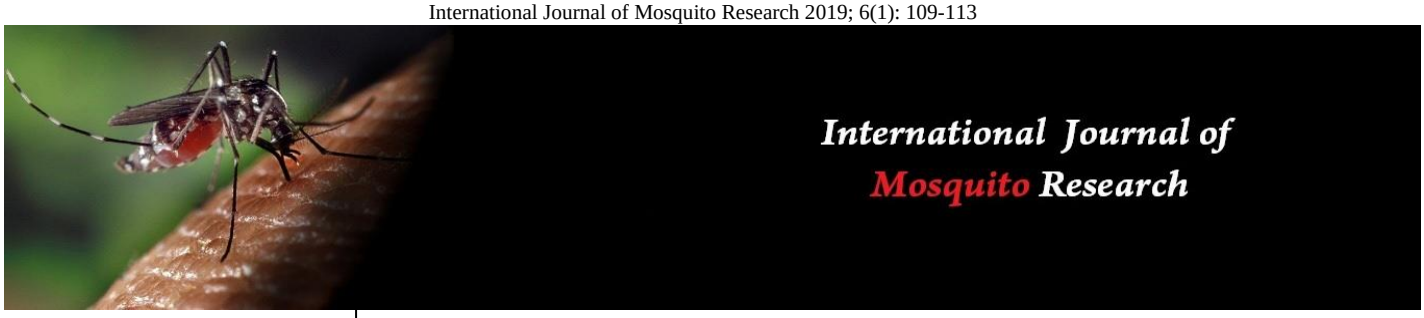

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Optimizing of maseration with ethanol and water solvents against the toxicity of extract of wuluh starfruit (*Averrhoa bilimbi* L.) in controlling larva of *Aedes aegypti*

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Abstract
The control of *Aedes* sp. mosquito need to be conducted appropriately and environmental friendly. Wuluh starfruit (*Aaverrhoa bilimbi l.*) contains alkaloids, saponins, and flavonoids, can be used as insecticides. In its usual use through maceration, good maceration will produce good toxicity as well as the death of *Aedes aegypti* larvae. In this study an optimization of the composition of ethanol and water was carried out as a dancer in the maceration process, on fruit and tested for toxicity to *Aedes* mosquito larvae. Aim: To determine the toxicity of carambola fruit in killing *Aedes aegypti* larvae using the difference maceration method on the ratio of ethanol-water dancers (100% -0%, 75% -25%, 50% -50%, 25% -75% and 0% -100%).
Materials and Methods: This was an experimental study, the object of the research was the extract of Wuluh starfruit and the difference in maceration on the spread between ethanol and water. Mortality data were analyzed by ANOVA, if there was a real effect of the treatment, it was followed by LSD test, and phytochemical analysis of Wuluh starfruit.
Results and Discussions: The results obtained by extract of carambola fruit (*Averrhoa bilimbi l.*) The highest number of dead mosquitoes was obtained through an extraction process with the composition of ethanol and water solvents with a ratio of 100%: 0% with an average number of deaths of 25 (100%), significant influence on the average mortality of *Aedes aegypti* larvae from various variations of ethanol and water solvents in the extraction of fruit and wuluh starfruit leaves (*Averrhoa bilimbi l.*).

Keywords: maseration, wuluh starfruit extract, *Aedes aegypti,* water solvent

Introduction
Dengue hemorrhagic fever (DHF) is a public health problem in Aceh province as well and one of DHF endemic province in Indonesia. In 2017, the total number of cases was 2,591 cases (incidence rate/IR: 49.93 per 100,000 population), and 12 were dead (case fatality rate/CFR: 0.46%). The IR was high compare to the national DHF program (<49 per 100,000 population) [1, 2]
Aedes aegypti is a mosquito that can act as a vector of various diseases including DHF [3] The main preventive way to control DHF cases by controling *Aedes aegypti* [4, 5] The control can be conducted on adult mosquitoes or larva [6] Control of larvae can be done by chemical, biological, and physical methods. One of the chemical eradication that can be done is by larvacide [7]
Indonesia has a diversity of plants that have active ingredients as natural larvacides, one of them is Wuluh starfruit (*Averrhoa bilimbi l.*) It is usually used as traditional medicine, not as larvacide. Secondary compounds produced by alkaloids, saponins, and flavonoids [8] Bioactive compounds contained in plants can be used as a synthetic insecticide. The difference is that the active ingredients in vegetable insecticides are synthesized by plants and the types can be more than one type (mixture) [9, 10] In Aceh Province, Wuluh starfruit is a plant which is widely planted in Aceh, because the fruit is a typical mixture in the whole of Aceh. The fruit is abundant and very easy to get in the province.
The study conducted by Nopianti *et al*. concluded that extracts of Wuluh starfruit affected the mortality rate of *Anopheles aconitus* instar III with a dose of 4.5% by 100%.[11] Oktavia's study 2012) also concluded that there was an effect of Wuluh starfruit extract as *Aedes aegypti* larvacide with a 3% dose of (larval mortality as much as 100% [5].

The study continued by Lisa concluded that the most effective concentration of Wuluh starfruit extract (*Averrhoa bilimbi l.*) in the form of granules 200 mg / 100 ml with 100% larvae death from the overall test larvae with LC50 values of 91.667 mg and LC90 values of 142.399 mg. [12]

The technique for getting plant extracts can be done by several methods such as maseration and extraction [13]. The chemical compound of plants can be obtained by extraction. The principle of this extraction is to separate the components in the material extracted using a particular solvent [14]. In the search process it is determined by using fluids such as water, ethanol, a mixture of water-ethanol, or ether [15]. To improve the search, the taken steps are by using a mixture of water and ethanol.

This chemical compound was different from the research conducted by Roy A, *et al*. [16] This is because there are several factors that influence differences in the active ingredient content of medicinal plants, namely the geographical location or place to grow plants, climate, cultivation methods, the way and time of harvesting, drying methods and storage methods. [17] In addition, the extraction method and the type of solvent used also affect the chemical content in the extract. Saponins are a class of triterpennoid compounds that can be used as insecticides [18]

Extraction by using solvents such as ethanol, methanol, ethyl acetate hexane and water can separate important compounds in the material. The selection of solvents to be used in the extraction process must consider the nature of the content of the compounds to be isolated. Important properties are polarity and polar groups of a compound. In principle, a material will easily dissolve in the solvent with the same polarity [19] so it will affect the physicochemical properties of the extract produced. The extraction method used is also thought to affect the physicochemical properties of the extract. This study aims to determine the toxicity of extract of starfruit leaves (*Averrhoa bilimbi l.*) in the maceration process with ethanol-water runners (100% - 0%, 75% - 25%, 50% - 50%, 25% - 75%, and 0% - 100%) in killing larvae of *Aedes aegypti*.

Materials and Methods

This was a quasi experimental with a post-test only design with control group design. The object of the research was the fruit and leaves of Wuluh starfruit obtained from the Aceh Besar district. The method of making extracts by macerating with ethanol and water solutions. The research subject was larvae of *Aedes aegypti* that were hatched. The sample size for each treatment was 30, with consideration for experiments using 25-30 grains of larvae in each treatment. [20] According to Supranto, the control group and treatment group each received 5 times repetition for each test. [21] The dosage used based on the Bagas with a 4.5% dose of starfruit extract with *Aedes* larvae mortality of 100%. [15]

Wuluh starfruit were washed with running water to remove dirt. The fruits cut into pieces and dried without drying directly in the sun. After drying, it blended with a blender and then poured into prepared glass jars. The simplicia powder was weighed as much as 100 grams and then put in a glass jar on each powder result on a different jar. The maceration process can be shown in Table 1.

Table 1: Percentage (%) of ethanol-water composition in the extraction process [22]:

Trials	Etanol	Water
I	100% (750 ml)	0% (0 ml)
II	75% (562.5 ml)	25% (187.5 ml)
III	50% (375 ml)	50% (375 ml)
IV	25% (187.5 ml)	75% (562.5 ml)
V	0% (0 ml)	100% (750 ml)

The liquid stored for 5 days and protected from light by stirring occasionally. After 5 days the marinade was filtered through a glass funnel coated with filter paper, so the pulp and juice are separated. Dregs are added to ethanol and then stirred and dispersed until extracts of 1000 mL are obtained. The juice was then stored in a closed container and protected from light for 2 days. After 2 days, the extract of ethanol extract was concentrated using a vacuum rotary evaporator at a temperature of 40-50^{o}C until a thick extract was obtained [23].

Data analysis by calculating the percentage of death test larvae and data obtained from the calculation of the simplex lattice design (prediction) compared with the actual test data was analyzed by ANOVA in one direction to find out whether there were significant differences between treatments. If the results of the significant ANOVA test, $p<\alpha$, then proceed with the last significant difference test (LSD), and testing of the secondary metabolic content of the extraction was carried out at the organic chemistry instrument laboratory.

Results

The results of the observations at the time of the study carried out four repetitions of the treatment of larvae of *Aedes aegypti* for 24 hours based on the dosage levels used with different ethanol-water sprays on the star fruit (*Averrhoa bilimbi l.*) in killing of larvae of *Aedes aegypti*. The number of deaths of larvae of *Aedes aegypti* can be shown in Table 2:

Table 2: Number of deaths of larvae of *Aedes aegypti* against differences in ethanol spreaders - Wuluh starfruit water *(Averrhoa bilimbi l.)*

Treatment	No. of larvae of *Aedes*	Repitition					Total	Average	%
		1	2	3	4	5			
		N	N	N	N	N			
Averrhoa bilimbi l.									
I	25	25	25	25	25	25	125	25	100
II	25	21	24	24	24	23	116	23	92,8
III	25	21	21	23	22	22	109	22	87,2
IV	25	20	20	21	23	21	105	21	84
V	25	15	16	17	17	20	85	17	68
VI	25	0	0	0	0	0	0	0	0

- I : 100%-0% (etanol – water)
- II : 75%-25% (etanol – water)

- III : 50%-50% (etanol – water)
- IV : 25%-75% (etanol – water)
- V : 0%-100% (etanol – water)
- VI : control

Based on Table 2, the highest number of dead mosquitoes were obtained through an extraction process with a composition of ethanol and water with a ratio of 100%: 0% (on each experiment) with an average the number of deaths is 25 (100%). The larval mortality data were then processed using ANOVA to find out whether the treatment with variations in etaonol and water solvents at different extractions would produce different mosquito larvae deaths. The test results for each Wuluh starfruit (*Averrhoa bilimbi L*.) are presented in Table 3:

Table 3: Anova test results on the average mortality of *Aedes aegypti* larvae with differences in optimization of ethanol and water solvents for Wuluh starfruit extract *(Averrhoa bilimbi l.)*

Variable	Average	SD	SE	95% CI		P value
				Lower	Upper	
100%-0%	25.00	0.000	0.00	25.00	25.00	0,000
75%-25%	23.20	1.304	0.58	21.58	24.82	
50%-50%	21.80	0.837	0.37	20.76	22.84	
25%-75%	21.00	1.225	0.55	19.48	22.52	
0%-100%	17.00	1.871	0.84	14.68	19.32	
Control	0	0	0	0	0	
Total	21.60	2.944	0.59	20.38	22.82	

Based on Table 3, the ANOVA test results obtained P value = 0,000 (p<0,05) which means that there is a significant effect on the average mortality of *Aedes aegypti* larvae from various ethanol and water solvents in extracting the Wuluh starfruit (*Averrhoa bilimbi l.*). Then, LSD continued testing to determine the most effective dose. The results of the LSD test are presented in Table 4 below:

Table 4: LSD test results on the average number of deaths of *Aedes aegypti* larvae by using different ethanol and water solvents for extracts of Wuluh starfruit

(I) % extract etanol – water	(J) % extract etanol - water	Mean difference (I-J)	SE	p value	95% Confidence Interval	
					Lower	Upper
100%-0%	75%-25%	1.800*	0.769	0.030	0.20	3.40
	50%-50%	3.200*	0.769	0.000	1.60	4.80
	25%-75%	4.000*	0.769	0.000	2.40	5.60
	0%-100%	8.000*	0.769	0.000	6.40	9.60
75%-25%	100%-0%	-1.800*	0.769	0.030	-3.40	-0.20
	50%-50%	1.400	0.769	0.084	-0.20	3.00
	25%-75%	2.200*	0.769	0.010	0.60	3.80
	0%-100%	6.200*	0.769	0.000	4.60	7.80
50%-50%	100%-0%	-3.200*	0.769	0.000	-4.80	-1.60
	75%-25%	-1.400	0.769	0.084	-3.00	0.20
	25%-75%	0.800	0.769	0.311	-0.80	2.40
	0%-100%	4.800*	0.769	0.000	3.20	6.40
25%-75%	100%-0%	-4.000*	0.769	0.000	-5.60	-2.40
	75%-25%	-2.200*	0.769	0.010	-3.80	-0.60
	50%-50%	-0.800	0.769	0.311	-2.40	0.80
	0%-100%	4.000*	0.769	0.000	2.40	5.60
0%-100%	100%-0%	-8.000*	0.769	0.000	-9.60	-6.40
	75%-25%	-6.200*	0.769	0.000	-7.80	-4.60
	50%-50%	-4.800*	0.769	0.000	-6.40	-3.20
	25%-75%	-4.000*	0.769	0.000	-5.60	-2.40

*. The mean difference is significant at the 0.05 level.

Based on Table 4, only the ethanol and water solvents 50%: 50% compared to 75% -25% not significant with a value of p = 0.084> of the alpha value 0.05. and with 25%: 75%, p value = 0.311. Whereas in other solvents the p value < 0.05. The death graph can be shown in figure 1.

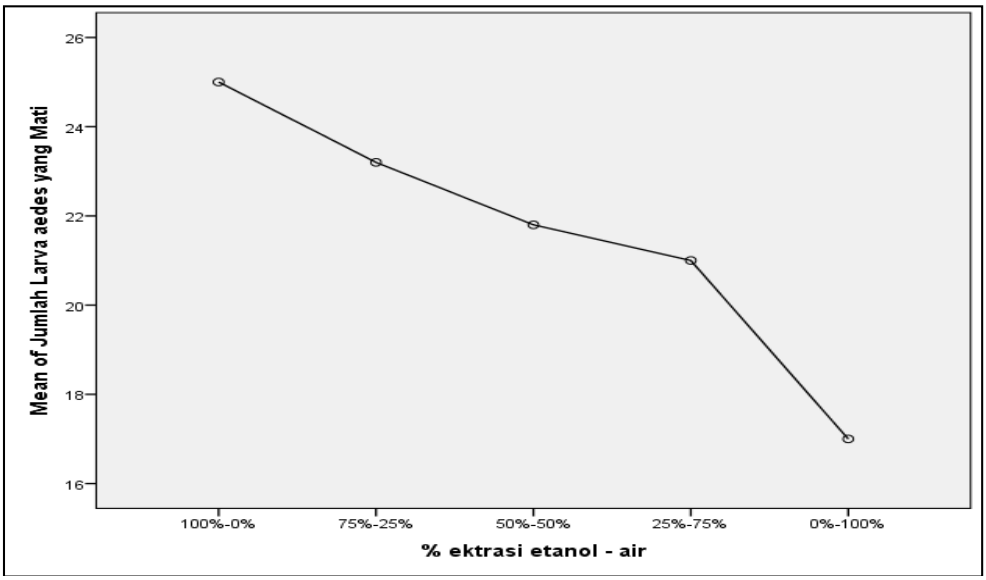

Fig 1: Graph of concentration of the death response of *Aedes aegypti* larva at various types of extractive solvents after 24 hours treatment with Wuluh starfruit

Based on Figure 1, it can be seen that the higher ethanol solvent in the fruit and leaves of the starfruit (*Averrhoa bilimbi l.*) causes a high percentage of *Aedes aegypti* larvae deaths. The test results on the killing power based on the simplex lattice design approach are as follows:

Table 5: SLD equation

Extract	SLD Equation
Wuluh starfruit	Y=25(A) + 17 (B) + 4 (A)(B)

(A) : the value of death at 100%-0% (etanol – water)
(B) : the value of death at 0%-100% (etanol – water)

The above equation shows that in starfruit with ethanol: water (100% -0%) solvent with (coefficient = 25) gives a greater influence on the mortality of *Aedes aegypti* larvae compared to ethanol: water solvent (0% -100%) with (coefficient value = 17). However, the solvent interaction between ethanol: water (50%: 50%) has a large effect (coefficient value = 4) ethanol and water only.

Discussions

The results of the study show that the more ethanol solvents to make extracts from the fruit and leaves of Wuluh starfruit (*Averrhoa bilimbi l.*), the more larvae die. The experiment by using of Wuluh starfruit extracct (*Averrhoa bilimbi l.*) resulted that the highest number of dead larvae of *Aedes aegypti* obtained through an extraction process with the composition of ethanol and water solvents with a ratio of 100%: 0% (in each experiment) with an average number of deaths of 25 (100%). By the leaves of Wuluh starfruit (*Averrhoa bilimbi l.*) resulted that the highest number of dead larvae of *Aedes aegypti* obtained through the extraction process with the composition of ethanol and water solvents with a ratio of 100%: 0% (in each experiment) to the number of deaths of 18 (73.6%). Soluble phenol compounds are affected by the solvent polarity while the antioxidant power of the solvent is influenced by compounds which are soluble in the mixture. Antioxidant compounds besides phenolics include alkaloids, vit. C, vit. E, beta carotene. and others.

Research conducted by Nopianti *et al*. resulted that the extract of Wuluh starfruit affecting the mortality rate of *Anopheles aconitus* instar III larvae with a 4.5% dose of 100%. [24] Another research conducted by Oktavia also concluded that there was an effect of Wuluh starfruit extract as larvacide of *Aedes aegypti* with a 3% dose of larval mortality as much as 100%. [5] The study continued with Lisa (2015) with the concentration of the most effective starfruit extract (*Averrhoa bilimbi l.*) in the form of granules 200 mg / 100 ml with 100% larvae death from all test larvae with LC50 values of 91,677 mg and LC90 values of 142,399 mg. [25]

The content of these chemicals that affect the difference in the active ingredients of medicinal plants. namely the geographical location or place to grow plants. climate. how to cultivate. the way and time of harvest. drying methods and storage methods. [17] Other than that. extraction methods and types of solvents used also affect the chemical content in extracts [26] Extraction using solvents such as ethanol, methanol, ethyl acetate hexane and water are able to separate important compounds in a material. The selection of solvents to be used in the extraction process must consider the nature of the content of the compounds to be isolated. Important properties are polarity and polar groups of a compound. In principle, a material will easily dissolve in the solvent with the same polarity [19] so that it will affect the physicochemical properties of the extract produced. The extraction method used is also thought to affect the physicochemical properties of the extract. So that in this study resulted in the death of *Aedes* larvae was greater in extracts with ethanol than the dancer from water. This is in consistent with research that states that the higher the larvicidal dose given, the higher the average mortality of Anopheles aconitus mosquitoes. It can be said that the death of the test larvae is due to the content of chemical compounds in the juice of the star fruit (*Averrhoa bilimbi l.*). The chemical compounds in the wuluh starfruit (*Averrhoa bilimbi l.*) consist of alkaloids. saponin. and flavonoid [10] Based on Mawuntyas' research resulted that alkaloids can also be used as insecticides [27] Alkaloids in fresh leaves or fruit taste bitter on the tongue. Alkaloid in the form of salt can degrades the into cell wall and damage it. Saponins are a class of triterpennoid compounds that can also be used as insecticides. Saponins are found in plants which are then consumed by insects. having a working mechanism can reduce the activity of digestive enzymes and absorption of food. It is a stomach poison. Flavonoids are phenol compounds as anti-microbial, anti virus, antifungal and work against insects. In this study fruit extracts and leaves of Wuluh starfruit (*Averrhoa ilimbi l.*) were using by extraction method.

Ethanol solvents are universal solvents that are able to bind all chemical components found in natural material plants, both non-polar, semi polar, and polar. Methanol is a fluid that easily enters the cell through the material cell wall. The secondary metabolites found in the cytoplasm and will be dissolved in the solvent and the compound will be fully extracted. [28] The high yield of matoa leaf extract with methanol solvent showed that ethanol solvent on the leaves of matoa was able to extract compounds better because the acquisition of compounds is based on the similarity of the polarity of the solvent. Viewed from the data above, the matoa leaves extracted with methanol solvent had the highest total flavonoids compared to other solvents. This is due to the ability and nature of the solvent to dissolve different flavonoids. depending on the level of polarity of the solvent and the extracted compound. According to the principle of polarization. a compound will dissolve in a solvent which has the same polarity [18].

Conclussions

The composition of ethanol and water solvents with a ratio of 100%: 0% was the highest number of dead *Aedes aegypti* larvae against the Wuluh starfruit extract (*Averrhoa bilimbi l.*) with an average number of deaths 100%. There was a significant effect on the average mortality of *Aedes aegypti* larvae from various variations of ethanol and water solvents in extracting the star fruit (*Averrhoa bilimbi l.*) (p value = 0.000 (p<0.05).

Suggestion

In making extracts of Wuluh starfruit, just added water because it is already effective to kill the *Aedes aegypti* larvae.

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