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Formulation of Antibacterial Liquid Soap Based on Butterfly Pea Flower (*Clitoria ternatea* L.) Extract with Ultrasound-Assisted Extraction Technique

Nofran Putra Pratama[1*], Kurnia Rahayu Purnomo Sari[1], Budi Rahayu[2]

[1]Pharmacy Study Program, Universitas Jenderal Achmad Yani, Yogyakarta, Indonesia
[2]Midwife Study Program, Universitas Jenderal Achmad Yani, Yogyakarta, Indonesia
[1]nofranputrapratama@gmail.com*; [1]kurniarahayupurnamasari@gmail.com; [2]budiayu_88@yahoo.co.id
*corresponding author

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ABSTRACT

Butterfly pea flower (*Clitoria ternatea* L.) exhibits antibacterial properties due to bioactive compounds like phenolics and flavonoids. These compounds were extracted using the UAE (Ultrasound Assisted Extraction) method, which is more efficient than conventional methods. This study aims to address antibacterial resistance by formulating an antibacterial liquid soap using butterfly pea flower extract as the active ingredient. Results showed the liquid soap demonstrated good antibacterial activity and physical stability; however, its pH exceeded skin safety standards. A favorability test involving 20 respondents indicated positive responses regarding texture, scent, and skin-softening effects. In conclusion, liquid soap containing butterfly pea flower extract has potential as an effective antibacterial product but requires pH improvement to enhance safety.

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1. Introduction

One way to obtain chemical compounds contained in plants is by extraction methods. Currently, conventional extraction methods have been abandoned because of their many weaknesses such as long extraction time and the solvent needed is quite a lot (Sudarwati et al., 2019). The transition to modern methods such as ultrasonic methods has been done quite a lot nowadays. The UAE method is a novelty of the maceration method, where the UAE method is carried out using ultasonic waves (*ultrasound*) with a frequency, and uses high vibrations of 20kHz. The use of ultrasonic waves can accelerate extraction and can increase the efficiency of the extraction process of a compound from a plant (Wijngaard et al., 2012).

One of the plants that contain many chemical compounds is butterfly pea flowers, butterfly pea flowers are flowers with characteristic purple petals. Butterfly pea flowers usually grow vines and are often found in home yards, in rice fields, or in plantation areas. (Handito et al., 2022). Butterfly pea flowers are known for their ability as antibacterials because they contain several chemical compounds (Budiasih, 2017). These compounds include flavonoids, phenolics, tannins and alkaloids (Sitorus et al., 2020).

Flavonoid compounds are able to inhibit bacterial activity by breaking down proteins in the cell membrane (Sitorus et al., 2020b). Phenol compounds can inhibit antibacterial activity by activating proteins in the bacterial cell membrane. Tannins can destroy bacterial cell membranes and DNA. Alkaloids can damage the work of enzymes by synthesizing bacteria, so that it can cause disruption

of bacterial metabolism and the energy needed is reduced which will result in bacterial cells will die (Riyanto et al., 2019a). Based on research (Frisca et al., 2021), he growth of Escherichia coli ESBL bacteria can be inhibited by ethanol extract of butterfly pea flowers at different concentrations. According to (Pertiwi et al., 2022), ethanol extract of butterfly pea flowers with various concentrations can inhibit the development of *Staphylococcus epidermidis* bacteria. The growth of Pseudomonas aeruginosa and Bacillus cereus bacteria using ethanol extracts of butterfly pea flowers can inhibit bacterial growth (Riyanto et al., 2019b). Another advantage of butterfly pea flowers is their nature as an environmentally friendly natural ingredient and are easy to integrate into modern product formulations, such as liquid soap.

In the context of increasing bacterial resistance to conventional antibiotics, this study aims to develop alternative hygiene products based on natural ingredients. The formulation of liquid soap with active ingredients of butterfly pea flower extract is expected to meet the need for effective, safe, and environmentally friendly hygiene products, while offering the advantages of ease of use and comfort. Therefore, this study focuses on optimizing the UAE extraction method to obtain a liquid soap formulation that not only has antibacterial activity, but is also physically stable and suitable for use on the skin.

2. Methods

This research was carried out using an experimental design in the laboratory. The research stages were carried out starting from sample collection, plant determination and sample extraction. Butterfly pea flowers were obtained in Glagah Village, Kulon Progo, Yogyakarta Istimewah Region (7°54′10.616″S 110°4′21.917″E). Extraction of butterfly pea flower (*Clitoria ternatea* L.) using ultrasonic method and using 96% ethanol solvent with variation of solvent ratio and extraction time. The extract obtained was then determined as % yield, total phenolic and flavonoid content. The most optimal results were continued by testing the inhibition of *Escherichia coli* and *Staphylococcus aureus* bacteria using the disc diffusion method and formulating solid soap preparations followed by testing the physical properties of soap preparations.

2.1 Research Design

The research design used was Randomized Group Design (RAK) with two factors, namely solvent ratio and ultrasonic extraction time. The solvent ratio consisted of 1:5, 1:10 and 1:15, while the extraction time consisted of 10, 20 and 30 minutes. Each research design was repeated 3 times, resulting in 27 experiments. The research design is presented in Table 1.

Table 1. Research design

Solvent Ratio	Extraction Time (minutes)		
1:5	10	20	30
1:10	10	20	30
1:15	10	20	30

2.2 Extract Preparation

Preparation of butterfly pea flower extract using a ratio of 1:5; 1:10; and 1:15. Each comparison uses 2 grams of butterfly pea flower powder, so that the solvent used is 10 mL, 20 mL, and 30 mL. Put the weighed butterfly pea flower powder into an Erlenmeyer flask and dissolve it with 96% ethanol solvent according to the ratio. Extraction was carried out by ultrasonic method using *ultrasonic bath* based on the research design in (Table 1). The obtained suspension was filtered using Whatman paper number 1 and then concentrated using a waterbath to evaporate the solvent in the extracted sample and obtained a thick extract (Anjani, 2019). The results of the thickened extract were weighed and the yield was calculated.

2.3 Antibacterial Activity Test

The medium used for the antibacterial activity test was Meller Hinton Agar. Enter 0.1 mL of bacterial suspension of *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 in a petri dish using a micropipette and flatten it using a sterilized L rod. The disc paper that will be used is soaked in the test solution of butterfly pea flower ethanol extract that has been made with various concentrations for 10 minutes (Nor et al., 2018). The soaked disc paper was placed on MHA media that had been inoculated with bacteria. As a positive control using 10µg ampicillin antibiotic and for negative

control using distilled water. Incubated in an incubator for 24 hours at 37ºC. The zone of inhibition formed in the form of a clear area on the disc paper media was observed and measured using a caliper on a mm scale. The results obtained were calculated and the classification was determined.

2.4 Liquid Soap Formulation

The formula used in this study is based on research conducted by Amelia (2017) which has been modified. All ingredients that will be used are weighed first according to the recommended dosage. Olive oil was put 15 mL into a hot mortar, then added with 40% potassium hydroxide as much as 8 mL little by little while continuing to heat at 50℃ to get soap paste. Weighed 1 g of Na-CMC and then developed it in 20 mL of water on a hotplate stirrer and then mixed it in the soap paste mixture. The mixture was added 0.25 g stearic acid, stirred until homogeneous. Added 0.5 g SLS, stirred until homogeneous. Add 0.5 g BHA, then stir until homogeneous. Add flavoring to taste and mix until homogeneous. Add bay flower extract and mix until homogeneous. Liquid soap is added with the remaining distilled water to a volume of 50 mL, put into a clean container that has been prepared. The manufacture of butterfly pea flower extract liquid soap is adjusted to each concentration. Soap preparations will be made into 3 formulas and 1 control formula. The complete formulation can be seen in Table 2.

Table 2. Liquid soap preparation formula

Material	Unit	F0 (control)	F1 (2,5%)	F2 (5%)	F3 (7,5%)
Extract	g	0	0.25	0.5	1
Olive Oil	mL	15	15	15	15
KOH	mL	8	8	8	8
Na-CMC	g	1	1	1	1
SLS	g	0.5	0.5	0.5	0.5
Stearic acid	g	0.25	0.25	0.25	0.25
BHA	g	0.5	0.5	0.5	0.5
Scent		qs	qs	qs	qs
Aquadest	mL	ad 50	ad 50	ad 50	ad 50

2.5 Physical stability and favorability test

2.5.1 Organoleptical Test

Organoleptical examination includes observation of shape, color and odor, which are stable and should show the same characteristics in the form of shape, color, and odor of the soap.

2.5.2 pH test

The pH measurement is carried out by dipping the pH meter calibrated with a solution that has a pH of 7, then the pH meter electrode is dipped until the tip of the electrode is all immersed in the preparation and the number read becomes stable. The number that shows the pH value is then recorded.

2.5.3 Foam stability test

Foam height testing aims to measure the stability of soap in forming foam. The test was carried out by means of purple sweet potato extract soap preparation put in a 100 mL measuring cup and added distilled water to 50 mL and shaken vigorously for 20 seconds. The foam height formed is observed for stability for 5 minutes.

2.5.4 Taste test

The liking test was conducted using the level of liking method. This test was conducted on viscosity, texture, scent, non-sticky impression, moisture, and comfort. Rating scale 1-5 with 20 respondents.

3. Results and Discussion

3.1 Preparation of Butterfly Flower Extract

Butterfly pea flowers are wet sorted to select flowers that will be used as samples. Flowers that have been wet sorted are then washed and dried using an oven for 3 days at a temperature of 45ºC. Drying aims to reduce the water content so that the stability of the sample will increase. The dried samples were then pollinated using a grinder and sieved using a sieve number 40 mesh to obtain suitable and uniform particles. Pollination aims to reduce the particle size of the sample, this will

cause more contact with the solvent so that the extraction process will be more effective. 7 Kg of wet bayang flower samples produced 600 grams of bayang flower powder.

The powder was then extracted by ultrasonic wave-assisted extraction method, by dissolving 70% ethanol solvent with a solvent-sample ratio of 1:10 i.e. (10 grams:100 mL) for 20 minutes. The extraction process was carried out in three replicates. The filtrate obtained from the extraction was then thickened by evaporating using a waterbath. The extract yield obtained is 52.875%, this result is in accordance with the requirements of the viscous extract yield value which is more than 7.5%.

3.2 Antibacterial Activity

The average diameter of the inhibition zone formed is then classified by the bacterial growth inhibition response according to Pangouw et al. (2020), namely if the inhibition zone is more than 20 mm, it is classified as a very strong growth inhibition response, the diameter of the inhibition zone is 11-20 mm, it is classified as a strong growth inhibition response, the diameter of the inhibition zone is 5-10 mm, it is classified as a moderate growth inhibition response, and the diameter of the inhibition zone is less than 5 mm, it is classified as a less growth inhibition response. The average results of the inhibition zone of *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 can be seen in Table 3 and Table 4.

Table 3. Average diameter of inhibition zone of ethanol extract of butterfly pea flower against *E.coli* ATCC 25922

Concentration	Average Zone of Inhibition Diameter (mm)	Growth Inhibition Respone
20%	6.57	Medium
40%	7.42	Medium
60%	8.62	Medium
80%	9.16	Medium
100%	10.05	Medium
Positive control	21.63	Very strong
Negative control	0	None

Table 4. Average diameter of inhibition zone of butterfly pea flower ethanol extract against *S. aureus* ATCC 25923

Concentration	Average Zone of Inhibition Diameter (mm)	Growth Inhibition Respone
20%	7.47	Medium
40%	8.53	Medium
60%	9.41	Medium
80%	9.82	Medium
100%	11.06	Medium
Positive control	33.93	Very strong
Negative control	0	None

Based on the results of the diameter of the inhibition zone in Table 3 and Table 4, ethanol extract of butterfly pea flowers with a concentration variation of 20%, 40%, 60%, 80% and 100% can inhibit the growth of *E. coli* ATCC 25922 and *S. aureus* ATCC 25923. The higher the concentration, the greater the inhibition zone formed. The inhibition zone of butterfly pea flower ethanol extract at a concentration of 100% against *E. coli* ATCC 25922 bacteria has a moderate growth inhibition response, while the *S. aureus* ATCC 25923 bacteria has a strong growth inhibition response.

The results of this study indicate that butterfly pea flower extract with a higher concentration provides a larger inhibition zone diameter against *E. coli* and *S. aureus*. At a concentration of 100%, the inhibition zone against *E. coli* reached 10.05 mm (moderate category), while against *S. aureus* it reached 11.06 mm (strong category). These results are in line with the research of Pertiwi et al., (2022), which reported that ethanol extract of butterfly pea flowers at high concentrations effectively inhibits the growth of *S. epidermidis*, another gram-positive bacteria, with a mechanism of action involving phenolic and flavonoid compounds that damage bacterial cell membranes. The results of this study indicate that butterfly pea flower extract with a higher concentration provides a

larger inhibition zone diameter against *E. coli* and *S. aureus*. At a concentration of 100%, the inhibition zone against *E. coli* reached 10.05 mm (moderate category), while against *S. aureus* it reached 11.06 mm (strong category).

3.3 Liquid Soap Formulation

Liquid soap preparations have an average weight of 50 mL with varying concentrations of butterfly pea flower extract used as an active ingredient in the preparation. Liquid soap with a concentration of 0.25% (F1) is yellow, 0.5% concentration (F2) is light yellow, and 1% concentration (F3) is green. The appearance of the preparation can be seen in Figure 1

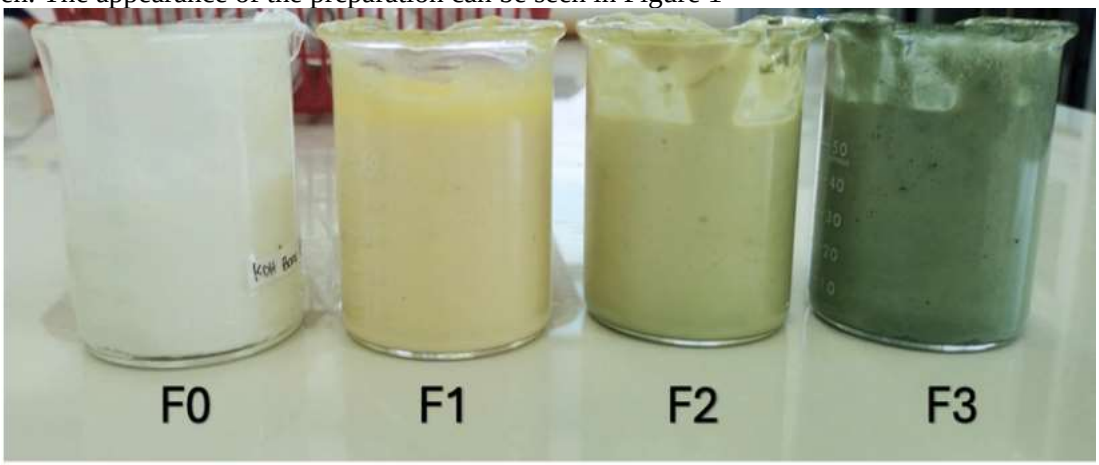

Figure 1. Liquid soap preparation. F0 : Control, F1 : 0,25% concentration, F2 : 0,5% concentration, F3 : 1% concentration

3.4 Physical Stability and Taste Test of Preparations

3.4.1 Organoleptical Test

Based on the organoleptic test results on days 1, 3, and 5, all formulas did not change. Organoleptic results can be seen in Table 5.

Table 5. Organoleptic observations

Day-	Formula	Organoleptic Observations		
		Texture	Color	Scent
1	F0	Viscous	White	Typical
	F1	Viscous	Light Yellow	Typical
	F2	Viscous	Yellow	Typical
	F3	Viscous	Green	Typical
3	F0	Viscous	White	Typical
	F1	Viscous	Light Yellow	Typical
	F2	Viscous	Yellow	Typical
	F3	Viscous	Green	Typical
5	F0	Viscous	White	Typical
	F1	Viscous	Light Yellow	Typical
	F2	Viscous	Yellow	Typical
	F3	Viscous	Green	Typical

3.4.2 pH and Foam Stability Test

The pH of the liquid preparation was observed on days 1, 3, and 5, using a universal pH and pH meter. In general, the pH of the preparation tends to decrease from the first day to the fifth day. On the first day, the F0 preparation showed a pH of 10 with a universal pH and 10.8 with a pH meter, while the other formulas (F1, F2, F3) had a lower pH with a range of 9 to 8.5. On the fifth day, the pH of F0 remained the highest with a universal pH value of 10 and a pH meter of 10.1, while the pH of the other formulas dropped to around 8. These results are not in accordance with the

provisions of the pH of liquid soaps for use on the skin usually have a pH between 5 and 7. This pH range is considered safe and suitable for human skin, maintaining the natural moisture of the skin and preventing irritation There are several factors that affect the pH of soap to be very alkaline, including excessive alkali content, incomplete saponification process, lack of buffering agents, use of high alkaline-based ingredients, and storage effects. Soap with a pH that is too high (too alkaline) Soap with a pH that is too high (too alkaline) can risk causing skin irritation, dry skin, and disruption of the skin microbiome (Rosmainar, 2021).

For foam stability, the foam height tended to increase over time in all formulas. On the first day, the highest foam height was achieved by F0 (40 mm) and the lowest by F1 (12 mm). On the third and fifth days, foam stability increased, with F0 reaching a maximum foam height of 65 mm on the fifth day, while the other formulas (F1, F2, F3) increased gradually with foam heights of 16 mm, 20 mm, and 36 mm, respectively. Overall, F0 had the highest pH and foam stability compared to the other formulas. This indicates that formula F0 has better pH and foam stability during the observation period than the other formulas (F1, F2, F3) (Rosmainar, 2021). Observations of the pH and foam stability of the preparation were carried out on days 1, 3, and 5 on Table 6.

Tabel 6. Observation of pH and Foam Stability

Observation		Day 1				Day 3				Day 5			
		F0	F1	F2	F3	F0	F1	F2	F3	F0	F1	F2	F3
pH	Universal	10	9	9	9	10	9	8	8	10	8	8	8
	pH Meter	10.8	9.3	8.9	8.5	10.5	8.7	8.5	8.2	10.1	8.3	8.2	7.8
Foam Height (mm)		40	12	14	26	56	15	18	30	65	16	20	36

3.4.3 Taste Test

The favorability test was conducted on 20 respondents who were asked to assess the soap preparation in terms of scent, texture, cleaning ability, foam quality, softening effect, and comfort. The preference test assessment is divided into 5 levels, namely very like, like, ordinary, dislike, very dislike. The demographics of the respondents used include age, gender and frequency of use of liquid soap. From the demographics of the respondents, it can be seen that the majority are women (16 people) with an age range of 20-30 years (14 people) who use liquid soap every day (16 people). Respondent demographic data can be seen in Table 7.

Table 7. Respondent demographics

Demographics		
Age	<20 years	6
	20-30 years	14
	31-40 years	0
	41-50 years	0
	>50 years	0
Gender	Male	4
	Female	16
Frequency of use of liquid soap	Every day	16
	3-5 times per week	4
	1-2 times per week	0

The results of the favorability test showed that the scent of liquid soap received a positive response, with most respondents stating "Like" (10 respondents) and "Really Like" (6 respondents). Only a few respondents (4 people) felt that the scent was "Ordinary", and no one felt dislike. On the aspect of the texture of the soap when held, ratings also tended to be high, with 16 respondents feeling "Liked" and 3 others "Liked Very Much"; only one respondent considered the texture "Ordinary".

In terms of the ability to cleanse the skin, 11 respondents rated it as "Liked" and 7 chose "Liked", indicating that the cleansing function of this product is quite effective. Meanwhile, regarding the amount and quality of foam produced, ratings varied somewhat. A total of 10 respondents found it "Very Like" and another 10 "Like", while 8 people found it "Average". For the aspect of the effect of liquid soap on the skin such as softness after use, there was a very positive response, with 14

respondents choosing "Liked Very Much" and 5 "Liked". On the aspect of overall comfort of use, the results were very satisfactory, with 12 respondents stating "Liked Very Much" and 8 others "Liked". The results of the favorability test can be seen in Table 8.

Table 8. Taste test results

Parameters	Strongly dislike	Dislikes	Ordinary	Like	Very Favorable
Liquid Soap Scent	0	0	4	10	6
Liquid Soap Texture (When Held)	0	0	1	16	3
Skin Cleansing Ability	0	0	2	7	11
Quantity and Quality of Foam Produced	0	0	8	10	10
Effect of liquid soap on skin (e.g Softness after use)	0	0	1	5	14
Overall comfort of use	0	0	0	8	12

In terms of liquid soap formulation, physical stability and user preference for the preparation have been tested. Organoleptic and preference tests showed positive results, where the majority of respondents liked the scent, texture, and softness of the skin produced after use. This study is consistent with the study (Rosmainar, 2021)., which reported that liquid soap formulations with natural active ingredients showed high appeal to consumers, especially due to their comfort of use and moisturizing properties. However, the pH of the liquid soap preparation in this study was still above the safe standard for skin (5–7), similar to the report by Megawati and Nugroho (2021), who found that the alkali content in the saponification process affected the increase in soap pH.

4. Conclusion

Liquid soap with butterfly pea flower extract has potential as an effective antibacterial product with adequate physical stability, but needs improvement in the pH aspect to increase the safety of its use.

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