Conference Paper

The Effectiveness of Citrus Hystrix As Rapelant against Aedes Aegypti

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Abstract

Indonesia is the country with the highest cases of dengue fever in Southeast Asia. The WHO estimates a 50–100 million dengue infections worldwide every year. Dengue Hemorrhagic Fever (DHF) is transmitted through the bite of female Aedes mosquitoes infected with the Dengue virus. Mosquitoes *Aedes aegypti* is the main vector of the dengue virus. At the time, there is no cure for dengue so that the control efforts are focused on breaking the chain of life cycles. One effort to prevent the transmission of the dengue virus is to avoid vector mosquito bites. Repellent can reduce exposure to the bite of mosquitoes infected with the dengue virus. The aim of this study was to determine the effectiveness of the extract of *citrus hystrix* leaf as a repellent against the *Aedes aegypti* mosquitoes. This research is a laboratory experimental study with a one-shot case study design. In this study, the extract of *citrus hystrix* solution was made at a concentration of 10%, 20%, and 30%, and then the extract solution was used as a stock to make a 100-gram base lotion that would be used as a repellent. Repellent effectiveness is seen from the percentage of repellent protection power. The percentage of repellant protection used with the basic ingredients of *Citrus hystrix* leaf extract at concentrations of 10%, 20%, and 30%, respectively, at 93.33% 94.67%, and 97.33%. The extract of *citrus hystrix* leaf was found to be effective as a repellent against *Aedes aegypti* mosquitoes.

Keywords: Citrus hystix leaf, *Aedes aegypti*, repellant

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1. Introduction

Dengue Hemorrhagic Fever (DHF) is a very important health problem in the world. Dengue Hemorrhagic Fever was first reported by in 1779 in Asia, Africa and North America in the medical literature in 1780 (Tawatsin & Thavara, 2010). At present, DHF is an Endemic disease in more than 100 countries in Africa, America, the Eastern Mediterranean, Southeast Asia and the Pacific (WHO, 2012). Dengue fever is found in tropical and sub-tropical regions. Data from all over the world shows Asia ranks first in the number of dengue sufferers each year (WHO, 2012). Indonesia is the country with the highest cases of dengue fever in Southeast Asia. Indonesia is one of the countries in the world that has a tropical climate with a total of 156,086 cases of dengue in

Indonesia with 1,358 deaths due to dengue. Thus the morbidity rate (Incidence Rate = IR) of Dengue Hemorrhagic Fever is 65.7 per 100,000 population and the mortality rate (Case Fatality Rate = CFR) is 0.87%.

The prevalence of this disease has developed very rapidly in the last decade. It is estimated that around 50 million - 100 million people are infected with the dengue virus which results in dengue and 22,000 deaths each year, especially among children. Dengue hemorrhagic fever is caused by the dengue virus which consists of four dengue virus serotypes namely DENV 1, DENV 2, DENV 3, DENV 4 (Tawatsin & Thavara, 2010). Dengue is transmitted by the bite of female Aedes mosquitoes infected with the Dengue virus. *Aedes aegypti* is the main vector of the dengue virus (CDC, 2012). Factors that influence the increase and spread of dengue cases include the absence of effective mosquito vector control in endemic areas and increased transportation facilities (Gubler, 2010). At present there is no cure for dengue so that denguecontrol efforts are hemorrhagic feverfocused on terminating the chain of life cycles.

Aedes aegypti mosquito is the most efficient vector mosquito for dengue because of its domestic habits. Ae mosquito. Aegypti acts as a DHF vector in urban areas (in homes) while Ae. Albopictus acts as a DHF vector in rural areas (outside the home) (Nimmanitya, 2003). Morbidity and mortality of dengue virus infection are influenced by various factors including host factors, namely vulnerability and immune response, environmental factors, namely geographical conditions and demographic conditions, the viral factor itself and the type and density of mosquitoes as vector transmitters of the disease.

Southeast Sulawesi Province with a population of 2,417,962 there are a number of cases of DHF 838 with an Incidence Rate of 34.66 and the number of people who died as much as 0.95% (Ministry of Health, 2015). Kendari City is a city with an area of 300.89 $km^2$and an area with the highest number of cases of Dengue Fever in Southeast Sulawesi and cities with high endemicity status. Kendari City is a city consisting of 10 sub-districts with a total of 64 kelurahan among these villages with 57.81% of villages with an Endemic category with a number of DHF cases for 3 consecutive years averaging over 3 cases, and 42.18% with regional categories or Non Endemis Village.

Based on data from the Kendari City Health Office reporting dengue cases in 2010 amounted to 168 cases with an incidance rate: 0.57%, in 2011, 33 cases with an incidence rate of 0.10% and in 2012 became 100 cases with an insidance rate of 0.32 % (Kendari City Health Office, 2012). Besides the data of Kendari City Health Office Reporting on DHF cases in 2013 were 231 cases with incidance rate of 0.73% with the highest dengue cases namely Tobuuha Village, Puuwatu District with 19 cases, Bonggoeya Village, Wua

- Wua District with 14 cases and in 2014 as many as 30 cases with an incidence rate of 0.10% with the highest DHF cases, namely Kambu Sub-District Kambu District with 6 cases following Podambea Sub-District Kadia District with 3 cases and in 2015 with 78 cases with an insidance rate of 0.26% with the highest DHF cases namely Tobuuha Sub-District, Puuwatu Sub-district with 10 cases following the Podambea Sub-District of Kadia Sub-District with 6 cases which prove that the DHF vector chain termination program has not been effective in Kendari City (Kendari City Health Office, 2015). Based on data from 2013-2015, the regions with the highest DHF were Tobuuha Sub-District, Puuwatu Sub-district with 24 cases, followed by Bonggoeya Sub-District Wua-Wua District with 18 cases, Podambea Sub-District with 17 cases, Watulondo Sub-District, Puuwatu District with 13 Cases and Mandonga and Sub-Districts. Guava with 12 cases, with a case fatality rate of 0%. Data from Kendari City Health Office reported that throughout 2013 - 2015 out of ten Subdistricts, only one Subdistrict was not an endemic area of DHF, Abeli Subdistrict, followed Kendari District with a slight prevalence of Endemic DHF.

Currently the dengue control effort that has been carried out in Kendari City is instructing the community to implement a Clean and Healthy Life Behavior (PHBS) pattern. But dengue cases in the city of Kendari from year to year are still high. One effort to prevent the transmission of dengue virus is to avoid vector mosquito bites. *Repellent* can reduce exposure to mosquito bites that may be infected with the dengue virus (Kazembe*et al*., 2012).

Repellent that is widely used by the public is *repellent* synthetic. An example of *repellent* syntheticis*N, N*-diethyl-meta-toluamide (DEET) which is used to reject mosquitoes. Many reports on the toxicity of DEET, ranging from mild effects, such as urticaria and skin eruptions, to severe reactions, such as *toxic encephalopathy* (Tawatsin, 2006). This has led to the need for research and development of *repellents* naturalderived from plant derivatives.

One plant that is often used by the public as a *repellent* is kaffir lime (*Citrus hystrix*). Lime leaves contain several active compounds are reported to have activity mosquito repellent*(repellent)*aschampene, *pinene,*alkaloids, terpenoids, saponins, and sineol (Widiani, 2012; Maia, 2011). Some previous studies showed that citrus plants have activities as a *repellent*.

This study aims to determine the effect of citrus leaf extract (*Citrus hystix*.) As a *repellent* on *Aedes aegypti mosquitoes*.

2. Methods

This study is astudy laboratory experimentalbased on procedures recommended by the *World Health Organization Pesticides Evaluation Scheme* (WHOPES, 2009) with a *one-shot case study* design, namely a research design with treatment of independent variables followed by observation or measurement of independent variables (Sugiyono, 2011). This study uses a completely randomized design.

The materials used were *Aedes aegypti mosquitoes* femaleand mosquito eggs obtained from the Laboratory of Health Polytechnic Department of Kendari Ministry of Health, aquades, larvae in the form of fish pellets, mosquito feed in the form of sugar water, kaffir lime leaves, ethanol 96% as solvents extract of kaffir lime leaves, 70% alcohol as a thinner, gloves, and *syringe* 1 ml.

3. Research Procedure

3.1. Rearing mosquitoes

Aedes aegypti eggs obtained from the Department of Health Analyst were hatched in plastic tubs filled with distilled water as high as 3/4 plastic tubs. Hatched larvae are maintained until they become the adult mosquito stadium. After the stock of mosquitoes is obtained, female mosquitoes are selected from the stock population to separate them from male mosquitoes. Female mosquitoes are transferred to the test cage using an aspirator.

3 cages of test mosquitoes are needed, with 25female *Aedes aegyptimosquitoes* in each mosquito reservoir. Mosquitoes are maintained until they reach the age of 5-7 days *post-emergence*, during which time mosquitoes are fed a sugar solution (WHOPES, 2009).

3.2. Preparation of Test Solution

Citrus hystrix leaves are dried at room temperature and smoothed using a *blender*. Leaves of crushed kaffir lime dissolved with 96% ethanol. The maceration results are then evaporated with the evaporator. The evaporation results are a *stock solution* which is then diluted with 70% alcohol to obtain a concentration of 10%, 20%, and 30% in 1 ml.

3.3. Making lotion as a rapellant

From a stock solution of 10%, 20%, 30% lime extract, then 5 ml was taken and mixed with a lotion base to 100 grams. Stir until homogeneous until evenly formed lotion consistency. The result is obtained a lotion of 10%, 20%, 30% *Citrus hystrix*.

3.4. Testing Repellent

testing *Repellent* is based on WHOPES (2009) which extract lime leaves will be applied to the forearm of volunteers. Before and after the experiment each test area (forearm) was washed with soap and rinsed with water, then dried. The part of the hand is covered by gloves. First, the left arm as a control was applied with 1 ml of 70% alcohol and then put into a mosquito cage and observed and noted the number of mosquitoes perched within a period of 30 seconds. Within 30 seconds it will be ensured that the mosquitoes perch> 10 to begin testing. After 30 seconds the arm is carefully removed from the mosquito cage. Then the same arm is smeared with the lowest dose of 10% extract of kaffir lime leaves. Then put it back into the cage to be observed for 30 seconds. During testing, the test arm is attempted not to move. This procedure is repeated on the same arm for each dose increase. Tests were carried out sequentially and had to be carried out with each other without delay and thedose *repellent* for each test was calculated as the sum of the doses to get the cumulative dose on each test. At the end of the dosage test, 1 ml of alcohol was applied to the right arm and then dried for approximately 1 minute. The volunteers' right arm was inserted into the same cage to ensure that the number of mosquitoes perched on the arm ≥ 10 mosquitoes in the 30-second period.

WHOPES (2009) recommends that the test be carried out with a minimum of 3 repetitions. The second and third tests were carried out on different days, the next day at the same test time. The mosquitoes used in each test were different samples from the mosquito samples used in the previous tests.

3.5. Data Analysis

At the end of the testing the percentage of protection power was assessed as the proportion of mosquitoes perched on treatment with the number of mosquitoes perched on the control arm, calculated by the following formula:

Percentage of protection power (%) = $\frac{C-T}{C} \times 100\%$

Description: C = number contact Masquitoes in the control arm

T = number of contact mosquitoes in the treatment arm

4. Results

Table 1: Rapelant testing of the Aedes aegypti mosquito.

Concentration	Total Aedes aegypti perched on the arm		
	R1	R2	R3
10 %	2	1	2
20 %	2	1	1
30 %	1	1	0
Ket: R: Repetition			

Table 1 shows that the number of mosquitoes that perched on the arms during testing both on repetition 1, repetition 2 and repetition 3 only showed a small number (<of 5 mosquitoes that reached the arm per treatment).

The results of the percentage of rotection power can be seen in table 2 below:

Table 2: Percentage of protection power.

Dosis	Persentase daya proteksi			Rata rata
	R1	R2	R3	
10 %	92 %	96 %	92 %	93,33 %
20 %	92 %	96 %	96 %	94,67 %
30 %	96 %	96 %	100 %	97,33 %

Based on the results in table 2, it can be seen that the average protection power at a concentration of 10% is 93.33%. The average protection power at a concentration of 20% was 94.67% and the average protection power at a concentration of 30% was 97.33%.

5. Discussion

Based on the results of testing the rapellant protection against the number of *Aedes aegypti mosquitoes* that perch, it can be seen that the concentrations of 10%, 20% and 30% concentrations of the extract of kaffir lime leaves provide great protection against the resistance of *Aedes aegypti mosquito*. All protection power shows protection in the range of 93 to 97%. This means that the rapelant from the kaffir lime leaf extract lotion is effective in providing protection> 90%.

This research is in line with the research conducted by Joni (2013) who reported the protection power of orange peel extract against *Aedes aegypti* and *Aedes albopictus mosquitoes,* except that in the study the protection power was smaller at <50%. Research using other plants conducted by Kardinan (2007) reported the protection of basil leaves againstmosquitoes *Aedes sp* but the protection power was only 57.59%. Likewise the research conducted by Yuliasih (2010) which shows the percentage of protection power that is different from theplant *Ocinum sanctum* against *Aedes sp*. These differences in results indicate a difference in protection power between each plant. This difference in results is thought to be due to differences in research material as well as the response of different species ofmosquitoes *Aedes* to certain ingredients (Roestaman, 2003).

According to Wager (2011) and Menegristek (2010), the skin of citrus plants has the potential as a repellent, because it contains essential oils and other components such as limonene, linalool, octanal, decanal, citronellol, geraniol, valensen, sinnsial and sinential. Inayah (2007) reports that linalol, citronellal and geraniol are compounds that are repellant to arthropods.

The use of natural ingredients from citrus leaf extract can be one of the alternative alternatives that are safer for the environment compared to the use of chemicals *containing N, N-Diethylmeta-toluamide* (DEET). The results of this protection power study were followed by the kruskal wills test conducted to determine the differences between treatments with the average cumulative amount of the repelling power of the rapelant against the mosquitoes that perched on the arms. From the test results obtained P> 0.001, which means that there is no difference in the cumulative amount of mosquito protection power using rapelant extract of kaffir lime leaves between each concentration. This is because in all three concentrations namely 10% concentration, 20% and 30% have shown protection power that has the same range of + 90%.

6. Conclusion

Based on the results of the study it can be concluded that:

1. The concentration of orange leaf extract (*Citrus hystrix*) of 10%, 20%, and 30% is effectively used as a rapellant for the prevention of the bite of *Aedes aegypti mosquito.*

2. Percentage of rapellant protection used with the basic ingredients of kaffir lime extract (*Citrus hystrix*) of 93.33% for concentrations of 10%, 94.67% for concentrations of 20%, and 97.33% for concentrations of 30%.

7. Suggestion

This research should be continued with a viscosity test to determine whether or not it is good if the research material has been reacted with chemicals used for the skin.

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