



# Efficacy of Biopesticides from the Leaves of *Dioscorea bulbifera* L. in the Control of Drywood Termites (*Cryptotermes cynocephalus* Light)

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## ABSTRACT

Damage to building wood due to termite infestation, especially the dry wood termite (*Cryptotermes cynocephalus* Light) leads to considerable economic losses. Among the potential biopesticides considered for termite control is the yam *Dioscorea bulbifera*; the leaves contain bioactive compounds such as alkaloids, flavonoids, saponins, tannins, steroids, and terpenoids, which are thought to have potential as biopesticides. The goal of this study was to determine the effectiveness of *D. bulbifera* leaves as a biopesticide in the control of *C. cynocephalus*. The experimental approach was based on a completely randomized factorial design, with factor A being the type of solvent (*n*-hexane, ethyl acetate, and methanol) and factor B the concentration of the extract (1%, 1.5%, 2%, 2.5%, and 3%). Contact toxicity tests were performed (five replicates). The observed parameters included wood retention, termite mortality, wood weight loss, degree of damage, and qualitative and quantitative phytochemical tests (through gas chromatography-mass spectrometry). The most effective *D. bulbifera* leaf extract was that prepared with methanol, with the following results: wood retention, 0.33 kg/m<sup>3</sup>; termite mortality, 76%; test weight reduction, 2.88 g; and degree of damage, 56.55%. The phytochemical test results showed that the secondary metabolites in the methanol extract of *D. bulbifera* leaves contained alkaloids, flavonoids, phenols, steroids, terpenoids, tannins, and saponins. The methanol extract also contained 11 compounds with potential biopesticide activity. Further studies are required to determine the appropriate concentration for application as a biopesticide.

**Keywords:** biopesticides, *Cryptotermes cynocephalus*, *Dioscorea bulbifera* leaves, efficacy

Date Received October 24, 2024; Date Revised November 25, 2024; Date Accepted January 10, 2025; Published May 25, 2025

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## 1. INTRODUCTION

Termites inflict damage to buildings and other wood structures on a massive scale (Mishra *et al.*, 2021). Damage to building wood owing to termite attacks, especially dry wood termites (*Cryptotermes cynocephalus* Light) causes substantial economic losses (Nandika *et al.*, 2015).

Synthetic insecticides are a means of controlling dry wood termites. However, their excessive use and consistent application have side effects on the environment and human health (Shabana *et al.*, 2017; Subekti *et al.*, 2019). The harmful effects of pesticides on humans include neurological toxicity, chronic neurodevelopmental damage, potential immunological and reproductive malfunctions, and cancer (Matisová and Hrouzková, 2012). Owing to their high toxicity and poor biodegradability—leading to contamination of soil, water, and croplands—the application of pesticides at excessive levels or over long periods has resulted in environmental issues and financial losses (Lima *et al.*, 2013). Thus, the search for and development of alternative insecticides, with minimal harmful effect on the environment and human health, is warranted. One such alternative in the control of dry wood termites is the use of botanical insecticides. Many plant species are suitable as sources of pest control material, given that plants are generally rich in bioactive compounds that function as natural defenses against pests (Lengai *et al.*, 2020). A number of secondary metabolites, such as alkaloids, terpenoids, and phenolics, serve to deter or kill insects. The potential of botanical pest control, is widely recognized, although continued research and refinement of these materials is needed.

Biopesticides are natural products derived from plants containing secondary metabolites such as alkaloids, terpenoids, and phenolics. Yams, plants in the genus *Dioscorea* (Dioscoreaceae), are considered a potential source of biocontrol agents. Besides other compounds,

*Dioscorea* spp. contain various toxic compounds such as dioscorin, diosgenin, dioscin, saponins, and tannins (Ningsih *et al.*, 2013). These compounds are distributed in all plant organs; therefore, every part of the plant is a potential source of biopesticides or biocontrol material (Gajger and Dar, 2021; Muhidin *et al.*, 2020). Various studies have investigated the insecticidal activity of *Dioscorea hispida* with respect to specific pests—*Plutella* sp. (Utomo, 2017); *Spodoptera litura* larvae (Ningsih *et al.*, 2013); *Aedes aegypti* and *Aedes albopictus* larvae (Dewi, 2018); Anopheles, Culex, *Aedes aegypti* (Wardianti and Septiani, 2017); *Nillavarpata lugens* Stall (Muhidin *et al.*, 2020); *S. litura* F. larvae (Darmanto *et al.*, 2019); *Spodoptera exigua* (Mustarsidin *et al.*, 2021); *Pamacea canaliculata* (Alfaizal *et al.*, 2021); and *Aedes aegypti* (Kasman *et al.*, 2020).

Another species of *Dioscorea* that has potential as a biopesticide is the subject of the present study, the yam *Dioscorea bulbifera* Light, which is widely distributed through Asia and Africa (Ghosh, 2015). Indeed, *D. bulbifera* is considered an invasive species that contributes to environmental issues in many parts of the world; the species is listed in the Global Weed Compendium (Oksari *et al.*, 2019). It has been reported that *D. bulbifera* leaves contain bioactive compounds, such as alkaloids, flavonoids, saponins, tannins, steroids, and terpenoids, which are thought to have potential as botanical insecticides Oksari *et al.* (2021). An aqueous extract of *D. bulbifera* leaves has been shown to produce the best results, with a termite mortality rate of 70.97% at a concentration of 50% (Oksari *et al.*, 2023). To the best of our knowledge, there are as yet no published studies of the effectiveness of *D. bulbifera* extracts of different polarities at specified concentrations. The aim of the present study was to determine the effectiveness of *D. bulbifera* leaves as a biopesticide for controlling dry wood termites (*C. cynocephalus* Light).

## 2. MATERIALS and METHODS

### 2.1. Materials

*D. bulbifera* leaves were randomly collected from the Bogor Botanical Gardens-BRIN, Bogor, West Java, Indonesia. The chemicals used for extraction were *n*-hexane, ethyl acetate, and methanol (technical grade). The materials used in the bioassay were: rubberwood samples [*Hevea brasiliensis* (Willd. ex A.Juss.) Müll. Arg.], Tween 80 (technical grade), acetone (technical grade), and the dry wood termite *C. cynocephalus* Light.

### 2.2. Methods

#### 2.2.1. Sample preparation

*D. bulbifera* leaves (15 kg) were cleaned, cut into cubes, dried in air, and then placed in an oven at 60°C for 72 h. The dried leaves (1.5 kg) were crushed and placed in a 40-mesh sieve to produce 750 g of *D. bulbifera* leaf powder (Oksari *et al.*, 2021).

#### 2.2.2. Determination of water content

Water content was determined using the modified SNI 01-3182-1992. *D. bulbifera* leaves were weighed at  $\pm 2$  g ( $m_0$ ) in a Petri dish and placed in the oven for 5 h at a temperature of  $103 \pm 2^\circ\text{C}$ . The samples were then placed in a desiccator for approximately 15 min and weighed again to obtain the final weight ( $m_1$ ). The percentage of water content was calculated as follows:

$$\text{Water content} = \frac{m_0 - m_1}{m_1} \times 100 \quad (1)$$

Where  $m_0$  = initial weight (g);  $m_1$  = weight after drying (g).

#### 2.2.3. *Dioscorea bulbifera* leaf extraction

*D. bulbifera* leaf extract was prepared following a

previously published procedure (Asmaliyah *et al.*, 2020). *D. bulbifera* leaves were extracted with different solvents, including *n*-hexane, ethyl acetate, and methanol, using a multistage maceration method with a powder-to-solvent ratio (based on dry weight) of 1:6 (w/v). 1.5 kg of *D. bulbifera* leaf powder was soaked in *n*-hexane (9 L) for 24 h. The *n*-hexane extract was then filtered until clear using a glass funnel lined with filter paper, and the macerate was dissolved in 9 L ethyl acetate for 24 h. The ethyl acetate macerate was then filtered and dissolved in 9 L of methanol for 24 h. The resulting filtrate was placed in an evaporation flask in a rotary evaporator (50°C–60°C and 500–700 mmHg vacuum) to evaporate the solvent. The resulting extracts, including *n*-hexane, ethyl acetate, and methanol extracts, were stored in the refrigerator (4°C) until use (Asmaliyah *et al.*, 2010).

#### 2.2.4. Phytochemical qualitative test

*D. bulbifera* leaf extract was tested for bioactive content using the method described by Harborne (1987). Phytochemical tests were performed on the alkaloids, flavonoids, phenolics, tannins, terpenes, saponins, steroids, and terpenoids.

##### 2.2.4.1. Alkaloids

*D. bulbifera* leaf extract was dissolved in a few drops of 2N sulfuric acid and tested using three alkaloid reagents. The results are positive for alkaloid presence if the precipitate from the Drgendorff test is red to orange. In the case of the Mayer and Wagner tests, positive results are obtained if the product is yellowish-white and brown, respectively.

##### 2.2.4.2. Phenolic compounds

Ten drops of 1% FeCl<sub>3</sub> were added to 50 g of the extract. An extract is positive for phenol if it produces a green, red, purple, blue, or dark black color.

#### 2.2.4.3. Flavonoids

A 50 g sample of extract was added to 100 mL of hot water; the mixture was then boiled for 5 min and filtered. Next, 0.05 mg of Mg powder and 1 mL of concentrated HCl were added to 5 mL of the filtrate, and the mixture was shaken vigorously. A positive test is indicated by red, yellow, or orange precipitate.

#### 2.2.4.4. Saponins

A 50 g sample of extract was added to 10 mL of water, and 2 drops of 1 N HCl were added. If the foam formed remains stable for  $\pm 7$  min, the extract is positive for the presence of saponin.

#### 2.2.4.5. Steroids and terpenoids

Ten drops of glacial  $\text{CH}_3\text{COOH}$  and two drops of concentrated  $\text{H}_2\text{SO}_4$  were added to 50 g of the extract. The solution was shaken gently and left for several minutes. A positive test is indicated by the color of the precipitate: blue or green indicates steroids and red or purple, terpenoids.

#### 2.2.4.6. Tannins

One milliliter of extract was added to 10 mL of distilled water, heated in a water bath, and 2–3 drops of 1% FeCl were added. The formation of green, dark blue, or greenish-black color indicated a positive result.

#### 2.2.5. Gas chromatography-mass spectrometry analysis

Crude extracts from leaves of *D. bulbifera* were subjected to phytochemical screening by gas chromatography-mass spectrometry (GC-MS) using an Agilent GC model 19091S-433: 93.92873 DB-5MS UI 5% Phenyl Methyl Silox. Extraction was performed using methanol, *n*-hexane, and ethyl acetate. A 1- $\mu\text{L}$  sample was injected into the GC-MS apparatus equipped with a glass column 30 m long, 250  $\mu\text{m}$  diameter, and 0.25  $\mu\text{m}$  thickness. The column temperature was 40°C and the

injection temperature 300°C. Compounds were identified based on the similarity index and compound fragmentation patterns (Idu *et al.*, 2021.).

#### 2.2.6. Provision of termites

The dry wood termite *C. cynocephalus* from the Ministry of Environment and Forestry (KLHK) Bogor Central Laboratory for Standardization of Sustainable Forest Management Instruments was used for testing. Each replicate in all treatments contained as many as 50 termites (worker caste). The selected termites were active, healthy, and equal in size (Zulkahfi *et al.*, 2017).

#### 2.2.7. Bait provision

Rubberwood samples [*Hevea brasiliensis* (Willd. ex A.Juss.) Müll.Arg.] were each cut with 5 cm  $\times$  2.5 cm  $\times$  2.5 cm, and five test samples were provided as replicates for each treatment. The rubberwood samples were soaked in these extracts for 24 h and then dried. Each test piece of wood was then installed in an acrylic tube 1.8 cm in diameter and 3.5 cm in height. Fifty healthy and active worker termites (*C. cynocephalus*) were placed in an acrylic tube, and the test samples were stored in the dark for 12 weeks (Badan Standardisasi Nasional, 2014).

#### 2.2.8. Mortality test

This test used *n*-hexane, methanol, and ethyl acetate extracts from multilevel maceration extraction. Each extract was treated with six concentration levels, namely 0%, 1%, 1.5%, 2%, 2.5%, and 3% (control/without extract). For each concentration and the control, five replications were performed, each with 50 termites. The test solution was prepared as a stock solution of 21 g of extract in 700 mL of a mixture of solvent, acetone, Tween 80 emulsifier, and distilled water (5:5:1:3). The tests were performed using the contact method. The number of dead termite larvae was recorded weekly for 12 weeks (Asmaliyah *et al.*, 2010).

### 2.2.9. Measured parameters

The efficacy parameters of *D. bulbifera* leaf extract on dry wood termites were wood retention (Vachlepi *et al.*, 2015), termite mortality rate, wood weight loss, and degree of damage (Oksari *et al.*, 2023). The scale of the level of damage and wood resistance indicates a change in the degree of damage (Azis *et al.*, 2013).

### 2.2.10. Data analysis

In the randomized factorial design, the combination of factor A was the type of solvent (*n*-hexane, ethyl acetate, and methanol) and factor B was the concentration of the extract (1%, 1.5%, 2%, 2.5%, and 3%) with five replicates. Statistical analysis was performed using the Statistical Tool for Agricultural Research (STAR 2.0.1). Further quantitative tests were performed using Duncan's test at  $\alpha = 5\%$  if the treatment had a significant effect. Abbot's equation was used to correct mortality when the control mortality was higher than 5% but lower than 20% (Abbott, 1925).

## 3. RESULTS and DISCUSSION

### 3.1. Secondary metabolites of *Dioscorea bulbifera*

The bioactive compounds (steroids, terpenoids, and tannins) were obtained based on the results of the studies on *n*-hexane and ethyl acetate extracts. The methanol extract contained bioactive compounds, such as alkaloids, flavonoids, phenols, saponins, steroids, terpenoids, and tannins (Table 1). Research conducted by Oksari *et al.* (2021) reported that the methanol extract of *D. bulbifera* leaves contained alkaloids, flavonoids, phenolic compounds, saponins, tannins, steroids, and terpenoids. According to research conducted by Sami and Fata (2019) and Wardianti and Septiani (2017), the members of *Dioscorea* contain the alkaloid compounds dioscorin, diosgenin, dioscin, saponins, and tannins,

**Table 1.** Results of phytochemical tests of *Dioscorea bulbifera* leaf extract

No	Qualitative test	Extract type		
		<i>n</i> -Hexane	Ethyl acetate	Methanol
1	Alkaloids			
	Mayer	–	–	+
	Wagner	–	–	+
	Dragendorf	–	–	+
2	Flavonoids	–	–	+
3	Phenols	–	–	+
4	Steroids	+	+	+
5	Saponins	–	–	+
6	Tannins	+	+	+
7	Terpenoids	+	+	+

(+): positive for secondary metabolites, (–): negative for secondary metabolites.

which have potential as botanical insecticides.

The potential of some of these chemical compounds can be observed in the results of several chemical compounds reported in previous studies. The alkaloids (dioscorin), steroids (diosgenin), and saponins (dioscin) are thought to cause nervous disorders, such as seizures, when consumed by humans and animals, and have properties that inhibit eating activity and the growth of insect eggs (Obidiegwu *et al.*, 2020). The leaves of *Murraya koenigii* L. (flavonoids), *Phyllanthus niruri* L. (tannins), and *Syzygium cumini* L. (saponins) contain chemicals that are poisonous and may be used as botanical pesticides, according to research by Mishra *et al.* (2021). Saponins, being neurotoxins, interfere with normal feeding behavior and digestion across a wide variety of animals and are thus applied as pesticides against rats, caterpillars, and sucking pests (Qasim *et al.*, 2020). Alkaloids inhibit growth and development of pathogenic fungi. Furthermore, tannins are known to

have the potential as an astringent that can harden the skin of pests (War *et al.*, 2012). These results indicate that *D. bulbifera* extract contains compounds that have potential as botanical insecticides.

The results of the GC-MS analysis showed several peaks representing compounds with potential as biopesticides (Table 2). The toxic potential of these compounds was determined based on previous research. Eleven active compounds were found in the methanol extract of *D. bulbifera* leaves, that have potential as biopesticides. They are bis(2-ethylhexyl) phthalate (13.3288%); 9,12,15-octadecatrienoic acid, ethyl ester (9.5474%); n-hexadecanoic acid (9.3031%); 9,12,15-octadecatrienoic acid (8.6313%); 9,12-octadecadienoic acid, methyl ester (4.7349%); phytol (4.3512%); linoleic acid ethyl ester (4.0186%); hexadecanoic acid, ethyl ester (3.0095%); cyclododecane (0.6826%); neophytadiene (0.5287%); and squalene (0.4755%). Thirteen active compounds in the ethyl acetate extract were found to have potential as biopesticides: 9,12,15-octadecatrienoic acid (23.4595% and 0.4076%); n-hexadecanoic acid (19.0431%); bis(2-ethylhexyl) phthalate (15.1595%); 9,12,15-octadecatrienoic acid, ethyl ester (7.1435%); linoleic acid ethyl ester (3.7708%); squalene (2.7998%); nonacosane (1.9255%); neophytadiene (1.0709%); methylene chloride (0.8235%); 3,7,11,15-tetramethyl-2-hexadecen-1-ol (0.5430%); hexadecanoic acid, methyl ester (0.3808%); 9,12-octadecadienoic acid (Z, Z)-, methyl ester (0.2314%); and eicosane (0.3896%; 0.4769%; 3.1479%). Fifteen active compounds in the hexane extract were found to have potential as biopesticides: 9,12,15-octadecatrienoic acid (30.1129%; 1.4116%; and 0.9645%); n-hexadecanoic acid (17.5116%; 0.6185%; and 0.6349%); hentriacontane (4.4677%); nonacosane (2.9074%); squalene (2.5324%); octacosane (1.2690%); triacontane (1.0540%); tetracosane (0.9710%); eicosane (0.6310%; 1.9426%; 1.0637%; and 0.2965%); oxalic acid, heptadecyl hexyl ester (0.5952%); docosanoic acid, docosyl ester (0.5295%); 2,4-di-tert-butyl-phenol

(0.3648%); hexadecanoic acid methyl ester (0.1959%); 1-docosene (0.1755%); and 9,12-octadecadienoic acid, methyl ester (0.1548%; Table 2).

The most active compound obtained using multilevel maceration (ethyl acetate and hexane) was 9,12,15-octadecatrienoic acid (Table 2). 9,12,15-Octadecatrienoic acid (linolenic acid) possesses antiparasitoid properties (Dalei *et al.*, 2022). Conjugated linoleic acid is an insecticide with antifeedant effects, increased larval mortality, decreased larval development, and egg viability, as it manipulates fatty acid composition in non-mammalian systems, leading to embryonic mortality (Clements *et al.*, 2019). Other functions include biopesticides, insectifuges, and nematocides (Banu and Nagarajan, 2013; Nishanthini *et al.*, 2014; Rency *et al.*, 2015; Zayed and Samling, 2016). Based on the GC-MS analysis (Table 2), 42 compounds were detected in the *n*-hexane extract, with the highest percentage being fatty acids and their esters. This result is in line with previous findings of that the *n*-hexane extract of *Azadirachta excelsa* seed kernels contains several fatty acids (Adfa *et al.*, 2023). The fatty acid compounds with high percentages in the *n*-hexane extract of *D. bulbifera* leaves were 9,12,15-octadecatrienoic acid and n-hexadecanoic acid (Table 2). Fatty acid compounds are toxic to insecticide-treated larvae (Perumalsamy *et al.*, 2015). *Robinia pseudoacacia* L. seed extract contains a fatty acid comprising 9,12-octadecadienoic acid, 9,12,15-octadecatrienoic acid methyl ester, and 9,12-octadecadienoic acid methyl ester that has potential as a bioinsecticide (Jiang *et al.*, 2018). Based on the results of GC-MS analysis (Table 2), *n*-hexane extract from *D. bulbifera* leaves also contained 9,12,15-octadecatrienoic acid methyl ester and 9,12-octadecadienoic acid methyl ester, but with smaller percentages, namely 0.5032% respectively and 0.1548%. These compounds were identified in relatively large quantities in the methanol extract of *D. bulbifera* leaves, namely 9,12,15-octadecatrienoic acid methyl ester with an area of 16.1615% and 9,12-octadecadienoic

**Table 2.** Analysis results of secondary metabolites present in the extract of *Dioscorea bulbifera* leaves using the multilevel maceration method

	Compound	Retention time (min)	%Area
Solvent: Methanol			
1	1-Butanol, 3-methyl-, acetate	9.7459	15.1372
2	2a,4a,6a,6b-tetrahydrocyclopenta[cd]pentalene	10.8801	1.7136
3	Neophytadiene	18.6058	0.5287
4	Pentadecanoic acid, 14-methyl-, methyl ester	19.5132	6.3691
5	n-Hexadecanoic acid	19.9165	9.3031
6	Hexadecanoic acid, ethyl ester	20.1812	3.0095
7	9,12-Octadecadienoic acid, methyl ester	21.1516	4.7349
8	9,12,15-Octadecatrienoic acid, methyl ester	21.2146	16.1615
9	Phytol	21.3028	4.3512
10	Heptadecanoic acid, 16-methyl-, methyl ester	21.4415	0.8574
11	9,12,15-Octadecatrienoic acid	21.6305	8.6313
12	Linoleic acid ethyl ester	21.7566	4.0186
13	9,12,15-Octadecatrienoic acid, ethyl ester	21.8196	9.5474
14	Bis(2-ethylhexyl) phthalate	24.9073	13.3288
15	Cyclododecane	25.1594	0.6826
16	Squalene	26.9868	0.4755
17	Vitamin E	29.7847	1.1497
Solvent: Ethyl acetate			
1	Methylene chloride	4.5536	0.8235
2	trans-2,3-Epoxydecane	18.2531	0.9730
3	Neophytadiene	18.6186	1.0709
4	Dodecanal	18.8707	0.1944
5	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	19.0723	0.5430
6	Hexadecanoic acid, methyl ester	19.5512	0.3808
7	n-Hexadecanoic acid	19.9293	19.0431
8	Octadecanoic acid	20.1940	4.0329
9	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	21.1518	0.2314
10	9,12,15-Octadecatrienoic acid, methyl ester	21.2148	0.8327
11	7-Oxabicyclo[4.1.0]heptane, 1,5-dimethyl-	21.3157	0.5909
12	9,12,15-Octadecatrienoic acid	21.6433	23.4595

**Table 2.** Continued

	Compound	Retention time (min)	%Area
Solvent: Ethyl acetate			
13	Linoleic acid ethyl ester	21.7694	3.7708
14	9,12,15-Octadecatrienoic acid, ethyl ester	21.8324	7.1435
15	9,12,15-Octadecatrienoic acid	21.9836	0.4076
16	Cyclooctene, 3-ethenyl-	23.4456	0.4276
17	Cyclooctene, 3-ethenyl-	23.6472	0.6253
18	gamma.-Sitosterol	24.3026	4.8910
19	Octadecane, 1-chloro-	24.6177	0.4288
20	Bis(2-ethylhexyl) phthalate	24.9075	15.1595
21	1-Octadecanesulphonyl chloride	25.3990	0.3438
22	9,12,15-Octadecatrienoic acid, methyl ester	25.6385	0.3688
23	Hexacosane	26.1426	0.7054
24	Eicosane	26.8610	0.3896
25	Squalene	26.9870	2.7998
26	1H-Indene	27.4155	0.5609
27	Nonacosane	27.6046	1.9255
28	Eicosane	28.4112	0.4769
29	Eicosane	29.3564	3.1479
30	Vitamin E	29.7849	4.2510
Solvent: <i>n</i> -Hexane			
1	2,4-Di-tert-butylphenol	14.9510	0.3648
2	1-Docosene	18.1900	0.1755
3	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-(1alpha,2beta,5alpha)-	18.6185	0.4712
4	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	19.0722	0.4031
5	Hexadecanoic acid, methyl ester	19.5385	0.1959
6	<i>n</i> -Hexadecanoic acid	19.9796	17.5116
7	<i>n</i> -Hexadecanoic acid	20.1939	0.6185
8	<i>n</i> -Hexadecanoic acid	20.2443	0.6349
9	9,12-Octadecadienoic acid, methyl ester	21.1517	0.1548
10	9,12,15-Octadecatrienoic acid, methyl ester	21.2147	0.5032
11	Undec-10-ynoic acid, dodecyl ester	21.3029	0.6579
12	Ethyl 2-butyramido-3,3,3-trifluoro-2-(4-fluoroanilino)propionate	21.3660	0.9229



**Table 2.** Continued

	Compound	Retention time (min)	%Area
Solvent: <i>n</i> -Hexane			
13	9,12,15-Octadecatrienoic acid	21.7062	30.1129
14	9,12,15-Octadecatrien-1-ol	21.8322	8.0197
15	9,12-Octadecadienoic acid	22.0465	1.0640
16	Z,Z-11,13-Hexadecadien-1-ol acetate	22.0969	4.8523
17	9,12,15-Octadecatrienoic acid	22.6137	1.4116
18	Tetratetracontane	22.9665	1.5370
19	4,8,12,16-Tetramethylheptadecan-4-olide	23.4455	0.6625
20	Heptasiloxane, hexadecamethyl-	23.7731	0.5533
21	Tetracosane	23.8109	0.9710
22	Oxalic acid, heptadecyl hexyl ester	24.1890	0.5952
23	Tridecane	24.4663	0.7691
24	Tetratetracontane	24.6175	1.3573
25	Docosanoic acid, docosyl ester	24.8570	0.5295
26	Docosyl octyl ether	24.9074	0.8193
27	1-Pentacontanol	25.1091	0.6993
28	Tritetracontane	25.3989	1.3404
29	1,2-Epoxy-5,9-cyclododecadiene	25.5501	0.4893
30	9,12,15-Octadecatrienoic acid	25.6132	0.9645
31	Eicosane	25.8652	0.6310
32	Eicosane	26.1425	1.9426
33	Eicosane	26.3694	1.0637
34	Octacosane	26.8609	1.2690
35	Squalene	26.9869	2.5324
36	1-Pentacontanol	27.1633	0.4462
37	1-Bromo-11-iodoundecane	27.4028	0.6352
38	Nonacosane	27.6045	2.9074
39	Triacontane	28.4110	1.0540
40	Hentriacontane	29.3563	4.4677
41	Vitamin E	29.7596	3.3919
42	Eicosane	30.4401	0.2965

acid methyl ester with an area of 4.7349%. The fatty acid and ester compounds in the ethyl acetate extract were almost identical to those in the *n*-hexane and methanol extracts. The most abundant compounds were 9,12,15-octadecatrienoic acid and *n*-hexadecanoic acids.

The compound bis (2-ethylhexyl) phthalate was not found in the *n*-hexane extract but was found in the ethyl acetate and methanol extracts, and *D. bulbifera* had a higher area percentage in the ethyl acetate extract (Table 2). This compound is one of the compounds that have larvicidal activity (Javed *et al.*, 2022; Ravi *et al.*, 2018), which is related to the inhibition of acetylcholinesterase activity (Rajamanikyam *et al.*, 2017). Inhibition of this enzyme, which is typically expressed in the nervous system, can result in death (Colovic *et al.*, 2013). Taken together, the results of our chemical analysis of *D. bulbifera* leaf extract shown that it has potential as a control agent against drywood termites. Further study is warranted.

### 3.2. Effect of *Dioscorea bulbifera* extract on dry wood termites

There were significant differences among the different solvents in terms of the measured parameters (retention, mortality, reduction in test weight, and degree of damage). In addition, there were significant differences in retention and mortality based on the concentration of the extract (Table 3). The difference in the influence

of the factors on some of these parameters is likely due to different mechanisms. Based on the results shown in Table 3, it is suspected that there is a mechanism of action for certain botanical insecticides that inhibits the reproductive process of female insects; reduces appetite; causes insects to refuse to eat; damages the development of eggs, larvae, and pupae; disrupts insect reproduction; and inhibits skin molting (Campos *et al.*, 2019). Based on the results of this study, the mechanism of action of *D. bulbifera* leaf extract can lure termites to eat the test wood. Therefore, *D. bulbifera* leaf extract is believed to be a member of a class of insecticides known as attractants, specifically botanical insecticides that can draw insects, act as components of insect traps, and inhibit the growth of fungi and bacteria (Kumar *et al.*, 2021; Mishra *et al.*, 2021; Ngegba *et al.*, 2022; Rahayu *et al.*, 2023).

### 3.3. Retention

The wood retention value of the *n*-hexane extract of *D. bulbifera* leaves was significantly greater than that of the control and the other extract treatments (ethyl acetate and methanol). The best retention was obtained for extract using *n*-hexane extract (1.13 kg/m<sup>3</sup>) and at a concentration of 2% (1.08 kg/m<sup>3</sup>) according to the results of the DMRT test (Table 4). Because it binds strongly to substances in sapwood, the *n*-hexane extract of *D. bulbifera* leaves at a concentration of 2% had the

**Table 3.** Effects of *Dioscorea bulbifera* extract on drywood termites

Factor	Parameter			
	Retention	Mortality	Weight loss test	Degree of damage
Type of solvent	*	*	*	*
Concentration	*	*	ns	ns
Type of solvent: Concentration	ns	ns	ns	ns

\* Significantly different ( $F < 0.05$ ); ns not significantly different ( $F > 0.05$ ).

**Table 4.** Effect of *Dioscorea bulbifera* leaf extract on test wood retention

Factor		Mortality
Type of solvent ( $F = 8.11$ ; $p = 0.0001$ )	Control	$0.00^c \pm 0.67$
	<i>n</i> -Hexane extract	$1.13^a \pm 0.67$
	Ethyl acetate extract	$0.87^{ab} \pm 0.67$
	Methanol extract	$0.33^{bc} \pm 0.67$
Concentration ( $F = 3.83$ ; $p = 0.0075$ )	0%	$0.00^b \pm 0.67$
	1%	$0.38^{ab} \pm 0.67$
	1.5%	$0.43^{ab} \pm 0.67$
	2%	$1.08^a \pm 0.67$
	2.5%	$1.06^a \pm 0.67$
	3%	$0.92^a \pm 0.67$

<sup>a-c</sup> Numbers followed by the same letter indicate that the results were not significantly different at  $p < 0.05$  using DMRT.

best retention value. The results of this study are consistent with those of Vachlepi *et al.* (2015), who stated that preservatives with high retention capabilities indicate that these preservatives can be appropriately absorbed into wood. The amount of extract absorbed into the wood determines the level of protection of the wood from attack by wood-destroying organisms. According to research conducted by Santos *et al.* (2022) and Syahidah and Yunianti (2021), the solvent concentration affects wood durability; the higher the concentration of the extract, the deeper the penetration and the better the retention. However, the wood retention values at concentrations of 2.5% and 3% decreased; this is related to the chemical composition of the cell wall, particularly the bonds between the active ingredients and the number of free hydroxyl groups (-OH; Alara *et al.*, 2021). According to Tascioglu *et al.* (2012), concentration affects retention, further supporting the effect of concentration on attractiveness. The quantity of material (chemical compounds) that makes up the concentration

directly affects the retention. This is consistent with the assertion of Arinana *et al.* (2024) that the quantity of materials (chemical compounds) that create concentrations directly affects retention.

In the ethyl acetate and methanol extracts, the retention values were lower than that in the *n*-hexane extract (Table 4). This is due to several factors, such as the wood species, which affect the retention value of wood, especially the anatomical structure of the constituent cells, the proportion of constituent cells, the thickness and condition of the cell wall layer, the contents of the cell cavities, and the chemical composition of the cell wall, which indicates whether the wood is reactive or not chemicals (Zhang *et al.*, 2022).

### 3.4. Dry wood termite mortality

The termite mortality rate, measured over the 12 weeks of testing, is a measure of the level of toxicity of the extract against dry wood termites. Based on the results of the DMRT test (Table 5), mortality following extract treatment was significantly different from that of the control. However, the difference in mortality between the treatments was not significant. The highest mortality rate was obtained from the ethyl acetate extract (80.56%, equivalent to a corrected mortality of 78.5%) and at a concentration of 2.5% (86.8%, equivalent to mortality corrected of 85.4%). Ethyl acetate extract contains a bioactive tannin compound, which has potential as a botanical insecticide (Ningsih *et al.*, 2013). According to Aflah *et al.* (2021), the mechanism of action of these compounds is to attack the nervous tissue in the insect's body, causing a loss of appetite and the inability of insects to damage and eat the plant material; as a result, the insect loses energy and eventually dies. Termites in the control group died because they could not adjust to their new surroundings and had no other food source save the test paper (Zalsabila *et al.*, 2024). Therefore, the significant termite mortality shown in each treatment

**Table 5.** Effect of *Dioscorea bulbifera* leaf extract on drywood termite (*Cryptotermes cynocephalus*) mortality

Factor		Mortality
Type of solvent ( $F = 37.49$ ; $p = 0.000$ )	Control	$9.6^b \pm 13.95$
	<i>n</i> -Hexane extract	$75.36^a \pm 13.95$
	Ethyl acetate extract	$80.56^a \pm 13.95$
	Methanol extract	$76^a \pm 13.95$
Concentration ( $F = 2.67$ ; $p = 0.0399$ )	0%	$9.6^b \pm 13.95$
	1%	$72.8^a \pm 13.95$
	1.5%	$77.6^a \pm 13.95$
	2%	$72^a \pm 13.95$
	2.5%	$86.8^a \pm 13.95$
	3%	$77.33^a \pm 13.95$

<sup>a,b</sup> Numbers followed by the same letter indicate that the results were not significantly different at  $p < 0.05$  using DMRT.

of the *D. bulbifera* extract indicated that the extract had anti-termite efficacy, according to the data.

Several factors contributed to the high mortality of drywood termites in the different treatments (Table 5). The first possible cause of termite death is the disruption of cellulase enzymes due to the death of protozoa, which are symbionts in the termite stomach. These protozoa produce cellulases, which break down cellulose into simple sugars that serve as energy sources for termites. The death of protozoa disrupts cellulase enzyme activity; therefore, termites do not obtain food and energy sources, resulting in death. Termites also engage in cannibalism (eating weak or diseased termites), which is thought to cause death (Ali *et al.*, 2019; Nalepa, 2020; Scharf and Peterson, 2021).

Another possible mechanism of death is damage to the nervous system caused by the bioactive compounds. The dioscorin present in *D. bulbifera* has insecticidal effects on pests. Apart from dioscorin, other saponin compounds found in *Dioscorea* plants are known to

repel pests, making these species of yam more effective for controlling pests (Gajger and Dar, 2021; Salem *et al.*, 2020). Saponin compounds have low surface tension, thus damaging cell membranes, inactivating cell enzymes, and damaging cell proteins. The *D. bulbifera* compound that affects pest mortality is diosgenin, which affects pest mortality through the nervous system and causes disorientation and eventual death (Butarbutar *et al.*, 2013). Methylene chloride is a pesticide (chemical used to kill pests such as rodents, insects, and plants). Animal studies have shown increases in liver and lung cancers and benign mammary gland tumors following the inhalation of methylene chloride. Animal studies have demonstrated that methylene chloride crosses the placental barrier, resulting in minor skeletal variations and lowered fetal body weights have been noted (ATSDR, 2022).

### 3.5. Wood weight loss and degree of damage

As a result of the dry wood termites consuming the test samples, the weight of the wood decreased. Weight loss determines the resistance of a test sample to attack by harmful organisms. Wood weight loss and the degree of damage in the methanol extract of *D. bulbifera* leaves were significantly different from those in the control and other treatments (*n*-hexane and ethyl acetate extracts). Methanol extract was most effective in terms of wood weight loss (2.88%) and the degree of damage (56.55%) based on the results of the DMRT test (Table 6). The methanol extract, which contains poisonous secondary metabolites, including alkaloids, flavonoids, phenols, steroids, terpenoids, saponins, and tannins, can cause termites to reject them as food and reduce the test weight and level of damage. This aligns with the findings of Mishra *et al.* (2021) who stated that leaf tannins, saponins, and flavonoids have toxic properties.

Termites were not deterred from eating the wood because there were no toxic substances in the control

**Table 6.** Effects of *Dioscorea bulbifera* leaf extract on wood weight loss and degree of damage

Type of solvent	Parameter	
	Wood weight loss ( $F = 8.24$ ; $p = 0.0001$ )	Degree of damage ( $F = 8.24$ ; $p = 0.0001$ )
Control	5.10 <sup>ab</sup> ± 4.03	100 <sup>ab</sup> ± 79.15
<i>n</i> -Hexane extract	8.14 <sup>a</sup> ± 4.03	159.54 <sup>a</sup> ± 79.15
Ethyl acetate extract	7.30 <sup>a</sup> ± 4.03	143.14 <sup>a</sup> ± 79.15
Methanol extract	2.88 <sup>b</sup> ± 4.03	56.55 <sup>b</sup> ± 79.15

<sup>a,b</sup> Numbers followed by the same letter indicate that the results were not significantly different at  $p < 0.05$  using DMRT.

test sample (Table 6). Termites eat all parts of wood more freely without any coating or protective material on the wood surface or inside the wood (Hassan and Morrell, 2021). Termite infestations are more effectively prevented using a concentrated mixture of preservatives (Larasati and Sulisty, 2022). Extracts with high toxicity result in reduced consumption of termite food and a smaller percentage of weight reduction in the test sample because antifeedant compounds inhibit insect-eating power (Arbaiatusholeha *et al.*, 2016).

The results of the DMRT test on the degree of damage showed that the damage caused by termite attacks was minimal in the methanolic extract (Table 6). The damage to each type of extract can be categorized by the level of resistance and wood damage based on Azis *et al.*, 2013. In the present study, we classified the extracts based on the degree of wood damage, following the Azis *et al.*, 2013. The three types of extracts were classified into the groups of wood damage and hefty attack, this is because the value of the degree of damage was  $> 50\%$  (Azis *et al.*, 2013). The methanol extract was more effective than either the *n*-hexane and ethyl acetate extracts because it was associated with a smaller degree of damage. In addition, based on the level of wood resistance reported by Sornnuwat (1996), the results of this study indicate that the methanol extract of *D. bulbifera* leaves is included in wood resistance class II (resistant).

## 4. CONCLUSIONS

The methanol *D. bulbifera* leaf extract was found to be the most effective of the different *D. bulbifera* leaf extracts in controlling dry wood termites. The effectiveness of the extract stems from the secondary metabolites that result in high mortality rate of the termites, less loss of wood mass, and lower degree of damage. The wood species affects the retention value of wood. Further studies are required to determine the appropriate concentration for application as a biopesticide.

## CONFLICT of INTEREST

No potential conflict of interest relevant to this article was reported.

## ACKNOWLEDGMENT

We thank The Ministry of Education, Culture, Research and Technology of Indonesia for funding this study (grant number 106/E5/PG.02.00.PL/2024) for Penelitian Kerjasama Dalam Negeri (PKDN) and the International Scientific Article Writing Clinic. The authors acknowledge the facilities and scientific and technical support of the Advanced Characterization Laboratories Serpong, National Research and Innovation Agency through E-Layanan Sains, National Research

and Innovation Agency.

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