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Analysis of the Resistance of Nipah Salt (*Nypa fruticans* Wurmb.) Against *Staphylococcus aureus* ATCC 25923 and *Candida albicans* ATCC 10231 by Well Diffusion and Disc Diffusion Methods

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ABSTRACT: *The midrib of the nipa palm is a part of the nipa palm plant which has various benefits in the treatment of diseases, one of which is as an antibacterial and antifungal. From these parts can be processed into antibacterial and antifungal salts. The aim of this study was to analyze the activity of nipah salt in inhibiting the growth of Staphylococcus aureus ATCC 25923 and Candida albicans ATCC 10231. The nipah fronds were processed and purified into salt and tested for its antibacterial and antifungal activity using the disc and well diffusion method. The test samples were amoxicilin and ketoconazole as positive controls, CMC as negative controls and nipah salt with concentrations of 15%, 25%, 50%, 75% and 100%. The test results showed nipah salt which produced an amendment of 3%. In the antibacterial test with the disc and well diffusion methods, the greatest inhibition was found at the same concentration, namely 100% nipah salt, 11.7 mm and 13.5 mm respectively in the strong inhibition category. In the antifungal test, the disc and pitting methods had the same category of inhibition, namely moderate. The conclusion shows that in the bacterial inhibition test, the disc diffusion method has a larger area of inhibition than the well method, while in the antifungal test, the disc and well methods have an area of inhibition that does not differ in area of inhibition.*

Keywords: *Nipah; antibacterial; antifungal.*

I. INTRODUCTION

Skin infections often caused by bacteria and fungi are a type of disease that has a high number of infected populations in Indonesia. To overcome this, prevention and treatment are needed through various activities such as counseling, guidance, environmental cleaning and administering antibiotics. By administering antibiotics, appropriate management is needed to avoid the occurrence of resistance to bacteria and fungi (Kurniati et al., 2017). The use of antibiotics has a weakness in the irrationality of their use. Therefore, the use of antibiotics is one of the logical ways to overcome microbial resistance.

Skin diseases are usually caused by the bacteria *Staphylococcus aureus* and the fungus *Candida albicans*. This type of bacteria has the characteristics of toxin virulence, invasiveness, and resistance to antibiotics. Apart from that, these bacteria cause various health problems, such as mild skin infections, food poisoning, and systemic infections. (Karimela et al., 2017). Meanwhile, one part of the normal flora in the form of *Candida albicans*, which is pathogenic, can cause infections, ranging from superficial mucous membrane infections to invasive diseases in the form of *candidiasis* (Lestari, 2010).

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To treat fungal and bacterial infections, treatment is needed other than antibiotics which are very susceptible to resistance. Treatment can be done by replacing synthetic drugs with drugs from plants. Lots of drugs Natural materials i have potential as antibacterial and antifungal , one of which is the nipah plant (Nypa *fruticans* Wurmb.). This plant is a type of *palmae* and grows in river water environments that are influenced by sea tides. This type of plant is part of the mangrove forest plant (Subiandono et al., 2011). This plant has benefits for the body such as antioxidant, anti-inflammatory, insulin secretion stimulus, and cytoprotective (Khairi et al., 2020). The fronds of this plant can be processed into salt which functions as a food flavoring, natural food preservative, and also has potential as an antibacterial and antifungal. Therefore, this research analyzes the processing of palm leaf fronds into salt as an antibacterial and antifungal agent, especially *Staphylococcus aureus* and *Candida albicans* using disc and well diffusion methods. This was done to determine the differences in inhibition results so that the appropriate inhibition method can be obtained to inhibit the test bacteria and fungi.

II. MATERIAL AND METHODS

2.1. Tools and Ingredients

Tools include chemical glasses (Pyrex), measuring glasses (Pyrex), test tubes, tube clamps, measuring flasks (Pyrex), dropper pipettes, funnels (Pyrex), water bath, watch glass, ruler, evaporating cup, spatula, funnel , analytical balance *(BEL)*, pestle and mortar, incubator (Memmert), petri dish, autoclave (GEA). Ingredients include distilled water, HCl *(Merck)*, CaO (merck), $Ba(OH)_2$ *(Merck)*, $(NH_4)_2CO_3$ *(Merck)*, HNO_3 *(Merck)*, Nutrient Agar (NA), Sabouraud Dextrose Agar (SDA), amoxicillin, ketoconazole, litmus paper and filter paper *(Whattman No. 1)*, Midrib Nipah was obtained from the Pamulutan area, Ogan Ilir, South Sumatra, Palembang, Indonesia. The test bacteria and fungi were *Staphylococcus aureus ATCC 25923 and Candida albicans ATCC 10231* from the Balai Besar Laboratorium Kesehatan (BBLK), Palembang, Indonesia.

2.2 Research Methods

2.2.1 Purification of Nipah Salt

Nipah plants carried out species identification in Herbarium Laboratory, Andalas University, Padang, Indonesia. Nipah fronds are squeezed and filtered. The resulting filtrate was left for 24 hours at room temperature. Then it is evaporated at a temperature of 100^oC to obtain nipah salt and purification is carried out. A total of 5 g of brown nipah salt was dissolved in 50 mL of distilled water by heating. Then 5 g of CaO was added to the solution and the $Ba(OH)_2$ solution was dropped until it did not form sediment. Next, add drop by drop $(NH_4)_2CO_3$ while stirring. Then the mixture was filtered and neutralized through dilute HCl using litmus paper and evaporated until salt was obtained and weighed.

2.2.2 Test antibacterial and antifungal activity

The antibacterial and antifungal testing methods are well and disc diffusion, each with a diameter of 6 mm. The growth media for bacteria and fungi are Nutrient Agar (NA) and Saboraud Dextrose Agar (SDA), respectively . The test samples were amoxicillin, ketoconazole, CMC, and nipah salt (15%, 25%, 50%, 75%, and 100%). Then incubated for 24 hours at 35^oC with three replications and the inhibition zone was measured in the form of the clear diameter of each test sample.

III. RESULT

The results of the first stage of this research are the results of the identification of nipah root species through the determination method. The determination results show that the nipah root has a species in the form of *Nypah fruticans* Wurmb. The second stage is the purification of nipah salt from nipah fronds with various chemical reagents. Nipah fronds that have been squeezed will obtain nipah liquid. Then from the evaporation of 100 mL of nipah water there are 3 g of brown nipah salt so that the percentage yield is 3%. In the third stage, antibacterial testing was carried out on the purified nipah salt with two different inhibition methods, namely the well diffusion method and the disc method. Table 1 showed that the concentration of 100% purified nipah salt

had the largest inhibition diameter of 11.7 mm in the disc method and 13.5 mm in the well method.

Concentration (%)	Inhibitory method (average resistance diameter)			
	Disc (mm)		Well (mm)	
15	6.4	Moderate	9.3	Moderate
25	7.8	Moderate	12.5	Strong
50	10.1	Moderate	12.9	Strong
75	11.3	Strong	11.4	Strong
100	11.7	Strong	13.5	Strong
Amoxicillin (+)	13.5	Strong	17.8	Strong
CMC (-)	0	-	0	-

Table 1: Antibacterial test of nipah salt

The last stage is the antifungal test with Candida albicans against nipah salt that has been purified by the same two methods before. Table 2 shows that both disc methods have a better inhibitory ability than the wells method in the antifungal test against nipah salt.

Concentration (%)	Method (average diameter)			
	Disc (mm)		Well (mm)	
15	6. 4	Moderate	6.0	Moderate
25	7.0	Moderate	6.0	Moderate
50	6.7	Moderate	6.0	Moderate
75	6.9	Moderate	6.0	Moderate
100	6.3	Moderate	6.0	Moderate
Ketoconazole (+)	15.8	Strong	13.2	Strong
CMC (-)	0	-	0	-

Table 2: Antifungal test of nipah salt

IV. DISCUSSION

To determine the type of plant used for exploration, determination is required. Determination is the process of determining the type of test plant based on the suitability of the morphology of the plant under study to the plant that has been determined. The purpose of the determination is to determine the identity of the plant being specifically studied so that errors in selecting the plant to be tested can be avoided (Darma & Marpaung, 2020). The results of the determination that have been carried out show that the sample in the form of nipah plants is a genus of *Arecaceae* with the type *Nypah fruticans* Wurmb.

From 100 mL of evaporated nipah water, 3 g of brown nipah salt was obtained. From these results, the percentage of evaporation from nipa palm fronds was 3%. To purify the nipah, it is dissolved using several solvents. The technique used is crystallization through evaporation and precipitation because it is one of the best separation methods. Basically, the purpose of crystallization is to separate and purify crystals, and the result is crystals with the desired quality. Crystal quality can be measured by three parameters, namely purity, size distribution and crystal shape (Umam, 2019).

From this purification technique, nipah salt is dissolved with CaO powder. This powder functions to whiten salt from impurities originating from Ca^{2+} ions because it can increase the differentiation of the solubility of NaCl with impurities. The Ca^{2+} ions will bind carbonate ions (CO_3^{2-}) to form a precipitate in the form of calcium carbonate ($CaCO_3$) (Gemati et al., 2013).

Next, add the $Ba(OH)_2$ solution to the nipah salt. The aim is to ionize chlorine ions from $CaCl_2$ and bind impurities of Mg^{2+} or Fe^{2+} ions . The reaction that occurs when the solution is added is that $Ba(OH)_2$ experiences

ion decomposition into Ba^{2+} ions and OH^- ions . Then the OH^- ions bind to Fe^{2+} ions and Mg^{2+} ions respectively producing $Fe(OH)_2$ and $Mg(OH)_2$ precipitates. Next, dissolve ammonium carbonate $(NH_4)_2CO_3$ in the sample until no more precipitate forms. The aim is to bind excess Ba^{2+} and Ca^{2+} ions in the sample to produce $BaCO_3$ and $CaCO_3$ precipitates (Maulana et al., 2017). Next, it is filtered to obtain a filtrate and diluted HCl is added. This aims to neutralize the solution from the addition of $Ba(OH)_2$ (Maulana et al., 2017). To test neutrality properties, use litmus paper or a pH meter.

Antibacterial and antifungal testing begins with making *Nutrient Agar* (NA) media. Nutrient Agar is a medium that generally contains mostly water with nutrients in the form of 0.8% protein and 1.2% agar. This media is used to grow and rejuvenate microbes and test the purity of an isolate in the laboratory(Juariah & Sari, 2018). The antibiotic amoxicillin is used as a positive control in antibacterial tests because this substance is a broad spectrum penicillin derivative antibacterial. This shows that it can inhibit various types of Gram-positive and Gram-negative bacteria. By binding to one or more amoxicillin-protein bonds, the drug inhibits bacterial cell wall formation. This causes transpeptidase to occur, namely the formation of peptidoglycan in the bacterial cell wall is blocked so that the formation of the cell wall is biologically hampered which results in the breakdown of this part which is called lysis (Nursin et al., 2019). The role of CMC functions as a comparison that does not provide the effect of a treatment.

Methods in this research are disc diffusion and well diffusion. In the well method, *holes are* made vertically in solid agar that has been inoculated with the test bacteria. The hole is filled with test samples. At the end of incubation for 1 x 24 hours at a temperature of 35°C, bacterial growth was observed to see the area of resistance around the hole. This method allows relatively easy measurements of the zone of inhibition because microbes not only move on the top surface of the media but also on the bottom surface. Apart from that, this method has disadvantages, namely the presence of agar residues in the media and the possibility that the media will crack or break around the wells. This can interfere with the absorption of the test sample into the medium which will impact the formation of the clear zone diameter during the sensitivity test (Nurhayati et al., 2020).

In the disk diffusion method , the disk paper absorbs the saturated test sample . The paper disc is then placed on the surface of the media that has been inoculated with the test microbial culture . Next, incubate for 24 hours at 35°C. The clear area or zone around the paper disc is observed to indicate the presence or absence of microbial growth. The diameter of the clear area or zone is proportional to the number of test microbes added to the paper disc. This shows that the method has a faster test on paper disc preparation (Katrin et al., 2015).

From the results of measuring the inhibitory diameter *of Staphylococcus aureus ATCC* 25923 using the well and disc diffusion methods, it shows that as the concentration of nipah salt increases, the resulting inhibitory diameter becomes larger. These results also show that the solution with a nipah salt concentration of 100% in both the well and paper disc methods has the highest inhibitory diameter, namely 13.5 mm and 11.7 mm respectively in the strong inhibitory category (Table 1). The average categories for the diameter of the inhibition zone formed by *Staphylococcus aureus* are the inhibition zone <5 mm (weak), 6-10 mm (moderate), 11-20 mm (strong), and >21 mm (very strong) (Surjowardojo et al., 2015). Table 1 also shows that the well resistance method has a higher level of sensitivity than the disc diffusion method.

The results obtained by nipah salt showed that the inhibition zone against *Staphylococcus aureus bacteria* at a concentration of 100% was the largest in the well method at 13.5 mm, which was categorized as strong. Basically, the diameter of the inhibition zone tends to be directly proportional to the increase in extract concentration. However, in this study, at some concentrations, the diameter of the inhibition zone formed did not always increase along with increasing extract concentration. This is influenced by several aspects, namely the speed of diffusion of antibacterial compounds on different agar media, and the types and concentrations of different active compounds, each of which provides a different diameter of the inhibition zone at a certain time.

Candida albicans fungus inhibition test against nipah salt, the resulting inhibition zone was not very visible or very small. The largest zone of inhibition produced by nipah salt against the *Candida albicans fungus* was 7 mm at a concentration of 25% using the disc method. Meanwhile, the average zone of inhibition for the welling method is 6 m m and categorized as moderate (Table 2). The mechanism for inhibiting fungal growth is the degradation of cell membranes by active antifungal substances. This event will disrupt the integrity of cellular

components which are the part responsible for the immune system and result in fungal respiration not occurring (Eddy, 2009). Several aspects can influence the mechanism of inhibition of microorganisms by antimicrobial compounds, such as disruption of the compounds that make up cell walls, increased permeability of cell membranes, which can cause loss of components that make up cells, inactivation of enzymes, and damage to the function of genetic material. (Ernawati & Sari, 2015).

V. CONCLUSION

In the bacterial inhibition test, the disk diffusion method had a greater resistance area than the well method, whereas in the antifungal test, the disk and well methods had an obstacle area that was not different. In addition, the researcher would like to thank the Ministry of Education, Culture Research and Technology, Republic of Indonesia for the grant for the Penelitian Dosen Pemula (PDP) in 2022.

VI. REFERENCES

1. Darma, W., & Marpaung, MP (2020). Analysis of the type and content of saponin in yellow root extract gravimetrically. *Journal of Chemical Education and Chemical Sciences*, *3* (1), 51–59.
2. Eddy, S. (2009). Inhibitory effect of antimicrobial substances from bitter leaf extract (Andrographis paniculata (Burm.f.) Ness) on the growth of the fungus Candida albicans in vitro. *Science*, *6* (1), 9–15.
3. Ernawati, E., & Sari, K. (2015). Chemical compound content and antibacterial activity of avocado peel extract (Persea americana P.Mill) against Vibrio alginolyticus bacteria. *Journal of Veterinary Studies*, *3* (2), 203–211.
4. Gemati, A., Gunawan, G., & Khabibi, K. (2013). Purification of NaCl Salt via the Cross Salt Recrystallization Method with the Addition of Na2CO3, NaOH and Polyaluminium Chloride for Removal of Ca2+ and Mg2+ Impurities. *Journal of Chemical Science and Applications*, *16* (2), 50–54.
5. Juariah, S., & Sari, WP (2018). Utilization of Tofu Industrial Liquid Waste as an Alternative Media for the Growth of Bacillus sp. *Journal of Clinical Health Science Analysis*, *6* (1), 24–29.
6. Karimela, El. J., Ijong, FG, & Dien, HA (2017). Characteristics of Staphylococcus aureus isolated from smoked Pinekuhe fish traditionally processed in Sangihe district. *JPHPI*, *20* (1), 188–198.
7. Katrin, D., Idiawati, N., & Sitorus, B. (2015). Test the antibacterial activity of Malek (Litsea graciae Vidal) leaf extract against Stapylococcus aureus and Escherichia coli bacteria. *JKK*, *4* (1), 7–12.
8. Khairi, I., Bahri, S., Ukhty, N., Rozi, A., & Nasution, M.A. (2020). Potential Use of Nipah (Nypa Fruticans) as Functional Food and Pharmaceuticals. *Laot Journal of Marine Science*, *2* (2), 119–128. http://jurnal.utu.ac.id/JLIK/article/view/3146
9. Kurniati, NF, Garmana, AN, & Aziz, N. (2017). Antibacterial and antifungal activity of turi (Sesbania grandiflora L. POIR) root, flower and leaf extracts. *Acta Pharmaceutica Indonesia*, *42* (1), 1–8.
10. Lestari, PE (2010). The role of virulence factors in the pathogenesis of Candida albicans infection. *Stomatoggenetics* , *7* (2), 113–117.
11. Maulana, KD, Jamil, MM, Putra, PEM, Rohmawati, B., & Rahmawati, R. (2017). Improving the Quality of Bledug Kuwu Salt Through a Recrystallization Process with CaO, Ba(OH)2, and (NH4)2CO3 Impurity Binders. *Journal of Student Creativity*, *2* (1), 42–46. http://journal.unnes.ac.id/nju/index.php/jcs
12. Nurhayati, LS, Nadhira, Y., & Hidayatulloh, A. (2020). Comparison of Yogurt Starter Antibacterial Activity Testing Using the Well Diffusion Method and the Disc Diffusion Method. *Journal of Animal Products Technology*, *1* (2), 41–46. https://doi.org/10.24198/jthp.v1i2.27537
13. Nursin, N., Nurliana, Laily, Imran, I., & Musta, R. (2019). Antibacterial Activity Test for Staphylococcus aureus and Salmonella typhi from Microencapsulation Results of Rogo (Premna Serratifolia Linn) Essential Oil. *Hydrogen: Journal of Chemical Education*, *7* (2), 73–81.
14. Subiandono, E., Heriyanto, N.M., & Karlina, E. (2011). *as a food source from mangrove forests*. *5*, 54–60.

15. Surjowardojo, P., Susilorini, TE, & Sirait, GRB (2015). Inhibitory effect of Manalagi Apple peel decoction ((Malus sylvestrs Mill.) on the growth of Staphylococcus aureus and Pseudomonas sp. Causes of Mastitis in dairy cattle. *Journal of Tropical Livestock*, *16* (2), 40–48.
16. Umam, F. (2019). Salt Purification using the Recrystallization Method in Bunder Pamekasan Village to Achieve SNI for Kitchen Salt. *Pangabdhi Scientific Journal*, *5* (1), 24–27.