EFFICACY TEST OF ARABICA COFFEE LEAF ETHANOL EXTRACTAS A BIOLARVASIDE AGAINST THE VECTOR OF DENGUE HEMORRHAGIC FEVER (DHF)

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ABSTRAK

Demam Berdarah Dengue (DBD) disebabkan oleh virus dengue yang ditularkan melalui vektor nyamuk *Aedes aegypti* yang banyak ditemukan di daerah tropis, termasuk Indonesia. Penggunaan larvasida kimiawi sebagai pembasmi jentik nyamuk menimbulkan populasi yang resisten, berefek toksik bagi manusia dan lingkungan. Daun kopi arabika (*Coffea arabica*) mengandung senyawa kimia alkaloid, saponin, flavonoid, terpenoid, polifenol yang memiliki peran penting dalam membunuhlarva *Aedes aegypti*. Penelitian ini menggunakan Rancangan Acak Lengkap (RAL) dengan masing-masing konsentrasi di uji bertingkat yaitu 12,5%, 25%, dan 50%, dengan metode maserasi menggunakan pelarut etanol 96%. Penelitian ini bertujuan untuk mengetahui potensi biolarvasida ekstrak etanol tanaman daun kopi arabika dengan sediaan granul terhadap larva *Aedes aegypti* dalam LT 50 dan LT 95 di analisisregresi probit dan linear regresi menggunakan aplikasi SPSS. Pada penelitian ini ada tiga kelompok perlakuan, masing-masing kelompok ada 3 kali pengulangan dengan konsentrasi masing-masing 12,5%, 25%, dan 50%. Setiap pengulangan diamati dalam satuan menit selama 24 jam. Hasil yang diperoleh pada konsentrasi 25% tingkat kematian larva yang paling tinggi dengan persentase 50,6%, konsentrasi 50% dengan persentase 29,3%, selanjutnya pada konsentrasi 12,5% dengan persentase 9%.

Kata kunci: *Demam Berdarah, Aedes aegypti, Biiolarvasida, Mortalitas*

ABSTRACT

Dengue fever (DHF) is caused by the dengue virus transmitted through the mosquito vector *Aedes aegypti*, which is commonly found in tropical areas, including Indonesia. The use of chemical larvicides to eradicate mosquito larvae has resulted in resistant populations, which are toxic to humans and the environment. Arabica coffee leaves (*Coffea arabica*) contain chemical compounds of alkaloids, saponins, flavonoids, terpenoids, polyphenols that have an important role in killing *Aedes aegypti*larvae. This study used a completely randomized design (CRD) with each concentration tested in stages, namely 12.5%, 25%, and 50%, with the maceration method using 96% ethanol solvent. This study aims to determine the biolarvicidal potential of ethanol extract of Arabica coffee leaf plants with granule preparations against *Aedes aegypti* larvae in LT 50 and LT 95 in probit regression analysis and linear regression using the SPSS application. In this study there were three treatment groups, each group had 3 repetitions with concentrations of 12.5%, 25%and 50% respectively. Each repetition is observed in minutes for 24 hours. The results obtained at a concentration of 25% had the highest

larval death rate with a percentage of 50.6%, a concentration of 50% with a percentage of 29.3%, then at aconcentration of 12.5% with a percentage of 9%.

***Keywords**: DHF, Aedes aegypti, Biolarvicide, Mortality*

INTRODUCTION

Dengue hemorrhagic fever (DHF) is a viral infection transmitted by the *Aedes aegypti* mosquito that usually occurs in tropical and subtropical climate (Maulana *et al.*, 2021). World Health Organization (WHO) notes that dengue fever tends to peak during and after the rainy season. There are several factors that influence the spread of dengue fever, including rainfall, humidity, mosquito population, and the incubation period of the dengue virus (Marcellia, Ulfa and Azizah, 2022). Viruses transmitted by mosquitoes can be treated with herbs taken from plants that contain chemicals (biologically active). The way to control *Aedes aegypti* larvae is by using synthetic insecticides such as temidol, a mosquito killer. Long term use of synthetic insecticides can lead to insecticide resistance in mosquito larvae (Rusli Abdullah *et al.*, 2023).

The use of synthetic insecticides can have harmful effects, poisoning organisms and damaging the environment. These effects can be reduced by using natural larvicides to control *Aedes aegypti* larvae (Anita Dyah Listyarini and Erni Rosiyanti, 2021). New insecticides are being developed that are more environmentally friendly and less harmful. The use of bioinsecticides derived from plants that contain chemicals that can poison insects but are easily decomposed by humans in nature (biologically active). Natural insecticides derived from plants are good materials to be developed because of their potential in controlling disease vectors. The power of natural insecticides comes from the toxic substances contained in plants. These substances act as stomach poisons and contact poisons (Marcellia, Ulfa and Azizah, 2022).

Vector control can be done through physical, chemical, environmental and biological means. Physical vector control uses physical materials to reduce the number of vectors, namely by changing the salinity or acidity (pH) of water bodies, setting traps, using electric beating devices, and others (Anggraini, Huda and Agushybana, 2023). Chemical vector control uses chemicals (insecticides) to reduce

vector populations. Vector control through environmental management, such as installation of water systems directly connected to the house, replacement of water storage containers that can become breeding habitats for mosquitoes, and biological vector control through the use of predators, parasites, and bacteria that act as natural enemies of mosquitoes, the causative agent of dengue fevers (Yupita,Frsilia and Indriani, 2022).

Some studies prove that the active compounds contained in arabica coffee leaf waste extract include flavonoids, alkaloids, saponins, polyphenols, quinones and terpenoid(Panuluh, 2020)(Wila and Nusa, 2020)(Azzahra, Narsa and Gama, 2023)(Aliyyu, 2023). Arabica coffee leaf extract contains active compounds in the form of flavonoids which are theoretically respiratory toxic to Aedes aegypti mosquitoes, so it has great potential as a natural mosquito repellent, and contains many active compounds in the form of polyphenols which can be toxic to Aedes aegypti larvae when in contact so that it has high larvicidal potential. (Amini, Santjaka and Setiawan, 2018)

RESEARCH METHODS

Time And Place Implementation

This research began in August 2023 and is planned to last until May 2024. Samplesof Ae. Aegypti for the larvicide efficacy test was taken from clean water reservoirsin people's homes in Banda Aceh City. Species identification and efficacy tests will be carried out at the Parasitology Laboratory, Faculty of Medicine, Abulyatama University.

Arabica Coffe Leaf Extraction

Extraction begins by preparing simplicia by chopping coffee leaf samples and drying them in an incubator at 37°C for 24 hours to reduce the water content. Thenmaceration was carried out by simply soaking it in 96% ethanol for 72 hours and storing it at a temperature below -10°C. The soaking results are filtered for evaporation. Evaporation was carried out at the Chemistry Laboratory at Syiah Kuala University, using certain method.

Testing of Arabica Coffee Leaf Phytochemical Compounds

Phytochemical content tests were carried out at the FKIP Chemistry Laboratory at Syiah Kuala University using reagents for each compound using certain method.

Granulation of Arabica Coffee Leaves

Method for making wet granulation of biolarvicide with the addition of ethanol as a solvent to obtain Arabica coffee leaf extract. The resulting extract in thick form after the evaporation process is mixed with ingredients in the form of aqua bidestillate, lactose, magnesium stearate, polyvinyl piropidol/PVP so that they bindtogether and form lumps, then molded using a 20 mesh sieve. Then an oven is carried out with the aim of evaporating the water content and organic solvent to form dry granulations. The drying temperature is 40^0C for 8 hours.

Test the Efficacy of Arabica Coffee Leaves

Efficacy tests were carried out to obtain LT50.95 and LC50.95 values. The test used a Completely Randomized Design (CRD) with each concentration in a stratified test, namely 12.5%, 25% and 50%. The test was carried out by placing 25 test larvaein an extract concentration solution of 50 ml for each repetition. Observation of larval mortality started from 10, 20, 30, 40, 50, 60, 2 hours, 4 hours, 6 hours, 8 hours and 24 hours after contact. If the control mortality is more than 10%, it will be corrected using the Abbot formula (Susanti and Boesri, 2012).

Data Analysis

Analysis of $LC_{50,95}$ and $LT_{50,95}$ in probit regression and linear regression analysis using the SPSS application. Granule evaluation is analyzed univariately and interpreted in tabular form.

RESULT AND DISCUSSION

Phytochemical Test Results

After testing Arabica coffee leaf extract, positive results were obtained for alkaloids, saponins, flavonoids, quinones, polyphenols and triterpenoids.

Efficacy Test Result

Tabel 1. Average and Percentage Mortality of Aedes Aegypti Larvae at Various Concentrations of Ethanol Extract of Arabica Coffee Leaf.

Concentration (%)	Treatment	Sample	Mean and percentage of larval mortality after arabica coffe leaf extract exposure at minute												Mean mortality at 1440 ± standard deviation	Percentage of death at 1440th minute
			10	20	30	40	50	60	120	180	240	360	720	1440		
12.5	1	25	0	0	0	0	0	0	0	0	0	1	1	2	2,33 ± 0,58	9%
	2	25	0	0	0	0	0	0	0	0	0	1	1	2		
	3	25	0	0	0	0	0	0	0	0	0	1	1	3		
Total			0	0	0	0	0	0	0	0	0	3	3	7		
Average death per time			0	0	0	0	0	0	0	0	0	1	1	2,33		
\	1	25	0	0	0	0	0	0	0	1	1	3	7	13	12,67 ± 0,58	50,6%
25	2	25	0	0	0	0	0	0	0	2	2	5	5	13		
	3	25	0	0	0	0	0	0	0	0	1	3	5	12		
Total			0	0	0	0	0	0	0	3	4	11	17	38		
Average death per time			0	0	0	0	0	0	0	1	1,33	3,67	5,67	12,67		
50	1	25	0	0	0	0	0	0	0	2	2	3	6	8	7,00 ± 1,00	29,3%
	2	25	0	0	0	0	0	0	0	1	2	4	5	6		
	3	25	0	0	0	0	0	0	0	1	2	3	5	7		
Total			0	0	0	0	0	0	0	1	2	3	5	7		
Average death per time			0	0	0	0	0	0	0	1,33	2,00	3,33	5,33	7,00		

The research consisted of three treatment groups as shown in table one. Each group was repeated three times, with concentrations of 12.5%, 25%, and 50%, respectively. Observations were made every minute for 24 hours. The highest larvalmortality rate was found at 25% concentration with a percentage of 50.6%. The 50% concentration produced a mortality rate of 29.3%, while the 12.5% concentration produced a mortality rate of 9%.

Linear Regression Analysis Results

The assumption test results must have been done, so the probit data generated in this study can be continued with a simple linear regression test.

Tabel 2. Regression test results of the effect of concentration on probit.

concrentration (%)	Melliliters (ml)	Mortality	Mortality (%)	probit	P_value	Regression equation	R^2
12,5	0,625	2	8	3.5949	0,013	Y= 3,971+1,355x	0,380
12,5	0,625	2	8	3.5949			
12,5	0,625	3	12	3.8250			
25	1,25	13	52	5.0502			
25	1,25	13	52	5.0502			
25	1,25	12	48	4.9408			
50	2,5	8	32	4.5323			
50	2,5	6	24	4.2937			
50	2,5	7	88	4.4172			

Based on the table above, coffee leaf ethanol extract is effective as a larvicide against dengue fever vectors with a sig value <0.05 and the effect is 0.380, meaningthat coffee leaf ethanol extract has a positive effect on larval mortality by 14.4%. The model obtained is Y = 3.971 + 1.355x, meaning that if the concentration increases by one unit, the percentage of larvicide mortality rate increases. Based on this, it appears that the ethanol extract of coffee leaves has less acute toxicity and isincluded in the toxic criteria in the weak category.

Tabel 3. Regression test results of the effect of time on probit

Time	Probit		
	Group 1	Group 2	Group 3
10	0	0	0
20	0	0	0
30	0	0	0
40	0	0	0
50	0	0	0
60	0	0	0
120	0	0	0
180	0	3.2493	2.7738
240	0	3.3836	3.05569
360	3.2493	3.9463	3.2493
720	3.2493	4.2479	3.4027
1440	3.6775	5.1050	3.6775
P-Value	0,000	0,002	0,006
regression equation	Y=0,003+0,003x	Y = 0,568+0,004x	Y = 0,541+0,003x
R^2	0,848	0,802	0,741

Based on the table above, ethanol extract of coffee leaves effectively controls dengue fever vectors as a larvicidal agent with a sig value <0.05. The intensity of influence in treatment group 1 was 0.848 (72.0%), in treatment group 2 was 0.802 (64.4%), and in treatment group 3 was 0.741 (54.9%). This means that the ethanol extract of coffee leaves has a strong influence on larval mortality at any time, so that the longer it is used, the higher the larvicide mortality rate. The model obtainedis Y=0.003+0.003x, Y=0.568+0.004x, Y=0.541+0.003x which means that if one unit of time is added, the percentage value of the probability of larval death will also be

greater. From this point of view, the ethanol extract of coffee leaves has acute toxicity and is included in the standard of severe toxicity.

Results of Probit Analysis

Tabel 4. Results of Probit Analysis of Lethal Time (LT) 50.95 and Lethal Concentration (LC) 50.95 for Ethanol Extract of Coffee Leaf

CONCENTRATION %	P-VALUE	LT	LC
12,5	**P1**= 0,000	**LT50:** 1537.509501 **LT95:** 2598.217555	LC 50: 0.183417 LC 95: 0.580861
25	**P2**= 0,000	**LT50**: 395.515414 **LT95**: 664.002432	
50	**P3**= 0,000	**LT50**: 1476.308417 **LT95**: 2674.716398	

Based on the table, it can be explained from the three treatment groups, the results obtained that within 24 hours of the experiment in treatment group 1 with a concentration of 12.5%, the estimated value of LT50 = 1537.509501 and LT95 = 2598.217555, meaning that to kill 50% of 75 larvae takes ± 64 hours, while to kill 95% larvae takes ± 108 hours. Furthermore, in treatment group 2 with a concentration of 25%, the LT50 = 395.515414 and LT95 = 664.002432 values were obtained, meaning that to kill 50% of 75 larvae takes ±16 hours, while to kill 95% larvae takes ± 27 hours. In treatment group 3 with a concentration of 50%, the LT50 = 1476.308417 and LT95 = 2674.716398 values were obtained, meaning that to kill50% of 75 larvae takes ± 61 hours, while to kill 95% larvae takes ± 111 hours. Thepercentage of concentration which is needed to kill 50% of larvae is 18.34%, and to kill 95% of larvae is 58.06%. Thus, it can be concluded that the high and low percentage of concentration used will affect the death of larvae. The results of statistical testing with a confidence level of p (0.05) obtained based on significant time (p-value < 0.05) and significant oncentration can be known from the p-value
< 0.05.

CONCLUSION

The ethanol extract of Arabica coffee leaves is proven to have potential as a biolarvicide with its compounds until there is mortality of Aedes aegypti larvae. The utilization of Arabica coffee leaves in the future can be a new product so that it is more beneficial for education and society. Based on data analysis in this study, it can be explained that this study has three treatment groups, each group has 3 repetitions with concentrations of 12.5%, 25%, and 50% respectively. Each replicate was observed in minutes for 24 hours. The results obtained at a concentration of 25% the highest larval mortality rate with a percentage of 50.6%,50% concentration with a percentage of 29.3%, then at a concentration of 12.5% with a percentage of 9%.

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