Formulation and Antibacterial Activity Test of Patikala Fruit Ethanol Extract Hand Sanitizer Gel (Etlingera elatior) Against the Growth of Staphylococcus aureus Bacteria

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Abstract

Patikala fruit samples were used in this research because people only think that patikala fruit can only be used as a cooking spice. This research aims to formulate ethanol extract of patikala fruit (Etlingera elatior) into a hand sanitizer gel preparation, as well as test the antibacterial activity against Staphylococcus aureus bacteria using the paper disk method. In this research, 4 hand sanitizer gel formulations were made with different concentrations, namely F0 (0%), F1 (20%), F2 (25%), F3 (30%). Then a physical stability test was carried out on the hand sanitizer gel preparation for 4 weeks with test parameters including organoleptic test, pH test, homogeneous test, spreadability test, hedonic test, irritation test, viscosity test, each of the 4 formulas made showed satisfactory results. requirements for the stability of hand sanitizer gel preparations. Furthermore, testing the antibacterial activity of hand sanitizer gel preparations with concentrations of 20%, 25%, 30%, obtained results of inhibition zone diameter values of 20% 12.25 mm, 25% 12.5 mm, 30% 12.66 mm. It can be concluded that the hand sanitizer gel preparation of patikala fruit ethanol extract can inhibit the growth of staphylococcus aureus bacteria.

Key words: Antibacterial, hand sanitizer, patikala *fruit*

1. Introduction

Indonesia is a developing country with a tropical climate and this can affect the number of infectious diseases that occur in Indonesia. In recent decades, infectious diseases have continued to increase, in developing countries are still very vulnerable to skin infections, one of the bacteria that cause infections in humans is Staphylococcus aureus. Staphylococcus aureus is one of the bacteria found on the skin such as on the surface of the hands. Staphylococcus aureus can enter the body through the mouth, nose, eyes and skin, and can cause skin infections such as boils, pus and acne [1].

One of the efforts that can be made to reduce bacterial contamination through hands is awareness in maintaining hand hygiene by using hand antiseptics. Various kinds of hand sanitizer products have been circulating in the community generally using active ingredients in the form of alcohol to provide antibacterial capabilities. In addition to being flammable, the continuous and excessive use of alcohol can cause irritation and trigger dryness of the skin. Efforts to reduce the use of chemicals such as alcohol, an innovation is needed by using bioactive compounds from

plant extracts in the manufacture of hand sanitizer preparations[2].

The use of plant extracts in making hand sanitizer is considered safer and will not irritate the skin. Plants that can be used in making hand sanitizers are plants that have antibacterial properties. As previously explained, patikala fruit has chemical compounds that have potential as antibacterials so that the possibility of this plant can be used as a hand sanitizer gel preparation. Therefore, in this study, a test was conducted on the manufacture of hand sanitizer gel from patikala fruit extract which was tested on Staphylococcus aureus bacteria. This study aims to determine the effect of patikala fruit extract (Etlingera elatior) on the growth of Staphylococcus aureus bacteria and determine the concentration of patikala fruit extract (Etlingera elatior) in the preparation of hand sanitizer gel that is optimal in inhibiting the growth of Staphylococcus aureus [2].

Hand sanitizer is a hand sanitizer with antibacterial properties that can kill and prevent bacteria. Hand sanitizer gel is a gel (jelly) shaped hand sanitizer that is used to clean and remove bacteria from hands and contains 60% alcohol as the active ingredient. Many hand sanitizers are derived from ethanol or alcohol mixed with thickeners such as glycerin, carbopol, and can be formulated as gels, jellies, or foams for easy use. This gel has become popular because it is very easy and practical to use without soap and water, this disinfectant gel is a great choice for people [3].

Patikala plant (Etlingera elatior) is one of the wild plants that thrives in some parts of Indonesia. Among them in Mario village, Baebunta sub-district, North Luwu district, South Sulawesi. The utilization of patikala in Mario village is still less than optimal, the community only makes patikala plants as a typical South Sulawesi food which is usually only made a mixtureof Kapurung, because many people do not know the content and properties of patikala plants andthe lack of public insight makes people not care about the existence of patikala plants, therefore,to increase public knowledge, it is necessary to conduct research on this plant, which will be studied, namely the fruit by making formulations and testing the antibacterial activity of patikalafruit ethanol extract hand sanitizer gel preparations against the growth of staphylococcus aureus bacteria

The content of secondary metabolite compounds in patikala plants has antibacterial properties so that it can inhibit bacterial growth. For example alkaloids, flavonoids, tannins and saponins have various biological activities related to protection, communication, or adaptive responses of organisms to environmental changes [2].

In this study Yusran & Muhammad (2018), ethanol extract of patikala fruit was carried out with different concentrations, namely 10%, 15%, 20%, 25%, 30%, and 35%. The results of antibacterial activity of ethanol extract of Patikala fruit against Staphylococcus aureus at concentrations of 10%, 15%, 20%, 25%, 30%, and 35% respectively, namely at a concentration of 10% has a 5 mm inhibition zone, at a concentration of 15% has a 9 mm inhibition zone, 20% has an 11 mm inhibition zone, at a concentration of 25% has a 12 mm inhibition zone, at a concentration of 30% has a 14 mm inhibition zone and at a concentration of 35% has a 15 mm inhibition zone. This shows that ethanol extract of patikala fruit has antibacterial activity against Staphylococcus aureus [4].

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: is a variable that is the cause of the emergence or change of the dependent variable [5]. The independent variable in this study is the formulation of patikala fruit ethanol extract (Etlingera elatior) hand sanitizer gel with various concentrations, namely 20%, 25% and 30%.

The dependent variable: adalah variable yang menjadi akibat, karena adanya variable bebas [5]. Variabel terikat pada penelitian ini adalah sifat fisik sediaan gel *hand sanitizer.*

Tools and materials

The tools used in this study are: Autoclave, Petri dish, porcelain cup, analytical balance, incubator, autoclave, ruler/mystar, glass object, oven, ose, beaker, measuring cup, micro pipette, pH meter, stamper (mortar and pestle), stirring rod, brookfield viscometer. The materials used in this study are: Parchment paper, aquadest, carbomer 940, patikala fruit extract (Etlingera elatior), ethanol 96%, glycerin, methyl paraben, Staphylococcus aureus bacterial culture, TEA (triethanolamine), vanilla (flavor).

Sample processing

Samples in the form of patikala fruit are kept in the garden of Mario village, Baebunta sub-district, North Luwu district, South Sulawesi. Patikala fruit was taken randomly on each patikala tree, patikala fruit samples were taken using knife/machete equipment and sacks for storage of patikala fruit samples.

Preparation of ethanol extract of patikala fruit

The extraction process was carried out by maceration method, namely 10: 1 patikala fruit simplisia powder as much as 500 grams was put into the maceration container and added 5 liters of 96% ethanol until the simplisia powder was completely submerged and allowed to stand for 5 days while stirring occasionally. The extract obtained was filtered using filter paper, after the first extraction process was completed the pulp was macerated again with a new liquid. The filtrate obtained is separated from the solvent by evaporating it using a fan so that a concentrated extract is obtained [6].

Preparation of hand sanitizer gel

Making hand sanitizer gel according to Shu (2013), mortar and stamper (mortar and pestle) were prepared. Carbomer 940 was weighed as much as 1 gram and sprinkled over 20 mL of heated distilled water. Carbomer 940 that has been sprinkled is stirred quickly in the mortar until a gel period is formed and 1.25 ml of TEA is added. Methyl paraben was weighedas much as 0.1 gram and dissolved in distilled water as much as 5 mL, put into a mortar, stirreduntil homogeneous. Glycerin weighed 5.125 ml was added to the mortar, stirred until homogeneous. Patikala fruit extract was weighed as much as 10 grams and dissolved into 5 mL of distilled water and stirred until dissolved. Patikala fruit extract that has dissolved is putinto a mortar, mixed until homogeneous and crushed until a gel is formed and stirred until homogeneous [7].

Evaluation of hand sanitizer gel preparation

Organoleptical test

Visual assessment included color, odor and gel shape, ease of application and free of coarse grains. Rating scale 1-4 as follows: not suitable, suitable, very suitable.

pH test

0.5 grams of gel is placed in the center of a Petri dish lined with graph paper. Gel spreadability was measured by the diameter of the gel spreading from both sides after being left for one minute. Measurement of the gel diameter started without load, then 50 grams, 100 grams, 150 grams, 200 grams were added until constant spreadability was achieved, and the diameter of the spreading gel was recorded after 1 minute.

Homogeneity test

The homogeneity test of the gel preparation is carried out by spreading the gel on glass or other transparent materials whose composition must be homogeneous. Homogeneous gel is characterized by the absence of lumps in the application results, the same structure and even color from the starting point of application to the end point of application.

Spreadability test

0.5 grams of gel is placed in the center of a Petri dish lined with graph paper. Gel dispersion was measured by the diameter of the gel spreading from both sides after being left for one minute. The measurement of gel diameter was started without load, then 50 grams, 100 grams, 150 grams, 200 grams of load were added until uniform dispersion was achieved, and the diameter of gel dispersion was recorded after 1 minute.

Hedonic test

The liking test was conducted on 14 panelists and given a questionnaire containing an assessment of the liking of the organoleptic test carried out visually on the hand sanitizer gel preparation, including color, odor and texture. The assessment results were given a scale of 1-4 as follows: 1 (dislike), 2 (less like), 3 (quite like), and 4 (like) [8].

Irritation test

The skin irritation test was conducted to determine the side effects of using hand sanitizer gel on hand skin. The skin irritation test was carried out by applying hand sanitizer gel with a concentration variation of 20%, 25% and 30% on the palms of 14 volunteers. The skin irritation test was carried out with special criteria including healthy, aged 20-25 years, no wounds on the palms of the hands, not using cosmetics on the palms of the hands and not using topical antibiotics.

Viscosity test

The tool used to measure viscosity is a brookfield LV viscometer. The way to do the viscosity test is to put the gel preparation in a 100 ml beaker, then a size 4 spindle is attached to the viscometer and the rotor is run at a speed of 30 rpm. After the speed shows a stable number, the results are recorded [9].

Tool sterilization

The tools to be sterilized were first washed and dried. Petri dishes are wrapped in aluminum foil. For glassware (test tube, beaker, erlenmeyer), the mouth is covered with sterile

cotton wrapped in gauze and then wrapped in aluminum foil, sterilized in an autoclave at 121ºC, for 15 minutes. For tools such as ose, and tweezers are sterilized by the Flambir method, which is soaked with 70% alcohol for 5 minutes then ignited with a bunsen flame. Tools made of rubber such as rubber pipettes, sterilized by soaking them in 70% alcohol for 5 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 37ºC for 1 x 24 hours.

Preparation of Test Media

9.5 g NA solid media was dissolved in 250 mL sterile distilled water and heated until dissolved. Then sterilized with an autoclave 121ºC for 15-20 minutes. Sterilized media was put into Petri dishes and then allowed to stand until solid.

Antibacterial Activity Test using the Disc Diffusion Method

In paper discs, a filter disc paper is used which functions as a place to accommodate antimicrobial substances. Filter paper containing antimicrobial substances is placed on agar plates that have been inoculated with test microbes and then incubated at a certain time and temperature, according to the optimum conditions of the test microbes, namely at 37ºC for 18-24 hours. There are 2 kinds of inhibition zones formed from the Kirby Bauer method. Radical zone is an area around the disk where there is absolutely no bacterial growth. Antibacterial potential is measured by measuring the diameter of the radical zone. Irradical zone is an area around the disk where bacterial growth is inhibited by antibacterials, but not killed [10].

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 Harita (2019), the criteria for antibacterial power strength are as follows: the diameter of the inhibition zone is 5 mm or less then the inhibitory activity is categorized as weak, the diameter of the inhibition zone is 5-10 mm then categorized as moderate, the diameter of the inhibition zone is 10-20 mm categorized as strong and if the diameter is 20 mm or more then 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 [11].

3. Result and Discussion

3.1. Result

The results of the water content determination test of patikala fruit simplisia powder (Etlingera elatior) are 4%, this meets the requirements for water content in accordance with the requirements for water content in general, which is not more than ≤10% [12]. Penetapan kadar air bertujuan untuk memberikan batas maksimal atau rentang besarnya kandungan air didalam bahan. Pada umumnya penentuan kadar air bahan pangan dilakukan dengan mengeringkan bahan dalam oven pada suhu 105℃ selama 5 jam atau sampai diperoleh berat konstan [12].

The drying shrinkage result obtained from patikala fruit (Etlingera elatior) simplisia

powder is 6.8%, which meets the requirements for a good drying shrinkage value. Drying shrinkage is one of the non-specific parameters 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 105oC for 30 minutes to a constant weight and then expressed in percent. A good requirement for drying shrinkage is ≤10% [12].

This test shows that formulation I, formulation II, formulation III and formulation 0 have the same aroma results, which have a distinctive vanilla aromatic odor. The gel preparation of hand sanitizer formulation I, formulation II, and formulation III also has the same color, which is brown obtained from ethanol extract of patikala fruit and F0 is colorless because no ethanol extract of patikala fruit is added, where the negative control only contains the base of the gel preparation. Then in terms of observations in terms of formulation F0, FI, FII, FIII have the same shape, namely gel (semi-solid), formulations F1, FII, FIII are thicker than formulation F0 due to the addition of various active substances in formulations FI, FII, FIII. The results of organoleptical observations of hand sanitizer gel preparations in Table 5 show that before and after storage have not changed, namely with a brown gel-shaped color (semi-solid) and a distinctive vanilla smell. This shows that in this parameter the preparation is said to be good before and after storage, or the components in the preparation during storage do not experience reactions between one material and another, so there are no signs of reaction from color changes [13].

The results of pH testing of patikala fruit extract hand sanitizer gel preparations can be seen that all formulas have a good pH value This shows that the preparation is in accordance with the pH of normal skin, namely 4-7. The pH test was carried out with the aim of knowing how much the pH of the gel produced and changes during storage. The pH test results explain that the patikala fruit (Etlingera elatior) hand sanitizer gel has met the requirements of SNI No. 06-2588-1992 with a pH of 4-7. The change in pH from time to time from the first week of testing to the fourth week has changed. This is because at the time of storage indicates the lack of stability of the preparation, which causes the product to be damaged during storage. Changes in pH can be influenced by decomposition media such as storage temperature which can increase acid or base levels [14]. Another factor is light rays from outside, where light is a catalyst in oxidation reactions by transferring energy from light waves to reactive through the ability to increase energy as a precaution against accelerating oxidation reactions. Therefore, packaging preparations in dark bottles can withstand direct light entry [15]. But these changes are still reasonable because the changes do not occur significantly [16].

Based on the homogeneity testing that has been done, it shows that the results of the four formulations show good homogeneity because the observation does not show the presence of small particles that are not well distributed in the hand sanitizer gel base, on the application of the gel preparation looks smooth evenly there are no clumped particles. The homogeneity test results show that the formulated hand sanitizer gel has met the requirements of SNI No. 06-2588-1992. This observation aims to see significant changes in the final preparation that has been made. A good preparation must be homogeneous and free of particles that are still clumped [17].

The results obtained in this spreadability test, formula I and formula II have a fairly wide spread compared to formula III. The test results that have been carried out show that all preparations meet SNI No. 06-2588 standards. Good spreadability is one indicator that the gel preparation is easy to apply. The spreadability test aims to determine the speed of spread and determine the softness of the gel preparation on the skin. Based on the results, there is no significant difference in the spreadability value of each formulation. These results indicate that all formulations made meet the spreadability requirements, which range from 5-7 cm in diameter [17].

Based on the data from the table above, the hedonic test results have been carried out using a google form questionnaire with each respondent asked to observe each formula made. The most preferred aroma, color and texture assessment in preparations F0, FI, FII, and FIII is FI with an aroma assessment of 11 people like and 3 people really like. Color assessment as many as 9 people like and really like 4 people. Texture assessment 12 people like and 2 people really like. The hedonic test is conducted to determine the panelists' personal responses to liking or disliking based on their level of a product or sample [18]. This test uses 14 respondents who are calculated based on the population using the slovin formula. In this study were 7th semester students of S1 Pharmacy Study Program, Faculty of Health Sciences, University of Muhammadiyah Palopo.

Skin irritation testing was conducted to explore the future effects of the hand sanitizer gel on the skin of the hands. In this test, it was seen that none of the 14 respondents felt irritation on the hands, either in the form of redness, itching or burning. This is because the pH of the hand sanitizer gel preparation is still within the allowable range of 4.5-6.5, if the pH is outside the allowable range, the effect is that the hands will experience irritation and the hand sanitizer gel preparation cannot be used long-term [19]. Uji iritasi kulit yang dilakukan untuk menegtahui terjadinya efek samping pada kulit, dengan memakai sediaan gel *hand sanitizer* dibagian lengan atau belakang telinga dan dibiarkan selama 24 jam [13].

In testing the viscosity of hand sanitizer gel preparations, Formula 0 viscosity value is 7431 Cps higher than formulations I, II, and III and does not meet good requirements, Formula 1 viscosity value is 3027 Cps, Formula II 3651 Cps Formula III 3879 Cps and meets the requirements of 2000-4000 [20]. The results of the viscosity measurement of the patikala fruit extract (Etlingera elatior) hand sanitizer gel preparation can be seen in table 11 where the longer the gel storage time, the viscosity of the preparation increases. The change in the viscosity of the gel preparation is due to the presence of air bubbles in the preparation that arestill trapped when making the gel. Bubbles in gel preparations affect the viscosity value, the more bubbles, the more the viscosity of the preparation will increase, this is in accordance withthe literature [17].

Based on the table of antibacterial effectiveness test results of hand sanitizer gel preparations of ethanol extract of patikala fruit (Etlingera elatior) above, it can be seen that each concentration can form an inhibition zone against the growth of Staphylococcus aureus bacteria except k- which does not form an inhibition zone. At 20% concentration ethanol extract with an average diameter of 12.25 mm has a strong inhibition zone, 25% concentration

ethanol extract with an average diameter of 12.5 mm has a strong inhibition zone, and 30% concentration with an average of 12.66 mm has a strong inhibition zone. From the three concentrations above, it can be seen that the average inhibition zone formed in formula 1, formula 2 and formula 3 is categorized as strong, while K + is categorized as moderate, this is in accordance with the literature Yusran & Muhammad (2018), which states that the diameter of the inhibition zone of 5-9 mm is categorized as moderate. This is due to technical factors that affect the size of inhibition in the disc diffusion method, including: inoculum density, disc mounting time, incubation temperature, incubation time, plate size, thickness of agar media and media composition [1].

The data from the antibacterial activity test of several concentrations of ethanol extract of patikala fruit (Etlingera elatior) followed by data analysis using SPSS aims to see the inhibition of hand sanitizer gel preparations of ethanol extract of patikala fruit (Etlingera elatior) can inhibit the growth of Staphylococcus aureus bacteria.

3.2. Discussion

Samples in the form of patikala fruit are maintained in the garden of Mario village, Baebunta sub-district, North Luwu district, South Sulawesi. Patikala fruit was taken randomly on each patikala tree, patikala fruit samples were taken using knife/machete equipment and sacks for storage of patikala fruit samples.

The extraction process was carried out by maceration method, namely 10: 1 patikala fruit simplisia powder as much as 500 grams was put into the maceration container and added 5 liters of 96% ethanol until the simplisia powder was completely submerged and allowed to stand for 5 days while stirring occasionally. The extract obtained was filtered using filter paper, after the first extraction process was completed the pulp was macerated again with a new liquid. The filtrate obtained is separated from the solvent by evaporating it using a fan so that a concentrated extract is obtained [6].

4. Conclusion

Ethanol extract of patikala fruit (Etlingera elatior) can be formulated into hand sanitizer gel preparations that meet physical stability evaluation tests including organoleptic test, pH test, homogeneity test, spreadability test, hedonic test, irritation test, viscosity test.

Ethanol extract of patikala fruit (Etlingera elatior) which is formulated into hand sanitizer gel with 3 different concentrations, namely 20% concentration with an average value of 12.25 mm is categorized as strong, 25% concentration with an average value of 12.5 mm categorized as strong and a concentration of 30% with an average value of 12.66 mm is categorized as strong.

Test the antibacterial activity of hand sanitizer gel preparations with ethanol extract of patikala fruit (Etlingera elatior), all concentrations have an activity effect on the growth of Staphylococcus aureus bacteria

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