

Termiticidal Activity of N-Hexane Extract of *Tetracera indica* Leaves and Determination Chemical Compounds using GC-MS

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Abstract

Termiticidal activity of n-hexane extract of *Tetracera indica* leaves against *Coptotermes curvignatus* Holmgren has conducted research using the *no-choice test* method, and determination of chemical compounds using GC-MS. The results of the termiticidal activity test showed that the percentage of termite mortality increased with increasing extract concentration. The results of the chemical compounds analysis using GC-MS showed 34 peaks in the n-hexane extract of *T. indica*. A total of 29 peaks were successfully identified as tentative compounds based on the similarity of their mass spectra with the NIST database. The main compounds of the n-hexane extract of *T. indica* are Oelic acid (40.77%) and Cis-9-octadecenal (33.78%). The presence of these peaks is suspected to be active against anti-termite activity. The presence of other compounds such as triterpenoids/steroids based on phytochemical screening may also contribute to its activity.

Keywords: Antitermite; *Tetracera Indica*; *Coptotermes curvignathus*; GC-MS

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INTRODUCTION

Termites are a type of insect that can cause significant disadvantage. These insects can detriment various building components and materials generally made from wood or cellulose, such as furniture, paper, fabric, and even plantation crops [1]. Termite attacks can reach high parts of buildings and cause major losses [2]. *Coptotermes curvignathus* Holmgren is one of the most destructive termite species in Indonesia. This organism attacks building foundations and wood by burrowing through burrows, causing building components and wood to become porous and destroyed. [3].

Currently, termite control in Indonesia generally still uses chemical pesticides such as synthetic termiticides, which are quite expensive and have the potential to have a negative impact on the environment. Such as fipronil and imidacloprid compounds (nitro guanidine group) which are often used to overcome termite attacks on plants [4]. Repeated use of the same termiticide can cause pests to become resistant. Therefore, an alternative is needed in the form of natural termiticides or organic termiticides (derived from plants) which are more environmentally friendly to replace synthetic termiticides [5]. One of the plants that can be used as a natural pesticide is the mempelas plant (*T. indica*).

T. indica is a climbing shrub with red flowers. Phytochemical tests have shown that the mempelas plant contains secondary metabolites such as alkaloids, saponins, tannins, flavonoids, triterpenoids, and steroids [6]. Based on the GC-MS results, the methanol extract of mempelas plants contains allelopathic compounds consisting of fatty acids, flavonoids and their derivatives (in every part of the plant) and steroids so that they can be used as raw materials for bioherbicides. Secondary metabolite compounds such as flavonoids and their derivatives, apart from acting as allelopathy, also have the ability to protect against attacks by insects and other pests as insecticide and pesticide compounds. [7]. Methanol extract from the stems and leaves of the mempelas plant is also used as a bioherbicide, and has been proven effective in controlling the weeds *Eleusine indica* and *Digitaria sanguinalis* in broccoli cultivation, without having a negative impact on plant height, number of leaves, or the level of phytotoxicity of broccoli [8].

Based on the secondary metabolite described and data from previous studies, the mempelas plant (*T. indica*) has the potential to be developed as a raw material for producing natural termiticides. Currently, there have been no reports on the use of n-hexane extract of mempelas leaves as a natural termiticide. Therefore, this study was conducted to evaluate the termiticide from mempelas leaf extract against *C. curvignathus* termites and to identify its chemical components using GC-MS.

METHODS

Materials and Instrument

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: technical n-hexane, filter papers Whatman No. 1 with a diameter of 9 cm as termite bait, and distilled water. Fresh leaf samples were cleaned, cut into small pieces, and air-dried in a room without direct sunlight. After air-drying, the dry sample was obtained, which was then weighed.

The equipment used was: GC-MS, rotary evaporator (Heidolph), oven, analytical balance, petri dish with inner diameter 90 x 20 mm (Normax), desiccator, micro pipette, and general glassware.

Sample Extraction

T. indica leaf samples were cleaned, cut into small pieces, and air-dried in a room without direct sunlight. After air-drying, the dried sample was obtained and then weighed. A total of 430 g of air-dried mempelas leaves were placed in a dark bottle and then 2.5 L of n-hexane solvent was added. The process of extracting the sample was carried out using the maceration method. The maceration results were then filtered, and the solvent was evaporated using a rotary evaporator at a temperature of 45-50°C to obtain a concentrated n-hexane extract. The sample was then re-macerated using the same procedure as the first maceration. The re-maceration process was repeated 7 times until the solvent became clear (colorless) [9].

Phytochemical Screening

Profiling of secondary metabolites using phytochemical tests on n-hexane extract of mempelas leaves includes tests for flavonoids, alkaloids, saponins, tannins and steroids.

Determination of Chemical Compounds of N-Hexane Extract of *T. indica* Leaves

The chemical compounds of the n-hexane 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 of Technology (NIST)-62 data bank (62 entries), Willey 229 library and pesticides.

Anti-termite Activity of N-Hexane Extract of *T. Indica*

The termites tested in this study were taken from the University of Jambi's forest area, on trees infested by the termite *Coptotermes curvignatus* Holmgren. Prior to testing, the termites were brought to the laboratory and placed in jars to allow them to adapt to the laboratory environment. Twenty-two termites were used for the test: 20 workers and two soldiers. The method used to test termiticide activity was a forced baiting/*no-choice test* [5][10].

The n-hexane extract of mempelas leaves was formulated in six different concentrations, based on the percentage ratio of the extract mass (g) to the mass of the feed filter paper (g) multiplied by 100%. The concentrations used in the test included 0% (solvent only as a negative control), 2.5%, 5%, 10%, 15%, and 20% w/w. Each concentration was tested with four replications. The extract was dissolved using n-hexane, then the solution was dripped evenly onto the feed filter paper. The n-hexane extract of memelas leaves was formulated in six different concentrations, based on the percentage ratio of the extract mass (g) to the mass of the feed filter paper (g) multiplied by 100%. The concentrations used in the test included 0% (solvent only as a negative control), 2.5%, 5%, 10%, 15%, and 20% w/w. Each concentration was tested with four replications. The extract was dissolved using n-hexane, then the solution was dripped evenly onto the feed filter paper. Humidity and water availability in the petri dish were monitored daily, and 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 [10]:

$$\text{Termite Mortality (\%)} = (\text{Number of Dead Termites} / \text{Total Number of Test Termites}) \times 100$$

RESULTS AND DISCUSSION

Extraction and Determination of Chemical Components

The mempelas leaves of 430 g was macerated using 2.5 L of n-hexane solvent for 3-4 days, maceration was carried out 7 times until the extract was no longer colorless, after which the evaporation process was carried out using a rotary evaporator to obtain a thick extract that was blackish green. The mass of the thick n-hexane extract of mempelas leaves was 8.9153 g with a yield of 2.07%. The results of phytochemical screening of the n-hexane extract of mempelas leaves showed positive results for terpenoids and steroids, while for tannins, alkaloids, flavonoids, and saponins showed negative results. This is because the use of solvents with different polarities can affect the optimal extraction of secondary metabolite compounds in phytochemical screening tests [11].

Analysis of the chemical compounds of the n-hexane extract of *T. indica* using GC-MS showed 34 peaks indicating the number of volatile chemical compounds contained in the extract Table 1. The GC-MS analysis technique is very effective for analyzing compounds that have volatile properties. Based on the TIC data of the GC-MS chromatograph compared with the comparative MS spectrum from NIST, Willey 229 Library and Pesticid Library, 29 chemical components of the n-hexane extract of *T. indica* were identified, while 6 other components could not be identified due to the low similarity index value. The identified compounds consist of fatty acids (41.62%), aldehydes (36.10%), esters (6.72%), diacylglycerols (6.6%), ketones (2.97%), hydrocarbons (0.29%), alcohols (0.24%) and triterpenoids (0.21%). This is supported by previous research that *T. indica* plant extracts consist of fatty acids and steroids [7]. Based on GC-MS data analysis, the n-hexane extract of mempelas leaves has five main components with the largest area concentrations at peaks number 9, 31, 15, 33 and 7 with area concentrations respectively: 32.61%; 9.60%; 8.62%; 8.24 and 6.75%, the five chemical components at peak number 9 were identified as Oleic acid compounds while peaks 31, 15, 33, and 7 were identified as Cis-9-octadecenal compounds. Based on the data in Table 1, it can be seen that in addition to the five main components above, there are 24 other minor compounds identified.

Table 1. Chemical Content of N-Hexane Extract of *Tetracera indica* Leaves Detected by GC-MS

No peak	Chemical Compounds	RT (Minute)	(%) Area	No peak	Chemical Compounds	RT (Minute)	(%) Area
1	Hexadecanal	14.047	0.13	18	1,3-Dipalmitin	20.863	0.21
2	Heptadecan-2-one	15.002	0.24	19	1,9-Tetradecadiene	21.258	0.18
3	Methyl Palmitate	15.239	0.14	20	Oleyl alcohol	21.332	0.24
4	Cis-9-octadecenal	16.052	0.57	21	Erythrodil	21.757	0.21
5	Tidak teridentifikasi	16.317	0.54	22	2-Pentyl-2-nonenal	21.849	0.28
6	Oleic acid	16.583	3.82	23	Tidak teridentifikasi	22.172	1.39
7	Cis-9-octadecenal	16.930	6.75	24	Tidak teridentifikasi	22.347	0.24
8	Methyl Oleate	17.126	5.83	25	Tidak teridentifikasi	22.951	1.76
9	Oleic acid	17.782	32.61	26	Tidak teridentifikasi	23.165	0.60
10	Tidak teridentifikasi	18.225	0.07	27	2,6,10,15,19,23-hexamethyltetracosane	23.544	0.77
11	1,2-	18.630	4.05	28	Diisononyl Phthalate	23.762	0.11

<i>Dipalmitoylglycerol</i>							
12	<i>1,3-Dipalmitin</i>	19.058	1.67	29	<i>9-Octadecenal</i>	24.574	0.24
13	<i>Methyl ricinoleate</i>	19.214	0.85	30	<i>16-Hentriacontanone</i>	27.525	1.73
14	<i>Diocetyl adipate</i>	19.964	0.64	31	<i>Cis-9-octadecenal</i>	29.903	9.60
15	<i>Cis-9-octadecenal</i>	20.283	8.62	32	<i>16-Hentriacontanone</i>	30.092	1.00
16	<i>Glyceryl 1,3-distearate</i>	20.457	0.67	33	<i>Cis-9-octadecenal</i>	33.023	8.24
17	<i>Oleic acid</i>	20.691	4.34	34	<i>2,2-Dideutero Octadecanal</i>	33.258	1.65

Anti-termite Activity of N-Hexane Extract of *T. Indica*

The termiticide activity test in this study used NDT samples (n-hexane extract of mempelas leaves). The test results showed that the NDT extract had termiticide activity against *Coptotermes curvignathus* Holmgren termites, this is shown by the graph of the relationship between the percentage of termite mortality and the observation time (days) when administering n-hexane extract of mempelas leaves at various variations in the % mass of extract per mass of bait filter paper. Figure 1 shows that the percentage of termite mortality in each n-hexane extract treatment is directly proportional to the increasing concentration given, so that the greater the concentration of n-hexane extract of mempelas leaves given, the higher the percentage of *Coptotermes curvignathus* Holmgren termite mortality.

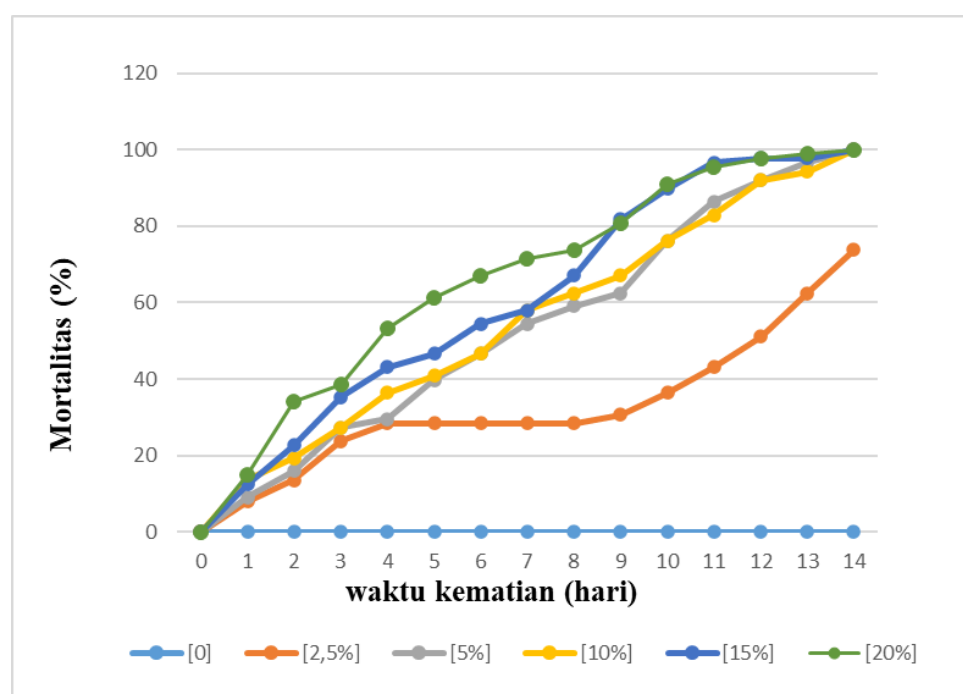


Figure 1. Termiticidal activity of N-hexane extract *Tetracera indica* against *Coptotermes curvignathus*

The test of termiticide activity, one negative control and five variations in n-hexane extract of mempelas leaves (NDT) extract concentrations were used. Based on Figure 1, all five concentrations showed higher effectiveness in killing termites compared to the control. The control used from the first to the fourteenth day did not cause termite mortality, while the five concentration variations (2.5%, 5%, 10%, 15%, and 20%) showed higher mortality rates. Termite mortality increased each day with increasing concentration variations. Concentrations of 5%, 10%, 15%, and 20% were able to cause 100% termite mortality on the

14th day, while the 2.5% concentration did not reach 100% even on the 14th day, but had reached 73.86%. These results indicate that the n-hexane extract of *T. indica* leaves has the potential to be a termiticide, because even the lowest concentration was able to cause more than 50% termite mortality on the 14th day. The N-hexane extract of *mempelas* leaves (NDT) showed the ability to kill termites, allegedly due to the relationship between secondary metabolite compounds contained in the extract, such as Oleic acid and Cis-9-octadecenal compounds identified in GC-MS in killing *Coptotermes curvignathus* Holmgren termites. Li et al. [12], stated that oleic acid can trigger termite death through two main mechanisms: inhibiting the insect's cuticle tissue, accelerating dehydration and physiological failure in the insects. Adfa et al. [13], reported that the compound Cis-9-octadecenal was detected in the n-hexane extract of *Azadirachta excelsa* seeds, and the extract showed significant antitermite activity against subterranean termites.

CONCLUSIONS

The research results concluded that the n-hexane extract of *mempelas* leaves has termiticidal activity against *Coptotermes curvignathus* Holmgren. This was demonstrated by the higher concentration of the n-hexane extract of *mempelas* leaves added to the bait filter paper, the higher the termite mortality. Concentrations of 5%, 10%, 15%, and 20% were able to cause 100% termite mortality on the 14th day. This termiticidal activity is related to the chemical content of the n-hexane extract of *T. indica*. The suspected active compounds are Oleic acid and Cis-9-octadecenal.

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AUTHOR CONTRIBUTIONS

S, H., M, M., T.E.R., and M.I.N.W., carried out the research and inquiry; U, B., and N.P., processed the research data; M.E., applied statistical methods; D, E., wrote, reviewed, and edited; oversaw and secured funding.

CONFLICT OF INTEREST

The authors declares that there are no conflicts of interest.

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