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Anti-Termite Activity of *Melia azedarach* Extracts

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ABSTRACT

Natural preservatives have been widely considered as non-synthetic materials that can prevent the biodegradation of wood due to subterranean termite attacks, namely *Coptotermes curvignathus*. *Melia azedarach* is a fast-growing species that has been widely studied for its antifeedant compounds and shows good potential to be developed as a biopesticide, especially as an anti-termite. This study aimed to identify the termiticide and antifeedant properties of the wood, leaves, and bark extracts of *Melia azedarach* against subterranean termites and identify their bioactive compounds. The acetone extract from the three parts of the tree was fractionated. The most active fractions were selected from each part based on the percentage of termite mortality and antifeedant activity and analyzed for its bioactive compounds by pyrolysis GCMS. The ethyl acetate fraction of *Melia azedarach* bark exhibited strong toxic properties compared to other fractions. In the leaf section, the diethyl ether fraction showed the best termite repellent (antifeedant) properties. The anti-termite properties influenced phenolic compounds and organic acid that dominated the bark ethyl acetate fraction and leaf diethyl ether fraction. These findings show the great potential of bioactive compounds from biomaterials to be developed as a promising biopesticide.

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1. Introduction

Most of the wood preservatives used today are synthetic preservatives that negatively affect the environment and human health. Wang et al. (2021) reported that commercial wood preservatives such as 2,4-dichlorophenol and 2,4,6-trichlorophenol have been shown to cause cancer. Likewise, water that has been contaminated and washed by wood preservatives had a high level of toxicity to aquatic biota (Łebkowska et al. 2003). Natural compounds with a better level of safety and the same effectiveness of preservation as a substitute for synthetic preservatives should be developed to overcome these requirements.

One of the wood preservation purposes is to prevent wood damage caused by microorganisms and insects, including wood-destroying termites. *Coptotermes curvignathus* is one of the termites with a very high level of attack on lignocellulosic materials (Nandika et al. 2003). Termites can attack the various lignocellulosic materials in buildings in Indonesia and cause

economic losses reaching approximately IDR 8.67 trillion per year (Nandika 2015). Several studies have shown the efficacy of secondary metabolites from plants against termite attacks, including the extracts, fractions, and isolates of leaf compounds of *Protium javanicum* (Adfa et al. 2010), the resin of *Dacryodes edulis* (Bédounguindzi et al. 2020), extracts of the heartwood of *Hymenaea stigonocarpa* (Maranhão et al. 2013), and extracts of the bark and leaves of *Lawsonia inermis* (Adedeji et al. 2017). Therefore, it can be considered that the compounds and isolates of plant metabolites have good properties as substitute materials for synthetic preservatives to overcome economic problems due to termite attacks.

Melia azedarach is a fast-growing tree species that is extensively produced in Indonesia. Statistics Indonesia (2019) reported that *Melia azedarach* tree log production in 2019 was 15,995 m³. Research using extracts, fractions, or components of compounds from this plant shows good potential as a biopesticide. For example, methanol extracts from *Melia azedarach* leaf and seeds showed a biopesticide effect against the destroying insect of *Hyblaea puera* (Nathan and Sehoon 2006). In addition, extracts from *Melia azedarach* fruit have potential as nematicides (Cavoski et al. 2012), parasitoid-related biopesticides (Defagó et al. 2011), and larvicides (Al-Mehmadi and Al-Khalaf 2010).

As mentioned above, *Melia azedarach* could be promising as a substitute material for synthetic preservatives. In particular, the leaf extract of *Melia azedarach* was toxic to the *Microtermes obesi* and *Odontotermes obesus* termite species (Qureshi et al. 2015). However, there is still no information on studies that reported the potential of the extracts of the heartwood and bark of *Melia azedarach* from Indonesia as an anti-termite *C. curvignathus*. To date, there have also been no studies on identifying the components of *Melia azedarach*'s bioactive compounds as an anti-termite. Therefore, this study was conducted to determine the potential of *Melia azedarach* trees from Indonesia in inhibiting the activity of subterranean termites and identify the bioactive components in the most active fractions.

2. Materials and Methods

2.1. Materials

A 6-years old *Melia azedarach* tree was obtained from the Cibereum area, Bogor, Indonesia. The leaves, bark, and heartwood were separated and converted to a powder. The test termites were *Coptotermes curvignathus*, obtained from *Kawasan Hutan Dengan Tujuan Khusus* (KHDTK) Yanlapa, Bogor, Indonesia. The chemicals used were acetone, n-hexane, diethyl ether, ethyl acetate, 70% ethanol, distilled water, filter paper, dental cement, tissue, and cellulose paper.

2.2. Methods

2.2.1. Extraction and fractionation

A total of 2 kg of leaf, bark, and heartwood powders were immersed in acetone with a powder-solvent ratio of 1:3. The mixture was allowed to stand for 48 h and then filtered to obtain a separate filtrate. This process was repeated until a clear filtrate was obtained. The filtrate was concentrated using a rotary evaporator (Heidolph, Germany), producing a concentrated extract. The extract was weighed to obtain the yield of the extract. The extracts were then fractionated using n-hexane, diethyl ether, and ethyl acetate (Fig. 1) to obtain the hexane soluble fraction (HF),

diethyl ether soluble fraction (DF), ethyl acetate soluble fraction (EF), and water soluble fraction (WF). The yield of the fraction was measured against the initial weight of the powder.

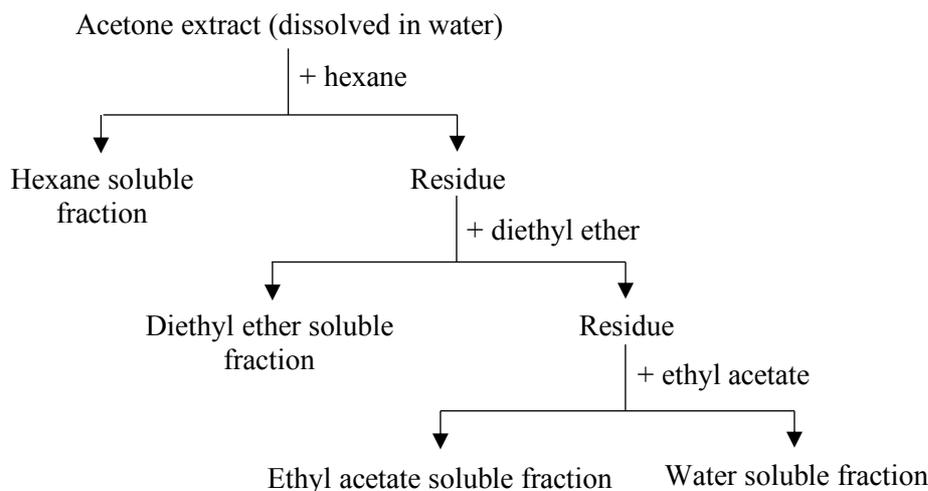


Fig. 1. Fractionation process of the extract of *Melia azedarach*'s leaf, bark, and heartwood.

2.2.2. Anti-termite test

The anti-termite test was performed using the cellulose paper method (Mukai et al. 2017). The test determined the termiticidal (termite mortality) and antifeedant activity (the weight loss of cellulose paper after the test). Cellulose paper (Whatman 42, 1.5 cm in diameter) was oven-dried at $103 \pm 2^\circ\text{C}$ for 24 h and then weighed. The cellulose paper was then soaked with fractions with concentrations of 2, 4, 6, 8, and 10% (w/w). The control group was also made by soaking cellulose paper with and without acetone. Each test group consisted of three papers for three replications. The preparation of the anti-termite test is presented in Fig. 2.

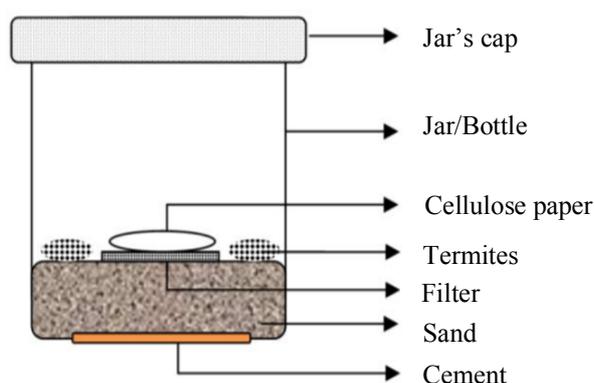


Fig. 2. Anti-termite test preparation.

Cellulose paper treated with and without fractions was placed in glass jars. A total of 50 termites consisting of 45 workers and 5 soldiers were used for testing. The test was performed for 21 days in a dark room. After 21 days, the termiticidal activity was measured using equation 1 and the antifeedant activity was calculated using equation 2.

$$M = \left(\frac{I_0 - I}{I_0} \times 100\% \right) - Mc \quad (1)$$

where M is mortality (%), M_c is the mortality percentage of the control group (%), I_o is the initial number of alive termites (individual), and I is the number of alive termites after 21 days of treatment (individual).

$$WL = \left(\frac{W_0 - W}{W_0} \right) \times 100\% \quad (2)$$

where WL is cellulose paper weight loss (%), W_0 is the initial weight of cellulose paper (g), and W is the weight of cellulose paper after 21 days of treatment (g).

The results were reported as the relationship of concentration to the percentage of termite mortality and feed activity. The LC_{50} value (concentration value that causes the death of individual termites by 50% of the total individual) was also analyzed using probit analysis.

2.2.3. Identification of compounds by Py-GCMS

The best fractions from leaf, bark, and heartwood were selected as three fractions with the highest mortality percentage and the best antifeedant activity. The compound analysis was conducted using a pyrolysis gas chromatography-mass spectrometry (Py-GCMS) type QP2010 (Shimadzu, Japan). The pyrolysis temperature used was 400°C for 1 h. The injected temperature was 280°C and the column temperature was gradually conducted from 50–280°C with a heating rate of 15°C/min. Electrospray ionization (mass range of 15–1000 m/z) was used as the ionization mode, and the weight analyzer was performed by a high-performance quadrupole mass analyzer. The resulting mass fragmentation was identified using the National Institute of Standards and Technology (NIST) database to obtain the type of compound.

2.2.4. Statistical analysis

Data related to mortality and weights of cellulose paper were presented in the average of three replications using the 2016 Microsoft Excel. In addition, probit analysis was also analyzed using the same application as the probit value table guidelines (Finney and Stevens 1948).

3. Results and Discussion

3.1. The Yield of Acetone Extract and Its Fraction

Extraction yields in each section of leaf, bark, and heartwood showed various levels of secondary metabolite or extractive components in each section and fraction. *Melia azedarach* leaf extract showed the highest yield percentage compared to bark and heartwood (Table 1).

Table 1. Extract yields and extract fractions from the leaves, bark, and heartwood of *Melia azedarach*

Extract/fraction	Yield (%)		
	Leaf	Bark	Heartwood
Acetone extract	10.53	7.54	0.74
HF	6.70	1.28	0.33
DF	2.80	0.69	0.25
EF	0.08	2.95	0.12
WF	0.95	2.62	0.04

Notes: HF is hexane soluble fraction, DF is diethyl ether soluble fraction, EF is ethyl acetate soluble fraction, and WF is water soluble fraction (WF).

The percentage yield of the fraction showed a different tendency in each section. The fraction of the leaf extract showed the highest percentage of the n-hexane fraction. The same phenomenon was also obtained from the fraction of the heartwood. The fraction of *Melia azedarach* bark extract showed a different trend. The ethyl acetate and water fractions showed higher yields than other fractions.

The leaf and heartwood extract of *Melia azedarach* probably contained higher nonpolar compounds due to the higher percentage of hexane soluble fraction yield than other fractions. It might be because n-hexane is a nonpolar solvent capable of dissolving nonpolar compounds (Abarca-Vargas et al. 2016). This heartwood and leaf probably contain compounds that are usually found in nonpolar fractions, such as terpenoids and limnoids (Sharma and Singla 2013; Zeng et al. 2019). In contrast to heartwood and leaf, the bark was dominated by polar compounds indicated by higher yields of ethyl acetate and water fractions. These two types of solvents are polar solvents capable of dissolving polar compounds (Abarca-Vargas et al. 2016). Our results were in line with the previous studies that succeeded in isolating flavonoid compounds from *Melia azedarach* bark (Sharma and Singla 2013).

3.2. Anti-termite Properties of the Extract Fraction

The anti-termite properties of the fraction of *Melia azedarach*'s leaf, bark, and heartwood showed an increased mortality percentage with increasing concentration. The tendency is shown for all sections (leaf, bark, and heartwood) and all fractions (Fig. 3).

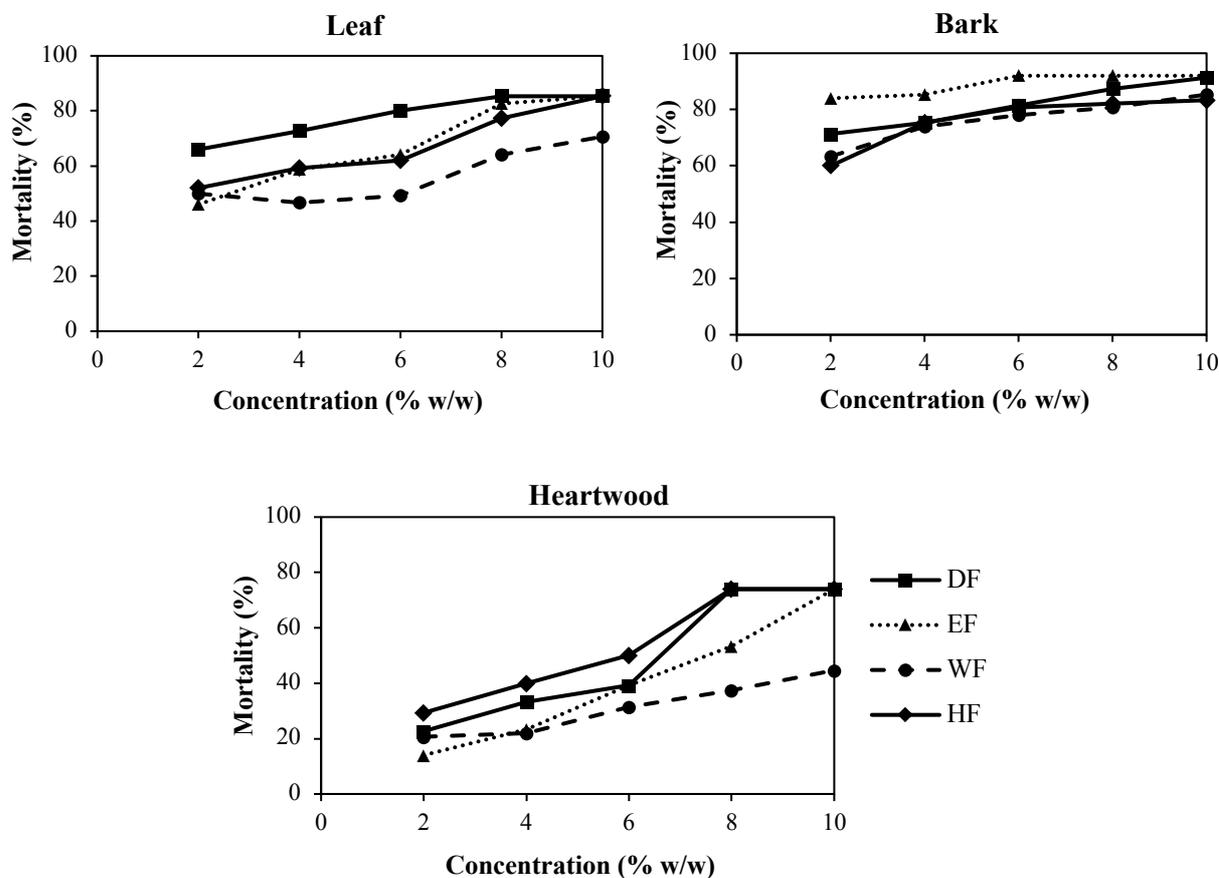


Fig. 3. The relationship between the termite mortality percentages and the test concentration.

The percentage of deaths, in this case, indicated the toxicity of the fraction to termites. The fraction that was able to produce the highest percentage of mortality at the lowest test concentration showed the high toxicity of the fraction to termites. The DF leaf showed the highest mortality percentage at the lowest concentration compared to other leaf fractions. The highest to lowest toxicity of the *Melia azedarach* leaf fraction was DF, HF, AF, and EF, respectively. Different things were shown in the bark and heartwood fractions. The fractions with the highest toxicity from bark and heartwood extracts were EF and HF, respectively. Based on the mortality percentage, the fraction of leaf and bark had similarity with the mortality percentage range at the lowest concentration above 50%. However, a different phenomenon occurred in the heartwood fraction. Only HF had a mortality percentage above 50% at the lowest concentration. [Mishra et al. \(2017\)](#) also reported that the mortality of *Microcerotermes beelsoni* termite increased up to 20% as increasing the concentrations of *Punica granatum* fruit rind fractions.

Toxicity analysis can also be predicted based on the LC₅₀ value, which describes the concentration at 50% mortality of the total termite individual. Based on this method, the fractions from all sections showed quite diverse toxicity to termites and were in line with the curve depiction of the concentration and mortality relationship. The LC₅₀ value of each fraction showed high toxicity, which was less than 20% (w/w) (**Table 2**). Trends in the values of leaf fractions with increased toxicity, namely the WF, EF, DF, and HF. Two types of fractions, namely the DF and HF, were more toxic than other fractions. The heartwood HF (LC₅₀ 4.62%) also showed high toxicity compared to the other heartwood fractions. In addition, the bark fraction also showed similar toxicity to the leaves fraction. The order of bark fractions from high to low toxicity was EF, DF, WF, and HF. Likewise, [Qureshi et al. \(2015\)](#) also reported that *Melia azedarach* leaf's aqueous extract and methanol showed high toxicity values in *Odontotermes obesus* and *Microtermes obesi* termites.

Table 2. Toxicity of *Melia azedarach*'s leaf, bark, and heartwood extract fractions

Fraction type	LC ₅₀ (% w/w)		
	Leaf	Bark	Heartwood
HF	0.78	1.01	4.62
DF	0.82	0.78	5.42
EF	2.63	0.09	6.84
WF	3.20	0.91	17.35

Notes: HF is hexane soluble fraction, DF is diethyl ether soluble fraction, EF is ethyl acetate soluble fraction, and WF is water soluble fraction (WF).

The decrease in weight of the cellulose paper after 21 days of the experiment showed the fraction's anti-termite properties. The decrease in the weight of cellulose paper was inversely proportional to the increase in concentration (**Table 3**). At the highest concentration (10% w/w), the weight reduction percentage of cellulose paper showed the lowest value compared to other concentrations. The tendencies were shown to all types of fractions in the three extracts. The leaf DF had the lowest weight loss of cellulose paper compared to all fractions. It indicated that the leaf DF was the best fraction to prevent termites from consuming cellulose paper or had the best antifeedant activity. Increasing concentration, which causes a lower percentage of cellulose paper weight loss, was also observed in longicamphenylone and isolongifolic acid compounds ([Mukai et al. 2017](#)).

Table 3. Percentage of weight loss of cellulose paper after 21 days of treatment

Part/fraction type	Percentage of weight loss of cellulose paper (%) in concentration					
	2%	4%	6%	8%	10%	
Leaf	Control	42.17	42.17	42.17	42.17	42.17
	HF	23.63	21.45	13.44	3.43	3.22
	DF	11.63	7.99	5.73	5.37	0.63
	EF	26.04	14.77	12.07	5.61	1.15
	WF	24.64	24.64	22.63	14.22	14.17
Bark	Control	39.50	39.50	39.50	39.50	39.50
	HF	21.70	19.50	18.40	16.20	14.50
	DF	20.80	19.00	18.70	15.30	12.50
	EF	15.50	13.80	11.60	9.80	6.30
	WF	28.00	22.10	19.60	16.90	15.60
Heartwood	Control	30.36	30.36	30.36	30.36	30.36
	HF	21.21	18.54	16.65	13.50	12.29
	DF	23.58	20.56	19.79	17.34	15.62
	EF	24.27	21.43	20.54	18.59	17.07
	WF	24.15	23.37	22.80	20.85	18.30

Notes: HF is hexane soluble fraction, DF is diethyl ether soluble fraction, EF is ethyl acetate soluble fraction, and WF is water soluble fraction (WF).

The anti-termite properties of the fraction can also be described in terms of the percentage of prevention of consumption (antifeedant) of cellulose paper at a concentration of 2–10% (w/w). The percentage value of prevention was obtained by reducing the percentage of weight loss on the control cellulose paper against the fraction treatment (Fig. 4).

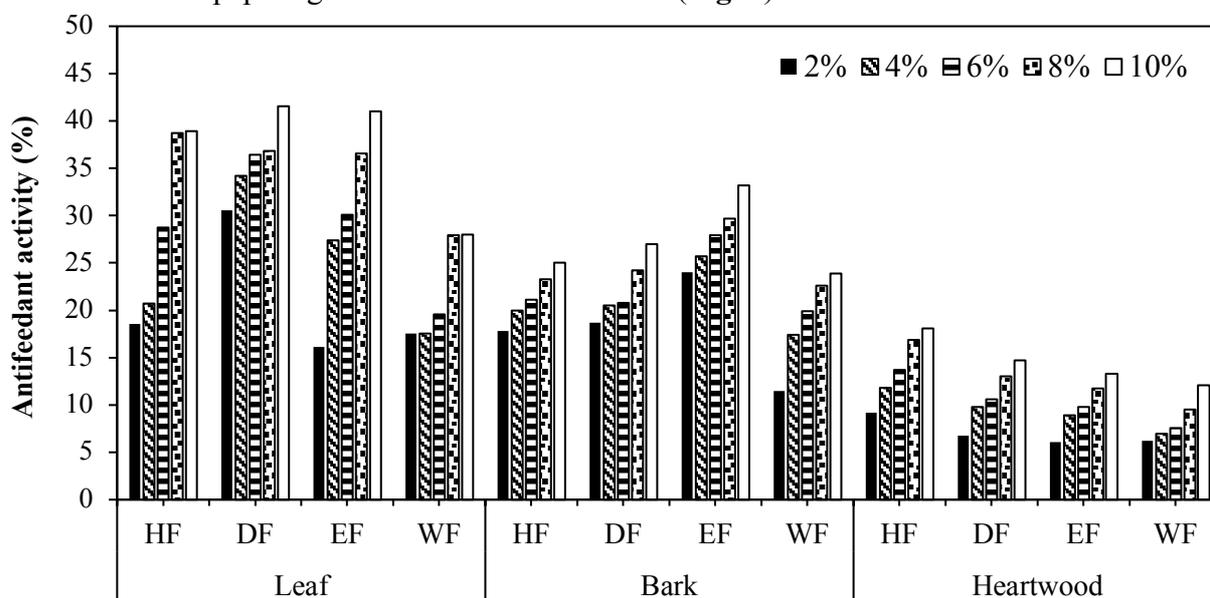


Fig. 4. Percentage of antifeedant activity to concentration in *Melia azedarach*'s leaf, bark, and heartwood extract fractions.

The highest percentage showed that the difference in the weight loss was high between the treatment fraction and control, indicating good antifeedant properties (Fig. 4). The percentage of antifeedant shows an increasing trend which is in line with the increase in concentration. A similar

phenomenon was also observed in the treatment effect of longifolene compounds from essential pine oil (Mukai et al. 2017). The tendency was also found in all types of *Melia azedarach* extract fractions. Overall, the lowest and highest antifeedant percentages were produced in 10% (w/w) of leaf DF and 2% (w/w) heartwood EF with 41.54% and 6.09%, respectively. When reviewed in each section, the trend of the lowest and highest weight loss percentages is different for each section. Leaf DF had the best antifeedant effectiveness because it had the highest antifeedant percentage (30.54%) at the lowest concentration. Similar phenomena also showed in other parts but differed in terms of the type of fraction. The ethyl acetate and n-hexane soluble fractions had the highest antifeedant percentage at a concentration of 2% (w/w) in the bark (24%) and the heartwood (9.15%). It also showed that these two fractions had the best effectiveness as antifeedants.

Mortality and antifeedant activity of all fractions showed a relationship that could predict the anti-termite mechanism of the best fraction in each type of extract. Based on the high mortality value and the best-antifeedant activity, the most active fractions from each part were leaf DF, bark EF, and heartwood HF. The low mortality percentage and high antifeedant activity indicate an anti-termite mechanism in preventing consumption (antifeedant). This mechanism was observed in the leaf diethyl ether fraction because the toxicity value of the leaf diethyl ether fraction (LC_{50} : 0.82% w/w) was lower than that of the bark ethyl acetate fraction (LC_{50} : 0.09% w/w), but the antifeedant percentage of the bark ethyl acetate fraction was higher than the other fractions. On the other hand, the bark fraction probably contained an anti-termite mechanism through its toxicity (termicide) because the toxicity value of the bark EF was higher than that of the leaf fraction, but its antifeedant activity was lower compared with the other fractions. The anti-termite mechanism through antifeedant activity has previously been observed in norlongilactone, longifolic acid, and isolongifelic acid compounds, while a toxic mechanism with low antifeedant activity was observed in longicamphenylon compounds (Mukai et al. 2017). However, these two types of mechanisms were not observed in the heartwood extract fraction, which had low toxicity and antifeedant activity.

3.3. Metabolite Components in the Best Fraction of Each Type of Extract

The three fractions with the high toxicity based on mortality percentage and the best-antifeedant activity, namely leaf DF, bark EF, and heartwood HF, showed different constituents based on pyrolysis-GCMS analysis (Table 4). Leaf DF was dominated by long-chain organic acids (lipids) and short chains (cinnamic acid, benzoic acid, benzenepropanoic acid, and acetic acid). Meanwhile, bark EF was dominated by phenolic compounds with a dominance of pyrocatechol compounds. Heartwood HF was composed of nonpolar compounds with long carbon chains, namely lipids, aldehydes, steroids, and sterols. These three extracts can be arranged based on the increasing polarity, namely HF of heartwood, DF of leaves, and EF of bark if sorted by polarity.

The anti-termite properties of the three fractions were related to the composition of the compound in the fractions. As previously discussed, the three fractions could be sorted by increasing their anti-termite properties, namely bark, leaves, and heartwood (based on extract toxicity) and leaves, bark, and heartwood (based on termite activity). If it is related to the compound content of each of these fractions, qualitatively, phenolic compounds play an essential role in anti-termite properties in this study. It was shown in the bark EF, which has anti-termite properties through the toxic mechanism with a dominant and more excellent content of phenolic

compounds than in leaf DF and heartwood HF. In addition, the acidic compounds also showed potential as anti-termite components through the antifeedant mechanism, which was responsible for the anti-termite properties of the leaf DF, especially carboxylic acids with benzene and lipid structures. Compound with long-chain carbon also showed a similar effect as shown in heartwood HF. Consequently, it revealed that the constituents that play an essential role in the anti-termite properties of the *Melia azedarach* tree fraction are compounds with phenolic structure, a carboxylic acid group, and a long-chain carbon. Adfa et al. (2010) also explained that flavonoid and coumarin compounds have good anti-termite properties.

Table 4. Metabolite constituents from leaf DF, bark EF, and heartwood HF

Compounds name	Abundance (%)			Group of compounds
	Leaf	Bark	Heartwood	
Cinnamic acid	18.54	-	0.30	Organic acid
Benzoic acid	12.82	-	1.38	Organic acid
Benzenepropanoic acid	9.07	-	0.53	Organic acid
Acetic acid	-	7.49	-	Organic acid
Palmitic acid	4.98	3.83	13.10	Lipids
Stearic acid	1.78	-	-	Lipids
Lauric acid	2.01	-	1.42	Lipids
Myristic acid	1.48	0.80	0.71	Lipids
Fitol	4.01	-	-	Phenolic
p-cresol	1.69	-	-	Phenolic
Carvacrol	1.58	-	-	Phenolic
Pyrocatechol	-	10.39	-	Phenolic
4-methylcatechol	-	5.00	-	Phenolic
Guaiacol	-	1.40	0.32	Phenolic
o-ethylphenol	-	0.30	-	Phenolic
2-methoxy-4-methylphenol	-	0.44	-	Phenolic
P-Ethyl guaiacol	-	1.10	-	Phenolic
2,6-dimethoxyphenol	-	1.28	-	Phenolic
p-benzyl-phenol	-	2.03	-	Phenolic
Dioctyl Phthalate	-	4.37	-	Esther
Sitosterol	-	3.26	-	Steroids
18-Hydroxydeoxycorticosterone	-	-	3.45	Steroids
Limonene	-	-	0.65	Terpenoids
Alpha-Cadinol	-	-	2.39	Terpenoids
Cis-9-Octadecenal	-	-	19.71	Aldehyde
Octadecanal	-	-	7.88	Aldehyde
17beta-Methyl-5alpha-androstan-16-one	-	-	11.78	Steroids

4. Conclusions

The best fractions from the leaves, bark, and heartwood were the leaf diethyl ether, bark ethyl acetate, and heartwood hexane fractions, respectively. The ethyl acetate fraction of *Melia azedarach* bark showed a toxic anti-termite mechanism, while the diethyl ether fraction showed an anti-termite mechanism through antifeedant activity. The hexane fraction of heartwood had weaker termite-resistant properties than the leaf and bark fraction. Components of organic acids (simple, aromatic, and long-chain), phenolic compounds, steroids, and terpenoids affected the bioactivity. Based on these results, the fraction of *Melia azedarach* leaf, bark, and heartwood acetone extract can be developed as an active ingredient of termite biopesticide.

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