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Potential topical lotion bischofia javanica leaf extract as a repellent for aedes aegyptil

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Abstract

Dengue Hemorrhagic Fever (DHF) or Hemorrhagic Fever (HF) is a disease caused by the Dengue virus, transmitted through the bites of Aedes aegypti L and Aedes albopictus mosquitoes. Both types of mosquitoes are found in almost all corners of Indonesia. One way to avoid these mosquitoes is to use natural repellents with a repelling or killing effect. The purpose of this study was to analyze the repellent efficacy of Bischofia javanica leaf extract lotion against A. aegypti L mosquitoes. The method used was an experiment based on the procedure recommended by the World Health Organization Pesticides Evaluation Scheme. Leaf samples of B. javanica were extracted by maceration. The test for the extract lotion was carried out on the organoleptic parameters, homogeneity, pH, centrifugation, patch test, spreadability, adhesion, and effectiveness as a repellent. The results showed that the 50 percent concentration of B. javanica leaf extract lotion had 100 percent protection power (at the 1st to the 2nd hour). However, after 5 hours, the protection power decreased to 83.21 percent.

***Keywords*:** Repellent, lotion, *Bischofia javanica* leaf extract, *Aedes aegypti* L.

1. Introduction

Dengue Hemorrhagic Fever (DHF) or Hemorrhagic Fever (HF) is the most reported tropical disease. More than 100 countries and 2.5 billion people in the world live in dengue-endemic areas (1). This disease is caused by the dengue virus, which belongs to the Arthropod-Borne Virus, the genus Flavivirus, and the Flaviviridae family transmitted through the mosquito bites *A. aegypti* and *A. albopictus* (2). Dengue virus (DENV) is a single positive stranded RNA (3). Four virus serotypes have been identified namely DEN-1, DEN-2, DEN-3, and DEN-4 which are found in the same region of the world (4). These four viruses are called serotypes because each has a different interaction with antibodies in human blood serum. The dengue virus can only replicate within the host organism (5).

In 2020, dengue fever continued to hit several countries, with reports of increasing numbers of cases in Bangladesh, Brazil, Cook Islands, Ecuador, India, Indonesia, Maldives, Mauritania, Mayotte (Fr), Nepal, Singapore, Sri Lanka, Sudan, Thailand, Timor-Leste, and Yemen (6). Based on these data, efforts are needed to eradicate *A. aegypti* mosquitoes, one of which is by using natural ingredients. Natural materials usually use plant-based ingredients (7). These essential ingredients work by repelling mosquitoes made in lotions, creams, or clothes to protect the body from mosquito bites. Currently, mosquito eradication research is focused on natural-based repellents with a repellent or killing effect. One of the plants from the Euphorbiaceae family that is believed to repel mosquitoes and insects is *B. javanica*. This plant is widespread from western India, southern Japan to the archipelago and east to Australia and the Pacific (8). In Indonesia, especially in Central Sulawesi, *B. javanica* plants are known as pepolo. The public does not widely know the benefits of this plant. The main ingredients in *B. javanica* are tannins, β-amyrins, betulinic acid, friedelan-3α-ol, epifriedelinol, friedelin, luteolin, and glucosides, quercetin, beta-sitosterol, stigmasterol, ursolic acid (9). Traditionally, this plant's properties treat various chronic conditions such as inflammation, tuberculosis, ulcers, fractures, and dislocations. (10). Besides, the results of other studies indicate that *B. javanica* has intense antiparasitic activity (11) and has antimicrobial activity (12), anti-leukemic (13). Apart from the secondary metabolite content possessed by plants, the mosquito repellent activity found in plants is also highly dependent on product formulations, such as creams, lotions, gels, and sprays which affect the duration of effectiveness (14)(15). Some essential oils have been reported as potential natural sources of

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insect repellent; however, if they are used without using a formulation technique, the repellant effect is low because essential oils have high volatility to evaporate quickly (16). Therefore it is necessary to have a formulation technique using a lotion that allows controlling the volatility of essential oils, thereby extending the repellent duration. Based on the content and activity of the compounds possessed by *B. javanica*, it is expected that leaf extract can be used as a mosquito repellent.

2. Material & Methods

2.1 Material

2.1.1 Preparation of Test Solutions

The leaves of *B. javanica* were obtained from Napu Village, Central Sulawesi, and then the leaves were dried at room temperature and mashed using a blender. The crushed leaves are dissolved with 96% ethanol. An evaporator then evaporates the maceration results. The evaporation product is a stock solution that is then diluted with 70% alcohol to get a concentration of 25% and 50 in 1 ml.

2.1.2 Rearing Aedes aegypti L. Mosquitoes.

Adult A. aegypti mosquitoes were collected from the Donggala Health Research and Development Center. Mosquitoes were transferred from the breeding grounds to a 50 x 50x50 cm mosquito cage made of a wooden frame covered with fine wire. The bottom of the cage is lined with thick cardboard. The cage door is equipped with a cotton cloth to prevent adult mosquitoes from escaping. Adult mosquitoes were reared at a laboratory temperature of 27 ± 2º C and relative humidity between 75 and 85%. Adult mosquitoes were fed a 10% sugar solution for three days before being treate

2.1.3 Preparation of the Lotion Formulation Extract

Na-lauryl sulfate is dissolved in a certain amount of water in a water bath, then gradually methylparaben and propylparaben are dissolved into Na-lauryl sulfate solution. Added distilled water and maintained at a temperature of 70-75 ^{0}C. Separately the oil phase was dissolved in a water bath, gradually starting from stearic acid, cetyl alcohol, lanolin, and adding liquid paraffin; the temperature was kept at 70-75 ^{0}C. Leaf extract *B. javanica* that has been weighed is placed on top of the lotion preparation. Mortar is heated with hot water and covered with aluminum foil. The Extract and aqueous phase were mixed gradually with the oil phase in a heated mortar to form a stable and homogeneous lotion until the temperature reached 40-45 C. The lotion preparation was stored in a closed container. The extract lotion was made in two formulations: formula 1 with an extract content of 0.10 g and a formula with an extract content of 0.20 g. As a comparison, lotion preparations without extract were also tested (Table 1).

Table 1: The formulation of the *B. javanica* leaf extract lotion

Material	F1[(a)]	F2[(b)]	C-[(c)]
B. javanica leaf extract	0.10 g	0.20 g	-
Liquid paraffin (v/v)	3 mL	3 mL	3 mL
Stearic acid	2 g	2 g	2 g
Lanolin	2 g	2 g	2 g
Na lauryl sulfate	2 g	2 g	2 g
Methylparaben (w/v)	0.12 g	0.12 g	0.12 g
Propylparaben (w/v)	0.10 g	0.10 g	0.10 g
Cetyl alcohol	2 gr	2 gr	2 gr
Aquadest add	100 mL	100 mL	100 mL

[a]Formula 1 lotion preparation with extract content of 0.10 g

[b]Formula 2 lotion preparation with extract content of 0.20 g

[c](C-) negative control, lotion without the addition of extract

2.2 Methods

2.2.1 Experimental design

The study used an experimental laboratory method based on the procedure recommended by the World Health Organization Pesticides Evaluation Scheme (WHOPES). The guidelines provide guidance and procedures on laboratory studies, field trials and evaluation of technical material used in mosquito repellent products and on the methods used to determine their application rate(s) and effectiveness (17). Evaluate the physical quality of the lotion was carried out after the preparation of extract lotion was formed (1)

Organoleptic

The organoleptic test was performed visually on the extract lotion of *B. javanica* leaves to determine the color of the preparation, consistency of the preparation, and the smell of the lotion preparation.

Homogeneity

The homogeneity test of the lotion preparation was carried out by observing the results of lotion rubbing on a glass plate. A total of 0.5 lotions of *B. javanica* leaf extract was rubbed on a glass slide, then closed with another slide, then observed the lotion's homogeneity.

pH

A total of 0.5 grams of *B. javanica* leaf extract lotion was diluted with 5 mL of distilled water. Determination of the properties of the acid/base preparation of the *B. javanica* leaf extract lotion can use pH paper.

Centrifugation

A total of 2 grams of *B. javanica* leaf extract lotion was put into a centrifuge tube, then rotated at 3000 rpm for 30 minutes, then observed whether there was separation.

Patch test

The safety test of *B. javanica* leaf extract lotion was carried out on ten volunteers who had applied 300 mg of lotion on their hands for 15 minutes, then saw the reaction whether they were irritated/allergic or not.

Spreadability test

A total of 0.5 grams of *B. javanica* leaf extract lotion is placed on the watch glass, and then on top of the lotion, another watch glass is placed and left for 1 minute. Lotion in the watch glass is then added with 50, 100, and 150 g, left to stand for 1 minute, and then the diameter of the distribution is recorded.

Adhesion test

A total of 0.25 grams of *B. javanica* leaf extract lotion was placed on a glass object and pressed with a load of 1 kg for 5 minutes. Furthermore, a load of 80 grams is released, recorded the time when the two glass objects were released.

2.2.2 Test the protection power of lotion

The test was carried out in a mosquito cage measuring 30 x 30 cm, the walls of which were made of nylon gauze. The confinement provided is III for sample testing, control (-), and control (+). Each of them was inserted with 30 *A. aegypti* mosquitoes that had not yet sucked blood. Then the arm was applied with 300 mg of *B. javanica* leaf extract lotion from each test formula. Then the arm that has been smeared with lotion is put in a mosquito cage for 15 minutes also done for the (+) and (-) controls. The formula can determine the effectiveness against mosquito infestation (17) recommends that the test be carried out at least three times. The second and third tests were carried out on different days, namely the next day at the same test time. The mosquitoes used in each replication were different samples from the mosquito samples used in the previous test. At The end of the test, the percentage of protective power is assessed as the proportion of the number of mosquitoes perching on the treatment arm with the number of mosquitoes perching on the control arm, calculated by the following formula:

$$\text{Percentage of Protection Power (\%)} = \left(\frac{\sum C - \sum T}{\sum C}\right) X 100\%$$

Information:

C= number of contact mosquitoes on the control arm

T= number of contact mosquitoes on the treatment arm

After obtaining the percentage of protective power at each concentration rated the effective concentration to obtain a protective power percentage of 50% and 99%.

2.2.3. Statistical analysis

Data are presented as mean ± SE. Intergroup differences were evaluated using one-way ANOVA analysis (ANOVA), using SPSS software (SAS Institute Inc., Cary, NC). Differences were considered statistically significant at probability values less than 0.05.

3. Result

3.1 Evaluation Results of Lotion Preparations

Lotion evaluation includes inspection of appearance or organoleptic, homogeneity examination, examining the degree of acidity (pH), examining the effect of centrifugation, safety testing (Patch Test) on the lotion spreadability testing, and adhesion testing.

1. Organoleptic test:
   On organoleptic examination, the base formula produces a brownish, odorless lotion when the formula is added with a fragrant aroma, the smell changes to smell nice.
2. Homogeneity test
   The homogeneity test of the formula lotion shows that the lotion has good homogeneity. They are indicated by the presence of a consistent dosage color and the mass of the lotion form. The homogeneous lotion preparation indicated that the mixture of the lotion ingredients and the *B. javanica* leaf extract mixed well because there were no lumps in the preparation in the test formula. The aroma and color of the lotion evenly show that the evaluation of the *B. javanica* leaf extract lotion formula is good and meets the physical qualification requirements.
3. pH test
   The pH value for the four test lotions was 6.4 and was within the human skin pH range of 5.5-6.5. Measuring the degree of acidity of the *B. javanica* leaf extract lotion needs to be done because the repellent lotion is intended for topical use, which helps avoid skin irritation. This lotion is safe to use because it is in the pH range of human skin.
4. Centrifugation test
   The centrifugation test aims to see the stability of the lotion. From the results of the centrifugation test, it was found that the *B. javanica* leaf extract lotion test showed a good level of stability so that it met one of the lotion preparation requirements.
5. Test the patch test
   Testing the safety of lotion preparations by doing a patch test on ten panelists who had been smeared with *B. javanica* leaf extract lotion on the hands in the open air for 15 minutes against the panelists did not show any allergic reactions. Therefore, the Patch Test results showed that the lotion formula was safe to use as a topical preparation because the ten volunteers did not show any signs of allergy.
6. Test scatter ability
   Testing the spreadability of the *B. javanica* leaf extract lotion was carried out to see the ability of the lotion to spread evenly on the skin. The measurement of the scattering power was carried out using a watch glass, which was smeared with

lotion and then marked using a ruler. The lotion's dispersion test result showed that in 20 minutes, the spreadability reached 6.6 x 6.5 cm.

7. Test adhesion
   Observation of the adhesion test is carried out to determine how far the lotion can stick to the skin to get the desired therapeutic effect. The result of the lotion adhesion test showed an average adhesion of 3.3 seconds. In summary, the results of testing the parameters for physical properties are presented in table 2.

Table 2: The results of testing the physical properties of the *B. javanica* leaf extract lotion preparation

Parameters (Physical Properties)	Result of measurement of lotion formula (quantitative and qualitative)
Organoleptic:	
Smell	Typically aromatic smell
Colour	Light brown
Shape	Thick
pH	6.4
Centrifugation	Stable (Good)
Homogeneity	Evenly (Good)
Patch Test	No allergic reactions
Spreadability (cm)	6.5 x 6.7
Adhesion (seconds):	
Testing 1	3
Testing 2	4
Testing 3	3

3.2 Effectiveness test as a repellent

3.2.1 Aedes aegypti Mosquito Perching Frequency

The frequency of perching on the *A. aegypti* mosquito on the hands of optimistic control respondents, standard control, and respondent's hands *B. javanica* leaf extract lotion smeared with time intervals can be seen in the following figure1. In Figure 1, it appears that the *B. javanica* leaf extract lotion formula at 0 to 5 hours can repel *A. aegypti* mosquitoes when compared to negative controls (arms smeared with lotion without Extract). The average frequency of mosquitoes perching on respondents' arms smeared with *B. javanica* leaf extract lotion Formula 1 (F1) was 2.13 birds/hour, while formula 2 (F2) was 1.65 birds/hour (attachment 1). In the negative control (lotion without Extract), the average frequency of mosquitoes perching on the arm was 9.80 or 9 per hour. Meanwhile, in the positive control, the average frequency of mosquitoes that reached the respondent's arm was 0.56 individuals/hour.

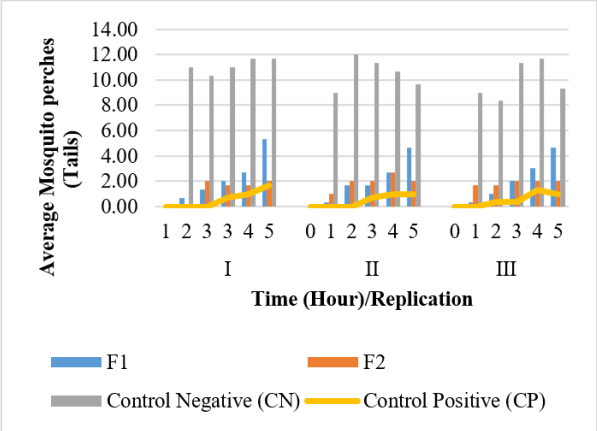

Fig. 1. The average frequency of perching on the mosquito *A. aegypti* on the respondent's hand was based on time and

3.2.2 Protection Power of B. javanica Leaf Extract Lotion

To determine the protective power of *B. javanica* leaf extract lotion, count the number of perches on the control arm minus the number of perches on the treatment arm divided by the number of perches on the control arm, then multiplied by 100%. The results of the calculation of the percentage of the protective power of *B. javanica* leaf extract lotion against contact with *A. aegypti* mosquitoes in formulas 1 and 2, negative control, and positive control are presented in Figure 2. Based on Figure 2, the protective power of *B. javanica* leaf extract lotion Formula 1 (F1) against *A. aegypti* mosquitoes is 82.20% on average, formula 2 (F2) is 86%, 0% negative control, and 95.35% positive control. Thus, the results indicate that the percentage of the protective power of *B. javanica* leaf extract lotion formula 1 and 2 is still under positive control.

3.2.3 Repellent Efficacy Test for B. javanica Leaf Extract Lotion Against A. aegypti L. Mosquitoes.

Based on the data from the calculation of the protective power of *B. javanica* leaf extract lotion, then perform the efficacy test of the protective power of *B. javanica* leaf extract against *A. aegypti* mosquitoes using oneway ANOVA (one-way ANOVA). The results of the analysis can be seen in table 3. Based on table 3, the analysis results of the variant formula 1 (extract concentration 25%) on the positive control sig value, 0.01 (<0.05), show that formula 1 is significantly different from positive control (repellant). On the other hand, the ANOVA results in formula 2 (F2) are sig. 0.712 (> 0.05); thus, the *B. javanica* leaf extract lotion in formula 2 (extract concentration 50%) was not significantly different from the positive control.

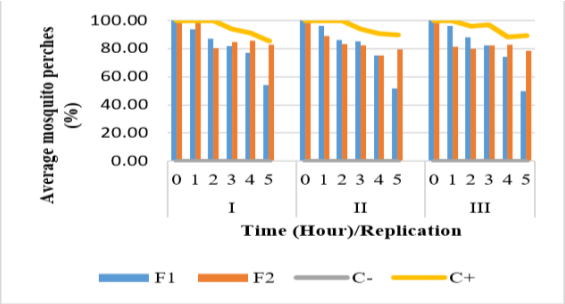

Fig. 2. Protection Power of *B. javanica* Leaf Extract Lotion against *A. aegypti* mosquitoes.

Table 3: Analysis of Variants (ANOVA) Protection Power of *B. javanica* Leaf Extract Lotion

		Sum of Squares	df	Mean Square	F	Sig.
F1	Between Groups	4232,328	10	423,233	13.409	.001
	Within Groups	220,951	7	31,564		
	Total	4453,278	17			
F2	Between Groups	573,225	10	57,322	.692	.712
	Within Groups	580,021	7	82,860		
	Total	1153,246	17			

4. Discussion

In the lotion extract formula *B. javanica*, 2 (50% extract concentration) had almost the same ability as the positive control. The ability of volatile compounds at a concentration of 50% extract is quite a lot to repel *A aegypti* mosquitoes. Plants that contain lots of volatile compounds can act as insect repellents (7). Most plants that contain active compounds can be used to prevent attacks by plant-eating insects (phytophages) (18). The active compounds are divided into several categories: repellents, food inhibitors, toxins, and growth regulators. Although the primary function of the active compounds contained is as protection against phytophagous insects, many of these compounds are effective against mosquito bites and other Diptera, and some of these compounds are hematophagous insect repellents (19). *Bischofia javanica* plants have many active compounds that have various biochemical activities. *B. javanica* leaves contain active compounds including carbohydrates, flavonoids, proteins, amino acids, tannins, β-amyrins, betulinic acid, friedelan-3α-ol, epifriedelinol, friedelin, luteolin and glucosides, quercetin, beta-sitosterol, stigmasterol, ursolic acid (9). So far, and there has been no research on the repellant activity of *B. javanica* leaves against *A. aegypti* mosquitoes. Based on the research results, the leaf extract lotion of *B. javanica* has biological activity as a repellent. They were based on various kinds of active substances, including alkaloids, terpenoids, triterpenoids, saponins, sterols (20). The research data showed that the 50% concentration of *B. javanica* leaf extract lotion showed the highest protection. It can be explained that the higher the concentration of *B. javanica* leaf extract, the higher the volatile components it contains. Volatile components can bind to odour receptor proteins in insects and interfere with insects' olfactory process so that insects stay away from the sample (21) (22). Based on the results of observations on the frequency of perching on the *A. aegypti* mosquito that landed on the respondent's arm after giving the leaf extract lotion formula *B. javanica* (F1), the protective power in the first hour was 94%, and at the fifth hour, the protective power was 75% to 50%. In formula-2, the protection power at the 1st to the 2nd hour reaches 100, and at the 5th hour, the protection power reaches 83.21%. The results of Abiy et al (2015) study using DEET (N, N-diethyl-1,3-methylbenzamide), neem oil and chinaberry oil against *Anopheles arabiensis* mosquitoes with 98% DEET repellent per cent with a protection time of 8 hours. When compared with the protection of *B. javanica* extract lotion against *A. aegypti* mosquito bites, the protective effect is still below DEET. However, compared to the protection obtained from neem oil and chinaberry oil, the entire protection time for neem was 3 hours, while chinaberry oil was one hour. So the lotion formulation of *B. javanica* extract was still more effective than the two oils. The decrease in the protective power of *B. javanica* leaf extract lotion from the 2nd to the 6th hour in formula 1 (25% concentration) was due to the evaporation of chemical compounds in the *B. javanica* leaf extract lotion. Over time, the potential/protective power of *B. javanica* leaf extract lotion decreased. In addition, the activity of the *A. aegypti* mosquito has decreased from time to time (23) stated that with increasing time, the protection against mosquitoes or the effectiveness of the repellents decreased. The decrease in mosquito activity was caused by differences in age and oviparity of each mosquito. Length of time protection against mosquito landings and bites at each concentration is influenced by the amount of compound content that functions as a repellent in the lotion.

5. Conclusion

This study concluded that *B. javanica* leaf extract lotion could protect against *A. aegypti* mosquito bites. Formula 1 (25 percent extract concentration) provides 75 percent protection for 5 hours. Formula 2 (50% concentration) provides 83.21 percent protection for 5 hours.Thus the *B. javanica* leaf extract lotion can make repellant against the *A. aegypti* L. mosquito.

6. Conflicts of interest

The authors declare that they have no competing interest.

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