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Investigation of leaves of *Xylocarpus granatum* as a larvicide agent against *Aedes aegypti* and its associated anti-bacterial properties

Sunelsya Surya, Delianis Pringgenies *, Wilis Ari Setyati and Muhammad Syaifudien Bahry

Department of Marine Science, Faculty of Fisheries and Marine Science, Diponegoro University, Jl. Prof. H. Soedarto, S.H. No.1. Tembalang, Semarang, Central Java, 50275, Indonesia.

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Abstract

Mangrove is a coastal plant that has rich benefits. This research aimed to investigate *X. granatum* leaves extract as a mosquito larva eradicator and find the antibacterial activities of its bacteria. The eradicator test was done for 24 and 48 hours. As many as 10 pieces of cups, filled with 50 ppm, 100 ppm, 250 ppm, 500 ppm, and 1000 ppm of *X. granatum* leaves extract. Each cup contains 10 mosquitoes (*Aedes aegypti*). Probit LC_{50} test was carried out from *X. granatum* leaves extract and an antibacterial test was carried out by isolating bacteria from *X. granatum* leaves. The survival rate percentage of larvae in *X. granatum* leaves extract, the highest was 50% at 24 and 48 hours and the lowest was 0% at 48 hours. The result of the probit test shows 426,618 mg/L at 24 hours and 100,193 mg/L at 48 hours, categorized as a toxic compound. There were a total of 7 pure isolates obtained. Antibacterial tests showed the antibacterial activity of isolates against Staphylococcus aureus, Vibrio harveyii, and Vibrio alginolyticus, with the result being 3 isolates (XGL1, XGL2, and XGL3), 2 isolates (XGL6 and XGL7), and 4 isolates (XGL2, XGL3, XGL6, and XGL7). Based on the results, 1000 ppm *X. granatum* leaves extract has the potential as a mosquito larva eradicant or Phyto-insecticide. In addition, there were symbiont bacteria in *X. granatum* leaves that were potentially used as larva eradicant.

Keywords: Antibacterial; *Xylocarpus granatum* Leaves; Phyto-insecticide; *Aedes aegypti*

1. Introduction

Mangroves are plants that were often found on sloping muddy beaches and river estuaries [2]. The mangrove ecosystem has a relationship with various components: biotic and abiotic components [15]. The mangrove community also consists of microorganisms, including bacteria [14].

Mangrove forests in Indonesia are classified as the largest in the world, covering more than 50% of the mangrove forest area in Asia [4]. Most *Xylocarpus* sp. can be found in the Karimunjawa [8]. Mangrove *X. granatum* has thick leaves with leaves arranged in pairs or alone and has an elliptical to inverted egg shape [5]. *X. granatum* leaves contain antioxidants that have many benefits [9]. In addition, the bark and fruit of *X. granatum* can be used as a medicine for stomach and liver pain [1]. In addition, *X. granatum* also has antibacterial ability [12]. Secondary metabolites produced by *X. granatum* and its microorganism can be used as anti-inflammatory, antiviral, and anti-cancer.

The *Aedes aegypti* mosquito is a vector of Dengue Hemorrhagic Fever (DHF), transmitted through its bite [3]. The spread of DHF is caused by several factors: humans, viruses, the environment, and infectious vectors [13]. The countermeasure for increasing *A. aegypti* mosquitoes in the biosphere is using natural insecticides as alternative materials to eradicate vectors [10]. Putri & Hidajati [7] stated that *Xylocarpus* sp. was popularly used as Phyto-insecticides. Previous research

* Corresponding author: Delianis Pringgenies
Department of Marine Science, Faculty of Fisheries and Marine Science, Diponegoro University, Jl. Prof. H. Soedarto, S.H. No.1. Tembalang, Semarang, Central Java, 50275, Indonesia.

by Pringgenies [8] claims that *X. granatum* mangrove extract could potentially eradicate *A. aegypti* mosquito larvae. This study aimed to determine the potential of *X. granatum* leaves extract as an anti-larval mosquito *A. aegypti*. The survival rate of *A. aegypti* mosquito larvae was investigated after the addition of Xylocarpus mangrove leaves extract with different concentrations and times to eradicate *A. aegypti* mosquito larva.

2. Material and methods

The research material used was the leaves of the *Xylocarpus granatum*. Mangrove samples in this study were taken in the waters of Semarang. Drying of samples was carried out with the help of indirect sunlight for 4-7 days at the beginning of the research, followed by making flour. The sample weighed as much as 500 g and extracted using the maceration method with 5L technical ethyl acetate until all the content was extracted. The solution obtained was evaporated using a rotary evaporator until the solvent was exhausted, then the partition process was carried out using a separatory funnel. The partition results should be evaporated again. Biological activity and toxicity assays of Neem leaves extract were carried out on *A. aegypti* mosquito larvae. Based on a method by Nurhayati [6], the level of toxicity was analyzed and produced data in the form of percent mortality.

A. aegypti mosquito larvae were bred by laying their eggs in distilled water on trays. The growth medium was placed in a humid place until the egg larvae hatched and were classified as instar 4. The test was carried out using 14 small transparent cup bottles, each at 50 ppm; 100 ppm; 250 ppm; 500 ppm; 1000 ppm; negative control; and positive control. All tests were carried out with 2 samples for each treatment. Each bottle was given extract diluted with DMSO appropriate to the test concentration and then added with aquadest until it reached a volume of 10 ml. Then, each bottle was then given 10 *A. aegypti* larvae for toxicity test observations. The positive control used was abate solution according to WHO, which was 100ppm/10ml. The negative control used was aquadest. The observation was made after 24 and 48 hours. The variable observed was the number of mosquito larvae that survived and are still alive.

The qualitative antibacterial test was performed by isolating the bacteria symbiont of *X. granatum,* referring to the Pringgenies [17] method. Symbiont bacteria isolates were isolated using Zobell 2216E media in a petri dish and incubated at room temperature for 24 hours. The pure isolate of bacteria was tested using the paper disk diffusion method using *Staphylococcus aureus, Vibrio harveyii,* and *Vibrio alginolyticus* as pathogens. Antibacterial activity will then be determined by observation of the inhibition zone formation of the isolate.

3. Results and discussion

The results of the test showed the percentage of *A. aegypti* larvae's survival rate at 24 hours of testing on the concentration of mangrove leaves *X. granatum* extract was as follows: 50 ppm was 50%, 100 and 250 ppm were 40% (60% mortality), 500 ppm was 20% (80% mortality), and 1000 ppm was 0% (100% mortality). The results of the tests carried out at 48 hours were as follows: 50 ppm was 50%, 100 and 250 ppm were 20% (80% mortality), 500 and 1000 ppm were 0% (100% mortality). The effect of 50 ppm *X. granatum* mangrove leaves extract on the survival rate of *A. aegypti* larvae at 24 and 48 hours showed that the highest survival rate was 50%. The larvae of *A. aegypti* died entirely at 1000 ppm *X. granatum* leaves extract at 24 and 48 hours (100% mortality), as shown in Figure 1.

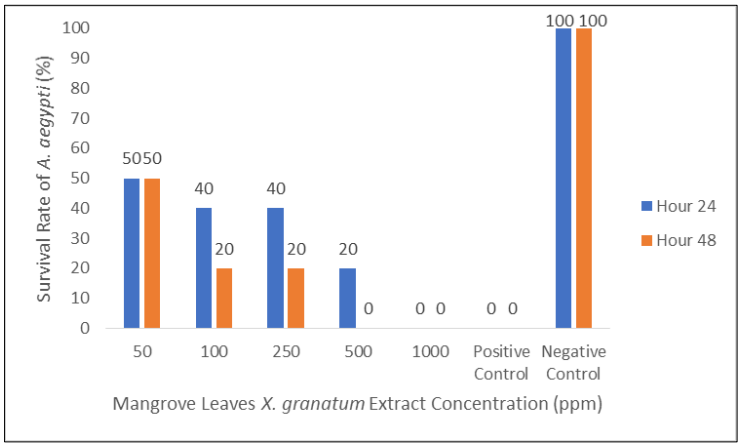

Figure 1 Survival rate (%) of *X. granatum* mangrove leaves with different concentrations (ppm) against *A. aegypti* mosquito larvae within 24 and 48 hours

The results showed that the higher of concentration of *X. granatum* extract-treated against *A. aegypti* mosquito larvae, the lower its survival rate. Higher solvent concentration will increase the mortality rate. Based on the data collected, 1000 ppm of *X. granatum* leaves extract at a 0% survival rate was proven to have the potential to kill mosquito larva. In addition, the 1000 ppm of *X. granatum* mangrove extract also had higher effectiveness in killing *A. aegypti* larvae than the Pugun Tanuh herb extract. Putri [7] stated that Avicennia marina bark could kill *A. aegypti* mosquito larvae at a concentration level of 3000 ppm. In contrast to Beluntas leaves extract, which was extracted using ethyl acetate, it contained compounds that were toxic and capable of killing larvae. According to Rochmat [11] Beluntas leaves (Pluchea indica Less) contain quinic acid compounds and have the potential as biolarvicides.

Table 1 LC_{50} Toxicity Classification (Source: Wagner 1993)

LC_{50} Value (mg/L)	**Toxicity Level**
≤ 30	Very toxic
31 ≤ LC_{50} ≤ 1000	Toxic
> 1000	Non-toxic

The relationship between LC_{50} and the classification of chemical toxicity levels which stated in Table 1. The result of the research showed that the *X. granatum* mangrove leaves extract had an LC_{50} value of 426,618 at 24 hours of observation and 100,193 at 48 hours of observation. These results are classified as toxic compounds, as shown in Table 2.

Table 2 LC_{50} Result of *X. granatum* Leaves Extract

Extract	**Duration of Contact Time (hours)**	**LC_{50} Value (mg/L)**
Xylocarpus granatum Leaves	24	426,618
	48	100,193

The level of toxicity of *X. granatum* mangrove leaves extract using methanol solution is directly proportional to the concentration. This result affects the survival phenomenon of *A. aegypti* larvae, shown in Figure 1 and Figure 3. The condition of *A. aegypti* mosquito larvae shows that it will die completely at the 1000 ppm *X. gratanum* leaves extract after 24 hours and 48 hours. The toxic compound can interfere with *A. aegypti* larvae organs resulting in death. Rochmat [11] stated that the mechanism of death of *A. aegypti* larvae was caused by the presence of an active substance from the extract solvent fraction that entered the larva's body and interfered with its digestive organs.

Table 3 Colony Morphology of *X. granatum* Symbiont Bacteria

Isolate Number	**Colony Morphology**				
	Shape	**Color**	**Margin**	**Elevation**	**Size**
Isolate 1	Circular	White	Undulate	Flat	Moderate
Isolate 2	Irregular	White	Undulate	Flat	Large
Isolate 3	Irregular	White	Undulate	Flat	Large
Isolate 4	Circular	White	Entire	Flat	Large
Isolate 5	Filamentous	Cream	Rhizoid	Flat	Moderate
Isolate 6	Circular	White	Entire	Flat	Punctiform
Isolate 7	Circular	White	Entire	Convex	Punctiform

The colony morphology of *X. granatum* symbiont bacteria showed that there were total 7 pure isolates that were symbiotic bacteria present in the mangrove leaves of *X. granatum*: XGL1, XGL2, XGL3, XGL4, XGL5, XGL6, and XGL7. There are various characteristics that differ in terms of shape, color, margin, elevation, and size of the colony. Almost all pure isolates had flat elevation, except XGL7 which had convex elevation. In addition, all isolates are white except for XGL5. There are several margins found in isolates, such as undulate margin on XGL1, XGL2, and XGL3; entire margin on XGL4, XGL 6, and XGL7; and rhizoid margin at XGL5. Circular shape dominates in colony morphology, namely circular shape owned by 4 of the 7 isolates, which were XGL1, XGL4, XGL6, and XGL7, shown in Table 3.

Table 4 Antibacterial Test Result of *X. granatum* Leaves Symbiont Bacteria

Isolate Number	Pathogenic Bacterial		
	S. aureus	***V. harveyii***	***V. alginolyticus***
Isolate 1	V	-	-
Isolate 2	V	-	V
Isolate 3	V	-	V
Isolate 4	-	-	-
Isolate 5	-	-	-
Isolate 6	-	V	V
Isolate 7	-	V	V

The qualitative results of the antibacterial test show an antibacterial activity of each isolate on the bacterial pathogens *S. aureus*, *V. harveyii*, and *V. alginolyticus*. The inhibition zone indicates the presence of antibacterial activity. The XGL 1, XGL 2, and XGL 3 isolates showed antibacterial activity against *S. aureus* bacteria. Antibacterial activity against *V. harveyii* was found in XGL6 and XGL7 isolate. There was the most antibacterial activity in the tests obtained, where XGL2, XGL3, XGL6, and XGL7 were able to become antibacterial agents against the pathogen *V. alginolyticus*, as shown in Table 4.

Based on the purification results on the symbiont *X. granatum*, various characteristics differ from one isolate to another. The results of antibacterial activities potentially indicate that the isolates have different bacterial species. Pulungan [18] in his research stated that the perfect identification of microorganisms could be done by gram staining and biochemical tests.

The results of the antibacterial test in the study showed that the XGL2 and XGL3 isolates were able to inhibit the pathogenic bacteria *S. aureus* and *V. alginolyticus*. In addition, XGL6 and XGL7 were able to inhibit the bacterial pathogens *V. harveyii* and *V. alginolyticus*. The occurrence of the clear zone in the test can be influenced by various types of secondary metabolites produced by each microbe. XGL2 and XGL3 isolate have the potential to produce the same secondary metabolites in inhibiting these pathogenic bacteria, as well as XGL6 and XGL7 isolate. Setyati [19] stated that the inhibitory power of each isolate was caused by differences in the content of secondary metabolites that had diffused into the agar medium. Antibacterial test in this study could be correlated with its ability as Phyto-insecticide. Bacteria can produce secondary metabolites and have the potential to kill mosquito larvae as well. Research conducted on mollusks by Syaifudien and Pringgenies [20] showed there are similarities between secondary metabolites in symbiotic microorganisms and mollusks as their hosts.

4. Conclusion

The extract of *Xylocarpus granatum* leaves has the potential as a botanical insecticide for the *Aedes aegypti* mosquito. At 1000 ppm, the extract of *Xylocarpus granatum* leaves has the ability to eradicate all *Aedes aegypti* larvae, therefore it could be utilized as a Phyto-insecticide. In addition, the symbiotic bacteria present in *X. granatum* leaves have the potential as Phyto-insecticide.

Compliance with ethical standards

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Disclosure of conflict of interest

Author declares that there are no conflicts of interest in any form. All authors have approved the final article.

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