Larvicidal activity of *Syzygium polyanthum* W. leaf extract against *Aedes aegypti* L larvae

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ABSTRACT

Introduction: *Aedes aegypty* is one of mosquito species, especially *Aedes* genus which can cause dengue fever to humans. This species acts as dengue virus vector through the bite of mosquitoes which enters the human bloodstream. Salam leaf extract is well known for its various functions, one of them is to fight against *Aedes aegypti* L. larvae.

Purpose: This research aims to know the larvicidal activity of *Syzigium polyanthum* W. leaf extract against *Aedes aegepty* L larvae.

Materials and methods: This study is an experimental research with *Posttest Only Control Group Design* method using ANOVA test. The samples were *Aedes aegypti* L. larvae instar III and *Syzigium polyanthum* W. extract. Maceration method was used in the extraction process. The dosages of the extract tested were 0% (control), 0.25%, 0.5%, 0.75%, and 1%.

Results: The results showed there were no larval mortality in 0% concentration (control). The percentage of larval mortality was on the average of 18.68% in 0.25%, 32% in 0.5%, 54.68% in 0.75%, 78% in 1% concentration. The *Analysis of Variance* obtained from F count = 1414.86 which was bigger than F table = 2.75, so H_0 was rejected. The analysis of *Reed and Muench* obtained was LC_{50} = 6576.68 ppm or around 0.66 %.

Conclusion: Based on that result, it can be concluded that the extract of salam leaf is potential as a larvacide on *Aedes aegypti* L. larvae.

Key words: Salam leaf extract (*Syzygium polyanthum* W.), larvicide, *Aedes aegypti* L.

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INTRODUCTION

Based on the survey conducted in Samarinda these recent years, there were 1028 cases in January to July 2012, 1020 cases in 2013, and 1027 cases in 2014. The cycle of DHF in Samarinda is also related to rainwater discharge level and the number of rainy days in a month. The higher the discharge of rainwater and the fewer number of rainy days per month will increase the risk of the DHF spread 3x higher. Based on the analysis of UPT Surdatinkes (Data Surveillance and Health Information Technical and Operational Unit), the beginning of August 2014 is the beginning of the next annual cycle. Therefore, precautionary measure is needed so that the number of patients will not increase compared to the previous years [1].

Aedes aegypti, is a mosquito that can spread the dengue fever, chikungunya, yellow fever virus, and other diseases. The mosquito can be recognized by white markings on its legs and a marking in the form of a lyre on the thorax. The mosquito is originated in Africa, [2] but is now found in tropical and subtropical regions throughout the world [3].

Aedes aegypti is a vector for transmitting several tropical fevers. Only the female bites for blood, which she needs to mature her eggs. To find a host, these mosquitoes are attracted to chemical compounds emitted by mammals. These compounds include ammonia, carbon dioxide, lactic acid, and octenol. Scientists at the Agricultural Research Service have studied the specific chemical structure of octenol to better understand why this chemical attracts the mosquito to its host [4]. They found the mosquito has a preference for "right-handed" (dextrorotatory) octenol molecules.

Aedes aegypti L. is a species that breeds in clean water shelters inside or outside the house. It is very threatening to human because *Aedes aegypti* acts as the vector of dengue disease. One of the vector control efforts is at the stage of larvae, either biologically using natural enemies or chemically using chemicals that affect larvicides [5]. The restraint that is popular today is chemical restraint using insecticide because it works faster and more effective than biological restraint, but it has negative impact such as environmental pollution and targeted insect resistance. Due to this condition, it is necessary to do an effort to get an alternative larvicides, that is using natural larvicide which is produced by toxic plant to the mosquito's larvae but has no impact to the environment and harmless to human.

Indonesia has very diverse flora, contains quite a lot of types of plants which are the source of larvicides material that can be used to control disease vectors. Today, researches on potential plant family as the natural larvicides have been reported. Wijaya [5] examined the devil's trumpet seed extract (*Datura metel*) containing alkaloids and *saponins* that work as larvicides against *Aedes aegypti L*. Moreover, the clove extract containing *eugenol, saponins, flavonoids* and *tannins* can kill the larvae of *Aedes aegypti* L [2].

Syzygium polyanthum W. is a tree species commonly known as salam in Indonesia. It belongs to the *Myrtaceae* family and is commercially used as a source of spices. Salam leaf itself contains saponins, triterpenes, alkaloids, and 0.05% essential oil consists of citral, tannins, flavonoids, sesquiterpenes, lactone, and phenol [6]. The largest content of salam leaf is eugenol and methyl chavicol [7].

Based on Gunawan and Sri [8], saponin is found in the plants that are usually consumed by insects and can decrease digestive enzyme activity as well as the food absorption, so saponin mechanism works as stomach poison. Saponins can lower the surface tension of the mucosal lining of the digestive tract so that the larval digestive tract wall becomes corrosive [1].

In line with the statement of Haridas et al. [9] Saponins have a cytotoxic and haemolytic [10], and are able of inhibiting the proteases activities [11]. It represents a constraint if we attempt to apply these substances as agricultural products such as larvacide, because in fact, the saponins are poisonous for the larvae of pests.

Based on the research by Cania [12], alkaloid is in the form of salt that can degrade the cell membrane to get into cells and damage them, and also disrupt the nervous system's work by blocking the action of larvae acetyl cholinesterase enzyme. Flavonoid works as a strong inhibitor of respiratory or as a respiratory poison. Flavonoids work by entering the body of larvae through the respiratory system which then will cause withering on the nerves and damage to the respiratory system, and the result is the larvae cannot breathe and eventually die. Based on the research result by Farida et al. [13], tannin generally inhibits the enzyme activity by forming a complex bound with the protein to the enzyme and the substrate which can cause indigestion and destroy cells in insects, so it works as a stomach poison in insects. Eugenol may affect the nervous system that are typical on the insects that are not on warm-blooded animals [2].

Based on those explanations above, a research using salam leaf extract was conducted which aimed to determine the effect of salam leaf extract (*Syzygium polyanthum* W.) as larvicide against *Aedes aegypti* L. considering salam leaf extract also contains saponins, flavonoids, alkaloids and tannins compounds.

MATERIALS AND METHODS

The climate in the world today, especially in Indonesia, has become erratic. Climate anomalies

and poor handling of the environment make the dengue fever expands in the society. Dengue fever is an acute febrile disease caused by the dengue virus, which enters the human bloodstream through the bite of mosquitoes of *Aedes genus*, for example *Aedes aegypti* or *Aedes albopictus.* Dengue hemorrhagic fever (DHF) is still one of the most important health problems in Indonesian society.This study was an experimental research with *the posttest only control group design,* which aimed to find out the effect of salam leaf extract (*Syzygium polyanthum* W.) as larvicide against *Aedes aegypti* L. larvae with treatments consisted of 6 concentrations (0%, 0,25%, 0,50%, 0,75% and 1%) with 5 replications.

This research was conducted in Pharmaceutical Research and Development Laboratory of Tropical Pharmacy of Mulawarman University Samarinda. The population used in this study was the third instar larvae of Aedes aegypti obtained from the Provincial Health Office of East Borneo. Based on the reference from WHO [14], the sample used were 25 larvae per replication.

The production of the solution extract to be tested were using salam leaf (*Syzygium polyanthum* W.) and also methanol solvent which were then extracted by maceration to get 100% of the extract. Then, the salam leaf extract was diluted using distilled water until the concentration reached 0%, 0,25%, 0,50%, 0,75% and 1% in 100 ml volume.

This maceration process was simple, only by leaving the pulverized salam leaf to soak in a suitable solvent in a closed container. Simple maceration was performed at room temperature by mixing the ground drug with the solvent (drug solvent ratio: 1:5 or 1:10) and left the mixture for several days with occasional shaking or stirring. The extract was then separated from the salam leaf particles by straining. The process was repeated for once or twice with fresh solvent. Finally, the last residue of the extract is pressed out of the salam leaf particles using a mechanical press or a centrifuge.

The length of the treatment of *Aedes aegypti* larvae to the solution tested was 24 hours. Then, the data was obtained by observing the total mortality of larvae after the treatment. After that, the data was analyzed using ANOVA (*Analysis of Variance*) with automatic statistical calculation on Microsoft Excel (Data Analysis Tool Pack activated) and LSD-test with manual calculation based on the formula below:

$$\text{LSD} = t_{v,\alpha}\sqrt{MS_{S(A)}\frac{2}{S}}$$

Before doing ANOVA test, the data were analyzed by Liliefors Normality Test to calculate the normality test. Liliefors test uses criteria: $L_0 < L_t$ at 5% significant level.

The last analysis was *Reed and Muench* analysis to get the toxicity value of a larvicide to *Aedes aegypti* larvae. Here is the formula:

$$h = \frac{50\%-a}{b-a}\ ; \quad i = \log\frac{k}{s}\ ; \quad g = h \times i\ ;$$

$$y = g + \log s\ ; \quad LC_{50} = \text{antilog } y$$

RESULTS

The data tabulated based on the result of the research to find out the effect of salam leaf (*Syzygium polyanthum* W.) extract as larvicide to *Aedes aegypti* L. larvae is as follows:

Table 1. Total amount of mortality of *Aedes aegypti*L. larvae in various concentration of salam leaf extract (*Syzygium polyanthum* W.) after 24 hours of treatment

Replication	Control	Treatment (ppm)			
		2500	5000	7500	10000
I	0	4	8	14	19
II	0	5	9	13	20
III	0	5	7	14	20
IV	0	5	8	13	20
V	0	4	8	14	19
VI	0	5	8	14	19
Total of Mortality	0	28	48	82	117
Mean	0	4,67	8,0	13,67	19,5
SD	0	0.516398	0.632456	0.516398	0.547723
Total of LarvaeTested	25	25	25	25	25
Percentage of Larval Mortality (%)	0	18.68	32	54.68	78

The data obtained from the research were then analyzed using Liliefors mormality test. The result of the analysis showed that Lt (0.161761) was bigger than Lv (0.133289). Because Lt > Lv, so the result of the normality test proved that the data were distributed normally and could be analyzed using Analysis of Variance (ANOVA).

The results of ANOVA test obtained that F_{count} 1414.86 > F_{table} 2.75 with the significant level (α) at 5%. It showed that hypothesis null (H_0) was rejected and it could be concluded that there was larvicidal activity of *Syzigium polyanthum* W. leaf extract against *Aedes aegepty* L. larvae.

Then, the LSD test was conducted to find out the significant differentiation level from each treatment with the result of the deviation value was 0.59. The result of LSD Test at 5% significance level showed that P_5 was significantly different to P_4 treatment. In 1% significance level, it can be seen that P_4 was most significantly different to P_3, P_2, P_1, and P_o treatments. P_3 was most significantly different to P_2, P_1, and P_o treatments. P_2 was most significantly different to P_1 and P_o treatment. P_1 was most significantly different to P_o treatment.

The next analysis is Reed and Muench analysis to get the toxicity value of a larvicide to *Aedes aegypti* larvae.

Table 2. Reed and Muench calculation analysis

Lethal Concentration (LC)	Distance Perserving (h)	Logarithm of The Concentration Increase (i)	g =h x i	Results of Lethal Concentration Equation (y)	Lethal Concentration value (ppm)	Lethal Concentration Value(%)
25	0.886	0.301	0.266	3.665	4623.81	0.46
50	0.675	0.176	0.118	3.818	6576.58	0.66
75	0.497	0.125	0.062	3.937	8649.68	0.87

The result of Reed and Muench analysis showed that the value of LC_{50} = 6576.68 ppm or around 0.66%. LC_{50} (Lethal Concentration 50) was the extract concentration that was able to kill 50% of the total larvae tested.

DISCUSSION

According to the result of this study, it was found that the salam leaf extract can be used as larvicide. It is possible because the chemical substances contained in salam leaf include saponin, alkaloids, flavonoids, tannin, and eugenol.

Based on observations made in this study, the typical salam leaf aroma wafted in the extract. Distinctive salam leaf aroma was determined by the presence of eugenol. The foam showed when the salam leaf extract was dissolved in the water, which was then shaken. The emergence of the foam showed saponins in the extract. The presence of saponins in the extract was also seen in larvae. There were also some larvae that looked dead, but if they were touched, weaker body movements were shown and the body size of dead larvae became longer than before treatment.

This is consistent with the results research of Aminah et al. [1] who stated that the saponins made the mortality of larvae size grew about 1-2 mm longer than before treatment.

The color of the solution in this study turned yellow, which caused the larvae became pale. It was possible because of the flavonoids that existed in the salam leaf extract. In mosquitos, several groups have reported on PDP's (Plant Derived Products) and inhibition: plant flavanoids are potent inhibitors of CYP6Z2; thymol and eugenol appear to inhibit both cytochrome P450 and Glutatione S-transferase activities; and essential oils of several native Columbian plants that possess repellent activity are the inhibitors of cytochrome P450 activity [15].

Thus, this study proved that salam leaf extract (*Syzygium polyanthum W.*) was a potential larvicide to *Aedes aegypti* L. Larvae since there was significant difference between the treatment groups and the control group. The effective concentration in this study was 1% salam leaf extract concentration with the average larval mortality percentage around 78%.

CONCLUSIONS

Based on the research and data analysis, as well as the discussion the researchers did on the research of larvicidal activity of *Syzigium polyanthum* W. leaf extract against *Aedes aegepty* L larvae, it can be concluded that; There was larvicidal activity of *Syzigium polyanthum* W. leaf extract against *Aedes aegepty* L larvae. The value of LC_{50} from the salam leaf extract (*Syzygium polyanthum W.*) as larvicide to *Aedes aegypti* L. larvae was LC_{50} 6576.68 ppm or around 0.66%.

Conflicts of interest

None

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