Formulation and Anti Bacterial Activity Test of Liquid Soap Extract from Cocoa Beans (*Theobroma cacao* L.) against *Staphylococcus Aureus* and *Escherichia Coli* Bacteria

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Abstract: Cosmetics are ingredients or materials mixtures that have functions to clean, maintain, add attraction or change appearance. Antibacterial liquid soap is used to cleanse and soften skin. Many people are not aware of making antibacterial liquid soap which can be made traditionally by using cocoa beans (*Theobroma cacao L.*). This study aimed to formulate liquid soap extracts from cocoa bean extracts which are used as basis for making liquid soap and tested the antibacterial activity against Staphylococcus aureus and Escherichia coli. The research method was an experimental method; Simplisia was extracted by using maceration method with 80% ethanol solvent. Testing antibacterial activity used disk diffusion method and statistical analysis used one way ANOVA test method. The evaluation results of soap formulation quality testing showed that pH and froth ability had good results and fulfills SNI standard 06-3532-1996. The results showed that formulation III with concentration of 20 g cocoa bean extract had great antibacterial activity in each bacterium with values of 12.93 mm and 10.90 mm. In positive control, antibacterial activity showed values of 15.70 mm and 15.26 mm. The statistical analysis results used one way anova test method showed significant results $p < 0.05$, with significant value of 0.015 found in *Staphylococcus aureus* bacteria so that the results showed that it affected the antibacterial activity test. Antibacterial liquid soap containing cocoa bean extract has antibacterial potential but not as much as antibacterial potential such as Dettol bath soap.

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1 INTRODUCTION

Cosmetics are materials or mixtures that rubbed, glued, poured, sprinkled or sprayed on, put in, it used on the body or human body parts to clean, maintain and add the attraction or change the shape, protecting to keep it in good condition repairing body odor but it is not intended to treat or cure an illness (Mu and Sprando., 2010).

Cosmetics have been widely used by the public and the main purpose of their usage is to beautify themselves, increase attractiveness, self-confidence, and protect hair skin from UV damage, pollution and other environmental factors, prevent aging and help someone enjoy and appreciate life more (Mu and Sprando, 2010).

There are many types of cosmetics that used to protect the skin such as creams, gels, lotions, ointments, powders and one of them is soap. Currently in the market there are still many soaps that use synthetic ingredients such as (diethanolamine (DEA) and triclosan), but not all usage of active ingredients in cosmetics are suitable for every skin condition, which can cause skin irritation (Górnaś and Rudzińska, 2010).

Today, there are many people do not realize soap making can also be made traditionally. Cocoa beans is one of the traditional ingredients used (Tamarkin et al., 2018). Cocoa plant which has Latin name *Theobroma cacao L*. It is tree plantations that grow in areas that have soil and tropical climate that are suitable for cultivating cocoa plants (Arzhavitina and Steckel, 2010). The contents of secondary metabolites found in cocoa beans are flavonoids, saponins, and tannins which are chemical compounds that have potential as antibacterial (Santana et al., 2016).

Most people know that cocoa beans are only used in food / beverage processing, but currently cocoa beans can also be used in the pharmaceutical (medicine) and cosmetics industries which one of them in making liquid soap (Brito-vega, 2018).

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Liquid soap is a liquid form that is generally made by using oils or fats intended to clean the skin, made with additives namely surfactants, preservatives, foam stabilizers, deodorizers and dyes that are allowed, and used for bathing without causing irritation to the skin. Liquid soap has the ability to emulsify oily dirt so that it can eliminate bacteria and dirt that sticks to the skin (Foddai, Grant, and Dean., 2016).

Based on research conducted by Baharium (2014) states that ethanol extract of cocoa beans (*Theobroma cacao L.*) has an antibacterial effect in inhibiting Pseudomonas aeruginosa bacterium which is carried out in vitro. Therefore, this research conducted on the formulation and antibacterial activity test of liquid soap extract from cocoa beans (*Theobroma cacao L.*) against Staphylococcus aureus and Escherichia coli bacteria. This research included extracts making from cocoa beans by maceration and liquid soap formulation that is tested on Staphylococcus aureus & Escherichia coli bacteria to determine the inhibitory power of bacteria to the formulation of liquid soap preparations from cocoa bean extract with alkali (KOH), and then testing soap evaluation namely organoleptic test, pH, foam ability test and irritation test.

2 MATERIALS AND METHODS

Ripe cocoa beans and fresh yellow color are taken from Lake Rambai Village, Batang Gansal Sub District, Gansal District, Inhu, Riau. Cocoa beans that have been taken are sorted to get fresh seeds, then washed with running water and dried by direct exposure to sunlight.

2.1 Making Simplicia Powder

Cocoa beans that have been dried are mashed by pounding them before blend to get more uniform size. Then the powder is weighed as much as 500 grams, and then put into a container for extraction purposes by using the maceration method.

2.2 Research Tools and Materials

The tools used in this study include laboratory glassware, aluminum foil, autoclaves, 65 mesh sieves, stirring rods, blenders, bushes, petri dishes, hotplates, incubators, calipers, ose needles (diameter 1, 78 dm), cotton, disc paper, filter paper, LAF (Laminar Air Flow), magnetic stirrer, 20 µl micropipette, microscope, oven, pH meter, pycnometer, rotary evaporator, digital scales and vortex.The materials used in this study include stearic acid, aquadest, cocoa beans, Butyl Hydroxy Toluene (BHT), 80% ethanol, ethyl acetate, $FeCl_3$, glycerin, H_2SO_4, HCl 2N, hydroxypropyl methyl cellulose (HPMC), Ethanol 80%, ethyl acetate, FeC_{l3}, glycerin, H_2SO_4, HCl 2N, hydroxypropyl methyl cellulose (HPMC), indicators of phenolphthalein, potassium hydroxide (KOH) 0.1 N, chloroform, 0.9% NaCl solution, n-hexane solution, Mueller-Hinton Agar, coconut oil, castor oil, olive oil, Nutrient agar, Na_2SO_4 anhydrous, NaOH 2 N , and Dragendorff reagent, Lieberman-Bouchardat reagent, lead (II) acetate 0.4 N. The bacteria used were pure cultures of *Escherichia coli* and *Staphylococcus aureus* bacteria.

2.3 Phytochemical Screening Test

Phytochemical screening is carried out to analyze bioactive content which is useful as an antibacterial or as a treatment. The phytochemical screening test of this cocoa bean powder, namely:

2.3.1 Flavonoids Test

10 g of simplicia powder were added with 100 ml of hot water. The mixture is then boiled for about 5 minutes, and then filtered when it is hot. As much as 5 ml of filtrate was obtained, added 0.1 g of Mg powder, 1 ml of concentrated HCL and 2 ml of amyl alcohol, shaken, and allowed to separate. Flavonoids are positive if there is red, yellow, or orange color in the amyl alcohol layer (Cocan et al., 2018; Delazar, Asgharian, and Asnaashari., 2017).

2.3.2 Tanin Test

0.5 g of simplicia powder sample was added with 10 ml of distilled water. The extraction result is filtered then the filtrate obtained is diluted with distilled water until it is colorless. The results of this dilution are taken as much as 2 ml, and then added with 1-2 drops of iron (III) chloride. A blue or blackish green color indicates tannins (Cocan et al., 2018;, Delazar, Asgharian, and Asnaashari., 2017).

2.3.3 Test of Saponins

As much as 0.5 g of simplicia powder was put into a test tube and 10 ml of hot aquadest was added, chilled, then shaken vigorously for no less than 10 minutes to as high as 1-10 cm of the froth obtained. Furthermore, with the addition of 2N hydrochloric acid, if the foam does not disappear, the results obtained indicate the presence of saponins contained in a simplicia (Cocan

et al., 2018; Delazar, Asgharian, and Asnaashari., 2017).

2.3.4 Alkaloide Test

The simplicia powder of cocoa beans was weighed 0.5 g then added 1 ml HCL 2 N and 9 ml aquadest, heated over water bath for two minutes, cooled and filtered. The resulting filtrate is used for testing. 10 drops of filtrate are taken into test tube with 2 drops of Meyer reagent added and white / yellow precipitate is formed. Next 10 drops of filtrate were added into test tube and 2 drops of bouchardate reagent were added to form a brown to black precipitate. Then 10 drops of filtrate were put into test tube and then added 2 drops of dragendrof tuning and an orange to red brown formed. If at least 2 out of 3 reagents produce the same precipitate then it positively contains alkaloids (Cocan et al., 2018; Delazar, Asgharian, and Asnaashari., 2017).

2.3.5 Steroid / Triterpenoida Test

A total of 1 g of simplicia powder was macerated with 20 ml of n-hexane for 2 hours, and then filtered. The filtrate is evaporated in a vaporizer cup. To remaining 2 drops of anhydrous acetic acid and 1 drop of concentrated sulfuric acid are added. The purple or red color then turns green blue indicating the presence of steroids / triterpenoids (Cocan et al., 2018; Delazar, Asgharian, and Asnaashari., 2017).

2.3.6 Glycoside Test

Simplisia powder was weighed as much as 3 grams, then mixed with 30 ml mixture of 7 parts by 80% ethanol volume and 3 parts by volume of distilled water (7: 3), refluxed for 10 minutes, chilled and filtered. To 20 ml filtrate, 25 ml of lead (II) acetate 0.4 N were added, shaken, allowed to stand for 5 minutes and then filtered. The filtrate was extracted 3 times, each time with 20 ml mixture of 3 parts by volume of chloroform (p) and 2 parts by volume of isopropanolol (p). To the chloroform layer, sodium sulfate anhydrous (p) is added to taste, filtered and evaporated at temperature of no more than 50^0 C. Dissolve the rest with 2 ml of methanol, then take 0.1 ml of the experimental solution put into test tube, evaporated on a water bath. To remaining 2 ml of water added and 5 drops of molish reagent, carefully added 2 ml of sulfuric acid formed purple ring at boundary of both liquids indicating the presence of sugar bonds (Cocan et al., 2018; Delazar, Asgharian, and Asnaashari., 2017).

2.3.7 Test of Anthraquinic Glycosides

A total of 0.2 g cocoa bean simplex powder was weighed, and then 5 ml of 2 N sulfuric acid was added, heated briefly, after being cooled, added 10 ml of benzene, shaken and allowed to stand. The benzene layer is separated and filtered, shaken the benzene layer with 2 ml of NaOH 2N, allowed to stand. The red water layer and the colorless benzene layer show anthraquinone (Cocan et al., 2018; Delazar, Asgharian, and Asnaashari., 2017).

2.4 Making Cocoa Bean Extract

The method of making cocoa bean extract is done by using cold extraction method, namely maceration. Simplisia which has been in the form of powder weighed 500 grams, then macerated by soaking 10 parts of simplicia, then put in a vessel and poured 75 parts of the liquid, then covered and allowed to stand for 3-5 days in place which is protected from sun light. Shaken repeatedly, filtered then squeezed. The pulp from maceration is washed by using 25 parts of liquid until the juice is obtained. The vessel is closed and left for 2 days in a cool place and protected from sunlight, then separated the precipitate obtained. The filtrate that has been produced is then evaporated by using rotary evaporator until it thickens slightly, and then evaporates over water bath to form a thick extract (Baharium, 2014).

2.5 Sterilization of Tools and Materials

The tools used for antibacterial testing are first sterilized by heating process carried out to kill all forms of organisms. Non-glass tools are sterilized by using an autoclave at 121^0 C for 15 minutes. Whereas glassware is sterilized using an oven at 170^0 C for 1-2 hours (Widyaningsih, et al., 2018).

2.6 Soap Formulation

According to Saryanti and Setiawan (2018), formulations used in making liquid soap preparations can be seen in Table 1.

Table 1: Formulations of Liquid Soap

Ingredient	FI (g)	F II (g)	F III (g)	FIV (Comparison)
Cocoa bean extract	5	10	20	Conventional
Castor Oil	10	10	10	
Olive Oil	15	15	15	
Coconut Oil	10	10	10	
KOH	8	8	8	
HPMC	3	3	3	
Stearic acid	2	2	2	
Glycerin	18,75	18,75	18,75	
BHT	0,02	0,02	0,02	
Aquadest	ad 100	ad 100	ad 100	

2.6.1 Soap Making

Castor oil is first mixed with olive oil and coconut oil, then stir slowly until homogeneous. Then KOH solution is added little by little to the oil mixture at temperature 60-70^0 C to form a paste. Then added stearic acid and BHT which had previously been melted, then put into the mixture and stirred until homogeneous, then put HPMC which has been developed in hot aquadest into mixture. Furthermore, glycerin and cocoa bean extract are added and stirred until homogeneous. Then add up to 100 ml of distilled water and then stir until homogeneous and put into a container (Saryanti and Setiawan., 2018).

2.7 Evaluate of Liquid Soap Preparations

2.7.1 pH Test

Liquid soap pH requirements based on SNI meet the requirements namely 8 - 11. pH Conditions that are too low can cause irritation to the skin (Widyaningsih, et al.,2018)

2.7.2 Irritation Test

The irritation test technique is an open sample test (Patch Test) on the back of the ear against 20 panelists. A positive irritation reaction is characterized by redness, itching, or swelling in the skin area of the inner ear that is treated. The irritation test parameters include swelling, itching, redness, peeling and feeling dry with numerical assessment (1) irritation, (2) slight irritation, (3) absence of irritation.

2.7.3 Determination of Antibacterial Activity

Antibacterial activity test used disc diffusion method, as much as 15 ml media Nutrient agar (NA) is poured into the cup until evenly distributed, then homogenized and allowed to stand at temperature room until the media is solidified. Then as much as 0.1 ml of Staphylococcus aureus bacterial inoculum suspension is inserted into sterile petri dish, then put disc paper into petri dish, then dropped 3 drops of cocoa bean extract test sample soap with various formulations (FI, FII, FIII). In this study also used Aquadest as negative control and Dettol liquid bath soap as positive control. The cup is left at temperature room for 10-15 minutes, and then incubated at 37° C for 24-48 hours, then the diameter of the inhibition zone around the disc is measured by using calipers. Tests carried out with three repetitions (triplo). The same treatment was carried out on Escherichia coli bacterial inoculums (Ditjen POM, 1995).

3 RESULTS AND DISCUSSIONS

3.1 Phytochemical Screening

Phytochemical screening results of cocoa beans are carried out to determine the class of secondary metabolite compounds contained in it. The examination of secondary metabolites is carried out on groups of flavonoid compounds, tannins, saponins, alkaloids, steroids / triterpenoids, glycosides and anthraquinone glycosides. The results of phytochemical screening of cocoa beans can be seen in Table 2.

Table 2: Results of phytochemical screenings

Compound Class	Reactor	Result	Conclusion	Reference (Indira *et al*., 2014) dan (Dedy, 2016)
Flavonoid	Mixture 0.1 g of Mg	Ring is formed	(+) Flavonoid	(+) Flavonoid

	powder, 1 mL HCl (p), 2 mL Amil alcohol	Coloured Red in the amyl layer alcohol		
Tanin	FeCl3 (blue or blackish green)	Blue color is formed	(+) Tanin	(+) Tanin
Saponin	Aquadest is hot and whipped strong	Foam formed	(+) Saponin	(+) Saponin
	With the addition of 1-2 drops of HCl 2N	Froth / foam is not lost		
Alkaloids	Meyer reagent (sediment yellow / white)	Was formed precipitate yellow / white	(+) Alkaloid	(+) Alkaloid
	Reactor Bouchardat (sediment brown-black)	Reactor Bouchardat (sediment brown-black)		
Steroids	H_2SO_4 (p) (purple and red, and or change to green / blue)	First of all purple, then change go green	(+) Steroid	(+) Steroid
Glycosides	Lead (II) acetate 0.4 N	A purple ring forms at the second fluid boundary	(+) Glikosida	(+) Glikosida
Anthracinone Glycosides	2 N sulfuric acid, benzene, 2N NaOH	No red water layer formed	(-) Anthracinone glycosides	(-) Anthracinone glycosides

3.2 Soap Quality

Soap testing is carried out to determine the physical appearance and quality of cocoa bean liquid soap preparations, organoleptic test examinations which include the shape, odor and color of the preparation, pH test, foam ability test and irritation test on volunteers.

3.2.1 pH Test and Froth Capability

pH testing of liquid soap is done because the high and low pH values of the soap preparations can affect the absorption rate of the skin. Testing the ability of the foam is done by fast shaking using a test tube, aiming to determine the stability of the soap scum caused. The testing results of pH analysis can be seen in Table 3.

Table 3: pH value and foam stability

NO.	Preparation	pH	Froth Ability (cm)
1.	Formula I	10,32	6,8
2.	Formula II	10,14	7,2
3.	Formula III	9,5	7,2

pH testing of liquid soap is done because the high and low pH values of the soap preparations can affect the absorption rate of the skin. According to SNI, good pH of soap is used which is alkaline in the range of 8-11 to be able to clean the dirt on the skin. The pH testing result on each formulation of cocoa beans extract liquid soap has values ranging from 9.5 - 10.25. The addition of strong alkaline KOH can increase pH value and the addition of high cocoa bean extract to the formula results in decrease the pH value. When compared with the comparator Dettol

soap which has pH 9 available on the market, the pH of liquid soap with cocoa bean extract additive is also close to the commercial standard.

The observations showed that each formula produced relatively similar foam because it had value of 6.4 cm while Dettol soap had value of 8.2 cm. The difference occurred due to the surfactant used by Dettol soap, namely sodium lauryl sulfate (SLS), so that the resulting bubble foam was more numerous. The bubbles shape produced in the liquid soap extract of cocoa beans is very thin so that the rate of decline in the foam very quickly disappears within ± 180 seconds. Measurement of foam height after 5 minutes decreases with each formulation. It is due to the small amount of surfactant that is added to the liquid soap preparation so that there is no repulsion between bubbles resulting in lower voltage and the bubbles break easily (Politova, et al., 2018).

3.2.2 Irritation Test

The questionnaire results found that liquid soap extract of cocoa beans showed no irritation. The results of irritation testing data on 15 volunteers can be seen in Table 4.

Table 4: Result of irritation test

Formula	**Volunteer**	**Skin Condition**		
		Swelling	Reddish	Itching
F I	I	1	1	1
	II	1	1	1
	III	1	1	1
	IV	1	1	1
	V	1	1	1
	VI	1	1	1
	VII	1	1	1
	VIII	1	1	1
	IX	1	1	1
	X	1	1	1
	XI	1	1	1
	XII	1	1	1
	XIII	1	1	1
	XIV	1	1	1
	XV	1	1	1
FII	I	1	1	1
	II	1	1	1
	III	1	1	1
	IV	1	1	1
	V	1	1	1
	VI	1	1	1
	VII	1	1	1
	VIII	1	1	1
	IX	1	1	1
	X	1	1	1
	XI	1	1	1
	XII	1	1	1
	XIII	1	1	1
	XIV	1	1	1
	XV	1	1	1
FIII	I	1	1	1
	II	1	1	1
	III	1	1	1
	IV	1	1	1
	V	1	1	1
	VI	1	1	1
	VII	1	1	1
	VIII	1	1	1
	IX	1	1	1
	X	1	1	1
	XI	1	1	1
	XII	1	1	1
	XIII	1	1	1
	XIV	1	1	1
	XV	1	1	1

Irritation testing is done to determine safety when using antibacterial liquid soap extract of cocoa beans. The irritation test on liquid soap is done by applying preparations to the volunteers' inner arms. The inner arm skin is more sensitive because the horny layer is thin enough so that the absorption of soap preparations by the skin is faster (Widyaningsih, Chasani, and Diastut., 2018). The results of observing the irritation test by spreading the questionnaire to 15 volunteers gave choice number 1 which indicated that liquid soap extract of cocoa beans did not cause irritation in the form of redness, swelling and itching. It can be attributed to the fact that the liquid soap extract of cocoa beans is formulated with natural ingredients that are not harmful so it is safe to use.

3.2.3 Antibacterial Activity Test for Liquid Soap Cocoa (*Theobroma cacao* L.) Extract

The results of antibacterial activity testing on liquid soap of cocoa bean extracts were carried out by using three different formulations namely FI 5 g, FII 10 g, and FIII 20 g. The results of inhibitory zones of liquid soap extract of cocoa beans on the bacteria

Staphylococcus aureus and *Escherichia coli* can be seen in Figures 1, 2, and Table 5.

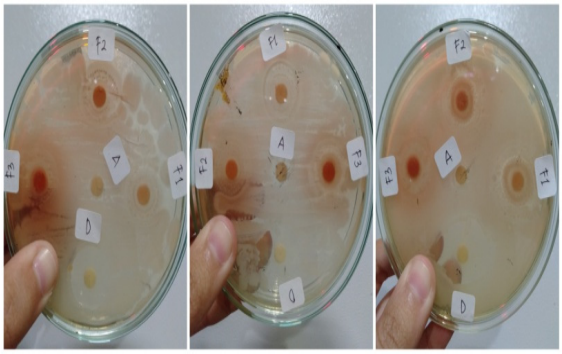

Figure 1: Area of circle of inhibition zone of *Staphylococcus aureus*

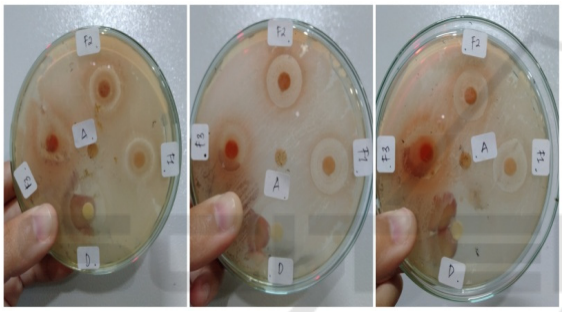

Figure 2: Area of circle of inhibition zone of *Escherichia coli*

Table 5: Inhibition Zone of *Theobroma Kakao* L Extraction

No.	Treatment	Average diameter of resistance (mm)	
		Staphylococcus aureus	*Escherichia coli*
1.	Aquadest	-	-
2.	Dettol	15,70	15,26
3.	FI	10,33	9,23
4.	FII	11,70	10,53
5.	FIII	12,93	10,90

The antibacterial activity results of liquid soap preparations from cocoa bean extracts showed that diameter of inhibitory zones in Staphylococcus aureus and Escherichia coli bacteria were categorized as having strong activity in inhibiting bacterial growth. Inhibitory zones on positive control of Dettol soap are 15.70 in Staphylococcus aureus bacteria and 15.26 in Escherichia coli bacteria and are categorized as having strong inhibitory zone activity and the results of aquadest as negative control do not form inhibitory zones because aquadest does not have the ability to inhibit bacterial growth.

The antibacterial activity of liquid soap extract of cocoa beans showed that Gram positive testing of Staphylococcus aureus bacteria had a greater inhibition zone than Gram negative testing of Escherichia coli bacteria from several variations of liquid soap preparation formulas. According to Volk (1992), it is due to differences in both of bacteria testing where they have different cell wall compositions and structures so that gram-positive bacteria are more susceptible to chemical compounds than gram-negative.

A Gram-positive bacterium has a simpler cell wall structure, which is single layer that has low lipid content (1-4%) so that bioactive materials more easily enter the cell. While gram-negative bacteria have more complex cell wall structure, which is three layers consist of the outer layer of lipoprotein, the middle layer of lipopolysaccharide that functions as a barrier to the entry of antibacterial bioactive material, and the inner layer of peptidoglycan which has high lipid content (11-12%). In addition, according to (Arafat and Rahman., 2017) certain antibacterial compounds can increase their activity from bacteriostatic to bacteriocidal if a concentration of the compound in the preparation is increased. The greater the concentration contained in an antibacterial agent, the stronger the work activity.

4 CONCLUSIONS

Liquid soap extract contains cocoa beans that have antibacterial activity but it is not greater than antibacterial activity of Dettol soap.

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