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Antibacterial Activity of Katang-Katang Leaf Extract Gel against Staphyloccus Aureus

Eti Haryati[1], Anto Sugiarto[1], Ahmad Azrul Zuniarto[1], Atini Sabila Hakkin[1]

[1]*Fakultas Farmasi, Universitas YPIB Majalengka*

**Corresponding Author: Eti Haryati*
E-mail: etiharyati99999@gmail.com

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Abstract

Infectious diseases are one of the health problems faced by developing countries such as Indonesia. Various methods are used to treat skin infections caused by Staphylococcus aureus bacteria, one of which is traditionally using plants, namely katang-katang leaves which have active compounds of flavonoids, alkaloids, saponins and tannins as antibacterials. This study aims to determine the antibacterial activity of the gel preparation of katang-katang leaf extract (Ipomea pes-caprae) to determine the concentration that has the greatest antibacterial activity and to determine the physical properties. Gel preparations were made with concentrations of 15%, 20%, and 25%. The test method was carried out using the in vitro disc diffusion method and physical properties tests were carried out which included organoleptic tests, homogeneity tests, pH tests, spreadability tests, adhesion tests and viscosity tests. Data analysis was carried out using normality tests, homogeneity tests, anova and post hoc test. The results of research testing the antibacterial activity of katang-katang leaf extract gel at a concentration of 15% was 3.27 mm, 20% was 4.97%, 25% was 5.97 mm, K+ was 10.97 mm and K- was 0 mm. Katang-katang leaf extract gel has antbacterial activity that is not equivalent to the positive control, katang-katang leaf extract gel does not meet the preparation evaluation requirements and is unstable during storage. From these results it can be concluded that the katang-katang leaf extract gel has antibacterial activity against Staphylococcus aureus and the katang-katang leaf extract gel does meet the preparation evaluation requirements and is unstable during storage.

Introduction

Infectious diseases are one of the health problems faced by developing countries such as Indonesia. One of the causes of infection in Indonesia is Methicillin-Resistant Staphylococcus aureus (MRSA) with a fairly high prevalence (23.5%). Infectious diseases can occur in all parts of the body, one of which is the skin. Microorganisms that inhabit the skin without causing disease in the host are called normal flora. One of the normal flora on the skin is Staphylococcus aureus. However, a weakened immune system can trigger serious infections from Staphylococcus aureus (Afifurrahman et al., 2014; Widiastuti et al., 2019).

Various methods are used to treat infectious diseases, both modern, such as antibiotics and traditional, such as using plants. Treatment with antibiotics is recommended for diseases caused by bacteria (Cheesman et al., 2017; Pancu et al., 2021). The development of bacterial resistance is known to be related to inappropriate use of antibiotics and changes in the spectrum of antibiotics used in treatment (Aziz et al., 2016; Lieberman, 2003; Alanis, 2005). Staphylococcus aureus bacteria have extraordinary adaptability so they can be resistant to many

antibiotics (Afifurrahman et al., 2014; Tigabu & Getaneh, 2021). Therefore, treatment with natural sources is necessary. One of them is by using traditional medicine from plants.

The use of traditional medicine is recommended by World Health Organization (WHO) in maintaining public health, prevention, and treatment of disease, especially for chronic and degenerative diseases (Pan et al., 2013; Chali et al., 2021). Traditional medicine is in great demand because it is easy to obtain near where you live and is economically more affordable when compared to modern medicine and treatment. Besides that, Traditional medicines are relatively safe because they are not mixed with chemicals so they do not have side effects like modern medicines (Nisfiyanti, 2012; Choudhury et al., 2023). One of the medicines that coastal communities often use is medicine from the katang-katang plant.

Lombok Island is an island that is famous for its worldwide beach tourism. Along the road in the coastal area you can find many wild plants growing along the beach, This plant has trumpet-like flowers and is known as horse tapak or katang-katang (Ipomoea pes-caprae (L)R.Br.). According to the place where it grows, it turns out that this plant is empirically used to treat residents or tourists who are stung by jellyfish and sea urchins while playing on the beach. The benefits of katang-katang leaves that are squeezed and placed on the sting site can relieve pain and swelling caused by poison from sea urchins and jellyfish. Katang can also be used as a source of natural antioxidants because it is able to inhibit free radicals (Kathiresan, 2014). Apart from that, the katang-katang plant also functions as an antibacterial (Anandhi & Ushadevi, 2013), because katang-katang leaves contain phytochemical compounds alkaloids, flavonoids, tannins and saponins (Anandhi & Ushadevi, 2013).

Empirical use by the public is done by boiling and grinding (Andayani & Hardiyanti, 2018). Due to its use by processing it first, it needs to be designed to be made in powder dosage form so that it is easier to use for the public, namely in gel dosage form. Gel preparations are preparations that have many advantages compared to other topical preparations. The gel feels light when used on the skin, thereby increasing the comfort of use. Gel has soft physical properties, is soft and easy to apply and does not leave an oily layer on the skin surface (Jones, 2010; Azizah, 2024).

According to research results Julianti et al. (2023) Inhibitory Power Test of Ethanol Extract of Tapak Kuda Leaves (Ipomea pes-caprae(L) R.Br) Against Propinibacterium acne starting from concentrations of 5%, 10%, 25%, 50, and 100%. Able to inhibit the growth of Propinibacterium acne bacteria only at a concentration of 100%, namely 1.14 mm (weak category inhibitory power).

Based on the description and data related to the number of cases of infection, the impact of resistance, the researchers were interested in carrying out a test entitled antibacterial activity test of katang-katang leaf extract gel. (Ipomoea pes-caprae (L.) R.Br) against Staphylococcus aureus.

Methods

The research method used in this research is the experimental method (*experiment*), namely carrying out treatments or experiments on the object being researched, with the aim of knowing the effects arising from the treatment that has been given and to obtain data (Wijaya-Nim, 2011). The population in this research was the katang-katang plant (*Ipomea pes-caprae (L) R. Br*) and gram-positive bacteria. The sample is part of the number of characteristics possessed by the population (Sugiyono, 2013). In this research, The sample used was katang-katang leaves (*Ipomea pes-caprae* (L) R. Br) obtained from Beraringan Beach in Kayangan Village, Kayangan District, North Lombok Regency, and Bacteria *Staphylococcus aureus*. The sampling technique in this research used the purposive sampling method. The purposive sampling method is sampling based on certain considerations made by the researcher himself

based on previously known characteristics or characteristics of the population (Wijaya-Nim, 2011).

In this research, tools were used: Petri dishes, pipettes, filter paper, Erlmeyer, stirring rod, osse needle, glass funnel, glass beaker, measuring cup, evaporating cup, 1 cc syringe, mortar and stamper, bunsen, tripod, abscess gauze. , Caliper, Test Tube, Test Tube Rack, Rotary evaporator, Sterile Gauze, Flannel Cloth, Paper Discs, Maceration Vessel, Autoclave, Autoclave Rack, Blender, Scales, Cotton, Incubator, Parchment Gauze, Microscope, Water Bath, Cotton, Gauze Parchment, Microscope, Watch Glass, Oven, Refrigerator, Micro Pipette, Brookfield Viscometer, Spatula, Water Bath, Glass Object, Tweezers, Scissors, pH Meter, Plano Kraft Paper (brown paper), Aluminum Foil, Mesh 40 Sieve.

Plant Determination

Plant determination aims to confirm the Katang-Katang plant (*Ipomoea pes-caprae* (L.) R.Br) which was carried out in the botany laboratory at YPIB University by comparing and matching the morphological characteristics of plants using literature

Making Simplicia

After the raw materials are collected, wet sorting is carried out which aims to separate dirt or foreign materials from the plants, then washing and chopping are carried out to facilitate drying in the sun. After the simplicia is clean, the simplicia is ground using a blender, then sifted using a No. 40 sieve, after that packing and packaging is carried out so that the simplicia is protected from microbial contamination, as well as retaining other compounds.

Preparation of Katang-Katang (*Ipomoea pes-caprae (L.) R.Br*) Leaf Extract

Put 200 grams of dried katang-katang leaves into a maceration vessel, add 2000 mL of 70% ethanol solvent/until submerged then stir occasionally for 6 days, squeeze the extraction results and filter with a planel cloth so that no dregs remain at all. Making a thick extract using an evaporator until a thick extract is obtained. Maceration was chosen because the active substance cannot withstand heating, and this method is simple but is able to produce good extracts

Calculating Yield

$$rendemen = \frac{bobot\ ektrak\ kental}{berat\ simplisia} x100\ \%$$

Yield requirements: The yield requirement for thick extract is that the value is not less than 10% (farmakope Herbal Indonesia 2017).

Making Katang-Katang Leaf Extract Gel (*Ipomoea pes-caprae* (L.) R.Br)

Table 1. Formulation of Katang-Katang Leaf Extract Gel Preparation

Materials used	Information	Formulasi %				Condition
		X1	X2	X3	K(-)	
Katang-Katang Leaf Extract	Active substance	15	20	25	0	-
Na-CMC	Gel base	3,5	3,5	3,5	3,5	3%-6% (H Abdelkader dkk, 2014)
TEA	Pemberi basa	1	1	1	1	1%-5% (Belinda, 2020)
Gliserin	Humektan	30	30	30	30	30% (Depkes RI, 2014)
Metil parabean	Preservative	0,2	0,2	0,2	0,2	0,2%-0,3% (Rowe et al., 2009)
Aquades add	Solvent	100	100	100	100	-

Na-CMC is developed using sufficient hot water into a mortar and crushed until transparent, then added TEA (Trieethanolamine) until homogeneous, then added methyl paraben which has been dissolved in distilled water, stirred until homogeneous, the extract is mixed with glycerin and mixed into the base and homogenized is added the remaining water into the base and homogenized again

Making Slant Agar Media for Cultivating *Staphylococcus aureus* Bacteria

Weigh 1 gram of nutrient agar, put it in an Erlenmeyer, add 17 ml of distilled water to 3 test tubes, heat the nutrient agar solution while stirring until the nutrient solution is clear and homogeneous, plug the mouth of the Erlenmeyer with fat cotton and sterile gauze then wrap it in parchment gauze, then tie it with thread, carry out sterilization, put 5 ml of nutrient solution into sterile test tubes each.

How Bacterial Rejuvenation Works

Flamb the tip of the tube needle until it is reddish orange, apply the tube needle to the pure bacterial culture, instill the inocula into the agar medium in a zig-zag shape starting from the bottom of the tube, cover the tube again with cotton wool, incubate the culture for 24 hours at a temperature of 30-37ºC, do it regularly. aseptic. Measurement using a caliper after incubation. The diameter measurement is vertical, horizontal and diagonal so as to increase the validity of the data. The results of the three measurements will be averaged as accurate data.

Antibacterial Activity Test

Prepare a petri dish that has been sterilized first using an autolkaf, on the outside of the cup, mark the group or treatment number, pour the sterilized NA solution into 5 lukewarm petri dishes of 17 mL each, put the *Staphylococcus aureus* bacterial culture suspension into the petri dish. 0.2 mL each using a 1 cc syringe, shake the cup containing the bacterial culture suspension so that it is evenly distributed on the surface of the media, then cover the culture dish until it is cold and solid, place a paper disc with a diameter of 6 mm into the media containing *Staphylococcus aureus* bacteria, Add the Katang-Katang leaf extract gel from each concentration and add 0.2 mL each to the negative and positive control groups, close the cup, incubate again for 2 x 24 hours at a temperature of 30º - 37ºC. Measure and observe the clear zone on the media using a caliper which is marked by the presence of a clear zone in each treatment.

Result and Discussion

Determination of katang-katang plants(*Ipomoea pes-caprae* (L.) R.Br) carried out at the Botany Laboratory of YPIB Majalengka University, using key determinations from the literature showed that the plants used in this research were actually katang-katang species. *Ipomoea pes-caprae* L. R.Br. Determination is carried out with the aim of finding out the truth of the plant to be studied and avoiding errors in collecting material (Maria & Rosa, 2021).

Results of Extraction of Katang-Katang Leaves (*Ipomoea pes-caprae* (L.) R.Br)

Extraction was carried out using the maceration method using 2,000 ml of 70% ethanol. Katang-katang leaf simplicia powder (*Ipomoea pes-caprae* (L.) R.Br) 200 g is used and put into a macerator, then soaked and left for 6 days. The resulting maserate was 1,600 ml. Then the resulting macerate was evaporated using a water bath and a thick extract of 63.16 g was obtained. The extract yield was 31.58%. The greater the yield value indicates the greater the value of the extract produced. The yield calculation aims to find out how much extract is obtained from the fresh simplicia used. Whether maceration was chosen for its cost-effectiveness, simplicity, or suitability for the plant material would have added depth to the methodological discussion. In addition, the solvent used for extraction, 70% ethanol, is mentioned, but no explanation is given as to why this particular concentration was chosen.

Ethanol is a common solvent in phytochemical extraction due to its ability to dissolve a wide range of polar and nonpolar compounds, but it would be useful to discuss why 70% ethanol was chosen over other concentrations, or why ethanol was preferred over other solvents such as methanol, acetone, or water. Different solvents and concentrations can affect the composition of the extract by selectively extracting certain compounds. Higher concentrations of ethanol (e.g., 95%) may extract different compounds than a 70% ethanol solution, which would affect the antibacterial activity observed in the study. The yield obtained is quite high because the material used as a simple medicine is leaves, leaves provide a lot of content. Good requirements according to the Herbal Pharmacopoeia are a yield of no more than 30%, this time it was obtained more than 30%. Weighing simple medicine or extract may be wrong because it is not weighed using an analytical balance

Results of Antibacterial Activity Test of Katang-Katang Leaf Extract Gel (*Ipomoea pes-caprae* (L.) R.Br)

Antibacterial activity testing was carried out with the aim of determining whether or not there was an effect of katang-katang leaf extract gel in inhibiting bacterial growth *Staphylococcus aureus* (Baktiar et al., 2023). The antibacterial activity test in this study was carried out using the disc diffusion method, namely placing a paper disc on a medium with a paper disc diameter of 6 mm.

Tabel 2. Clear Zone Recapitulation Results

Formula	Clear Zone Diameter		Average (mm)
	First day	The second day	
Katang-katang leaf extract gel with a concentration of 15%	3	3,9	3,4
Katang-katang leaf extract gel with a concentration of 20%	4,7	5,4	5
Katang-katang leaf extract gel with a concentration of 25%	5,8	6,9	6,3
Positive control (Clindamicyn gel)	10,3	11,7	11
Negative control (Gel base)	0	0	0

The selection of positive control is Clindamycin gel is Clindamycin is sensitive to Stapylococcus aureus bacteria. In addition, Clindamycin gel has the same dosage form with the sample of this study, namely katang-katang extract gel. The use of placebo gel is expected to provide information that the effectiveness is indeed due to the katang-katang extract and not due to the substances in the gel base. In this study, the gel base did not provide antibacterial activity, so it is clear that the antibacterial activity in the katang-katang extract gel is due to the active substances in the katang-katang leaves. Factors such as humidity, light exposure, and even slight variations in incubation temperature can affect bacterial activity and the formation of inhibition zones. By controlling these variables, this study can reduce potential sources of variability in results. A stable, dark environment with controlled humidity during incubation can help maintain uniform bacterial growth conditions across samples. Such environmental controls will ensure that the observed antibacterial effects can be attributed to the Katang-Katang extract and not external factors, thus ensuring that the growth activity is due to the Katang-Katang leaf extract.

Results of Analysis of the Antibacterial Activity of Katang-Katang Leaf Extract Gel

To strengthen the data obtained and to answer the hypothesis, the data was processed using statistical calculations which began with a normality test and a homogeneity test. If the data is normally distributed and homogeneous then proceed using the one-way ANOVA test and post hoc test. However, if one of the data is not normal or homogeneous then proceed with a non-

parametric test. Based on Noor Fajriyati's criteria, an inhibition zone diameter measuring ≤ 5 mm has weak inhibition power, 5-10 mm has moderate inhibition power, 10-20 mm has strong inhibition power, and ≥ 20 mm has very strong inhibition power. So the inhibition zone obtained by the 15% concentration of katang-katang leaf extract gel is weak, namely 3.64 mm, the 20% concentration is in the medium category, namely 5.3 mm, the 25% concentration is in the medium category, namely 6.24 mm, and in the positive control the strong category is 11.7mm. From these results it can be seen that the katang-katang leaf extract gel has the potential to be antibacterial, this is due to the presence of alkaloids, tannins, saponins and flavonoids contained in the katang-katang leaf extract (Carolia & Noventi, 2016).

Based on the results of the data normality test, the katang-katang leaf extract gel (*Ipomoea pes-caprae* (L.) R.Br) value obtained (sig) > 0,05. These results show that the data obtained is normally distributed. The results of the homogeneity test calculation show that the sig value obtained is 0.151 > 0,05 meaning that the data is homogeneous. Based on the results of the anova test, data from katang-katang leaf extract gel (*Ipomoea pes-caprae* (L.) R.Br) This value is obtained (sig) 0,000 < 0,05 meaning H0 is rejected and H1 is accepted, which means Katang-katang leaf extract gel (*Ipomoea pes-caprae* (L.) R.Br).) has antibacterial activity against bacteria *Staphylococcus aureus*.

Based on the results of post hoc tests, it is known that katang-katang leaf extract gel has the potential to be antibacterial against bacteria *Staphylococcus aureus*, This can be seen from the results of the post hoc test, namely sig 0.000 < 0.05 which means it has a difference with the positive control or is not equivalent. The three concentrations of katang-katang leaf extract gel 15%, 20% and 25% has antibacterial activity.

Conclusion

From research data regarding the antibacterial activity test of katang-katang leaf extract gel against bacteria Staphylococcus aureus The following conclusions were obtained: 1) Katang-katang leaf extract gel (Ipomoea pes-caprae (L.) R.Br) has antibacterial activity against bacteria Staphylococcus aureus; 2) Katang-katang leaf extract gel (Ipomoea pes-caprae (L.) R.Br) has the greatest antibacterial activity at a concentration of 25% against bacteria Staphylococcus aureus.

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