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Formulation and anti-bacterial of liquid soap combination of Citronella (*Cymbopogon nardus* L. Rendle), Cinnamon (*Cinnamomum burmanni* Ness Ex Bi.), and Orange Lemon (*Citrus lemon* L.) Essential Oils on *Staphylococcus epidermidis*

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ABSTRACT

The condition of our community in the midst of a pandemic makes us aware of the importance of cleanliness. One way to maintain cleanliness is to clean our self with soap. Cinnamon has antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*. Lemongrass has antibacterial activity against *Staphylococcus aureus*, while lemons are able to inhibit the growth of *Staphylococcus epidermidis* and Propionibacterium acnes bacteria. The aims of this study are to determine the formulation and physical stability of liquid soap with a combination of lemongrass, cinnamon, and lemon essential oils and to determine its antibacterial activity against *Staphylococcus epidermidis*. Physical evaluation of soap includes organoleptic, homogeneity, viscosity, pH, and specific gravity which is compared with SNI, and hedonic test is performed. Antibacterial test was performed against *Staphylococcus epidermidis*.The results of the study obtained homogeneous liquid soap preparations, thick liquid form, a yellow-orange color, distinctive smell of citronella. Specific gravity (g/ml) test results meet the requirements of SNI, which is between 1.01 – 1.10 at room temperature. The results of the pH test meet the requirements of SNI, which are between 8 – 11. The results of the viscosity (cPas) test show that there is an increase in viscosity from week 1 to week 6. The increase in viscosity is related to particle size during storage. The foam height (cm) test results obtained F1: 11.76 ± 0.67, F2: 11.67 ± 1.25, F3: 11.33 ± 1.02, F4: 11.60 ± 0.79, F5: 11.18 ± 0.84. The results of the hedonic test obtained that the average respondent liked the foam produced, the shape, color, comfort when used, and the smell. The lowest value of the hedonic test is the smell of the liquid soap that is produced. From the results of antibacterial test against *S. Epidermidis*, it is known that liquid soap has antibacterial activity with strong inhibitory power.

1. Introduction

Health begins with public awareness of the importance of maintaining personal hygiene, family, community, and surrounding environment. Health is not only measured from physical, mental, and social aspects, but also by economic and social productivity (Notoatmodjo, 2010). So that this awareness of health must be continuously built and nurtured, considering that health conditions will greatly affect our productivity. One of the things that can support health is public hygiene.

The current condition of society, in the midst of a disease pandemic caused by the Covid-19 virus that has swept the world, has made people aware of the importance of cleanliness. People must maintain personal hygiene to avoid exposure to the virus. One way to maintain cleanliness is to clean yourself with soap. The skin covers the surface of the body and has a function as a protector from various disturbances and external stimuli. Bacteria that are found on the skin can cause several diseases including boils, acne, pneumonia, meningitis, and arthritis. One of the natural ingredients that

can be efficacious as an anti-bacterial is lemongrass, cinnamon, and lemon. Cinnamon bark oil has antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis* with very strong inhibition (Aqmarina et al., 2016; Tiran & Nastiti, 2014). Cinnamon bark has antioxidant activity equivalent to the antioxidant activity of α-tocopherol (Latief et al., 2013). Meanwhile, citronella has excellent antibacterial activity against *Staphylococcus aureus* and *E. coli* (Rita & Vinapriliani, Ni Putu Eka Gunawan, 2018). Lemons are able to inhibit the growth of *Staphylococcus epidermidis* and *Propionibacterium acnes* bacteria (Eliana Kadek, Habibah Nur, 2020; Hartin & Rini, 2019). The lemon essential oil contains 59.7% limonene (Sokovicǵ et al., 2010). Limonene is a compound that functions as an antibacterial.

From the description above, the authors are interested in conducting research on the antibacterial activity and physical stability of liquid soap with a combination of essential oils of citronella (*Cymbopogon nardus* L.), cinnamon (*Cinnamomum burmanni* Ness ex BI.), and lemon (*Citrus lemon*). Liquid bath soap is a liquid skin cleansing preparation made from synthetic detergent active ingredients and or from the saponification or neutralization process of fats, oils, waxes, rosin, or acids with organic or inorganic bases without causing irritation to the skin (SNI, 1996).

Citronella has been widely developed into soap dosage forms, as well as cinnamon. However, liquid soap with a combination of essential oils has not been widely developed in a soap dosage form. Liquid soap with a combination of essential oils of citronella (*Cymbopogon nardus* L.), cinnamon (*Cinnamomum burmanni* Ness ex BI.), and lemon (*Citrus lemon*) is expected in addition to have better antibacterial activity, the soap also has advantages as antioxidants. The results of this study are expected to get a good soap preparation so that it can be adopted and used by the community.

2. Materials and Methods

2.1. Preparation of samples

The material used in this research were virgin coconut oil, virgin olive oil (borges), stearic acid (Dwilab Mandiri scientific), sodium lauric sulfate (Brataco), cocamide DEA (Dwilab Mandiri scientific), KOH (Brataco), Aqua demineralized, propylene glycol (Brataco), glycerin (Brataco), EDTA Na (Brataco), lemongrass essential oil (Indonesian atsiri house), cinnamon essential oil (Indonesian atsiri house), lemon essential oil (Indonesian atsiri house), nutrient agar medium (Merck), brain heart infusion media (BHI)) (Merck), sterile distilled water, S. epidermidis, acetone (Brataco), diethyl ether (Brataco).

Meanwhile the tool used in this research was Beaker glass (Pyrex), measuring cup (Pyrex), stir bar, water bath (Faithful), mixer (Miyako), soap bottle, flakon, pH meter (Ohaous), digital viscosimeter (NDJ 8S), test tube, tube rack, pycnometer (Pyrex), incubator (Memmert), petri dish, erlenmeyer (Pyrex), measuring flask (Pyrex), Bunsen, autoclave (KK Gauges), dropper pipette, laminar airflow (Biobase).

2.2. Processes of Soap Making

Making liquid bath soap was by weighing each ingredient. Component 1 (VCO, Virgin Olive Oil, and stearic acid) was heated in a water bath. Component 3 (KOH and Aqua DM) was dissolved until dissolved. Component 4 (Propylene glycol, glycerin, EDTA Na, and Aqua DM) was dissolved until dissolved. Component 1 was put into a container, then part of component 4 was added, component 3 was added to the container, and shaken with a mixer speed 1 until homogeneous. Add the remaining component 4 into the container, and shaken until homogeneous. Added component 2 (SLS and cocamide DEA), stirred until homogeneous, and added component 5, stirred until homogeneous. Put in a closed container (Indriaty et al., 2019) (Table 1).

Table 1. Liquid soap formula

Substance		Unit	FI	FII	FIII	FIV	FV
Component 1	VCO	g	10	10	10	10	10
	Virgin Olive Oil	g	10	10	10	10	10
	Acid stearic	g	3	3	3	3	3
Component 2	SLS	g	15	15	15	15	15
	Cocamide DEA	mL	8	8	8	8	8
Component 3	KOH	g	4	4	4	4	1.2
	Aqua DM	mL	4	4	4	4	4
Component 4	Propilen glycol	g	5	5	5	5	5
	Glycerine	g	10	10	10	10	10
	EDTA Na	g	0.2	0.2	0.2	0.2	0.2
	Aqua DM	mL	100	100	100	100	100
Component 5	Citronella Essential oil	mL	0.25	0.25	0.5	0.5	0.75
	Cinnamon Essential oil	mL	0.25	0.5	0.25	0.5	0.75
	Lemon Essential oil	mL	0.1	0.1	0.1	0.1	0.1

2.3. Organoleptic Test

Organoleptic test was carried out with the aim of knowing the visual appearance of the shape, color, and smell of liquid soap preparations of a combination of lemongrass, cinnamon, and lemon essential oils. Organoleptic test of liquid soap was carried out for 6 weeks at room temperature (25°C) with weekly evaluation (Rasyadi et al., 2019).

2.4. Homogeneity Test

The homogeneity test was carried out by observing the soap particles and the color of the soap by applying it to the watch glass (Rasyadi et al., 2019).

2.5. Specific Gravity Test

Specific gravity test of liquid soap using a pycnometer. The pycnometer was cleaned by rinsing with acetone, then with diethyl ether, dried and the pycnometer was weighed. Liquid soap was put in the pycnometer, cooled by immersing the pycnometer in ice water, allowed to come to 25^{o}C, and adjusted to the calibration line. The pycnometer was removed from the ice water bath, allowed to stand at room temperature, and weighed. It is also done using distilled water (Depkes, 2020).

2.6. Viscosity Test

Viscosity test was carried out using a brookfiled viscosimeter (Rasyadi et al., 2019).

2.7. pH test

pH test was carried out using a pH meter (Indriaty et al., 2019). The test was carried out using an OHAUS type ST 350 electrode at 25^{o}C.

2.8. Foam Height Test

The foam height test was carried out by pouring 2 ml of the soap preparation into a 10 ml measuring cup, then closed, shaken for 20 seconds and the height of the foam was calculated (Ardina & Suprianto, 2017).

2.9. Hedonic Test

The hedonic test was carried out to determine the extent to which respondents received the formulated preparations (Prayadnya, I.G.Y., M.W. Sadina, N.L.N.N. Kurniasari, 2017). The hedonic

test was carried out on 20 respondents, by providing an assessment of hand soap for color, aroma, comfort, shape, and foam. Each respondent gave an assessment from the number 1 to 4 (1 = dislike, 2 = dislike less, 3 = like, 4 = like very much).

2.10. Antibacterial Activity Test Against *S. epidermidis*

Tools sterilization

All tools to be used are washed and then dried. Glassware tools were sterilized using an oven at 200ºC for 60 minutes. Tools that cannot withstand heating are heated using the wet heat method (autoclave) and heated at 121ºC for 15 minutes. Laminar Air Flow (LAF) sterilization uses 70% alcohol which was sprayed around the walls of the LAF and the UV lamp is turned on for 30 minutes before the LAF is used. NA and BHI media were sterilized using an autoclave at 121ºC for 15 minutes.

Inoculation Media

Nutrient Agar (NA) media was dissolved using distilled water, heated until dissolved, and put into an autoclave at 121ºC for 15 minutes, then poured NA media into sterile petri dishes and left at room temperature until hardened.

Bacterial suspension was carried out by taking 3-5 bacterial colonies into 10 mL sterile BHI media, then incubating at 37ºC until the turbidity was the same as Mc.Farland's standard solution (Naibaho et al., 2013).

Test Media

The NA medium was dissolved with sterile distilled water. Heat until completely dissolved. Sterilization was carried out in an autoclave at 121°C for 15 minutes. Removed and poured into a petri dish. Left at room temperature, the surrounding area was given Bunsen to avoid contamination.

Antibacterial Activity Test

The antibacterial activity test used a good method.

Two hundred microliter of bacterial suspension was taken using a micropipette and a sterile yellow tip. Poured into a solid NA medium in a petri dish and leveled using a spreader glass, the test cup was incubated at 37ºC for 24 hours and then measured the diameter of the resulting inhibition zone. The inhibition zone that occurs is indicated by the presence of a clear zone around the well.

3. Results and Discussion

3.1. Organoleptic Test Result

Organoleptic test was carried out with the aim of knowing the appearance of liquid soap preparations in terms of shape, color, and visual aroma of liquid soap preparations a combination of lemongrass, cinnamon, and lemon essential oils. Organoleptic examination was carried out within 6 weeks at room temperature with an evaluation every week. The results of the organoleptic test of the five liquid soap formulas obtained liquid soap preparations with a thick liquid form, yellow-orange color, and a distinctive smell of citronella (Figure 1 and Table 2).

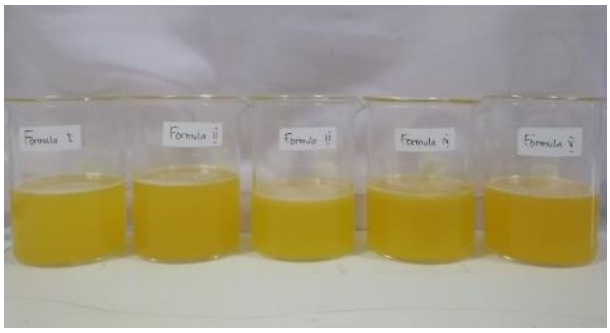

Fig. 1. Liquid Soap is a combination of lemongrass, cinnamon, and lemon essential oils

Table 2. Organoleptic test result

Test	Formula				
	F1	F2	F3	F4	F5
Shape Colour Smell	Thick liquid yellow-orange citronella	Thick liquid yellow-orange citronella	Thick liquid yellow-orange citronella	Thick liquid yellow-orange citronella	Thick liquid yellow-orange citronella

3.2. Homogeneity Test Result

Homogeneity test was carried out to determine that the liquid soap produced was homogeneous, where the added ingredients were evenly dispersed. The results of the homogeneity examination carried out for 6 weeks, obtained a homogeneous liquid soap preparation. Liquid soap preparations must show a homogeneous arrangement and no visible grains (Table 3).

Table 3. Homogeneity test result

Test	Formula				
	F1	F2	F3	F4	F5
Homogeneity	homogeneous	homogeneous	homogeneous	homogeneous	homogeneous

3.3. Specific Gravity Test Result

Specific gravity is the ratio of the density of a substance to the weight of water in the air at the same temperature (25^{o}C) and volume. The value of the specific gravity of a substance is caused by the type and concentration of the material added to the solution. The density of liquid soap was checked using a picnometer. The results of the specific gravity test of liquid soap combination of lemongrass, cinnamon, and lemon essential oils meet the requirements in the Indonesian National Standard for liquid soap preparations, which are between 1.01 – 1.10 at room temperature. The specific gravity of the soap produced at F1 : 1.04 ± 0.01, F2 : 1.04 ± 0.01, F3 : 1.04 ± 0.02, F4 : 1.03 ± 0.01, F5 : 1.04 ± 0.00 (Table 4).

Table 4. Specific gravity test result

Test (g/mL)	Formula (±)				
	F1 ± SD	F2 ± SD	F3 ± SD	F4 ± SD	F5 ± SD
Specific Gravity (g/ml)	1.04 ± 0.01	1.04 ± 0.01	1.04 ± 0.02	1.03 ± 0.01	0.00

3.4. Viscosity Test Result

The results of the viscosity test showed that there was an increase in viscosity from week 1 to week 6. The increase in viscosity was related to particle size during storage. The particles tend to reduce the surface area by merging between particles, so that larger particles are obtained and a smaller surface area, so that the viscosity increases (Lachman et al., 1994) (Table 5).

Table 5. Viscosity Test Result (cPas)

Test	Formula				
	F1	F2	F3	F4	F5
Week-1	859.03	889.27	816.4	894.5	908.4
Week-6	1399.27	1219.83	1280.73	1294.63	972.00

3.5. pH Test Result

pH test aims to determine the safety of a preparation. A pH that is too low causes the nature of the preparation to become acidic, so that it can irritate the skin, while a pH that is too high causes the nature of the preparation to become alkaline, so that it can make the skin scaly. The pH of the liquid soap was checked using a pH meter. The pH test results obtained that the pH was in accordance with

the requirements of the Indonesian National Standard regarding the pH of liquid soap, which was between 8 - 11 (SNI, 1996) (Table 6).

Table 6. pH Test Result

Test	Formula				
	F1 ± SD	F2 ± SD	F3 ± SD	F4 ± SD	F5 ± SD
pH	9.80 ± 0.32	9.73 ± 0.30	9.69 ± 0.30	9.70 ± 0.33	9.67 ± 0.32

3.6. Foam Height Test Result

The test of foam height is carried out to test the foaming properties of liquid soap preparations, to determine whether liquid soap preparations can produce foam when used. The measure of the ability to form foam can be seen from the foam height in the container as soon as the foam is produced. The foam height test was carried out because most consumers expect the cleaning preparation to produce foam as a sign of proper cleaning (Baki and Alexander, 2015). The results of the foam height test obtained F1: 11.76 ± 0.67 cm, F2 : 11.67 ± 1.25 cm, F3 : 11.33 ± 1.02 cm, F4 : 11.60 ± 0.79 cm, F5 : 11.18 ± 0.84 cm (Table 7).

Table 7. Foam height test result (cm)

Test	Formula				
	F1 ± SD	F2 ± SD	F3 ± SD	F4 ± SD	F5 ± SD
Foam Height (cm)	11.76 ± 0.67	11.67 ± 1.25	11.33 ± 1.02	11.60 0.79	11.18 ± 0.84

3.7. Hedonic Test Result

The hedonic test was carried out on 20 respondents, where each respondent assessed 5 formulas for comfort in use, foam formed, dosage form, dosage color, and aroma of the preparation. Respondents gave an assessment of the numbers 1 to 4, where the larger the number, the better the assessment (1 = dislike, 2 = dislike, 3 = like, 4 = very much like). The results of the hedonic test showed that in the comfort category the most preferred was formula 1, in the foam category the most preferred was formula 4, in the most preferred form category were formulas 1 and 4, and in the color category the most preferred was formula 3 and in the aroma category, the most preferred in preference is formula 4 (Table 8).

Table 8. Hedonic test result

Formula	Comfort in Use	Foam Formed	Dosage Form	Colour	Aroma
1	3.25	2.9	3.2	3	2.95
2	3.1	3.05	3.1	3	2.8
3	3.1	3.05	3.1	3.15	2.9
4	3.1	3.1	3.2	2.95	2.75
5	3.05	3	3.1	2.95	3

3.8. Antibacterial Activity Test Result

The classification of bacterial inhibitory responses with an inhibition zone diameter of >20 mm includes strong inhibition, 16-20 mm has moderate inhibition, and <15 mm has weak inhibition (Wardania et al., 2020). From the results of the antibacterial activity test against *S. Epidermidis*, it is known that liquid soap has antibacterial activity with strong inhibition in formulas 1, 2, 3, and 4 (Table 9).

Table 9. Antibacterial activity test result

Formula	Antibacterial Activity (mm) Mean ± SD
1	25.83 ± 3.05
2	31.17 ± 5.39
3	23.83 ± 3.81
4	20.50 ± 3.60
5	13.83 ± 1.25
Control +	12.00
Control -	0.00

4. Conclusion

This research can be concluded that liquid soap can be made with 5 formulas with different concentrations of the active ingredients of essential oils. The results of the physical evaluation of liquid soap with a combination of lemongrass, cinnamon, and lemon essential oils produced liquid soap that met good soap parameters in accordance with SNI for liquid bath soap. The results of the hedonic test in terms of comfort, shape, foam, color, and aroma showed that the respondents liked it on average. The antibacterial test resulted from the liquid soap combination of citronella essential oil, cinnamon, and lemon can inhibit *S. Epidermidis* bacteria with strong inhibitory power. Further research is needed to evaluate the stability of the preparation, as well as to test the activity against other bacteria that support soap preparations.

Author Contributions: Umi Nafisah conceived and designed the study. Umi Nafisah performed all data analysis. Umi Nafisah, Iin Suhesti, and Puput Albetiainterpreted the result and revised the paper. Umi Nafisah wrote the manuscript. All authors read and approved the final manuscript.

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Competing Interests

The authors declare no conflict of interest.

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