

Formulation And Antibactory Activity Test Of Deodoran Spray Etanol Extract Of Mangrove Leaves (*Rhizophora Mucronata*) Against The Bacteri *Staphylococcus Aureus*

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Abstract

Mangrove plants that grow abundantly along the coast are widely known for blocking waves and protecting coastal ecosystems. However, the use of mangrove plants is still limited and tends to focus on the function of physical protection against coastal abrasion so that parts of the mangrove plant such as leaves are rarely known about their benefits among the public This research aims to formulate ethanol extract of mangrove leaves (*Rhizophora mucronata*) into a deodorant spray preparation, as well as test the antibacterial activity against *Staphylococcus aureus* bacteria using the paper disk method. In this research, 4 deodorant spray formulations were made with different concentrations, namely F0 (without extract), F1 (10%), F2 (15%), F3 (20%). Then a physical stability test was carried out on the spray deodorant preparation for 4 weeks with test parameters including organoleptic test, pH test, clarity test, irritation test and hedonic test for each of the 4 formulas made showing that the results met the stability requirements for the spray deodorant preparation. Furthermore, testing the antibacterial activity of spray deodorant preparations with concentrations of 10%, 15%, 20% and K-, K+ showed that the average diameter of the inhibition zone formed was 12,75 mm, 15,16 mm, 18,25 mm 0 mm, 1.5 mm it can be concluded that mangrove leaf extract (*Rhizophora mucronata*) can be formulated into a deodorant spray preparation and provides maximum antibacterial effect, namely at a concentration of 20% at 18,25 mm.

Keywords: Deodorant spray, Mangrove leaves, *Stapylococcus aureus*

1. Introduction

Body odor is one of the problems that disrupt daily life. Unpleasant body odor often makes a person feel less confident and makes people around them uncomfortable. The unpleasant smell usually appears when someone starts sweating. Body odor comes from a combination of sweat and bacteria. Actually, sweat is odorless but it is the bacteria that make the body odor because the bacteria perform activities in moist and wet environments. The process of releasing sweat is a natural activity carried out by the body (Handayani *et al.*, 2022).

The dosage form used to treat body odor caused by bacteria is deodorant. Deodorant is a product that is believed to reduce body odor caused by the mixing of bacteria. Currently, there are many deodorant products with a variety of different dosage forms. One of them is deodorant spray. Deodorant spray is a cosmetic product that absorbs sweat, covers body odor and reduces body odor by spraying on certain body parts. The main advantage of deodorant spray compared to other deodorants is that in the active system of deodorant spray there is no contact between the deodorant and the user's skin, which means that the level of hygiene is high (Oktaviana *et al.*, 2019).

Some of the bacteria that cause body odor are *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Corynebacterium acnes*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes*. *Staphylococcus* is able to convert certain amino acids into highly fragrant short-chain volatile fatty acids, namely isovaleric acid which contributes to underarm odor (Siskawati *et al.*, 2014).

One of the plants that has properties that are utilized is the mangrove plant (*Rhizophora mucronata*) which has antibacterial properties. Mangrove leaves (*Rhizophora mucronata*) have antibacterial potential because they inhibit the growth of *Staphylococcus aureus* bacteria. This is because the leaves of mangrove trees (*Rhizophora mucronata*) contain flavonoids, alkaloids, tannins, triterpenoids, and flavonoids (Egra *at al.*, 2019). Based on previous research Kurnianingsih *et al.*, (2021), stated that mangrove leaf extract (*Rhizophora mucronata*) has antibacterial activity against *Staphylococcus aureus* bacteria, mangrove leaf extract has inhibition against *Staphylococcus aureus* bacteria at a concentration of 5% (7.53 mm), concentration 10% (11.76 mm) and 20% (12.26 mm) classified as strong.

The beauty of mangrove trees that thrive on the coast of Palopo city is widely recognized as a barrier to waves and maintaining coastal ecosystems. The use of mangrove trees is still limited and tends to focus on the function of physical protection against coastal abrasion. Based on the antibacterial activity of mangrove leaves (*Rhizophora mucronata*), it is necessary to develop a pharmaceutical preparation to increase its use, one of which is a deodorant spray preparation. Based on the description above related to the benefits of mangrove leaves (*Rhizophora mucronata*), this study was conducted to determine the antibacterial activity of deodorant spray preparation formulations of ethanol extracts of mangrove leaves (*Rhizophora mucronata*).

2. Methodology

This research was conducted from September to October at the Microbiology Laboratory, Faculty of Health Sciences, University of Muhammadiyah Palopo.

Research Variables

Independent variables: variations in concentration of 10%, 15%, 20%, ethanol extract of mangrove leaves (*Rhizophora mucronata*) as an active substance in the preparation of deodorant spray preparation formula. The dependent variable: characteristics of formulations F1, F2, F3, deodorant spray preparation of ethanol extract of mangrove leaves (*Rhizophora mucronata*) including organoleptic test, pH test, clarity test, irritation test, hedonic test and activity test against *Staphylococcus aureus* bacteria.

Tools and materials

The tools used are analytical balance, magnetic stirrer, pH paper viscometer, stirring rod, volumetric flask, test tube, ose wire, jar, petri dish, fan, beaker, deodorant spray container, oven, blender, porcelain cup, analytical balance, autoclave, tweezers. The materials used are mangrove leaf extract (*Rhizophora muncronata*), propylenglycol, glycerin, 70% alcohol, vanilla oil, distilled water, *Staphylococcus aureus* bacteria, deodorant spray (brand

X) in the market, gauze, aluminum foil, sodium agar (NA) media, wrap plastic, 96% ethanol, filter paper, NaCl 0.9%.

Sample processing (*Rhizophora mucronata*)

Mangrove leaves (*Rhizophora mucronata*) taken are washed using running water until clean. then mangrove leaves that have been washed then drained. Mangrove leaves (*Rhizophora mucronata*) are cut thinly or chopped. Then dried in the sun with a black cloth covered as a cover until the simplisia dries for 5-7 days. Mangrove leaves that have been dried are pulverized using a blender. Then sifted using a mesh sieve no.40 (Maulidah, *et.al* 2022).

Preparation of Mangrove Leaf Extract (*Rhizophora mucronata*)

The extraction process is carried out by maceration. 500 grams of simplisia powder was weighed, then put into a maceration container. Soaked using 96% ethanol solvent as much as 5000 ml. soaking was carried out for 3x24 hours, in a maceration container with stirring once. Then filter the soak for the first 3 days with a funnel lined with filter paper (filtrate 1).Simplisia was again soaked for 2 days with 96% ethanol with 500 ml and then filtered (filtrate 2). The results of filtrate 1 and filtrate 2 were mixed.The filtrate obtained was then aerated using a fan (Paputungan & Yamlean, 2014).

Preparation of Positive Control and Negative Control

The preparation of the control solution is by using a marketed deodorant spray (brand X), while the negative control uses the preparation base.

Preparation of Deodorant spray

Prepare the tools and materials to be used Put enough 70% ethanol into the beaker glass Add propylenglycol stir until homogeneous Add glycerin little by little Add mangrove leaf extract (*Rhizophora mucronata*) into the beaker glass stir until homogeneous. Add vanilla oil to taste stir until homogeneous add the remaining 70% alcohol until the limit mark and stir until homogeneousPut into a container (spray bottle)

Evaluation of Deodorant spray preparation

Organoleptic Test

Prepare the deodorant spray preparation that has been made, observe the shape, color, aroma of the preparation made (Wulandari *et al*, 2019).

pH test

Prepare deodorant spray preparation, spray deodorant spray preparation on pH paper, Measure the pH by matching it to the pH indicator, record the pH result (Afriani, 2017).

Clarity test

Pour the deodorant spray preparation into a glass beaker, observe using a sodium lamp (clear / or not), record the results of the workability test (Wulandari *et al*, 2019).

Irritation Test

A population of 14 respondents, each respondent sprayed the preparation that had been made, left for 24 hours to observe the symptoms that arose (Dewi *et al.*, 2019).

Hedonic Test

Population of 14 respondents, each respondent sprayed the deodorant spray preparation, Recording each assessment from the respondent (Dewi *et al.*, 2019).

Tool sterilization

The tools to be used in the study were washed thoroughly and then dried, then, the tools were wrapped in aluminum foil, after that, sterilize NA media using an autoclave at a pressure of 1 atm, using a temperature of 121⁰C for 15 minutes

Bacteria Rejuvenation

Each *Staphylococcus aureus* bacteria was taken using a sterile wire loop 1 dose was taken from the pure culture, then inoculated by streaking on slanted nutrient agar (NA) medium, incubated in an incubator at 37⁰C for 1 x 24 hours (Afriani, 2017).

Preparation of Test Bacteria Suspension

Put 10 ml of 0.9% NaCl solution into a test tube, then take the bacteria with a sterile tube, suspend them in 10 ml of sterile 0.9% NaCl solution, then homogenize using a vortex (Afriani, 2017).

Making NA Test Media

Weigh 20 grams of NA media (nutrient agar) using an analytical balance, then put it in an Erlenmeyer flask, stir it with 150 ml of distilled water until homogeneous, heat it on a magnetic stirrer until the media dissolves homogeneousl, cover the media with cotton, then sterilize using an autoclave at 121⁰C for 15 minutes, pour the sterile media into a sterile petri dish, wait until the media solidifies (Meisani *et al*, 2018).

Antibacterial Activity Test using the Disc Diffusion Method

A total of 0.1 ml of the test bacterial suspension was inserted into a Petri dish containing sterile NA media, the disc paper used was 0.5 cm in diameter, placed the disc paper that had been dripped with the test solution with a concentration of 10%, 15%, and 20% on the surface of the NA media that had been planted with bacteria, Place the disc paper that has been dripped with deodorant spray solution (brand X) as a positive control and deodorant spray base as a negative control, incubated for 24 hours at 37^oC, the antibacterial diameter was observed based on the diameter of the inhibition zone indicated by the clear area formed around the disc paper and measured using a ruler, the measurement results were recorded (Atikah, 2013).

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 Davis and Stout (1971) in (Harita, 2019) the criteria for the strength of antibacterial power, namely the diameter of the inhibition zone of 5 mm or less, the inhibitory activity is categorized as weak, the diameter of the inhibition zone of 5-10 mm is categorized as moderate, the diameter of the inhibition zone of 10-20 mm is categorized as strong and if the diameter is 20 mm or more, 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 (Harita, 2019)

3. Result and Discussion

3.1. Research result

Mangrove leaves (*Rhizophora mucronata*) were taken in the city of Palopo. Processing of samples through the simplisia processing stage begins with the collection of mangrove leaves (*Rhizophora mucronata*). Then the wet sorting process is carried out, namely separating the simplisia attached to the simplisia. Then washed in running water then kneaded and dried for 5-7 days in the sun, covered with black cloth and dry sorting is done to separate the simplisia from attached particles or foreign particles during the drying process (Maulidah, *et.al* 2022). Weighed 500 grams of mangrove leaf (*Rhizophora mucronata*) simplisia powder, then soaked with 96% ethanol solvent, for 3x24 hours in a maceration container with occasional stirring. Filtered soaking results for the first 3 days using a funnel lined with filter paper (filtrate 1), simplisia again soaked for 2 days with 96% ethanol with 500 ml and then filtered (filtrate 2). The results of filtrate 1 and 2 were mixed, the filtrate obtained was aerated using a fan (Paputungan & Yamlean, 2014).

Determination of water content aims to determine the percentage of water content left in simplisia. This is important to know the maximum limit of water content in simplisia 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 simplisia (Depkes RI, 2000). The test results of determining the water content of mangrove leaf simplisia powder (*Rhizophora mucronata*) are 9%, this meets the requirements of water content in general, which is not more than 10% (Depkes RI, 2017). Drying shrinkage is a non-specific parameter that aims to provide a maximum limit (range) of the amount of compounds lost in the drying process. Basically, drying shrinkage is the measurement of substances after drying at a temperature of 105°C until constant weight is then expressed in percent. The test results of the determination of drying shrinkage of mangrove leaf simplisia powder (*Rhizophora mucronata*) are 8.69%, this meets the requirements of drying shrinkage in general, which is not more than 10% (Depkes RI, 2000).

Organoleptical test results of deodorant spray preparations of mangrove leaf extract (*Rhizophora mucronata*) were carried out on 4 preparations of various concentrations. The organoleptic test aims to see the physical appearance of the preparation by observing the shape, color and aroma of the preparation (Wulandari *et al*, 2019). Based on the observations that have been made, the texture of the three dosage formulations is liquid and the distinctive smell of vanilla obtained from the addition of flavoring. From observations for 4 weeks, the four formulas did not change either in shape or aroma, while the color formed from the four formulations had differences, namely formula 0 had a clear color, formulas 1 and 2 had a brown color while formula 3 had a dark brown color, this was due to differences in the concentration of extracts used in each formula.

The pH test results of the deodorant spray preparation of mangrove leaf extract (*Rhizophora mucronata*) were carried out using a pH stick. From the measurements that have been made, the data obtained in table 5. The pH test results of the four preparation formulas carried out for 4 weeks obtained stable preparation pH results that have met the skin pH requirements of 4.5-7.0 (Afriani, 2017). Changes in the pH value of the formula are caused by environmental factors such as changes in temperature because storage is carried out at room

temperature and storage containers that are less impermeable, allowing air to enter (Afni, N., & Said, N., 2015).

The results of the deodorant spray preparation clarity test of mangrove leaf extract (*Rhizophora mucronata*). Obtained data in the table 6. The purpose of the clarity test is to determine the clarity of the deodorant spray made and observe whether the preparation is clear or cloudy. The results of the clarity test of the four dosage formulations carried out for 4 weeks obtained clear stable preparations that have met the requirements of clear preparations and are free of foreign particles (Wulandari *et al*, 2019). Hedonic test results on deodorant spray preparation of ethanol extract of mangrove leaves (*Rhizophora mucronata*). This parameter plays an important role because it is directly related to acceptability to consumers. The irritation test was conducted by 14 respondents. Irritation testing was carried out to determine the irritating effect of the deodorant spray preparation of ethanol extract of mangrove leaves (*Rhizophora mucronata*). After being applied to the skin, the safety level of the preparation can be determined. This irritation test has the aim of preventing side effects on the skin. The results obtained in the preparation of deodorant spray ethanol extract of mangrove leaves (*Rhizophora mucronata*) with a concentration of 0%, 10%, 15%, 20% there are no side effects in the form of skin redness, itching, and swelling caused by the preparation of deodorant spray preparations from the four formulas have a pH that matches the pH of the skin, namely 4.5 - 7.0 (Afriani, 2017). From the results of the irritation test, it can be concluded that the deodorant spray preparation made for the skin is suitable for the skin.

The results of the hedonic test of deodorant spray preparation of ethanol extract of mangrove leaves (*Rhizophora mucronata*) can be seen in the table 8. The results obtained in the hedonic test based on the aroma with an assessment of the category of likes in F3, this is obtained from the distinctive aroma of vanilla that respondents like. The results in the hedonic test based on the color assessment category liked in F1, this was obtained from the relatively low concentration of mangrove leaf extract (*Rhizophora mucronata*) used so as to produce a preparation color that was not concentrated. Hedonic test results based on the impression of softness in the category liked in F3, this is due to the use of glycerin as a moisturizer with a higher concentration in formula 3. Based on table 9, the results of the antibacterial activity test of deodorant spray preparations of ethanol extracts of mangrove leaves (*Rhizophora mucronata*) with each concentration can form an inhibition zone on the growth of *Staphylococcus aureus* bacteria. The large inhibition zone is found in F3 with a concentration of 20%, which is 18.25 mm categorized as strong. The results of the antibacterial activity test of ethanol extracts of mangrove leaves (*Rhizophora mucronata*) which inhibit *Staphylococcus aureus* bacteria are in line with research Kurnianingsih *et al.*, (2021) with a concentration of 20% the inhibition zone area formed by 12.26 mm which is categorized as strong. This is in accordance with the content of secondary metabolites in mangrove leaves (*Rhizophora mucronata*) namely tannins, flavanoids, saponins.

Based on the research of David and Strout (1971) in (Harita, 2019) the criteria for the strength of antibacterial inhibition are the diameter of the inhibition zone of 5 mm or less, the inhibitory activity is categorized as weak, the diameter of the inhibition zone of 5-10 mm is categorized as moderate, the diameter of the inhibition zone of 10-20 mm is categorized as strong and if the diameter is 20 mm or more, the inhibitory activity is categorized as very

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The positive control (brand X deodorant spray) which is claimed to have antibacterial properties showed an inhibition zone. Brand X deodorant spray as a positive control contains aluminum Chlorhydrate as an active ingredient that functions as an antibacterial by inhibiting bacterial growth and reducing the amount of sweat released from the armpits by clogging pores (Rossalinda *et al.*, 2021). The data analysis used in this study is a one-way variant test (One Way Anova). The One Way Anova test aims to determine whether there are differences in the average of the test samples. Before analyzing the data with One Way Anova, normality test and homogeneous test must first be carried out. From the results of the normality test data on the diameter of the antibacterial inhibition zone of the deodorant spray preparation of ethanol extract of mangrove leaves (*Rhizophora mucronata*) against staphylococcus aureus bacteria. From the normality test data using Shapiro-Wilk can be seen (attachment 7). The results obtained for the value of F1 P = 0.122, F2 p = 0.463, F3 P = 1,000, K- P = 0, K + P = 0.363 which is greater than 0.05. So it can be said that the data is normally distributed. Data is normally distributed if the sig p value is > 0.05. Furthermore, the data homogeneity test using the Levene statistic obtained a significant value of P = 0.009 can be seen in (Appendix 7), so it can be said that the data is homogeneous. Homogeneous data if the sig value of P > 0.05 means that the data in the study have the same variance so that One Way Annova can be continued.

The One Way annova test obtained was <0.000 because the p value <0.05, so the average value between comparisons is significantly different. Testing is continued with the Post-Hoc Test test, significant or significantly different data marked with a star (*) can be seen in (attachment 7). F1 concentration is significantly different from F3, F2 concentration is significantly different from KP (positive control) and KN (negative control), F3 concentration is significantly different from F1, KP (positive control), KN (negative control), KP (positive control) concentration is significantly different from F2, F3, KN (negative control), KN (neative control) concentration is significantly different from F1, F2, F3, KP (positive control).

3.2 Discussion

From the research that has been carried out, the results of the water content test are obtained which meet the water content test requirements, namely no more than 10%. According to BPM (2014). Then the drying shrinkage test yielded results that also met the drying shrinkage test requirements, namely 8.69%. After testing the water content and drying loss, the preparation was made according to the formulation, then the first test was carried out on the mangrove leaf ethanol extract spray deodorant, namely the organoleptic test with the results obtained, namely a liquid preparation, odorless (preparation without extract). The typical aroma is vanilla (10%, 15% and 20% turmeric rhizome extract gel preparation) and the clear color (gel without extract) is brown (10%, 15% and 20% mangrove leaf extract preparation). In the clarity test, results were obtained that met the requirements for a good

spray deodorant preparation, there were no fine grains and the base was mixed evenly with the active substance.

The pH test on the mangrove leaf extract deodorant preparation obtained results that met the pH test requirements that were acceptable to the skin, namely 4.5-7.0. In the test results obtained on the deodorant spray preparation of ethanol extract of mangrove leaves (*Rhizophora mucronata*) with concentrations of 0%, 10%, 15%, 20%, there were no side effects in the form of reddish skin, itching and swelling caused by the deodorant preparation. sprays from the four formulas have a pH that matches the skin's pH, namely 4.5 - 7.0 (Afriani, 2017). The results of the hedonic test are based on the color rating for the liking category in F1, this is obtained from the relatively low concentration of mangrove leaf extract (*Rhizophora mucronata*) used, resulting in the color of the preparation. which is not thick. The hedonic test results are based on the impression of softness in the like category in F3, this is due to the use of glycerin as a moisturizer with a higher concentration in formula 3. The results of the antibacterial activity test of the deodorant spray preparation of ethanol extract of mangrove leaves (*Rhizophora mucronata*) with each concentration can form an inhibitory zone for the growth of *Staphylococcus aureus* bacteria. A large inhibitory zone is found in F3 with a concentration of 20%, namely 18.25 mm, which is categorized as strong

4. Conclusion

Mangrove leaves (*Rhizophora mucronata*) can be formulated into a deodorant spray because it can meet good physical stability standards including organoleptical tests, which do not experience changes in aroma, taste, and shape during testing. The pH test before and after testing for 4 weeks meets the skin standard requirements of 4.5 - 7.0. In the clarity test, the results obtained meet the criteria for the clarity test, namely a clear preparation. From the results of the irritation test did not cause any side effects, so the deodorant spray made is safe to use for the skin. In the hedonic test results that are preferred from aroma, color, softness based on the percentage preferred in formula F3.

Ethanol extract of mangrove leaves (*Rhizophora mucronata*) formulated into deodorant spray preparations with different concentrations can inhibit *Staphylococcus aureus* bacteria. The resulting antibacterial activity test results from concentrations of 10%, 15%, 20% have an inhibition zone. Antibacterial activity test of deodorant spray preparations of ethanol extract of mangrove leaves (*Rhizophora mucronata*) using different concentrations, namely 10% has an inhibition zone of 1.75 mm, 15% has an inhibition zone of 2.58 mm, and 20% has an inhibition zone of 5.75 mm.

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