

FORMULATION AND EVALUATION LIQUID HAND SOAP OF CELERY LEAF (*Apium graveolens*) EXTRACT

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Abstract: Washing hands with soap is one of the new habits during the COVID-19 pandemic, which is very important to continue to apply. Therefore, alternative natural ingredients are needed to manufacture liquid hand soap that is safe to use in the long term. Celery leaves contain flavonoids, saponins, tannins, essential oils, choline, lipase, and alkaloids. The content of these secondary metabolites has antimicrobial activity. This study aims to formulate liquid hand soap preparations from celery leaf extract following SNI 2588.2.2017. This research is experimental laboratory research that formulates soap preparations with concentrations of 5%, 10%, and 15% and is followed by an evaluation of the preparations based on the referenced SNI quality requirements. This study resulted in an extract yield of 37.12% with organoleptic test results in a thick liquid dosage form, characteristic odor of celery, and green color. In the evaluation test of preparations based on SNI 2588:2017, the results obtained that liquid hand soap preparations meet the requirements. PH for FI, FII, and FIII respectively 9.4, 9.5, and 9.3. In the total active ingredient test, the FI 34.6% was obtained; FII 32.2% and FIII 32.6%. Then in the ethanol insoluble material test, the FI results were 0.094%, FII 0.086%, and FIII 0.112%. Free alkali test obtained FI 0.044%, FII 0.0472%, and FIII 0.0488%. Then the total plate number test was obtained in FI, FII, and FIII preparations; namely, there was no colony growth in samples with dilutions of 10-1, 10-2, and 10-3. The formulation of liquid hand soap meets the quality requirements of SNI 2588.2.2017.

Keywords: *Celery, Essentials Oil, Formulation, Liquid Handsoap*

INTRODUCTION

The world, including Indonesia, was shocked by the outbreak of a new virus at the end of 2019. This virus is a new type of Coronavirus (SARS-CoV-2) which can then cause the disease we know as COVID-19 [1]. This virus is sensitive to heat and can be inactivated by disinfectants containing: chlorine, lipid solvents at 56°C for 30 minutes, ether, alcohol, peroxyacetic acid, nonionic detergents, formalin, oxidizing agents, and chloroform [2-4].

To do prevention, the World Health Organization (WHO) recommends doing essential protection, including washing hands regularly with soap or hand sanitizer. Water is often referred to as the universal solvent. Still, washing hands with water alone is not enough to eliminate the coronavirus because it is an RNA virus with a lipid bilayer envelope. Soap can lift and break down hydrophobic compounds such as fats or oils [5-6]. Thus, the use of and the need for soap is getting higher. Liquid soap is a liquid preparation intended to clean the skin, made from soap-based ingredients added with surfactants, preservatives, foam stabilizers, fragrances, and dyes that can be used without irritating the skin. When used frequently for a long time, antiseptic soap on the market can cause side effects and skin irritation [7]. Thus, liquid soap formulation is formulated from natural ingredients that need to be developed.

Indonesia has many natural resources that can be developed as essential ingredients for soap celery (*Apium graveolens*). Celery plants contain

essential oils (alanine and allicin), apiin glycosides, iso-quercetin, umbelliferon, asparagine [8]. It also contains Vitamin B, Vitamin PP, and E and folic acid, phosphorus, potassium, and Zn [9]. Especially on the celery leaf contains flavonoids, saponins, tannins, essential oils, choline, lipase, alkaloids [8]. Flavonoids, saponins, and tannins in celery are antibacterial [10]. Flavonoids have various activities such as anti-inflammatory, antioxidant, antimutagenic, and anticarcinogenic properties [11]. The presence of these compounds makes this plant often used in different traditional medicines and can maintain our fitness and health [12].

The celery essential oil has antifungal activity against many bacteria, including *Staphylococcus aureus*, *Streptococcus faecalis*, *S. spyogenes*, and *P. solanacearum* [13]. Because celery has an antimicrobial effect, this celery plant can be used as a primary ingredient in making antiseptic liquid soap. Essential oils can be used for healing various skin problems. It is because essential oils have antiseptic properties, so they are relatively faster in curing various skin problems or wounds, and this oil also prevents wounds and infections. In addition, the development of cosmetic products from celery leaves has not been explored much, making it an alternative natural ingredient that can be developed as an antiseptic. The purpose of this study was to formulate liquid hand soap preparations from celery leaf extract in accordance with the latest Indonesian National Standard (SNI), namely SNI 2588:2017 [14].

METHODS

This research is experimental laboratory research by making formulations of soap preparations and evaluating the practices based on the Indonesian National Standard [14].

Place and Time

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

Materials

The tools used in this research are: 200 mesh sieve, magnetic stirrer, thermometer, upright cooler, water bath, glass filter, pH meter (Emeltron), measuring cup (pyrex® Iwaki), stirring rod, dropper pipette, erlenmeyer (pyrex® Iwaki), analytical balance (BB Adam), measuring flask (pyrex® Iwaki), separating funnel (pyrex® Iwaki), filter paper with a porosity of 20µm (Whatman), stative, burette, erlenmeyer sharpening lid (pyrex® Iwaki), petri dish (pyrex® Iwaki), incubator (Ecocell MMM Group), autoclave (ALP), oven, blender (Philips), hair dyer, glass beaker (pyrex® Iwaki), measuring pipette, test tube, tube rack, bunsen, loop needle, tweezers, micropipette (Eco pipette CAPP), vortex, scale ruler.

The materials used in this study were celery leaves, olive oil, potassium hydroxide (KOH), carboxyl metal cellulose (CMC), stearic acid, butyl hydroxyanisole (BHA), Sodium Lauryl Sulfate (SLS), Stearic acid, BHA, aquadest, ethanol. 95%, 99.5% ethanol, petroleum ether, anhydrous sodium sulfate, 0.5 M sodium hydroxide solution, 1% phenolphthalein (PP) indicator, neutral ethanol, 0.1 N HCl standard solution, Nutrient agar (NA), NaCl physiological 0.9%, PCA media (Plate Count Agar).

Research Procedure

1. Sampling

The sample used in this study was fresh celery leaves obtained in a celery plant from the Mataram area of NTB Province.

2. Sample Processing

The samples were sorted wet, washed with running water, drained, then air-dried for six days, then dried in an oven at 400C. The dried samples were then blended and sieved using a 200 mesh sieve.

3. Extracting

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

treatment was the same as the first maceration to produce filtrate II and residue II. Then residue II was macerated with 500 ml of 96% ethanol. After a day, sample II was filtered to produce filtrate III and residue III. The filtrates I, II, and III were combined, then evaporated to obtain a thick extract of celery leaves.

4. Formulation

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

Materials	Unit	Formula I (5%)	Formula II (10%)	Formula III (15%)
Celery leaf extract	gram	2,5	5	7,5
Olive oil	ml	15	15	15
KOH	ml	8	8	8
CMC	gram	0,5	0,5	0,5
SLS	gram	0,5	0,5	0,5
Stearic acid	gram	0,25	0,25	0,25
BHA	gram	0,5	0,5	0,5
Aquades	ml	Add 50	Add 50	Add 50

5. Making Liquid Soap Ethanol Extract Celery Leaves

All ingredients are weighed in advance according to the dose. Put 15 ml of olive oil into a beaker, then add 8 ml of 40% KOH little by little while continuing to heat at 50°C until a soap paste is obtained, then add 15 ml of distilled water. Then add Na-CMC, which has been developed in hot distilled water was stirred until homogeneous. Then added stearic acid was stirred until homogeneous. Added SLS until homogeneous. BH was added until homogeneous. Enter the celery leaf extract, mix until homogeneous, add 50 ml of distilled water. Making liquid soap ethanol extract of celery leaves adjusted to each concentration.

6. Liquid Soap Quality Test 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 was homogenized with CO2-free aquadest then added. This solution was allowed to stand 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.

7. Total Active Ingredients Test

a. Determination of ethanol-soluble ingredients: A total of 5 grams of sample was weighed and put into a 300 ml Erlenmeyer, then added 100 ml of ethanol (99.5%). Connect the Erlenmeyer to the upright cooler, then heated for 30 minutes on a water bath with occasional stirring. The warm solution was then filtered using a glass filter, and

the remaining solution attached to the Erlenmeyer was rinsed with 50 ml of ethanol (95%). Transfer the filtrate into a 250 ml volumetric flask and add 95% ethanol. The 100 ml volume pipette is then transferred to a 200 ml beaker whose empty weight is known. Heat on a water bath to evaporate the ethanol. Dry in the oven at 105 °C for 1 hour. Cool in a desiccator to a constant weight and then weigh and calculate the content of the ethanol-soluble material.

- b. Determination of materials soluble in petroleum ether: A total of 10 grams of sample was weighed and put into a 300 ml Erlenmeyer. Dissolve the sample in 200 ml of the water-ethanol mixture solution. Filter if there is an insoluble material, then add 5 ml of 0.5 mol/l NaOH solution and a few drops of PP indicator to make sure the solution is alkaline. Transfer to a 500 ml separatory funnel and extract 50 ml of petroleum ether three times. If the emulsion is getting thicker, add ethanol to remove it. Wash three times in petroleum ether with 30 ml each of the water-ethanol mixture solutions and wash twice with 30 ml each with distilled water. Dry with anhydrous sodium sulfate until there is no water film. Filter using dry filter paper into a 300 ml Erlenmeyer of known weight, rinse the filter paper with a bit of petroleum ether, leave the Erlenmeyer in a desiccator to room temperature. Heat the solution in a water bath to evaporate the petroleum ether, leaving it until the smell of petroleum ether is gone. Blow-dry air over the Erlenmeyer to remove any residual petroleum ether until the petroleum ether odor is gone. Weigh until the weight is constant. Calculate the content of the substance dissolved in petroleum ether.

8. Determination of Ethanol Insoluble Material

A total of 5 grams of the sample was dissolved (b1) with 200 ml of neutral ethanol into the Erlenmeyer sharpening lid. It installed an upright cooler heated over a water bath until completely dissolved the soap. Dry the filter paper in the oven at (100-105) 0C for 30 minutes, then let it cool. Weigh the filter paper or gooch dish. Repeat drying of filter paper until the weight remains (b0). Place the filter paper or gooch dish in the funnel above the Erlenmeyer flask, assembled with a vacuum pump. When the soap is completely dissolved, pour the liquid onto a filter paper or gooch dish and cover it with an upright cooler to protect the solution from carbon dioxide and acids during the process. Wash the insoluble material in the first Erlenmeyer with neutral ethanol, pour the washing solution into filter paper or gooch dish, then wash the residue on filter paper or gooch dish with neutral ethanol until completely free of soap. Save the filtrate for the free alkali test. Dry the filter paper or gooch dish and residue in the oven at (100-105)0C for 3 hours. Let it cool and weigh the filter paper or gooch cup (b2).

9. Free Alkali or Free Fatty Acid Test

The filtrate from the determination of the insoluble material in ethanol is heated. When it is almost boiling, 0.5 ml of 1% PP indicator is added. If the solution is acidic, there will be no color change. Titrate with standard KOH solution to the endpoint. If the solution is alkaline, the PP indicator will change the color of the solution to red. Titrate with standard HCl solution until the red color disappears. Count to NaOH if it's a base and oleic acid if it's an acid.

10. Microbial Contamination: Total Plate Number (ALT)

Weigh 1 ml of sample into a test tube, add 9 ml of physiological NaCl solution (10-1), and then homogenize. Then do the 10-2 and 10-3 dilutions. 1 ml of the diluted sample was taken and put into a sterile petri dish. Add sterile PCA media at a temperature of 45-55°C, poured into a petri dish as much as 10-15 ml. The petri dish was moved and allowed to solidify. Incubate in an incubator at room temperature (35-37°C) for 48 hours with the cup upside down. Make observations every 24 hours. The number of colonies was Observed and counted that grew was calculated as the total plate number.

RESULT AND DISCUSSIONS

The simplicia extraction of celery leaves produced a thick green extract with a distinctive celery aroma with an extract yield of 37.12%. It was stated that the yield of celery leaf extract (using ethanol as solvent) was not less than 24.6% [15]. The results of organoleptic observations of Formula I (5%), Formula II (10%) and Formula III (15%) liquid handwashing soap celery leaf extract are shown in Table 2.

Table 2. Organoleptic assay

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

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

The quality requirements for liquid hand soap refer to the latest SNI, namely SNI 2588:2017, which is a revision of SNI 06-2588-1992. This quality

requirement begins with a pH test. The results of the pH test can be seen in Table 3.

Table 3. pH Test Result

Liquid Hand Soap Formulation	pH
FI (5%)	9,4
F2 (10%)	9,5
F3 (15%)	9,3

The pH value is one of the quality requirements in the manufacture of liquid soap because pH affects the skin's condition after use. The pH quality requirements according to [14] are 4-10. Testing the pH of liquid hand soap ethanol extract of celery leaves FI, FII and FIII gave the results respectively 9.4., 9.5 and 9.3. It shows that the liquid hand soap preparations FI, FII, and FIII 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 due to alkali, which does not react with fatty acids in the saponification process [16]. 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's constituents, a strong base, namely KOH [17].

The next test is the total active ingredient test. The total active ingredient is the ethanol-soluble material minus the petroleum ether soluble material. Active ingredients (anionic, nonionic, cationic, and amphoteric surfactants) and active ingredients (unreacted organic materials, perfumes, alkanol amide fats, free fatty acids, and waxes) can be dissolved in ethanol. Materials other than the active ingredients can also dissolve in petroleum ether [14]. The results of the total functional ingredient test are shown in Table 4.

Table 4. Total Active Ingredients Test Results

Formulation	a	b	c=a-b	Description
FI (5%)	35,5	0,9	34,6	Qualify
FII (10%)	33	0,8	32,2	Qualify
FIII (15%)	33,5	0,9	32,6	Qualify

Keterangan:

- a : Soluble materials in ethanol
- b : Soluble materials in petroleum ether
- c : Total active ingredients (%)

The ingredients/compounds in soap that are saponified are included in the soluble materials in the ethanol (polar) category. In contrast, the ingredients in soaps that are not saponified are categorized as soluble materials in petroleum ether (non-polar). The number of fatty acids affects the saponification of ingredients/compounds in soap. High amounts of fatty

acids can interfere with the soap emulsion and cause dirt on the soap, so the ingredients/compounds in soap are not soapy. Therefore, the amount of free fatty acids is required according to the SNI standard [18]. The total test of active ingredients FI (5%), FII (10%), and FIII (15%) in Table 4 is 34.6%, 32.2%, and 32.6%. The total active ingredients of the three formulas were not significantly different. It is because the amount of olive oil as a soap base is the same in FI, FII, and FIII soap preparations. Based on the quality requirements of SNI 2588:2017, the total required active ingredients is a minimum of 10%. Thus, it can be said that the formulation of liquid hand soap preparations with ethanol extract of celery leaves FI, FII, and FIII meets the requirements of SNI 2588:2017.

The ethanol-insoluble material test on liquid hand soap preparations FI, FII, and FIII obtained results of 0.094 %, 0.086 %, and 0.112 %. These results meet the quality requirements of liquid hand soap based on SNI 2588:2017, namely, the material that is insoluble in ethanol is a maximum of 0.5%. The ethanol-insoluble material test determines how much of the soap is insoluble in ethanol. Materials that are not soluble in ethanol can cause lumps that interfere with the appearance of soap [19].

In this study, the free alkali test (calculated as NaOH) was carried out because based on the results of the initial examination, when the sample solution (soap) was dripped with phenolphthalein (PP) indicator, it changed color to red. It indicates that the soap is too alkaline, so a free alkali test is carried out. The free alkali test is carried out by titrating the sample solution using a standard acid solution (0.1 N HCl) until the red color of the sample solution disappears. Free alkali test results FI 0.044; FII 0.047, and FIII 0.048. The requirement for alkali-free hand sanitizer according to SNI 2588:2017 is a maximum of 0.05%. It means that FI, FII, and FIII preparations meet the quality requirements of SNI 2588:2017.

Free alkali test was carried out to determine the presence or absence of free alkali in liquid soap [17]. The presence of free alkali indicates the presence of alkali metals such as Li, Na, and K. It is not reacted in olive oil during the saponification process. They are still in the form of alkali metals. Soap preparations containing high levels of free alkali can irritate the skin and cause dry skin [20-22]. The lack of free alkali content in liquid soap FI, FII, and FIII is due to prolonged heating in the manufacture of the liquid base until the soap becomes a dry paste so that KOH is one of the soap bases has reacted with olive oil.

According to the pouring method, the Total Plate Number (ALT) test is intended to calculate the growth of aerobic mesophyll bacterial colonies after the sample is planted on a media plate and then stored for 24-48 hours at a predetermined temperature [23]. The results of the ALT test for liquid hand soap preparations FI, FII, and FIII are shown in Table 5.

Tabel 5. Total Plate Number Result

Dilution	Total Plate Number		
	FI (5%)	FII (10%)	FIII (15%)
10 ⁻¹	0	0	0
10 ⁻²	0	0	0
10 ⁻³	0	0	0

The total plate count test results on liquid hand soap preparations of celery leaf extract with three concentrations of 5%, 10%, and 15% showed no colony growth. It is because celery leaf extract can be antibacterial. The quality requirements for hand sanitizer liquid soap are SNI 2588:2017, a maximum ALT of 1x10³. Thus, hand sanitizer liquid soap celery leaf extract complies with SNI 2588:2017.

CONCLUSION

Based on the research results, it can be concluded that the ethanolic extract of celery leaves can be formulated in the form of liquid handwashing soap, and the results of the evaluation of the preparation meet the quality requirements of SNI 2588:2017.

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