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QUALITY OF ETHANOL EXTRACT OINTMENT OF ARECA SEEDS (ARECA CATECHU L.) AS ANTI BACTERIAL STAPHYLOCOCCUS AUREUS QUALITY OF PINANG SEEDS EXTRACT ETHANOL (ARECA CATECHU L.) OINTMENT AS AN ANTI BACTERIAL STAPHYLOCOCCUS AUREUS

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ABSTRACT

The areca nut plant (Areca catechu L) is a traditional plant that has long been used in traditional medicine for various types of diseases such as a stimulant, stomach disorders, worms, smallpox, cholera, venereal diseases and wounds or skin diseases. One of the factors causing skin infections is the bacteria Staphyloccocus aureus. Areca nut (Areca catechu L.) is a natural ingredient that contains antibacterial compounds, namely phenols, alkaloids, terpenoids, saponins, flavonoids, glycosides and tannins. Objective; to determine the antibacterial activity of an ointment preparation of ethanol extract of areca seeds ((Areca catechu L.) against Staphylococcus aureus. Method; this research used experimental research methods, namely the extract was obtained by maceration method with 96% ethanol, and concentrated using a rotary evaporator. Ethanol extract of seeds areca nut is mixed into the ointment preparation with a concentration of 20%, 30%, and 40%. Results; research shows that the average zone of inhibition in each sample of 20% areca nut extract is 7.86 mm, 30% is 12.23 mm, and 40% is 14.3 mm, positive control is 18.36 mm, negative control is 0 mm. Conclusion; from this research is that the ethanol extract of areca nut seeds can be formulated in ointment preparations with concentrations of 20%, 30%, and 40 % which can inhibit the growth of Staphyloccocus aureus bacteria. The concentration of areca nut ethanol extract which has the strongest antibacterial activity against the growth of Staphyloccocus aureus bacteria is a concentration of 40% with an inhibition zone of 14.3 mm.

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1. INTRODUCTION

Indonesia is one of the countries with the greatest biodiversity in the world, having more than 30,000 species of higher plants. Various medicinal plants and thousands of potentially medicinal plants in Indonesia contain various types of natural chemical compounds(1). Utilization of natural resources in the form of plants is used as healing and a form of a healthy lifestyle(2). Empirically, Indonesian people have used herbs as reliable traditional medicine(3). Traditional medicine is of sufficient concern to continue to be developed and strived to become formal medicine in Indonesia(4). Various community efforts to develop traditional healing services have provided healing options for users to maintain health or treat disease(5).

The areca nut plant (Areca catechu L) is a traditional plant that has long been used in traditional medicine for various types of diseases such as a stimulant, stomach disorders, worms, smallpox, cholera, venereal diseases and wounds or skin diseases. Areca nut has many uses, especially in medicine, from the roots, fruit, leaves to betel nut skin which has antioxidant activity(6). Natural ingredients such as areca nut are potential sources of metabolite compounds that have antibacterial effects(7).

In previous research, testing secondary metabolite compounds (phytochemical screening) of areca nut ethanol extract contained phenols, flavonoids, tannins, saponins, triterpenoids, glycosides

and alkaloids.(8). Areca nut seeds contain these compounds which are known to have antibacterial properties(9). Infectious diseases are still a global health problem, including in developing countries like Indonesia. Infectious diseases are diseases caused by pathogenic microbes and are dynamic. In general, infectious diseases can be cured using antibiotics. Irrational use of antibiotics can cause pathogenic bacteria to become resistant(10).

Bacteria are microorganisms that cannot be seen with the naked eye, but can only be seen with the help of a microscope(11).Pathogenic bacteria that often cause infections in humans in the community and nosocomially are Staphylococcus aureus bacteria. Staphylococcus aureus bacteria are bacteria that live on the surface of the body of healthy individuals without causing harm, especially around the nose, mouth, genitals and rectum. However, when our skin is injured or punctured, these bacteria will enter through the wound and cause infection(12).

*Staphylococcus aureus*is a pathogen that causes various skin and soft tissue infections as well as causes more serious and more invasive diseases. Staphylococcus aureus causes disease by invading tissues and producing toxins. This organism can spread from the site of origin to the site of infection through skin wounds, including surgical wounds or skin abrasions(12). Based on previous research, Mifttakhul Baiti in 2018, stated that ethanol extract of areca nut seeds (Areca catechu L.) was able to inhibit the growth of Staphylococcus aureus bacteria. Concentrations of 20%, 30%, and 40% are concentrations that are effective in inhibiting the growth of Staphylococcus aureus in the strong category(13).

One pharmaceutical preparation that is easy to use is ointment. The ointment preparation was chosen because it is the most suitable pharmaceutical preparation for skin treatment because the contact between the drug and the skin is longer(14). Ointment is a semi-solid preparation intended for topical use on the skin or mucous membranes. The medicinal substance must be dissolved or homogeneously dispersed in a suitable base or ointment base. Ointments can contain drugs or not contain drugs which are called ointment bases.(15). Based on the background which states that the ethanol extract of areca seeds has anti-bacterial activity, the researchers were interested in examining the antibacterial activity test of the ethanol extract of areca seeds in the form of ointment with concentrations of 20%, 30% and 40%. The ointment preparation was chosen because it is a pharmaceutical preparation that is used topically for the treatment of various skin diseases.

2. METHOD

This type of research is carried out experimentally, namely using the diffusion method to determine a symptom or influence that arises as a result of certain treatments(30). The samples used were old areca nut plants taken purposively, that is, without comparing plants from other areas. The tools used in this research were Erlenmeyer tubes (Pyrex), Petri dishes (Pyrex), dropper pipettes, evaporating dishes, stirring rods, water baths, horn spoons, measuring flasks (Pyrex), measuring cups (Pyrex), test tubes. (pyrex), test tube rack, tweezers, incubator, object glass, microscope, water bath, scales, incandescent lamp, filter paper, label paper, tissue, 65 mesh sieve, mortar and stamper, rotary evaporator, oven and caliper.

Material

Old areca nut seed extract (Arecacathecu L.), staphylococcus aureus bacteria, aquadest, ceraalba, adeps lanae ethanol 96%, filter paper, white vaseline, stearyl alcohol, Mc.farland solution, NaCl, and Nutrient Agar (NA).

Making Simplicia Powder

6 kg samples of areca nut were collected, then wet sorted and then washed with running water. The areca nut that has been cleaned is split and the seeds are cut into small pieces then dried using a drying cabinet. The dried simplicia is sorted dry and made into powder by blending and sifting. Simplicia powder is stored in a clean and tightly closed container(31).

Preparation of Ethanol Extract of Areca Nut Seeds

The material extraction process used in this research uses the maceration method with 96% ethanol solvent in a ratio of (1: 10). The simplicia powder was macerated for 5 days, where 500 g of simplicia areca seeds were put into a glass jar then soaked using 3,750 ml of 96% ethanol solvent covered with aluminum foil for 5 days (stirring occasionally) then filtered using filter paper and

obtained filtrate 1 and dregs. The dregs were re-soaked using 1,250 ml of 96% ethanol solvent for 2 days (stirring occasionally) then filtered using filter paper and obtained filtrate 2 and dregs. Next, combine filtrates 1 and 2 and concentrate them in a rotary evaporator until a thick extract is obtained. The thick extract has been weighed and stored in a closed container before use(32).

How to Make Ointment

Making ointment preparations using the melting process. Prepare the tools and materials then weigh all the necessary ingredients. Put vaseline album and adeps lanae into a porcelain cup then melt over a water bath (mass 1). Then melt cera alba and stearyl alcohol in a water bath (mass 2). Before homogenizing the preparation, first heat the mortar with hot water. In a dry mortar, add mass 1, little by little, grind until homogeneous and add mass 2, grind until homogeneous until an ointment mass is formed. After the ointment mass is formed, add the areca nut extract according to the concentration, grind until homogeneous and then put into a well-closed container.

Stock Evaluation

EvaluationThe preparations in this study used organoleptic tests, homogeneity, pH, spreadability and preparation tests.

Antibacterial Activity Test

Antibacterial testing was carried out by pouring 15 mL of NA into a petri dish and adding 0.1 mL of bacterial suspension, homogenizing it and then leaving it to solidify. Then a well is made using a well tool with a diameter of ± 6 mm, the well is made perpendicular to the surface of the media with a depth of 0.1 g. Each well is spaced so that the observation areas do not overlap. Then put the ethanol extract of areca nut ointment into each well and mark it. Incubated for 24 hours at 37°C(37).

Observation and Measurement

Observations were made after 1 x 24 hour incubation period and the inhibition zone formed was observed and interpreted by looking at the clear area around the well and then calculating the diameter of the inhibition zone. The clear zone is an indication of the sensitivity of bacteria to antibiotics or other antibacterial agents used as test materials which is expressed as the diameter of the inhibition zone. The diameter of the inhibition zone is measured using a ruler or caliper by measuring the inhibition zone around the well hole.

Data analysis

Analysis of data obtained from research results in the statistics laboratory used the ANOVA (Analysis of variance) test with the SPSS program(38).

3. RESULTS AND DISCUSION

Preparation Evaluation and Inhibitory Power Test

Table 2. Data from Evaluation Results of Stocks and Inhibition Zones

Ointment Formula	Organoleptic Test			Homogeneity Test (+/-)	Average pH	Spreadability Test				Obstacles zone	
	Form	Color	Smell			No burden	50 gr	100 gr	150 gr	Average	Category
Negative control	Half solid	White	No smell	+	4.9	4.0cm	4.1cm	4.2cm	4.4cm	0	There isn't any
Positive control	-	-	-	-	-	-	-	-	-	18.36	Strong
F1	Half solid	Red	Distinctive smell	+	5.0	4.0cm	4.1cm	4.4cm	4.5cm	7.86	Currently
F2	Half solid	Dark red	Distinctive smell	+	5.3	4.1cm	4.3cm	4.5cm	4.6cm	12.25	Strong
F3	Half solid	Deep dark red	Distinctive smell	+	5.5	4.3cm	4.6cm	4.8cm	5.0cm	14.30	Strong

Based on the results of observations of the ointment preparations, it shows that the three formulas have a distinctive areca nut smell, red color, and are semi-solid in form. Checking the homogeneity of the preparation can be carried out by smearing the preparation on two pieces of glass or other suitable transparent material. The preparation must show a homogeneous composition and no visible coarse grains.

The results of measuring the pH of the areca nut extract ointment were pH 4.9 for formula 0 (ointment base), pH 5.0 for formula 1, pH 5.3 for formula 2, and pH 5.5 for formula 3. Based on the results of the spreadability test of the areca nut ethanol extract ointment preparation, the spreadability must meet the spreadability parameters, namely around 5-7 cm. The results of the antibacterial activity test of the ethanol extract of areca nut seeds (Areca catechu L.) and the ethanol extract ointment of areca nut seeds (Areca catechu L.) can be seen in table 4.6. Based on the results of the antibacterial activity test, it was found that the negative control was 0 mmm, the control (+) was 18.36 mm and in the areca nut ethanol extract ointment the concentrations of 20%, 30% and 40% were 7.86 mm, 12.23 mm, and 14.3 mm.

Stock Stability Check

The results of observing the stability of areca nut extract ointment preparations can be seen in table 4.5

Table 2.Observation data on stock stability

No	Ointment	Observations during storage Completed 1 week 2 weeks 3 weeks 4 weeks made XYZXYZXYZXYZXYZ
1	Blank	- - - - - - - - - - - - - - -
2	F1	- - - - - - - - - - - - - - -
3	F2	- - - - - - - - - - - - - - -
4	F3	- - - - - - - - - - - - - - -

Information :
X: Color change
Y: Change in Smell
Z: Breakage of the emulsion
-: No change occurred

Data analysis

Staphylococcus aureus bacteria

ANOVA

staphylococcus aureus

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	591,605	4	147,901	79,503	,000
Within Groups	18,603	10	1,860		
Total	610.208	14			

Data analysis of the resistance diameter results in table 4.8 uses the One Way ANOVA parametric statistical test. In calculating the results of this research, a confidence level of 95% (α = 0.05) was used. The data analyzed is Formula I with a concentration of 20%, Formula II with a concentration of 30%, Formula III with a concentration of 40%.

Discussion

Organoleptic Test

From the organoleptic test observations, the ethanol extract of areca nut which was formulated in the form of an ointment met the ointment quality test parameters, namely from the organoleptic test, the form of the ointment was semi-solid, the color and smell of the ointment were red and had a distinctive smell of areca nut. The higher the concentration of the extract, the more the distinctive smell of areca nut seeds increases and the color of the ointment becomes deep dark red.

Homogeneity Test

From table 1 it is known that all formulas have good homogeneous properties. This is indicated by the results of observations which show that there are no coarse grains that clump or particles that have not been dissolved in the ointment are evenly dispersed on the slide and there is no clumping of particles when observed. The purpose of carrying out a homogeneity test is to determine whether the components of the preparation are mixed well and do not contain grains or coarse particles that have not been dissolved.

Test pH

Based on the results of this pH examination, it is carried out to determine the pH stability of the ointment which must match the pH of the skin so that irritation does not occur on the skin. The pH value of the preparation must be stable during storage. The third value of the ointment formulation meets the requirements because it is in the pH range between 4.9 – 5.5 so it can be comfortably used topically without causing skin irritation or dry skin. The pH value of the three ointment formulas meets the skin pH criteria, namely 4.5 – 6.5. So the pH of the ointment preparations of the three formulas was declared stable during storage.

Spreadability Test

Based on the results of the spreadability test experiments that have been carried out on areca nut extract ointment preparations, they must meet the spreadability parameters, namely 5 -7 cm. Blank ointment preparations and areca nut extract ointments at concentrations of 20% and 30% did not meet the spreadability parameters, but at a concentration of 40% they met the spreadability parameters because they were within a range of 5 cm. The spreadability of the ointment is used to determine the extent of the spread of the ointment when applied to the skin. The results of the spreadability test of the areca nut extract ointment preparation at a concentration of 40% on an absorption basis can be said to meet the requirements for good spreadability. Good dispersing power causes extensive contact between the drug and the skin, so that absorption of the drug into the skin occurs quickly(39).

Preparation Stability Test

Based on the results of stability tests of areca nut extract ointment preparations with concentrations of 20%, 30%, 40%, and blanks which were carried out for 4 weeks at room temperature (25°C) that each formula which had been observed for 28 days showed a stable condition because there is no color change, odor, and emulsion breaking. This shows that the areca seed extract ointment is stable in storage. The stability of a preparation can be seen from whether there is a change in color or odor during storage. A preparation becomes unstable due to clumping of globules from the dispersed. Whether a preparation is damaged or not can be seen from the change in color and change in odor.

Antibacterial Activity Test

From the observation data which can be seen in table 4.6, it is found that FI, FII and FIII have an inhibiting effect. This shows that areca nut extract (Areca catechu L.) is able to inhibit Staphyloccocus aureus bacteria. The zone of inhibition for the positive control Staphyloccocus aureus bacteria was 18.36 mm, while the blank blank did not obtain an inhibition zone at all, the ointment with 20% ethanol extract of areca nut was 7.86, the ointment with 30% ethanol extract of areca nut was 12.23 mm and ointment with 40% areca nut ethanol extract is 14.3 mm. From table 4.6 it can be seen that the largest inhibitory zone for Staphyloccocus aureus bacteria occurs at a concentration of 40% with an inhibitory power of 14.3 mm.

This study used samples of areca seed extract (Areca catechu L.) with the aim of testing the antibacterial activity of ointment preparations in inhibiting the growth of Staphylococcus aureus bacteria using the agar diffusion method, namely the well method. This research was carried out in three repetitions. Tests regarding the power of areca nut extract (Arecs catechu L.) against Staphylococcus aureus bacteria showed that betel nut extract (Arecs catechu L.) had inhibitory power against the growth of Staphylococcus aureus bacteria. This was proven by the presence of a diameter of the inhibition zone around the well containing areca nut extract.

One of the factors that influences the diameter of the inhibition zone is concentration. The higher the concentration of the extract, the more microorganisms that can be inhibited, so the diameter of the inhibition zone is also larger(40). The existence of an inhibition zone in the extract formulation made is most likely due toAreca nut plants contain compoundsphenols, flavonoids, tannins, saponins, triterpenoids, glycosides, and alkaloids(8). The mechanism of action of phenol is by increasing the permeability of the cytoplasmic membrane, causing leakage of intracellular components and coagulation of the cytoplasm, resulting in cell lysis. Phenolic compounds are antibacterials that are bactericidal. Phenolic compounds have broad spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria(40).

The mechanism of action of flavonoids is by damaging the bacterial cell membrane in the phospholipid section thereby reducing permeability which results in the bacteria being damaged.(41). Terpenoids have an antibacterial mechanism by destroying bacterial cell membranes. Cell membrane damage can occur when antibacterial active compounds react with the active sites of the membrane or by dissolving lipid constituents and increasing their permeability.(42) The mechanism of saponin's action as an antibacterial is by causing leakage of proteins and enzymes from the cells of the Porphyromonas gingivalis bacteria. Saponin is an active substance that can increase membrane permeability resulting in hemolysis in cells. If saponin interacts with bacterial cells, the bacteria will burst or lyse(43). The chemical compound content of glycosides has the potential to act as an antibacterial by penetrating into cell walls, thereby causing damage to bacterial cell walls.(44)

Alkaloid compounds have the ability to inhibit bacterial growth. The ability of alkaloids to inhibit bacterial growth is associated with their ability to intercalate with DNA, thus inhibiting DNA synthesis and reverse transcriptase, as well as by releasing lipoteichoic acid adhesins from the cell surface thereby disrupting membrane permeability.(45). These results explain that the higher the concentration, the wider the inhibition zone formed. The diameter of the zone of inhibition of areca nut (Areca catechu L.) seed extract ointment tends to increase with increasing concentration. The effectiveness of antibacterial substances is influenced by the concentration of these substances. Increasing the concentration of substances causes an increase in the content of active antibacterial compounds so that their ability to inhibit bacteria also increases.

4. CONCLUSION

Based on the research results obtained above, it can be concluded that the areca nut ethanol extract ointment can be formulated as an ointment and has a dark red color, a distinctive areca nut smell, and a smooth shape. The concentration that is effective in inhibiting the growth of Staphylococcus aureus bacteria is a concentration of 40% with an inhibition zone of 14.3 mm. Suggestion From the results obtained in making areca nut (Areca catechu L.) ethanol extract ointment, it can be recommended to develop a formulation of areca nut (Areca catechu L.) ethanol extract ointment in other pharmaceutical dosage forms and in the use of positive controls using materials made from natural plants.

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