Formulation And Testing Of Antibacterial Activity Of Rambutan Leaves (Nephelium Lappaceum Linn) Ethanol Extract Ointment Against The Bacteria Staphylococcus Aureus

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ABSTRACT

Rambutan leaf samples were used in this research because people only think that rambutan leaves are just trash that is often thrown away and burned. This research aims to formulate the ethanol extract of rambutan leaves (Nephelium lappaceum Linn) into an ointment preparation, as well as testing the antibacterial activity against Staphylococcus aureus bacteria by using the paper disk method. In this research, 4 ointment formulations were made with different concentrations, namely F0 (0%), F1 (10%), F2 (20%), F3 (30%). Then a physical stability test was carried out on the ointment preparation for 4 weeks with test parameters including organoleptic test, pH test, homogeneity test, adhesion test, spreadability test, viscosity test, irritation test and hedonic test for each of the 4 formulas made which showed the results. meet the requirements for the stability of ointment preparations. Furthermore, testing the antibacterial activity of ointment preparations with concentrations of 10%, 20%, 30% and K-, K+ resulted in inhibition zone diameter values of 10.66 mm, 13.33 mm, 22 mm and 0 mm, 11 mm. It can be concluded that the ethanol extract ointment preparation of rambutan leaves can inhibit the growth of staphylococcus aureus bacteria. The maximum antibacterial activity effect of the ethanol extract of rambutan leaves (Nephelium lappaceum Linn) is at a concentration of 30%. With an inhibition zone of 22 mm.

Key words: Ointment, Rambutan Leaves, Stapylococcus aureus

1. Introduction

Ointment is a drug applied to the skin and is intended for external use. Ointments are usually used to treat acute or chronic skin diseases by penetrating the skin layers to develop the desired effect. Ointments have a composition consisting of medicinal or active ingredients and ointment base, or commonly known as active ingredient carrier (Turnip & Dwicahya, 2020).

The formula for a good ointment must determine the correct base, the previous bases were hydrocarbon-based, absorbent and water-washable. Hydrocarbon ointment base at 8% concentration is a better ointment base based on the physical stability test results of the product. The use of an appropriate ointment base must be considered, because the selection of the right ointment base can affect the physical stability and therapeutic effects of the ointment preparation. Based on this, researchers want to study which ointment base is suitable for the active ingredients of rambutan leaf ethanol extract based on the results of evaluating its physical properties [1].

Staphylococcus aureus is a spherical gram-positive bacterium and is a major human pathogen. Staphylococcus aureus can also cause several infections such as acne and boils in humans and animals, Staphylococcus aureus bacteria can also cause serious and fatal

infections which are usually the most common postoperative wound contamination in patients (Turnip et al., 2020).One of the bacteria causing to several antibiotics, such as penicillin group drugs [1].

Bacteria that cause infections and diseases can be prevented with antibacterial drugs. Antibacterials are substances that can inhibit the growth of bacteria and kill pathogenic bacteria. antibacterials are divided into two, namely: bacteriostatic which inhibits bacterial growth and bactericidal which can kill bacteria (Magani et al., 2020).

Extracts from rambutan leaves have antibacterial activity against the growth of Staphylococcus aureus at concentrations of 5%, 20%, 50% and 75%, providing inhibition zones of 0 mm, 16 mm, 21 mm, and 26 mm respectively (Kumar et al., 2021).

The reason for using rambutan leaf samples, because people only think that rambutan leaves are just garbage that is often thrown away and burned. Rambutan leaves have medicinal content and benefits, rambutan leaves contain compounds such as steroids, flavonoids, polyphenols, hydroquinone, saponins and tannins (Rizky & Tanjung, 2019).

In research conducted (Harahap, 2022) has tested antibacterial activity against Staphylococcus aureus and Eschericehia coli. Based on the above background, the author wants to make ointment preparations and test the antibacterial activity of rambutan leaves in inhibiting the growth of Staphylococcus aureus bacteria.

2. Methodology

This research was conducted in August-September 2023 in the Microbiology Laboratory of the Faculty of Health Sciences, University of Muhammadiyah Palopo, South Sulawesi.

Research Variables

Independent variables, namely variables that are the cause of the emergence or change of the dependent variable (dependent variable). In this study, which has a position as an independent variable is the ethanol extract of Rambutan leaves (Nephelium lappaceum Linn) with several concentrations, namely 10%, 20%, 30% concentration. As an active substance in making ointment preparation formulas.

The dependent variable: The characteristics of the formulations (F1, F2, F3) of rambutan leaf ethanol extract ointment preparations (Nephelium lappaceum Linn) include organoleptic test, ph test, spreadability test, adhesion test, homogeneity test and hedonic test and also activity test against Staphylococcus aureus bacteria.)

Tools and materials

Autoclave stirring rod Petri dish porcelain dish Erlenmeyer Test tube measuring cup beaker pH paper incubator Magnetic stirer Oven Wire ose round paper disc tweezers pipette drops Scales glass jar Ointment container Blender vaselin album Nipagi Cera alba Almunium foil Aquadest, Bacteria Staphylococcus aureus Ethanol extract of rambutan leaves 96% ethanol,Green tea honey Marketed ointment brand (X) Filter paper Nutrient agar (NA) label Nacl 0.9 Cotton gauze Wrab paper

Sample processing

Samples of rambutan leaves (Nephelium lappaceum Linn) obtained were wet sorted. c. Samples of rambutan leaves are then washed thoroughly with running water and then dried at room temperature by drying for several days Samples of rambutan leaves that have been dried are sorted dry and weighed then mashed using a blender to obtain rambutan leaf powder (Nephelium lappaceum Linn). Rambutan leaf powder is then stored in a clean container for the next step.

Preparation of Rambutan Leaf Extract (*Nephelium lappaceum* Linn)

The extraction process was carried out by maceration method. 500 grams of simplisia powder was put into a container and added 96% ethanol solvent as much as 3 liters. Then soaked for 5 days with occasional stirring Filtered the results of soaking for 5 days using flannel cloth to separate the extract with the filtrate (filtrate 1) The extract was again soaked for 3 days with 96% ethanol with a solvent of 1.5 liters and then filtered (Filtrate 2) The results of filtrate 1 and filtrate 2 were mixed The filtrate obtained was then aerated using a fan at room temperature until a thick extract was formed.

Preparation of Positive Control and Negative Control

The preparation of the control solution will be carried out by making a positive control solution, namely by using Mupirocin ointment on the market, while the negative control uses the preparation base,

Preparation of Ointment

Hydrocarbon Ointment for F1, F2, and F3 as follows: Phase 1 Cera alba according to the calculation of ingredients and vaseline album according to the calculation of ingredients are melted on a 75 - 80^{0}C water bath using a porcelain cup, stirred until homogeneous. Phase 2 nipagin is added to the main ingredient, namely rambutan leaf extract (Nephelium lappaceum Linn) stirred until homogeneous After phase 3 is homogeneous, phase 1 is added and mixed until both phases are homogeneous. The finished ointment preparation is put in an ointment container/pot and carried out physical evaluation tests and antibacterial tests against Staphylococcus aureus bacteria [2].

Evaluation of Deodorant spray preparation

Organoleptic Test

Organoleptic testing is carried out by observing the ointment preparation from the shape, smell and color of the preparation. ointment specifications that must be met are choosing a semi-solid form, the color must match the specifications at the time of the initial preparation of the ointment and the smell is not rancid [3].

Homogeneity Test

Homogeneity testing of ointment preparations is carried out by applying ointment to a piece of glass or other transparent material which must show a homogeneous arrangement. Homogeneous ointment is characterized by the absence of lumps in the result of smearing, an even structure and has a uniform color from the starting point of smearing to the end point of smearing. The tested ointment was taken in three places, namely the top, middle and bottom of the ointment container [3].

Spreadability Test

A total of 0.5 g of ointment was placed on a round glass with another glass placed on top and left for 1 minute. The spread diameter of the ointment was measured. Afterwards, 100 grams of load was added and allowed to stand for 1 minute and then a constant diameter was measured. The diameter of good ointment spreadability is between 5-7 cm [3].

Adhesion Test

A total of 0.25 grams of ointment is placed on a glass object that has been determined. Another object glass was placed on top of the ointment. After that, a 1 kg load was added for 5 minutes to the object glass and mounted on the test device. The 80-gram weight was released and the time was recorded until both glasses were released. The experiment was repeated 5 times [4].

pH test

Measurement of pH value using a universal pH stick tool dipped in 0.5 g of ointment. The pH value of a good ointment is 4.5-6.5 (Sari et al, 2017).

Irritation Test

Irritation testing of ointment preparations made was carried out on 14 male and female volunteers. The preparation was applied to the volunteer's forearm and left for 5 minutes. Then the back of each volunteer's hand was observed, if there was a reaction (no red and no swelling) given a sign (-), if there was a reaction (red skin) given a sign (+), then if there was swelling given a sign (++) (Rosyiedi, 2011).

Viscosity Test

The device used for the viscosity test is a viscosimeter with a suitable rotor (rotor number 4). The rotor is placed in the center of the glass container containing the ointment, then the device is turned on so that the rotor starts to rotate. The needle indicating viscosity will automatically move to the right. After stabilizing, then read the viscosity on the scale on the viskotester [5].

Hedonic Test

The hedonic test was carried out by distributing questionnaires to 14 respondents to observe the level of preference for ointments from texture, aroma, color and impression when applied to the skin. The range of values in the hedonic test is 1 (dislike), 2 (less like), 3 (like) and 4 (very like) [1].

Tool sterilization

The tools to be used in the test were washed thoroughly and then dried. After that, it was wrapped in aluminum foil and then sterilized together with NA media using an autoclave at a pressure of 1 atm with a temperature of 121^0C for 15 minutes.

Preparation of Test Media 20 gr of NA (Nutrient Agar)

Weighed 20 grams of NA (Nutrient Agar) media using analytical scales and then put into an Erlenmeyer flask Then dissolved with 150 ml of distilled water until homogeny Stirred and heated on a hot plate while stirring until the media dissolves well. Covered the media with cotton, after which it was sterilized by autoclaving at 121^0C for 15 minutes. Poured sterile media into sterile petri dishes, waited until the media solidified [6].

Bacteria Rejuvenation

A total of 0.1 ml of the test bacterial suspension was put into a Petri dish containing sterile NA media. The disc paper used had a diameter of 0.5 cm. Put the disc paper that had been smeared with the test ointment preparation with a concentration of 10%, 20%, and 30% on the surface of the NA media that had been planted with bacteria. Incubated for 24 hours at 37 oC. The antibacterial diameter was observed based on the inhibition diameter indicated by the clear area formed around the disc paper and measured using a ruler. The measurement results were recorded [7].

Zone of Inhibition Measurement

Antibacterial activity can be said to be positive if a clear zone of inhibition is formed around the disc paper. The part that is calculated with a caliper or ruler is the diameter of the inhibition zone formed. According to [8] the criteria for antibacterial power strength are as follows: the diameter of the inhibition zone is 5 mm or less then the inhibitory activity is categorized as weak, the diameter of the inhibition zone is 5-10 mm then categorized as moderate, the diameter of the inhibition zone is 10-20 mm categorized as strong and if the diameter is 20 mm or more then the inhibitory activity is categorized as very strong. The formation of the inhibition zone in the antibacterial activity test is influenced by several factors including the concentration of the extract, the content of antibacterial compounds and the type of bacteria [8].

The following is a picture of the antibacterial activity test of rambutan leaf extract ointment preparation against Staphylococcus aureus bacteria.

3. Result and Discussion

3.1 Result

Rambutan leaves used were obtained from the city of Palopo. The method of making rambutan leaf simplisia is rambutan leaf samples (Nephelium lappaceum Linn) obtained by wet sorting then the rambutan leaf samples are then washed thoroughly with running water and then dried at room temperature by drying for several days then the dried rambutan leaf samples are dry sorted and weighed then mashed using a blender to obtain rambutan leaf powder (Nephelium lappaceum Linn). Rambutan leaf powder is then stored in a clean container for the next step.

Organoleptic testing aims to determine the organoleptic preparation which includes color shape and aroma. This organoleptic test is very important because it determines the quality of the ointment preparation by eye and sees the physical results of the ointment. The ointment specifications that must be met are that it has a semi-solid shape, the color must match the specifications at the time of the initial manufacture of the ointment and the smell is not rancid [3].

From the results of the pH test of rambutan leaf extract ointment preparations (*Nephelium lappaceum* Linn) conducted for 4 weeks, it was found that the pH of the preparation was stable which met the requirements of a good pH of 4.5-6.5 or in accordance with the pH value of human skin (Sari et al, 2017).

The results of the homogeneity test of rambutan leaf ethanol extract ointment preparations from all formulas made show homogeneous results which are characterized by evenly mixed or dispersed particles and no lumps when the preparation is applied to the glass object

The results of the spreadability test of the four dosage formulations carried out for 4 weeks obtained unstable spreadability results. This is due to the consistency of the ointment preparation which is massy resulting in the spread of the ointment not being maximized [3].

Good adhesion allows the active substance in the preparation not to be easily released and the longer it is attached (contact with the skin) can produce a longer effect and as expected. The requirement for good adhesion for topical preparations is not less than 4 seconds, meaning that the results of the adhesion test of the four dosage formulations have met the requirements for good adhesion [4].

The viscosity test of the ointment was conducted to determine the viscosity of each ointment. Based on the table of viscosity test results conducted on each concentration of rambutan leaf ethanol extract ointment, different viscosity results were obtained. A good viscosity requirement for ointment preparations is 2000-50,000 cP [9].

The results obtained in the preparation of ointment of ethanol extract of rambutan leaves (Nephelium lappaceum Linn) with concentrations of 10%, 20%, 30% did not show any side effects in the form of skin redness, itching, and swelling caused by the ointment preparation. From the results of the irritation test, it can be concluded that the preparation of rambutan leaf ethanol ointment (Nephelium lappaceum Linn) made is safe to use on the skin (Sari et al, 2017).

The hedonic test results were carried out using a google form quiz. The hedonic test involved 14 respondents using the 20% error slovin formula. This is because the population used is small. The results obtained in the hedonic test based on aroma with an assessment of the likes category in F1 and F3. This is obtained from the distinctive aroma of green tea honey that respondents like. The results in the hedonic test based on color in the like category in F0, because F0 does not use extracts so that the color impression is more attractive when compared to formulas that have added extracts. The results of the hedonic test based on the texture impression of the category liked in F1, F2, this is due to the use of vaseline album as an emollient with a higher concentration.

The results of the antibacterial activity test of the ethanol extract ointment preparation of rambutan leaves with each concentration can form an inhibitory zone for the growth of Staphylococcus aureus bacteria. The large inhibitory zone found in F3 with a concentration of 30%, namely 22 mm, is categorized as very strong. The antibacterial activity test results obtained are in line with research (Kumar et al., 2021) that rambutan leaf extract can inhibit Staphylococcus aureus bacteria with a concentration of 75%, which shows that the inhibition zone area of 26 mm is classified as very strong. The criteria for the strength of antibacterial inhibitory power are an inhibition zone diameter of 5 mm or less, then the inhibitory activity is categorized as weak, an inhibition zone diameter of 5-10 mm is categorized as moderate, an

inhibition zone diameter of 10-20 mm is categorized as strong and if the diameter is 20 mm or more then the activity inhibition is categorized as very strong [8].

The data analysis used in this research is a one way variance test (One Way Anova). The One Way Annova test aims to find out whether there is a difference in the average of the test samples. Before analyzing data using One Way Anova, a normality test and homogeny test must first be carried out. From the results of the normality test data on the diameter of the antibacterial inhibition zone of the ethanol extract ointment of rambutan leaves against staphylococcus aureus bacteria. From the normality test data using Shapiro-Wilk it can be seen (attachment 7). The results obtained were F1 P=0.000, F2 p=0.000, F3 P=0.000 K- P= 0, K+ P= 1.000 which was greater than 0.05. Data is normally distributed if the sig p value is >0.05. Next, a data homogeneity test was carried out using Levene statistics, resulting in a significant value of P = 0.089, which can be seen in (Appendix 7), so it can be said that the data is homogeneous. Where the data is homogeneous if the sig P value is >0.05, meaning

The data in the research have the same variance so that One Way Annova can be continued. The One Way ANOVA test obtained was <0.000 because the p value was <0.05, so the average value between the comparisons had different meanings.

The test is continued with a Post-Hoc Test. Data that is significant or meaningfully different is marked with a star (*) which can be seen in (attachment 7). The F1 concentration has a different meaning from F3 and KN. The F2 concentration has a different meaning from F3 and Kn. F3 concentration has a different meaning from F1, F2, KP, KN. KP concentration has a different meaning from F3 and KN. KN concentration has a different meaning from F1, F2, F3, KP.

3.2 Discussion

The rambutan leaves used were obtained from the city of Palopo. The method for making rambutan leaf simplicia is that the sample of rambutan leaves (Nephelium lappaceum Linn) obtained is sorted wet, then the sample of rambutan leaves is then washed thoroughly with running water and then dried at room temperature by air-drying a few days later. The sample of dried rambutan leaves is sorted dry. and weighed then ground using a blender until rambutan leaf powder (Nephelium lappaceum Linn) is obtained. The rambutan leaf powder is then stored in a clean container for the next steps

In the first stage, the extraction process was carried out by the remaceration method. 500 grams of simplisia powder was put into a container and added 96% ethanol solvent as much as 3 liters. Then soaked for 5 days with occasional stirring Filtered the results of soaking for 5 days using flannel cloth to separate the extract with the filtrate (filtrate 1). The extract was again soaked for 3 days with 96% ethanol with a solvent of 1.5 liters and then filtered (Filtrate 2). The results of filtrate 1 and filtrate 2 were mixed. The filtrate obtained was then aerated using a fan at room temperature until a thick extract was formed.

This water content determination aims to determine the percentage of water content remaining in the simplicia. It is important to know the maximum limit of water content in

simplicia because if the amount of water contained is too high, it becomes a medium for the growth of bacteria and fungi which can damage the quality of simplicia (Ministry of Health of the Republic of Indonesia, 2000).

The drying shrinkage parameter is a non-specific parameter which aims to provide a limit (range) regarding the amount of compounds lost in the drying process, not only describing the water lost but other evaporated compounds. Basically, drying loss at a temperature of 105◦ C until constant weight is then expressed in percent (Ministry of Health of the Republic of Indonesia, 2017).

Organoleptic testing aims to determine the organoleptic preparation which includes color and aroma. This organoleptic test is very important because it determines the quality of the ointment with the naked eye and looks at the physical results of the ointment. The specifications that must be met for the ointment are that it has a semi-solid form, the color must match the specifications at the time of initial manufacture of the ointment and the smell must not be rancid [3].

The purpose of carrying out an ointment pH test is to see the acidity level of the ointment preparation produced using a pH meter.

The homogeneity test is carried out to determine whether the homogeneity of the preparation can be mixed evenly between the active substance and the ointment base or not [3].

Spreadability measurement aims to see the spreadability of the ointment. The parameters of the ointment used have a spreadability of 5-7 cm (Sari et al, 2017).

Adhesion testing is carried out with the aim of determining the time it takes for the preparation to stick to the skin. Good adhesion means that the active substance in the preparation does not come off easily and the longer it sticks (in contact with the skin) it can produce a longer and as expected effect.

The ointment viscosity test is carried out to determine the viscosity of each ointment.

each ointment.

The purpose of carrying out an irritation test is to meet skin sensitivity and prevent side effects on the skin. After use on the skin, the safety level of the preparation can be known. This irritation test aims to prevent side effects on the skin.

Hedonic (Like) testing is carried out to test physical quality organoleptically. This test is very important because it is directly related to acceptability for consumers. The hedonic test was carried out on 14 respondents.

The results of the antibacterial activity test of the ethanol extract ointment preparation of rambutan leaves with each concentration can form an inhibitory zone for the growth of Staphylococcus aureus bacteria. The large inhibitory zone found in F3 with a concentration of 30%, namely 22 mm, is categorized as very strong.

The data analysis used in this research is a one way variance test (One Way Anova). The One Way Annova test aims to find out whether there is a difference in the average of the test samples. Before analyzing data using One Way Anova, a normality test and homogeny test must first be carried out. Testing continues with a Post-Hoc Test. Data that is significant or

meaningfully different is marked with a star (*)

4. Conclusion

Rambutan leaves (Nephelium lappaceum Linn), can be formulated into an ointment. Physical stability tests include organoleptic tests, which do not experience changes in aroma, color and shape during testing. The pH test before and after testing for 4 weeks meets the skin requirements, namely 4.5-7.0. In the homogeneity test, results were obtained that met the requirements of the homogeny test, namely a preparation that was free from foreign particles. In the adhesion test, results were obtained that met the criteria. In the spreadability test, results were obtained that did not meet the criteria because the consistency of the ointment mass resulted in the spread of the ointment not being optimal. In the viscosity test, results were obtained that met the criteria. In the irritation test, the ointment applied to the skin did not show any irritating effect. In the test, hedonic results were obtained based on aroma, color, texture based on the hedonic test (Likes), namely F1.

The ethanol extract of rambutan leaves (Nephelium lappaceum Linn) which is formulated into ointments with different concentrations can inhibit Staphylococcus aureus bacteria, where the results of the antibacterial activity test resulting from a concentration of 10% are categorized as strong, while for 20%, it is categorized as strong, 30% has the inhibition zone is categorized as very strong.

Test the antibacterial activity of the ethanol extract ointment preparation of rambutan leaves (Nephelium lappaceum Linn) using different concentrations, namely 10% has an inhibition zone of 10.66 mm, 20% has an inhibition zone of 13.33 mm, and 30% has an inhibition zone of 22 mm. It can be concluded that the concentration of the maximum antibacterial activity effect of the ethanol extract of rambutan leaves (Nephelium lappaceum Linn) is at a concentration of 30%. .

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