Antibacterial Activity Of Liquid Soap Preparations From Leaf Extract Waru (*Hibiscus Tiliaceus* L) Against *Staphylococcus Aureus*

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Abstract

Skin infectious diseases in humans are a very frequent type of disease, in the human population there are about 30% colonized by Staphylococcus aureus. The skin is the main defense against bacteria if the skin is no longer protected, it will be very easily infected with bacteria, fungi, viruses, protozoa and many other small groups such as Rickettsia, Mycoplasma and Chlamydia.waru plants (Hibiscus tiliaceus L) contain saponins, flavanoids, polyphenols and triterpenoid compounds. The purpose of the study was to determine the physical quality of the waru leaf extract liquid soap formula and to determine which waru leaf extract liquid soap formula has the best inhibition zone against Staphylococcus aureus bacteria. This type of research is an experimental method. The physical quality test of liquid soap showed that the liquid soap was stable. Testing antibacterial activity using the disc diffusion method, the results obtained by the clear zone in F1 show an average value of 25.75, F2 shows an average value of 19.4, and in F3 shows 30.4. From this study, good results were obtained on the physical quality of waru leaf extract liquid soap preparations in all formulas. Of the three best formulas, F3 and provides the greatest antibacterial effect with an average inhibition zone of 30.4 with a very strong category.
Keywords: Antibacterial; Waru leaf; Liquid soap; Staphylococcus aureus.

1. Introduction

Skin infections in humans are a very common type of disease. Skin infections are often referred to as infectious diseases because they can infect from one individual to another, either through direct contact or not. The factors that cause the transmission of skin diseases are unclean environments and behaviors that do not support health. In the human population there are about 30% colonized by Staphylococcus aureus [1]. This bacterium is the cause of infections on the skin and in soft tissues, one of which is the skin found in the community and hospital infections (nosocomial infections) [2].

According to [3] Skin is the main defense against bacteria if the skin is no longer protected, it will be very easily infected with bacteria, fungi, viruses, protozoa and many other small groups such as Rickettsia, Mycoplasma and Chlamydia. To maintain skin health, you can use one of the pharmaceutical preparations, namely soap. Antiseptic soap is soap that has the efficacy to kill bacteria found on the skin. The requirements for good antiseptic soap have specific standards, namely that the soap must be able to remove dirt and bacteria, and the soap does not damage skin health [3].

The common formulation in soap making involves the use of triloxane as an antibacterial agent. Excessive and routine use of triclosan can result in the destruction of normal flora that serves as skin protection, including against fungal infections. To prevent the negative impact of using triclosan, an alternative is to utilize natural ingredients as antibacterial agents.

One of the plants that has properties that are widely used in Indonesia is the waru plant (Hibiscus tiliaceus L) because waru leaves contain saponin, flavanoid, polyphenol and triterpenoid compounds [4]. In waru leaves with concentrations of 5% to 20% have high antibacterial effectiveness. The content of waru leaves are saponins, steroids, tannins, polyphenols, and flavanoids. The antimicrobial activity of waru leaves is proven to be effective against various types of bacteria, both gram-positive and gram-negative. Bacteria such as Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, and Salmonella partyphi are some examples that are sensitive to the antimicrobial properties possessed by waru leaves [5]. In wonokerto village, south sukamaju sub-district, north luwu district, the utilization of waru leaves is still less than optimal, because many people do not know the content and properties contained in waru plants and the lack of knowledge makes people not care about the presence of waru plants around them. Therefore, to increase public knowledge I want to conduct research on waru leaves, namely by testing the physical quality and activity of waru leaves by sampling in wonokerto village, south sukamaju sub-district, north luwu district. Based on the description above regarding the benefits of waru leaves (Hibiscus tiliaceus L.), this study was conducted to determine the antibacterial activity of liquid soap preparation formulations of waru leaf extract (Hibiscus tiliaceus L).

2. Methodology

a. Type of Research

This study utilized the experimental research method, an approach that involves experimental activities to identify the impact of specific treatments or experiments. This research aims to explore the results of the experiment on the dependent variable being tested.

b. Time and Place of Research

This research was conducted from August to November at the Microbiology and Pharmaceutical Technology Laboratory, Faculty of Health Sciences, Universitas Muhammadiyah Palopo.

c. Population and Sample

1. Population The population in this study was waru leaves (Hibiscus tiliaceus L) taken in Wonokerto Village, South Sukamaju District, North Luwu Regency.
2. Samples The samples used in this study were waru leaves (Hibiscus tiliaceus L).

d. Research Variables

1. Independent variable: Variations in the concentration of waru leaf extract (Hibiscus tiliaceus L) as an active substance in the formulation of liquid soap preparations in terms of the number of % (0.8%, 1%, 1.2%) of waru leaf extract in liquid soap preparations.
2. Variation dependent: Characteristics of liquid soap formulations from waru leaf extract as antibacterial which includes: Organoleptical test, pH test, homogeneity test, foam height test, irritation test, hedonic test, and antibacterial activity test.

e. Tools and Materials

1. Tools

The tools used in this research are 250 ml beaker glass, autoclave, spirit lamp, ose needle, analytical balance, erlenmeyer, 100 ml measuring cup, hotplate, spoit, petri dish, blender, 50 ml measuring cup, test tube, evaporator, spatula, horn spoon, test tube rack, ruler, label, pump bottle, porcelain cup, glass plate, round glass, hot plate magnetic stirrer, wooden rake, digital balance, tweezers, vial bottle, L rod, pH universal, and oven.

f. Material

Waru leaf extract, coconut oil, 70% ethanol, potassium hydroxide (KOH), sodium carboxymethyl cellulose (CMC), stearic acid, methyl paraben, glycerin, vanilla ice, 0.9% Nacl, nutrient agar media, distilled water, sodium lauryl sulfate and Staphylococcus aureus bacteria.

g. Researc Procedure

1. Sample processing

Simplisia sample processing used in this study was waru leaves taken as much as 2 kg [6]. According to [7] the criteria for the leaves used are fresh waru leaves that are dark green in color because green waru leaves have chlorophyll content. Dark green waru leaves are able to produce higher flavonoid compounds compared to light green leaves because according to [8] in [7] the chlorophyll content found in leaves in intensifying the green color has a maximum carotenoid which causes increased accumulation of components such as chlorophyll and carotene to produce flavonoids. Furthermore, it is cleaned by washing using running water and then separating the leaves and dirt then wet sorted and then dried by aerating at room temperature protected from direct sunlight for 3-5 days to dry. After that, it is sorted dry so that the simplisia is protected from dirt [4].

2. Extract

Waru leaf powder was macerated every 50 grams of powder added with 375 ml of 70% ethanol solvent until the sample was submerged. By stirring at least 3 times a day for 5 days. The extraction results were filtered and the solvent was evaporated with a fan to produce a thick extract [9]. 70% ethanol is used as a solvent because it is able to dissolve polar and semi-polar compounds. In addition, the use of 70% ethanol is considered safe, neutral, and has the properties of inhibiting bacterial growth and bacteria. mold. Another reason for using 70% ethanol is that the concentration of flavonoid bioactive compounds is more easily detected with this solvent [8].

3. Determination of moisture content

The first step is to dry the empty porcelain cup in an oven at 40℃ for 30 minutes. After the drying process for 30 minutes, the porcelain cup was allowed to reach room temperature, then weighed to get the weight of the empty porcelain cup. A total of 2 grams of waru leaf fine powder was measured and put into a porcelain cup. The porcelain cup containing the fine powder of waru leaves was then placed in an oven at 40℃ for 3 hours. The choice of 40℃ temperature in the

drying stage aims to maintain the content of active compounds in the simplisia. It is important to avoid temperatures above 50℃ because it can cause damage to the active compounds in the simplisia [10].

3. Result and Discussion

3.1. Result

A. Simplisia Quality Standardization

1. Organoleptic test

Organoleptic test	Result
Shape	Fine powder
Color	Light green
Odor	Characteristic smell of the extract

2. Result drying shrinkage determination

		Range
1. Empty cup weight	41,6 grams	
2. Weight of cup + Sample before oven	43,6 grams	Drying shrinkage standard is <10% (Maryam et al., 2020)
3. Weight of cup + sample after oven	43,4 grams	
4. Sampel weight	2 grams	

3. Result of moisture content determination

		Range
1. Weight of cup + Sample before oven	1 grams	
2. Weight of cup + Sample after oven	3 grams	The requirement for water content of simplisia that meets is less than 10 % (Depkes RI, 2017)
3. Sample	10 grams	

B. Physical Quality test

1. Organolepsticsl test

NO.			Sunday 1	Sunday 2	Sunday 3
1.	F0	Shape	Thick	Thick	Thick

			liquid	liquid	liquid
		Color	Yellow	Yellow	Yellow
		Smell	Vanilla Ice	Vanilla Ice	Vanilla Ice
2.	F1	Shape	Thick liquid	Thick liquid	Thick liquid
		Color	Dark Red	Dark Red	Dark Red
		Smell	Vanilla Ice	Vanilla Ice	Vanilla Ice
3.	F2	Shape	Thick liquid	Thick liquid	Thick liquid
		Color	Dark Red	Dark Red	Dark Red
		Smell	Vanilla Ice	Vanilla Ice	Vanilla Ice
4.	F3	Shape	Thick liquid	Thick liquid	Thick liquid
		Color	Dark Red	Dark Red	Dark Red
		Smell	Vanilla Ice	Vanilla Ice	Vanilla Ice

2. Homogencity test

Formula	**Homogenity test**		
F0	Sunday 1	Sunday 2	Sunday 3
F1	Homogenius	Homogenius	Homogenius
F2	Homogenius	Homogenius	Homogenius
F3	Homogenius	Homogenius	Homogenius

3. pH Test

pH Test					
Formulation	**Sunday 1**	**Sunday 2**	**Sunday 3**	**Average**	**Range**
F0	10	10	10	10	101
F1	11	11	11	11	11
F2	11	11	11	11	11
F3	11	11	11	11	11

4. Foam height test

Formulation	**Sunday 1**	**Sunday 2**	**Sunday 3**	**Averange**
F0	75 mm	83 mm	100 mm	86 mm
F1	85 mm	85 mm	85 mm	85 mm

F2	70 mm	75 mm	73 mm	73 mm
F3	90 mm	90 mm	88 mm	88 mm

5. Anibacterial activity test

NO.	Concenaion	Diameter			Averange	Catttegory
		P2 (mm)	P2 (mm)	P3 (mm)		
1.	Positif control	14,75	17,5	18	16,75	Strong
2.	F0	5,5	6	8	6,5	Medium
3.	F1	29	25,25	23	25,75	Very strong
4.	F2	20,75	12	25,5	19,4	Strong
5.	F3	33	31,25	27	30,4	Very strong

3.2. Discussion

A. Sample Collection and Processing

In this study, the material used was 2 kg of waru leaves (Hibiscus tiliaceus L) with the criteria of fresh dark green leaves. Leaf collection was carried out at 09.00-12.00 by picking the fifth leaf from the top. Next, it is cleaned by washing using running water and then separating the leaves and dirt then chopped into small pieces to reduce the moisture content of the material so that it can inhibit the growth of unwanted bacteria [11]. Then dried by airing at room temperature protected from direct sunlight for 3-5 days to dry. After that, it is sorted dry so that the simplisia is protected from impurities [4].

B. Simplisia Quality Standardization

1. Organoleptical test

Test organoleptical powder simplia Retrieveda little bit, carried out organoleptical tests were carried out, namely odor, shape and color).

2. Result Drying shrinkage determination

The following are the results of the determination of drying shrinkage of waru leaf simplisia (Hibiscus tiliaceus L).Determination of drying shrinkage is carried out using an oven at 105ºC until a constant weight is obtained. The purpose of drying shrinkage is to provide a maximum limit (range) on the number of compounds lost during the drying process [12]. The compounds lost in the drying process are volatile compounds such as essential oils and water. The result of drying shrinkage determination is 0.1%. This result is stated to meet the requirements because the standard drying shrinkage is <10% [13].

3. Result of moisture content determination

The following is the determination of the water content of waru leaf simplisia (Hibiscus tiliaceus L).

Determination of water content is carried out to measure the amount of water contained in simplisia with the aim of setting a minimum limit for the water content in simplisia [12]. The water content in simplisia needs to be kept low, so that simplisia can be stored for a longer period of time and microbial growth that can affect the quality of simplisia can be inhibited. The results of the analysis of water content in simplisia showed 0.08%. This figure meets the requirements because the desired moisture content of simplisia must be less than 10%, in accordance with the regulations of the Ministry of Health of the Republic of Indonesia in 2017[12].

4. Extraction

Waru leaf simplisia was macerated every 50 grams of powder added with 375 ml of 70% ethanol solvent until the sample was submerged. By stirring at least 3 times a day for 5 days. The extraction results are filtered and the solvent is evaporated with a fan to produce a thick extract [9]. In the study, samples of waru leaves (Hibiscus tiliaceus L) obtained from wonokerto village, north luwu district were used. The extraction process was carried out by maceration method. The maceration method is the process of extracting simplisia using an appropriate organic solvent with several stirring. Maceration is a cold extraction process, so this method is suitable for the extraction process to retrieve flavonoid compounds, flavonoid compounds themselves are compounds that are not resistant to heating and are polar and semi-polar compounds, besides the use of 70% ethanol is non-toxic and harmless, neutral and inhibits the growth of bacteria and mold. The choice of 70% ethanol as a solvent is also based on the fact that the concentration of flavonoid bioactive compounds is more easily detected when using 70% ethanol [14]. After that, the waru leaves were dried for several days, after drying the waru leaves were pulverized using a blender and weighed as much as 500 g and then soaked with 70% ethanol in a glass jar with occasional stirring. The rendition of the extract from waru leaves (Hibiscus tiliaceus L) is as follows: Calculation of the yield of waru leaf extract (Hibiscus tiliaceus L).

From the maceration process, the percent yield was 14.68%. The greater the rendamen value of an extract shows the value of the extract produced is also more and more. The requirement for the percent yield of thick extracts is that the value is not less than 10% [15]. So it can be seen that the yield of the maceration using 70% solvent has met the requirements. The extract obtained was 73.4 g with a yield of 14.68%.

The results of the organoleptic test of liquid soap of waru leaf extract (Hibiscus tiliaceus L) did not occur physical changes during storage from week 1 to week 3 The organoleptic differences in each formulation did not differ, in F1 the color produced was dark red, F2 the color produced was dark red, and F2 the color produced was dark red. produced dark red, and F3 the resulting color is dark red. In line with research [16] that the resulting color change comes from the extract, the higher the extract, the

darker the color.

The homogeneity test is carried out to see whether the liquid soap preparation that has been made is homogeneous or not [17]. The results of the homogeneity test of preparations F0, F1, F2, and F3 which were observed for 3 weeks were homogeneous because there were no clumped particles. The homogeneous requirement is that there is no solid material or lumps on the glass plate. The homogeneity test is carried out to see whether the liquid soap preparation that has been made is homogeneous or not [17]. The results of the homogeneity test of preparations F0, F1, F2, and F3 which were observed for 3 weeks were homogeneous because there were no clumped particles. This meets the requirements. The homogeneous requirement is that there is no solid material or lumps on the glass plate.

The pH test is carried out because it is one of the quality requirements of liquid soap. This is because liquid soap is in direct contact with the skin and can cause problems if the pH does not match the pH of the skin. pH was measured on days 1, 7, and 14. The pH results obtained in F1, F2, and F3 have an average value of 11. This has met the criteria for liquid soap pH for liquid soap is 8-11 [18].

The foam height test was conducted to see the height of the foam produced liquid soap that complies with the liquid soap foam height standard set by the National Standards Agency. The foam height test meets the criteria for the average value of F0 86, F1 85, F2 73 and F3 88. This meets the foam height requirements in line with the liquid soap foam height standard of 12- 220 mm BSN, 1996).

The irritation test was carried out on 14 respondents obtained from the slovin formula using an error of 20%. This is because the population used is small (Patarianto, 2015). The irritation test obtained results from 14 respondents for the three formulas that did not occur irritation by looking at the symptoms that arose behind the ear in the form of itching, redness and roughening of the skin.

The hedonic test (liking test) was conducted on 14 respondents with the slovin formula using an error of 20%. This is because the population used is small [19]. The hedonic test obtained results from 14 respondents, namely F1, F2 and F3.

Waru leaves (Hibiscus tiliaceus L.) were chosen because they have compounds that have potential as antibacterials (Rawool and Parulekar, 2019). The test method used in the antibacterial activity test is the disc diffusion method.

The results obtained in the antibacterial test of liquid soap preparations of waru leaf extract (Hibiscus tiliaceus L) can inhibit the growth of Staphylococcus aureus bacteria. The test carried out has an average inhibition zone diameter of 0.8% concentration F1 25.7 mm, 1% concentration F2 19.4 mm, and 1.2% concentration F3 30.4 mm. F1 and F3 are included in the category of very strong inhibition zone. In waru leaves with concentrations of 5% to 20% have high antibacterial effectiveness (Lusiana, 2013). In the positive control using products on the market, namely liquid soap x has an average inhibition zone of 16.75 mm, which is included in the strong category and the negative control, namely the formula without extracts, has an average inhibition zone diameter of 6.5 mm, including the medium category explaining that the

inhibition zone against bacterial growth will be greater the higher the concentration added. The difference in the size of the inhibition zone at each concentration is due to the difference in the amount of active ingredients contained in each concentration. The higher the concentration, the greater the amount of active ingredients contained, so that the resulting inhibition zone is also greater. As for what affects the inhibition zone in F2, the 1% concentration in cup B treatment decreases, which is influenced by the 1% concentration that does not penetrate completely into the paper disk and is difficult to diffuse in the media so that the inhibition formed is smaller than the treatment of cup A and cup C [20].

The results of the data obtained were statistically analyzed with one way anova and tukey tests with SPSS IBM 24. shows that the results of the One Way Anova Test are significant for the waru leaf ethanol extract treatment group with a p value = 0.000. Because the p value <0.05, the average value between treatment groups of ethanol extract of waru leaves is significantly different. To find out which treatment group has a significant difference, then further post-hoc analysis is carried out.

Is the result of post hoc tests which shows if the data has $p < 0.05$, it means that the data is significant or significantly different from other concentrations. If $p > 0.05$, then the data is not significant or not significantly different from other concentrations. Post hoc tests The positive control had no significant difference with F1 0.8% concentration, F2 1% concentration, and negative control. But it has a significant difference with F3 concentration of 1.2%. For the negative control, there is no significant difference with F2 concentration of 1% and positive control. But it has a significant difference with F1 0.8% concentration and F3 1.2% concentration. From the results of the research conducted, the best preparation from the physical stability test and antibacterial activity is the preparation in formula 3 with an extract concentration of 1.2%.

4. Conclusion

A. Conclusion

1. In this study, good results were obtained in the physical quality of liquid soap preparations of waru leaf extract (Hibiscus tiliaceus L) in all formulas.
2. Of the three best formulas, the 1.2% concentration gave the greatest antibacterial effect against Staphylococcus aureus, namely an average inhibition zone of 30.4 with a very strong category.

B. Advice

In further research, it is hoped that the maker of the liquid soap preparation formula will improve the color of the preparation to make it look more attractive and in the physical quality test of the preparation for the pH test using a pH meter so that the results obtained are accurate.

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