Liquid Bath Soap Formulation and Antibacterial Activity Test Against *Staphylococcus aureus* of Kecombrang (*Etlingera elatior* (Jack) R.M.Sm.) Flos Extracts

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***Abstract*— The formulation of kecombrang flos extract (*Etlingera elatior* (Jack) R.M.Sm.) liquid bath soap has been established. The objective of this research was to formulate liquid bath soap of kecombrang flos extract (*Etlingera elatior* (Jack) R.M.Sm.) and to test its antibacterial activity to Staphylococcus aureus. Kecombrang flos extract was extracted by maceration method using 96% ethanol, and followed by minimum inhibitory concentration (MIC) test using hole method. Concentration variation of kecombrang flos extract was conducted as F1 (6%), F2 (8%), and F3 (10%). The formula of liquid bath soap of kecombrang flos extract was evaluated using several examinations such as organoleptic, pH, viscosity, density, foaming stability, antibacterial activity test, irritation test and hedonic test. The result shows the liquid bath soap of kecombrang flos extract F1, F2 and F3 can inhibit the growth of *Staphylococcus aureus*. Based on statistical test using SPSS 21 (for trial) ANOVA method continued by LSD shows that F0 (negative control), F1, F2, F3 and positive control (triclosan 2.5%) have difference meaningful result with significance value < 0,05.**

Keywords—Kecombrang; Liquid bath soap; Antibacterial; Staphylococcus aureus

I. INTRODUCTION

The skin is the part that covers and protects the entire outer surface of the body from environmental influences, such as to protect from the effect of microorganisms (Harahap, 2000). *Staphylococcus aureus* is a normal flora bacteria of the skin, but if it exists in large quantities it can cause skin disease (Brooks, *et al*., 2012). It triggers the need for additional protection to the skin, one is to use antibacterial soap preparations. The soap preparation is believed to cleanse the skin effectively and supported by its antibacterial properties (Gandasasmita, 2009; Muthmainah, *et al*., 2014).

The bath soap products based on natural ingredients are still rarely found in the market, synthetic materials most found as active ingredient in the market. Some studies suggest that synthetic active ingredient is dangerous and has negative effects on human skin, because of its potentiality to cause irritation in consumers with sensitive skin, for example triclosan as an active ingredient which found in nearly all antibacterial soaps on the market. If triclosan accumulates in human body fat, it would potentially cause thyroid dysfunction. Therefore, nowadays there are a lot of soap manufacturers turning in to natural products to be used as the active ingredient of making soap that is safe and does not irritate the skin (Nurhadi, *et al*., 2012; Fadillah, 2014).

One of the natural active ingredients that have antibacterial properties are kecombrang flos. The component of kecombrang floss has been known consisting of alkaloids, flavonoids, polyphenols, steroids, saponins and essential oil (Tampubolon, *et al*., 1983).

Antibacterial substances from ethanol and ethyl acetate extract of kecombrang flos can inhibit a variety of bacteria such as *Bacillus cereus, Pseudomonas aeroginosa, Salmonella typhimurium, Escherichia coli, Listeria monocytogenes, Staphylococcus aureus* and *Aeromonas hydrophilia*. Whereas, the water extract can inhibit the growth of *Escherichia coli* and *Staphylococcus aureus* bacteria (Hudaya, 2010; Sukandar, *et al*., 2010). Based on the above research, a formulation of

liquid bath soap of kecombrang flos extract and antibacterial activity test against *Staphylococcus aureus* is conducted.

II. MATERIALS AND METHODS

A. Materials

Kecombrang flos (Cibalong, Tasikmalaya), Mueller Hinton Agar media (Oxoid), Nutrient Agar (Oxoid), ethanol 96% (Brataco), physiological saline (Widatra bhakti), soap bases were purchased from local industry in Bandung, Indonesia.

The apparatus used included electric Oven (*Memmert*®), incubator (*Memmert*®), blender (*National*®), macerator, rotary evaporator (*EYELA OSB-2100*®), digital scale (*Mettler toledo,* JL 1502-6®), waterbath, pH strip (*Merck*®), viscometer (*Brookfield*), autoclave, pycnometer (*pyrex*®), Vernier calipertools laboratory glassware (*pyrex*®), and other supporting equipment.

The Bacterial Test is *Staphylococcus aureus,* obtained from the Laboratory of Microbiology, Institut Teknologi Bandung.

B. Methods

Determination and Sample Preparations

Kecombrang flos samples are collected from Cibalong, Tasikmalaya, West Java province of Indonesia, which was determined in the School of Life Sciences and Technology, Institut Teknologi Bandung. Kecombrang flos were collected, washed with clean water to remove impurities and foreign objects attached, then drained and dried in an oven at a temperature of 40-50^{o}C. Simplicia is dried and then made into a powder by means of a blender and sieved with a mesh sieve No. 40 (Munawaroh, 2014).

Extractions

Extraction of kecombrang flos was carried out using maceration method. First of all, kecombrang flos was macerated with n-hexane to remove oil and grease, then macerated again using 96% ethanol while was stirred several times during the first 6 hours and allowed to stand until 18 hours and then filtered, solvent replacement is done repeatedly. The filtrate was evaporated until it dry completely using rotary evaporator at 40^{o}C. The residues in the form of condensed extract weighed and the yield was calculated and were preserved in sterile glass bottles at room temperature until further use (DepKes RI, 2008).

Phytochemical Analysis

Preliminary screening of secondary metabolites performed on crude drugs and extracts kecombrang such as alkaloids, flavonoids, tannins, polyphenols, monoterpenes and sesquiterpenes (Febrianti, *et al*., 2014). The results were shown in Table 2.

Extract Quality Monitoring

Extract quality monitoring consists of moisture content determination and drying shrinkage.

Extract Antibacterial Activity Test

Antibacterial activity test was conducted using hole plate method. Extract was weighed and dissolved in 96% ethanol in order to obtain concentration in increments of 10 in the range of 0-100%. 1 ml bacterial isolation suspension was added to petri dish, which already contains 20 ml sterile media of Mueller Hinton Agar (MHA). The petri dish was rotated slowly so that bacteria and agar can homogenously mixed, then let it solidified. 50 µl Kecombrang flos extract and 50 µl ethanol 96% as negative control inserted into separated hole in petri dish which has been hollowed out and marked. The inhibition zone diameter around the hole was measured after incubating at 37°C for about 18-24 hours. The values were recorded by the average (mm) diameter. This assay was done triplo (Firdaus, 2014). The results were shown in Table 3.

Determination of Extract Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration determination of extract was conducted to find the lowest concentration of extract that still provide antibacterial activity against bacteria (*Staphylococcus aureus*) using hole plate method (Firdaus, 2014). The results were shown in Table 4.

Table 1. The Formula of Liquid Bath Soap of Kecombrang Flos Extract

Ingredients	Concentrations (%)			
	F0	*F1*	*F2*	*F3*
Kecombrang flos extract	-	6	8	10
Virgin coconut oil	30	30	30	30
KOH	8,1	8,1	8,1	8,1
Stearic acid	2	2	2	2
Glycerine	5	5	5	5
HPMC	3	3	3	3
BHT	0,1	0,1	0,1	0,1
Phenonip®	0,3	0,3	0,3	0,3
Aquadest add	100	100	100	100

Where: F0 = Formula 0, F1 = Formula 1, F2 = Formula 2, F3 = Formula 3
Phenonip® : phenoxyetol, methyl paraben, ethyl paraben, propyl paraben, iso-butyl paraben, n--butyl paraben.

Liquid Bath Soap Formulation of Kecombrang Flos Extract

The optimization was done based on texture

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and consistency. Selected based antibacterial activity test of kecombrang flos extract was added to the formula. The results were shown in Table 1.

Liquid Bath Soap Evaluation of Kecombrang Flos Extract

Physical Appearance Determination

The formulations appearance was determined by visual (colour and shape) and odor. The data were obtained from testing during one month storage period and were checked every week.

pH Determination

pH determination was done using pH-indicator strips (range : 6,5-10). The data were obtained from testing during one month storage period and were checked every week. The results were shown in Table 5.

Viscosity Measurement

The viscosity measurement were carried out using Brookfield viscometer and number 5 spindle on 60 rpm. The data were obtained from testing during one month storage period and were checked every week. The results were shown in Table 6.

Density Determination

Density determination was done using pycnometer (Muthmainnah, *et al*., 2014). The results were shown in Table 7.

Foam Stability Examination

1 gram formula will be examined by diluting the formula in a test tube with distilled water up to 10 mL, shaken with a vortex for 30 seconds and then foam height was measured and allowed to stand up until 5 minutes and the foam height result was measured again after 5 minutes (Rosdiyawati, *et al*., 2012). The results were shown in Table 8.

Calculation :

$$\% \text{ Foam Stability} = \frac{\text{Final foam height (mm)}}{\text{Initial foam height (mm)}} \times 100\%$$

Preparation Antibacterial Activity Test

Preparation antibacterial activity test was done the same as extract antibacterial activity test using hole method. 1 µl bacterial test suspension was put to petri dish which already contains 20 ml sterile media of Mueller Hinton Agar (MHA). The petri dish was rotated slowly so that bacteria and agar can homogenously mixed, then let it solidified. 5 holes were made and marked. Kecombrang flower extract preparation with variated concentration, soap base (without extract) as negative control, and antibacterial soap found in the market as positive control, 50 µl respectively were put into the hole using micropipette. The inhibition zone diameter around the hole was measured after incubating at 37°C for about 18-24 hours. The values of preparation antibacterial were analyzed using SPSS 21 ANOVA method (*for trial*). The results were shown in Table 9-12.

Hedonic Test

Hedonic test was carried out using 30 panelists with parameters as likes, rather likes and dislikes to liquid bath soap formulation of kecombrang flos extracts that have been formulated. Organoleptic assessment was carried out using viscosity, the amount of foam and the ease of cleaning. Hedonic test results were analyzed statistically using Friedman test by SPSS 21 (*For trial*). The results were shown in Table 13.

III. RESULT AND DISCUSSION

Phytochemical Analysis

Table 2. Phytochemical Analysis Results of Crude Drug and Extract of Kecombrang Flos

Secondary Metabolites Compound groups	**Determination results**	
	Crude drug	*Extract*
Alkaloids	+	+
Flavonoids	+	+
Polyphenols	+	+
Mono and Seskuiterpenes	+	+

Where : (+) : Positive

Alkaloids could inhibit the growth of bacteria by interfering with peptidoglycan component of the bacterial cell wall layer that are not fully formed and caused the death of these cells. Flavonoids as antibacterial forming a complex of the extracellular protein that has the integrity of the bacterial cell membrane. Polyphenols are derivatives of phenol which denature cell proteins and protein hydrogen bonds between phenol and protein resulted in defective protein structure. The potentialitty of Essential oil as an antibacterial agent is by interfering formation of membrane or cell wall so it is not formed or imperfectly formed (Rijayanti, 2014).

Extract Quality Monitoring

Extract quality monitoring aimed to find out the quality of the used extract, to obtain good quality extract. Determination of water content was performed to determine the water content in the extract, because a lot of water in the extract will trigger the growth of microorganisms that will also affect the quality of the preparation. The water content in the extract was obtained 2%. These results meet the requirement (not more than 10%) (DepKes RI, 2008). Drying shrinkage was conducted in order to determine the

percentage of the loss compound because of the heating process. Drying shrinkage values were obtained for 3,713%. These results meet the requirement (not more than 10%) (DepKes RI, 2008).

Antibacterial Activity Test of Kecombrang Flos Extract

Table 3 Antibacterial Activity Test Results of Kecombrang Flos Extract

Extract Concentration (%)	Diameter Zone of Inhibition (mm)
10	6,2
20	7,8
30	9,6
40	11,9
50	13,7
60	15,5
70	17,1
80	18,4
90	19,6
100	20,7

The table above shows that the higher the concentration of the extract, the bigger the inhibition zone diameter.

Minimum Inhibitory Concentration (MIC) of Kecombrang Flos Extract

Table 4 The Results of Minimum Inhibitory Concentration

Extract Concentration (%)	Diameter Zone of Inhibition (mm)
1	-
2	-
3	-
4	0,4
5	1,2
6	2,6
7	3,1
8	4,7
9	5,5

Preparations Evaluation

1. *Physical appearance Determination*

Evaluation of liquid bath soap has shown that all of the formulas have good stability during one month storage at room temperature (28 ± 2°C) in terms of shape, smell and color.

2. *pH measurement*

pH or degree of acidity is one of the requirement quality of liquid bath soap formulation. In general, soap products pH tend to alkaline. It is because the basic ingredients of the liquid soap is a strong alkaline. KOH is used to produce the saponification reaction with the fat or oil (Gandasasmita, 2009). The results shown in table 5.

Table 5. The results of pH Measurements

Week	pH			
	F 0	*F1*	*F 2*	*F3*
1	10	8,7	8,5	8,3
2	10	8,5	8,3	8,3
3	10	8,3	8,1	8,1
4	10	8,3	8,1	7,9

From the observation of the storage duration, all formulas showed pH range according to the requirements (8-11) (SNI, 1996). The differences in the pH value of each formula is affected by the amount of extract added. According Gandasasmita (2009), The liquid bath soap with an alkaline pH can be used to destroy the fat in the skin so that the dirt can be water soluble. However, if the pH is too high, long contact time with the skin will cause skin irritation.

3. *Viscosity measurement*

Table 6. The Result of Viscosity measurements

Formula	Viscosity (cP) Weeks			
	1	*2*	*3*	*4*
1	4831±1,5	4751±1,5	4846±1,5	4771±1,5
2	4681±1,5	4621±1,5	4684±1,0	4627±1,5
3	3561±1,5	3601±1,5	3615±1,5	3580±1,5
4	2931±1,5	2946±1,5	2945±1,5	2886±1,5

4. *Density measurement*

Table 7. Density Measurement Result

Formula	Density (g/mL)
F0	1,0634
F1	1,0583
F2	1,0567
F3	1,0542

Density results of formula is 1,0542 to 1,0634 g/mL, the results have met the requirements (range from 1,010 to 1,100 g / mL) (SNI, 1996). The value of material density is influenced by constituents, concentration and physical properties.

5. *Foam Stability measurement*

Soap contained sodium lauryl sulfate (SLS) as a foam enhancer. SLS is a surfactant, commonly used in the manufacture of soaps, and in large concentrations can irritate the skin (Rosdiyawati, *et al*., 2012).

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Table 8. Foam Stability measurement Results

Formula	High of Foam (mm)		%
	Initial	*Final*	Stability
F0	29,6	13,5	45,60
F1	28,5	12,4	43,50
F2	28,1	12,2	43,41
F3	27,5	11,4	41,45
Comparator	50,4	40,3	79,96

Preparations Antibacterial Activity Test

Table 9. Antibacterial Activity Test Results of Liquid Bath soap

Formula	Diameter Zone of Inhibition (mm)
F0 (Negatif control)	7,1 ± 0,11
F1	7,9 ± 0,15
F2	11,5 ± 0,20
F3	13,6 ± 0,15
Positive control	9,3 ± 0,40

Based on these results, the increase in the diameter of inhibition zone is proportional to the amount of extract concentrations that were added into the preparation. Soap preparations is surfactant that can lower the surface tension of the bacterial cell wall and damage the cell membrane permeability (Fadillah, 2014). The Data of antibacterial activity test results was analyzed using ANOVA test.

Table 10. Statistic Results of Homogeneity and Normality Test

Test	Sig.
Homogen (levene)	0,120
Normal (Kolmogorof-Smirnov)	0,775

Table 11. Statistic Analysis Variance Results

	N	Mean±s.d.	Sig.
F0 (- control)	3	7,13±0,1	0,000
F1	3	7,96±0,1	
F2	3	11,56±0,1	
F3	3	13,30±0,2	
(+) control	3	9,33±0,4	

Table 12. Statistic Least Significant Difference (LSD) Test Results

(I) Formula	(J) Formula	Mean Difference (I-J)	Sig.
Formula 1	Formula 2	-3,60000*	.000
	Formula 3	-5,33333*	.000
	(+) control	-1,36667*	.000
	(-) control	,83333*	.006
Formula 2	Formula 3	-1,73333*	.000
	(+) control	2,23333*	.000
	(-) control	4,43333*	.000
Formula 3	(+) control	3,96667*	.000
	(-) control	6,16667*	.000

Hedonic Test

Table 13 Statistic Hedonic Test Results

Parameters	Formula	Results	
		Ranks	*Sig*
Consistency	1	2,65	
	2	2,08	0,000
	3	1,27	
Foaming	1	2,46	
	2	1,87	0,000
	3	1,67	
Easy to clean	1	2.18	
	2	1,97	0,036
	3	1,85	

IV. CONCLUSIONS

Kecombrang flos extract can inhibit the growth of *Staphylococus aureus* bacteria, the higher the extract concentration, the bigger the inhibitory power. Based on the statistical test, F0 (negative control), F1, F2, F3 and positive control (0.25% triclosan) have significant differences $p<0.05$. Each formula has a different potency in inhibiting *Staphylococus aureus* bacteria. Formula 3 (F3) is the best formula with inhibition diameter 13,6 ± 0,15 mm, and Formula 1 (F1) is most preferred when compared to F2 and F3 with a significance value $p<0,05$ by hedonic test.

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