

Larvicidal Test of Butterfly Pea Flower Tea (*Clitoria Ternatea* L.) Brew on Instar III *Aedes Aegypti* Mosquito Larvae

Uji Larvasida Seduhan Teh Bunga Telang (*Clitoria Ternatea* L.) Terhadap Larva Nyamuk *Aedes Aegypti* Instar III

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Abstract

Aedes aegypti is a mosquito species capable of transmitting dengue fever to humans. Vector control aimed at disrupting the mosquito life cycle is an effective strategy for preventing dengue fever. Larvicides are a class of pesticides used to kill insects at the larval stage. Butterfly pea flowers (*Clitoria ternatea* L.) contain active compounds such as flavonoids, which have potential larvicidal properties. This study aimed to determine the larvicidal effect of butterfly pea flower infusion (*Clitoria ternatea* L.). Telang flower tea was prepared at concentrations of 10%, 5%, 2.5%, and 1.25% for the treatment groups, while abate powder was used as the positive control. The study employed a completely randomized design by placing *Aedes aegypti* larvae into plastic cups divided into six groups with three replications, each containing 25 larvae. The treatment groups received telang flower tea at concentrations of 10%, 5%, 2.5%, and 1.25%. Larval mortality was observed over a 24-hour period. The results demonstrated larvicidal activity, as indicated by larval mortality. The effective concentration causing 50% larval mortality was 10% after 6 hours of observation, and 5% and 10% after 12 hours. In conclusion, telang flower tea (*Clitoria ternatea* L.) has been proven to exhibit larvicidal activity against instar III *Aedes aegypti* larvae at concentrations of 10% and 5%. The phytochemical compounds present in butterfly pea flowers—namely saponins, flavonoids, and tannins exert a rapid effect on larval mortality by damaging cell membranes or disrupting larval metabolic processes.

Keywords: Butterfly flower, *Clitoria ternatea* L, Larvasida, *Aedes aegypti*.

Abstrak

Aedes aegypti adalah salah satu jenis nyamuk yang dapat menularkan penyakit demam berdarah kepada manusia. Pengendalian vektor untuk memutus siklus hidup nyamuk dapat dilakukan sebagai pencegahan penyakit demam berdarah. Larvasida merupakan golongan dari pestisida yang dapat membunuh serangga pada stadium larva. Bunga telang (*Clitoria ternatea* L.) mengandung bahan aktif Flavonoid, yang mempunyai potensi sebagai larvasida. Penelitian ini bertujuan untuk mengetahui pengaruh seduhan bunga telang (*Clitoria ternatea* L.) terhadap aktivitas larvasida. Seduhan Bunga Telang dengan konsentrasi 10%, 5%, 2,5%, 1,25% untuk kelompok perlakuan, serta bubuk abate untuk kelompok kontrol positif. Penelitian ini menggunakan rancangan acak lengkap dengan memasukan Larva *Aedes aegypti* ke dalam gelas plastik yang dibagi dalam 6 kelompok dan 3 ulangan yang berisi 25 ekor. Pada kelompok perlakuan diberikan seduhan bunga telang dengan konsentrasi 10%, 5%, 2,5%, dan 1,25% Kemudian dilakukan pengamatan larva yang mati selama 24 jam. Hasil penelitian menunjukkan adanya efek larvasida yang diketahui dari kematian larva dimana konsentrasi yang efektif untuk menyebabkan kematian larva mencapai 50% adalah pada konsentrasi 10% setelah 6 jam pengamatan dan pada konsentrasi 5% dan 10% setelah 12 jam. Kesimpulan seduhan teh bunga telang (*Clitoria ternatea* L) terbukti memiliki aktivitas larvasida *Aedes aegypti* instar III dengan konsentrasi 10% dan 5%. Kandungan fitokimia bunga telang memiliki respon cepat terhadap mortalitas larva *Aedes Aegypti* yaitu saponin, flavonoid dan tanin yang mampu berperan sebagai larvasida melalui mekanisme merusak membran sel atau mengganggu proses metabolisme larva.

Kata Kunci: Bunga Telang, *Clitoria ternatea* L, Larvasida, *Aedes aegypti*.



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Introduction

Dengue fever is a viral infection transmitted by the bite of the *Aedes aegypti* mosquito and has become a major global health problem, with a steadily increasing incidence over the past five decades [1]. Bhatt *et al.* (2013) estimated that 390 million dengue infections occur annually, with 96 million exhibiting clinical manifestations of varying degrees of disease severity [2]. In 2024, the Ministry of Health data recorded that on March 26, 2024, dengue fever cases in Indonesia reached 53,131 cases with 404 deaths [3]. One of the mass control programs for *A. aegypti* larvae is to use insecticides in the form of temephos/abate powder as a larvicide [4][5]. The use of insecticides can break the chain of disease transmission by killing the vector that carries the disease. However, its use must be done carefully because it can cause the death of non-target organisms, lead to environmental problems/pollution, and induce resistance to the vector. Resistance of *Aedes aegypti* larvae to temephos is common in several areas of Indonesia [6] [7]. Prevention strategies through effective, safe, and sustainable vector control are expected to be more effective than the use of chemical fogging, which is often not in accordance with procedures, thus triggering the potential for vector resistance to insecticides [8]. The condition of resistance to chemical insecticides encourages the need for alternative insecticides to eradicate *Aedes aegypti* larvae that contain phytochemicals and are safe for the environment [9] [10].

In Indonesia, the butterfly pea flower (*Clitoria ternatea* L.) is gaining popularity due to its believed health benefits. Many people have begun growing butterfly pea flowers for their families or selling them fresh or dried. Furthermore, butterfly pea flowers are more commonly found in lowlands than in highlands, indicating that they are easily adaptable to a variety of environments [11]. All parts of the butterfly pea flower are well-known. From the roots to the flowers, it is believed to have medicinal effects and strengthen organ function [12]. Phytochemical screening reveals that the plant contains tannins, carbohydrates, saponins, alkaloids, triterpenoids, glycosides, flavonoids, and steroids [9]. *Clitoria ternatea* is helpful as an antimicrobial, antiparasitic, anti-inflammatory, antioxidant, antidepressant, antidiabetic, and potentially plays a role in the nervous system [13] [14].

Butterfly pea flowers also contain active ingredients, namely flavonoids, which have potential as larvicides. Previous studies have shown that butterfly pea flower extract (*C. ternatea* L.) at concentrations of 4000 ppm, 6000 ppm, and 8000 ppm exhibits larvicidal activity against the death of *Culex quinquefasciatus* Say mosquitoes, which is more effective than abate or temephos [15]. A study conducted by Martini (2020) revealed that the interaction between excessively high temperatures and prolonged drying times affects the flavonoid content of butterfly pea flower herbal tea, resulting in lower total flavonoid levels. This statement is supported by research Kusuma *et al.* [16], which states that the higher the IC50 value, the lower the antioxidant activity. Butterfly pea flowers that are heated in the form of a brew or heated are considered easier to apply directly by the community and do not reduce their content much [16]. Therefore, this study used butterfly pea flower infusion to examine the effect of butterfly pea flower infusion (*Clitoria ternatea* L.) on the larvicidal activity of *Aedes aegypti* instar III.

Experimental Section

Material and apparatus

The instrument used in this study were an analytical balance, blender, measuring cylinder, plastic container, beaker, glass stirring rod, pipette, evaporator, butterfly pea flowers, distilled water, 70% ethanol, and *Aedes aegypti* mosquito larvae.

Methods

First stages of making butterfly pea flower infusion is making butterfly pea flower tea. The butterfly pea flowers are dried at room temperature. After the butterfly pea flowers are dry, they are blended into a powder. Then, 1 gram of the herb is weighed, placed in a beaker, and 50 ml of distilled water is added. Then, steep for 2 minutes and stir. Brewing procedure: Brew butterfly pea flowers at 100°C. Larvae are added to the brew after the water has cooled to room temperature. Then, filter and discard the dregs. This research is a purely experimental research conducted at the Integrated Laboratory of the Faculty of Medicine, Islamic University of Indonesia.

The subjects of the third instar *Aedes aegypti* mosquito larvae were 450, with each group consisting of 25 larvae, with three repetitions. The control group in this study consisted of a negative control group, which received distilled water (K1), was boiled to 100°C without butterfly pea flowers, then allowed to cool to room temperature. and a positive control group, which received 1% Abate (K2). A total 100 ml of the brewing solution was used to soak 25 larvae in each group. The treatment group consisted of individuals given a 10% concentration of butterfly pea flower infusion (K3), 5% concentration (K4), 2.5% concentration (K5), and 1.25% concentration (K6). In each group, 25 mosquito larvae were used, and the mortality of the larvae was observed after administration of the butterfly pea flower infusion at 6, 12, and 24 hours (Table 1). The criteria for dead larvae are that they do not move, even when touched with a pipette. The results obtained were analyzed using *Analysis of Variance* (ANOVA) and continued with *post hoc test* difference test to determine the differences in the treatments given. Meanwhile, to determine the effective concentration (LC₅₀), probit analysis was used.

Table 1. Treatment of brew (Butterfly pea) on *Aedes aegypti* sp. mosquito larvae.

	Concentration	Number of samples	Repetition
Negative control	Concentration 0% (K1)	25 tails	3 times
Positive control	Abate (K2)	25 tails	3 times
Brew leaf Butterfly pea	Concentration 10% (K3)	25 tails	3 times
	Concentration 5% (K4)	25 tails	3 times
	Concentration 2.5% (K5)	25 tails	3 times
	Concentration 1.25% (K6)	25 tails	3 times
	Total	150	450 tails

Results and Discussion

Based on the research carried out, the results are presented as the percentage of larval deaths in various groups, as shown in Tables 2, 3, and 4.

Table 2. Results of observations of larval mortality after 6 hours of administration of butterfly pea flower infusion

Treatment	Number of <i>A.aegypti</i> larvae	Number of deaths Replication			Average mortality of larvae	Presentation of larval death
		1	2	3		
Negative control (K1)	25 tails	0	0	0	0	0%
Positive control (K2)	25 tails	25	25	25	25	100%
Concentration 10% (K3)	25 tails	10	15	13	12.67	50.68%
Concentration 5% (K4)	25 tails	5	6	5	5.33	21.32%
Concentration 2.5%(K5)	25 tails	0	0	0	0	0%
Concentration 1.25% (K6)	25 tails	0	0	0	0	0%

Table 3. Results of observations of larval mortality after 12 hours of administration of butterfly pea

Treatment	Number of <i>A.aegypti</i> larvae	Number of deaths Replication			Average mortality of larvae	Presentation of larval death
		1	2	3		
Negative control (K1)	25 tails	0	0	0	0	0%
Positive control (K2)	25 tails	25	25	25	25	100%
Concentration 10% (K3)	25 tails	25	25	15	21.67	86.68%

Concentration 5% (K4)	25 tails	15	15	15	15	60%
Concentration 2.5%(K5)	25 tails	0	2	2	1.33	5.32 %
Concentration 1.25% (K6)	25 tails	0	1	1	0.67	2.68%

Table 4. Results of observations of larval mortality after 24 hours of administration of *Butterfly pea* flower

Treatment	Number of <i>A. aegypti</i> larvae	Number of deaths Replication			Average mortality of larvae	Presentation of larval death
		1	2	3		
Negative control (K1)	25 tails	0	0	0	0	0%
Positive control (K2)	25 tails	25	25	25	25	100%
Concentration 10% (K3)	25 tails	25	25	25	25	100%
Concentration 5% (K4)	25 tails	25	23	25	24.33	97.32%
Concentration 2.5%(K5)	25 tails	5	8	7	6.67	26.68%
Concentration 1.25% (K6)	25 tails	0	2	2	1.33	5.32%

Table 5. Average mortality of *Aedes Aegypti* larvae at various concentrations of brew *Butterfly pea* flower every 3 hours for 24 hours of treatment.

Time observation (hour)	K1 Distilled water	K2 Abate	K3 Butterfly pea 10%	K4 Butterfly pea 5%	K5 Butterfly pea 2,5%	K6 Butterfly pea 1.25%
6	0.0±0.00*	25±0.00	12.6±2.51*	5.3±0.57*	0.0±0.00*	0.0±0.00*
12	0.0±0.00*	25±0.00	21.6±5.77	15.0±0.00*	1.3±1.15*	0.6±0.57*
24	0.0±0.00*	25±0.00	25±0.00	24.3±1.15	6.6±1.52*	1.3±1.15*
Mortality(%)	0%	100%	79.12%	59.54%	10.66%	2,66%

(*) significantly different compared to the positive control group

Based on observations of larvae after 6, 12, and 24 hours, the results were obtained in the form of the number and percentage of larval deaths in various treatment groups and repetitions, as seen in Tables 2, 3, 4 and 5. This study used brewed butterfly pea flowers as an alternative insecticide. Butterfly pea flowers contain compounds that have the potential to act as larvicides. Based on the results of observations of larval deaths after 6 hours, as shown in Table 2, it was found that the highest mortality occurred in *A. Aedes aegypti* larvae after 6 hours of being given butterfly pea flower brew were at a concentration of 10% with an average percentage of larval deaths of 50.68% (12.67 larvae).

In comparison, the lowest mortality was observed at concentrations of 2.5% and 1.25%, with an average percentage of larval deaths of 0%, indicating that no larvae died (0 larvae). In terms of the quantity of larval deaths, an increase in larval mortality was found as the duration of the brewing period increased. Table 3 shows that the highest mortality rate of *A. aegypti* larvae after 12 hours of administration of the butterfly pea flower infusion was at a concentration of 10%, with an average mortality rate of 86.68% (21.67 larvae). The lowest mortality rate was at a concentration of 1.25%, with an average mortality rate of 2.68% (0.67 larvae).

In Table 4, it was found that the highest mortality of *A. aegypti* larvae after 24 hours of administration of butterfly pea flower infusion was at concentrations of 10% and 5% with an average percentage of larval mortality of 100% (25 individuals). In comparison, the lowest mortality was at a concentration of 1.25%. The results showed that LC50 was obtained at a concentration of 10% starting at 6 hours and a concentration of 5% starting at 12 hours. In comparison, the achievement of LC90 at a concentration of 10% and 5% was obtained after 24 hours. The conclusion is that butterfly pea flower (*Clitoria ternatea* L.) tea infusion has been proven to have larvicidal activity against *Aedes aegypti* instar III at concentrations of 10% and 5%. Butterfly pea flowers, containing compounds such as flavonoids, alkaloids, saponins, and tannins, exhibit insecticidal activity that can be utilized to control *Aedes* mosquitoes through the mechanism of inhibiting the development of larvae into pupae and adults, thereby preventing larval development and resulting in larval death [17]. Flavonoids enter the cuticle that coats the larva's body, thus damaging the larva's respiratory system. This is in line with Hayatie et al. (2015) who stated that flavonoids enter the insect's body through its respiratory system, then cause damage to the respiratory system, resulting in mortality [18]. The saponin content is suspected to be toxic in water and interfere with the larva's digestive process. Saponin also has detergent-like properties so it is considered capable of increasing the penetration of toxins because it can

dissolve lipophilic materials in water. Saponin can also irritate the digestive tract mucosa and contains steroid hormones that reduce the surface tension of the mucous membrane of the larva's digestive tract, causing damage to the digestive tract wall. Saponin is a bioactive compound as a toxin, included in the group of contact poisons because it can enter through the larva's body wall and stomach poisons through the mouth because larvae usually obtain food from their habitat. In addition, saponin also has a bitter taste, which reduces the larvae's appetite, which then causes the larvae to die of starvation [19].

Conclusions

This study concludes that the infusion of butterfly pea flower tea (*Clitoria ternatea L.*) exhibits the same effective larvicidal activity on *Aedes aegypti* instar III as abate powder, as evidenced by the 100% mortality of *A. aegypti* mosquito larvae after 24 hours at concentrations of 10% and 5%. Butterfly pea flower tea has been shown to have larvicidal activity, making it a potential natural alternative that is easy to make. The phytochemical content of butterfly pea flowers has a rapid response to *Aedes aegypti* larval mortality, namely saponins, flavonoids, and tannins, which can act as larvicides through mechanisms that damage cell membranes or disrupt larval metabolism. Further research is needed to test the stability of the tea and its toxicity to non-target organisms.

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