TEST OF PREPARATION FORMULATION SPRAY MANGROVE LEAF EXTRACT RHIZOPORA MUCRONATA AS REPELLENT AGAINST MOSQUITO ANOPHELES VAGUS

Nur'ain Dunda[1)], Sucia Pratiwi Tampoy[2)], Adnan Malaha[3)]
[1,2,3)]Bina Mandiri University Gorontalo, Indonesia
E-mail : dundanurain@gmail.com, Suciatampoy@gmail.com, adnanmalaha@ubmg.ac.id

ABSTRACT

The mangrove plant Rhizopora mucronata is a plant that belongs to the Family Rhizophoraceae and is often found in tidal areas, one of which is in Boalemo Regency, Bajo Village. This plant is used as a repellent on the leaves. The secondary metabolite compounds contained in the leaves of Rhizopora mucronata function as repellent namely alkaloids, flavonoids, tannins and terpenoids. This study aims to determine the activity and significant differences in the concentration of the preparation formulation spray mangrove leaf extract Rhizopora mucronata as repellent against mosquitoes Anopheles vagus. Laboratory experimental research method with the research object being mangrove leaf extract Rhizopora mucronata which includes the maceration and extraction stages with methanol solvent, identification of secondary metabolite compounds, preparation evaluation test, and repellent test with 6 test groups, namely negative control, F0%, F10%, F15%, F20%, and positive control (soffel spray). The results of the study showed that the formulation spray mangrove leaf extract Rhizopora mucronata had activity against mosquitoes Anopheles vagus due to mangrove leaf extract Rhizopora mucronata contains alkaloids, tannins, flavonoids and terpenoids which function as repellent . The preparation formulation spray mangrove leaf extract Rhizopora mucronata concentrations of 10%, 15%, and 20% do not have a significant difference in effect on mosquitoes Anopheles vagus, because at this concentration the compound content of mangrove leaves Rhizopora mucronata is equally effective as repellent against mosquitoes Anopheles vagus.

Keywords : Rhizopora mucronata, Repellent, Anopheles vagus

INTRODUCTION

Mosquitoes are small insects belonging to the order Diptera, known as two-winged insects. Research by Dila, Muhammad, and Ari (2020), mosquitoes are insects that act as vectors for various types of viruses, bacteria and protozoa that cause disease. Several diseases in tropical areas such as Indonesia are caused by microorganisms that are infected by mosquitoes when they suck blood. One of the diseases caused by mosquitoes is malaria. Malaria is a disease that is a global health problem, especially in tropical and subtropical areas. The *Anopheles vagus* mosquito is a species of mosquito that is a vector for malaria.

According to Endah (2020) Mosquitoes *Anopheles vagus* which are infected with the plasmodium parasite will spread the parasite to other people through subsequent bites, so that the plasmodium parasite will enter the human body through mosquito saliva and will reproduce in in human blood cells which causes the symptoms of malaria. Therefore, controlling the Anopheles vagus mosquito population is very important in preventing the transmission of malaria. There are many ways that can be done to

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prevent malaria. One of them is by using mosquito repellent (*repellent*) at home.

According to Irfayanti, Jasmiadi, and Tari (2022) currently *repellent* circulating in the community is made from synthetic chemicals containing *N,N-diethyl-meta-toluamide* (DEET) or *picaridin* . *Repellent* like this is available in the form of *spray* (spray), the preparation *spray* is preferred because its practical use is by spraying. However, long-term use of DEET will cause various dangerous side effects such as neurotoxicity or damage to the peripheral nervous system in the brain caused by exposure to toxic substances (Tavares, 2018).

However, along with the development of science *repellent* began to be developed through the use of natural ingredients. One of them is the mangrove plant *Rhizopora mucronata* . According to Ali et al (2017) mangrove plants *Rhizopora mucronata* contain secondary metabolite compounds which have potential as antimalarials. The part of the mangrove plant that is widely used as an antimalarial or *repellent* is the leaves. According to Syafrina et al (2023) mangrove leaves *Rhizopora mucronata* contain secondary metabolite compounds such as flavonoids, tannins, alkaloids and terpenoids. Each of these compounds acts as an inhibitor of the reproduction of the *Anopheles vagus mosquito*.

The use of natural ingredients from mangrove leaves *Rhizopora mucronata* as *repellent* needs to be applied effectively. However, before that, mangrove leaves need to be made in the form of a dosage formulation *spray* mangrove leaf extract *Rhizopora mucronata* so that it can be used by the public or is easy to apply. Because not many studies have tested the *spray* preparation formulation from *Rhizopora mucronata* leaf extract against *Anopheles vagus mosquitoes* mosquitoes, therefore it is necessary to test the *spray* preparation formulation. _> extract mangrove leaves *Rhizopora mucronata* right. In order to provide optimal control effect against *Anopheles vagus mosquitoes*.

Based on the background description above, the researcher is interested in testing *repellent* against formulation leaf extract *Rhizopora mucronat a* with the title "Test Formulation of Preparation *Spray* Leaf Extract Mangrove *Rhizopora mucronata* As *Repellent* Against Mosquitoes *Anopheles vagus*.

RESEARCH METHODS

The research method used in this research is quantitative with the type of research *experimental laboratory* which consists of 6 treatment groups with three repetitions . A tools used are blender, glass jar, analytical balance, measuring cup, *Stopwatch* , filter cloth, test tube, water bath, dropper pipette, *paper cup* mosquito, funnel chemicals, mosquito aspirator, and modified box measuring 40x40x 30 cm . B materials used are HCl, ethanol 96%, Glycerin, BHT *(Butylated Hydroxytoluene)* , Magnesium, $FeCl_3$, H_2SO_4 , Reagent *Liebermann Burchard* , Mayer's reagent, methanol, aluminum foil, mosquitoes *Anopheles vagus* , and mangrove leaf extract *Rhizopora mucronata*.

RESEARCH RESULT

1. Yield % Yield

Maceration of mangrove leaf simplicia *Rhizopora mucronata* with a sample size of 350 grams using 2 L methanol solvent obtained a good % yield of 13.7%. Can be seen in **Table 1.**

Table 1 . Results % Yield

Sample	Simplicia Weight (g)	Extract weight (g)	Yield (%)
Rhizopora mucronata mangrove leaves	350	48	13,7

2. Secondary Metabolite Compound Test Results

The results of the secondary metabolite compound test for mangrove leaf extract *Rhizopora mucronata* using different reagents showed that it positively contained

Alkaloid, Flavonoid, Tannin and Terpenoid compounds. Can be seen in **Table 2.**

Table 4.2
Secondary Metabolite Compound Test Results

Compound	Reagent	Results	Ket
Alkaloids	HCl and Meyer's Reagent	A precipitate is formed	(+)
Flavonoids	Concentratd HCl and Mg	Red	(+)
Tannin	FeCl3 and H2SO4	Brownish yellow color	(+)
Terpenoids	Liebermann Burchard's reagent	Red or purple color	(+)

3. Evaluation Test Results for Preparation *Spray*

a. Organoleptic Test

The organoleptic test of the preparation *spray* mangrove leaf extract *Rhizopora mucronata* was carried out by direct visual observation which included examining the shape, smell and color. The following organoleptic test results can be seen in **Table 3.**

Table 3. Organoleptic Test Results

Formulation	Parameter	Test Results
F0	Color	Clear
	Form	Liquid
	Smell	No smell
F1	Color	Brownish Green
	Form	Liquid
	Smell	Typical extract
F2	Color	Brownish green
	Form	Liquid
	Smell	Typical extract
F3	Color	Brownish green
	Form	Liquid
	Smell	Typical extract

b. Homogeneity Test

The homogeneity test results of the preparation *spray* mangrove leaf extract *Rhizopora mucronata* showed that the preparation did not contain particles. The following homogeneity test results can be seen in **Table 4.**

Table 4. Homogeneity Test Results

Formulation	Test Results
F0	Homogeneous
F1	Homogeneous
F2	Homogeneous
F3	Homogeneous

c. Clarity Test

The clarity test resulting from the preparation formulation *spray* mangrove leaf extract *Rhizopora mucronata* is clear and cloudy based on direct visual observation. Can be seen in **Table 5.**

Table 5. Clarity Test Results

Formulation	Test Results
F0	Clear
F1	Murky
F2	Murky
F3	Murky

d. Test pH

Based on pH measurements carried out using a universal pH dipped into each preparation formulation *spray* mangrove leaf extract *Rhizopora mucronata* for 3 seconds, it produces a good pH for use on the skin. Can be seen in **Table 6.**

Table 6. pH Test Results

Formulation	**Test Results**			**Average**
	I	**II**	**III**	
Negative Control	4,5	5,3	5	4,9
F0	7	6,5	7	6,8
F1	5	6	5,5	5,5
F2	6	5,5	6	5,8
F3	6	5	6	5,6
Positive Control	5	6	7	6

e. Irritation Test

The irritation test of the preparation *spray* mangrove leaf extract *Rhizopora mucronata* was carried out on 10 respondents to see the presence of itching, redness and burning reactions for 15 minutes. Irritation test results can be seen in **Table 7.**

Table 7. Irritation Test Results

Formulation	Reactions to Respondents			Number of Respondents
	Redness	Itchy rash	Stings	
F0	No	No	No	9
F1	No	No	No	9
F2	No	No	No	9
F3	No	No	No	9

4. Test Results *Repellent*

The test results of *repellent* preparation *spray* mangrove leaf extract *Rhizopora mucronata* against mosquitoes *Anopheles vagus* can be seen in **Table 8.**

Table 8. Test Results *Repellent*

Treatment	Number of Mosquitoes Landing on Hands (Tails)			Average
	The day I	Day II	Day III	
Negative Control	8	5	6	6,3
F0	1	2	1	1,3
F1	0	0	0	0
F2	0	0	0	0
F3	0	0	0	0
Positive Control	0	0	0	0

a. Normality Test

The normality test results from the formulation test for the preparation *spray* mangrove leaf extract *Rhizopora mucronata* as *repellent* against mosquitoes *Anopheles vagus* produced a significant value of <0.05 . The data is normally distributed (significant >0.05) and the data is not normally distributed (significant <0.05). From the results of the normality test it can be concluded that the data is not normally distributed. Can be seen in **Table 9.**

Table 9. Normality Test

Treatment Group	Significant
Negative Control	0,637
F0%	0,000
F10%	0
F15%	0
F20%	0
Positive Control	0

Based on the assessment of data distribution from the formulation test of the preparation *spray* mangrove leaf extract *Rhizopora mucronata* as *repellent* against mosquitoes *Anopheles vagus* the result was that the data was not distributed normal. This means that the data does not meet the requirements for parametric testing using the analysis of variance (ANOVA) test. Therefore, the non-parametric Kruskal-Wallis test was used as a substitute.

b. Kruskal Test W allis

The results of the Kruskal-Wallis test from the formulation test of *spray* mangrove leaf extract *Rhizopora mucronata* as *repellent* against mosquitoes *Anopheles vagus* produced a significant value <0 .05. There is an influence (significant <0.05) and there is no influence (significant >0.05).

Based on the results of the Kruskal-Wallis test, the data obtained a significant value of <0.05 or there was an influence from the formulation test of the preparation *spray* mangrove leaf extract *Rhizopora mucronata* as *repellent* against mosquitoes *Anopheles vagus* . Therefore, it was continued with the Post Hoc Duncan test to see any differences between the formulation concentration treatments.

c. Duncan's Post Hoc Test

Post test results t Hoc Dun can from preparation test formulation *spray* leaf extract mangrove *Rhizopora mucronata* as *repellent* against mosquitoes *Anopheles vagus* can be seen in **Table 10.** that there is no difference in effect between F 10%, F 15% , F 20%, and Con Positive control or the same value.

Table 10. Duncan's Post Hoc Test

Treatment Group	Significant
Negative Control	6,33
F0%	1,33
F10%	0,00
F15%	0,00
F20%	0,00
Positive Control	0,00

Based on the results of the Post Duncan test, it was found that there was no difference in the concentrations of 10%,

15%, 20% and the positive control, because they were in the same subset. So it can be concluded that from the formulation test of the preparation *spray* mangrove leaf extract *Rhizopora mucronata* against mosquitoes *Anopheles vagus* there is no significant difference at concentrations of 10%, 15%, and 20 %.

DISCUSSION

Samples used in testing the formulation of *spray* as *repellent* against mosquitoes *Anopheles vagus* namely mangrove leaves *Rhizopora mucronata* , were taken from the village Bajo, Tilamuta District, Boalemo Regency at 07:00-09:00 am. Because at that time plants are photosynthesising. These mangrove leaves have gone through several stages of processing until they have reached the stage of becoming a thick extract.

Mangrove leaves *Rhizopora mucronata* were wet sorted to remove foreign objects that were still attached to the leaf sample. Then, washing was carried out using running water so that residual dirt did not stick to the mangrove leaf samples *Rhizopora mucronata* (Suharti, Risa, and Ibnu, 2024). Next, the mangrove leaves *Rhizopora mucronata* are chopped to make drying easier.

Drying of mangrove leaf simplicia *Rhizopora mucronata* using sunlight, the samples were covered with black paranet netting to avoid damage to the compounds contained in the samples due to high temperatures resulting from sunlight, such as tannin compounds (Anita, Aristhasari, and Refida, 2021). After that, the dried mangrove *Rhizopora mucronata* leaves were ground using a blender and stored in the laboratory.

Mangrove leaves *Rhizopora mucronata* which have become fine powder are macerated using methanol solvent for up to 4×24 hours until the color of the solvent is no longer dark. Maceration for 4×24 hours can produce the most effective bioactive compounds because the length of contact time between the solvent and the extracted material has reached the limit of no extracted compounds. The basic principle of extraction is *like dissolves like* which means that the solubility of a compound in a solvent is based on the similarity of polarity between the solvent and the compound to be extracted (Saliny and Manju, 2019). After that, the soaked leaf powder *Rhizopora mucronata* was filtered using a filter cloth and the evaporation process was carried out using a water bath at a temperature of 40-50°C and obtained an extract yield of 13.7%. The yield can be said to be good if the value is >10% with the evaporation process at a temperature of 40-50°C (Ramli et al, 2023). The yield was obtained by comparing the weight of the extract obtained from the evaporation process and the weight of the dry simplicia then multiplying by 100%. So, it can be stated that the yield result **in Table 1.** is a good yield with a value of 13.7% or >10%.

Furthermore, the results of the identification of secondary metabolite compounds can be seen in **Table 2.** where mangrove leaves *Rhizopora mucronata* are positive for containing secondary metabolite compounds, namely alkaloids, flavonoids, tannins and terpenoids. This is also proven based on the research results of Sengodan et al, (2020) that mangrove leaves *Rhizopora mucronata* positively contain secondary metabolite compounds, namely alkaloids, flavonoids, tannins and terpenoids. Testing for flavonoid compounds, mangrove leaf extract *Rhizopora mucronata* mixed with concentrated HCl and Magnesium reagents got positive results because there was a red color change. The color change occurs because Magnesium powder and HCl react by forming bubbles which are H_2 gas, while Magnesium metal and concentrated HCl function to reduce the core contained in the flavonoid structure to form a red color (Salimi et al, 2020).

Alkaloid compound test, mangrove leaf extract *Rhizopora mucronata* mixed with HCl and Meyer's reagent obtained positive results indicated by the formation of a white

precipitate. Alkaloid compounds that contain nitrogen atoms and are basic in nature are reacted with HCl with the aim of extracting alkaloids that are basic in nature using acidic compounds. After that, Meyer's reagent is added so that the alkaloid compound reacts with the tetraiodomercurate (II) ion to produce a complex compound and a white to yellowish precipitate is formed (Indah, Diah, and Tony, 2020).

Tannin compound test, mangrove leaf extract *Rhizopora mucronata* mixed with $FeCl_3$ and added with H_2SO_4 solution got positive results with a blackish yellow color change. Tannin compounds are polar compounds because of the presence of OH groups. Therefore, when $FeCl_3$ and H_2SO_4 are added to the sample, the tannin compound will release the H atom, resulting in a blackish yellow color change (Indah, Diah, and Tony, 2020).

Testing for terpenoid compounds, Rhizopora mucronata mangrove leaf extract was reacted with Liebermann's *reagent* (acetic anhydride and concentrated sulfuric acid) resulting in a positive result with the formation of a red color. The purpose of adding acetic anhydride is to form acetyl derivatives, while the addition of H_2SO_4 aims to hydrolyze water which reacts with acetyl derivatives to form a color solution. Thus, the color change occurs due to oxidation of terpenoid compounds through the formation of conjugated double bonds (Indah, Diah, and Tony, 2020).

Based on the results of the phytochemical screening test, it can be concluded that mangrove leaf extract *Rhizopora mucronata* can be used as *repellent* against mosquitoes *Anopheles vagus* because it positively contains secondary metabolite compounds such as alkaloids, tannins , flavonoids, and terpenoids. These compounds have their respective functions which can prevent and protect against mosquito bites.

In **Table 8.** you can see the test results *repellent* preparation formulation *spray* mangrove leaf extract *Rhizopora mucronata* against mosquitoes *Anopheles vagus* . It was found that the average number of mosquitoes that landed on the formulation *spray* mangrove leaf extract *Rhizopora mucronata* 10% concentration was 0; 15% concentration of 0; and a 20% concentration of 0. The negative control average number of mosquitoes that landed was 6.3%. Based on research results Isnaini, Tutik, and Selvi (2022) that 96% ethanol produces a mosquito repellent percentage of 0%, meaning that 96% ethanol does not act as a *repellent* against mosquitoes . The *repellent* test is carried out 3 times for 30 seconds, because the landing speed and mosquito bite is only 30 seconds. Likewise, each treatment group test was carried out on different days with the aim of seeing the comparison of treatment groups during statistical analysis (WHOPES, 2009).

Based on the results obtained from the test *repellent* preparation formulation *spray* mangrove leaf extract *Rhizopora mucronata* , at a concentration of 10% it already has good mosquito repellent power against mosquitoes *Anopheles vagus* . This is also proven by previous research conducted by Sengodan et al, (2020) that a 10% concentration of the formulation *spray* mangrove leaf extract *Rhizopora mucronata* has effective mosquito repellent power because this dose can Prevent and protect from mosquito bites.

Formulation of the preparation *spray* mangrove leaf extract *Rhizopora mucronata* as *repellent* against mosquitoes *Anopheles vagus* from the results of the normality test in **Table 9.** indicates that the resulting mosquito repellent power is significant <0.05. Data is normally distributed if the significant value is >0.05 and not normally distributed if the significant value is <0.05. From these results, the resulting data is not normally distributed because the significant value is <0.05. This means that the data does not meet the requirements for a parametric test using ANOVA (*Analysis Of Variance*), so a non-parametric test using the Kruskal-Wallis test is used as an alternative to the ANOVA test (*Analysis Of Varience*).

The results of the statistical analysis of the Kruskal-Wallis test showed a significant value of <0.05, which indicates that there is an effect of the preparation formulation *spray* mangrove leaf extract *Rhizopora mucronata* as *repellent* against mosquitoes *Anopheles vagus* . Then, to determine the significant differences in concentrations of 10%, 15%, and 20%, the formulation test for the preparation *spray* mangrove leaf extract *Rhizopora mucronata* against mosquitoes *Anopheles vagus* , was carried out Duncan's Post Hoc test. From **Table 10.** shows that at formulation concentrations of 10%, 15%, and 20% there is no significant difference in effect on mosquitoes *Anopheles vagus* , because at these concentrations the compound content of mangrove leaves *Rhizopora mucronata* is equally effective as *repellent* against mosquitoes *Anopheles vagus* . Therefore, from the results of testing the preparation formulation *spray* mangrove leaf extract *Rhizopora mucronata* against mosquitoes *Anopheles vagus* it can be concluded that there is no significant difference at a concentration of 10%, 15%, and 20%.

CONCLUSION

Based on the results of research testing the formulation of *spray* mangrove leaf extract *Rhizopora mucronata* as *repellent* against mosquitoes *Anopheles vagus* , it can be concluded:

1. The preparation formulation *spray* mangrove leaf extract *Rhizopora mucronata* has activity as *repellent* against mosquitoes *Anopheles vagus*.
2. The formulation of the preparation *spray* mangrove leaf extract *Rhizopora mucronata* as *repellent* against mosquitoes *Anopheles vagus* at concentrations of 10%, 15% and 20% is not there is a significant difference, because at this concentration the compound content of mangrove leaves *Rhizopora mucronata* is equally effective as *repellent* against mosquitoes *Anopheles vagus*.

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