

Effectiveness of Ramania Leaves (*Bouea macrophylla* Griffith) from South Kalimantan as a Mosquito Larvae Biolarvicidal

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Abstract:

Indonesia itself as a tropical country has many plants that can be used as biolarvicides, one of which is the ramania plant (*Bouea macrophylla* Griffith). Ramania fruit epidermis contains secondary metabolites such as flavonoids, saponins, phenolic tannins, alcohol, steroids and terpenoids where these compounds have a larvicidal effect. The purpose of this study was to determine the activity of the ethanol extract of the epidermis of the ramania fruit as a biolarvicidal Mosquito larvae from the LC50 value. This research is a laboratory experimental research. The treatment concentrations used in this study were 25 ppm, 50 ppm, and 100 ppm. Temephos 1% was used as a positive control. Concentrations were then tested on 20 larvae instar III. The results of SPSS analysis with a significance of <0.05 showed that there was an effect of treatment on the number of larvae deaths, where the results of Tuckey analysis showed that concentrations of 50 ppm and 100 ppm and Temephos 1% showed differences. which means the number of deaths of test larvae. The results showed that the ethanol extract of ramania rind of ramania fruit (*Bouea macrophylla* Griffith) was effective as a biolarvicidal mosquito larvae. The results of probit analysis showed that the LC50 of ethanol extract of ramania rind against larvae was 20,844 ppm which is a low toxic group in aquatic environments.

Keywords: Biolarvicida; *Bouea macrophylla* Griffith; LC50; Larvae

Introduction

Dengue Hemorrhagic Fever (DHF) is commonly found in tropical and sub-tropical areas. Asia ranks first in the number of DHF sufferers each year. The number of sufferers and the area of spread of DHF is increasing along with increasing mobility and population density. In Indonesia, DHF was first discovered in the city of Surabaya in 1968, as many as 58 people were infected and 24 of them died with a mortality rate (AK) of 41.3%.

The eradication of dengue hemorrhagic fever is basically carried out in accordance with the eradication of infectious diseases in general, namely by eradicating mosquitoes as the vector of transmission. Eradication of this transmitting vector can be done by controlling adult mosquitoes or larvae by chemical means. Chemical control is carried out by killing the larvae with the aim of breaking the chain of transmission using temephos. However, the use of this chemical-based temephos can cause various negative effects on the environment so that other larvicides with natural ingredients that are safer for the environment are needed.

Ramania is used for its fruit, leaves and stems. Ramania fruit is green when it is young, and is often consumed as a salad or a mixture of Ramania sauce. Ripe ramania fruit is yellow, has a sour-sweet taste and can be eaten directly. Ramania stems can be used as boards (Harsono, 2017).

So far, the utilization of the part of the Ramania plant in the form of Ramania leaves is still limited, namely it is only consumed for food salad. Research is needed on the content of secondary metabolic compounds in Ramania leaves to find out other benefits of Ramania leaves (Arwita, 2013). Ramania leaf ethanol extract contains secondary metabolites of flavonoids, phenols,

alkaloids, steroids and terpenoids (Aryzki, 2019).

This research propose to see extract ramania activity to larvae in three concentration (25 ppm, 50 ppm and 100 ppm) and count LC50 with probit analysis.

Research Method

Sample preparation

Sample processing begins with simplicia processing from ramania leaves. The ready-made Ramania leaf simplicia was subjected to phytochemical screening to determine the content of secondary metabolites present in Ramania leaves. Then proceed with the extraction process with the maceration method. Extraction was carried out with 97% ethanolic solvent to obtain ethanolic extract of ramania leaves. Then a phytochemical screening was carried out on the ethanolic extract of ramania leaves to reconfirm the content of the compounds present in the ethanolic extract of ramania leaves (Aryzki, 2019).

Tools and Materials

The tools used in the study were knives, blenders, scales, plastic cups, 100 ml measuring cups, water baths, glass jars, evaporating cups, stirring rods, tube clamps, ovens, test tubes, Bunsen lamps, Buchner funnels, rotary evaporators.

The materials used in this study were third instar larvae, larvae, ramania leaves, distilled water, 96% ethanol, 1% abate, tap water, Mg metal powder, 0.5M FeCl₃, 2N HCl, chloroform, 1N NaOH, iodine 0.1 N, ethanol p.a (Merck), distilled water, n-hexane, HgCl₂, KI, distilled water, Bi(NO₃)₂, HNO₃, I₂, filter paper, HCl 2N, acetic anhydrous, concentrated H₂SO₄, 1% FeCl₃, gelatin 1%, 25 ppm NaCl, Mg band, 70% ethanol, concentrated HCl and 1N HCl.

Phytochemical Test Stage

The ethanol viscous extract was then taken a little and dissolved in 10 mL

ethanol and then tested for its phytochemicals.

1. Flavonoids

An 1 mL of ethanol extract was added with 3 mL of 70% ethanol, and shaken, then heated in a water bath, and shaken again then filtered. The filtered filtrate was added with 0.1 g of Mg band and 2 drops of concentrated HCl. A positive test containing flavonoid compounds is indicated by the presence of red color (Harborne, 1987).

2. Tannins

1 mL of ethanol extract dripped with 5 drops of 25 ppm NaCl and filtered. The filtrate obtained was added with 1% gelatin and 25 ppm NaCl. A positive test for the presence of tannins is indicated by the presence of a white precipitate (Tiwari et al., 2011).

3. Phenol

1 mL of ethanol extract plus 10 drops of 1% FeCl₃. A positive test for the presence of phenolic compounds is the formation of red, blue, purple, black or green (Harborne, 1987).

4. Flavonoids

1 mL of ethanol extract was added with 3 mL of 70% ethanol, and shaken, then heated in a water bath, and shaken again then filtered. The filtered filtrate was added with 0.1 g of Mg band and 2 drops of concentrated HCl. A positive test containing flavonoid compounds is indicated by the presence of red color (Harborne, 1987).

5. Saponins

1 mL of ethanol extract was mixed with 2 mL of distilled water and shaken for 1 minute, then added 2 drops of 1N HCl. Positive test for the presence of saponin compounds if a stable foam is formed $\pm$ 7 minutes (Harborne, 1987).

6. Tannins

1 mL of ethanol extract dripped with 5 drops of 25 ppm NaCl and filtered. The filtrate obtained was added with 1%

gelatin and 25 ppm NaCl. A positive test for the presence of tannins is indicated by the presence of a white precipitate (Tiwari et al., 2011).

7. Steroids and Triterpenoids

1 mL of ethanol extract was added (CH₃CHO)₂O and concentrated H₂SO₄. The presence of steroid compounds is indicated by the formation of green or blue color. The presence of triterpenoid compounds is indicated by the formation of a golden yellow, yellow or purple color (Harborne, 1987).

8. Alkaloids

1 mL of ethanol extract was added with 2 mL of 2N HCl and shaken. The mixture was then divided into 3 different tubes. In each tube, 1 drop of Mayer's reagent is added to the first tube, 1 drop of Dragendorff's reagent to the second tube, and 1 drop of Wagner's reagent to the third tube.

The presence of alkaloid compounds if the addition of Mayer's reagent forms a yellow precipitate, the addition of Dragendorff's reagent forms a red precipitate and the addition of Wagner's reagent forms brown or red precipitate (Tiwari et al., 2011).

Bio larvicidal Activity Test

The bio larvicidal test was carried out on 6 groups consisting of a solution of ramania leaf extract with a concentration of 25 ppm, 50 ppm, 100 ppm, positive control of 1% temephos, negative control of tap water and negative control of aquadest.

Then, 20 larvae third instar were added to each group. Each group was repeated 3 times. The number of dead larvae was counted after 24 hours of observation.

Data Processing

Data were analyzed using SPSS including data normality and homogeneity tests, comparative tests and Probit Tests.

Results

This researcher used the test material in the form of ethanol extract from the skin of the ramania fruit, the skin obtained was wet sorted to remove dirt and adhering dust. Then wash it using water. Washing is done to remove soil and other impurities attached to the simplicia material (Wati, 2010).

The skin is dried in the sun on a tray and covered with a black cloth, the drying process lasts about 3 to 4 days marked by leaves that are wrinkled and when crushed will crumble and the dry weight of the leaves is constant in 3 weightings. The dry weight of the leaves is 1 kg. The first weighing is 1.2 kg, the second and third are constant 1 kg.

Drying aims to prevent damage to the active substance content in the plant so that it can be stored for a long time. The damage is due to enzymatic decomposition of active substances such as hydrolysis, oxidation and polymerization, so that the yield decreases (Triayu, 2009).

The powder obtained was then weighed as much as 1 kg and added 4 liters of 25 ppm ethanol. The mixture was macerated for 1x24 hours while occasionally stirring. The function of stirring is to ensure a faster balance of

concentrations of extraction materials in the extractor liquid (Voight, 1994).

The maceration results were filtered using a Buchner funnel to separate the filtrate from the dregs. The remaining dregs were macerated again with 2 liters of ethanol and filtered again until a total filtrate of 4,500 mL was obtained. The filtrate obtained was then concentrated using a vacuum rotary evaporator at 50°C for 15-20 minutes. The temperature setting at 50°C aims to keep the nutritious compounds contained in the simplicia stable and not damaged. The results of the liquid extract were 2,317 ml of liquid extract. The yield of the extract obtained was 17.77%.

The extract was then subjected to ethanol-free testing. This test is carried out to ensure that no solvent is left behind and causes data bias. The test results will show the same results as testing for similar alcohol groups, that is, there will be no change color from orange to green (Kumalasari, 2011).

Phytochemical Screening

Phytochemical screening is an important first step in research on medicinal plants or in terms of liquefying new active compounds derived from natural ingredients. The phytochemical screening carried out included testing for flavonoids, tannins, phenols, alkaloids, saponins, steroids and terpenoids. The results of the phytochemical screening of ramania leaves can be seen in table 1.

Table 1 Results of Phytochemical Screening

Test	Results	Conclusion
Flavonoids	Intense Yellow Color	+
Tannins	No white precipitate formed	-
Phenol	A blackish green precipitate was Formed	+
Alkaloids	Yellow precipitate	+
Saponins	No foam formed	-
Steroids	A brown ring is formed	+
Terpenoids	Formed red	+

Bio larvicidal Test

The larvae used in the test were identified at the Laboratory of the Tanah Bumbu Health Research and Development Center. The larvae used were third instar larvae. The larvae have entered the third instar characterized by a larval age of 5-7 days and a larval size of 4-7 mm. Instar III is selected because in this phase the larvae have fairly good resistance to the external

environment, besides that in the third instar the larval organs are complete so that the larvae already have tools for eating and are active for their development (Prijadi, 2014). The results of the research on the bio larvicidal effect of ramania fruit extract on third instar larvae for 24 hours obtained the primary data presented in table 2.

Table 2 Total mortality of larvae at various concentrations of ramania leaf extract for 24 hours of treatment.

Treatment	Average	
	Tail	%
Control negative (aquadest)	0	0
Control negative (tap water)	0	0
Concentration 25 ppm	11	44
Concentration 50 ppm	14	56
Concentration 100 ppm	17	68
Control positive (Temephos 1%)	20	100

Based on the main test results in table 2 after 24 hours of treatment with predetermined concentrations, the average percentage of larval mortality at each concentration can be seen. It can be seen that there is a difference in the average number of deaths of test larvae in

each treatment group. However, from each replication, the number of deaths was slightly different in each treatment, this was influenced by the health of the larvae used during the test which was not known beforehand.

Table 3 Summary of Results of Tukey Post Hoc Analysis

	Group	p Value
Negative control (Aquadest)	Negative control (tap water)	0,000*
	Concentration 25 ppm	0,000*
	Concentration 50 ppm	0,000*
	Concentration 100 ppm	0,000*
	Positive control (Temeplos 1%)	0,000*
Negative control (tap water)	Concentration 25 ppm	0,000*
	Concentration 50 ppm	0,000*
	Concentration 100 ppm	0,000*
	Positive control (Temeplos 1%)	0,000*
Concentration 25 ppm	Concentration 50 ppm	0,000*
	Concentration 100 ppm	1,000
	Positive control (Temeplos 1%)	1,000
Concentration 100 ppm	Positive control (Temeplos 1%)	1,000

Description: * means

The groups that did not have a significant difference were the 50 ppm, 100 ppm and 1% Temephos concentration groups (positive control) because in the 25 ppm, 50 ppm and 100 ppm treatment groups there was a significant difference in the number of deaths of the test larvae. The results showed that the larvicidal activity of the ethanol extract of ramania

rind against larvae was lower than 1% Temephos.

The probit analysis aims to determine the 24-hour LC₅₀ value, where from these results it can be seen which concentration has larvicidal activity because the actual test did not obtain an LC₅₀ value. After performing a probit analysis using Minitab 18 software with a 95% confidence level, the results are in accordance with Table 5

Table 4 Probit Analysis Results

probability	95.0 % Fiducial CI		
	Estimate	Lower	Upper
0.500	20.844	-	-

The results of the probit analysis obtained an estimate of the concentration that resulted in 100 ppm larval death, which was a concentration of 20,844 ppm. So that it can be concluded that the 24-hour LC₅₀ value of Ramania leaf extract is 20,844 ppm, which at this concentration is considered low poison in aquatic environments.

The LC₅₀ value of the Ramania leaf extract indicated that the Ramania leaf extract was indeed toxic to larvae, especially to III larvae.

Based on the results of the study, it can be concluded that the higher the concentration of Ramania leaf extract used, the greater the number of deaths of test larvae in the experiment.

The larvicidal ability of ramania fruit extract is produced from several chemical compounds present in the plant. The phytochemicals in ramania fruit are flavonoids, saponins, phenols, alkaloids, steroids, tannins and terpenoids.

Flavonoids which are a group of phenols can cause protein clumping. The denaturation of these proteins causes the permeability of the cell walls in the digestive tract to decrease. This will result in disruption of nutrient transport resulting in stunted growth and eventually the mosquito larvae will die (Wati, 2010).

Another substance that may cause the death of the larvae is tannins. Tannins are "phenolic compounds" that can precipitate proteins. Tannins have the ability to precipitate proteins. In the larvae, this can inhibit the proteins needed by the larvae for growth, which can cause the larvae to die (Sastriawan, 2014).

Saponins themselves can cause corrosion of the walls of the digestive tract of larvae due to their ability to damage membranes, besides that saponins can also interfere with the lipid layer on the epicuticle and the protein layer on the endocuticle makes it easier for toxic substances to enter the body of the larvae. A similar mechanism was also shown by the methanol extract of lime on larvae (Musiam, 2018).

Conclusions

The concentration that can kill 100 ppm (LC₅₀) of larvae is in the interval with an estimated concentration of 20.844%.

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