J. Pijar MIPA, Vol. 18 No. 6, November 2023: 970-975
DOI: 10.29303/jpm.v18i6.5863

ISSN 1907-1744 (Print)
ISSN 2460-1500 (Online)

EFFECT OF *GAMAL* LEAVES (*Gliricidia sepium*) EXTRACT AGAINST TERMITE (*Coptotermes curvignathus*)

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Received: October 17, 2023. Accepted: : November 20, 2023. Published: November 30, 2023

Abstract: The World Health Organization (WHO) estimates around 3 million people working in the agricultural sector experience insecticide poisoning, and 18 thousand of them die every year. The massive use of synthetic insecticides can harm non-targeted organisms such as plants and livestock. Moreover, this may lead to ecological imbalance and increase pollution. Thus, there is a need for innovation in making natural insecticides. Indonesia is home to abundant plant diversity; one of those is the *Gamal* plant (*Gliricidia sepium*). Previous studies found that *Gamal* leaves contain secondary metabolites such as flavonoids, alkaloids, tannins, saponins, and steroids. Flavonoids are known to have a toxic effect on mealybugs in the papaya plant, and tannins are antimicrobial and often used as pest control. Based on this, the *Gamal* plant has potential as a natural insecticide. One of the most critical pests in agriculture and households is termites (*Coptotermes curvignathus*), which often cause losses by damaging plants and household furniture. Also, there is no research yet on *Gamal* leaves against termites. Therefore, research is necessary to test the effectiveness of *Gamal* leaf extract against termites. In this study, experimental research was used with a quantitative approach. The *Gamal* leaf extract was obtained using ethanol and methanol solvents through a maceration technique. Several termites were acclimatized, and 120 were taken randomly for toxicity testing. Four concentrations (10%, 25%, 50%, and 75%) were applied to each group of ten termites. This treatment was repeated three times, and the termite mortality time was measured. Afterwards, the value of LC50 was determined using a regression line between log concentration and probit mortality. The result shows that the higher the concentration, the shorter the termite death time. The mortality rate reaches 100% within 1 to 2 minutes after treatment. The LC50 value of *Gamal* leaves extract against termites obtained is 5,1%. This indicates that *Gamal* leaves are very effective in killing termites, even in the lowest concentration. It is likely caused by secondary metabolites, especially flavonoids, that have insecticidal effects. In brief, the ethanolic and methanolic extract of *Gamal* leaves has the potential to be a natural insecticide to substitute synthetic insecticide, which is safer, economical, and environmentally friendly.

Keywords: *Gamal Leaves, Termites, Natural Insecticide, Extraction, Toxicity Test*

INTRODUCTION

Termites (*Coptotermes curvignathus*) are household pests that can cause economic losses [1]. Termites often nest and damage wooden furniture and house frames [2]. This is possible due to the ability of termites to digest cellulose, an organic compound found in wood, leaves, stems, paper, and cardboard [3]. To repel termites, people use synthetic insecticides made from chemicals that can harm health and pollute the environment [4-5]. Like pesticides, insecticides are toxic not only to target pests but also to humans and other non-target organisms such as plants and livestock [6]. Insecticides can poison humans through direct contact with the skin, breathing air, and consuming food contaminated with insecticides [7-8].

The World Health Organization (WHO) estimates that around 3 million people who work in the agricultural sector experience insecticide poisoning, and 18 thousand of them die every year [9]. The massive use of synthetic insecticides can disrupt the ecological balance by killing non-targeted insects and even being corrosive to other objects, especially humans [10][7]. Also, repeated use of insecticides can increase the risk of pesticide residue contamination in water, such as drinking water. Based on this problem, it is necessary to develop alternative products in the form of natural insecticides that are cheap, safe for humans and livestock, biodegradable, environmentally friendly, and effective in repelling pests, especially termites.

Indonesia has abundant biodiversity, including a diversity of plant species. Plants contain various kinds of bioactive compounds that are beneficial for people's lives [6]. Apart from being known to provide curative or preventive effects [10], these compounds are also widely used in agriculture, for example, as natural fertilizers and insecticides [11]. Of the number of plants that grow in Indonesia, the *Gamal* plant (*Gliricidia sepium*) is often used by the community as a natural insecticide [7].

The *Gamal* plant (*Gliricidia sepium*) is generally found on roadsides as a fence, and the leaves are used for animal feed, biopesticide, compost, organic liquid fertilizer, and controlling weeds [12-13]. *Gamal* leaves applied to soil can control soil physical properties, aeration, and drainage [14]. *Gamal* leaves can treat scabies, wounds, itching, rheumatism, and bone fractures [15]. In Central America, *Gamal* plants are known as antifungal and rodenticide [16]. *Gamal* plants and leaves can be seen in Figure 1.

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Figure 1. *Gamal* plant and leaves. Source: https://manfaat.co.id/manfaat-daun-*Gamal*

The complete classification of the *Gamal* plant is shown below:

Kingdom	: Plantae (plants)
Subkingdom	: Tracheobionta (vascular plants)
Superdivision	: Spermatophyta (seed plants)
Division	: Magnoliophyta (flowering plants)
Class	: Magnoliopsida (two-limbed plants)
Order	: Fabales
Family	: Fabaceae or Leguminosae
Genus	: Gliricidia
Species	: *Gliricidia sepium*

According to studies that have been conducted, *Gamal* leaves were identified to have antibacterial, anti-inflammatory, antioxidant, and insecticide effects [17][18]. These are presumably due to bioactive compounds contained in *Gamal* leaves. Many studies have conducted phytochemical tests to detect secondary metabolites in *Gamal* leaves. Some of them identified flavonoids, saponin, phenols, terpenoids, tannin, steroids, sterols, and alkaloids in the extract of *Gamal* leaves (Some structures of secondary metabolites can be seen in Figure 2)[18][19][20]. A study demonstrated that the bioactive compounds identified in *Gamal* leaves can disrupt the digestive organs of the insect's body due to its toxicity [21]. Magani *et al*. found that 96% ethanol extract from *Gamal* leaves inhibited the growth of *Staphylococcus aureus*, which were resistant to ß -*lactam antibiotics* [17]. In addition, research shows that *Gamal* leaf extract can control aphids (*Aphis gossypii*), which threaten the growth of chili plants (*Capsicum annum*) [22]. Fuadella *et al.* found that *Gamal* leaf extract was more effective than synthetic insecticides in killing leafworms (*Spodoptera exigua Hubner*) on shallot plants [23].

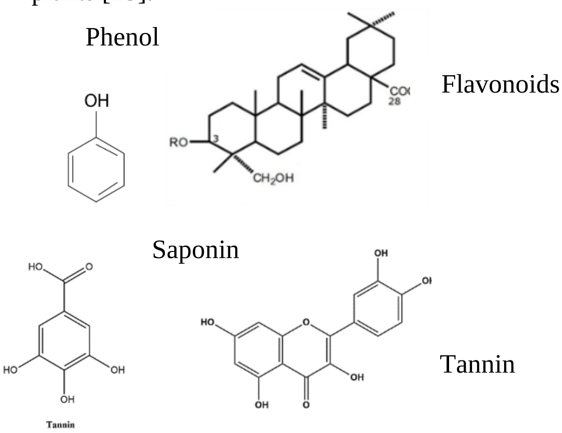

Figure 2. Secondary metabolite structures.

Based on this description, *Gamal* leaves have a great potential to be used as raw material for natural insecticides that are environmentally friendly and economical. Previous research has examined the manufacture of pesticides, but there has been no research on *Gamal* leaf extract against termites. Therefore, it is essential to determine the level of mortality and toxic effect of *Gamal* leaf extract as an insecticide against termites. Finally, this study is expected to be the primary step and reference for further research in developing natural ingredients into natural products such as natural insecticides. Also, the output of this study would be able to overcome the problem of termites that often cause losses.

RESEARCH METHOD

This research is green economy research to produce an insecticide product derived from *Gamal* leaf extract (*Gliricidia sepium*), with the sample object using termites (*Coptotermes Curvignathus*). The result of this study is projected to be able to produce a natural insecticide product that can be patented and commercialized for the community. The research will be conducted at the Pharmacy Laboratory of Bumigora University.

The research method is a scientific way to get data with specific purposes and uses [6]. In this study, the quantitative research method was used, which describes the problems identified by the author, where the object under study is explained from the author's point of view [7]. The type of design in this study is Post-test Only Control Group Design, in which the test is conducted after insects are given treatment, in this case by spraying *Gamal* leaf extract.

Tools and materials

In this study, several tools and materials were used in the laboratory. The tools utilized include beaker glass, measuring cylinder, graduated pipette, Erlenmeyer flask, petri dish, test tubes, sprayers, water baths, rotary evaporators, analytical balances, etc. Meanwhile, the materials used include *Gamal* leaves, termites, ethanol 96%, methanol 96%, glacial acetic acid (CH3COOH), concentrated sulfuric acid (H2SO4), distilled water, aquadest, aluminum foil, filter paper, etc.

Making a sample of Gamal leaf extract

The sample used was *Gamal* leaves taken from Jelantik Village, Central Lombok. The *Gamal* leaves were collected and dried under sunlight until they could be crushed into powder. *Gamal* leaves were extracted using the maceration method with ethanol and methanol solvents. The maceration began by mixing the *Gamal* leaves powder with either ethanol or methanol by comparison 1:5 (100 gram *Gamal* leaves powder and 500 ml solvent). Ethanol and methanol were chosen because they are universal solvents that distill or extract polar, semi-polar, or non-polar compounds. Ethanol is commonly used to extract flavonoids, saponins, tannins, anthraquinones, terpenoids, and alkaloids [23].

Meanwhile, methanol is very good at dissolving saponins [24]. The maceration process took 5-7 days. Afterwards, the extract mix collected was filtered and evaporated until a thick extract was obtained. Four different concentrations, 10%, 25%, 50%, and 75%, for the treatment of termites were made by diluting the thick extract with ethanol or methanol solvent.

Alcohol-free test

The alcohol-free test aims to confirm the sample no longer contains alcohol. An alcohol-free test was conducted by mixing a few drops of the model with glacial acetic acid and concentrated sulfuric acid in a heated test tube. The absence of an ester aroma indicates that the sample does not contain alcohol.

Table 1. The result alcohol-free test

Solvent	Extract concentration (%)	Average time of termite mortality (seconds)
Ethanol	Control-	0
	10	120
	25	97
	50	70
	75	63
Methanol	Control-	0
	10	110
	25	90
	50	78
	75	67
Control+	0	27

Termite sample preparation

Termites (*Coptotermes curvignathus*) were obtained from a broken door frame from Kuripan Village, West Lombok. Termites are acclimatized in a clear jar containing wood flakes for 3-5 days. For the toxicity test, 120 termites were required.

Toxicity test and probit analysis

In the toxicity test, each prepared concentration was sprayed three times on ten termites in a petri dish. The treatment was repeated three times. As a positive control, synthetic insecticide was used, while termite samples were sprayed with ethanol and methanol for negative controls. Next, the termite death time was calculated after spraying with *Gamal* leaf extract. The LC50 value was determined using probit analysis IBM SPSS version 22.

RESULTS AND DISCUSSION

The alcohol-free test shows no ester aroma, so it can be confirmed that the sample no longer contains ethanol or methanol. The results of the toxicity test of ethanolic and methanolic *Gamal* leaf extract against termites can be seen in Table 1. The concentration of the extract is directly proportional to the average time of termite death. The higher the extract concentration given, the shorter the termite death time. For instance, at the concentration of 75% and 10% with ethanol, termites died 63 seconds and 120 seconds after treatment, respectively. This is likely because the higher the concentration, the higher the bioactive compounds in *Gamal* leaf extract. Thus, the highest extract toxicity affects termites' shorter mortality time [25] [26]. However, in this experiment, ten termites in each repetition sprayed with ethanolic and methanolic extract of *Gamal* leaves died within 1-2 minutes after treatment. Also, the mortality rate of termites reached 100% (Table 2). This result aligns with research conducted by Nukmal et al., in which the fresh *Gamal* leaves extract causes 100% mortality on the imago of *Quadrastichus erythriane* after 72 hours of treatment.

To determine the LC50 value, the extract concentration is converted into x (log ten concentration), and the mortality of termites on each repetition is converted into probit scale. Then from this data, the linear line between extract concentration and mortality probit of termites obtained. The graph can be seen in Figure 3. Moreover, based on the regression analysis between the log concentration of extract and mortality probit of termites, a linear regression equation is acquired, namely $y=4{,}3453x - 0{,}059$. The regression line shows the LC50 value of 5,1%. This indicates that *Gamal* leaf extract is very effective in killing termites even at a deficient concentration, namely 10%.

This result is possibly due to secondary metabolites contained in *Gamal* leaves. A study has reported bioactive compounds in *Gamal* leaf extract, which have anti-insecticide effects, including alkaloids, terpenoids, phenolics, Tannins, Saponins, Flavonoids, Quinones, Proteins, and Sterols [27]. Tannin, saponins, free fatty acids, and alkaloids are among the secondary metabolites identified in *Calotropis gigantea* extract with a high bioinsecticidal effect against papaya mealybug. Many studies have demonstrated that flavonoids have toxic effects on different organisms. Among flavonoids known to be harmful to insects are isoflavonoid, kumarin, biochanin, and pinocembrin. Isoflavonoids such as rotenon have been used widely as insecticides [28]. *Kumarin* irritates insect skin and inhibits leucin transport [28]. Biochanin reduces fertility in primary reproductives of *Coptotermes formosanus* [29].

Meanwhile, pinocembrin treatment inhibited feeding and adversely affected the survival of *Spodoptera frugiperda* larvae [30]. Nukmal, *et al.* believe that flavonoid kills mealybug pests on papaya plants [34]. In addition, a study identified flavonoids as the highest amount of bioactive compounds in the water extract of *Gamal* leaf through the maceration technique [31]. Therefore, it is believed that secondary metabolites in *Gamal* leaf extract have toxic activity against termites.

Table 2. The result of the toxicity test on *Gamal* leaf extract against termite

Solvent	Extract concentration (%)	The number of termites	The number of termite died			The average of mortality	The percentage of mortality (%)
			U1	U2	U3		
Ethanol	Control-	10	0	0	0	0	0
	10	10	10	10	10	10	100
	25	10	10	10	10	10	100
	50	10	10	10	10	10	100
	75	10	10	10	10	10	100
Methanol	Control-	10	0	0	0	0	0
	10	10	10	10	10	10	100
	25	10	10	10	10	10	100
	50	10	10	10	10	10	100
	75	10	10	10	10	10	100
Control+	0	10	10	10	10	10	100

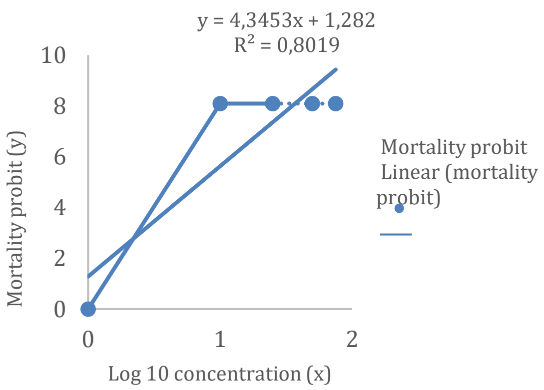

Figure 3. The mortality probit

During the toxicity test, termites' response to *Gamal* leaves extract sprayed by avoiding the extract and moving to the edge of the *petri* dish slowly became less mobile and finally died. This extract might cause termites to die in several ways. According to Nukmal *et al.*, flavonoids in Gamal leaves will enter the insect body through the respiratory system and cause damage to the spiracle. Flavonoids might also cause a reduction in nervous system function. Moreover, flavonoids can decrease insects' ability to digest food. In this case, flavonoids work as antifeedants by inhibiting taste receptors around the mouth of insects and causing them to fail to receive stimulation [32]. Robinson also stated that flavonoids may irritate insect skin after exposure to *Gamal* leaf extract [28]. Tannins can be used as astringent, substances that shrink body tissues and cover proteins on the skin and mucosal tissue. These cause the skin tissue to be constricted and dry, which could lead to a reduction of insect growth [Permadi dan Fitrihidajati, 2019]. Therefore, secondary metabolites play a crucial role in the mortality of termites.

For the positive control, treatment with synthetic insecticide shows the shortest termite death time, namely 27 seconds after spraying. Meanwhile, for the negative control, treatment with ethanol or methanol solvent shows that no termites died within an observation time of 1 hour. Moreover, for the use of solvents, there was no significant difference in the death time of termites given ethanol or methanol extract of *Gamal* leaves. These two solvents are universal so that they can extract polar and non-polar compounds from *Gamal* leaf extract [33].

CONCLUSION

According to the research, it can be concluded that the ethanolic and methanolic extracts of Gamal leaves have insecticidal effects against termites, with a mortality rate reaching 100%. This is possible due to secondary metabolites contained in *Gamal* leaves. Thus, the ethanolic and methanolic extract of *Gamal* leaves can be used as a natural insecticide as an alternative to synthetic insecticides, which are more economical and environmentally friendly.

ACKNOWLEDGMENT

We want to thank the Ministry of Education, Culture, Research and Technology of the Republic of Indonesia for its financial support in this research. Also, thanks to the head of The Department of Pharmacy, Bumigora University, for allowing us to conduct this research in the Pharmacy laboratory.

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ISSN 2460-1500 (Online)

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