

**EFFICACY TEST OF LIME LEAF ETHANOL EXTRACT
(*Citrus aurantifolia*) AGAINST *Aedes aegypti* LARVAE**

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ABSTRAK

Nyamuk *Aedes aegypti* merupakan vektor utama penyakit Demam Berdarah *Dengue* yang disebabkan oleh virus *dengue*. Upaya penanggulangan pemberantasan jentik dapat dilakukan dengan menggunakan larvasida alami atau biolarvasida. Ekstrak daun jeruk nipis (*Citrus aurantifolia*) diketahui memiliki aktifitas menghambat kutikula larva dengan kandungan tannin. Penelitian ini bertujuan untuk mengevaluasi pengaruh ekstrak daun jeruk nipis terhadap pertumbuhan larva *Ae aegypti*. Jenis penelitian ini ialah eksperimental, ekstrak daun jeruk nipis dibuat dengan metode maserasi menggunakan etanol 90% dilanjutkan evaporasi ekstrak. Penelitian dilakukan pada empat kelompok yang berdasarkan konsentrasi ekstrak jeruk nipis yaitu kontrol negatif (aquades), 12,5%, 25%, dan 50%. Pengujian dan pengamatan dilakukan selama 24 jam. Hasil penelitian menunjukkan ekstrak etanol daun jeruk nipis pada berbagai konsentrasi dapat menghambat pertumbuhan dan bersifat toksik 100% terhadap larva *Ae. aegypti*. Penelitian ini dapat disimpulkan bahwa penggunaan ekstrak daun jeruk nipis dapat dikembangkan sebagai biolarvasida.

Kata Kunci: *Aedes aegypti*, biolarvasida, *Citrus aurantifolia*, ekstrak, uji efikasi

ABSTRACT

The primary carrier of the dengue virus-caused disease dengue hemorrhagic fever is the *Aedes aegypti* mosquito. By eliminating the flick using larvacides, the effort of seeding can be avoided. Tannin concentration in lime leaf extract (*Citrus aurantifolia*) is known to suppress the growth of larval cuticles. The objective of the study was to assess how *C.aurantifolia* lime leaf extract affected the development of *Ae. aegypti* larvae. This form of research is experimental, and this extract is produced by macerating lime leaves in 90% ethanol before extracting it via evaporation. The study included four groups: aquades used as a negative control for testing on larvae and concentrations of 12.5%, 25%, and 50% lime leaf extract. Testing and observations have been conducted for 24 hours. The findings showed that different concentrations of lime leaf ethanol extract (*C. aurantifolia*), which is 100% poisonous to *Ae. aegypti* larvae, can impede its growth. To conclude, biolarvasida can be created by using the lime leaf extract. No

morphological modifications were made to the *Ae. aegypti* larvae used in this investigation.

Keywords: *Aedes aegypti*, biolarvacida, *Citrus aurantifolia*, efficacy test, extract

INTRODUCTION

In Indonesia, the *Aedes aegypti* mosquito is a disease-carrying insect. Climate parameters, such as temperature, humidity, rainfall, and wind speed, are one of the factors that help *Ae. aegypti* live in Indonesia [1]. Indonesia has a climate that supports the breeding of *Ae. aegypti* and the spreading of the DHF virus (Dengue Hemorrhagic Fever) [2].

Controlling mosquito vectors is one method for prevent dengue, among many others. Larval eradication with pesticides is an effective method of vector control. Long-term usage of insecticides will create a population of resistant vectors; therefore, biolarvacide is required to avoid the establishment of resistant vector populations [3].

Plants play a crucial role in sustaining the existence of mosquitoes, but they also have the potential to be employed as insect repellents. [4] Plants provide *Ae. aegypti* and *Ae. albopictus* with a food source and a place to relax. Plants may be able to

regulate *Ae. aegypti* by employing secondary metabolites that can function as both repellents and attractants [5].

This lime is a common yard plant. Lime is an integral element of local knowledge in Indonesia, where it is employed as a cooking spice and in ancient ceremonies due to its distinct scent [6]. Lime has the potential to be employed as a bactericide due to its fragrance and secondary metabolite content. Lime leaves include the secondary metabolites limonoids, saponins, and essential oils, which can act as biolarvicides [7]. Secondary metabolites are derived from terpenoid molecules, and they function in tandem to combat insect pests. Limonoids are poisonous and restrict insect development. Insects' digestive tracts convert saponins into toxins. Saponins react with water in the insect's body to produce a toxin, resulting in insect death [8]. Limonoids present in lime leaves are mimics of insect hormones

that regulate the formation of the cuticle on insect larvae [9].

The active ingredient in lime leaf extract can stop *Ae aegypti* larvae from growing. It can also be used as an insecticide and contact poison. This is because the more active molecules there are in lime leaf extract, the more

effect it has when it gets into the body, slowing the growth of *Ae. aegypti* and even killing it [10]. Based on what was said in the above reference, the secondary metabolites in lime could stop *Ae. aegypti* from growing.

METHOD

1. Making use of simplicity and extraction

Fresh lime leaves (*Citrus aurantifolia*) were chosen on the basis of their dark green color, washed, and then air-dried at room temperature. The leaves are subsequently macerated.

Lime leaf simplicia was steeped in ethanol containing 96% at a ratio of 1:2 for three cycles of 24 hours. After soaking, the evaporator is used to condense the extract to generate a concentrated extract.

2. Upkeep of the larvicide efficacy test and *Ae. aegypti*

At the Laboratory of Parasitology, Faculty of Medicine, Abulyatama University, *Ae. aegypti* eggs were hatched and raised as a research sample. The third-instar larvae

are those which are employed for assessing efficacy.

There are three concentrations of pure lime leaf extract available: 12.5%, 25%, and 50%. The solution was separated in each paper cup by as much as 50 ml for each repetition. Twenty-five instar III larvae were added to each copy of the lime leaf ethanol extract concentration in each replicate. The first hour, two hours, four hours, six hours, twelve hours, and twenty-four hours after exposure were observed periodically every ten minutes. Probit regression was used to assess the test findings [11].

RESULTS AND DISCUSSION

Based on the results of the investigation of the biolarvicidal efficacy test using a lime-leaf ethanol extract with three concentrations during 24 hours of exposure, it was determined that each concentration resulted in repeated larval mortality. In each repetition, 100% of the larvae had

died after 4 hours at a concentration of 12.5%. At 2 hours, each repetition of the 25% concentration resulted in the death of all larvae. Each replicate revealed concentrations of 50% larval mortality and 100% mortality at 25 minutes (Fig. 1).

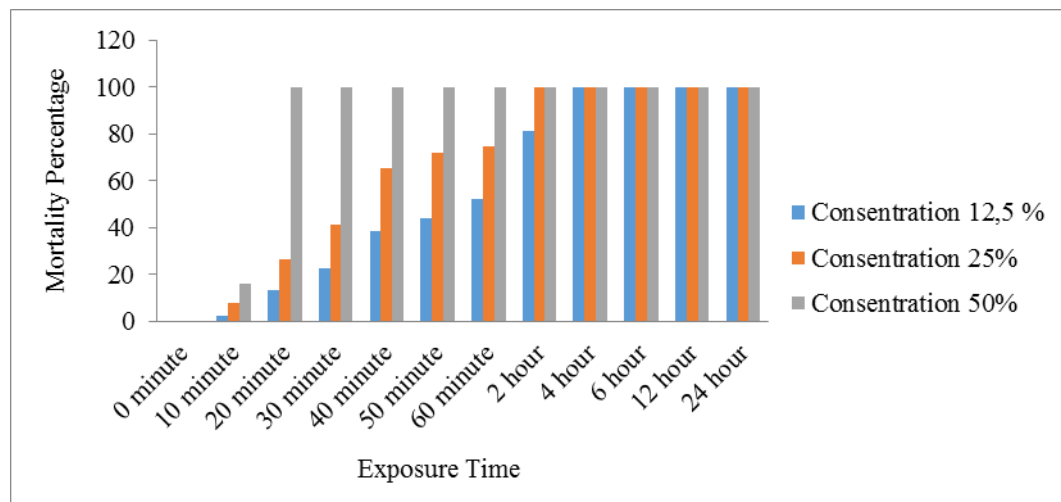


Figure 1. The Average Mortality of *Aedes aegypti* Larvae when being Exposed to an Ethanol Extract of *Citrus aurantifolia*.

The use of distilled water as a negative control did not result in any mortality (0%) within 24 hours. The concentration of the insecticidal chemical compound within the target species' body influences the toxicity of insecticides. The treatment demonstrated that the time and proportion of *Ae. aegypti* increased

with the concentration of lime leaf ethanol extract administered.

In a study conducted on *Anopheles* spp. larvae, 10% *C. aurantifolia* leaf extract led to the death of 83% of mosquito larvae six hours after exposure [12]. *Citrus hystrix* leaf extract is effective as a biolarvicide and can be used as an alternative biolarvicide, according to a study

conducted on *Ae. aegypti* [13]. At a concentration of 37.466%, the *C. aurantifolia* leaf juice was used to test the efficacy of biolarvicides against *Ae. aegypti* having a 90-percent mortality rate [14].

When *Ae. aegypti* larvae consume food, they are exposed to secondary metabolites in the form of limonoids and saponins contained in the ethanol extract of lime leaves. This exposure occurs through the larva's body wall or mouth. Secondary metabolites that enter the body of *Ae. aegypti* larvae at certain concentrations can act as contact poisons, stomach poisons, and respiratory poisons, causing the damage to the larvae's entire body system [10].

Saponin compounds function by irritating the larvae's digestive tract. Saponins have a bittering effect on the larvae, thereby reducing their appetite.

They can also damage the waxy layer that protects the insect's outer body, resulting in a significant loss of body fluids and death [15], [16]. Their compounds can be used as a mosquito larvacide against *Ae. aegypti*, which is more environmentally friendly than chemical abatement [15].

Limonoids that enter *Ae. aegypti*'s digestive system is absorbed by the digestive wall and then circulates throughout the body. *C. aurantifolia* leaves contain flavonoids that cause protein clumping. In the larval digestive tract, protein denaturation makes cell wall permeable, which disrupts nutrient transport. This can result in activity disruptions and death [17]. *C. aurantifolia* has a lethal dose range of 0.108% to 40.087% [18].

Table 1. LT50, 95, and Linear Regression Test Results for the Effectiveness of Lime Leaf Ethanol Extract Against *Ae. aegypti*.

Concentration (%)	LT50 (minutes)	LT95 (minutes)	Linear Regression
12,5	66.91	368.98	$y=0,16 x^{-2}$
25	66.87	361.73	$y=0,16 x^{-2}$
50	27.91	74.63	$y=0,16 x^{-2}$

The probit study of various amounts of ethanol extract from lime leaves yielded really positive findings. The quickest deadly dose of 50 happened in 27.91 seconds at a concentration of 50 percent. A 50% concentration of lime leaf ethanol produces the most fatal dose of 95 the quickest. Almost simultaneously, lethal dosages of 50 and 95 at concentrations of 12.5% and 25% were administered. At 66 minutes, the lethal dosage 50 (LT50) concentrations were 12.5% and 25%. At these concentrations and 25%, the 95th percentile lethal dosage (LD95) occurred at 368.98 and 361.73 minutes, respectively. There are variances in the mortality rates of

larvae at each concentration. At a concentration of 50%, the lethal dose value of 95 resulted in the quickest death rate compared to values of 12.5% and 25%.

Methanol extract of lime leaves is lethal to *Ae. aegypti* instar III and can induce mortality. The greater the concentration of the extract administered, the greater the mortality of the larvae. *Ae. aegypti* can be killed with as little as 2.197 mg of extract per 1,000 mililitre distilled water [17]. The application of *C. aurantifolia* extract as a biolarvicidal has potential [19].

CONCLUSION

A wide range of the ethanol extract's concentrations of lime leaves (*Citrus aurantifolia*) can inhibit *Ae.*

aegypti's growth and are toxic to the mosquito, according to the results of an efficacy test.

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