

# Termiticidal

*by Astuti Arif*

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## Termiticidal Activities of The Bark Crude Extract of Derris Elliptica Benth Against Coptotermes sp

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## 1 Termiticidal Activities of The Bark Crude Extract of *Derris Elliptica* Benth Against *Coptotermes* sp

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**Abstract.** Tuba (*Derris elliptica*) is one of the insecticide-producing plant which can reduce the pest population due to its active compound, rotenone (C<sub>23</sub>H<sub>22</sub>O<sub>6</sub>). The rotenone is deposit in all parts of tuba plant, as such as roots, stems, and leaves. The aim of this research aims was to evaluate the effectiveness of tuba bark extract against subterranean termite *Coptotermes* sp. The powder of tuba bark extract with 40-60 mesh size macerated with the ethanol by solvent obtains the extract. The extract was made into various concentration namely 0,5%, 1%, and 1,5%, and applied to the paper disc. There are two types of termite test in this study, namely toxicity test and respiratory test. The mortality of termite and the weight loss of the paper disc were used as indicators of extract activity. The result showed that the concentration of 1,5% indicated the highest mortality from both of two types of test. This study was similar for the lowest weight loss of sample which was also given by concentration 1,5%.

### 1. Introduction

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In the tropics, termites are very easily found in various habitats [1-4] which play a crucial role in the decomposition of organic matter and improve the physical properties of chemical soils. However, it cannot be denied that termites can damage buildings and plants, with losses reaching trillions 13 per year [5]. Termites can enter buildings in various ways, such as creating a hole through a wall, crawling through cracks in the foundation, and climbing through the roof [6]. Once entering the building, subterranean termites will damage unprotected cellulose materials, such as wood, underground cables, and farming equipment. These termites are also found to invade agricultural and forestry plants. Although the termite species that have been identified reached 3,106 species [7], currently 28 species are known to be invasive species [8]. The existence of these pests has become a constraint on the cultivation of both forestry crops, plantations and agriculture in the field, especially subterranean termites *Coptotermes* sp. [9] The genus *Coptotermes* is well known as a very destructive termite in attacking wood components in buildings and plants. At least 6 20 species of *Coptotermes* were identified as invasive species, namely *C. acinaciformis*, *C. curvignathus*, *C. formosanus*, *C. frenchii*, *C. gestroi*, *C. sjostedti*, and *C. truncates* [8]. Another species of the genus is also reported to cause damage to buildings and plants, namely *Coptotermes* sp. [10-12].

6 integrated pest management, the application of synthetic insecticides is the main choice because of fast action, low cost, easy application and efficiency against a wide range of harmful species. However, consideration of the negative effects of synthetic chemicals such as toxicity to humans and animals, environmental contamination, and toxicity to non-target insects has shifted the paradigm 2 in integrated pest control with the use of alternative chemicals that are more environmentally friendly [13].



Many studies have been conducted to examine the biological activities of various plants as insect controllers, including rotenone-based botanical insecticide. This bioactive compound has been introduced since 1850, which was isolated from *Derris elliptical* (Wall.) Benth [14]. *Derris elliptica*, locally well known as tuba, is an insect-producing plant that can reduce pest and fish populations [15]. All parts of tuba plants such as roots, barks, and leaves are identified to contain rotenone as active compounds [16]. The compound has been widely used by farmers in agriculture as a safe insecticide to eradicate pests in plants and vegetables such as long bean vegetables [17]. In the field of the fishery, tuba root extract serves as a poisoning agent for fish, and anesthetized fish in freshwater fishing [18] and flavonoid content in tuba root extract can kill *Aedes aegypti* larvae [19] and *Aedes* sp. [20]. Referring to the utilization of tuba root extract as a natural insecticide, this study will evaluate the effectiveness of tuba bark extract against termite attack of *Coptotermes* sp. This research is expected to contribute to the development of bio-pesticides that are more environmentally friendly.

## 19 2. Material and Method

### 2.1. Samples collection and preparation

Tuba plant was taken from the area of the people's garden in Cabbengnge Village, Tadang Pali Village, Sibulue District of Bone Regency. Plants took the stem, then barked and cleaned with a brush to remove dirt. The cleaned bark that has been separated from the stem, then cut into 1 cm in length, then dried without exposure to direct light. The air-dried bark was milled using a hammer mill, then sieving with a sieve 40-60 mesh and ready for extracting.

### 2.2. Determination of samples moisture content

Before the extraction process, the first step is determination of water content from tuba bark powder. This moisture content is used to uniform the initial weight of the material to be extracted so that it can be known the weight of the result of each extraction. The procedure for determining the moisture content was based on SNI 01-3182-1992 which was modified by the following stages: Tuba bark powder was taken 2 g ( $m_0$ ) with 3 replicates weighed to calculate moisture content then wrapped with aluminum foil paper and put into the oven for 5 hours with a temperature of  $103 \pm 2^\circ\text{C}$ . After drying, the sample is inserted into the desiccator for  $\pm 15$  minutes. The sample is weighed again to obtain the final weight ( $m_1$ ).

### 2.3. Extracting of tuba bark

A total of 100 g of tuba bark powder was inserted into the beaker, then macerated with a ratio of 1 : 3 between the material and the solvent, then macerated and filtered every 3 days, to obtain the filtrate. Then the filtrate is solidified by using a rotary vacuum evaporator.

### 2.4. Preparation of the concentration of crude extracts

Preparation of the concentration of tuba bark extract based on a comparison between the weight of the crude extract and ethanol solvent, with a target concentration of 0.5%, 1%, and 1.5% (v/v). Furthermore, I also prepared a positive control (termiticide) and negative control (sample without extract).

### 2.5. Preparing of samples

The sample used for termite bioassay was Whatman paper with 2.5 cm in diameter. The sample preparation was carried out with the following stages: the paper was soaked until all parts were immersed in the crude extract and then air-dried for 24 hours to remove the solvent. The paper was oven-dried at  $60^\circ\text{C}$  for 5 hours, then put into the desiccator for  $\pm 15$  minutes. Furthermore, the paper is weighed to obtain weight before testing ( $B_0$ ).

## 2.6. Termites collection

A colony of termites *Coptotermes* sp. was collected from termite rearing in the Integrated Laboratory of the Forestry Faculty of Hasanuddin University. A total of 55 healthy and active termites consisting of 50 workers and 5 soldiers were placed in each container with 7 cms in diameter.

## 2.7. Bioassay of Antitermite

**Oral test:** Each container is filled with samples that have been soaked with extractor without extract, then 55 individual termites are inserted. All plastic container was placed into a large and dark shelf. Below the test paper was first given plastic wire netting. Termite mortality was observed 3 hours after testing. Further observed again at 6 hours, 9 hours, 12 hours, 24 hours (1 day), 3 days, 5 days, 7 days, and 14 days respectively. At the end of observation, samples were taken and cleaned from contaminants, then oven-dried at temperature  $103 \pm 2$  °C for 5 hours and put in a desiccator for 15 minutes. Finally, samples were weighed again to provide the weight after testing ( $B_1$ ).

**Respiratory test:** Procedure for this test was similar to the oral test, except for the addition of a block under the paper sample. Each container is filled with samples that have been soaked with extractor without extract, then 55 individual termites are inserted. All plastic container was placed into a large and dark shelf. Below the test paper was first given plastic wire netting. Termite mortality was observed 3 hours after testing. Further observed again at 6 hours, 9 hours, 12 hours, 24 hours (1 day), 3 days, 5 days, 7 days, and 14 days respectively. At the end of observation, samples were taken and cleaned from contaminants, then oven-dried at temperature  $103 \pm 2$  °C for 5 hours and put in a desiccator for 15 minutes. Finally, samples were weighed again to provide the weight after testing ( $B_1$ ).

## 2.8. Observed variables

**Mortality:** At the end of the observation, the percentage of termites mortality was calculated with the following formula:

$$MT = \frac{M_1}{M_2} \times 100$$

where MT represents the percentage of mortality,  $M_1$  represents the number of dead termites (individual), and  $M_2$  represents a total number of termites (55 individual).

**Weight loss:** The amount of paper consumed by termites were calculated with the following formula:

$$A = \frac{B_0 - B_1}{10} \times 100$$

Where A represents the percentage of weight loss,  $B_0$  represents the weight of the samples before testing (g), and  $B_1$  represents the weight of the samples after testing (g).

## 2.9 Statistical analysis

This experimental study was modeled in Completely Randomized Design (CRD), with the treatment of differences in extract concentrations consisting of:  $P_1$  (control or without extract),  $P_2$  (termiticide),  $P_3$  (concentration 0,5%),  $P_4$  (concentration 1%), and  $P_5$  (concentration 1.5%). Each treatment was done with 3 replicates. Data on mortality and weight loss were analyzed to determine the effect of treatment, and further test using Duncan's multiple range test (DMRT). The analysis was performed using SPSS software version 22.

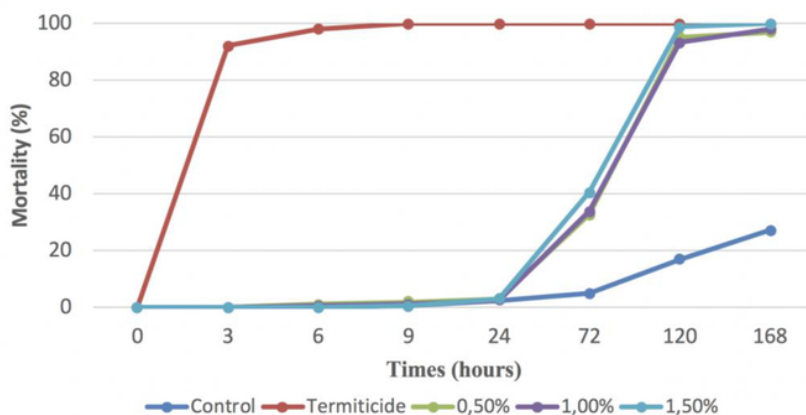
## 3. Results and discussion

### 3.1. Mortality

In this study, the percentage of termite mortality was obtained from testing the effect of crude extract of bark tuba which resulted in the death of termite due to defaunation of the gut symbionts (test of stomach toxicity) and nervous system disorder (respiratory test).

*Defaunation of termite symbionts*

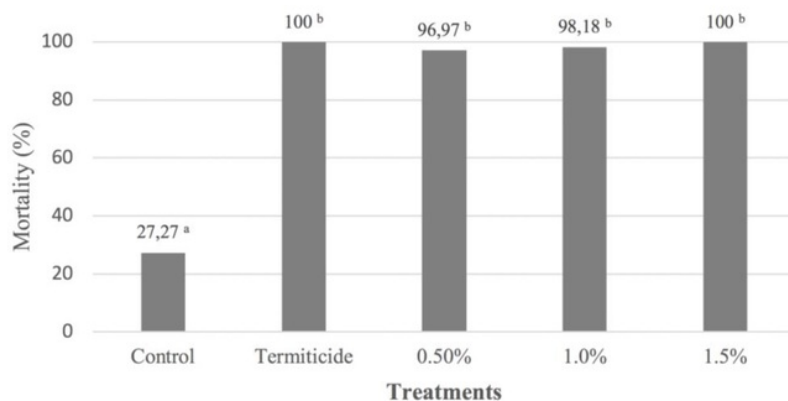
The mortality can be caused by the ability of termite to obtain an energy source from decomposition by the gut symbionts. The inability of the symbionts to produce decomposition products may occur because the input of food or substrates is unsuitable or poisonous, which affects the death of the symbionts, known as defaunation. The exposure period of crude extract test based on starvation for 2 weeks 2 days. The observation results indicate that at the beginning of observation the individual termites dead tend to occur slowly, and followed by a large number of mortality at the end of the observation that is 120 hours for all treatments. This tendency does not occur to the treatment of termiticide which has a large number of individual mortality values at the beginning of observation (at the 3rd hour first). The average termite mortality rate during observation per treatment can be seen in Figure 1.



**Figure 1.** Average of *Coptotermes* sp. mortality rate

In Figure 1, it can be seen that the average termite mortality rate at all treatments is relatively low on the first day, except in the treatment of termiticide. At the end of the observation, the lowest average mortality rate occurred in the control or feeding paper without tuba bark extract, was 27.27%. In other treatments, the average mortality rate reached the range 96.97-100% although the death rate per day varied, in contrast to the termiticide treatment causing very vast mortality at the beginning of observation. Based on the graph of the trend of the average rate of mortality rate mentioned in figure 1, the effect of active ingredient contained in bark tuba extracts in killing *Coptotermes* sp. can be indicated as an extract with slow toxic action.

The average of percentage mortality indicates an increase in mortality values which was directly proportional to the concentration of extract given. Average percentage mortality of *Coptotermes* sp. after feeding of tuba bark extract at various concentrations can be seen in Figure 2.

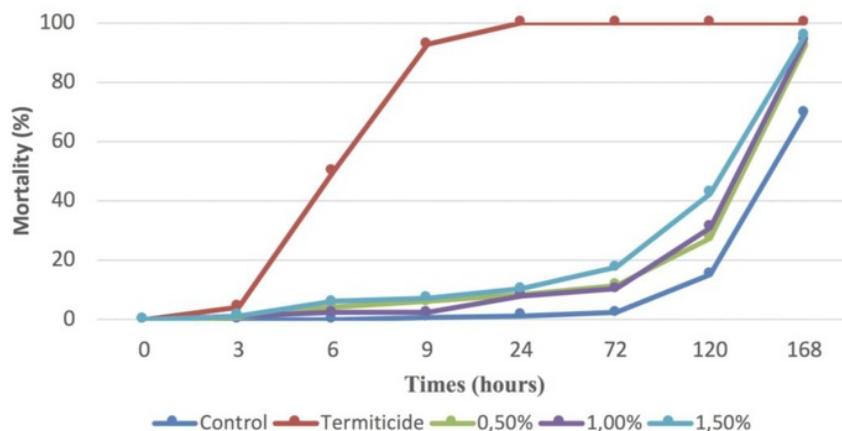


**Figure 2.** Average of *Coptotermes* sp. mortality

In Figure 2, it can be seen that the higher average mortality is indicated by termiticides and concentrations of 1.5%, and then followed by a concentration of 1% with a value of 98.18%, a concentration of 0.5% with a value of 96.97%, and the lowest value in control (27.27%). The results of the variance analysis on termite mortality data indicate that the treatment of feeding crude extract of *D. elliptica* bark at various concentrations has a very significant effect on the mortality of termites. To find out the different mean value of treatment, Duncan Multiple Range Test (Duncan Multiple Range Test) was performed with the result shown in Figure 2. The result of Duncan's test showed that the mean of control has a very significant difference with all other treatments. Meanwhile the mean of other treatments than control was not significantly different.

*Testing the nervous system*

Mortality in termites can be caused by inhibition of the nervous system' working mechanism. In general, termite symptoms exposed to neural toxins cause spasticity, paralysis, and death. At the beginning of observation, the death of the individual termites tended to occur slowly and followed by a large number of mortality at the end of the observation (168 hours) for all treatments. This phenomenon does not discover the termiticide treatment which has many individual deaths at the beginning of observation up to 6 hours. The mortality rate of termites during observation per treatment can be seen in Figure 3.



**Figure 3.** Average mortality rate

In Figure 3, it can be seen that the termite mortality rate in all treatments at the beginning of the observation is relatively low, except for the termiticide treatment. At the end of the observation, the lowest mortality rate occurred in control or without crude extract was 69.70%. In other treatments, the average mortality rate reaches the range of 92.12-100%, although the mortality value per day varies, where the termiticide treatment caused the death of individual termites faster at the beginning of the observation. Based on the graph of the mortality rate trend in Figure 3, the effect of the active ingredients of *D. elliptica* bark extract on the death of *Coptotermes* sp. can be expressed as a toxic slow action extract.

The average of percentage mortality indicates an increase in mortality values which was directly proportional to the concentration of extract given. Average percentage mortality of *Coptotermes* sp. after feeding of *D. elliptica* bark extract at various concentrations can be seen in Figure 4.

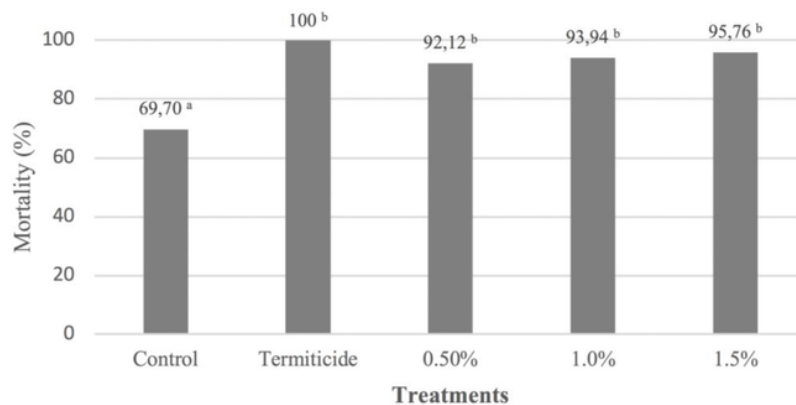


Figure 4. Average of *Coptotermes* sp. mortality

The average of the percentage mortality can be seen in Figure 4 with the highest mortality (100%) occurring at the termiticide treatment and the lowest in control (69.70%). For the three concentrations of the extract applied the average value of the mortality rate is relatively the same. The variance analysis on termite mortality data indicated that the treatment of feeding crude extract of *D. elliptica* bark at various concentrations has a very significant effect on the mortality of termites. To find out the difference of treatments mean value, the Duncan Multiple Range Test (Duncan Multiple Range Test) was performed with the result shown in Figure 4. The result of Duncan's test showed that the mean of control has a very significant difference with all other treatments. Meanwhile the mean of other treatments than control was not significantly different.

### 3.2. Weight Loss

The results showed that *D. elliptica* bark extract affected the weight loss of the samples (test paper). The highest of weight loss for each treatment was found in control, which was indicated by the relatively high difference in value compared to other treatments. The average weight loss of samples for each treatments can be seen in Figure 5.

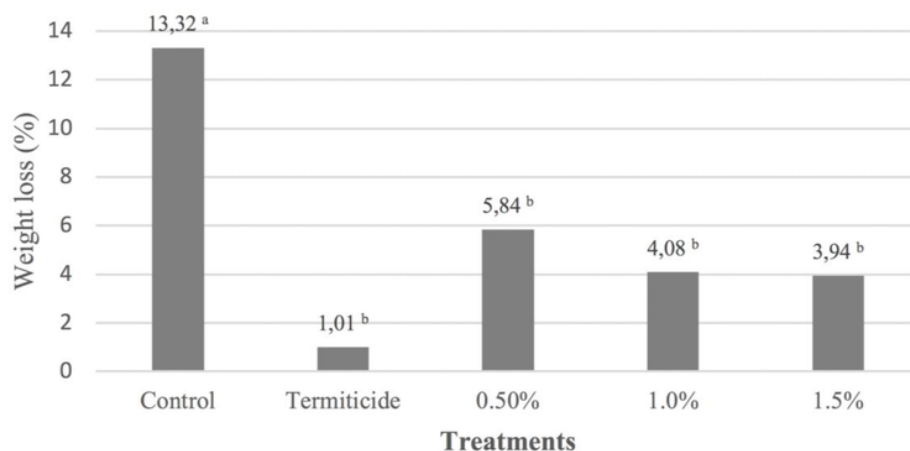


Figure 5. Average weight loss

The histogram in Figure 5 showed that the largest weight loss was found in control (13.32%) and the lowest in termiticide (1.01%). For the treatment of the extract showed decreasing weight decreasing with the concentration of extract applied to samples. The results of the variance analysis on weight loss data indicated that the treatment of feeding crude extract of *D. elliptica* bark at various concentrations is a very significant effect on the weight loss. To find out the difference of treatments mean value, the Duncan Multiple Range Test (Duncan Multiple Range Test) was performed with the result shown in Figure 5. The result of Duncan's test showed that the mean of control has a very significant difference with all other treatments. Meanwhile the mean of other treatments than control was not significantly different.

### 3.3. Discussion

Assessment of the effectiveness of *D. elliptica* bark extract against *Coptotermes* sp. has been carried out on a laboratory scale for 2 weeks 2 days based on starvation. In this study, mortality values vary depending on the concentration of extract applied. The value of termites mortality increases with the amount of concentration given, although the increase in value occurs slowly at the beginning of observation. In other words, the toxic extract effects worked slowly. Tu bark extract containing an active ingredient in the form of rotenone is assumed to cause mortality in termites.

The results of this study are similar to the findings of [15] that *D.elliptica* roots extracts contain a chemical compound containing rotenone which poison termites. Rotenone serves as a strong neurotoxic and an antifeedant that causes termites not to be eager to eat and makes the termites weaker and eventually leads to death. Terminal mortality occurs several hours to several days after contact with the rotenone. Also, rotenone is known to be toxic contact and stomach poisons that work slowly against insects and based on how it works Rotenon was a respiratory toxin. The findings of [21] also proved that rotenone serves as an inhibitor of a respiratory, inhibitor of feeding, and inhibitor of the development of *anopheles* sp. (Diptera: Culicidae). The utilization of *D. elliptica* stem extract as an organic pesticide studied [22] proved that the applied extract could accelerate the death of wetland mollusca (*Pila ampullacea*) because of containing toxic substances of rotenone (C<sub>23</sub>H<sub>2</sub>O<sub>6</sub>).

The mechanisms of termite poisoning can occur in termite digestion by destroying or interfering the bacteria activity in the termites gut. Bacterial symbiont plays a role in producing enzymes to breakdown cellulose in the termite gut. The death of bacteria will cause termites to not digest wood or other cellulose material that will lead to termite deaths. The death of bacteria was assumed similarly with the death of protozoa because of both them were gut symbionts. The findings of [23] studies using *D. elliptica* root

extracts proved that termite mortality might be caused by two things: (i) extractive substances from *D. elliptica* roots causing protozoal death in the stomach of termites when consuming cellulose paper, and (ii) the extract has caused damage to the nervous system in termites. Protozoa contained in the stomach of termites in charge of digesting cellulose cannot eat the paper, so the death of protozoa in the stomach of termites, termites, will become dead because the termite eaten bait which consists mainly of cellulose can not be absorbed by the termite body. It was further mentioned that the active rotenone substances inhibit the respiratory enzyme, glutamate oxidase enzyme. This enzyme functions in the catabolism of amino acids and their biosynthesis.

Figure 1 and Figure 4 show that the lowest termite mortality occurs in control. This condition is possible because termites in the control treatment are made in such a way that it resembles its natural state including foods that do not contain toxic materials as it is known that termites in nature have behaviors that will choose the most appropriate environment for their lives, where termites are exposed to a variety of food choices. Termites that can survive and adjust will do the orientation of food. Conversely, termites that are not able to adjust will die because the food provided does not meet the termite requirements and choose to fast. Under these circumstances, the condition of termites will be weak and gradually die or sick.

The next parameter used in showing the toxicity of *D. elliptica* bark extract is by calculating the weight loss of the test paper after being fed to termites. The average percentage mortality of termites is inversely proportional to the average percentage reduction in weight of the test paper. The more concentration the percentage decrease the weight of the test paper decreases. This can be expected because of active compounds in toxic tube skin extracts that are rotenone. Increased concentrations are in line with the increasing amount of these toxic compounds, thereby decreasing the feeding activity of termites against the samples.

#### 4. Conclusion

Tuba bark extract is effective in inhibiting the activity of ground termites *Coptotermes* sp. which was characterized by 100% mortality for abdominal toxicity test and 95.76% mortality for respiratory toxicity test, and the lowest decrease weight was 3.94% which occurred at a concentration of 1.5%. Tuba bark skin extracts are potential to be used as a termite control material, but further research is needed to reduce the concentration value by considering the threshold value and looking for the possibility of other active compounds other than the rotenone contained in tuba bark extract.

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