions exhibited significant inhibition effects towards fungal growth compared to the control. The data showed that *n*-hexane fraction was more toxic than methanol fraction against *T. versicolor*. At 0.25% concentration, *n*-hexane and methanol fractions inhibited the fungal growth to 100% and 78.33%, respectively. The GC-MS profiling showed that most of the compounds detected from both fractions consisted of phenylpropanoid, aldehyde, fatty acids, long-chain hydrocarbons, sesquiterpenes, and lignans. The major component of *n*-hexane fraction were safrol (46.8%), octadecanal (14.35%), and elemicin (9.55%). While the main compounds of methanol fraction included 9,12-octadecadienoic acid, methyl ester (8.29%), safrole (7.98%), and octadecanoic acid, methyl ester (7.01%). The preliminary phytochemical test showed that *n*-hexane fraction contains terpenoids and coumarins, while the methanol fraction contains flavonoids, saponins, and tannins. Therefore, the presence of these compounds might be responsible for their antitermite and antifungal activities. It was observed that the antitermite potential of *C. parthenoxylon* was useful in controlling termite pests and wood-rotting fungi for now and near future.

Keywords: *Cinnamomum parthenoxylon*, Fungicidal, GC-MS, Safrole, Termiticidal activity

Introduction

Laurus, *Persea*, and *Cinnamomum* are known as the three most economically valuable genus in the Lauraceae family. The genus Cinnamomum is reported to have excellent biopesticide capabilities (Ong *et al.*, 2020). The trans-cinnamaldehyde, isolated from *Cinnamomum cassia* showed a strong insecticidal activity against *Sitophilus zeamais* and *Tribolium castaneum* (Huang and Ho, 1998). Dichloromethane extract of *Cinnamomum champora*

[*] **Corresponding Author**: Adfa, M.; **Email**: morina@unib.ac.id; morinaadfa@gmail.com

showed strong antifungal activity against *Curvularia lunata*, and *Alternaria alternate* (Guleria and Kumar, 2006). Also, the *Cinnamomum zeylanicum* essential oil, which major compound is cinnamaldehyde (77.51%), inhibited *Fusarium oxysporum* growth at 200 ppm concentration (Behtoei *et al.*, 2012), and caused mortality to larva and adult of *Alphitobius diaperinus* at concentrations of 5 and 10% (Volpato *et al.*, 2016).

However, there are few reports on the *Cinnamomum spp*. towards the control of termite pests and wood rot fungi, since several species of termites and rotting fungi are wood destroying organisms causing quite high economic losses on building materials and in the agricultural sector. The *Cinnamomum camphora* showed an effective repellent performance and resistance towards termites, such as *Coptotermes curvignathus* (Roszaini *et al.*, 2013).

The negative effects of synthetic pesticides towards non-targeted pests, human health, and the environment cause the need for an alternative natural sources that have the potential of controlling pests and being environmentally friendly. Due to the high diversity level in the plant kingdom, various metabolites are produced, which are used as biopesticides. Therefore, various extracts, fractions, essential oils, wood vinegar, and single compounds from plants are used as an alternative material in replacing persistent synthetic pesticides (Giraldo-Rivera and Guerrero-Alvarez, 2019).

Furthermore, this research explores the potential of *Cinnamomum* genus that grows in Bengkulu, Sumatra Island-Indonesia in inhibiting the growth of termites and wood-rotting fungus. Synonyms of *Cinnamomum parthenoxylon* are *C. porrectum*, and *C. inunctum*, in Bengkulu-Indonesia are known as the *Kayu Gadis*, with distinct characteristics, such as fragrant smell from the trunk and roots when cut. Therefore, they are used by the Indonesian residents as building and other woodworking materials, since they are highly resistant towards termite attacks (Hani *et al.*, 2010).

In previous studies, we have reported the antitermite activity of crude methanol extract of *C. parthenoxylon* leaves and its fraction against *C. curvignathus* by forced feed method. The results showed that the crude methanol extract and its fractions could reduce termite survival and showed feeding deterrent activity when compared to the control. Therefore, the presence of several compounds, such as terpenoids, flavonoids, coumarins, phenolics, benzenoids, chromenes, phenylpropanoids, fatty acid methyl esters, might be influenced the antitermite activity (Adfa *et al.*, 2017).

The liquid smoke produced from *C. parthenoxylon* wood also showed very effective anti-termite and anti-wood-rotting fungal activity. The carboxylic acid content from the liquid smoke, contributed greatly to its termiticidal activity and it other phenolic compounds highly inhibited the growth of *Schizophyllum*

commune and *Fomitopsis palustris* (Adfa *et al.*, 2020). However, there have been no reports of antitermite or anti wood decay fungi activity from the *C. parthenoxylon* roots.

Therefore, the goal of this research was to determine the antitermite and antifungal activity of the non-polar and polar fractions of *C. parthenoxylon* root towards termites (*C. curvignathus)* and wood rotting fungus (*Trameter versicolor*), as well as analyzing their chemical components using GC-MS.

Materials and methods

Plant materials

The roots of *Cinnamomum parthenoxylon* were collected from Bengkulu University. These samples were identified at Herbarium Bogoriense-LIPI, and *C. parthenoxylon* herbarium (MAKG-01) was stored in the organic chemistry laboratory, chemistry department, Bengkulu University.

Extraction

Fresh roots of *C. parthenoxylon* weghing 1.8 kg was finely chopped and macerated with 5.7 L *n*-hexane for 5 days at rt. The obtained mixture was filtered and the solvent was evaporated, while the residue was inserted back into the bottle for re-maceration, which was repeated 5 times (until the *n*-hexane extract became clear). All the concentrated *n*-hexane extracts were called the *n*-hexane fraction of *C. parthenoxylon* root. The residue from this process was further macerated using 5.5 L methanol for 4 days, and was carried out 4 times (until the methanol extract becomes clear). The obtained mixture was filtered and the solvent was evaporated, while all the concentrated methanol extracts were called the methanol fraction of *C. parthenoxylon* root (Adfa *et al.*, 2015).

Preliminary phytochemical screening and analysis of chemical components by GC-MS

The qualitative phytochemical screening of the *n*-hexane and methanol fractions of *C. parthenoxylon* roots used a standard method (Harborne, 1998). While their chemical components were analyzed using a GC-MS Shimadzu Qp-2010S according to Adfa *et al.* (2017). The percentage of the components was calculated using the GC-MS TIC peak regions.

Termiticidal activity

The experimental termites were obtained from trees attacked by *Coptotermes curvignathus* around the main campus of Bengkulu University, Kandang Limun. The no-choice test was used to evaluate the termiticide activity. The concentrations of *n*-hexane and methanol fractions were calculated based on the mass ratio of the sample to the filter paper multiplied by 100%, with a series test concentrations of 0%, 2.5%, 5%, 10%, 12.5%, and 15 %. After weighing, the sample was dissolved in 500 µL *n*-hexane or methanol and the resulting solution was dropped on filter paper (Whatman No. 3 and 90 mm diameter), then it was dried overnight and placed in a desiccator for 1 hour. The test filter paper was placed in a Petri dish (90 mm dia. × 20 mm high), then thirty worker and three soldier termites from active *C. curvignathus* were inserted into the test Petri dishes, and placed in a plastic tray with a damp cloth and stored in a dark room (80% ± 5 Relative Humidity) for 14 days. The test was carried out 3 replicates. The average termite mortality rate (%) was determined daily, due to the influence of both fractions using the equation 1 (Adfa *et al.,* 2017).

$$\text{(Eq.1)}\quad \text{Termite Mortality (\%)} = \frac{\text{Number of Dead Termites}}{\text{Total Number of Test Termites}} \times 100\%$$

Antifungal activity

The solid PDA media (7.8 g PDA in 200 mL distilled water) was placed (10 mL) in Petri dishes as a lower layer and allowed to solidify. After the solidification, 9.5 mL of semi-solid medium (3.9 g PDA in 200 mL distilled water) was poured and mixed with a solution of the *n*-hexane and the methanol fractions of various concentrations (0.5 mL). The mass variations of both fractions used were 0, 0.005, 0.01, 0.015, 0.02, 0.025, and 0.03 g, which yielded the test concentrations of 0, 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3% w/v. After the media solidified and cooled, the *Trametes versicolor* fungus, previously cultured was cut using a 5 mm diameter cork-borer, and placed in the middle of the test medium. The antifungal activity test was repeated 3 times. The fungal cultures were incubated at 25 ℃ for 5 days (Sekine *et al.,* 2009). After the incubation, the diameter of each angle of fungal growth was measured in a petri dish, and the anti-fungal activity test was repeated 3 times. From the measurement results of the fungal mycelium growth, the inhibition percentage was calculated using the equation 2 (Wang *et al.,* 2005).

$$\text{(Eq.2)}\quad \text{\% Fungal Growth Inhibition} = \frac{\text{Da-Db}}{\text{Da}} \times 100\%$$

Description:
Da = the growth diameter of fungal mycelium in control (cm)
Db = the growth diameter of the fungal mycelium in the sample (cm)

Results

Extraction and preliminary phytochemical screening and GC-MS analysis

From the *C. parthenoxylon* roots maceration, as much as 1.8 kg was mixed with *n*-hexane solvent to yield 44.2 g (2.45%) of a concentrated dark brown extract. This was referred to as the *n*-hexane fraction. Furthermore, the sample residue was macerated again using methanol solvent, which yielded a dilute extract with brownish-red colour. After the solvent was evaporated, 42.3 g (2.35%) concentrated extract of methanol with a brownish-red colour was obtained. This was referred to as the methanol fraction.

The qualitative chemical screening of *n*-hexane and methanol fractions of *C. parthenoxylon* roots mentioned in Table 1, showed the presence of various secondary metabolites. The standard procedures was carried out to identifying the presence of tannins, flavonoids, alkaloids, saponins, steroids, and triterpenoids. Methanol fraction contains flavonoids, saponins, and tannins, while the triterpenoids and coumarins groups were presented in *n*-hexane fraction (Table 1). The presence of flavonoids in the methanol fraction was observed when a reddish-brown colouration formed after treating the sample with magnesium powder and concentrated HCl (Shinoda test). Tannins were presented when green precipitation was formed after the addition of ferric chloride solution. The formation of steady foam after shaking the fraction with hot distilled water was a positive test for the presence of saponins. The presence of triterpenoids in *n*-hexane fraction was observed when a reddish coloration was formed, indicating a positive reaction for the presence of triterpenoids (the Liebermann-Burchard's test). Coumarins were identified using NaOH in a filter paper, then examined under UV light, the formation of yellow-blue fluorescence indicated their presence.

The components of the *n*-hexane and methanol fractions of *C. parthenoxylon* roots was investigated further using GC-MS (Table 2). A total of 34 compounds were found in *n*-hexane fraction, and 26 compounds in methanol fraction. It can be understood that there was larger amount of compounds found in *n*-hexane compared to that found in methanol fraction detected by GC-MS, seeing that the GC-MS technique performed well for analysing volatile compounds.

23 components were identified from the *n*-hexane fraction, while 10 were from methanol as shown in Table 2. After comparing the similarity index MS spectrum of each component with the data base NIST 62 library, 6 constituents were detected in both of fractions, namely safrol, octadecanal, 1-dodecanal, pentadecanoic acid,14-methyl-,methyl ester, bis (2-ethylhexyl) phthalate, and hinokinin. The highest peak region of *n*-hexane fraction was obtained in

number 2 (46.8%), and was identified as safrole from the phenylpropanoid group. The others above 5% were number 12 (14.35%), and 11 (9.55%), and were identified as octadecanal and elemicin. The highest peak region of methanol fraction was obtained in number 13 (8.29%), and was identified as 9,12-octadecadienoic acid, methyl ester (*E,E*). The others above 5% were number 2 (7.98%), 11 (7.01%), 15 (6.94%), 12 (5.89%), 20 (5.11%), and 5 (5.01%), and were identified as safrole, octadecanoic acid,methyl ester, 9-octadecynoic acid,methyl ester, pentadecanoic acid,14-methyl-,methyl ester, bis (2-ethylhexyl) phthalate, and gamma-cadinene, respectively.

Table 1. Phytochemical screening of *n*-hexane and methanol fractions of *C. parthenoxylon* roots

Compounds Group	**Reagent**	***C. parthenoxylon* roots fractions**	
		***n*-Hexane**	**Methanol**
Alkaloids	Mayer ($HgCl_2$ + KI)	-	-
	Wagner (I_2 + KI)	-	-
Flavonoids	(Mg powder + concentrated HCl)	-	+
Saponins	Foam test (H_2O, shake vigorously)	-	+
Tannins	$FeCl_3$	-	+
Steroids	(Concentrated H_2SO_4 + anhydrous acetic acid)	-	-
Triterpenoids	(Concentrated H_2SO_4 + anhydrous acetic acid)	+	-
Coumarins	NaOH 10%, fluorescence under UV	+	-

Note: (+) Contain the tested compound and (-) does *not*

Termiticide and fungicidal activities of n-hexane and methanol fractions of C. parthenoxylon roots

In this study, the daily termite mortality of *C. curvignathus* treated with *C. parthenoxylon* roots fractions was determined for 14 days by using the no-choice feeding method. The results showed that both fractions had strong termiticidal activity with nearly similar average mortality at all tested concentrations (Figures 1 and 2). Furthermore, both fractions showed similar mortality pattern, except when they were given at 5% concentration. At the lowest concentration (2.5%), 56.6% to 63.6% of termites were dead after treatment with both of fractions, compared to the corresponding control. While at the highest concentration (15%), all the termites dead after 6 to 7 days subject to *n*-hexane and methanol fractions, respectively. Starting on day 8, termite mortality caused by the two fractions at concentrations of 15, 12.5, and 10% no significant difference.

The *n*-hexane and methanol fractions of *C. parthenoxylon* roots showed inhibition against wood rotting fungi growth. The mycelium growth of *T.*

versicolor on the test medium and the control was shown in Table 3, while the antifungal activity was summarized in Figure 3. This data showed that the *n*-hexane fraction was more toxic than the methanol fraction against *T. versicolor* using the agar media test. At the concentration of 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3% w/v, the *n*-hexane fraction inhibited fungal growth by 51.11, 63.08, 75.30, 93.01, 100, and 100%, respectively. While, the growth inhibition of fungi, treated with methanol fraction were 61.67, 67.83, 70.56, 76.67, 78.33, and 81.11 %, respectively. At the concentrations tested, the growth inhibition of the *n*-hexane fraction steadily increases from 51.11% to 100%, while the methanol fraction increased slightly ranging from 61.67% to 81.11%.

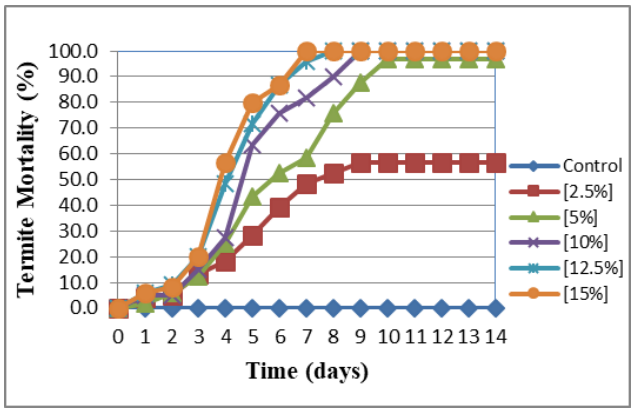


Figure 1. Termiticide activity of *n*-hexane fraction of *Cinnamomum parthenoxylon* roots against *Coptotermes curvignathus*

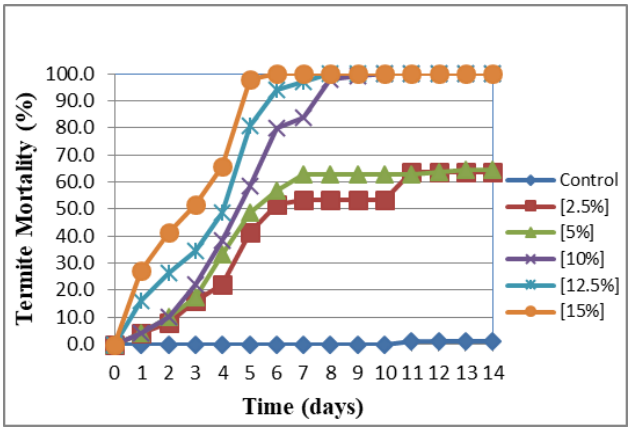


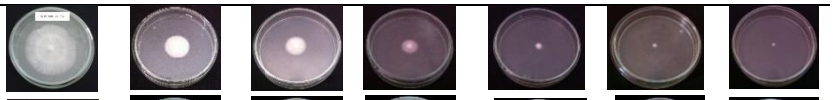
Figure 2. Termiticide activity of methanol fraction of *Cinnamomum parthenoxylon* roots against *Coptotermes curvignathus*

Table 2. The chemical components of *n*-hexane and methanol fractions of *C. parthenoxylon* roots detected by GC-MS

***n*-Hexane Fraction**			**Methanol Fraction**		
Peak No	**Compound**	**Area %**	**Peak No**	**Compound**	**Area%**
1	*Not identified*	0.76	1	*Not identified*	1.76
2	Safrol*	46.80	2	Safrol*	7.98
3	Methyl eugenol	0.73			
4	1-Dodecanal*	0.27	3	1-Dodecanal*	3.43
5	*Not identified*	0.28	4	*Not identified*	2.18
6	*Not identified*	2.08			
7	*Not identified*	1.13			
8	Cis,cis,trans-3,3,6,6,9,9-hexamethyl-tetracyclo[6.1.0.0(2,4).0(5,7)]nonane	3.34	5	Gamma-cadinene	5.01
9	Dehydroaromadendrene	0.70			
10	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	0.83	6	*Not identified*	1.92
11	Elemicin	9.55			
12	Octadecanal*	14.35	7	Octadecanal*	4.56
13	Bisabolol	1.30			
14	*Not identified*	0.93			
15	Isobornyl acetate	1.72	8	*Not identified*	1.96
16	Tridecan-2-one	0.59	9	*Not identified*	1.84
17	*Not identified*	1.83	10	*Not identified*	3.25
18	Cyclopropanepentanoic acid, 2-undecyl-, methyl ester, trans-	0.76	11	Octadecanoic acid, methyl ester	7.01
19	*Not identified*	1.72			
20	Pentadecanoic acid, 14-methyl-, methyl ester*	0.28	12	Pentadecanoic acid, 14-methyl-, methyl ester*	5.89
21	Undecane	0.98			
22	Heptanoic acid, ethyl ester	1.03	13	9,12-Octadecadienoic acid, methyl ester, (E,E)-	8.29
23	*Not identified*	0.45			
24	1-Tetradecanol	0.36	14	*Not identified*	4.83
25	Bicyclo 3.1.1 heptan-3-ol, 2,6,6-trimethyl-, (1.alpha.,2.beta.,3.beta.,5.alpha.)-	2.15	15	9-Octadecynoic acid, methyl ester	6.94
			16	*Not identified*	4.74
26	Heptane, 3,3-dimethyl-	0.52	17	*Not identified*	1.67
27	9-Octadecenoic acid (Z)-, hexyl ester	0.69			
28	9,12-Octadecadienal	0.32			
29	Pentadecanoic acid, 4,6,10,14-tetramethyl-, methyl ester	0.64			
30	*Not identified*	0.80			
31	*Not identified*	0.43	18	*Not identified*	1.92
32	*Not identified*	0.44	19	*Not identified*	3.27
33	Bis(2-ethylhexyl) phthalate*	0.42	20	Bis(2-ethylhexyl) phthalate*	5.11
			21	*Not identified*	1.82
			22	*Not identified*	5.17
			23	*Not identified*	1.72
34	Hinokinin*	0.80	24	Hinokinin*	3.87
			25	*Not identified*	1.75
			26	*Not identified*	2.12

Noted: * these compounds contained in both fractions

Table 3. The growth of *Trametes versicolor* fungus after the administration of *n*-hexane and methanol fractions of *Cinnamomum parthenoxylon* roots at various test concentrations

Roots fractions *C. parthenoxylon*	**Concentrations tested**						
	[0%] control	[0.05%]	[0.1%]	[0.15%]	[0.2%]	[0.25%]	[0.3%]
n-Hexane							
Methanol							

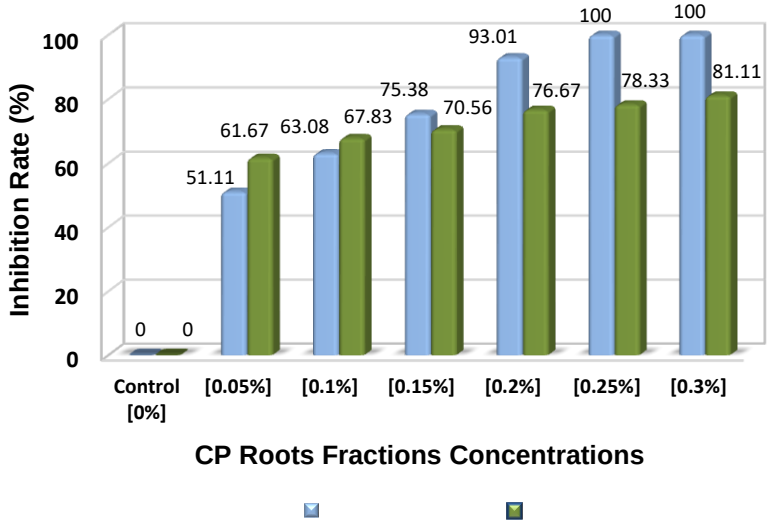


Figure 3. Fungicidal activity of *n*-hexane and methanol fractions of *Cinnamomum parthenoxylon* (CP) roots against *Trametes versicolor*

Discussion

Most of the compounds detected from *n*-hexane and methanol fractions of *C. parthenoxylon* roots by GC-MS consisted of phenylpropanoid, phenyl propene, aldehyde, fatty acids, long-chain hydrocarbons, sesquiterpenes, and lignin. Currently, there were limited information based on *C. parthenoxylon* roots constituents, however, only their essential oil were reported. Tisserand and Young (2013), stated the essential oil present in the roots of *C. porrectum*, which was safrole (85.5-97.0%), while the main constituent of *C. parthenoxylon* roots bark oil growing wild in Vietnam was benzyl benzoate (52.0%). The main constituent of *C. parthenoxylon* wood essential oil was

reported as safrole (90.3%), which was almost similar to that of *n*-hexane and methanol fractions of the roots in this study (46.80 and 7.98%) (Dũng *et al.,* 1995).

According to the chemical components of *n*-hexane fraction as detected by GC-MS, the safrole might be greatly contributed to the termiticidal activity in addition to other minor components, as well as terpenoids and coumarins that detected by preliminary tests. Safrole showed fumigant and contact toxicities against *Sitophilus zeamais* and *Tribolium castaneum* (Huang *et al.,* 1999).

The safrole concentration in methanol fraction was lower than that in n-hexane fraction, however, both had nearly similar termiticidal activity. Safrole and flavonoids, tannins, saponins in a synergistic effect might be responsible for this activity. In addition, the methanol fraction contains lignan compound (hinokinin) higher than n-hexane fraction may contribute as well. The hinokinin isolated from heartwood of *Chamaecyparis obtusa* showed repellent and antifeedant activities against Japanese termites (*Reticulitermes speratus*) (Morikawa *et al.,* 2014).

Regarding the chemical components of *n*-hexane and methanol fractions, Table 2 showed the main constituents of *n*-hexane fraction detected by GC-MS, namely safrole, elemicin, and octadecanal, while others, such as aldehyde, fatty acids, long-chain hydrocarbons, and sesquiterpenes were its minor compounds. Safrole is a major constituent of *Piper auritum* essential oil that inhibited important postharvest fungal pathogens of fruits, namely *Colletotrichum gloeosporioides*, *C. acutatum* and *Botryodiplodia theobromae* at 400 µg/mL (Pineda *et al.,* 2012). Tavares *et al.* (2008) reported that the essential oil of *Daucus carota* with high amounts of elemicin had strong antifungal activity against *Trichophyton mentagrophytes*, *Microsporum canis*, *T. rubrum*, *Epidermophyton floccosum*, and *M. gypseum*. The antifungal activity was shown by *n*-hexane fraction might due to its major content, namely safrole and elemicin, as well as the contribution of other minor compounds.

Meanwhile, the antifungal activity of the methanol fraction was caused by its composition, namely fatty acids, safrole, and hinokinin which were detected by GC-MS and possibly the influence of other compounds that could not be detected by GC-MS such as flavonoids, saponins, and tannins which were detected during preliminary phytochemical screening. As much as 28.13% fatty acids were detected in the methanol fraction, which consisted of four compounds, namely 9,12-octadecadienoic acid, methyl ester (*E, E*) (8.29%), octadecanoic acid, methyl ester (7.01%), 9-octadecynoic acid, methyl ester (6.94%), and pentadecanoic acid, 14-methyl-, methyl ester (5.89%). Moreover, fatty acid methyl ester has been reported to inhibit various bacterial and fungal. Secondary metabolic antifungal effectiveness depending on the type of active

molecule, includes direct interactions with fungal enzymes, disruption of cell walls and cell membrane structure, antioxidant activity, or causes changes in the morphology of hyphae.

In conclusion, an environmentally friendly pesticide is therefore recommended in order to avoid the negative effects on human health and non-targeted pests. The findings indicated that *Cinnamomum parthenoxylon* which is a natural resource from Bengkulu-Indonesia, showed its potential as an anti-termite and antifungal. Both the non-polar and the polar fractions of *C. parthenoxylon* roots exhibited strong termiticide effect against *Coptotermes curvignathus*, and also inhibited the growth of *Trametes versicolor* at all concentration tested. Revealed the presence of phenylpropanoids, aldehydes, fatty acids, long-chain hydrocarbons, terpenoids, lignans, flavonoids, coumarins, saponins and tannins may play an important role for their antitermite and antifungal activity. This research is ongoing to isolate the active compounds of *C. parthenoxylon* roots fractions, and to determine their molecular structure, as well as investigating their fungicidal, termiticidal, and repellent activities singly or in combination.

Acknowledgements

The authors are grateful to the Ministry of Research and Technology/BRIN Republic of Indonesia for their financial support in the second year fundamental grant (165/SP2H/AMD/LT/DPRM/2020).

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(Received: 8 June 2021, accepted: 30 December 2021)