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Research Article

THE EFFECTIVITY TEST OF *ALOE VERA* LEAF EXTRACT TO LARVAE *CULEX SPECIES*

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ABSTRACT

Objective: The purpose of this study was to investigate the effectiveness of *Aloe vera* extract as a *Culex* sp larvicide.

Methods: This study used experimental post-test only one group design, for to the results of study used by probit analyzed to assessed toxicity. The experimental animals used are *Culex* sp (species) instar III/IV with 700 total samples larvae, and each treatment contained 25 larvae with 200 ml of water. The study was conducted with seven treatments including extract concentration used are 20, 40, 60, 80, and 100 part per million (ppm), negative control (water), and positive control (abate®1 ppm), with the different observation times 180, 360, 540, 1440, and 2880 min.

Results: This study which showed lethal concentration median % at 68.8 ppm concentration with 1440 min observation time.

Conclusion: The results obtained in this research work that the extract of *A. vera* has larvicidal against instar III/IV *Culex* sp larvae.

Keywords: *Aloe vera*, *Culex* sp, Larvae, Larvicide.

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INTRODUCTION

Mosquitoes are insects that have slim and small body size, where each type of mosquito has a different size [1]. Mosquitoes are most of the major vectors that cause disease in humans [2], including *Culex*, *Anopheles*, *Aedes*, and *Mansonia* [3].

The *Culex* sp mosquitoes are scattered almost all over the world, especially in tropical and subtropical regions [3]. The *Culex* sp mosquitoes can proliferate either in a difficult environment of clean water or high pollution levels. The habitat of *Culex* sp mosquitoes is located around the house and is therefore often called home mosquitoes [4]. According to the World Health Organization (WHO) 2006 [5], some species of *Culex* sp mosquitoes that play an important role in the spread of diseases such as *Culex quinquefasciatus* is the principal vector of *Filariasis bancrofti*. This mosquito species is also a potential vector of several arboviruses such as West Nile virus and Japanese Encephalitis (JE) [6].

Indonesia is one of the potential countries to the residence and breeding is good enough for *Culex* sp mosquitoes because it has a tropical climate [7]. Therefore, many cases of disease were caused by the vector of mosquitoes; one of which is filariasis. Filariasis in Indonesia is widespread in almost all provinces, and cases of filariasis patients in Indonesia are increasing every year. Based on district reports in 2009, there were three districts with the most filariasis cases, namely, Aceh Utara (91353 cases), Manokwari (667 cases), and Mapping area (652 cases) [8]. In addition to filariasis, JE disease can also cause death. Based on a study conducted by Kari *et al.* [9], JE is an endemic disease in Asia. In Indonesia exactly Bali, Kari *et al.* [9] do prospective research in the hospital with 599, 120 children <12 years old in July 2001–December 2003. Furthermore, in several provinces of Indonesia have reported JE cases [10].

Based on the number of cases of disease caused by *Culex sp* mosquitoes, it is necessary to control the vector of *Culex sp* mosquitoes, one way by controlling the mosquito vector larvae [11]. The control population of larvae is usually applied by insecticides to the breeding ground, but the use of excessive synthetic insecticides has created new problems of resistance and side effects [12]. How to control mosquito vectors by utilizing plants is one of the alternative ways of environmentally friendly and harmless control [13]. Conventionally, plant-based products have been used in human communities for many centuries for managing insects [14]. Many studies used plants as larvacides by observing lethal concentration (LC) and effective time to kill mosquito larvae [15]. One of the plants that have the potential as a larvacide is *Aloe vera* plant.

A. vera is an important medicinal plant [16], and that is often used in life and the world of health such as cosmetic ingredients [17]. *A. vera* is a family plant of *Liliaceae* and belongs to the genus *Aloe*. *A. vera* is commonly known as *Aloe barbadensis*. This plant grows well in dry conditions which pretty much get sunlight [18]. *A. vera* plants contain secondary metabolites that can be used as a larvacide. Research conducted by Ramesh *et al.* shows the presence of larvicidal potential of *A. vera* extract in *Culex salinarius* larvae [17]. Based on the above description, due to the many cases of disease that have been reported with concerns about the resistance of *Culex* sp mosquitoes to chemicals, therefore researchers interested in conducting larvacide test research by utilizing natural ingredients such as *A. vera* is easy to get the community, especially those living in the tropics.

MATERIALS AND METHODS

Materials

Instar III/IV *Culex* sp larvae, 700 sample larvae *Culex* sp for four repetitions each glasses, the glass consisting of 7 glasses of research, consisting of extract *A. vera* with 20, 40, 60, 80, and 100 ppm, negative control and positive control (abate®1 ppm). Each test group contained 25 *Culex* sp larvae in each study glass [5]. *A. vera* washed and weighing 1000 g then cut and then dried with 50°C temperatures. Once dried, crushed it until powdered and store in a container. Criteria inclusion: The larva *Culex* sp alive, active, and instar III/IV were included in this study.

Methods

The process to make A. vera extract

A. vera was soaking in ethanol 96% for 3 h, then insert it into the percolator tool slowly [19], and close the percolator leave to soak

for 24 h. Open the percolation tap then let it flow at a speed of 1 ml/min then add back ethanol 96% repeatedly so that the simplicial remain submerged. Percolation had stopped when the dripping liquid is colorless again. The result of percolation is called liquid extract, then the liquid extracts evaporated so obtained the viscous extract, and then the condensed extract in the freeze dryer for 24 h [20]. The research was conducted after containing the Ethics Committee from the Health Research Ethical Committee Universitas Sumatera Utara.

Observation of larvicidal effect

25 *Culex* sp larvae inserted into each research glass that had been mixed *A. vera* extract with concentration 20 ppm; 40 ppm; 40 ppm; 60 ppm; 80 ppm; and 100 ppm, negative control and positive control (abate®1 ppm). Observe the total of dead larvae in 180; 360; 540; 1440; and 2880 min after being treated [12]. Record the result of the research to the observation sheet. The result data from observation were processed by computerized with one-way analysis of variance (ANOVA), then least significance different (LSD) test to find the mean significant difference pairs and probit analysis to assess the larvicidal toxicity of *A. vera* extract to larvae expressed with LC.

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical screening was conducted to determine the active compound [21] of *A. vera*, and this is presented in Table 1.

The average of larval mortality rates is presented in Table 2.

Table 2, mortality of larvae has started to occur in A. vera extract with a concentration of 20 ppm at 180 min and higher mortality rate of larvae at increasing concentration of A. vera leaf extract. In conducting probit analysis, normality test is used to find out the distribution of research data, followed by one-way ANOVA, homogeneity and post hoc analysis using LSD. In the data normality test used is Shapiro–Wilk because of the amount of data <50. It is also stated by Dahlan [22] that on the small amount of data (<50) the Shapiro–Wilk normality analysis is used. If the value of $p>0.05$ (greater value than the level of significance used [5%]), then it can say that the data are the normal distribution. Here are the results of data distribution analysis (Table 3).

After the data are a normal distribution, one-way ANOVA test can be performed. Here is the result of one-way ANOVA test (Table 4).

Table 1: Phytochemical screening

Active compounds	Result
Steroid/terpenoid	Negative
Alkaloid	Positive
Glycosides	Positive
Saponin	Positive
Tannin	Positive
Flavonoid	Positive
Essential oil	Negative

To know which groups have differences, a *post hoc* test is performed. Before performing the *post hoc* test, a variant test is necessary to determine whether the data are homogeneous or not. The following test results variant data.

Table 5 shows that the $p>0.05$ overall data at various observations of variant time of the homogeneous data.

The one-way ANOVA test is significant, and the homogeneous variant, the *post hoc* analysis to be used is LSD. The LSD test was used to determine whether there was a significant difference in the number of larval mortality in each pair tested. Furthermore, in determining effective concentrations killing 50% of the total number of test larvae, a probit analysis was performed to determine LC median (LC_{50}).

Table 6 shows overall that the smaller the concentration, the longer the observation time required.

This study was conducted to determine the effectiveness of *A. vera* extract on mortality of *Culex* sp Instar III and IV larvae. *A. vera* plant is a natural insecticide material containing 99.5% water; then there is content such as vitamins, minerals, enzymes, sugar, and secondary metabolic including anthraquinone, saponin, flavonoid, and tannin [23]. These secondary metabolites are toxic to mosquito larvae, causing the death of test larvae such as alkaloids, saponins, tannins, and flavonoids, this is by research Ramesh *et al.* study [17].

To know the content of secondary metabolite on *A. vera*, hence in this research screening phytochemical, it is necessary to see the secondary metabolite compounds found in plants [24]. The results of phytochemical screening (Table 1) contain alkaloids, glycosides, saponins, tannins, and flavonoids in accordance with the previous studies, Ramesh *et al*. [17] and Maurya *et al*. [25], from the results of phytochemical screening of secondary metabolite compounds toxic, are alkaloids, saponins, tannins, and flavonoids [26].

Alkaloids and saponins act as a stomach poison. Alkaloids are contained in many roots, seeds, wood, and leaves of plants [27]. According to research Wardani, *et al*. (2010) that extract of Shepherd also contains a secondary metabolite similar to the secondary metabolite of A. vera that is alkaloids [28]. Alkaloids can inhibit the growth hormone of insects. The lack of such hormones can lead to metamorphosis failure. How alkaloid works are to act as a stomach poison. When the compound enters the body of the mosquito larvae, it is digestive apparatus will become disturbed.

Glycosides are secondary metabolites in stomach-poisoned plants, in which the action of glycosides by suppressing appetite from these mosquito larvae [4]. Saponin is a stomach poison to the larvae. Saponins are characterized by froth and have higher solubility properties in water when saponin is shaken with water it will form a long-lasting foam, saponins entering through the cuticle, and trachea through the mouth of the larvae. The mechanism of saponins is to inhibit the action of enzymes that result in the decreased surface tension of the mucous membranes of the larval digestive system so that the walls of

Table 2: Average of larvae mortality at the particular time

Average mortality					
Concentration extract (ppm)	**180 min mean±SD***	**360 min**	**540 min**	**1440 min**	**2880 min**
20 ppm	1±0.82	2±0.82	3±0.82	6.25±0.50	8±0.82
40 ppm	2±0.82	4±0.82	5.25±1.26	8.5±0.58	10.25±1.26
60 ppm	3.25±1.26	6±0.82	6.75±0.96	9.75±0.50	13±0.82
80 ppm	4.75±0.96	5.75±0.96	7±0.82	14.5±1.29	18.25±1.71
100 ppm	5±0.82	6.75±0.96	7±0.82	19.75±1.71	23.75±1.26
Abate	25±0.00	25±0.00	25±0.00	25±0.00	25±0.00
Water	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00

*Mean±SD, SD: Standard deviation

Table 3: Test result of data normality

Observation	Shapiro–Wilk		
	Concentration (ppm)	Significant (p)	Information
180 min	20	0.683	Normal
	40	0.683	Normal
	60	0.406	Normal
	80	0.272	Normal
	100	0.683	Normal
360 min	20	0.683	Normal
	40	0.683	Normal
	60	0.683	Normal
	80	0.272	Normal
	100	0.272	Normal
540 min	20	0.683	Normal
	40	0.406	Normal
	60	0.272	Normal
	80	0.683	Normal
	100	0.683	Normal
1440 min	20	0.683	Normal
	40	0.683	Normal
	60	0.683	Normal
	80	0.850	Normal
	100	0.572	Normal
2880 min	20	0.683	Normal
	40	0.406	Normal
	60	0.683	Normal
	80	0.850	Normal
	100	0.406	Normal

Shapiro–Wilk normality test on the overall data above-obtained ($p>0.05$) at various concentrations (the data in each concentration are a normal distribution)

the digestive system become damaged [28]. Another study conducted by Anita and Cahyati [29], also mentions the same, that saponins can reduce the surface tension of the larval digestive system to corrosive. The pupa is not affected by saponin because it has a body wall structure consisting of a hard cuticle so that saponin compounds cannot penetrate the pupa wall.

Tannins are toxic by blocking the digesting of foods causing growth disturbances [30], also causing a water-absorption disorder in the larvae, thus causing death to the larvae [31]. Flavonoids are compounds which are also toxic to insects. According to Cania and Setyaningrum [32], flavonoids work as a strong inhibitor of respiration or as a respiratory toxin. Flavonoids have a way of working that is by entering into the body of the larvae through the respiratory system which will then cause wilting on the nerves as well as damage to the respiratory system and cause the larvae cannot breathe and eventually die. The position of the larval body that changes from normal can also be caused by flavonoid compounds due to its way through the siphon causing damage so that the larvae must be by its position on the surface of the water to facilitate the taking of oxygen.

Overall decrease of LC_{50} (%) value it can see (Table 6) that in general the longer time observation of *A. vera* leaf extract given the smaller the concentration required to kill 50% of test larvae in the study. The LC_{50} value in the 180 min up to the 540 min cannot be used because it is above the WHO standard value or 100 ppm. The death of the test larvae began to occur in the 1440 min until the 2880 min with the LC_{50} values sequentially being 68.8 ppm and 51.6 ppm, while in Maurya *et al*. efficacy extract of carbon tetrachloride *A. barbadensis* can be killed mosquito larvae *Culex* sp with result of LC_{50} 15.31 ppm after 24 h and 11.01 ppm after 48 h [25]. The occurrence of death of *Culex* sp larvae at various concentration of *A. vera* leaf extract is caused by the number of active compounds that directly contact with larvae on the media. The higher concentrations of active compounds received by Culex sp larvae are also more numerous, in accordance with research conducted by Yuni (2006) on tobacco leaf extract [33].

Table 4: One-way ANOVA test

Observation (min)	Significant (p)	Information
180	0.000	Significant
360	0.000	Significant
540	0.000	Significant
1440	0.000	Significant
2880	0.000	Significant

The p value of one-way ANOVA showed 0.000 ($p<0.05$) in the 180–2880 min observation, it can be concluded that there was a significant difference in mean larval mortality. ANOVA: Analysis of variance

Table 5: Variant data test

Observation (min)	Significant (p)	Information
180	0.139	Homogenous
360	0.102	Homogenous
540	0.139	Homogenous
1440	0.071	Homogenous
2880	0.070	Homogenous

Table 6: LC_{50} value at various observation time

Time (min)	LC_{50}(ppm)
180	179.8
360	134.6
540	131.1
1440	68.8
2880	51.6

LC_{50}: Lethal concentration median

In this study, it can be seen that by increasing the concentration of *A. vera* leaf extract can accelerate the time required to kill *Culex sp* larvae, where abate®1 ppm only takes a short time to kill the larvae. Abate®1 ppm commonly used in the form of sand grains (sand granules) and sown in a place that holds water. The dosage used is 10 g for every 100 L of water or equivalent to a dose of 1 ppm. Chemicals from abate®1 ppm can kill larvae for 3 months [34] this is because abate®1 ppm is a pesticide class of organophosphate compounds that are toxic in which it works by inhibiting or binding to the enzyme cholinesterase that will cause continuous muscle spasms and eventually the larvae will die [35].

CONCLUSION

The potent concentration to kill *Culex* sp larvae in this study is expressed by LC_{50} where *A. vera* extract concentration of 68.8 ppm takes a 1440 min observation time. It can be said to be more potent because by WHO [12] is natural larvacide which has effectivity value at the concentration below 100 ppm, while in minutes 180 to 540 min it takes *A. vera* extract concentration above 100 ppm to kill *Culex* sp larvae. LC_{50} at 2880 min was considered less effective than LC_{50} at 1440 min although the required a concentration was equally below 100 ppm, this is because regarding time LC_{50} at 2880 min takes longer time, so it can be declared less efficient.

CONFLICTS OF INTEREST

All authors have none to declare.

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