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Efficacy Test of Ethanol Extract From Matoa *(Pometia pinnata)* Stem Bark on *Aedes aegypti* Larval Mortality As An Effort to Control Dengue Fever

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Abstract
Dengue Hemorrhagic Fever (DHF) is an infectious disease caused by the dengue virus and transmitted primarily through the bite of the Aedes aegypti mosquito, the disease's primary vector. Controlling the population of this mosquito species is considered a strategic measure in preventing the spread of DHF. One potential biological control agent is the matoa plant (Pometia pinnata), a member of the Sapindaceae family widely distributed in tropical regions. This plant contains secondary metabolites such as flavonoids, tannins, and saponins, which exhibit natural insecticidal activity. This study aims to evaluate the efficacy of ethanol extract derived from the bark of P. pinnata against A. aegypti larvae. The extraction process was conducted using ethanol as a solvent, and the resulting extract was tested against mosquito larvae at concentrations of 25 ppm, 50 ppm, 70 ppm, and 90 ppm. Larval mortality data were analysed using Analysis of Variance (ANOVA) via SPSS software version 24.00. The results demonstrated that the ethanol extract of P. pinnata bark had a significant dose-dependent effect on larval mortality. These findings suggest the potential of the extract as a natural active ingredient in the formulation of plant-based larvicides, contributing to environmentally friendly and sustainable dengue vector control strategies.

INTRODUCTION

Infectious diseases remain a significant public health issue in the present era. Mosquitoes and other insects are among the primary contributors to serious health problems. As a tropical country, Indonesia is particularly vulnerable to mosquito-borne diseases (Moniharapon *et al*., 2021; Pakpahan *et al*., 2023). One such mosquito like *Aedes aegypti*, is a vector for several diseases, including chikungunya, yellow fever, Zika virus, and dengue virus (Gubler *et al*., 2014; Kraemer *et al*., 2019). According to Nurdin and Zakiyuddin (2018), dengue fever (DF) is an acute infection caused by the dengue virus that can result in shock and potentially death (Halstead, 2019).

Dengue fever (DF) is an infectious disease caused by the dengue virus, transmitted through the bite of *Aedes aegypti* (Singh and Ruzek, 2013; Moniharapon et al., 2020; Kaihena and Ukratalo, 2021; Ukratalo *et al*., 2024). According to the World Health Organization (WHO), dengue fever remains a significant public health challenge worldwide (WHO, 2009). This is evidenced by reports of a 30-fold increase in the incidence of dengue worldwide over the past 50 years. Dengue epidemics were recorded in the 19th and early 20th centuries in several regions, including the Americas, southern Europe, northern Africa, the eastern Mediterranean,

Asia, Australia, and several islands in the Indian Ocean, the South Pacific, and the Caribbean (Utami and Porusia, 2023).

Based on the Indonesian Health Profile data, the number of DF cases and deaths has decreased annually. In 2021, the number of cases and deaths dropped compared to 2020. In 2021, there were 73,518 reported cases with 705 deaths, whereas in 2020, there were 108,303 cases and 747 fatalities (Kemenkes RI, 2022).

Efforts to control the *Aedes aegypti* mosquito vector remain a primary strategy in the fight against DF (Donateli *et al.,* 2019; Ukratalo *et al*., 2024; Samsudin *et al.,* 2024). Despite the development of various methods, including the use of chemical insecticides, these approaches have led to resistance issues, environmental damage, and health risks for humans (Ambarita, 2015; Widiastuti *et al*., 2021; Anindya *et al*., 2023). As a result, there has been increased exploration of natural substances as more environmentally friendly alternatives to control *Aedes aegypti* larvae populations (Kardinan, 2003; Debboun *et al*., 2014; Mudaningra *et al*., 2023; Haidah *et al*., 2024).

Matoa (*Pometia pinnata*) is a plant from the Sapindaceae family found in tropical regions, including Indonesia (Fathoni *et al.,* 2024). The plant is widely distributed worldwide, and various parts of Matoa are used as traditional medicine in several countries. In Papua New Guinea, the chewed bark is used to treat burns. In Fiji, extracts from the leaves and bark are used to treat a variety of ailments including stomach disorders, diarrhea, dysentery, bone, muscle, and joint pain. In Tonga, an infusion of the bark is used to treat childhood diarrhea, stomach disorders, coughs accompanied by fever, and constipation, while the leaves are also used in treatments. In Sarawak, Malaysia, Matoa is used as a traditional remedy for chickenpox, where patients are bathed in hot bark extract (Lim, 2013; Lumintang *et al*., 2015).

According to Razoki (2023), matoa contains chemical compounds such as flavonoids, tannins, and saponins. This is further supported by research by Rochaeni *et al*. (2021), who found that *Matoa* contains secondary metabolites such as flavonoids, tannins, terpenoids, and saponins. The flavonoids and saponins found in Matoa stem bark are believed to have the potential to disrupt larval membranes, inhibit endocrine function, trigger chemical reactions that disturb the larval metabolic process, and affect their respiratory system. These effects result in decreased growth rates and increased mortality of mosquito larvae (*Aedes aegypti*) (Moniharapon *et al*., 2019; Musiam *et al*., 2020; Ayal *et al*., 2021; Ananda *et al*., 2023). This study aims to evaluate the potential of ethanol extract from Matoa stem bark to control *Aedes aegypti* larvae.

RESEARCH METHODS

Type of Research

This study is classified as laboratory experimental research.

Research Design

This study used a completely randomized design with 5 treatments. Each treatment was replicated three times. The treatment groups are as follows:

P0: No treatment (negative control)

P1: Given ethanol extract of *P. pinnata* stem bark at 25 ppm
P2: Given ethanol extract of *P. pinnata* stem bark at 50 ppm
P3: Given ethanol extract of *P. pinnata* stem bark at 75 ppm
P4: Given ethanol extract of *P. pinnata* stem bark at 90 ppm

Tools and Materials

The equipment used in this study consists of pipettes, plastic trays, 15 plastic containers (as test vessels), beakers, cloth, mixers, glass stirring rods, labels, and knives. The materials used include *P. pinnata* stem bark extract, ethanol, clean water or equates, *A. aegypti* larvae, and fish food.

Procedure

Preparation of Mosquito Larvae

Larvae of *A. aegypti* were collected from bathtubs and drinking water containers. They were placed in plastic trays containing approximately 1000 mL of clean water. Before this, the trays were filled with clean water and larval food. The larvae were then divided into observation groups, each consisting of 20 individuals. The larvae used were third-instar, measuring approximately 4–5 mm in length, characterized by visible thoracic spines and brownish-black spiracles.

Preparation of Test Materials

The sample was taken from the stem of the *P. pinnata* tree, located 50 cm above the ground. One kilogram of the stem bark was obtained by peeling the tree. After the collection, the sample was cleaned to remove impurities and unwanted parts, then washed with running water. The bark was cut into small pieces to facilitate the drying process, which was carried out for 10 days in a shaded area, away from direct sunlight. After drying, the sample was ground using a dry blender to obtain powder, which was sieved (Kakisina and Ukratalo, 2011; Kaihena *et al.,* 2024).

Two hundred grams of the dry powder were weighed and placed in a 4-liter conical flask. Two litres of 96% ethanol were then added until the powder was fully immersed. The mixture was homogenized and allowed to soak for 6 hours. It was then filtered through filter paper, and the extract was concentrated using a rotary evaporator until a thick extract was separated from the solvent. The concentrated extract obtained was weighed and recorded.

Larvicidal Activity Testing

The larvicidal activity test of the ethanolic extract of *P. pinnata* stem bark was adapted from the method described by Cheng *et al*. (2004), with modifications in the number of test animals and the duration of exposure. Three hundred third instar *A. aegypti* larvae were used, with 20 larvae placed in each container containing the ethanolic extract at specified concentrations. Each treatment was conducted in triplicate. Larvicidal activity was monitored for 24 hours from the time the larvae were introduced into the containers.

Data Collection

Data were collected by counting the number of dead larvae in each container and recording the result in a table. A larva was considered dead if it showed no response to water movement and did not react when gently prodded with a stick.

Data Analysis

Observational data will be analyzed using analysis of variance (ANOVA) with SPSS version 24.0. If a significant effect is found, further analysis will be conducted using the Least Significant Difference (LSD) test at a 0.5% significance level.

RESULTS AND DISCUSSION

Table 1 shows the average mortality rate of *A. aegypti* larvae in the control group and the groups treated with an ethanol extract of *P. pinnata* stem bark at concentrations of 25 ppm, 50 ppm, 70 ppm, and 90 ppm.

Table 1. Average Mortality Rate of *A. aegypti* Larvae

Treatment	Average Larval Mortality (%)					Total ± SD
	0 hours	6 hours	12 hours	18 hours	24 hours	
Control	0,00	0,00	0,00	0,00	0,00	0,00 ± 0,00[a]
Concentration 25 ppm	0,00	23,33	30,00	46,67	-	100 ± 17,77[b]
Concentration 50 ppm	0,00	31,67	33,33	35,00	-	100 ± 15,37[c]
Concentration 70 ppm	0,00	38,33	43,33	18,33	-	100 ± 21,13[d]
Concentration 90 ppm	0,00	48,33	51,67	-	-	100 ± 19,82[a]

Note: Superscripts with the same letter indicate no significant difference (α < 0,05)

Based on the results presented in Table 1, the average mortality rate of *A. aegypti* larvae at 25 ppm was 23.33% after 6 hours of observation, 30.00% after 12 hours, and 46.67% after 18 hours. At 50 ppm, the average larval mortality was 31.67% at 6 hours, 33.33% at 12 hours, and 35.00% at 18 hours. At the 70 ppm concentration, the average mortality rate was 38.33% at 6 hours, 43.33% at 12 hours, and 18.33% at 18 hours. At the 90 ppm concentration, the average mortality rate was 48.33% at 6 hours and 51.67% at 12 hours. These results indicate that the mortality rate of *A. aegypti* larvae generally increased with higher concentrations of ethanol extract. The data in Table 1 are illustrated in Figure 1.

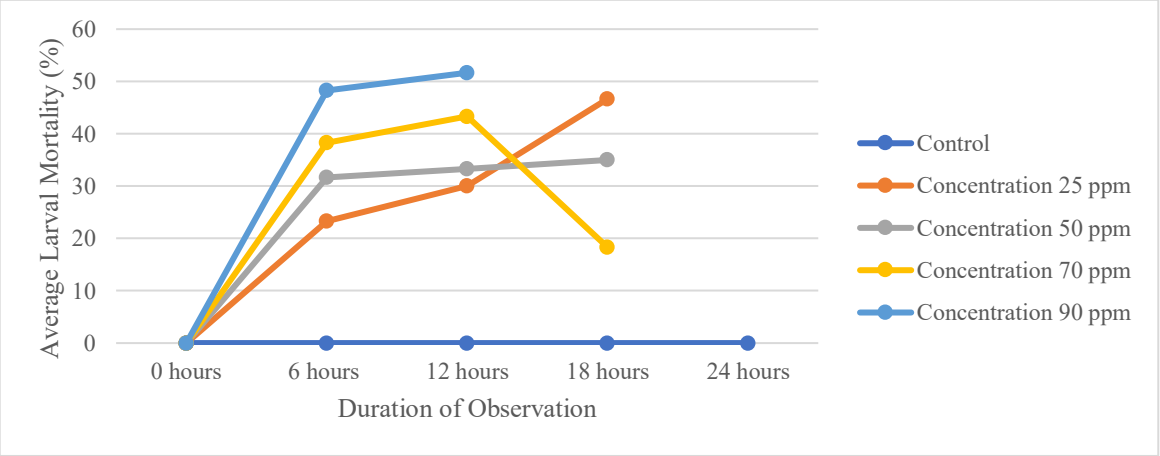

Figure 1. Graph of Average Mortality Rate of *A. aegypti* Larvae

The results of the ANOVA indicated that the administration of an ethanolic extract of *P. pinnata* stem bark had a significant effect on the mean mortality rate of *A. aegypti* larvae. The post hoc BNT test also revealed significant differences between the 25 ppm, 50 ppm, and 70 ppm. However, no significant difference was observed between the control and 90 ppm groups.

From the results presented in Table 1, it can be observed that at the beginning of the observation period (0 hours), no mortality was recorded in *A. aegypti* larvae. After 6 hours, mortality was observed in the groups treated with the ethanolic extract of the stem bark of *P. pinnata*, with the highest average mortality at 90 ppm (48.33%) and the lowest at 25 ppm (23.33%). At the 12-hour observation, the highest mortality was observed at 90 ppm (51.67%) and the lowest at 25 ppm (30.00%). However, at the 18-hour observation, the highest mortality occurred at 25 ppm (46.67%) and the lowest at 70 ppm (18.33%).

These results indicate that the ethanol extract of *P. pinnata* stem bark has a significant effect on the mortality of *A. aegypti* larvae. This is evident from the increased mortality rate associated with higher extract concentrations over different observation periods. For example, the highest mortality was observed at 90 ppm during the 6-hour observation period, while the highest mortality was observed at 25 ppm during the 18-hour observation period. Furthermore, the effect of ethanol extract on *A. aegypti* larval mortality is influenced by both concentration and observation time. Higher concentrations, especially at 90 ppm, generally resulted in greater mortality, although the variation depended on the observation period.

The higher concentrations of extracts resulted in higher mortality rates in mosquito larvae. This effect is attributed to the greater presence of secondary metabolites at higher concentrations, which enter the larvae's bodies, weaken their physiological resistance, and increase their vulnerability, ultimately leading to higher mortality. The stem bark extract of *P. pinnata* is known to contain secondary metabolites such as flavonoids, saponins, and tannins, all of which contribute to its larvicidal activity (Sirait et al, 2023).

Flavonoid compounds are recognized as part of the natural defence mechanisms of plants against attacks from various organisms, including insects (Harborne & Grayer, 2017). According to Riddick (2024), in mosquito larvae, flavonoids can inhibit feeding activity by disrupting the gustatory stimulus detection system, impairing the larvae's ability to recognise and consume food efficiently. This disruption ultimately results in the larvae failing to attain the critical body mass required to progress to the next developmental stage. Furthermore, flavonoids exhibit direct toxic effects on larvae, significantly increasing larval mortality rates within mosquito populations (Utami & Porusia, 2023).

Saponins, classified as secondary metabolites, play a crucial role in impeding the growth and survival of insect larvae through two primary mechanisms (Shakeel *et al.,* 2025). First, saponins stimulate the mucosal lining of the larval digestive tract, producing a bitter sensation that reduces feeding behaviour and appetite. As a consequence, larvae experience nutritional deficiencies that compromise their survival. Second, saponins can damage the protective wax layer covering the insect's body, maintaining internal fluid balance. Disruption of this barrier leads to excessive water loss, ultimately resulting in larval death. The toxic efficacy of saponins

has also increased with higher concentrations, as the larvae absorb more of the compound (Ardiansyah *et al*., 2023).

In addition to flavonoids and saponins, tannins contribute significantly to plant defence mechanisms against insect larvae (Iqbal & Poór, 2025). Tannins exert their effects by suppressing the activity of key digestive enzymes in larvae, specifically protease and amylase (Sagu *et al.,* 2021; Feng *et al.,* 2024). These enzymes are vital for digesting proteins and carbohydrates, and their inhibition impairs nutrient absorption. As a physiological response to this disruption, larval growth is retarded and nutritional imbalance ensues, which may lead to mortality or incomplete metamorphosis (Handayani *et al*., 2023).

The presence of secondary metabolites such as flavonoids, saponins, and tannins demonstrates substantial potential for the natural control of mosquito larvae populations. These compounds' distinct modes of action produce strong synergistic effects, leading to physiological impairment and developmental disruption in larvae. Moreover, increased concentrations of these compounds have been consistently associated with higher larval mortality rates, positioning them as promising candidates for developing environmentally friendly and sustainable botanical larvicides.

CONCLUSION

Based on the results and discussion, the ethanolic extract of *P. pinnata* stem bark has a significant effect on *A. aegypti* larval mortality. This provides a promising alternative for vector control, particularly in areas vulnerable to the spread of mosquito-borne diseases.

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