Jurnal Saintika Medika
Jurnal Ilmu Kesehatan dan Kedokteran Keluarga
Faculty of Medicine UMM

Vol. 16 No. 2 December 2020
p-ISSN : 0216-759X e-ISSN : 2614-X476X
http://ejournal.umm.ac.id/index.php/sainmed

The Effectiveness Of Squeeze Of Sambiloto *(Andrographis Paniculata)* Leaveson The Mortality Oflarvae *Aedesaegypti*

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Receive : Feb18th2020. Revised : Jun6th2020. Published: Dec27th2020

DOI: https://doi.org/10.22219/sm.Vol16.SMUMM2.11120

ABSTRACT

Dengue hemorrhagic fever has become a deadly disease since 2013. Dengue fever is a disease caused by dengue virus transmitted to humans through the bite of the Aedes aegypti. Eradication using chemical larvacides still has many disadvantages so that natural larvacides from plants are needed, one of which is larvacide from the squeeze of the sambiloto (Andrographis paniculata) leaves. The squeeze of the sambiloto leaf (Andrographispaniculata) contains flavonoids, alkaloids, tannins and saponins which have natural larvacidal activity. This research aims to determine the killing power of the squeeze of sambiloto (Andrographis paniculata) leaves against Aedes aegypti larvae and determine the most effective concentration as larvicide. This research was experimental laboratory with post test only controlled group design method. There are 600 instar III Aedes aegypti Larvae randomly divided into 6 groups (5%, 10%, 15%, 20%, aquadest and abate). Using 4 repetitions each group was observed every 6 hours for 24 hours. Based on the results of the Kruskal-Wallis non-parametric statistical test obtained p-value = 0,001 which means there are significant differences in larvicidal effects between groups. The concentration of 15% and 20% are the most effective in killing the larvae of Aedes aegypti.

Keywords : Aedesaegypti, The Leaf of Sambiloto, Andrographis paniculata, Larvacide

INTRODUCTION

One of infectious disease in Indonesia is dengue hemorrhagic fever and the disease is becoming a serious health problem. Dengue hemorrhagic fever (DHF) is an infectious disease caused by the dengue virus. DBD arise as extraordinary events, which caused anxiety in the community because it can cause death and spread very quickly. Indonesia reported as the 2nd ranked country with the largest dengue cases among the 30 endemic countries. Dengue morbidity rate increased from 27.67 in 2011 to 78.85 in 2016/100,000 population in Indonesia (KEMENKES, 2018).

DHF is still a serious problem in Central Java , proved 35 districts have ever been infected by dengue. Number of DHF cases in Central Java province in 2017 amounted to 21.68 / 100,000

population, while in 2015 the number of dengue fever patients as many as 315 cases spread over 12 districts with the highest people in the district of Sukoharjo, 54 patients(DKK Sukoharjo, 2015; Dinkes Jateng, 2018).

Dengue Hemorrhagic Fever (DHF) is an infectious disease caused by one of four different dengue virus and transmitted through mosquitoes, especially *Aedes aegypti* and *Aedes albopictus* were found among them in Indonesia until the Australia (Mau, Ira, & Bule, 2014).

The government has made various efforts to control the *Aedes aegypti* vector development, among others by means of chemical insecticides such as fogging and larvicidal on water reservoirs that are difficult to clean. Larvicides known by other terms that abatisasi. Larvicides used is temefos. Insecticides of chemicals turned out to cause many side effects and are not environmentally friendly include environmental pollution such as water contamination and insect resistance to insecticides so it needs insecticides are safer for the environment (Istiana*et al.*, 2012; Tennyson *et al.*, 2013).

Sambiloto (*Andrographispaniculata*) is one of the nine traditional medicine are favored to be studied until the stage of clinical trials. Sambiloto reported to have a variety of pharmacological effects such as anti-hyperlipidemia, analgesic, anti-diabetic, anti malaria. Sambiloto useful as larvicidal because they contain several active compounds, namely: alkaloids, flavonoids, saponins, and tannins. Sambiloto leaf effective as larvicides because it can kill the larvae of *Aedesaegypti*(Fathia, Argadireja, &Ismawati, 2019).

Prior studies ofSambiloto as a natural larvicidal used extraction methods. Here, we were interested in knowing the power to kill the squeeze of the sambiloto (*Andrographispaniculata*) leaves against *Aedesaegypti* larvae.

METHODS

This study is apost-test only with controlled group design experimental laboratory methods. This research was conducted in the laboratory of Parasitology, Faculty of Medicine, GadjahMada University on 28 October to 21 November 2019. The subjects used in this study were instar III larvae of *Aedesaegypti*.

The procedure of making the squeeze of sambiloto leaf, with modifications. Sambiloto leaf selected in the young leaves are still green. Sambiloto leaf in this study obtained from the District Sumberlawang, Sragen, Central Java, with an average rainfall of 3287 mm per year. Sambiloto leaf is weighed 1000 grams and washed throughly and then chopped, blended, and then squeezed and filtered by the filter cloth. Then filter results is concentrated squeeze with a concentration of 100%.

Sample required as many as 600 larvae of Federer's formula and 25 larvae/cup according to WHO guidelines, concentration of 4 times repetition each by referring to Federer's formula:

$(t - 1)(r - 1) \geq 15$

$(6-1)(r - 1) \geq 15$

$r \geq 4 = 4$ x replications

t: the number of intervetion, r: the number of repetitions, 15: constant (fixed number),

The negative control (given Aquadest), positive control (given abate 1 ml in 99 ml of water), treatment 1 (concentration of the squeeze of sambiloto leaf 5%), treatment 2 (concentration squeeze of sambiloto leaf 10%), treatment 3 (concentration squeezesambiloto leaf 15%), treatment 4 (concentration of the squeeze of a sambiloto 20%), lasting for 24 hours with four repetitions and observed every 6 hours.

Data analysis was done gradually, starting with the normality test by test Shapiro-Wilk and variance homogeneous (tested with Levene test), the difference in each variable treatment of the death of the mosquito will be analyzed using Kruskal Wallis test because the results of the data analysis are not qualified to do OnewayAnova test in that the data should be normally distributed and homogeneous. Next step is further post hoc test by Mann Whitney.

RESULTS AND DISCUSSION

Table 1. The number of *Aedes aegypti* larvae mortality of exposure of the squeeze of sambiloto (*Andrographispaniculata*).

Treat / Concentration	Repetition	Larvae mortality Leaf Squeeze Having given Sambiloto				Average Mortality Per Concentration	Death Percentage PerConcentration
		6th Hour	12th Hour	18th Hour	24th Hour		
P0 (CONTROL -)	I	0	0	0	0	0	0%
	II	0	0	0	0		
	III	0	0	0	0		
	IV	0	0	0	0		
Average Mortality Per Time		0	0	0	0		
P1 (5%)	I	1	8	16	23	23	92%
	II	1	7	16	22		
	III	2	8	18	24		
	IV	2	10	17	23		
Average Mortality Per Time		1.5	8.25	16.75	23		
P2 (10%)	I	4	12	19	24	24.5	98%
	II	5	14	20	25		

	III	4	13	21	25		
	IV	4	14	19	24		
Average Mortality Per Time		4.25	13.25	19.75	24.5		
P3 (15%)	I	8	17	22	25	25	100%
	II	6	18	23	25		
	III	10	16	23	25		
	IV	10	18	22	25		
Average Mortality Per Time		8.5	17.25	22.5	25		
P4 (20%)	I	16	22	25	25	25	100%
	II	14	20	25	25		
	III	13	19	24	25		
	IV	17	22	25	25		
Average Mortality Per Time		15	20.75	24.75	25		
P5 (CONTROL +)	I	25	25	25	25	25	100%
	II	25	25	25	25		
	III	25	25	25	25		
	IV	25	25	25	25		
Average Mortality Per Time		25	25	25	25		

(Source : Primary Data , 2019)

Seen in Table 1 that in the negative control group was not found Aedes aegypti larvae mortality, while the highest mortality rate obtained at a concentration of 15% and 20%. Based on this it can be seen that the higher the concentration of the squeeze of the leaves sambiloto (Andrographis paniculata) is given, the higher the death rate of larvae of Aedes aegypti.

Of normality test results showed the presence of data with $p<0.05$ so that the data distribution is not normal. Results of homogeneity test showed $p<0.05$ means the data is not homogeneous. Furthermore, to analyze differences in the effect of the concentration of the squeeze of sambiloto leaf against Aedes aegypti larvae mortality of non-parametric statistical test Kruskal-Wallis.

Data obtained from the nonparametric Kruskal-Wallis test showed the value of $p = 0.001$ which means that there is a significant difference between the mortality of larvae of Aedes aegypti with the squeeze of the leaves sambiloto (Andrographis paniculata).

Furthermore, the post hoc test used was the Mann-Whitney Test can be seen in Table 2.

Table2. Post Hoc Test Results Mann-Whitney.

Group	p-value	Test Results
P0 to P1	.013*	Significantly difference
P0 to P2	.013*	Significantly difference
P0 to P3	.008*	Significantly difference
P0 to P4	.008*	Significantly difference
P0 to P5	.008*	Significantly difference
P1 to P2	.008*	Significantly difference

P1 to P3	.036*	Significantly difference
P1 to P4	.013*	Significantly difference
P1 to P5	.013*	Significantly difference
P2 to P3	.127	Not Significantly difference
P2 to P4	.127	Not Significantly difference
P2 to P5	.127	Not Significantly difference
P3 to P4	1.000	Not Significantly difference
P3 to P5	1.000	Not Significantly difference
P4 to P5	1.000	Not Significantly difference

*Significantly difference($p < 0,05$).

Lethal Concentration 50%(LC_{50}) value is determined based on the number of test larvae mortality obtained at each concentration. Here is presented LC_{50} and LC_{90} values at each time of observation is based on the analysis of probit.

Table3. LC_{50} dan LC_{90}*Aedes aegypti*larvae at various times of observation

No.	Time (Hours)	LC_{50} (%)	LC_{90} (%)
1	6	18,624	34.906
2	12	13,221	28,962
3	18	11,983	18,147
4	24	8,108	11,424

(Source: Primary Data, 2019)

The data obtained in table 3 shows the LC_{50} and LC_{90} values which have decreased from the 6h hour to the last observation (24 hour). This shows that the longer the exposure to cause 50% larvae death and 90% of the total test larvae, the less concentration is needed. The results of probit analysis conducted at each time of observation, it appears that the LC_{50} value up to the 24h hour requires a concentration of 8,108%.

LT_{50} is the long time it takes to cause the death of 50% of the total larvae test. LT_{50} is used to determine whether an effective larvicides for use on a 24h period. Based on probit test LT_{50} values obtained at each concentration of such treatment in the following table:

Table 4. The values of LT_{50}*Aedes aegypti* larvae at various concentrations

No.	Concentration (%)	LT_{50} (Hours)
1	5	12,335
2	10	10,437
3	15	9,278
4	20	8,656

(Source: Primary Data, 2019)

The treatment group were given the squeeze of sambiloto leaf can be seen in the group P1 compared with P2, P3, P4, P5 has a p-value that is significantly different, and the group P0 compared with P1, P2, P3, P4, P5 also has value p different meaning. This is due to differences in larvae mortality rates vary on P0 group when compared to P1, P2, P3, P4, P5 ,. This is because the number of doses given concentration in each group had a significant difference and it is known that the higher the dose given, the higher the death rate of larvae. Therefore, the dose to the group P2, P3 and P4 which has the highest percentage of death between 98% to 100% more effective than the dose with the P1 group average mortality rate of 92%. On the positive control group (abate) with treatment 3 and 4 have the same power to kill the larvae of 100% within 24 hours, because at a dose of 15% and 20% contain bioactive compounds that were optimal.Death of larvae due to the inability of larvae to detoxify toxic compounds that enter the body(Yunita, 2009).

Based on the test results probit shows that the LC_{50} value is the concentration of the squeeze of the leaves sambiloto (*Andrographispaniculata*), which can cause the death of the larvae of *Aedes aegypti* by 50% within 6 hours is 18.624%, while the value of LC_{90} is the concentration of the squeeze of the leaves sambiloto (*Andrographispaniculata*) that can cause death of *Aedes aegypti* by 90% within 6 hours is 34.906%. LC_{50} values within a period of 12 hours was 13.221% and LC_{90} within 12 hours is 28.962%. Meanwhile, within 18 hours of sambiloto leaf squeeze has the LC_{50} and LC_{90} of 11.983% and 18.147%. And the last one LC_{50} and LC_{90} value over a period of 24 hours is 8.108% and 11.424%. Based on research that has been done, it is known that the higher the concentration of the squeeze of sambiloto leaf, it can increase the number of *Aedes aegypti* larvae mortality. This is because the larger the number concentration of larvicides, the greater the number of compounds in the larvicides that can kill larvae test (Minarni*et al*, 2013).

Test Results probit LT_{50} at concentrations of 5%, 10%, 15% and 20% showed results of 12.335 hours, 10.437 hours, 9.278 hours and 8.656 hours. Thereby granting the squeeze of the leaves sambiloto (*Andrographispaniculata*) is effective when used as larvicides against the third instar larvae of Aedes aegypti. In this study, LT_{50} values declining when compared with the increase in the concentration of the squeeze of the leaves sambiloto (*Andrographispaniculata*). These data shows that the higher the concentration given on the test larvae, the more the chemical constituents of larvae exposed to the test, so that the time required to kill the larvae becomes faster (Minarni*et al*, 2013) .

The high concentration of sambiloto (*Andrographis paniculata*) leavessqueeze lead time to achieve 50% mortality of larvae test more quickly, so that with the high concentration of sambiloto leaf squeeze (*Andrographis paniculata*) then also increase its toxic substances. Increased toxicity of substances contained in causing substances absorbed by the larvae of *Aedes aegypti* larvae as test exceeds the tolerance limit, resulting in damage to cells and tissues of the body so as to accelerate larvae mortality (Afrindayanti, 2017).

The results are consistent with a study in Phetchaburi, Thailand, in 2018 showed that a mixture of extracts of *A. paniculata*, *V. cinerea*, and *R. nasutus* effectively used as the *Aedes* mosquito larvicides. This study declares that an active substance that plays a role in killing the larvae are flavonoids so the larvae will die

because of poisoning of free radicals or other toxic substances in the environment (Duangkaew*et al*., 2018), Another study in Tamil Nadu, India, in 2016 concluded that the content in the extract of *A. paniculata* effective as larvicides at a concentration of 25 ppm by inhibiting the enzyme alpha and beta karboksilesterasein larvae, causing the larvae can not detoxify toxins and free radicals in the body , resulting in death(Edwin *et al*., 2016). Besides as larvicides, *A. paniculata* has also proven effective as a repellent. This was stated by a study in Bangkok, Thailand, in 2019. In the experimental study, showed that the extract of *A. paniculata* is more effective against mosquitoes are active during the day, such as *Aedes aegypti*. However, these studies can not disclose the content of the plant extract that can work as an active ingredient an antidote to mosquito bites (Sukkanon*et al*., 2019).

CONCLUSION

Squeezeof sambiloto (*Andrographis paniculata*) leaves has a killing effect on the instar III larvae of *Aedesaegypti* with a dose of the most effective in killing mosquito *Aedesaegypti* is at a concentration of 15% and 20%.

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