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Phytochemical Screening And Larvicidal Activity Of Kebiul (*Caesalpinia Bonduc. L*) Seed Kernel Against Aedes Aegypti Mosquito

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Abstract

Dengue Hemorrhagic Fever (DHF) is caused by an infected A. aegypti mosquito, which can cause serious bleeding, a sudden drop in blood pressure and even death. Kebiul seed kernel extract is thought to contain flavonoid compounds, terpenoids, saponins and steroids that can be used as larvicides. The purpose of this study was to determine the toxicity of the extract, n-hexane fraction, and ethanol fraction of kernel seed (C. bonduc. L) against A. aegypti mosquito larvae. This study used the methods of extraction, phytochemical screening, and toxicity tests by calculating larval mortality. Based on the results of phytochemical screening, extract of kernel seed (C. bonduc. L) contains tannins, flavonoids, alkaloids, steroidal saponins and terpenoids. Toxicity test (LC_{50} values) of extract, n-hexane fraction, and ethanol fraction of kernel seed (C. bonduc. L) are 368,566 g/mL, 483,010 g/mL, 338,361 g/mL. The LC_{50} value < 1000 ppm has a toxic effect, so the kernel seed has the potential to be used as a larvicide.

Keywords: *aedes aegypti; caesalpinia bonduc. l; dengue hemorrhagic fever*

Introduction

The Aedes aegypti mosquito is a type of mosquito that can carry the dengue virus and is the main vector that causes Dengue Hemorrhagic Fever (DHF) (Weaver *et al*, 2016; Sazalia *et al*, 2020). According to the Indonesian Ministry of Health (Kemenkes), there were 110,921 cases of dengue hemorrhagic fever (DHF) in Indonesia from January to October 31 2019. This number has risen dramatically since 2018, when it stood at 65,602 cases. Indonesia has the highest number of dengue cases in Southeast Asia and the second highest number of cases worldwide, after Brazil (Kaunang, 2015) (Pasaribu *et al.*, 2021).

One method of controlling mosquito larvae is to dry the area where the larvae develop or to use larvicides. Larvicides are pesticides that are used to kill immature insects or as larvae killers. Excessive use of larvicides is extremely hazardous. because it will harm non-target organisms such as humans, wildlife, fish, and arthropod species (Roghelia, 2017). Furthermore, the feared negative effect may result in insects that are resistant to the compound itself (Garcia *et al*, 2018; Goindin *et al*, 2017).

A good larvicide is an environmentally friendly larvicide and has a low toxicity to non-target organisms. Larvicides are made from natural ingredients because they are safer and have fewer side effects. Because of

the rapid decomposition by sunlight, air humidity, and other natural components, larvicides derived from natural ingredients are very safe to use,and also able to reduce the risk of soil and water pollution. Furthermore, because natural larvicides are non-toxic to non-target organisms, they are safe to use in baths and fish ponds (Nurhikma Sari, 2019). One of the plants used to make natural larvicides is the *kebiul* plant (*C. bonduc. L*).

Previous researchers found that *kebiul* leaf has larvicidal activity with a 100% mortality value at 1% concentration of patroleum ether and ethanol extract, while 55.0% at a concentration of 2.5% aqueous extract and 92.6% at a fixed oil concentration of 2.5% against *culix pipiens* larvae (Sundare, 2007). However, as far as research has been carried out, there have been no researchers who have tested the potential of the *kebiul* seed kernel as a larvicide, even though the *kebiul* seed kernel contains positive flavonoids, terpenoids, saponins and steroids (Sembiring, 2018). Because the *kebiul* seed kernel compound is nearly identical to the *kebiul* leaf compound, the *kebiul* seed kernel is thought to be used as a larvicide as well. Based on this, it is necessary to test the toxicity of the *kebiul* seed kernel fraction (C. bonduc. L) as a larvicide against Aedes aegypti mosquitoes.

Research Methodology

Materials

The tools and materials used are analytical balance, vacuum rotary evaporator. The material used is *Kebiul* seed kernel (*C. bonduc. L*) taken from Bengkulu Selatan district. Themephos (over-the-counter product), Ethanol (Merck), n-hexane (Merck), $FeCl_3$ (Merck KGaA), Chloroform (Merck KGaA), H_2SO_4 (Merck KGaA), Magnesium powder (Merck KGaA), Dragendorff reagent (Merck KGaA), HCl (Merck KGaA), Lieberman Burchard reagent.

Work procedures

Sample Preparation

Kebiul seeds (*Caesalpinia bonduc* L) were taken from South Bengkulu Regency by peeling the *kebiul* fruit and then took the seeds. *Kebiul* seeds are washed with running water and then dried in the sun. The dried *kebiul* seeds are crushed by using a hammer. The *kebiul* seed shell and the *kebiul* seed kernel are separated, then the *kebiul* seed kernel is mashed using a blender and a fine powder of the *kebiul* seed kernel is obtained.

Sample extraction and fractionation

A total of 180.7 g of kebiul seed kernels were put into a large glass jar with the addition of 3 liters of 96% ethanol then allowed to stand for 3x24 hours. The extract obtained was evaporated using a rotary evaporator at a temperature of 40^{o}C and then evaporated again using an oven at a temperature of 40^{o}C until the solvent was completely evaporated to obtain a thick ethanol extract of the kebiul seed kernel which was then fractionated.

The ethanol extract of 12 g thick kebiul seed kernel was dissolved with 100 mL of ethanol and then put into a separatory funnel with a closed tap. A total of 100 mL of n-hexane solvent was added to the extract solution and shaken until homogeneous. The n-hexane fraction was separated from the ethanol fraction by opening the separatory funnel faucet and accommodated into the Erlenmeyer. The treatment was repeated 2 times with the same solvent. The n-hexane and ethanol fractions were evaporated with a rotary evaporator at 40^{0}C to obtain ethanol extract, n-hexane fraction, and ethanol fraction of kebiul seed. Then the yield of each was calculated and tested on A. aegypti mosquito larvae. Storage of the test sample is done by inserting the test sample into a glass beaker covered with plastic wrap.

Phytochemical Screening

Phytochemical screening of kebiul seed kernel extract was carried out based on the method presented by Hernanda (2022) which included testing for alkaloids, flavonoids, saponins, terpenoids and steroids.

a. Alkaloids

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The thick extract, the ethanol fraction, and the n-hexane fraction of the kebiul seed kernel were dissolved in a solvent and then 2 mL was pipetted into a test tube with 2 drops of Mayer's reagent. After 30 minutes, changes were observed. If a yellow solution was formed with a white precipitate, the results were positive.

b. Flavonoids

The thick extract, the ethanol fraction and the n-hexane fraction of the kebiul seed kernel were dissolved in a solvent then 2 mL was pipetted into a test tube, then 2 mg magnesium powder was added and 3 drops of concentrated HCl were added. The sample was shaken to see if any changes occurred, such as the formation of a red, yellow, or orange color in the solution, indicating the presence of flavonoids.

c. Saponins

The thick extract, the ethanol fraction and the n-hexane fraction of the kebiul seed kernel were dissolved in a solvent and then 2 mL was pipetted into a test tube and hot water was added. The changes that took place were observed. The presence of saponins was indicated by the formation of a stable foam.

d. Terpenoid and Steroid Test

The viscous extract, ethanol fraction, and n-hexane fraction of kebiul seed kernels were dissolved in a solvent and pipetted into a test tube as much as 2 mL, then dripped with Lieberman-Burchard reagent until a purple, red, or reddish-brown color was formed. These findings indicate that the test is positive for terpenoids, and the formation of a brown, green, or blue ring indicates a positive steroid test.

e. Tannins

After dissolving the viscous extract, ethanol fraction, and n-hexane fraction of kebiul seed kernels in a solvent, a 2 mL pipette was placed in a test tube, and a few drops of 1 percent $FeCl_3$ solution was added. Based on the observed changes, the test was positive if a dark blue or greenish black color was formed.

Toxicity Test

Preparation of test larvae; The A. aegypti mosquito eggs used in this study were obtained from the Baturaja Health Research and Development Institute. A. aegypti mosquito eggs delivered in the form of paper eggs. A. aegypti mosquito larvae were prepared by soaking paper eggs in a container filled with water. Eggs hatch within ±24 hours into 1^{st} instar mosquito larvae and reach 3^{rd} instar at 4-5 days of age.

Each fraction, extract, and temephos (positive control) were prepared with a stock solution of 2000 ppm by dissolving 1 gram of the fraction, extract, and temephos into 500 mL of distilled water, then diluting to concentrations of 250 ppm, 500 ppm, 750 ppm, 1000 ppm, and 1250 ppm.

Using a pipette, 20 Aedes aegypti larvae were put in a vial filled with a positive control test solution at concentrations of 250 ppm, 500 ppm, 750 ppm, 1000 ppm, 1250 ppm, and 2000 ppm. Counting the dead larvae after 1x24 hours, indicated by sinking or immobile larvae, was used to make observations.

Results and Discussion

Phytochemical test aims to find out or identify what compounds are present in plants (Paputungan, Wonggo and Kaseger, 2017). Phytochemical tests were carried out qualitatively on 3 samples, namely ethanol extract, n-hexane fraction, and ethanol fraction of *kebiul* seed kernel (C. bonduc. L) as shown in Table 1.

Table 1. Phytochemical Test Results of Ethanol Extract, n-Hexane Fraction, and Ethanol Fraction.

Secondary Metabolic Compound	Ethanol extract	n-hexane fraction	Ethanol fraction	Positive color
Alkaloids (Dragendorff)	+	+	-	Reddish brown precipitate
Flavonoids	+	+	+	Bright yellow
Saponins	+	-	+	There is foam
Steroids	+	+	+	There is a brown ring
Terpenoids	-	+	+	Reddish brown
Tannins	-	-	+	Greenish black

Based on the phytochemical test, the ethanolic extract of the *kebiul* seed kernel contains flavonoid compounds, saponins and steroids where the results show the same compounds in the phytochemical test conducted by Dirmansyah (2021). According to Rino (2019), secondary metabolites contained in a plant are influenced by several factors such as the location of growth, climate, temperature, rainfall, age, allelopathy, nutrition and light.

Table 1. shows the positive test of alkaloid compounds in the extract and n-hexane fraction. The ethanol fraction showed negative results. This shows that the secondary metabolites contained in the *kebiul* plant are non-polar alkaloid compounds.

The positive test of flavonoid in the extract, the polar fraction (ethanol) and the non-polar fraction (n-hexane). It can occur because flavonoid compounds are a large group of secondary metabolite compounds and have very diverse types depending on their biosynthesis (Wongso, 2014). According to Simon (2018), flavonoids bound to sugar tend to be polar soluble, while less polar aglycones such as flavanones and isoflavones tend to be more soluble in semi-polar to non-polar solvents.

The results of the phytochemical test were negative for tannin and terpenoid compounds in the kebiul seed kernel extract. However, after separation with non-polar solvents (n-hexane) and polar solvents (ethanol) the results were positive for terpenoids, and positive for tannins for the ethanol fraction. It can occur because the tannin and terpenoid compounds contained in the extract have a large weight or are still in polymer form so that the compound is separated using two different solvents, where the ethanol solvent (polar) will attract polar terpenoid compounds and n-hexane solvent (non-polar) will attract terpenoid compounds that are non-polar. In tannin, it is only positive in the polar fraction after separation because the tannin compounds found in plants are polar so that they are soluble in polar solvents (ethanol fraction) (Wang *et al.*, 2016). The results of the saponin test were negative in the n-hexane solvent because saponins have a sugar (hexose) group that can be dissolved in polar solvents (Lindeboom, 2005).

Kebiul Seed Kernel Toxicity Test

In the toxicity test for larvicides, concentrations of 250ppm, 500ppm, 750ppm, 1000pm, 1250ppm, 2000ppm were used as well as positive control (temephos) and aquades negative control with a concentration of 0 ppm (without test solution). Positive control was used to compare as well as proving the killing power of commercial larvicides on test animals. Negative control was used to prove that the media and treatment did not cause death in the tested animals (Mayang,2021)(Ariani, 2020). The results of the larvicide toxicity test can be seen in the Table 2.

Table 2. Extract Toxicity Test Data, Ethanol Fraction, n-Hexane Fraction of *Kebiul* Seed and Temephos Kernel Against Aedes Aegypti Larvae

No	Concentration (ppm)	Number of Larvae with 3 times Test	Larval mortality recurrence (%)			
			Extract	Ethanol fraction	n-Hexane Fraction	Temephos
1	0	60	0	0	0	0
2	250	60	45	35	52	100
3	500	60	60	48	57	100
4	750	60	68	58	65	100
5	1000	60	75	67	72	100
6	1250	60	98	82	87	100
7	2000	60	100	92	95	100

Table 2 shows that on the extract, the ethanol fraction, the n-hexane fraction of the kebiul seed kernel had a mortality rate of *A. aegypti* larvae which was directly proportional to the increase in the concentration used. Kebiul seed kernel extract has the greatest killing power because it reaches 100% larval death after 24 hours. The n-hexane fraction and ethanol fraction reached 95% and 92% killing power, whereas the n-hexane fraction had higher killed power than the ethanol fraction. This is because secondary metabolites in the n-hexane fraction were more visible during the phytochemical test. It is marked by the emergence of a lighter or brighter color. The light or bright color indicates the number of compounds present in the sample. The more compounds are present, the higher the killing power of the larvae.

In addition to the extract, the ethanol fraction and the n-hexane fraction in this study were also used temephos as a positive control and aquades with a concentration of 0 ppm as a negative control. In Table 2, the percentage of larval mortality for temephos as a positive control reached 100%. Temephos has been specially designed for larval killing so that its killing power is much different than the extract, ethanol fraction and n-hexane fraction.

As for the negative control, it can be seen in the table that the number of larval deaths is 0% so that it can be ascertained that the treatment carried out during the test did not affect the mortality of the larvae.

Figure 1. Comparison of control larvae with dead larvae exposed to the test solution. Figure Captions a) and b) Larvae Exposed to Test Solution, c) Control Larvae.

According to Kurniawan *et al* (2015) larval death with various concentrations was caused by larvae that were in direct contact with the active compounds contained in the test solution. The difference between control larvae and larvae exposed to the test solution can be seen in Figure 1. Ramayanti (2016) said that tannins will inhibit the performance of enzymes that cause metabolic processes to be disrupted and larvae will experience nutritional deficiencies and finally dead. In addition, tannins will interfere with the digestive process because tannins will bind to the protein needed by the larvae so that the absorption of the protein needed by the larvae will be disrupted.

Flavonoids are strong respiratory inhibitors and respiratory poisons for larvae. The way flavonoids work is by entering the larva's body through the respiratory system

which will later cause damage to the nerves and damage to the respiratory system. The larvae will be unable to breathe and then die. It can be seen from the change from the normal position of the larvae when breathing becomes abnormal (Ramayanti, 2016).

Saponins and alkaloids act as stomach poisons where saponins can reduce the surface tension of the mucous membranes of the digestive tract of larvae, causing the walls of the digestive tract to become corrosive. Alkaloids will enter and damage cells and disrupt the larval nervous system by inhibiting the action of the acetylcholinesterase enzyme. Alkaloid compounds caused the occurrence of movement that slows down when stimulated by touch and always bends the body and changes in color to become more transparent ((Ramayanti, 2016).

Table 3. LC_{50} Value and Linear Regression Equation Data from Extract, Ethanol Fraction, and n-Hexane Fraction of *Kebiul* Seed

No	Sample	Regression equation	Value of LC_{50} (ppm)
1.	Extract	y= 2.8871x – 2.4099 R^2 = 0.809	368.566
2.	Ethanol fraction	y= 1.9981x - 0.3629 R^2 = 0.930	483.010
3.	n-Hexane Fraction	y= 1.7688x + 0.5261 R^2 = 0.819	338.361

Calculation of LC_{50} Value

The value of LC_{50} can be determined by using the mortality percentage of the test larvae and analyzed probit by using Microsoft Excel application. The calculation of the LC_{50} value is used to determine the toxicity of a test solution used (Wulandari, 2014). In this study, the test solutions used were extract, ethanol fraction and n-hexane fraction of *kebiul* seed kernel. The LC_{50} value of them can be seen in Table 3.

If seen from Table 3, it is known that the LC_{50} value of the extract, the ethanol fraction, and the n-hexane fraction of the *kebiul* seed kernel is 368.566; 483.010; 338.361 ppm consecutively. The data above show that the three solutions tested on aedes aegypti larvae were toxic to larvae so that they could be used as larvicides, because the LC_{50} value of the extract, n-hexane fraction, and ethanol fraction <1000 ppm.

Conclusions

Extract, ethanol fraction and n-hexane fraction of *kebiul* seed kernel have toxic properties that can be used as larvicides. Mortality values of *kebiul* seed kernel extract, n-hexane fraction and ethanol fraction were 100%, 95%, and 92%, respectively.

The LC_{50} value of the extract, the ethanol fraction and the n-hexane fraction of the *kebiul* seed kernel was 368.566; 483.010; 338.361ppm. it means that it can be used as a larvicide because <1000 ppm.

Suggestion

It is recommended in further research to isolate the secondary metabolite compounds contained in the *kebiul* seed kernel.

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