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Potential of methanol extract of *Tetracera indica* leaves in controlling *Coptotermes curvignathus* Holmgren

Dina Erliana[1*], Munifilia Ekasari[2], Uni Baroroh[3], Nurul Pratiwi[4], Sindi Haryani[5], Melyanda[6], Tio Ersa Relyansyah[7], Muhammad Irfan Nur Widad[8]
[1,2,4,5,6,7,8]Department of Chemistry, Faculty of Science and Technology, University of Jambi, Jl. Jambi-Muara Bulian Km15, Mendalo, Jambi 36361, Indonesia.
[3]Department of Biology, Faculty of Science and Technology, University of Jambi, Jl. Jambi-Muara Bulian Km15, Mendalo, Jambi 36361, Indonesia.
*Email: dinaerliana123@unja.ac.id

ABSTRACT

Subterranean termites (Coptotermes curvignathus Holmgren) are pests that damage building components and cause economic losses. The use of synthetic termiticides such as pyrethroids and organophosphates has the potential to cause residues, pest resistance, and environmental pollution. The development of plant-based biotermiticides is needed as alternative. One plant that has the potential to be developed as a termiticide source is Tetracera indica. This study aims to determine the potential of methanol extract of mempelas leaves (T. indica) as a biotermiticide against C. curvignathus termites through a no-choice test and to identify its chemical compounds using GC-MS analysis. The results of the termiticide activity test showed that the percentage of termite mortality increased with increasing concentration. The concentration of 25% extract can cause termite death to reach 100% on the 8th day. The results of chemical component analysis using GC-MS revealed 26 peaks, 19 of which could be detected as tentative compounds, based on the similarity of MS data with the NIST database. The main chemical compounds, namely methyl 9-octadecenoate (58.68%), methyl palmitate (16.13%), and methyl stearate (7.56%) as well as other bioactive constituents such as linoleic acid (4.39%), oleic acid (2.68%), and erythrodiol (0.09%), may contribute to the observed termiticidal activity through their reported biological activities.

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Antitermite, *Coptotermes curvignathus*, GC-MS, Methanol extract, *Tetracera indica*

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INTRODUCTION

The mempelas plant (*T. indica*) is a small woody climbing shrub with brightly colored flowers and many pink- and white-tipped stamens, found in low-altitude areas up to about 600 m. Based on phytochemical tests, *T. indica* contains secondary metabolites such as alkaloids, saponins, tannins, flavonoids, triterpenoids, and steroids (Ogunlakin & Sonibare, 2022).

T. indica has various biological activities that show its potential for use as a botanical pesticide. One of these is its potential as a bioherbicide. Adfa (2023) reported that the methanol extract from the stems and leaves of the mempelas plant was proven to be effective in controlling the weed *Eleusine indica*. Sukmawati et al. (2024) also reported that allelochemical compounds, such as wogonin present in *T. indica* extract, exhibited bioherbicidal activity by inhibiting weed germination of *Asystasia gangetica* and *Echinochloa crus-galli* through suppression of radicle and plumule growth. Furthermore, *T. indica* water extract also has anti-marine leech activity. Norhana, (2021) also reported that the water extract of *T. indica* was able to kill sea leeches at a concentration of 0.5 mg/ml within 5 minutes.

Other pharmacological activities of *T. indica* include antioxidant (Ladeska et al., 2022), anti-inflammatory (Ladeska et al., 2024), antibacterial (Muliyah et al., 2018), antidiabetic (Hasan et al., 2017), and toxic effects (Zarta et al., 2018). From a literature search, the use of the *T. indica* plant as an anti-termite has never been studied. Termites are pests that damage wooden structures, agriculture, and urban environments and can invade building structures (Oi, 2022). One of the types of termites that causes the most damage is *Coptotermes curvignathus* Holmgren (Isoptera: Rhinotermitidae) (Kamarudin et al., 2022). The average percentage of termite *C. curvignathus* attacks on buildings in large cities reaches over 70%. *C. curvignathus* attacks building components above ground level through burrows and can penetrate building foundations. Losses due to termite attacks not only include damage to building materials but also increase maintenance and installation costs for building structures (Subekti et al., 2017).

Termite control typically uses synthetic termiticide, which can have negative impacts on humans and the environment if used long-term (Kamarudin et al., 2022)**.** Continuous use of synthetic termiticides such as pyrethroids and organophosphates can cause residue accumulation, disruption to non-target organisms, and increase the risk of pest resistance. These conditions encourage the development of natural-based termiticides that are safer, biodegradable, and have different mechanisms of action. Therefore, the development of plant-derived natural termiticides has attracted increasing attention as a safer and more environmentally friendly alternative to conventional synthetic termiticides (Zhu et al., 2020). Based on the above description, *T. indica* has the potential to be developed as a natural termiticide, particularly its leaves, due to the presence of various secondary metabolites with diverse biological activities. To date, no studies have reported the use of *T. indica* leaf methanol extract as a natural termiticide. Therefore, this study was conducted to evaluate the termiticidal activity of the methanolic leaf extract of *T. indica* against *C. curvignathus*. Furthermore, to support its potential development as a natural termiticide, the chemical constituents of the extract were identified using Gas Chromatography–Mass Spectrometry (GC–MS) to determine the compounds that may contribute to its termiticidal activity.

METHOD

Tools and Materials

Mempelas leaves were collected from Pematang Gajah Village, Jambi Luar Kota, Muaro Jambi Regency, Jambi Province, Indonesia. The test termite is *Coptotermes curvignathus* Holmgren. Other materials were methanol, filter paper (Whatman No. 1, 9 cm diameter) as termite bait, and distilled water. The tools used were: GC-MS, rotary evaporator (Heidolph), oven, analytical balance, Petri dish with inner diameter 90 x 20 mm (Normax), desiccator, micropipette, and general glassware.

Sample Extraction

430 g of dried mempelas leaf sample was placed in a dark bottle, and 2.5 L of methanol was added. The extraction method used is maceration. The maceration results were filtered, and the solvent was evaporated from the macerate using a rotary evaporator at 45-50°C to obtain a concentrated methanol

extract. The remaining *T. indica* leaves were then remacerated using the same solvent-to-solid ratio as the initial extraction. The remaceration process was carried out until the filtrate became nearly colorless, indicating that extraction was complet (Erliana et al., 2022).

Determination of Chemical Compounds of Methanol Extract of *T. indica* Leaves

The chemical compounds of the methanol extract obtained were then determined using GCMS-QP2010SE SHIMADZU at the Integrated Laboratory of the Islamic University of Indonesia. The equipment conditions were as follows: Rtx-5MS column and Helium carrier gas. The GC chromatogram and mass spectrum obtained were compared with standard compounds in the *National Institute of Standards and Technology (NIST)-62 database (62 entries), the Wiley 229 library, and pesticides.*

Anti-termite Activity of Methanol Extract of *T. indica*

The termites tested in this study were collected from the University of Jambi's forest area, from trees infested by the termite Coptotermes curvignath*us* Holmgren. The method used to test termiticide activity is a *no-choice test*. Twenty-two termites were used for the test: 20 workers and two soldiers (Erliana et al., 2022).

The methanol extract of mempelas leaves was formulated in six different concentrations, based on the percentage ratio of the mass of the extract (g) to the mass of the feed filter paper (g) multiplied by 100%. The concentrations used were 0% (solvent only as a negative control), 5%, 10%, 15%, 20%, and 25% w/w. Each test was repeated 4 times. The extract was dissolved in methanol, then the solution was dripped evenly onto the feed filter paper. The number of termite deaths was recorded daily and calculated cumulatively on the 14th day. Termite mortality/death determined the anti-termite activity and the percentage of termite deaths was determined using the following formula (Adfa et al., 2023):

$$Termite\ Mortality\% = \frac{Number\ of\ Dead\ Termites}{Total\ Number\ of\ Test\ Termites} x100 \quad (1)$$

RESULTS AND DISCUSSION

Extraction and Determination of Chemical Components

The mempelas leaves (430 g) were macerated using 2.5 L of methanol for 3-4 days; the solvent was evaporated using a rotary evaporator until a thick green extract was obtained. The mass of the thick methanol extract of mempelas leaves was 49,139 g with a yield of 11,42%.

Analysis of the chemical compounds of the methanol extract of *T. indica* using GC-MS showed 26 peaks, indicating the number of volatile chemical compounds contained in the extract (Figure 1).

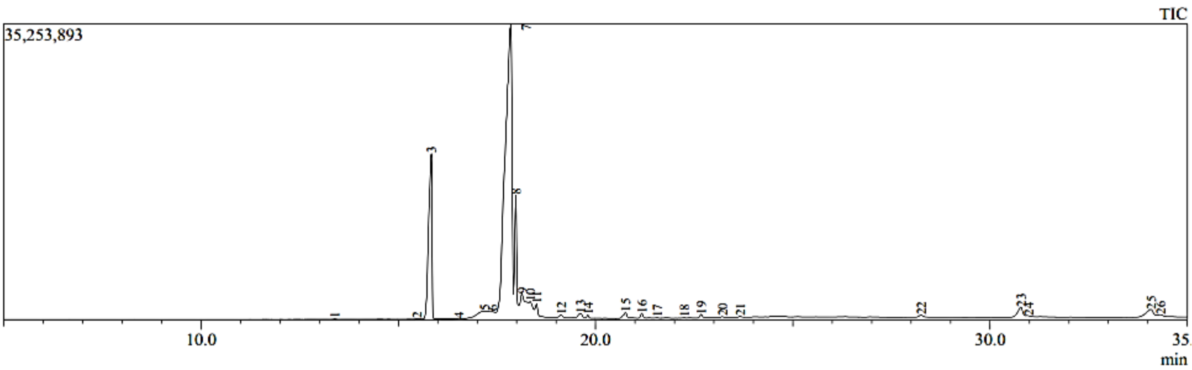

Figure 1. GC chromatogram of methanol extract of T. indica leaves

Based on the TIC data of the GC-MS chromatograph compared with the comparative MS spectrum from NIST, *Wiley 229* Library*, and Pesticide Library*, 19 chemical components of the methanol extract of *T. indica* were identified. In contrast, 7 other components could not be identified due to low similarity index values (Table 1)**.**

Based on the GC-MS data in Table 1, the methanol extract of mempelas leaves has three main components, with the largest area concentrations at peaks no. 7, 3, and 8. These main components have areas of more than 6%, appearing at peaks no. 7 is *Methyl 9-octadecenoate* (58.68%), no. 3 is *Methyl Palmitate* (16.13%), no. 8 is *Methyl Stearate* (7.56%). As shown in Table 1, in addition to the three main components mentioned above, 16 other minor compounds were identified.

Table 1. Chemical content of methanol extract of T. indica leaves detected by GC-MS

No. Peak	Chemical Compounds	RT (minutes)	Area (%)
1	*Methyl tetradecanoate*	13.387	0.02
2	*Cyclohexadecanone*	15.475	0.04
3	*Methyl palmitate*	15.838	16.13
4	Un-identified	16.533	0.07
5	*Oleic acid*	17.183	2.68
6	Un-identified	17.390	0.96
7	*Methyl 9-octadecenoate*	17.853	58.68
8	*Methyl stearate*	17.981	7.56
9	*Linoleic acid*	18.138	4.39
10	Un-identified	18.345	2.07
11	*9,11-Octadecadienoic acid*	18.506	1.23
12	*1,2-Dipalmitoylglycerol*	19.130	0.29
13	*Methyl ricinoleate*	19.622	0.52
14	*Methyl arachidate*	19.805	0.16
15	*Linoleoyl chloride*	20.761	0.56
16	*Cyclododecanepentanoic acid*	21.172	0.33
17	*Methyl behenate*	21.557	0.04
18	Un-identified	22.244	0.04
19	Un-identified	22.675	0.24
20	*Methyl tetracosanoate*	23.212	0.06
21	*Erythrodiol*	23.664	0.09
22	*16-Hentriacontanone*	28.245	0.22
23	Un-identified	30.781	1.46
24	Un-identified	30.975	0.14
25	*Cis-9-octadecenal*	34.076	1.65
26	*2-Bromooctadecanal*	34.317	0.36

The chemical components of the methanol extract of mempelas leaves identified via GC-MS can be classified into the following groups: fatty acid derivatives (92.03%), ketones (0.26%), aldehydes (2.01%), carboxylic acids (0.33%), terpenoids (0.09%), and glycerol (0.29%). This is in line with the study by Guntoro et al., (2020), which reported that, based on GC-MS analysis, the methanol extract of *T. indica* leaves contains 11 compounds, comprising four compounds classified as fatty acids (total area: 55.53%), one flavonoid (24.82%), and three steroids (6.34%). The research also reported that all parts of the methanol extract of the T. indica plant, based on GC-MS results, contained the following compounds: 9,12-octadecadienoic acid (Z,Z), flavone (5,7-dihydroxy-8-methoxy)*, and Hexadecanoic acid.*

Anti-termite Activity of Methanol Extract of T. indica

The results of the anti-termite activity test of the methanol extract of *T. indica* leaves against the test termite Coptotermes *curvignathus* Holmgren showed that administering the extract at various concentration levels was able to reduce the survival rate of termites. Figure 2, shows that the percentage of termite mortality in each treatment with methanol extract increased with increasing concentration. The higher the concentration of methanol extract of *T. indica* leaves, the greater the mortality rate of *C. curvignathus*. The anti-termite test in this study used 1 negative control and 5 test concentrations.

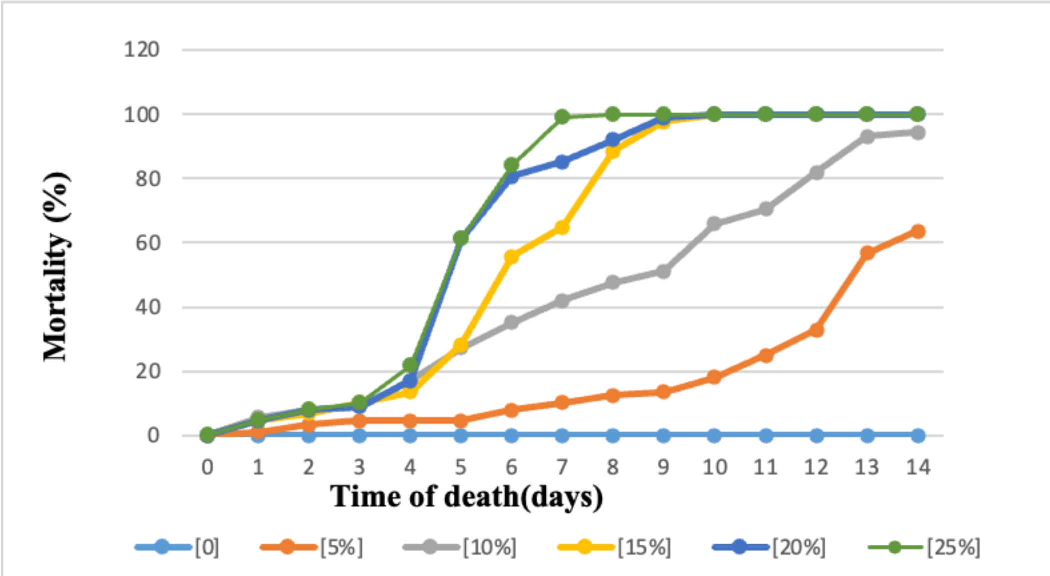

Figure 2. Daily mortality time of methanol extract *Tetracera indica* against *Coptotermes curvignathus*

Figure 2 explains that the control used on the first to 14th day did not cause termite mortality, while the five concentration variations (5%, 10%, 15%, 20%, and 25%) showed a higher mortality rate. At a concentration of 25%, termite mortality reached 100% on the 8th day. At concentrations of 20% and 15%, termite mortality reached 100% on the 10th day and at concentrations of 5% and 10%, termite mortality did not reach 100% even up to the 14th day, but had reached 56.82% and 93.18%. As shown in Figure 2, termite mortality showed a positive correlation with the concentration of the extract applied to the bait filter paper. The daily mortality rate of termites increased progressively with increasing concentration of the applied methanol extract of *T. indica*. Furthermore, *T. indica* leaf extract demonstrated potential as a termiticidal agent, as even the lowest concentration was able to induce more than 50% cumulative termite mortality after 14 days of exposure. Methanol extract of *T. indica* leaves shows the ability to kill termites, thought to be due to the relationship between the secondary metabolite compounds contained in the extract. GC-MS analysis revealed that the extract is rich in fatty acids and their derivatives, such as Methyl 9-octadecenoate (58.68%), Methyl Palmitate (16.13%), and Methyl Stearate (7.56%), linoleic acid (4.39%), and oleic acid (4.75%), which are thought to play a key role in killing termites. This is in line with research by Li et al. (2024), who reported that oleic acid is recognized by the odorant-binding protein CforOBP7 in the antennae of worker *C. formosanus* termites and triggers a burial behavior response, indicating that this compound exhibits specific interactions with the termite sensory system. Melo et al. (2018) according to his research, oleic acid, linoleic acid, and fatty acid methyl ester have bioactivity as natural insecticides that kill Culex quinquefasciatus larvae by affecting the larvae's fat body and midgut metabolism and shape. Recent research by Montanez-Palma et al. (2025) demonstrated that linoleic acid exhibits acaricidal activity against *Psoroptes cuniculi* by inducing high mortality through disruption of mite cuticular integrity and impairment of physiological functions. Methyl palmitate and methyl stearate have been reported to exhibit nematicidal activity against *Meloidogyne incognita* by inhibiting egg hatching, repelling second-stage juveniles (J2), reducing root gall formation and egg masses, and suppressing nematode populations (Lu et al., 2020). Methyl 9-octadecenoate (58.68%), commonly known as methyl oleate, may also contribute to termite mortality due to its insecticidal properties. Vasantha-Srinivasan et al. (2024) reported that methyl oleate isolated from *Phoenix pusilla* exhibited significant larvicidal activity against *Aedes aegypti* by inducing larval mortality and inhibiting the activity of key detoxification enzymes involved in larval development.

In addition to fatty acids and their derivatives, there are also minor compounds such as erythrodiol, a terpenoid compound at peak number 21. Erythrodiol, especially its derivative, 3β-(3,4-dihydroxycinnamoyl)-erythrodiol, has strong insecticidal activity, particularly as an antifeedant, growth inhibitor, and trigger for the death of *Pieris rapae larvae* (Wu et al., 2020). Erythrodiol identified in the methanolic extract of *Tetracera* indica belongs to the pentacyclic triterpenoid class. Lin et al. (2021) reported that plant-derived triterpenoids represent important bioactive molecules with insecticidal properties, including antifeedant activity, growth inhibition, and toxic effects on insect pests. Based on a review of the literature, a number of compounds derived from fatty acids and their derivatives, as well as terpenoids, have been reported to exhibit significant insecticidal activity; these compounds are also believed to be effective in exterminating termites.

Although GC-MS analysis successfully identified various volatile compounds in the *T. indica* methanol extract, non-volatile compounds which cannot be analyzed using this technique are also suspected of contributing to the termiticidal activity. Secondary metabolites such as alkaloids, flavonoids, and tannins are known to possess bioactivity capable of inhibiting or causing the death of termites (Rizqullah et al., 2023). In their study, Bezuneh et al. (2019) reported that secondary metabolites such as alkaloids, flavonoids, and tannins exhibit insecticidal and termiticidal activities by disrupting feeding behavior and physiological processes, ultimately causing termite mortality.

CONCLUSION

From the results, it can be concluded that the methanol extract of *T. indica* leaves has termiticidal activity against the termite *Coptotermes curvignathus* Holmgren. The highest concentration of 25% can cause 100% termite death on the 8th day. This activity is related to the chemical content of the methanol extract of T. indica leaves. The results of the analysis of the GC-MS methanol extract of T. indica leaves contained the main chemical compound components, namely Methyl 9-octadecenoate (58.68%), Methyl Palmitate (16.13%), and Methyl Stearate (7.56%). The suspected active compounds are major and minor compounds, such as Methyl 9-octadecenoate, Methyl Palmitate, Methyl Stearate, linoleic acid, oleic acid, and erythrodiol. Further research is needed to detect chemical constituents using LC-MS and test anti-termite activity using pure compounds. It is hoped that the potential of the *T. indica* plant as a biotermiticide can be developed in the future.

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