Phytochemical analysis ,Reppelant and Larvacide test of *Lansium domesticum* against Dengue Hemmoragic Fever Vector

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ABSTRACT

Prevention of dengue virus transmission as the cause of DHF can be done by using plant-based insecticides, both for larvae and as protection against mosquito bites (*rappelent*). . Larvicides are substances that can kill larvae while repellents are substances that work locally or at a certain distance to prevent insects from flying, landing or biting the skin of humans and animals.

This study aims to determine the potential of *Lansium domesticum* as a reppelant and larvicide against Aedes aegypti. A total of 5 variations in concentration, there are 10%, 20%, 30%, 40%, and 50% were tested with reppelants to determine their protective power. Furthermore, the larvacide test was carried out on Aedes aegypti at concentrations of 20%, 40%, 60%, 80% and 100%.

The test results showed that all concentrations of Lansium domesticum extract tested were effective as reppelants because they had a repellency of more than 50%. The larvicidal test showed that the highest concentration that was effective as a larvicide was 100% concentration.

Research shows that *Lansium domesticum* is effective as a reppelant and larvicide.

Keywords: *Lansium domesticum*, reppelant, larvicidal

INTRODUCTION

Dengue hemorrhagic fever (DHF) is caused by the dengue virus which is transmitted by the *Aedes sp*. mosquito, this mosquito is anthropophilic, lives close to humans and is often indoors. Data from all over the world states that Asia ranks first in dengue disease with a large number of sufferers every year. In Southeast Asia, WHO reported DHF that Indonesia

is the country with the highest DHF cases (1).

Vector control by chemical means is currently widely applied, one of which is fogging. Fogging has been widely reported to be less effective in killing targets and increasing vector resistance to insecticides (2); (3); (4); (5)

Eradication of dengue vector mosquito larvae that has been carried out by powder abate with Temephos. The use of this temefos raises new problems, one of which is is environmental pollution. Likewise, the use of DEET (N,N *Dimethyl-meta toluamide*) which *insect repellent* used today causes many losses. such as the level of toxicity to the skin and also has an impact on the central nervous system, if its use is not done properly (6). Widely traded chemicals for the basic material in synthesizing the *repellent* , it contains halogenated hydrocarbons which is known to have a relatively long half-life of decomposition and it is feared for its toxic properties (7) (8). The chemicals contained in insecticides have a negative impact others, such as toxic residues in food, water, air and soil, resurrection and resilience insect pests, and effects on non-target organisms. More than 645 species of insects and mites have developed resistance to insecticides with 542 species of resistant arthropods to at least one compound. Approximately 7,470 cases of resistance have been reported in insects against certain insecticides; The 16 arthropod species accounted for 3,237 (43%) (9).

Efforts to prevent the transmission of Dengue virus as the cause of DHF can be done by using plant-derived insecticides, both for adult mosquitoes, larvae and as protection against mosquito bites (*rappelent*). Larvicides are substances that can kill larvae while repellents are substances that work locally or at a certain distance to prevent insects from flying, landing or biting the skin of humans and animals. *Repellent* can reduce exposure to mosquito bites that may be infected with the dengue virus (10).

Considering the use of chemical larvicides and the use of synthetic repellents cause many negative effects, it is necessary to study and find alternatives. Langsat plant (*Lansium domesticum*) has potential as a natural insecticide. This is based on an empirical study which states that the use of langsat skin to repel mosquitoes has long been carried out by rural communities, including rural communities in
Southeast Sulawesi. This is supported by studies reporting langsat skin as a material that can be used as an electric mosquito repellent (12); (Yang *et al.*, 2004); (Worang et al., 2013).

Lansium domesticum (Meliaceae) is a medicinal plant that is widely grown in Southeast Asia (14). The L.Domesticum plant is a tree that grows to a height of up to 30 m, also known as langsat (14). Several bioactive compounds have been isolated from L.domesticum (15)(16)(11)(17)(18), of which some have shown potential as
antimalarial (19), antibacterial (20), antifeedant (14), antimutagenic (21) and insecticides (22). In a previous study, it was reported that the content of the bark of langsat stems is a compound of alkaloids, flavonoids, saponins, triterpenoids, and tannins, which have a larvicidal effect. Research on the potential of plants as repellents also shows that several plants in Indonesia, such as lemongrass (*Cymbopogon citrates*), zodia leaf (*Evodia suaveolans*), basil (*Ocimum sanctum* Linn) and cloves (Syzygium aromaticum), grapefruit (Citrus maxima) have potential as rapelant (Citrus maxima). 23);(24). The content of

L.domesticum in the variety in Southeast Sulawesi known as langsat needs to be studied by testing the content of secondary metabolites to determine its effectiveness as a reppelant and larvicide.

RESEARCH METHOD

This research is an experimental study with *post test only control group*. The research procedure begins with taking samples of the bark and stems of Lansium domesticum, taking samples in the Konawe Regency, Southeast Sulawesi. The bark and stems of Lansium domesticum were washed and dried and crushed to form a powder. Then preparations were made for phytochemical screening.

Sample preparation and extraction

The skin/peel of *Lansium domesticum* was washed under running water, drained and then chopped. Samples were dried by drying in the open air protected from sun exposure. Furthermore, it is mashed and then put into a beaker and ethanol solvent is added (1:4). The samples were soaked for 5 days and
homogenized by stirring occasionally. Then the liquid extract was filtered using filter paper and the filtrate was collected. The sample was continued by maceration for 2 days with a new ethanol solvent. The macerated sample was filtered and the filtrate obtained was collected into one with the macerated filtrate. The obtained filtrate was concentrated with a *rotary vacuum evaporator* to remove solvent. The same procedure was carried out for the bark extraction of Lansium domesticum.

Phytochemical Screening

This procedure has function to determination the chemical composition, included identification of Flavonoid, Alkaloid,Saponin,tannin , triterpenoid and steroid.

Identification of Flavonoid

A total of 0.1 g of thick extract was dissolved in 10 mL of ethanol then divided into four test tubes. The first tube is used as a positive control, the second tube contains the sample plus NaOH, the third tube contains the sample plus concentrated H_2SO_4 , and the fourth tube contains the sample plus Zn powder. The color changes that occurred in the second, third and fourth tubes were observed and compared with the positive control tubes. If there is a color change, then the sample is positive for flavonoids.

Identification of Alkaloids

In 0.5 g of thick extract, 2 mL of 70% ethanol was added and then stirred. The mixture is filtered and a little hot water is added to the filtrate. After cooling, the mixture was filtered and 2-3 drops of Mayer's reagent was added to the filtrate. If the sample becomes cloudy or a precipitate forms, it indicates a positive sample containing alkaloids (Gafur *et al*, 2013).

Identification of Saponins

A total of 0.1 g of thick extract was dissolved in 15 mL of hot water and heated for 5 minutes. The mixture was filtered and the filtrate was put into a test tube and shaken until foamy/foaming, then 2N HCl was added. The sample was said to be positive for saponins if the foam/foam persisted for 10 minutes.

Identification of Tannins

A total of 0.1 g of thick extract was dissolved in methanol, then 2-3 drops of 1% FeCl3 solution were$_{added}$. The sample is declared positive for tannin if a yellow precipitate is formed .

Identification of phenolic

A total of 0.1 g of thick extract was added to 20 mL of $FeCl_3$. A positive test for the presence of phenolics is the formation of a green to blue-black color.

Identification Terpenoid

The test was done by taking 2 mL each. Lansium domesticum samples were extracted with water and ethanol as solvents. After that, 3 drops of concentrated HCl and 1 drop of concentratedH SO was added to each extract. If each solution is colored or purple, then it is positive that it contains terpenoids.

Steroid Test

The test was done by taking 2 mL of samples that had been extracted with water and ethanol as solvents. After that, 3 drops of concentrated HCl and 1 drop of concentrated H_2SO_4 was added to each extract. If each solution forms a green color, it is positive that it contains steroids.

Rearing *Aedes aegypti*

Eggs *Aedes aegypti* obtained from the Department of Health Analysts, hatched in plastic tubs measuring $(35x25x5)cm^3$ filled with distilled water as high as plastic tubs. Larvae that have hatched are reared until they become adult mosquito stages, during their development the larvae are fed in the form of pelletizing, *rearing* carried out in mosquito cages. After obtaining the mosquito stock, female mosquitoes were selected from the stock population to separate them from male mosquitoes. Female mosquitoes were transferred to

the test cage using an aspirator. 3 test mosquito cages are required, with 25 female *Aedes aegypti mosquitoes* in each mosquito cage. Mosquitoes were reared until they reached the age of 5-7 days *post emergence*, during which time the mosquitoes were fed a sugar solution (25).

Procedure making reppelant formula Lotion

The sample used for lotion is Lansium domesticum skin extract. Lotion is made by weighing all the ingredients and preparing a mortar. The ingredients (*cetyl alcohol*, stearic acid , *methyl*

parabean, Adeps lanae TEA, glycerin, olive oil and aquadest) were put into a porcelain cup and melted on a water bath. In the manufacture of this lotion. The aqueous phase consisted of Meyl parabean, warm water, TEA and glycerin. While the oil phase consists of stearic acid, cetyl alcohol, and olive oil.

The lotion water phase was prepared by: putting methyl parabean into a glass beaker with 5-10 ml of water added and heated on a water bath at 70 o C until dissolved. Then added glycerin and triethanolamine until homogeneous. The oil phase was prepared by melting cetyl alcohol, lanolin, stearic acid, propyl parabean and patchouli extract on a water bath at 70 ^{o}C until the mixture was homogeneous. The water phase and the oil phase are put together in a container and then stirred to form a lotion preparation. The preparation is put in a container and labeled. Lotion preparations are made at concentrations of 10%, 20%, 30%, 40%, and 50%.

Physical Stability Test

Test of physical properties of peel Lansium domesticum ethanol extract repellant lotion. Organoleptic Observations The sample was put into a container and then observed for changes in the shape, color and odor of the peel ethanol extract repellant lotion preparation and the results of the observations were recorded. Homogenity Lotion is smeared on a slide, then covered with another slide and seen whether the preparation is homogeneous and the surface is smooth and evenly distributed.

The process of testing *rappelant* against *Aedes aegypti*

The process of testing *rappelant* against *Aedes aegypti*as follows: (1) Mosquitoes are included in the test container. Each container put 25 female mosquitoes; (2) *Rappelent* lotion preparation is applied to the left arm and the right hand is not smeared. This presentation was carried out for 5 minutes; (3) Furthermore, the number of mosquitoes that landed during the exposure was calculated, both on the test arm and the control arm; (4) The test is carried out for 6 series, where each test series is carried out for 35 minutes and exposure is 5 minutes (26). During the *repellent* , probandus was unable to wipe or wash hands.

The protection or repulsion power can be known how the level of effectiveness after calculated based on the formula of Schreck et al, namely: % (repellency) = [(*Ta- Tb*)/*Ta*] ×

100, where , Ta is the number of mosquitoes in the control and Tb is the number of mosquitoes on the test treatment (27).

Larvicide Test

The sample used for the larvicidal test was the stem of Lansium domesticum.many as 25 larvae of *A. aegypti* instar III mosquitoes were transferred from the container into a test bottle

containing extract (according to concentration), Temephos as a positive control and aquadest as a negative control. Larvicidal activity was observed for 24 hours. The time calculation starts after the larvae are put into the test bottle. Observation of the life path that the test larvae given extract was able to survive for a certain period of time but could not reach the next stage. The death effect in question is that the test larvae experience mortality due to the activity of the larvicidal extract given.

Data analysis

If the evaluation of larval mortality obtained an average larval mortality of 5%-20%, then a statistical analysis was carried out using the Anova test. Furthermore, probit analysis was carried out to determine the LC50 (16)

RESULTS AND DISCUSSION

Phytochemical Screening *Lansium domesticum* (langsat)

Content The results of phytochemical screening of Lansium domesticum peel and stem compound can be seen in table 1 below.

Table 1. Test results of secondary metabolites content of Lansium domesticum peel and stem extract

No	Parameters	Lansium domesticum peel	Lansium domesticum stem	Standar indicator
1	Alkaloid	+	-	An orange or red precipitate or a white precipitate is formed
2.	Flavonoid	-	+	Changes color from green to orange or yellow
3.	Saponin	-	+	Stable foam is formed
4.	Triterpenoid	+	+	Formed red or there is a brownish ring
5.	Steroid	-	-	A blue-green color is formed
6.	Tannin	+	-	Blackish brown and precipitate formed
7.	Fenol	+	-	Changing green to black

Note: + = Positive contains compound
- = Negative contains compound

The results of the phytochemical screening of *Lansium domesticum* show that the bark extract of *Lansium domesticum* contains Alkaloids, Triterpenoids, Tannins and Phenols. While the stem extract of *Lansium domesticum* contains flavonoids, saponins, and triterpenoids. The results of phytochemical screening were carried out to determine the chemical components of *Lansium domesticum* which were identified qualitatively.

Preparation *Lansium domesticum* and physical stability test

The composition of the lotion based on Lansium domesticum peel extract and the results of the physical stability test can be seen in table 2 and table 3 below.

Table 2. Composition lotion of *Lansium domesticum*

No	ingredient	Function	concentration				
			K1(10 %)	K2(20%)	K3(30%)	K4 (40%)	K5(50%)
1	Extract of Lansium domesticum	Active substance	10 %	20 %	30 %	40 %	50%
2	Cetyl alkohol	moisturizer	3 %	3 %	3 %	3 %	3 %
3	Asam stearat	emulsifier	10 %	10 %	10%	10%	10%
4	Metyl parabean	Preservative	0,15%	0,15%	0,15%	0,15 %	0,15 %
5	Adeps lanae	Addition substance	2 %	2 %	2%	2%	2%
6	TEA	Humektan	2 %	2%	2%	2%	2%
7	Gliserin	Humektan	10 %	10%	10%	10%	10%
8	Olive oil	Pelarut	10 %	10%	10%	10%	10%
9	Aquadest	Pelarut	100 %	100%	100%	100%	100%

Table 2 shows the chemical composition for each concentration of Lansium domesticum skin is the same, namely Cetyl alcohol 3%, stearic acid 10%, Methyl parabean 0.15%, adeps lanae 2%, TEA 2%, glycerin 10%, Olive oil 10% and aquadest 100%.

Table 3. Results of homogenity test and organoleptic lotion *Lansium domesticum*

Concentration ofreppelent	Homogenity test	Organoleptis test
10 %	Viscous form, light brown color and aromatic characteristic odor	Homogeneous, without air bubbles
20 %	Homogeneous, without air bubbles	Homogeneous, without air bubbles
30 %	Homogeneous, without air bubbles	Homogeneous, without air bubbles
40 %	Homogeneous, without air bubbles	Homogeneous, without air bubbles
50 %	Homogeneous, without air bubbles	Homogeneous, without air bubbles

Table 3 shows that all concentrations of Lansium domesticum skin reppelant lotion showed the results of the homogeneity test which were thick, light brown in color and characteristically aromatic. The organoleptic test results showed a homogeneous lotion consistency and no air bubbles.

Test of Reppelant Lotion *Lansium domesticum* against *Aedes aegypti*

In this research , a total of 5 variations in concentration, there are 10%, 20%,30%, 40%, and 50% were tested with reppelants to determine their protective power The result of reppelant test appear in table 2 below

Table 2. Test of Reppelant Lotion *Lansium domesticum* against *Aedes aegypti*

No	Formulation concentration of Lansium domesticum	Total of masquitoes	Protective power (%)						
			0	1	2	3	4	5	6
1	10 %	25	91%	89%	90 %	89 %	88%	87%	85%
2	20 %	25	92%	91%	90%	90%	87%	85%	85%
3	30 %	25	92%	89%	88%	89%	88%	87%	85%
4	40 %	25	92%	91%	88%	87%	87%	85%	85%
5	50 %	25	92%	89%	85%	82%	79%	76%	75%
6	K (+)	25	100%	100%	100%	98%	95%	94%	94%
7	K(-)	25	0	0	0	0	0	0	0

Table 3 shows that the protective power of Lansium domesticum peel extract for all concentrations is quite effective because it has a repulsion power above 50%. The effectiveness of langsat fruit peel extract in producing a repulsion of more than 50% against Aedes aegpti is due to the active substance content of the langsat fruit peel. Research has shown that the higher the concentration of *repellent* , the higher the repellency of mosquitoes (24). Furthermore, in another study, it was also stated that plants that had the highest repellent potential wereplants from the family *Asteraceae, Cladophoraceae, Labiatae, Meliaceae, Oocystaceae,* and *Rutaceae* (28). plant *Lansium domesticum* from the Meliaceae family. The main volatile oil components of plants with *repellent* are *monoterpenoids* such as *geraniol, citronellol, linalool, terpineol, thymol, q-cymene, -bulnesene, patchouli alcohol, -pinene, -patchoulene*and *carvone* (29)*;* (26)*;* (30)*;* (31).

From the Reppelency test, it can be seen that in the first hour the percentage of repulsion increases and then continues to decrease until the 6th hour. sixth. This is in accordance with the research of Pebrianti, et al (32).

Larvicide test of Lansium domesticum on the mortality of Aedes aegypti

The results of the larvacide test of Lansium domesticum stem extract can be seen in Figure 1 below.

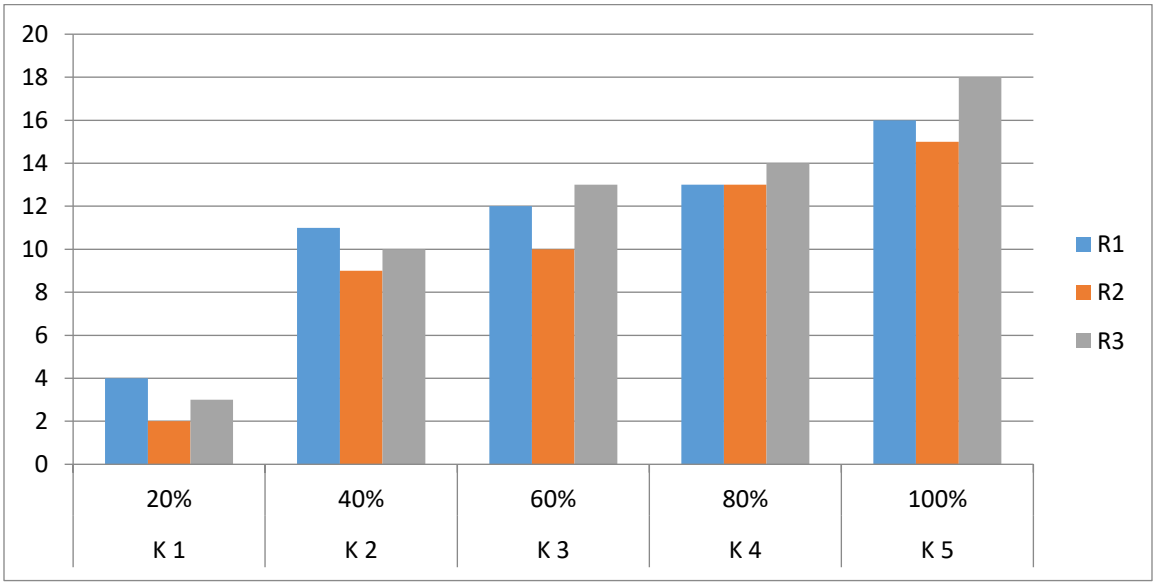

Figure 1. Larvicide test results of langsat bark on Aedes aegypti mortality

These results indicate that the highest percentage of *Aedes aegypti* was found in Lansium domesticum stem extract with a concentration of 100%, while the smallest larval mortality percentage was found in langsat stem extract at 20%.

Analysis of ANOVA test and Probit test

Data analysis to determine mortality mortality of Aedes aegypti larvae is using ANOVA test, which can be seen in table 4 below.

Table 4. Results of statistical tests using the Anova

	Sum of Squares	df	Mean Square	F	Sig
Between Groups	900,952	6	150,159	150,159	,000
Within Groups	14,000	14	1,000		
Total	914,952	20			

The table of Anova test results shows the calculated F value of 150,159 which is greater than the good F table at the 1% significance level of 2.85 and at the 5% significance level of 4.46. This means that there is a difference in the average mortality of mosquito larvae at each concentration of langsat skin infusion. The significance value shows 0.000 is smaller than 0.05 which means that each concentration of langsat skin infusion has an effect on the mortality of *Aedes aegypti*.

In the study, the 50% Probit analysis test was carried out which can be seen in the following table.

Table 5. Probit analysis of larvicidal power (LC 50)

Daya Larvasida (LC)	Waktu 24 jam (%)	Rentang batas	
		Bawah	Atas
LC50	47,571	10,001	85.286

Based on the table above, it shows that the LC50 value is 47.51, which means that the concentration required for the death of Aedes aegypti larvae by 50% is 47.571%.

The bark of langsat used in this study is the bark of old langsat which is still fresh, characterized by the bark of the stem being greenish brown or grayish, cracked and white. Observations of larval mortality showed that the percentage of larval mortality always increased with the high concentration used, this is in line with the theory that the higher the concentration of a larvicide, the higher the number of larval deaths, and the longer the exposure time, the higher the mortality of larvae. Alkaloids, terpenoids and flavonoids are plant defense compounds that can inhibit the eating process of insects and are also toxic.

Flavonoids and saponins themselves function as respiratory tract inhibitors. Flavonoids function as respiratory inhibitors, where flavonoids function to disrupt energy metabolism in the mitochondria by inhibiting the electron transport system so that obstacles in the electron transport system will block ATP production and cause a decrease in oxygen use by mitochondria and cause larvae to have difficulty breathing (33).

Triterphenoid compounds are one of the secondary metabolites that are found in large quantities and various molecular frameworks. Terpenoids are plant components that have an odor and can be isolated from plants by distillation called essential oils (34). This compound is a repellent (Reppelant) because it has an odor that insects do not like. These compounds will enter through the respiratory tract through the food eaten by insects and these substances are absorbed by the digestive tract (6).

The difference in the mortality of mosquito larvae at each concentration is due to the difference in the sensitivity of each larva to the concentration of langsat bark, where the higher the concentration used, the higher the level of viscosity and concentration of the langsat stem extract, so that the movement space of the larvae is not as limited as that of the larvae. being in the natural environment or in the outside environment, causing the larvae to have difficulty breathing and taking air on the surface of the water which results in insufficient oxygen for the carvae to grow, causing the death of the larvae (35).

The results of the probit analysis showed results that were in line with Handito's (2014) study which reported that the greater the concentration, the greater the toxicity of a solution to Aedes larvae, so that the number of mosquito larvae mortality also increased.

Conclusion

Lansium domesticum from the Meliaceae family that has the potential as a repellant and larvacide against *Aedes aegypti.*

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