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ANTIBACTERIAL ACTIVITY TEST OF CELERY LEAF (*Apium graveolens*) EXTRACT LIQUID HAND SOAP AGAINST *Staphylococcus aureus*

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Abstract: Personal and environmental hygiene must be considered to prevent the transmission of COVID-19. One of them is by washing hands with soap. Liquid soap is a skin cleanser made from soap-based ingredients and added surfactants, preservatives, foam stabilizers, fragrances, and dyes. The basic ingredients for soap can be natural, one of which is essential oil. Celery contains essential oils (alinin and allicin), flavonoids, protein, vitamin A, vitamin C, vitamin B, iron, calcium, sulfur, and phosphorus. Essential oil from celery has activity as an antifungal and is active against many bacteria, including *Staphylococcus aureus*. This study aims to determine the celery extract liquid hand soap has antibacterial activity against *Staphylococcus aureus bacteria* and the effective concentration of celery leaf extract liquid hand soap on the antibacterial activity of *Staphylococcus aureus*. The research method was laboratory experimental by testing the antibacterial activity of liquid hand soap formulations of celery leaf extract. The results of the organoleptic test were green in color, in viscous liquid form, and had a distinctive celery aroma. The pH test obtained a pH of 9.4 in the 5% formulation (FI), pH 9.5 in the 10% formulation (FII), and pH 9.3 in the 15% formulation (FIII). Antibacterial activity test FI, FII, FIII, negative control, and positive control resulted in a diameter of 17.1 mm inhibition zone; 23.2 mm; 17.4 mm; 0 mm, and 22.9 mm. The results of the *one-way* ANOVA test obtained a p-value <0.05, which indicated that H1 was accepted, i.e., liquid hand soap with celery leaf extract had antibacterial activity against *Staphylococcus aureus*. The *Tukey test* showed that the diameter of the FII inhibition zone (10% concentration) was close to that of the positive control inhibition zone. The most effective concentration is 10% (FII), with a very strong inhibition category.

Keywords: *Celery, Formulation, Liquid Hand Soap, Antibacterial*

INTRODUCTION

The COVID-19 pandemic is hitting Indonesia, and it was confirmed on March 3 2020. Particularly in NTB, on June 21, 2021, there were 1,671 active cases recorded, and according to data, on December 7, 2021, there were 27,754 positive cases with 910 deaths [1].

Personal and environmental hygiene must be considered to prevent transmission of COVID-19, apart from having to keep wearing a mask. Washing hands with soap is one of the most important new habits to continue to apply. Therefore, handwashing soap is essential always to provide. The need for liquid soap for washing hands is also increasing.

Using plant extracts as antibacterials is easier in the form of liquid soap that can be used daily. Soap is made by the saponification method, namely reacting triglycerides with caustic soda (NaOH) to produce soap and a byproduct of glycerin. Raw soap materials can be animal fat or vegetable fat/oil. The use of soap in everyday life is no stranger, especially according to its main function, cleaning. Various types of soap are offered in various forms, ranging from laundry soap (cream and powder), bath soap (solid and liquid), hand soap (liquid), and household cleaning soap [2].

Liquid soap is a liquid preparation intended for cleaning the skin, made from soap-based ingredients to which surfactants, preservatives, foam stabilizers, permissible fragrances, and dyes are added and can be used without irritating the skin. Antiseptic soaps on the market, when used frequently for a long time, can cause side effects and skin irritation [3]. Thus, the formulation of natural liquid soap needs to be developed.

Indonesia has various natural resources and is rich in ingredients that can be used as soap-base ingredients, one of which is essential oil. Essential oils are oils from plants whose components are volatile. Essential oils are also called ethereal oils and are also essential oils. Essential oils can be sourced from any part of the plant, from leaves, flowers, fruit, seeds, stems, or skin and roots. Various kinds of cultivated plants grown in various regions in Indonesia have great potential to be processed into essential oils, both superior and potential for development. One of them is the celery plant. Celery (*Apium graveolens*) is a type of plant that is well-known to the public. Apart from being used as a vegetable, empirically, people use it for treatment. The part of celery that is widely

used for research is the leaf, so researchers use celery leaves as a sample. Celery contains essential oils (alinin and allicin), flavonoids, protein, vitamin A, vitamin C, B vitamins, iron, calcium, sulfur, and phosphorus [4].

In general, celery's phytochemical compounds contain carbohydrates, phenols (flavonoids), alkaloids, and steroids. The existence of compounds such as limonene, selinen, pro coumarin glycosides, flavonoids, and Vitamins A and C, makes this plant often used in various traditional medicines and has the potential to maintain the fitness and health of our bodies [5]. Based on research, this plant contains vitamin C, which is twice as much as the vitamin C content in citrus fruits. It also contains B vitamins, PP and E, folic acid, phosphorus, potassium, and Zn [6].

There are three antibacterial mechanisms of flavonoids: inhibiting nucleic acid synthesis, inhibiting the function of the cytoplasmic membrane, and inhibiting energy metabolism. Saponins have the antibacterial ability to protect against potential pathogens; besides that, saponins will interfere with the surface tension of the cell wall. Tannins have antibacterial activity using bacterial walls that have been lysed due to saponins and flavonoids, thus causing tannin compounds to easily enter the bacterial cell and coagulate the protoplasm of the bacterial cell.

Essential oil from celery has antifungal activity and is active against many bacteria, including *Staphylococcus aureus, Staphylococcus albus, Shigella dysenteriae, Salmonella typhi, Sreptococcus faecalis, Sreptococcus pyogenes* and *Pseudomonas solanacearum* [7], so it is necessary to research to test the antibacterial activity of liquid hand soap preparations from celery leaf extract (*Apium graveolens*). Previously, research on the formulation and evaluation of liquid hand soap from celery leaf extract was carried out, and the results of the research showed that formulations with concentrations of 5%, 10%, and 15% met the quality requirements based on Standards National Indonesia (SNI), namely SNI 2588:2017 [8-9]. This research aimed to test the antibacterial activity of liquid handwashing soap formulations from celery leaf extract on *Staphylococcus aureus bacteria*.

METHODS

This research is an experimental laboratory study by making a soap preparation formulation and testing its antibacterial activity on *Staphylococcus aureus*.

Place and Time

This research was conducted at the Chemistry Laboratory and Clinic of the Medica Farma Husada Polytechnic, Mataram.

Tools and Materials

Tool

The tools used in the study were: 200 mesh sieve, *rotary evaporator*, filter paper, *aluminum foil*, magnetic stirrer, thermometer, water bath, pH meter (Emeltron), g e welding measure, stirrer, dropper pipette, erlenmeyer, weigh analytic, measuring flask, Petri dish, incubator, autoclave, oven, blender, bekerglass, measuring pipette, test tube, tube rack, bunsen, needle, tweezers, micropipette, vortex, calipers.

Ingredient

The materials used in the study were celery leaves, olive oil, potassium hydroxide (KOH), carboxyl metal cellulose (CMC), stearic acid, butyl hydroxy anisol (BHA), sodium lauryl sulfate. (SLS), Stearic Acid, BHA, distilled water, 95% ethanol, Nutrient agar (NA), 0.9% physiological NaCl, PCA (*Plate Count Agar*) media.

Research procedure

1. Sampling

The sample used in this study was fresh celery leaves obtained in the form of celery plants from the Mataram region, NTB Province.

2. Sample Processing

The samples were then sorted wet, washed with running water, drained, air-dried for 6 days, then dried in an oven at 40 ^{0} C. The dried samples were then blended and sieved using a 200-mesh sieve.

3. Extracting

The extraction process was carried out by continuous maceration method (remaceration) for 3x24 hours. Maceration day, first, as much as 500 grams of celery leaf simplicia powder is put into the container, then added 96% ethanol solvent, as much as 2000 ml. Covered with aluminum foil and left for a day (24 hours) while stirring occasionally. After 24 hours, samples that were macerated were filtered using filter paper to produce filtrate I and residue I. Residue I was then macerated with 1000 ml of 96% ethanol. The treatment was the same as the first maceration to produce filtrate II and residue II. Furthermore, residue II has macerated with 96% ethanol as much as 500 ml. after a day; sample II was filtered to

produce filtrate III and residue III. Filtrate I, II, andIII combined and evaporated to extract thick-leaf celery.

4. Formulation

The formulation will be shown in table 1 below.

Table 1. Formulation of liquid soap preparations made with various concentrations 5%, 10% and 15%.

Materials	Unit	The formula I (5%)	Formula II (10%)	Formula III (15%)
Extract leaf celery	grams	2.5	5	7.5
Oil Olive	ml	15	15	15
KOH	ml	8	8	8
CMC	grams	0.5	0.5	0.5
SLS	grams	0.5	0.5	0.5
Sour stearate	grams	0.25	0.25	0.25
BHA	grams	0.5	0.5	0.5
Aquades	ml	add 50	add 50	add 50

5. Making Liquid Soap Extract Celery Leaves

All ingredients are weighed in advance according to the measure. Entered 5 ml of oil into a beaker, then add KOH 40% 8 ml gradually while continuing to heat at 50°C until obtained paste soap, then add 15 ml of distilled water, then Enter Na-CMC, which has developed in aquades hot, stirred until homogeneous. Then add stearic acid and stir until homogeneous. SLS was added and stirred until homogeneous. Added with BHA, stirred until homogeneous. Add celery leaf extract, stir until homogeneous, and add 50 ml distilled water. Making liquid soap extract celery leaf ethanol adjusted to each respective concentration.

6. Testing Soap Quality Test

a. Organoleptic Test

Organoleptic tests were carried out to observe the shape, color, and smell of celery leaf extract liquid hand soap.

b. pH test

A total of 1 gram of the test sample (celery leaf liquid soap) was weighed and transferred to a 1,000 ml volumetric flask. The sample is homogenized with CO_2 -free aquadest and then added. This solution was left at room temperature. The pH meter that has been calibrated with a standard buffer solution is dipped into the sample solution to obtain the pH value of the solution.

c. Antibacterial Test

Antibacterial testing was carried out using the agar diffusion method (Kirby and Bauer diffusion modified using wells with two layers of agar media [10]. Observations were carried out after 1x24 hours of the incubation period. The clear area/zone indicates the bacteria's sensitivity to the test substance used as the test material, which is expressed by the diameter of the inhibition zone [11]. Measurement of the diameter of the inhibition zone formed using a caliper. Antibacterial testing was carried out with as many as 5 replications/repetitions. The average diameter of the inhibition zone was obtained from the results of measuring the inhibition zone to the 5 replications.

RESULTS AND DISCUSSIONS

The yield of celery leaf extract obtained 37.12%. It is in accordance with [12] which states that the yield of celery leaf extract (using ethanol solvent) is at least 24.6%. The organoleptic observations of Formula I (5%), Formula II (10%), and Formula III (15%) liquid handwashing soap with celery leaf extract are shown in Table 2.

The organoleptic test aims to see a preparation's physical appearance, including color, smell, and shape. Based on Table 2, it was stated that FII (10%) and FIII (15%) liquid hand soap had a dark green color, like the color of celery leaf extract, while FI (5%) was light green. It is because the FI concentration is the lowest, so of course, the amount of extract used is also the smallest, and the yellow soap base causes the soap color to turn light green. All Formulas (FI, FII, and FIII) have a characteristic celery odor and are in liquid form.

Table 2. Organoleptic Test Results

Organoleptic	Liquid hand soap formulation		
	FI (5%)	FII (10%)	FIII (15%)
Color	Light green	Deep green	Darker green
Odor	Celery smell	Celery smell	Celery smell
Shape	Liquid, thick	Liquid, thick	Liquid, thick

Next, a pH test of celery leaf extracts liquid hand soap was carried out. The results of the pH test can be seen in **t**able 3**.**

Table 3. pH Test Results

Liquid hand soap formulation	pH
FI (5%)	9.4
F2 (10%)	9.5
F3 (15%)	9.3

The degree of acidity (pH) is one of the quality requirements in making liquid soap because pH affects the condition of the skin after use. The pH quality requirements are 4-10. Testing the pH of liquid hand soap preparations of ethanol extract of celery leaves FI, FII and FIII gave successive results of 9.4., 9.5 and 9.3 [9]. It shows that FI, FII, and FIII liquid hand soap preparations meet the requirements of SNI 2588: 2017, and it can be said that these preparations are safe to use. A pH that is too low (strong acid) can cause skin irritation, while a pH that is too high (strong base) can cause scaly skin. High pH can cause skin irritation because it has a high level of free alkali. The level of free alkali in soap is caused by alkali, which does not react with fatty acids in the saponification process [13]. The pH test results at each concentration/formula are almost the same as the criteria for being a weak base. It is influenced by one of the soap ingredients, a strong base, KOH [14].

The next test was to test the antibacterial activity of celery leaf liquid hand soap. This test was carried out using the good diffusion method. The hole/well method is carried out by making holes in solid agar inoculated with bacteria. After incubation for 1x24 hours, observations were made, and the diameter of the inhibition zone was measured, which was indicated by the formation of clear/bright zones. The results of measuring the average diameter of the inhibition zone of the 5 replications/repetitions of the tests performed are shown in table 4.

Table 4. Antibacterial Test Results

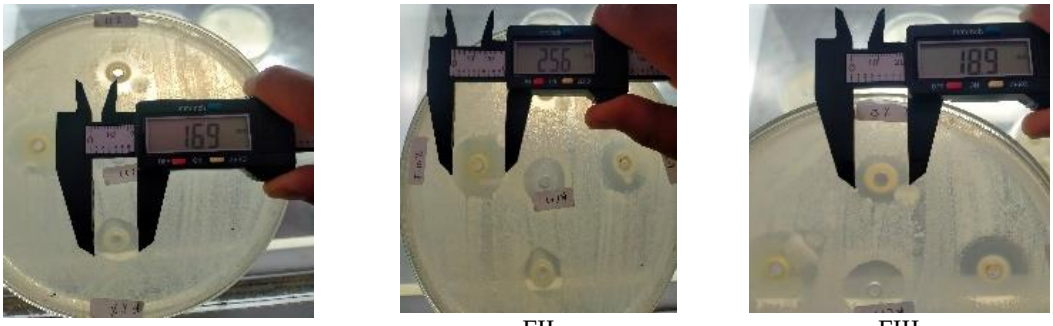
Formulas	Average Inhibition Zone Diameter (mm)	Resistance Category
FI (5%)	17.1	Strong
FII (10%)	23.2	Very strong
FIII (15%)	17.4	Strong
K (+)	22.9	Very strong
K (-)	0	Weak

Information:
FI : Formula with a concentration of 5%
FII : Formula with a concentration of 10%
FIII : Formula with a concentration of 15 %
K (+) : Positive control
K (-) : Negative control

Measurements of the diameter of the liquid hand soap inhibition zone of 5%, 10%, and 15% replication 1 can be seen in Figure 1.

Antibacterial test results at a concentration of 5% average diameter of the inhibition zone were 17.1 mm with the category of strong inhibition. The results at 10 % concentration mean the diameter of the inhibition zone is 23.2 mm with the category of very strong inhibition. At 15% concentration, the average diameter of the inhibition zone is 17.4 mm with the category

of strong inhibition. The positive control in this study showed that the average diameter of the inhibition zone was 22.9 mm with a very strong category of inhibition, while a negative control of 0 mm was in a weak category.



FI FII FIII

Figure 1. Inhibition zones of FI, FII, and FIII on replication 1

The results of measuring the average diameter of the inhibition zone were then analyzed using SPSS version 20. First, a data normality test was performed using the *Shapiro-Wilk test*. It was found that the p-value was smaller than the significant or error level of 5% ($p < 0.05$). The inhibition zone data for antibacterial activity followed the pattern, and the analysis results were carried out using a normal distribution. Next is the data homogeneity test using the *Levene test*. The results were $p < 0.05$, meaning that the inhibition zone data for antibacterial activity were not homogeneous. In the *one-way ANOVA test*, the p-value was lower than the 5% significant level ($p < 0.05$), which means that H1 was accepted (celery leaf extract liquid hand soap has antibacterial activity against *Staphylococcus aureus.* Then the last SPSS analysis was performed. *Tukey* test This test aims to determine the concentration formula of celery leaf extract, whichever is different, *using* Tukey's test. Based on the results of the *Tukey test*, it was found that differences in results were based on the zone of inhibition of antibacterial activity. They were divided into two groups: the first group with a concentration of 5 % celery extract formula and a 15% concentration of celery leaf extract formula. The second group is a 10% concentration of celery leaf extract formula and positive control (brand X soap). It means that the celery leaf extract formula with a concentration of 10% has an average value of the diameter of the inhibition zone that approaches the average diameter of the positive control inhibition zone. Thus it is said that the most effective formula/concentration of celery leaf liquid hand soap is 10%. The study [14] also found that 2%, 4%, and 8% concentrations of celery herb liquid soap had high antibacterial activity against *Staphylococcus aureus bacteria.* It is because celery contains active substances of flavonoids, saponins, tannins 1%, essential oils 0.033%, apiin, apigenin, choline, lipase, and aspargine which can inhibit bacterial activity [14-19].

Flavonoid derivatives contained in celery are flavones, such as luteolin, apigenin, and chrysoberyl [20]. Flavonoid content in 100 grams of fresh celery leaves is 5.3-16 µmol apigenin, 18-51 µmol apigenin glycosides, 7.1-21 µmol luteolin glycosides, and 13-38 µmol chrysoberyl glycosides [21]. Another component of the active ingredient in celery leaves is saponins, natural glycoside products with a high molecular weight [22]. Saponins have the antibacterial ability to protect against potential pathogens. Besides, that saponins will interfere with the surface tension of the cell wall. The mechanism of saponins as antibacterial agents is by interacting with cholesterol in the cell membrane, causing the cell membrane to undergo lipid modification which will interfere with the ability of bacteria to interact with the membrane [23]. Tannin compounds are active ingredients from celery plant extracts that can destroy bacterial colonies and inhibit microbial growth [24].

CONCLUSION

Based on the research results, it can be concluded that liquid hand soap with celery leaf extract concentrations of 5%, 10%, and 15% has antibacterial activity against *Staphylococcus aureus.* The most effective concentration was 10%, with an average inhibition zone diameter of 22.98 mm (very strong inhibition category).

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