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Pistia stratiotes as Effective Larvicide against *Aedes aegypti*

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ABSTRACT

World Health Organization (WHO) noted that Indonesia was the country with the highest cases of Dengue Hemorrhagic Fever in Southeast Asia. Control efforts include vector observation and monitoring, one of which is larvicides. This study aims to determine the effectiveness of Apu-apu (*Pistia stratiotes*) leaf extract to inhibit the growth of the third instar larvae of the *Aedes aegypti*. The design of this study was Completely Randomized Design (CRD). The treatment in this study was repeated 3 times using 4 treatments (negative control treatment, given extract of 10 ml, 30 ml, 50 ml). The results showed that Apu-apu leaf extract had different effects on each test concentration (10ml, 30ml and 50ml). The higher the concentration, the greater mortality of *Aedes aegypti* larvae. Anova test showed that Apu-apu leaf extract (*Pistia stratiotes*) had an effect with the $F=94.667$, $p=0.000$ on larvae mortality. The ability of Apu-apu leaf extract to kill larvae in LC50 value was 4.0% per minute, analyzed using probit regression. *P. stratiotes* could be an effective larvicide against *A. aegypti* third instar larvae ($p=0.000$).

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5 Introduction

Dengue Hemorrhagic Fever is commonly found in tropical and sub-tropical areas. Data from around the world shows that Asia ranks first in the number of dengue hemorrhagic fever sufferers every year. Meanwhile, from 1968 to 2009, the World Health Organization (WHO) recorded Indonesia as the country with the highest cases of Dengue Hemorrhagic Fever in Southeast Asia (WHO, 2006a; WHO, 2006b).

Indonesia is a country with a wet tropical climate with high rainfall during the rainy season. The rainy season is a season

that is liked by mosquitoes to breed. The disease that often becomes a problem every year in the rainy season is Dengue Hemorrhagic Fever (DHF). Data from the Surabaya City Health Office recorded the number of DHF sufferers in 2014 (816 cases), 2015 (640 cases), 2016 (938 cases), and September 2017 (302 cases). The vector of transmission of this disease is the mosquito. The dengue virus is infected by *Aedes aegypti* mosquito's bite (Raekiansyah & Sudiro, 2002; Shu et al, 2009; Pongsiri et al, 2012).

When the virus enters the body of *Aedes aegypti*, the virus will infect the mid

stomach of the mosquito and spread to the salivary glands within 8-12 days. During this period Aedes aegypti can transmit the virus by biting humans. Immature Aedes aegypti can generally be found indoors and in places filled with water, such as water storage areas and others. Adult Aedes aegypti can be found around housing and fly within a radius of 400 meters. Aedes aegypti is the first agent of the tropical disease dengue fever when a person digests the Aedes aegypti mosquito infected with the dengue virus, the symptoms of dengue fever will appear within 4-7 days afterwards. Dengue Hemorrhagic Fever Virus generally causes symptoms in the form of a rash, high fever, and joint and muscle pain (Gunawan et al, 2016; Thu et al, 2004; Azami et al, 2011).

However, other symptoms of Dengue Hemorrhagic Fever include pain behind the eyes, swollen lymph nodes, bone pain, nausea, vomiting and headaches. Severe dengue fever can cause damage and rupture of blood vessels and can be life-threatening. To a less severe degree, the handling of dengue fever is only in the form of fluids to maintain balance in the body and anti-fever drugs. The number of patients with Dengue Hemorrhagic Fever (DHF) per 100,000 population in Central Java in the last five years was 59.2 in 2008; 57.9 in 2009; 56.8 in 2010; 15.3 in 2011; and 19.29 in 2012 (Fidayanto et al, 2013; Oliveira et al, 2010).

Its spread not only in urban areas, but also spread to rural areas. Since 2007, 6 regencies/cities out of 35 regencies/cities in Central Java have been endemic areas for DHF. In 2008-2009, it has spread to all districts/cities with a fairly high number of cases. In 2010-2011, all regions experienced a decline in dengue cases. Global environmental changes or Global Environmental Change (GEC), especially Global Warming, more or less played a role in the incidence of DHF. Every season change, especially from the dry season to the rainy season, various health problems hit, including the most common is an increase in the incidence of dengue fever. Other risk factors for dengue infection include the level

of host immunity, population density, vector-host interactions and virulence (Lam, 1994; Marjorie, 1999). The clinical manifestations of DENV infection range from asymptomatic infection or a mild flu-like syndrome, also known as dengue fever (DF), to the more severe and life-threatening forms, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) (Ito et al, 2015).

Drugs and vaccines to control Dengue Hemorrhagic Fever are still in the research stage. Prevention of Dengue Hemorrhagic Fever is prioritized by breaking the chain of transmission through vector control. Control efforts include vector observation and monitoring activities, namely mosquito surveys, larval surveys, egg capture surveys, insecticide spraying, 3M, 3M plus and larvicidation (Kiat et al, 2006; Sánchez et al, 2000).

The only method of eradicating dengue hemorrhagic fever is vector control. Vector control is done by using chemical insecticides that can cause resistance and environmental pollution if used continuously. Therefore, controlling using natural insecticides derived from plant extracts is one of the solutions that researchers have developed so far. One of the plants that have the potential as natural insecticides is the apu-apu (*Pistia stratiotes*) (Che et al, 2009).

Pistia stratiotes referred to as tropical duck grass or apu-apu leaf is one of the most dominant aquatic weeds in freshwater, and polluted water. This plant is raised in ponds as a shelter for other animals. The use of the apu-apu plant, especially on the leaves, is done to take advantage of the apu-apu leaves which are generally only thrown away in vain. The leaves of the apu-apu plant are generally only disposed of as waste, even though this apu-apu has biological activities that can provide various benefits to the community (Harapan et al, 2019; Sucipto et al, 2018). Present study evaluated the phytoremediation potential *P. stratiotes* and organic pesticides.

Materials and Methods

This research was carried out on 7-14 September 2021. The extraction process was conducted at the Biochemistry Laboratory of UIN Raden Fatah Palembang. The treatment with extract testing was conducted at the basic Biology Laboratory of UIN Raden Fatah Palembang.

Mortality data of *Aedes aegypti* larvae were obtained by giving the Apu-apu leaf extract (*Pistia stratiotes*) 3 treatments with each different concentration. *Aedes aegypti* larvae were observed within 24 hours. Every 1 hour spraying based on the treatment dosage with apu-apu leaf extract is carried out. A 50ml spray bottle was used as a tool to administer the Apu-apu (*Pistia stratiotes*) leaf extract to *Aedes aegypti* larvae. After 24 hours it can be observed how many larvae experienced mortality.

The data obtained were analyzed using Analysis of Variance (ANOVA) with the SPSS software application. The results of the analysis showed a significant difference, then the data were further analyzed using the Post Hoc LSD test. The further test aims to determine the location of the differences between the pairs of groups. The degree of significance used is = 0.05 (a significant difference if p 0.05).

Results and Discussion

The results of the treatment with the Apu-apu leaf extract (*Pistia stratiotes*) with each dose of 10 ml, 30 ml, and 50 ml, once every 1 hour for 24 hours can affect the mortality of *Aedes aegypti* larvae. Measuring average larval mortality before and after treatment are presented in Table 1 and Figure 1.

Table 1. The Average Mortality of *Aedes aegypti* Larvae Every 1 Hour for 24 Hours

Concentration	P1	P2	P3	Average
Control (-)	0	0	0	0
P1 (10ml)	4	3	4	3,7
P2 (30ml)	5	4	5	4,7
P3 (50ml)	5	5	5	5
Average				4,4

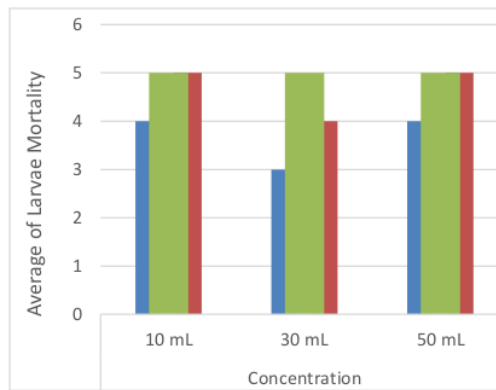


Figure 1. Percentage mortality of *Aedes aegypti* larvae due to concentration of *Pistia stratiotes* extracts

This study used 4 treatments, consisting of 1 negative control treatment and 3 treatments with concentrations of 10ml, 30ml, 50ml. Each treatment was tested by spraying every 1 hour for a period of 24 hours. Based on Table 1, it can be seen that the negative control treatment remained alive for a period of 24 hours. At a concentration of 10 ml repetition I caused the death of 4 larvae from a total of 5 larvae. The second repetition was 3 animals and the third repetition was 4 animals. So the number of larval deaths was 11 from the total test, which was 15 for 24 hours. Observations at a concentration of 30 ml repetition I caused the death of 5 larvae. In the second repetition 4 dead larvae and in the third repetition 5 dead larvae. So that the total number of larvae that died during the 24-hour observation was 14 larvae from a total of 15 larvae. At the observation concentration of 50 ml repetition I as many as 5 larvae died. In the second and third repetitions, 5 larvae died. So the number of larvae that died was 15 of the total test larvae of 15. According to Chang et al, 2011; Churrotin et al, 2006; King et al, 2008, the higher the concentration level given, the faster the death rate. Giving the extract concentration to a higher level will have a high effect as well. The working power of a

compound is largely determined by the amount of concentration.

Each concentration has a significant effect, as evidenced by the results of the

Anova analysis and tested further using Post Hoc LSD (Table 2 and Table 3).

Table 2. The Anova Analysis

	Sum of Squares	Df	Mean Square	f	Sig
Between Groups	47,333	3	15,778	94,67	0,000
Within Groups	1,333	8	0,167		
Total	48,667	11			

Table 3. LSD Post Hoc Test Results

Concentration (I)	Concentration (J)	Mean Difference	SD	Sig.	95% Confidence interval
					Lower Bound
P0 Negative control	10.00	-3.366	0.333	0.000	-4.4353
	30.00	-4.666	0.333	0.000	-5.4353
	50.00	-5.000	0.333	0.000	-5.7687
P1 Concentration 10 ml	.00	3.666	0.333	0.000	2.8980
	30.00	-1.000	0.333	0.017	-1.7687
	50.00	-1.333	0.333	0.004	-2.1020
P2 Concentration 30 ml	.00	4.666	0.333	0.000	3.8980
	10.00	1.000	0.333	0.017	0.2313
	50.00	-0.333	0.333	0.347	-1.1020
P3 Concentration 50 ml	.00	5.000	0.333	0.000	4.2313
	10.00	1.333	0.333	0.004	0.5647
	30.00	0.333	0.333	0.347	-0.4353

Based on the results of the Analysis of Variance (ANOVA) (Table 2.) shows that the value is significant ($F=94.667$, $p=0.000$). So it was concluded that there was a difference in the effect of apu-apu leaf extract.

In Table 3. the concentration of 30ml (P2) was not significantly different from the concentration of 50ml (P3) with a significant value of 0.347. But it was significantly different with the positive control concentration (P0) and 10ml concentration (P1). Meanwhile, at other concentrations, it was significantly different. This is because the probability value exceeds the standard value of the provision, which is <0.05 . So it can be said that all treatments greatly affect the positive control (P0) where the significance value is 0.00.

This apu-apu leaf extract gave different effects at each test concentration. The ability of apu-apu leaf extract to kill

Aedes aegypti larvae was also analyzed using probit regression analysis so that the LC50 value was known. Probit analysis was carried out to find out at what concentration animals experienced the most mortality. LD50 is defined as a statistical sign on the administration of a substance as a single dose that can cause the death of 50% of test animals (Paloucek et al, 2007). The results of the probit analysis are presented in Table 4.

Table 4. The Results of the Probit Analysis

Lethal concentration (LC)	Concentration %
50	4,064%

In Table 4. the results of the probit analysis showed that the LC50 value was obtained at a concentration of 4.064%. Signs of larvae experiencing mortality are marked by no movement, changes in body color to

transparent. The lower the LC50 value for a substance, it means that the substance has a higher activity in killing experimental animals. Andreas et al (2011), states that lower concentrations are needed to kill experimental animals in the same period of time.

Apu-apu leaf extract (*Pistia stratiotes*) was effective at LC50. In other words, *Pis. stratiotes* extract can be used as an insecticide and has an effect on mortality in *Aedes aegypti* larvae with the highest concentration level of 50 mL with a mortality of 15. Deus et al (2017), which states that the compounds contained in higher concentrations will have a higher effect on pest mortality as well. In addition, it is also due to the content of compounds in the apu-apu leaves (*Pistia stratiotes*) which are thought to cause mortality in *Aedes aegypti* larvae, namely flavonoids.

Flavonoids are chemical compounds that have insecticidal properties. Flavonoids attack the nerves in several vital organs of insects, causing a weakening of the nerves, such as breathing and causing death. According to Zandi et al (2011), flavonoids can work as strong respiratory inhibitors and inhibit oxidation reactions. This will cause an increase in CO₂ that exceeds O₂, so that the test larvae will move actively to look for fresh air. Ahdiyah & Purwani (2015), stated that flavonoids interfere with energy metabolism in the mitochondria by inhibiting the electron transport system. Flavonoids have a way of working, namely by entering the larva's body through the respiratory system which will then cause withering of the nerves and damage to the respiratory system and cause the larvae to be unable to breathe and eventually die.

In addition, there are also saponins which act as stomach poisons (Cania & Setyaningrum, 2013). Damage or defects in advanced stages of *Aedes aegypti* larvae are thought to occur due to toxic compounds that damage nervous tissue, such as alkaloids that can inhibit the process of larvae becoming pupae. Saponins found in leaves if consumed by insects can reduce the activity of

digestive enzymes and food absorption (Applebaum et al, 1969). Saponins can also reduce the surface tension of the larvae skin membrane and are able to bind free sterols in the digestion of food (Panche et al, 2016; Shen et al, 2009). Sterols are precursors of the hormone ecdysone, so that the decreasing supply of sterols will interfere with the molting process in insects.

Larvae can be said to be dead, it can be seen from their morphological conditions, namely if the larvae do not move anymore, the larvae settle to the bottom of the water, the larvae body is soft and the color changes from darker to pale and slightly transparent. If physiologically the death of the larvae is caused by some of the content contained in the mango peel extract. Flavonoids are chemical compounds with insecticidal properties that attack parts of the nerves in several vital organs resulting in weakening of the nerves, such as breathing to death. According to Cania & Setyaningrum (2013), saponins can act as stomach poisons and inhibit the action of the cholinesterase enzyme in larvae. Hayden et al (2015), stated that these compounds enter the larval body through the mouth as stomach poisons that can kill.

In this study, it was observed that extract of *P. stratiotes* rendered the *A. aegypti* larvae inactive and motionless. Furthermore, disturbance in the normal behaviour of the larvae was observed in treatments of *A. aegypti* suggesting that the plant possesses larvicidal properties that may affect the either behavioural or physiology of the larvae. This possibly may be interterm to the study of (Ito et al, 2015) which reported a decrease in the feeding behavior in *Anopheles* and *Culex* after treatment with neem extract.

Conclusion

Apu-apu (*Pistia stratiotes*) leaf extract as significantly effect on mortality of *Aedes aegypti* larvae. The ability of Apu-apu leaf extract to kill larvae in LC₅₀ value was 4.064% per minute.

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