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Research Article

Formulation and evaluation of liquid soap preparation of robusta green coffee extract (*Coffea canephora*) with virgin coconut oil (VCO) base as an antibacterial *Staphylococcus aureus*

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Keywords

Coffea canephora
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Abstract

Staphylococcus aureus can be found on the surface of the skin. One way to prevent *Staphylococcus aureus* on the skin is to clean the skin with soap. Liquid soap effectively removes dirt from the skin surface and protects the skin from bacteria. Determining the robusta green coffee bean extracts (*Coffea canephora*) appropriate activity was the study's major aim in a liquid soap that complies with SNI 06-4085-1996 for use as an antibacterial agent and meets the physical quality requirements for liquid soap preparations. The extraction is performed by a soxhlet extraction method with 96% ethanol. The results of the organoleptic test show a difference in color at each concentration, with the concentration of the extract, which is higher, affecting the color and odor, the results of the homogeneity show results that are homogeneous and free of coarse grains, the results of the pH test show results that meet the requirements, namely in the interval 8-11, the results of the viscosity show results that meet the SNI requirements of 400-4000Cps, the results of the foam height test show stable results and meet the SNI requirements of 13-220 mm. the results of the inhibition zone in the liquid bath soap preparation of robusta green coffee bean extract using the disk diffusion method show that F0 = 4.51mm (resistant), F1 = 11.11mm (resistant), F2 = 13.35mm (intermediate) and F3 = 20.57mm (suspicious).

Introduction

The skin, that covers the entirety of the human body, is the outermost layer of tissue and protects the body from risks outside, including bacteria. The skin also acts as a site of excretion, which is often mixed with dirt, leading to the growth of microorganisms such as viruses, fungi, and bacteria that can lead to infectious diseases (Legi et al., 2021).

One of the pathogenic microorganisms that cause infectious diseases is bacteria. Bacteria are microorganisms that cannot be seen with the naked eye, but only with the help of a microscope. Pathogenic bacteria are more dangerous and cause both sporadic and endemic infections, such as *Staphylococcus aureus*. *S. aureus* is a coccoid and gram-positive bacterium that is widespread and sometimes lives as normal flora in humans, in the groin and perineal area and the anterior nostrils. Approximately 25-30% of people carry *S. aureus* in the nasal cavities and on the skin. *S. aureus* can potentially cause respiratory, digestive tract, and skin infections, usually in the form of abscesses, i.e. pus or fluid formation in tissues. Specific types of abscesses include swelling and inflammation of the hair roots (folliculitis), and they can also cause diseases such as pimples and boils (Wardani et al., 2022).

Prevention of *S. aureus* bacteria on the skin can be done by washing the skin with soap. The long-chain fatty acid salt that is alkaline is soap. Soap is made of sodium and potassium salts of long-chain fatty acids that are obtained from saponified fat or oil. Soap is made from two main ingredients, alkali and fat or oil triglycerides.

Soap can emulsify water, dirt, or oil. Soap is very effective in removing dirt that adheres to the skin surface, both water soluble and fat soluble, and has the function of cleansing the skin and protecting it from bacteria and body odor (Dimpudus et al., 2017).

Much research has been done on the formulation and evaluation of antibacterial soap, such as Widyasanti's research (2017) which found that using VCO without adding Moringa seed oil as a soap base has a high lauric content, can produce a lot of foam and is the most requested by respondents, and, Rosmainar's research (2021) showed that a liquid soap formulation with Robusta black coffee extract and kaffir lime extract based on VCO derivatives, namely Cocomide DEA and Cocomidopropyl betaine, has met the standards of SNI 06-4085-1996, and the test results for microbial contamination show that *S. aureus* was detected.

In this study, the researchers developed an antibacterial soap formulation with different concentrations of green coffee bean extracts based on virgin coconut oil (VCO), which could later inhibit the growth of *S. aureus*. Robusta green coffee bean extract contains alkaloids, flavonoids, saponins, tannins, caffeine, phenolic chlorogenic acids, trigonelines, carbohydrates, lipids, amino acids, organic acids, volatiles, and minerals. The phenolic compounds in coffee act as antioxidants (Hasbullah et al., 2021) while caffeine has an antibacterial effect (Pawar et al., 2011) VCO is one of the oils that has a good saponification effect and contains fatty acids in the form of lauric acid (Hutasuhut, 2019 Lauric acid plays a role in saponification as it has high solubility that leads to excellent lathering of soap products and can act as an antiviral and antibacterial agent (Widyasanti et al., 2017).

In the liquid soap formulation of Robusta coffee extract with VCO base, the growth of *S. aureus* bacteria is tested by testing the inhibition of bacteria with the disc diffusion method using nutrient agar (NA) media. The antimicrobial substance soaked in the test sample was absorbed using disc paper as a medium in the disc diffusion method. Following the inoculation of the agar medium with the test microbial culture, the disc paper was placed on top of it, and the mixture was incubated for 18 to 24 hours at 35°C. The clear zone around the disc paper was observed to indicate the presence or absence of microbial growth. During test the microbes are introduced to the disc paper, the diameter of the clear area or zone increases proportionately. The advantage of this method is that the tests can be performed faster when preparing the discs (Nurhayati et al., 2020). Against this background, researchers are interested in exploring antibacterial soap formulations from VCO-based extracts of Robusta green coffee beans against *S. aureus*.

Method

Materials and Chemicals

The natural materials used in this study were green coffee Robusta (*Coffea canephora* Pierre ex A. Froehner), plant identification by the enclosed certified herbarium, virgin coconut oil (BE Organic), and other ingredients such as 96% ethanol (Planet Kimia), Aquadest (Pure Water), sodium laureth sulfate (Planet Kimia), TEA Lauryl Sulfate (medicine and laboratory supplier), Sodium Chloride, DMDM Hydantoin (Kimia Jaya), Tetrasodium EDTA (Citra Kimia), Citric Acid, Glyceryl Stearate, Robusta Coffee Extract, Robusta Green Coffee Fragrance (Kimia Jaya).

Soap Making Process

The soap formulation is done by mixing virgin coconut oil (VCO) heated to a temperature of 75°C, then adding as much sodium laureth sulfate and mixing the lauryl sulfate, then stirring at a constant temperature of 75°C until a soap paste is formed, then prepare DMDM hydantoin, tetrasodium, citric acid. the distilled water is mixed and stirred at a temperature of 75°C, then the temperature is lowered to 40°C for the addition of the coffee extract, while the temperature is slowly lowered again until it reaches room temperature, and then the fragrance and sodium chloride are added and stirred with a stirrer for 15 - 30 minutes until a homogeneous soap paste is formed. The formulation of the antibacterial soap is shown in Table 1.

Physical Characteristic of The Liquid soap

According to SNI 06-3734-2006 (BSN,2006), testing the liquid soap's physical qualities includes an antibacterial efficacy test against *Staphylococcus aureus* bacteria as well as an organoleptic test, homogeneity test, pH analysis, viscosity test, foam stability test, and a test for *Staphylococcus aureus* microbes using the disc diffusion method.

Table 1. Formulations for antibacterial soap preparations (Rosmainar, 2021).

Materials	Formulation			
	Robusta Coffee Extract			
	F0	F1	F2	F3
Sodium laureate sulfate	7%	7%	7%	7%
Virgin Coconut Oil	5%	5%	5%	5%
TEA lauryl sulfate	2.5%	2.5%	2.5%	2.5%
Sodium Chloride	1.5%	1.5%	1.5%	1.5%
DMDM hydantoin	0.1%	0.1%	0.1%	0.1%
Tetrasodium EDTA	0.05%	0.05%	0.05%	0.05%
Citric acid	0.05%	0.05%	0.05%	0.05%
Glyceryl stearate	1.5%	1.5%	1.5%	1.5%
Robusta Coffee Extract	-	2%	4%	6%
Fragrance	0.2	0.2	0.2	0.2
Aqua water	82.1%	80.1%	78.1%	76.1%
Total	100%	100%	100%	100%

Organoleptic Test

The organoleptic evaluation was performed by visual observation of the liquid bath soap, including odor, color, and shape (Rosmainar, 2021).

Homogeneity Test

Consider how the homogeneity test is carried out: when smeared on a glass plate and rubbed, the liquid soap mixture should have a homogenous structure, meaning no components should feel like particles or lumps on the glass (Maharani et al., 2021).

pH Analysis

The pH analysis is performed with a pH meter. The pH meter is calibrated using a buffer solution before each measurement is made. Soaking the electrode that has been cleaned beforehand in the material to be examined. The pH value is recorded on the pH meter's scale (Ircham, et al., 2022). The pH test is required because the liquid soap formulation must adhere to the SNI criteria, which include a pH range of 8 to 11, and because the soap comes into touch with skin (Maharani et al., 2021).

Test of the Foam Height

To measure the height of the foam, a simple method is to put one gram of soap in a tube with ten milliliters of distilled water and cover it. Determine the height of the foam that forms after 20 seconds of shaking. The SNI has established guidelines for the height of the liquid soap foam, which range from 12 to 220 mm (Maharani et al., 2021).

Diffusion Method Test of Bacterial Activity

Spread the bacteria on the MHA medium with a sterile cotton swab. Then insert a paper disk soaked with Robusta coffee bean extract at a concentration of 2%, 4%, and 6%. Using sterile forceps, place paper disks soaked in robusta coffee bean extract at various concentrations as a positive control and negative control aseptically on the surface of the solidified medium. Then incubate the media at 37°C for 18-24 hours (Nurhayati et al., 2023).

Results and Discussion

Identification of The Plants

To determine the identity of the plants used, a plant identification was carried out. The plant identification was conducted at BRIN (National Research and Innovation Agency) Plant Conservation Center at Jl. Raya Jakarta – Bogor KM.46 Cibinong Bogor, West Java 169110. Based on the identification letter numbered B-1043/IV/DI/05-07/4/2022, it is known that the plants used for the study are true green coffee bean plants Robusta (*Coffea canephora* Pierre ex A. Froehner) from the Rubiaceae family (Appendix).

Phytochemical Screening Result

The extraction method used was a Soxhlet extraction method. A Soxhlet extraction method is an extraction method using heat. The soxhlet extraction process involves heating a mixture to separate the contents while circulating the solvent. Compared to the maceration method, a Soxhlet extraction method provides higher extract yields (Irianty and Yenti, 2014). Robusta coffee contains alkaloids, tannins, saponins, and flavonoids. Phenolic compounds contain chlorogenic acid, a non-volatile organic acid that can prevent the growth of gram-positive and negative bacteria. These antibacterial compounds work by penetrating the cells and damaging the structure of the bacterial cell wall. Coffee contains antioxidant compounds that play a good role in health, including protection against various diseases, such as protection against various soft tissue diseases caused by the invasion of bacteria, viruses, antigens, and others. These antioxidant compounds include caffeine, phenol, and chlorogenic acid (Suryanti et al., 2023). Table 2 shows the results of the analysis of secondary metabolites in ethanol extract of green robusta coffee beans.

Table 2. Secondary metabolites of green Robusta coffee beans

Test	Result
Alkaloid	+
Flavanoid	+
tanin	+
Terpenoid	+
steroid	+

(+) = positive

Result of the organoleptic test

This test aims to present liquid bath soap preparations using the five senses (smell, color, and shape) (Rosmainar, 2021). Evaluation of the quality of the preparations and the average results of the observations made are shown in Table 3 and Fig.1.

Table 3. Observation results of liquid soap preparations

Observation	Liquid Soap formulation			
	F_0	F1	F2	F3
Organoleptic	The smell of coffee, whitish brown	The smell of coffee, light brown	Coffee smell, coffee brown color	The smell of coffee, mocha brown

F0: 0% Robusta Green Coffee Extract Formulation
F1: 2% Robusta Green Coffee Extract Formulation
F2: 4% Robusta Green Coffee Extract Formulation
F3: 6% Robusta Green Coffee Extract Formulation

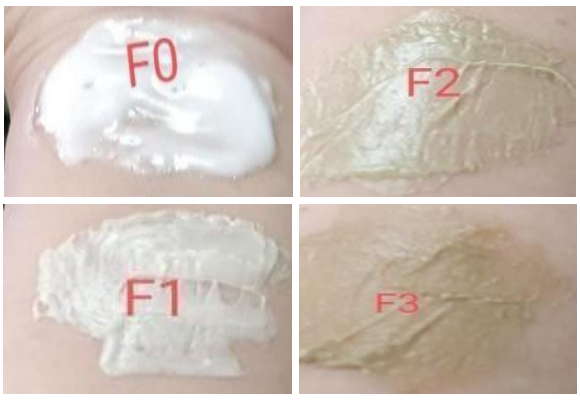

Fig.-1. The results of the organoleptic test

There are color variations at each concentration, as seen by Table 3 and Fig. 1, which show the organoleptic test results for the liquid soap made from Robusta green coffee bean extract. The color differences are because the concentrations of the extracts used in the formulation are different and lead to different color results. The resulting odor is the typical smell of coffee.

Result of The Homogeneity Test

The aim of the homogeneity test was to determine while the prepared liquid soap is homogeneous, that is, whether the additional ingridients are dispersed evenly (Nafisah, et.al., 2022), and whether the arrangements is homogenous and free of detectable grains (Table 4). The homogeneity test, which ran 6 weeks, revealed an equal formulation of liquid soap. The following characteristics are necessary for formulations of liquid soap.

Table 4. *Result of The Homogeneity Test*

Observation	Liquid Soap formulation			
	F_0	F1	F2	F3
Homogeneity	Homogeneous and free of coarse particles	Homogeneous and free of coarse particles	Homogeneous and free of coarse particles	Homogeneous and free of coarse particles

pH Test Result

The aim of the pH test is to evaluate the stability of a formulation of liquid soap. According to SNI 06-3734-2006, the pH value of liquid soap is in the range of 8-11, while a pH value that is too high can lead to skin dryness and, if it persists, to skin irritation such as itching, rash, redness, and scaling. This means that F0, F1, F2, and F3 meet the SNI requirements for liquid soap as they are within or below the specified interval limits. Statistically, the test of pH difference between the formulas using One Way ANOVA gives a significance value (0.447) > 0.05, which means that there is no difference in pH between F0, F1, F2, and F3. F0 shows a significant difference in pH to F1, F2, and F3, while there is no significant difference in pH between F1, F2 and F3. This indicates how the liquid soap's pH changes by adding more coffee extract (Table 5).

Table 5. pH test result

Observation	Liquid Soap formulation			
	F_0 ± SD	F1 ± SD	F2 ± SD	F3 ± SD
pH	8.1 ± 0.058	8.87 ± 0.189	9.08 ± 0.075	9.1 ± 0.00

Table 6. *Result of the test for antibacterial activity*

Formula	Zona Hambat (mm)	Description
F0	4.51 ± 2.489	resistant
F1	11.11 ± 0.573	resistant
F2	13.35 ± 0.021	intermediate
F3	20.57 ± 0.622	Susceptible
Control negative (ethanol)	0.00± 0.000	No inhibition
Control positive (kloramfenikol)	19.29 ± 0.019	Suspectible

Result of the foam height test

The height of liquid soap lather, according to SNI standards 06-3734-2006, is between 12 and 220 mm. Based on statistical analysis, the One Way ANOVA test for variations in foam height between formulas produced a significance value (0.000) <0.05, indicating significant differences between F0 (96.46mm), F1 (96.62 mm), F2 (98.4 mm), and F3 (98.4 mm). The Bonferroni test was used for the Post hoc analysis since the homogeneity test revealed a significant variation in foam height between F0 and F2 and F3, indicating that the data were not homogeneous (significance value > 0.05). This shows that increasing the coffee extract affects the foam height.

Result of the test for antibacterial activity

Staphylococcus aureus is a facultatively anaerobic spherical Gram-positive bacterium that has a diameter of 0.7-1.2 µm. It is grouped in irregular clusters similar to grapes, does not generate spores, and is immobile (Rahmi et al., 2015). The results of the morphology test by Gram staining are shown in Fig. 2.

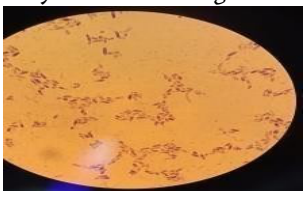

Fig.-2. *Staphylococcus aureus* bacterial staining

Bacterial inhibition reactions with an inhibition zone diameter of ≥18 mm are classified as Suspicious (Strong), Medium (13–17 mm), and Resistance (≤ 12 mm) (CLSI, 2018). Table 6 shows how the development of Staphylococcus aureus can be inhibited more when the concentration of Robusta green coffee bean extract is grew.

Conclusion

The organoleptic test results indicate differences in color at each concentration, with the extract concentration, which is higher, influencing the color and odor; the homogeneity test results demonstrate results that are homogenous and free of coarse grains; the pH test results demonstrate results that meet the requirements, specifically in the 8–11 range; the viscosity test results demonstrate results that meet the SNI requirements of 400–4000 Cps; and the foam height test results demonstrate stable results that meet the SNI requirements of 13-220 mm. Robusta coffee bean extract liquid soap preparation utilizing the disk diffusion method yielded the following results for the inhibition zone: F0 = 4.51mm (resistant), F1 = 11.11mm (resistant), F2 = 13.35mm (intermediate), and F3 = 20, 57mm (susceptible).

Conflict of Interests

The research and paper do not contain any conflicts of interest, as stated by the author (s).

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