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CHARACTERIZATION OF ORGANIC SOAP SYNTHESIZED FROM *Samia cynthia ricini* COCOON EXTRACT AND ITS ANTIBACTERIAL POTENTIAL

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ABSTRACT

In a modern era that increasingly values health and sustainability, the demand for natural and environmentally friendly personal care products continues to grow. One response to this trend is the development of organic soaps produced from various natural ingredients. In this study, organic soap using *Samia cynthia ricini* silkworm cocoon extract has been synthesized. This study aims to assess the quality of organic soap synthesized from silk cocoon extract based on the Indonesian National Standard (SNI) quality criteria and its antibacterial potential. Silk cocoon soap is made from a mixture of silk cocoon extract with palm oil, coconut oil, olive oil, sodium hydroxide, and water. The quality measurements include organoleptic tests, pH, moisture content, total fat, insoluble substance in ethanol, and free fatty acids, based on SNI No. 4085: 2017 and 3532: 2016. Antibacterial testing is conducted by disc diffusion. The results showed that the synthesized silk cocoon soap has a yellowish-white color, a hard density, and no odor. The quality criteria of the soap meet the established standards, with a pH of 9.66, a water content of 11.47%, total fat of 75.00%, ethanol-insoluble substances of 0.52%, and free fatty acids of 1.58%. The soap also demonstrates antibacterial potential that describes by a larger diameter of the inhibitory area against *Staphylococcus aureus* bacteria.

INTRODUCTION

Soap is a product utilized for cleanliness and maintaining skin health. In recent decades, there has been a growing public interest in natural and organic products due to heightened awareness of the detrimental effects of chemicals on health and the environment. Consequently, organic soap has become more popular among those seeking safer and more sustainable alternatives. Indonesia, a country rich in natural resources, possesses significant potential to produce various alternative materials, with silk cocoons as an example. While silk cocoons have traditionally served as primary raw materials for textiles, the untapped potential exists in utilizing them for soap production. This innovative approach could lead to the creation of organic soap, promoting safety for health. Such a transition is supported by the recognition that the protein content inherent in silk cocoons provides excellent antibacterial and antioxidant properties for the skin (Ulfa et al., 2020). Sericin, a protein component of silk cocoons, has been identified as a major contributor to the antibacterial properties of silk cocoon extracts. Sericin is known to have antimicrobial properties, which are essential for protecting the silkworm from biotic and abiotic hazards during its immobile pupal phase (Kaewkod et al., 2024; Biganeh, et al., 2022)

Soap is a skin cleansing bar or liquid made from the saponification or neutralization process of fats, oils, waxes, rosin, or acids with organic or inorganic bases without causing irritation to the skin (BSN, 2016). As an ingredient applied to the body, a soap must undergo a series of tests to verify that it meets quality standards for safe application. Additionally, quality testing is crucial because the results of these tests can determine whether the product can be manufactured on a larger scale (Cahyaningsih et al., 2019). The quality standards established by the Indonesian National Standards (SNI) 3532:2016 for bathing soap require a maximum water content of 15%, a minimum total fatty matter of 65%, a maximum of 2.5% free fatty acids measured as Oleic Acid, a maximum of 5.0% ethanol-insoluble material, and a maximum chloride content of 1% (BSN, 2016).

In a previous study, the quality of organic soap with a citronella oil additive was tested. As a result, it found 12.8% of water, 0.205% of free fatty acid, and a pH of 9.87 (Jalaluddin et al., 2019). Another study tested the quality of transparent solid soap made with palm oil and white tea extract as an active ingredient (*Camellia sinensis*). The test results indicated that the quality of the soap adhered to SNI solid bath soap No.06-3532-1994, except for the fatty acid content. The soap contained 12.17% water, 0.101% free alkali, 2.10% unsaponifiable fraction, and 35.67% fatty acid (Widyasanti et al., 2017). In this study, organic soap was successfully synthesized with the addition of *Samia cynthia ricini* silk cocoon extract. The soap derived from these silk cocoons must undergo a series of tests to ensure its quality meets the established standards (BSN, 2016). Therefore, soap quality testing including organoleptic evaluation, pH measurement, water content analysis, total fat assessment, ethanol-insoluble substance examination, and free fatty acid testing, is conducted to meet the quality requirements specified in the Indonesian National Standards (SNI) No. 4085:2017 and 3532:2016. Additionally, antibacterial testing is performed to assess the antibacterial potential of the soap using the disc diffusion method.

MATERIAL AND METHODS

Instrument and Materials

The materials used in this study are silk cocoon extract were obtained from silkworm farm *Samia cynthia ricini* in Lawang, Malang, East Java. olive oil, coconut oil, palm oil, sodium hydroxide (NaOH), sulfuric acid (H_2SO_4), potassium hydroxide (KOH), petroleum ether, phenolphthalein, methyl orange, distilled water, and *Staphylococcus aureus* bacteria were were collected from the biology laboratory of the Faculty of Mathematics and Natural Sciences at Universitas Negeri Malang. The instrument used in this research are a silicone soap mold, oven, desiccator, stirring rod, hand blender, extraction apparatus, beaker glass, analytical balance, pH meter, autoclave, and thermometer.

Methods

Silk Cocoon Soap Preparation

The soap was made by dissolving silk cocoons in sodium hydroxide solution and adding this to the oil mixture. The oils used were coconut oil, olive oil, and palm oil. These three types of oils were mixed and stirred while heated to 70^0C temperature. The oil mixture was then cooled out to room temperature. Next, the silk cocoon extract dissolved in sodium hydroxide solution was gradually added to the oil mixture, stirring until homogeneous and a trace occurs (a thickened soap mass solution). The soap mass, which was still liquid, was poured into molds and allowed to stand for 24 hours until it hardens. The composition of the ingredients is detailed in Table 1.

Characterization of soap

Organoleptic Test

The organoleptic test was conducted by observing the soap samples' shape, color, and smell on storage for two weeks.

pH Measurement

The pH measurement was carried out following SNI 4085:2017. Initially, the soap sample was mashed. Subsequently, one gram of the mashed soap was put into a 1000 mL volumetric flask, and then distilled water was added until reaching the mark. After sealing the volumetric flask, the solution was homogenized. The resulting soap solution was transferred to a beaker, and the pH of the solution was measured using a calibrated pH meter.

Water Content Measurement

The water content of the soap bar was determined using the gravimetric method following SNI 3532:2016. Five grams of soap bar sample were placed into a petri dish that had been dried in an oven at 105^0C for 30 minutes. Subsequently, the soap sample was heated in an oven at 105°C for 1 hour. Following this, the soap sample was cooled in a desiccator and weighed. The measurements were repeated until a constant weight was achieved.

Total Fat Test

Five grams of soap was dissolved in 100 mL of hot distilled water with a temperature between 70-80°C, then poured into a separating funnel. A few drops of methyl orange solution were added, and the mixture was shaken. Excess H_2SO_4 was added during shaking, followed by additional shaking and cooling to around 25°C. Subsequently, 100 mL of petroleum was added and the mixture was shaken again until a distinct separation of liquid layers was achieved. The liquid layer in the petroleum solvent was transferred to a chemical glass container, while the liquid in the separating funnel was re-extracted with 50 mL of petroleum solvent and repeated. The petroleum solvent extract was collected and washed with 25 mL of distilled water until neutral.

The resulting solvent extract was separated from the washings.

The petroleum solvent extract was evaporated using a water bath. The resulting residue was dissolved in 20 mL of 95% ethanol and titrated with alcoholic KOH using a phenolphthalein indicator until it turned pink. The titration solution was then evaporated in a water bath until the alcoholic solution disappeared, forming a thin layer of soap. This process was continued by heating at a temperature of 105°C for 15 minutes, followed by cooling and weighing. Lastly, the heating and cooling processes were repeated until the weight difference did not exceed 3 mg (BSN, 2016).

Ethanol-Insoluble Substances Test

Five grams of soap was dissolved in 200 mL of neutral ethanol in an erlenmeyer flask with a ground-glass stopper attached to an upright condenser. Then, it was heated in a water bath until the soap was completely dissolved. After complete dissolution, the liquid was poured onto pre-dried filter paper, weighed, and arranged with a vacuum pump. The residue on the filter paper was washed with neutral ethanol until entirely soap-free. Subsequently, the filter paper and the residue were dried in an oven for 3 hours at a temperature of 105°C, then left to cool and weighed (BSN, 2016).

Free Fatty Acid Test

Determination of free fatty acids was done by titrating the filtrate from the measurement of ethanol-insoluble substances with a standard alkali solution using phenolphthalein indicator, based on SNI 3532:2016. The testing began with heating the filtrate in a 250 mL erlenmeyer flask. Then, 0.5 mL of 1% phenolphthalein indicator was added and titrated with 0.1 N alcoholic KOH solution until a stable pink colour was formed.

Antibacterial Test

The antibacterial test on solid soap with silk cocoon extract started with the preparation of a nutrient agar medium, which was then autoclaved for sterilization. The test bacteria, Staphylococcus aureus, were isolated and cultured on an agar medium until they reached the desired growth phase. Solid soap containing 12% silk cocoon extract was prepared and transformed into discs or small pieces. Silk cocoon extract was aseptically injected into the soap discs using a sterile syringe. The injected discs were left to absorb the extract for several hours before being placed on the prepared agar medium. This process was followed by incubating the agar plates at a temperature and conditions suitable for the growth of the test bacteria. After incubation, bacterial growth inhibition zones around the soap discs were observed and measured. Positive controls (with standard antibiotics) and negative controls (without silk cocoon extract) were also compared in each experiment (Aminah et al, 2018).

RESULT AND DISCUSSION

The silk cocoon soap was synthesized using the active ingredient of silk cocoon extract, the composition of which can be seen in Table 1. In its production process, soap undergoes saponification, which is the hydrolysis reaction of triglycerides (fats or oils) using an alkaline solution, typically sodium hydroxide solution. Generally, the preparation of soap involves heating animal fat or vegetable oil in an alkaline environment (sodium hydroxide), followed by hydrolysis into carboxylate salts and glycerol, as illustrated in Figure 1 (The Royal Society of Chemistry, 2017).

Table 1. Composition of Silk Cocoon Soap Ingredients

No	**Measurement**	**Mass (g)**	
		F1 (Without Silk Cocoon Extract)	**F2 (With Silk Cocoon Extract)**
1	Silk cocoon	0.00	1.20
2.	Coconut oil	13.00	13.00
3.	Palm oil	12.00	12.00
4.	Olive oil	35.00	35.00
5.	Sodium Hidroxyde (NaOH)	8.34	8.34
6.	Destilled Water	19.45	19.45

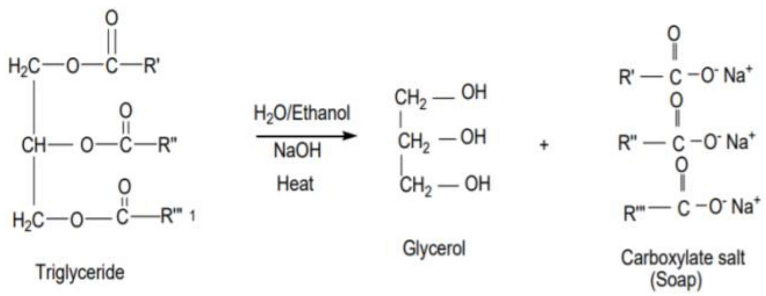

Figure 1. Saponification Reaction

The production of silk cocoon soap involves three types of oils: coconut oil, palm oil, and olive oil. Coconut oil is a semi-solid, white fat obtained from the flesh of coconuts. It contains short to medium-chain fatty acids, around 57% being caprylic acid (C8) and lauric acid (C12). Lauric acid exhibits antibacterial, antiviral, and antifungal properties(Riadi et al., 2020). Lauric acid also helps in maintaining skin moisture. Palm oil, with a relatively high content of palmitic acid (44.3%), contributes to the hardness of the soap and creates a stable lather(Sukawaty et al., 2016). Many users believe soap with abundant lather has better cleansing capabilities. Concurrently, adding olive oil can enhance the moisturizing effect of the soap. Each oil type plays a distinct role in the soap production process, contributing to its overall qualities and benefits for skincare.

This organic soap is made by adding silk cocoon to a sodium hydroxide (NaOH) solution and mixing it into a blend of oils that have been heated to a temperature of 70°C. NaOH is commonly applied in solid soap production because it imparts sparingly soluble properties in water (Kuo et al., 2020; Octora et al., 2020). Heating is performed to expedite the saponification reaction (Tarun et al., 2014).

Organoleptic Test

Organoleptic observations, including colour, shape, and aroma, were conducted on the soap after a storage period of 14 days. This storage time is necessary to ensure that the saponification reaction is complete, where the alkali base has fully reacted with the fatty acids (Sukeksi et al., 2021). The silk cocoon extract soap produced has a bar-shaped form with a relatively hard texture, appearing white to pale yellow, and it does not have the distinctive unpleasant odor characteristic of silk cocoons. The organoleptic observations indicate that the silk cocoon soap has a good appearance and aroma. Additionally, the soap containing silk cocoon extract is superior to those that do not contain the extract. The silk cocoon soap produced is presented in Figure 2.

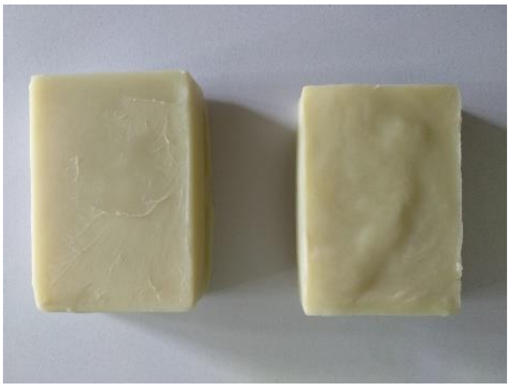

Figure 2. Silk Cocoon Extract Organic Soap

pH Measurement

The acidity level or pH is one of the crucial parameters in testing the suitability of soap for use. Measuring the pH of soap aims to determine its pH value to ensure it does not harm the skin (Vivian et al., 2014). The pH value of soap can affect the skin's response and is influenced by the amount of alkali in the soap. The higher the amount of alkali in the soap, the higher its pH will be. High alkali content can harm the skin by eroding the natural acidic mantle as a protective layer. Typically, the pH of the human skin surface ranges from 5 to 6.5, indicating slightly acidic properties (Febriani et al., 2020). Therefore, soap pH should not be too high (alkaline) as it can lead to dry skin and kill microorganisms that play a role in maintaining skin surface health (Betsy et al., 2021).

The research showed that the pH of the soap without silk cocoon (F1) was 9.57, and with the addition of silk cocoon (F2) was 9.66. The Indonesian National Standard (SNI 4085:2017) sets the safe pH range for soap at 4-10. Thus, the silk cocoon soap produced meets the quality requirements for safe use. This result is consistent with several studies indicating that soap typically has a pH range from 9.01 to 10.00, with only a few soap products having a pH matching that of the skin (Betsy et al., 2021).

Water Content Measurement

Water content analysis is performed to determine the amount of water present in a substance. Moreover, the water percentage serves as a criterion for evaluating the product's durability over time. Soap with high water content tends to experience quicker shrinkage during use, whereas soap with low water content may extended shelf life. Furthermore, the duration of soap storage can also affect its hardness as the water content evaporates over time (Vivian et al., 2014).

According to the findings, the proportion of water content in F2 soap was 11.47%, while F1 soap had slightly lower in percentage at 9.95%. The result represented the addition of silk cocoon extract increases the water content of the final solid soap. Notably, the percentage of water content aligned with the quality criteria for soap according to SNI 3532:2016, which allow a maximum limit of 15.00%.

Total Fat Test

Total fat is the amount of fat in soap that can be separated from the soap sample using a mineral acid such as HCl. The fatty acids typically found in soap include oleic acid, stearic acid, and palmitic acid (Betsy et al., 2021). The quality of total fatty acids is considered good if it exceeds 65.00% (SNI 3532:2016), and this parameter also related to the hardness of the soap. Meanwhile, low total fat that does not meet SNI standards tends to produce soft soap with minimal lather and poor durability, resulting in low-quality soap (Betsy et al., 2021). The reduction of total fat is caused by the water/soap ratio, where the proportion of soap composition decreases with high amount of water that are used (Betsy et al., 2021).

The result indicated that soap containing cocoon extract has 75.00% total fat. This result met the quality criteria of soap according to SNI standards. Compared to the total fat in soap without silk cocoon extract, which was 71.00%, it illustrated that adding silk cocoon extract to the soap increases the total fat content. The addition of silk cocoon extract can affect the total fat in the soap produced due to linoleic fatty acids in silk cocoon. Consequently, the greater the amount of silk cocoon extract added, the higher the total fat content in the produced soap.

Ethanol-Insoluble Substance Test

Another parameter tested is the ethanol-insoluble substance, an essential parameter for determining soap's purity. Soap with high levels of insoluble substance in ethanol show that it contains high levels of impurities caused by the levels of alkaline impurities used in soap ~~making~~ preparation (Vivian et al., 2014). Ethanol-insoluble substances include non-soap materials such as sodium silicate, sodium phosphate, and sodium carbonate and minor constituents like bleaching and brightening agents in the produced product (Popescu et al., 2011).[A1] The Indonesian National Standard sets the maximum allowable level of ethanol-insoluble substances at 5% (SNI 3532:2016).

The examination result of the ethanol-insoluble substance in F1 soap was 1.92%, and in F2 soap was 0.52%. The percentage of ethanol-insoluble substances in silk cocoon soap was almost three times lower than in the soap without silk cocoon extract. This describes that adding silk cocoon extract can reduce the number of impurities in soap. This result is in accordance with SNI standards and meets the quality requirements for safe soap.

Free Fatty Acid Test

Free fatty acids are acids in a soap sample that are not bound as sodium compounds or triglycerides. In other words, these fatty acids have not undergone saponification reactions and are still present in the soap. Free fatty acids are unwanted components in the cleaning process because a high percentage of them in soap can reduce the cleaning power. If free fatty acids are present in soap, they will bind to them when used. As a result, the soap's ability to bind or clean oil from oily substances is reduced (Jalaluddin et al., 2019).

In this study, the free fatty acid content in F1 soap is 2.61%, while in F2 soap, it is 1.58%. This result illustrates that the addition of silk cocoon extract aids in improving the saponification process, as indicated by the lower content of free fatty acids obtained. This content meets the quality requirements for bathing soap according to the SNI standards, with a percentage below 2.5%. Overall, the soap quality testing results can be seen in Table 2.

Table 2. Characteristics of Silk Cocoon Soap

Characteristics of Soap	SNI Standard	Soap Test Result	
		F1 (without silk cocoon extract)	**F2 (with silk cocoon extract)**
pH	4 – 10	9.58	9.66
Water content	max. 15%	9.95%	11.47%
Total fat	min. 65%	71.00 %	75.00%
Ethanol-Insoluble Substance	max. 5%	1.92%	0.52%
Free Fatty Acid	max. 2,5%	2.61%	1.58%

Antibacterial Potential Test

After conducting the feasibility test of soap based on SNI for solid soap, further testing is carried out regarding the antibacterial properties of silk cocoon soap. Studies have shown that silk cocoon extracts exhibit antibacterial activity against various bacteria, including skin pathogenic bacteria like *Staphylococcus* aureus, methicillin-resistant *Staphylococcus* aureus (MRSA), *Corynebacterium acnes*, and *Pseudomonas aeruginosa* (Kaewkod et al., 2024; Biganeh, et al., 2022). Previous research has shown that silk cocoons have antibacterial properties due to the presence of sericin (Ulfa et al., 2020). Sericin, a protein component of silk cocoons, has been identified as a promising antibacterial agent. It contains 18 amino acids, including serine, histidine, glycine, threonine, tyrosine, aspartic acid, and glutamic acid, which contribute to its antioxidant and antibacterial properties (Wang et al., 2021).

Sericin can be extracted using various methods. Physical methods include hot water degumming, where silk cocoons are boiled in hot water to extract sericin. Enzymatic methods involve the use of enzymes to break down sericin. Chemical methods include the use of alkaline or acidic solutions, soap, and organic solvents to extract sericin. Hydrothermal methods involve the use of high temperatures and pressures to extract sericin. Each method has its advantages and limitations, and the choice of method depends on the desired properties of the extracted sericin. For instance, hot water degumming can result in higher extraction yields and better sericin quality, while enzymatic methods can be more specific and efficient. Chemical methods can be more efficient and cost-effective but may also damage the sericin protein and result in lower extraction yields. Hydrothermal methods can be more environmentally friendly and result in higher extraction yields at higher temperatures. The choice of method can significantly impact the final product and its potential applications (Seo et al., 2022; Bungthong et al., 2021; Rangi, 2015; Yonesi, 2021; Wang, 2021).

The antibacterial testing of organic soap is conducted against *Staphylococcus aureus* bacteria using the Agar Diffusion method. *Staphylococcus aureus* bacteria are closely associated with the human body, particularly on the skin, as they can cause infections or damage to the skin or other organs if it overcome the body's defences. (Widyastuti et al., 2019). Due to this association, *Staphylococcus aureus* bacteria are used in this testing. Antibacterial testing of organic soap was performed against *Staphylococcus aureus* bacteria using the Agar Diffusion method. In addition to testing silk cocoon soap, a comparison was made with soap that did not contain silk cocoon extract. The results illustrated that the Diameter of the Inhibition Zone (DIZ) for organic soap with silk cocoon extract is larger than solid soap without silk cocoon extract.

The DIZ for organic soap with silk cocoon extract is 0.54 cm, while the DIZ for soap without silk cocoon extract is 0.48 cm. This suggests that the chemicals or enzymes in the silk cocoon extract can act against attackers such as microbes. This is likely because some fundamental amphipathic polypeptides lyse microorganisms, leading to their death

CONCLUSION

Organic soap has been successfully synthesized with silk cocoon extract. The quality of silk cocoon soap complies with the SNI 3532:2016, as indicated by the pH values, water content, total fat, ethanol-insoluble substances, and free fatty acids. In addition, silk cocoon soap demonstrates antibacterial potential against *Staphylococcus aureus* bacteria.

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