Sys Rev Pharm 2020; 11(3): 388 – 392
A multifaceted review journal in the field of pharmacy
E-ISSN 0976-2779 P-ISSN 0975-8453

Larvicidal Activity Test of Ethanolic Extract of (Euphorbia tirucalli Linn) Stem on Aedes aegypti Larvae

Yusnidar Yusuf[1], Kriana Efendi[2], Seta Diantasari[3]
[1,2,3]Faculty of Pharmacy and Science University of Muhammadiyah Prof. Dr. Hamka, Jakarta 12130, Indonesia
E-mail: yusnidaryusuf@uhamka.ac.id

Article History: *Submitted: 20.01.2020* *Revised: 10.02.2020* *Accepted: 11.03.2020*

ABSTRACT
Dengue virus is the cause of Dengue Hemorrhagic Fever (DHF) The mosquito Aedes aegypti as intermediator. This research examines the impact of the stem of Euphorbia tirucalli To prevent the transmission of Dengue virus to humans by control the development of mosquito larvae. *The research aimed at proving the larvicidal activity of the ethanolic extract of the stem of Euphorbia tirucalli Linn* the mosquito Aedes aegypti. Larvicide test above 7 treatments, ie group I as negative contro), group II as positive control by temephos 1% and the test group III, IV, V, VI, VII given extracts of the stem of the aveloz 10 ppm, 31.62 ppm, 99,98 ppm, 316,14 ppm, 1000 ppm. The results were analyzed by probit analysis using SPSS.17 application. The results showed that ethanolic extract of the stem of the aveloz (Euphorbia tirucalli Linn.) Effective as larvicides with LC50 171.480 ppm (0.0171%) and relatively safer to use than synthetic larvicides (Temephos).
Keywords: Euphorbia tirucalli Linn., Aedes aegypti L., larvicides, lethal concentration (LC50)
Correspondence:
Yusindar Yusuf
Faculty of Pharmacy and Science, University of Muhammadiyah, Prof. Dr. Hamka, Jakarta, 12130, Indonesia
E-mail: yusindaryusuf@uhamka.ac.id
DOI: 10.5530/srp.2020.3.48

INTRODUCTION

Dengue Haemorrhagic Fever (DHF) is caused by dengue virus that belongs to the Arthropod Virus B group (Arboviroses) which is now known as the genus Flavivirus, Flaviviride family, and has 4 types of serotypes: DEN-1, DEN-2, DEN-3, and DEN-4. The infection of one serotype will produce antibodies against the serotype, while the antybodies formed against the other serotype are so poor that they cannot provide the adequate protection against the other serotype[1][2].The clinical symptoms of DHF are high fever continuously for 2-7 days and the manifestation of bleeding is usually prceded by the appearance of red spots (petechiae) in the patient's body. The patients may experience shock syndrome and die. The main vector of dengue is Aedes Aegypti, and its potential vector is Aedes Albopictus [3] Rahuman, A.Abdul ;Gophalakhrisnan, Geetha Gopalakrishnan This is an ideal ecofriendly approach for the control of the dengue vector, *A. aegypti*, and the lymphatic filariasis vector, *C. quinquefasciatus* .[4]

The Dengue Hemorrhagic Fever (DHF) transmitted through the Aedes Aegypti mosquito vector is still an important health problem in the world. The spread of dengue in the world occurs very quickly, it is shown in the last 50 years that the incidence rate increased 30-fold and the spread area axtends both in urban and rural areas[5]. Based on WHO report, 78690 cases and more than 900 deaths were reported in Indonesia in 2004[6] Chemical composition

E. tirucalli contains white milky latex in any part of the shoot. According to Kapaczewski (1947), the latex contains about 28% solid matter whose composition is: 21 to 27% water soluble substances, 59 to 63% resin- soluble substances and 12 to 14% rubber-like substances. The chemical composition of the different parts of the plant has been extensively studied and a variety of chemical compounds have been isolated from them. This great variety of chemical substances listed reveals the complexity of E. tirucalli latex and may explain most of its functions. For example, low herbivore pressure, poisonous nature, pesticidal features and medicinal characteristics may all be attributed to this chemical constitution as follows: pests and desease.[7]

The increase and the spread of dengue fever cases is likely caused by the high population mobility, urban development, climate change and several other factors [8]. The results of the leaf extract of *M. citrifolia* are promising as good larvicidal activity against the mosquito vector *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus*.[9]

The prevention of mosquito development becomes the essential to reduce the incidence of dengue infection transmitted through the bite of Aedes Aegypti mosquitoes. One of the world's programs to sippress the mosquito transmition is by eradicating the larvae[5].

The synthetic larvacides are the most common way to break the chain of dengue virus transmission. But however, the use of abate (Temefos 1%) can continuously contaminate the condition of water especially the drinking water. In addition, the regular use of synthetic larvacides can make the mosquito vectors more resistant. In many parts of the world, it has been widely reported that Aedes Aegypti has been resistant to the various classes of insecticides[5].

One of the medicinal plants used by the community is the fracture plant (Euphorbia Tirucalli Linn) which is one of the species of the Euphorbiaceae family [10]. The Plant fracture contain of saponins, flavonoids and tannins. According to the information from the people of Central Sulawesi, this fracture plant is empirically a plant that is used as an anti-mosquito by burning the plant [11].Many people use the fracture plants as the safe botanical pestisides but are deadly to pests [12]. Based on the above exposure, the research will be conducted on the larvacidal activity of the bone fracture extract (Euphorbia Tirucalli Linn) against aedes aegypti mosquito larvae.

MATERIALS AND METHODS

Tool

The tool used in this research are: Reaction Tube, aluminium foil, oven, glass stir bar, tray for larvae, 250 ml beaker glass for larva test, measuring pipette, measuring cup, Pasteur pipette to take larva, measuring flask, waterbath, rotary evaporator, analytical scale, 250 ml round bottom flask, filter paper and larva filter..

Material

The materials used in this study were 600 g of dried branch twig (Euphorbia tirucalli Linn) which can be obtained from the Indonesian Spices and Medicinal Crops Research Institute (BALITRO) – Bogor, Aedes Aegypti mosquito egg obtained from Loka Litbang P2B2 Ciamis. Temephos 1% (as positive control), well water, 70% ethanol and aquadest.

Work Procedure

The Extraction of Twig fracture [13][14]

The main ingredient used in the larvacidal activity test is a fresh 1 kilogram twig fracture (Euphorbia tirucalli Linn.)obtained from the research institute for the spices and medicinal plants (BALITRO). The fresh leaves are collected, cleaned, washed with clean water and drained, then sorted and then dried in the oven at 50 c for about 24 hours. The dry matter is pollinated and then stored in a dry and tight place.

The Quality Examination of Fracture Plant Ethanol Extract

The examination of organoleptis includes the examination of shape, color and odor against the ethanol extract of fracture branch. The calculation of the yield is calculated from the weight ratio of the viscous extract obtained to the weight of the dry powder prior to the extraction

Phytochemical Screening of Fracture Branch Extract

Phytochemical screening test were performed to determine the presence or the absence of alkaloids, saponins, flavonoids, tannins and triterpenoids

Egg Maintenance for Becoming Larvae

The larvae used in this study were the instar larva III. The instar larva III were obtained by incubating Aedes Aegypti eggs using a tray/ jar containg 1500 ml of well water.The mosquito eggs are inserted in the tray/ jar and awaited 1-2 days to hatch into larvae, usually done 5 days after the eggs hatch

The Displacement of Larvae on Beaker Glass Treatment

The hatching eggs become instar larva III and IV and then transferred to a glass containing aquados, and remove for about 20 larvae using Pasteur pipette. Then these 20 larvae were transferred to a treatment glass containing the extract of the leaf and the fracture twig with each concentration using a larva filter

The Maintenance of Larvae as a Test Material

The eggs are put into a plastic tray containing the water for beeding. If the eggs have hatched into larvae, the larvae are fed in the form of fish food

Larva Maintenance

The selected larvae were instar larvae III/IV characterized by 5-7 day larvae and larval size is about 4-8 mm

Research of the Large Subject

The number of samples used in this study is 20 tails per treatment unit. At each concentration multiplied by the number of repetitions about 4 times.

Larvaside Test

The preliminary test conducted to obtain the lethal concentration is LC10 and LC90 fracture branch extract (Euphorbia tirucalli linn.). After obtaining the concentration in the preliminary test, we determine 5 concentrations of the test group, strating from the smallest concentration of LC10 to the largest concentration of LC90 with the same concentration range. Each treatment group made 4 replications.

The Test Parameters

The number of dead larvae that cannot rise to the surface is calculated in total. The calculation of the dead larvae number is done every 6 hours after the treatment for 24 hours.

The Data Analysis

After 24 hours observation, the data were analyzed by probit method using SPSS.17 application to determine the concentration of LC50 and LC90 ethanol extract of fracture branch

RESULTS AND DISCUSSION

The Extraction Result of Twig Fracture

The determination is the first step of research to get the correct identity of the examined plants, so as to provide certainty about the truth of the plant. It aims to avoid the errors on the used plants. The plant determination was done at Biological Research Center LIPI Bogor. The results show that the simplicia used in this study is a fracture twig (Euphorbia tirucalli L.) belonging to the Euphorbiaceae. The result of maceration of twig fracture obtained by viscous extract as much as 200,2 gram, it can be seen in the table below.

Table 3. The Result of Phytochemical Screening of twig fracture

The Test	Observation Result	Noted
Alkaloid	No sediment	-
Saponin	Not forming foam	-
Flavonoid	Yellow	+
Tanin Steroid	Greenish black color	+
Triterpenoid	Green Color	-

Larvaside Test Result

The research duration of the effectiveness test of twig fracture extract on Aedes Aegypti larvae instar III/IV was performed for 24 hours to see the larvacidal effect with the observation for 24 hours after treatment. Each of test group contains of 20 larvae of Aedes Aegypti instar III which included in each solution some extract concentration variant, temephos 1% as positive control and Aquadest 100 ml as negative control. To determine the concentration on larvacidal test of ethanol extract of twig fracture, a preliminary test was conducted. The result of the preliminary test is as follows:

Table 4. The Result of Preliminary test of Twig Fracture Ethanol extract

Concentration (ppm)	The Number of Dead Larvae				Average	
Replication	1		2			
	amount	%	amount	%	amount	%
0 ppm (0%)	0	0	0	0	0	0
10 ppm (0,001%)	1	5	3	15	2	10
100 ppm (0,1%)	7	35	8	40	7,5	37,5
1000 ppm (0,1%)	18	90	19	95	18,5	92,5

Based on the preliminary test obtained that LC10 and LC90 with the concentration respectively 10 ppm and 1000 ppm. After getting the concentration in the preliminary test, then determined 5 concentration using the logarithmic interval formula as follows:

$$\frac{N}{n} \qquad \frac{a}{n}$$

Log ^{n}= K (Log ^{n})

The next concentration after "a" can be calculated based on the formula :

$$\frac{a^2}{n} \qquad \frac{b^2}{a} \qquad \frac{c^2}{b}$$

b = ^{n} , dose c (next dose after b) = ^{a} , dose d = ^{b} and so on.

where :

N = deadly concentration of about 90% (over 50%) of the animal test

N = lethal concentration of about 10% (below 50%) of test animal

K = range/ variance -1 (k = number of group without -1)

A = dose or concentration n

each concentration is 10 ppm, 31,62 ppm, 99,98 ppm, 316,13 ppm, and 1000 ppm. The result is as follows:

Table 5. The Average Mortality Rate of Aedes Aegypti Larvae at Various Concentration of Twig Fracture Extract (Euphorbia tirucalli L) every 6 Hours after Treatment

Concentration (ppm)	The Number of dead Larvae							
Replication	1 hour		6 hours		12 hours		24 Hours	
	Amount	%	Amount	%	Amount	%	Amount	%
Negative Control	0	0	0	0	0	0	0	0
Positive Control	18,75	93,75	20	100	20	100	20	100
10 (0,001%)	0,05	0,25	1,25	6,25	1,75	8,75	2	10
31,62 (0,0032%)	1,5	7,5	3,5	17,5	4	20	5	25
99,98 (0,01%)	3	15	5	25	5,75	28,75	7,25	36,25
316,14 (0,032%)	5	25	7	35	7,75	38,75	9	45
1000 (0,1%)	10,5	52,5	15,5	77,5	16,75	83,75	18,5	92,5

From the above data is done a probit analysis which the obtained the following result:

Table 6. Probit Analysis Result

		95% Confidence Limits for Concentration (ppm)		
	Probability	Estimate	Lower Bound	Upper Bound
PROBIT	LC1	1.465	.492	3.114
	LC2	2.560	.976	5.005
	LC3	3.648	1.505	6.769
	LC4	4.761	2.084	8.501
	LC5	5.913	2.714	10.236
	LC10	12.439	6.690	19.461
	LC20	30.615	19.588	43.135
	LC30	58.610	41.412	78.592
	LC40	102.086	75.994	135.573

LC50	171.480	129.212	234.102
LC60	288.046	212.545	417.844
LC70	501.710	352.929	796.572
LC80	960.486	626.817	1.727.663
LC90	2.363.901	1.366.541	5.143.092
LC95	4.973.223	2.581.362	12.757.502
LC96	6.176.360	3.104.414	16.635.439
LC97	8.061.392	3.893.460	23.061.994
LC98	11.486.377	5.258.509	35.620.399
LC99	20.070.047	8.436.960	70.741.964

The larvae are also developed indoors with the optimum temperature for the larvae development that is 25-35oC, this temperature is suitable for the larvae development in outdoor[15].

This study used a positive control Abate which contains of Temephos with 1% concentration as a comparison of the larvacidal activity of the fractured twig extract to the temephos, so that it can be known whether or not the method used during the study. The temephos as the election used in this experiment as a comparison because the only larvaside used in Indonesia is Abate[3].

The result of this study in Table 10 showed that the extract of fractured twig had a larvicidal effect on aedes aegypti especially at the concentrations of 10 ppm, 31.62 ppm, 99.89 ppm, 316.14 ppm and 1000 ppm with the higher mortality rates at the higher extract concentrations in every 6 hours after treatment (graph 1). Then on the administration of Abate with concentration of 10000 ppm (1%) on the observation 6 hours after treatment obtained the mortality of larvae as much as 100%. This suggests that within 6 hours after the Abate

The twig fracture (Euphorbia tirucalli Linn.) are Euphorbiacea plant species from the previous studies known to contain the high level of flavonoids, tannins and steroids sufficiently qualitative[16]. The content of flavonoids and tannins are sufficiently qualitatively suspected as a substance larvas against aedes aegypti larvae.

Flavonoids work as a respitory inhibitors. Flavonoids enter the body of the larvae through the respiratory system which will then cause the weakness in the nerves as well as the damage to the respiratory equipment and cause the larvae cannot breathe. As a result of the flavonoids compound entry through siphon, causing the damage to the siphon so that the larvae should align its position with the water surface to facilitate the taking of oxygen[17].

Tannin can enter the larvae body in two ways: it penetrates the body wall of the larvae and enters through the digestive tract. Tannin that penetrate the larval body wall can affect the muscle activity which cause to the muscle weakness. While tannin that enter the digestive tract of larvae can decrease the activity of digestive enzymes and inhibit the absorption of food[18].

The most important mosquito control is to kill the larvae and kill only the adult mosquito if necessary. This is because the eradication of adult mosquitoes is only temporary, unsatisfactory and polluting in the environment, while larval handling is more localized at times and place resulting in the lower hazard levels. Synthetic larvasides are the most common way to break the chain of the dengue virus transmission. However, the use of Abate (temephos 1%) continuously can contaminate the water condition especially the drinking water[5].Therefore, the larvasides become an appropriate alternative is synthetic larvasides because it is hrelatively safe[19].

ACKNOWLEDGMENTS

This research was supported in part by Fakultas Farmasi dan Sains Universitas Muhammadiyah Prof. Dr. Hamka.

REFERENCES

1. A. Candra, "Demam Berdarah Dengue: Epidemiologi, Patogenesis, dan Faktor Risiko Penularan," ASPIRATOR-Journal Vector-borne Dis. Stud., vol. 2, no. 2, 2010.
2. D. K. R. Indonesia, "Tata Laksana Demam Berdarah Dengue di Indonesia." Ketiga. Jakarta: Departemen Kesehatan, 2004.
3. S. Gandahusada, H. D. Ilahude, and W. Pribadi, "Parasitologi kedokteran," Jakarta FKUI, 2008.
4. A. A. Rahuman, G. Gopalakrishnan, P. Venkatesan, and K. Geetha, "Larvicidal activity of some Euphorbiaceae plant extracts against Aedes aegypti and Culex quinquefasciatus (Diptera: Culicidae)," Parasitol. Res., vol. 102, no. 5, pp. 867–873, Apr. 2008.
5. W. H. Organization, S. P. for Research, T. in T. Diseases, W. H. O. D. of C. of N. T. Diseases, W. H. O. Epidemic, and P. Alert, Dengue: guidelines for diagnosis, treatment, prevention and control. World Health Organization, 2009.
6. I. Ahmad, S. Astari, R. Rahayu, and N. Hariani, "Status Kerentanan Aedes aegypti (Diptera: Culicidae) pada Tahun 2006-2007 terhadap Malation di Bandung, Jakarta, Surabaya, Palembang dan Palu," Biosfera, vol. 26, no. 2, pp. 82–86, 2009.
7. T. Mwine and P. Van Damme, "Euphorbia tirucalli L.(Euphorbiaceae): the miracle tree: current status of available knowledge," Sci. Res. Essays, vol. 6, no. 23, pp. 4905–4914, 2011.

8. R. I. Kemenkes, "Profil Kesehatan Indonesia," Jakarta Kementrian Kesehat. Republik Indones., 2010.
9. K. Kovendan, K. Murugan, S. P. Shanthakumar, S. Vincent, and J.-S. Hwang, "Larvicidal activity of Morinda citrifolia L. (Noni) (Family: Rubiaceae) leaf extract against Anopheles stephensi, Culex quinquefasciatus, and Aedes aegypti," Parasitol. Res., vol. 111, no. 4, pp. 1481–1490, Oct. 2012.
10. B. Budiman and R. Rahmawati, "Perbandingan Efektivitas Ekstrak Zodia (Evodia sauveolens) dan Serai (Cymbopogan citratus) Sebagai Repellent (Penolak) Nyamuk," Hig. J. Kesehat. Lingkung., vol. 1, no. 2, pp. 67–74, 2016.
11. S. Dalimartha, Ramuan Herbal Tumpas Penyakit. Penebar Swadaya Grup, 2013.
12. M. H. Toana and B. Nasir, "Studi Bioaktivitas dan Isolasi Senyawa Bioaktif Tanaman Euphorbia tirucalli L.(Euphorbiaceae) sebagai Insektisida Botani Alternatif," Agrol. J., vol. 17, no. 1, pp. 47–55, 2010.
13. R. I. DepKes, "Farmakope Herbal Indonesia," Ed. I. Jakarta, 2008.
14. M. Marlinda, M. S. Sangi, and A. D. Wuntu, "Analisis senyawa metabolit sekunder dan uji toksisitas ekstrak etanol biji buah alpukat (Persea americana Mill.)," J. Mipa Unsrat Online, vol. 1, no. 1, pp. 24–28, 2012.
15. A. Jacob, V. D. Pijoh, and G. J. P. Wahongan, "Ketahanan Hidup Dan Pertumbuhan Nyamuk Aedes spp Pada Berbagai Jenis Air Perindukan," J. e-Biomedik, vol. 2, no. 3, 2014.
16. M. H. Toana and B. Nasir, "STUDI BIOAKTIVITAS DAN ISOLASI SENYAWA BIOAKTIF TUMBUHAN Euphorbia tirucalli L.(EUPHORBIACEAE) SEBAGAI INSEKTISIDA BOTANI ALTERNATIF," AGROLAND, vol. 17, no. 1, 2012.
17. I. Chaieb, "Saponins as insecticides: a review," Tunis. J. Plant Prot., vol. 5, no. 1, pp. 39–50, 2010.
18. D. Wahyuni and I. Loren, "PERBEDAAN TOKSISITAS EKSTRAK DAUN SIRIH (Piper betle L.) DENGAN EKSTRAK BIJI SRIKAYA (Annona squamosa L.) TERHADAP LARVA NYAMUK Aedes aegypti L.," 2015.
19. E. Cania and E. Setyanimgrum, "Uji efektivitas larvasida ekstrak daun legundi (Vitex trifolia) terhadap larva aedes aegypti," J. Major., vol. 2, no. 4, 2013.