FORMULATION OF 70% ETHANOL EXTRACT OINTMENT OF KECAPI LEAVES (S*andoricum koetjape*) AND ANTIBACTERIAL ACTIVITY TEST AGAINST *Staphylococcus aureus* BACTERIA

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Abstract

Background: Kecapi's leaves are one part of a plant that thrives in Indonesia. Kecapi's leaves are known to have secondary metabolites such as alkaloids, flavonoids, tannins and terpenoids. These compounds can inhibit bacterial growth.

Purpose: This research aims to formulate kecapi's leaf extract with concentrations of 5%, 10%, and 15% to become an ointment and tested on the bacteria *Staphylococcus aureus*.

Methodology: The method used in the antibacterial test uses the agar well diffusion method.

Results: These results showed that kecapi leaf extract ointment had antibacterial activity and the largest inhibition zone was in the 15% concentration ointment with an average inhibition zone of 8.3 mm (medium category). Antibacterial test results data were tested using the *Kruskal-Wallis* with a value of 0.010 ($p < 0.05$) which stated that the ointment preparations with various concentrations had significant differences. The results of data processing were continued with the *Mann-Whitney* test and it was found that the negative control and positives control had significant differences with the other samples.

Keywords: Antibacterial, Kecapi extract, Ointment, *Staphylococcus aureus*

I. INTRODUCTION

Kecapi is a plant belonging to the Meliaceae family which originates from Southeast Asia such as Indonesia, Malaysia, Cambodia and South Laos (BPTH Sulawesi, 2013). The Kecapi plant is a plant that is commonly used as a traditional medicinal plant. Generally, the harp plant is used as medicine by boiling parts of the lute plant such as bark, leaves, fruit or other parts of the plant. Harp contains secondary metabolite compounds such as flavonoids, saponins and polyphenols which can be used as natural antibacterials (Sartika, 2020).

Specifically based on research conducted Fatmalia & Manalu (2019), It is known that lyre leaf extract (*Sandoricum koetjape*) has antibacterial activity against *Staphylococcus aureus* bacteria. *Staphylococcus aureus* is a gram-positive bacterium that is round and microscopically tends to be round, similar to grapes. These bacteria can survive with or without the help of oxygen. *Staphylococcus aureus* generally has an optimum growth temperature of 35 ^{0}C. forms large, slightly yellow colonies in good media.

Staphylococcus aureus is one of the bacteria that has the strongest resistance. On slanted agar, these bacteria can survive for months at room temperature or in the refrigerator (Radji, 2009). *Staphylococcus aureus* is a gram-positive bacteria that causes skin infections. Treatment therapy for skin infections generally uses topical preparations, including ointments (Fatmalia dan Manalu, 2019). Ointment is a semi-solid preparation that is easy to apply and is used for external use. The active substance in a suitable ointment base and the ointment should not smell rancid. Ointments are generally used for the therapeutic treatment of skin diseases because they have a suitable consistency (Naibaho

dkk., 2013). Based on this background, researchers were interested in testing the antibacterial activity of harp leaf extract ointment against Staphylococcus aureus bacteria.

II. METHODOLOGY

Tools and materials

The tools used in this research were Miyako, stirring rod, Erlenmeyer, knife, black cloth, measuring cup (Pyrex), test tube, test tube rack, dropper pipette, ointment pot, funnel, porcelain cup, mortar and stamper, spatula, sieve , analytical balance (Radwag AS220), gloves, hot plate (IKA C-MAG HS7), petri dish, autoclave (Hirayama HVE50), spatula, glass beaker (pyrex), Biological Safety Cabinet (nuAire), incubator (Memmert), (AMT20 Benchtop), oven (Memmert), tube needle, well spare, tweezers, Micropipette (Dlab), aluminum foil, filter paper, label paper, caliper and spirit.

The materials used in this research were lyre leaf extract (*Sandoricum koetjape*), PEG 400, PEG 4000, agar media, 70% ethanol, distilled water, 0.9% NaCl, cotton, Mg powder, FeCl3, H2SO4, HCl, Chloroform, reagents Mayer, Wagner, Dragendorff and Lieberman-Burchard, Mc Farland's solution and the test bacteria Staphylococcus aureus.The systematics of writing are arranged as follows: material, experimental design and treatment, research implementation procedures, laboratory analysis, and statistical analysis. This systematic is not rigid, it can be adapted to the characteristics of the scientific field. For example, for public research administration where there is no analysis laboratory, there is no need for laboratory analysis. On the other hand, other subsections can be added as needed.

Plant Extraction

Harp leaf extract is made using the maceration method. Put 350 grams of harp leaf powder into the container then add 3500 mL of 70% ethanol, then cover. Maceration is carried out for 2 days with occasional stirring. After the maceration is complete, filtering is carried out and the macerate is obtained. The resulting macerate is then concentrated using a rotary evaporator until a thick extract is obtained.

Phytochemical Screening

Alkaloid Test

0.5 gram of extract into a test tube then add 2 drops each of Dragendroff's, Mayer's and Wagner's reagents. positive for alkaloids if it produces an orange-red precipitate for Dragondroff's reagent, a white to yellowish precipitate for Mayer's reagent and light brown to yellow for Wagner's reagent (Sulistyarini, Sari dan Wicaksono, 2019).

Flavonoid Test

0.5 grams of harp leaf extract into a test tube then add 3 drops of concentrated HCl and shake vigorously. Then add 0.5 grams of magnesium powder and shake vigorously. The formation of a change in color of the solution from orange to purplish red in the sample contains flavonoids (Dyah & Pertiwi, 2021).

Saponin Test

A total of 1 gram of lyre leaf extract

Put 10 ml of distilled water into a test tube, shake vigorously for 10 seconds, then let it sit, add a few drops of HCL and let it sit for 10 minutes. Positive results are indicated by the formation of foam with a height of 1-10 cm. Positive results are shown by foam that remains stable (Sulistyarini, *et al* 2019).

Terpenoid Test

A total of 0.5 grams of lyre leaf extract is put into a test tube, add 2 drops of Lieberman Burchard reagent. If it changes to purple, this indicates the presence of terpenoids (Rumagit *et al.*, 2015).

Tannin Test

0.5 grams of harp leaf extract into a test tube then add 3 drops of FeCl3 solution. A positive result contains tannin if the color changes to blackish blue or greenish black (Dyah & Pertiwi, 2021).

Ointment Formula

Making the ointment begins by melting PEG 4000 and PEG 400 together and stirring until homogeneous, then pouring it into a mortar and stirring until an ointment mass is formed. The base of the finished ointment is added with lyre leaf extract little by little until it matches the formula.

Antibacterial Activity Test

Making Nutrient Agar (NA) Media

Weigh 23 grams then dissolve in 1000 ml of distilled water then heat over a water bath and stir using a magnetic stirrer until homogeneous (Azkiyah, 2020).

Sterilization of NA tools and media

Sterilization of tools uses 2 methods, namely aseptically and with the help of an autoclave. Glass equipment such as test tubes, erlenmeyer flasks and petri dishes are sterilized with the help of an autoclave at a temperature of 121℃ for 15 minutes (Allo, 2016).

Bacterial inoculation

A total of 10 ml of sterilized NA media was put into a test tube, then placed at an angle and allowed to solidify. After solidifying, take a stock colony of Staphylococcus aureus bacterial culture using a sterile loop needle, then plant it in slanted NA media by scratching, then incubate for 24 hours at 37℃(Mentari, 2016).

Preparation of bacterial suspension

Take 1 dose of bacterial culture that has been inoculated on slanted NA media and suspend it in 10 ml of 0.9% NaCl in a test tube. Compare the turbidity of the bacterial suspension with Mc Farland 0.5 solution until similar turbidity is obtained (Azkiyah, 2020).

Antibacterial Ointment Inhibition Test

This antibacterial activity test used the well diffusion method. This method begins by adding 100 µL of bacterial suspension then pouring 20 ml of liquid agar media, homogenizing it and letting it solidify, then make 5 wells in the NA media and enter each negative control, test sample with a concentration of 5%, 10% and 15% and 20 µL of positive control into the well, then the petri dish was incubated for 24 hours at 37℃. Measurements were carried out on the clear zone formed around the well which indicated the zone of inhibition of bacterial growth. This test was replicated 3x (Adhi, 2020).

III. RESULTS AND DISCUSSION

Extraction

The sample in this research was harp leaves taken from Sukamekarsari Village, Kalanganyar District, Lebak Banten Regency. From the extracted leaves, 47.6 grams of thick extract was obtained, which was then carried out by phytochemical screening.

Phytochemical Screening

Phytochemical screening aims to provide an overview of the secondary metabolite compounds contained in the plants used. Phytochemical screening is carried out by color testing (Susanti, Budiman dan Warditiani, 2015).The results of phytochemical screening carried out on kecapi leaf extract showed that it contained secondary metabolites including alkaloids, flavonoids and tannins. The antibacterial compounds in harp leaves have different mechanisms in inhibiting bacterial growth. Alkaloids and tannins have a mechanism of action that inhibits bacterial cell formation, flavonoids work by inhibiting bacterial cell protein synthesis (Zukhri, Murni Sari Dewi dan Hidayati, 2018).

The results of phytochemical tests in this study are different from previous research conducted by Heliawati (2018), which stated that harp has secondary metabolite activity such as flavonoids, terpenoids and tannins. Meanwhile, in research conducted by Sartika (2020), Kecapi leaves were found to contain secondary metabolite compounds such as flavonoids, saponins and polyphenols. Differences in secondary metabolite content are influenced by internal factors such as genes and external factors such as light, temperature, humidity, pH, nutrient content in the soil and altitude (Katuuk, Wanget dan Tumewu, 2019).

Antibacterial Activity Test

Testing of the antibacterial activity of harp leaf extract ointment was carried out using the well diffusion method. The well-drained diffusion method is used because the ointment samples used for testing have the same volume and are inserted into the well so that the test sample diffuses more optimally which is expected to provide greater results in inhibiting bacterial growth (Prayoga, 2013). Apart from that, the well method is used because it is easier to measure the area of the inhibition zone that is formed because bacteria are active not only on the top surface of the nutrient agar but down to the bottom (Haryati, dkk, 2017). Testing the antibacterial activity of lyre leaf extract ointment used varying concentrations, F0 as a negative control and 0.1% gentamicin ointment as a positive control. F0 or ointment base is used as a negative control to provide a comparison to see whether the ointment base used has antibacterial activity or not. Meanwhile, gentamicin was chosen as a positive control because it is an aminoglycoside antibiotic used for infections caused by gram-negative and gram-positive bacteria (Nur Azizah dan Samodra, 2022). The results showed that the ointment base or negative control did not have antibacterial activity and the three ointment formulas with varying concentrations produced had antibacterial activity. This research is in line with research conducted by Fatmalia & Manalu (2019), where the research results It states that lyre leaf extract has antibacterial activity against Staphylococcus aureus and Escherchia coli bacteria. FI with a concentration of 5% has an average inhibition zone and standard deviation of 3.3 ± 1.04 which is included in the weak inhibition criteria, FII with a concentration of 10% has an average inhibition zone and standard deviation of 6.0 ± 1.50; The inhibition zone is included in the criteria for moderate inhibition, FIII with a concentration of 15% has an average inhibition zone and standard deviation of 8.3 ± 0.76 which is included in the criteria for moderate inhibition and for the positive control, namely gentamicin ointment 0.1% The average inhibitory zone and standard deviation is 15.5 ± 0.87 which is included in the criteria for strong inhibitory power. Of the three ointments with varying concentrations, it is known that FIII has the largest zone of inhibition.

This shows that the greater the concentration of the extract, the greater the zone of inhibition that can be obtained. This research is in line with Nur Azizah & Samodra (2022), which states that the activity of antibacterial substances in killing or inhibiting bacteria depends on the concentration and type of material from the extract being tested. The greater the concentration of the extract, the greater the inhibition zone will be because there will be more compounds. bioactives contained in the extract. The higher content of antibacterial compounds can inhibit bacterial growth more optimally (Tansil *et al.*, 2016).

Data analysis is used to explain data from research results so that it is easier to understand. In this study, data analysis was used to see whether or not there was a significant comparison between the average inhibition zones of the samples tested. It is known that the results of the Kruskal Wallis analysis from the table obtained a sig value = 0.010 ($p<0.05$) which indicates there is a significant difference between treatments. To see how the data differs in the Kruskal Wallis analysis, proceed with the Mann-Whitney test.

Based on the results of the Mann-Whitney test, it was found that the average inhibition zone of the negative control had a significant difference to FI, FII, FIII and the positive control. FI does not have a significant difference with FII but has a significant difference with FIII and positive control. FII does not have a significant difference with FI and FIII but has a significant difference with the positive control, while FIII does not have a significant difference with FII but has a significant difference with FI and the positive control.

IV. CONCLUSIONS AND NEWNESS

Kecapi leaf extract has secondary metabolites such as alkaloids, flavonoids and tannins which can be used as natural antibacterials. Kecapi leaf extract ointment has antibacterial activity against Staphylococcus aureus bacteria. The largest inhibition zone is found in Formula III with a concentration of 15% which has the largest inhibition zone, namely 8.3 mm ± 0.76, which is included in the moderate inhibition category. Statistical showed that the results of the Kruskal Wallis analysis from the table obtained a sig value = 0.010 ($p<0.05$) which indicates there is a significant difference between treatments.

The implications of our research results in scientific development is an alternative preparation solution that is easy to use on the skin and has antibacterial properties and can add selling value in the economic sector.

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TABLES AND FIGURES

Table 1 Kecapi Leaf 70% Ethanol Extract Ointment Formula

Bahan (g)	F0	F1	F2	F3
Extract	-	5	10	15
PEG 400	60	57	54	51
PEG 400	40	38	36	34
Sum	100	100	100	100

Source : The ointment formulation refers to (Anief, 2015).

Table 2. Phytochemical Screening Results

Secondary metabolite	Result	Description
Alkaloid	+	Mayer & Wagner Reactor
Flavonoid	+	HCl + Mg powder
Saponin	-	HCl
Tanin	+	$FeCl_3$
terpenoid	-	Lieberman-Burchard reactor

Tabel 3. Antibacterial Test Results

Test	K – (0%)	FI (5%)	FII (10%)	FIII (15%)	K + (gentamicine 0,1%)
1	0	4,5	6	7,5	14,5
2	0	3	4,5	8,5	16
3	0	2,5	7,5	9	16
Average±SD	0±0	3,3±1,04	6,0±1,50	8,3±0,76	15,5±0,87