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Effectiveness of Solid Antibacterial Soap Formulated with Green Mussel Shell Waste (*Perna viridis*) against *Staphylococcus aureus*

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ABSTRACT: *Staphylococcus aureus* is a major cause of skin infections and remains a public health concern due to its high prevalence and the increasing emergence of antibiotic-resistant strains such as methicillin-resistant S. aureus (MRSA). These conditions highlight the importance of preventive approaches using non-antibiotic topical products. This study aimed to evaluate the antibacterial effectiveness of solid soap formulated with green mussel shell powder (*Perna viridis*), as well as its physical quality and consumer acceptance. A true experimental posttest-only control group design was applied using five formulations: F I (2%), F II (4%), F III (6%), F IV (0%, negative control), and F V (benzalkonium chloride as positive control). Antibacterial activity was assessed using the disc diffusion method, and the data were analyzed using one-way ANOVA. The results showed a concentration-dependent antibacterial effect, with higher concentrations of green mussel shell powder producing greater inhibition against *S. aureus*. All formulations met physical quality standards and demonstrated good stability and homogeneity. Sensory evaluation indicated high consumer acceptance across all formulations. Overall, these findings suggest that green mussel shell powder has strong potential as a sustainable and effective antibacterial ingredient for solid soap formulations intended for preventive skin hygiene.

Keywords: Antibacterial soap; Green mussel shell; *Staphylococcus aureus bacteria*

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INTRODUCTION

Skin infections caused by bacteria, especially *Staphylococcus aureus*, are a common health problem in many countries, including Indonesia. These bacteria can cause disorders ranging by itching, abscesses, impetigo to serious infections such as cellulitis (AL-kahfaji, 2022). In Southeast Asia, *S. aureus* and its methicillin-resistant variants (MRSA) are widely disseminated, with MRSA prevalence among *S. aureus* infections reported to range between approximately 20% and 30% across the region, reflecting a significant public health burden (Farook et al., 2022). Research in Java and Bali reported 45.3% of skin and soft tissue infection cases were caused by *S. aureus*, by 3.1% of them being MRSA (Santosaningsih, D., 2018). The high prevalence of skin infections, combined with the emergence of antibiotic-resistant strains such as MRSA, highlights that conventional antibiotic-based treatment alone is insufficient and must be complemented by effective preventive strategies.

Antibiotic resistance such as MRSA adds challenges to treatment so prevention is an important aspect (Pal et al., 2020). One of the prevention efforts is to maintain body hygiene by antibacterial soap. Topical antibacterial products, particularly soaps, play a crucial role in reducing bacterial load on the skin surface and minimizing the risk of infection transmission without contributing directly to systemic antibiotic resistance (Caioni et al., 2023). Green mussel shells *(Perna viridis)*, that have been considered waste, actually contain bioactive compounds by antibacterial activity (Prihanto et al., 2024; Rahman et al., 2024).

Previous studies have reported the antibacterial potential of shell-derived materials; however, most investigations focus on extracts or powders tested directly against bacteria, rather than their incorporation into practical consumer products such as solid soap formulations. The utilization of green mussel shells in solid soap formulations made by natural ingredients not only has the potential to inhibit the growth of *S. aureus*, but is also more environmentally friendly and safe to use (Choi et al., 2024; Ogedengbe, 2019)

Despite growing interest in natural and sustainable antibacterial agents, there remains a lack of studies that systematically evaluate the effectiveness of green mussel shell waste when formulated into solid antibacterial soap matrices, particularly against Gram-positive pathogens such as *Staphylococcus aureus*. This gap limits the translation of laboratory findings into applicable hygiene products. Integrating antibacterial performance with sustainability considerations is therefore essential, as the valorisation of aquatic shell waste not only addresses public health needs but also contributes to environmental waste reduction and circular economy principles.

Based on this, this study was conducted to evaluate the effectiveness of antibacterial soap made by green mussel shells against *Staphylococcus aureus* as an innovative effort in the prevention of skin infections as well as sustainable utilization of aquatic waste. Accordingly, this study aims to fill the identified research gap by assessing both the antibacterial activity and practical applicability of green mussel shell waste in solid soap formulation as a preventive, non-antibiotic, and sustainable approach to skin infection control.

METHODS

This type of research is included in experimental research. In the experimental group, antibacterial soap containing green mussel shells *(Perna viridis)* was used, while the

control group consisted of BKC (positive control) and aquadest (negative group). This experimental approach was designed to allow direct comparison of antibacterial activity between treatment and control groups under controlled laboratory conditions.

In this study, the design used was *The Posttest-Only Control Design*, *The Posttest-Only Control Design* design does not include a *pre-test*, but only conducts a *post-test* as a comparative measure of the effectiveness of the inhibition of green mussel shell soap *(perna viridis)* against the growth of *Staphylococcus aureus* bacteria. All antibacterial tests were performed under standardized in vitro conditions, where bacterial suspensions were prepared at uniform concentrations prior to testing.

Green mussel shells used as the raw material source, while the experimental units consisted of the formulated soap samples and bacterial cultures used in antibacterial testing. The samples used in this study were green mussels *(Perna viridis)* obtained in the waters of Pacitan, East Java. This location was selected as a representative source of green mussel shell waste. Calculation of the number of samples in each treatment used in this study utilizing the *Federer* formula and the resulting number of samples in each treatment group amounted to 4 samples. The number of replications was determined to provide sufficient data for statistical analysis of antibacterial activity.

This research was conducted at the Pharmaceutical Technology Laboratory, Natural Materials Chemistry Laboratory and Microbiology Laboratory of Kusuma Husada University Surakarta. This research was conducted in May-June 2025. The tools used in this study include test tubes, dropper pipettes, vortex, Bunsen, matches, ose, iron spatulas, Petri dishes, tube racks, scales, autoclaves, aluminum foil, sterile cotton swabs, erlenmeyers, time meters, incubators, rulers / vectors, paper discs, labels, McFarland 0.5%, tissue, tweezers and plastic wrap. The materials used in this study were Mueller Hinton Agar (MHA) media, NaCl, sterile distilled water, alcohol, *Staphylococcus aureus* culture, and soap made by green mussel shells *(Perna viridis)* to be tested.

Prior to formulation, green mussel shells were washed under running water, boiled for approximately 30 minutes, oven-dried at 105°C for 24 hours, ground into powder, and sieved using a 100-mesh sieve to obtain uniform particle size. The shell powder was sterilized at 160°C for 2 hours before being incorporated into the solid antibacterial soap formulation.

Antibacterial activity was evaluated using the disc diffusion method. Sterile paper discs were immersed in the test soap solution for 15 minutes, then placed on the surface of Mueller Hinton Agar inoculated with Staphylococcus aureus suspension adjusted to a turbidity standard of 0.5 McFarland. The plates were incubated at 37°C for 24 hours, and the diameter of the inhibition zones was measured using a caliper. The antibacterial activity was categorized based on the criteria of Davis and Stout, namely weak (<5 mm), moderate (5–10 mm), strong (10–20 mm), and very strong (>20 mm).

The hedonic (sensory acceptance) test was conducted to evaluate consumer preference toward the formulated soaps. A total of 20 adult panelists participated voluntarily in this study. The assessment was performed using a binary preference scale (Like/Dislike). The evaluated parameters included color, aroma, shape, and foam. Each panelist was asked to indicate their preference for each formulation and parameter. The results were expressed as the percentage (%) of panelists selecting "Like" for each criterion.

The data collected by this study were analyzed utilizing SPSS software version 22.0, utilizing one-way ANOVA. Data were considered significant if the p value was <0.05. Prior to ANOVA, the data were tested for normality and homogeneity of variance to ensure that the assumptions for parametric analysis were fulfilled. Post hoc analysis was conducted to identify significant differences among treatment groups.

RESULT AND DISCUSSION

Physical Quality of Preparations

Table 1 shows that based on the organoleptical test, the formulated soaps, both formulas 1, 2, and 3, do not have a different physical appearance. Based on the shape, color, and aroma, the formulated soap has met the standards as a general soap. These findings indicate that incorporating green mussel shell powder does not adversely affect the basic organoleptic characteristics of solid soap compared with conventional formulations. Similar observations have been reported in previous studies on natural-based antibacterial soaps, where the addition of mineral or shell-derived materials did not alter consumer-perceived physical quality (Rifkowaty et al., 2023; Neswita, 2021).

Table 1. Organoleptical Test Results

Formulation	Organoleptical Test		
	Shape	Color	Odor
F I (2%)	Solid	White	Aromatic
F II (4%)	Solid	White	Aromatic
F III (6%)	Solid	White	Aromatic
F IV (0%)	Solid	White	Aromatic

By Table 2, the results show that based on the stability test of the formulated soap, both formulas 1, 2, 3 and 4 are stable. This means that the three formulations even though they are stored for a long time do not change in terms of shape, color and aroma. As for the pH of antibacterial soap bars, it is in the range of 10.06 to 10.94. The formula devoid of extract (F IV) has the highest pH of 10.94, while the formula by the addition of green mussel shell powder (F I, F II and F III) shows a lower pH, ranging by 10.06-10.10. Furthermore, for the homogeneity test results, the formulated soaps, both formulas 1, 2, 3 and 4, are homogeneous. Taken together, these physical quality parameters demonstrate that the formulation process produced stable, homogeneous, and visually acceptable solid soaps.

Table 2. Stability, pH, and Homogeneity Test Results

Formula	Stability	pH*	Homogeneity
F I (2%)	Stable	10,10 ± 0.04	Homogeneous
F II (4%)	Stable	10,06 ± 0.05	Homogeneous
F III (6%)	Stable	10,06 ± 0.03	Homogeneous
F IV (0%)	Stable	10,94 ± 0.06	Homogeneous

*Mean ± SD, n =4. SD Standard deviation

The pH value, homogeneity, and physical stability of the soap not only reflect formulation quality but also play an important role in skin compatibility and antibacterial performance. Good homogeneity ensures a uniform distribution of active compounds, supporting consistent antibacterial activity across the soap matrix (Purwani et al., 2025). Physical stability during storage is also critical to ensure that antibacterial effectiveness is

maintained during actual use (Fatchiyah et al., 2023). Although the pH values fall within the Indonesian National Standard (SNI) for solid soap (pH 9–11), a pH approaching 11 may potentially cause irritation, particularly on sensitive skin when used repeatedly. Therefore, this soap is better positioned as an antiseptic soap for short-contact use, and further studies are needed to reduce the formulation pH or incorporate moisturizing agents to enhance skin safety.

The results of the organoleptic test revealed that all formulas had a solid form resembling commercial soap by a uniform milky white color and aromatic aroma that the panelists liked. This indicates that the mixing process is homogeneous and that the saponification reaction proceeds smoothly. The milky white color likely comes by the coconut oil and olive oil base devoid of the addition of synthetic dyes. In addition, the soap's aroma masked the distinctive smell of green mussel shell powder, thanks to the use of stable natural fragrances in the formulation.

The stability of the preparation was well maintained during storage, as evidenced by no change in color, odor, or texture, and no microbial contamination. This shows that the soap is able to maintain the quality and pharmacological activity of the active ingredients in it, so it has the potential to be developed into a safe and effective antibacterial product (Łusiak et al., 2024; Neswita, 2021). The pH test results revealed values ranging by 10.06-10.94 by an average of 10.29, that is still in accordance by SNI standards for solid soap (pH 9-11) (Panaungi et al., 2022). Although the soap is alkaline, the value is still safe for the skin because it is inside of the tolerance range, although it should be noted that too high a pH can disrupt the skin's protective layer (Ainun & Sylvia, N., 2024).

In addition, all formulas showed good homogeneity, with no separate particles or deposits in the soap. Homogeneity is important to ensure even distribution of active ingredients so that antibacterial activity against *Staphylococcus aureus* can take place optimally. Homogeneous products also provide a consistent aesthetic appearance and smooth texture, that improves the quality of the preparation (Wiyono et al., 2023). Overall, the physical quality results confirm that green mussel shell powder can be incorporated into solid soap formulations without compromising stability, safety, or aesthetic properties. Compared with similar natural soap formulations reported in the literature, the physical stability observed in this study is comparable or superior, indicating good formulation robustness.

Identification of Test Bacteria

The staining results (Table 3) in this study revealed that the test bacteria were purplish blue by a clustered cocci shape, that is in accordance by the characteristics of *Staphylococcus aureus* as a Gram-positive bacterium(Atmanto et al., 2022; Hamidah et al., 2019). The purple color that appears is due to the thick peptidoglycan layer on the Gram-positive cell wall, which retains crystal violet dye despite the alcohol's decolorization process (Figure 1).

Table 3. Bacterial Staining Test Results

Sample	Bacterial Color Test	Result	Types of Bacteria
Staphylococcus aureus	Gram Method (*Crystal violet*, Iodine, Alcohol and Safranin)	Purple	Gram Positive

Determining the type of bacteria is important because the structure of the cell wall affects the mechanism of action of antibacterial compounds. Gram-positive bacteria are generally more susceptible to agents that can damage the peptidoglycan layer than Gram-negative bacteria that have an additional outer membrane (Adnan et al., 2023). The selection of *S. aureus* as the test bacteria in this study is appropriate, given that this pathogen is often found on human skin and is a common cause of skin infections. In addition, the cosmopolitan and environmentally adaptable nature of *S. aureus* makes it a relevant model for assessing the effectiveness of natural-based antibacterial soaps (Hasbi & Rosyunita, 2024). Accordingly, Gram staining in this study served as a confirmatory step to support the relevance of subsequent antibacterial activity evaluation rather than as a primary analytical outcome.

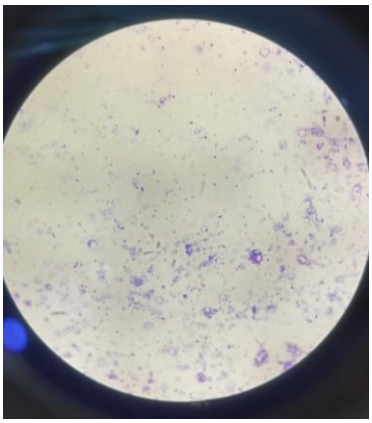

Figure 1. Bacterial Staining Result

Antibacterial Activity of Soap Formulation

Based on the results of the bacterial inhibition test drawn in Table 4, it is known that formulas by different concentrations give varying inhibition zone diameters against bacterial growth. Formula F0 (devoid of the addition of active substances) revealed the lowest inhibition by an average inhibition zone diameter of 10.87 mm. Increasing the concentration of active ingredients in formulas FI (2%), FII (4%) and FIII (6%) revealed a consecutive increase in inhibition by an average of 13 mm, 15.5 mm and 19.5 mm, respectively. This reveals the effect of concentration on antibacterial effectiveness.

Table 4. Inhibition Test Results

Formula	Bacterial Inhibition (mm)*	Category
F I (2%)	13.00 ± 0.41	Strong
F II (4%)	15.50 ± 0.41	Strong
F III (6%)	19.50 ± 0.41	Strong
F IV (0%)	10.87 ± 0.48	Medium
F V (BKC)	41.75 ± 6.65	Very Strong
F (Normal)	0.0 ± 0.00	Weak

*Mean ± SD, n =4. SD Standard deviation

The positive control showed the highest inhibition, with an average of 41.75 mm, while the negative control showed no inhibition zone (0 mm), indicating the absence of antibacterial activity. Statistical analysis using one-way ANOVA further confirmed that

formulation concentration had a significant effect on antibacterial activity ($p < 0.05$). This finding is consistent with previous studies reporting that increased concentrations of active substances contribute to stronger microbial inhibition (Asjur et al., 2025; Hayati et al., 2023).

Although the inhibition zones observed in this study fall within the moderate to strong category and do not exceed those of synthetic antiseptics, they indicate that shell-derived materials exhibit meaningful antibacterial potential. The antibacterial activity observed may be attributed to the presence of calcium carbonate, chitin, and chitosan in green mussel shells, which can disrupt bacterial cell walls and interfere with cellular metabolism, particularly in Gram-positive bacteria (Azizan et al., 2024; Lagmay & Agbayani, 2025). The novelty of this study lies in demonstrating that green mussel shell waste can be effectively integrated into solid soap formulations while maintaining physical quality, consumer acceptance, and antibacterial efficacy.

Hedonic Test

The hedonis test results (Table 5) revealed that all formulas (FI-FIV) obtained a very high level of liking in almost all parameters, especially color, shape, and aroma that reached 100% panelist liking. This reveals that the presence of natural active ingredients does not reduce the visual or sensory quality of the soap, and even still provides an attractive appearance and a favorable aroma (Das et al., 2024; Sujono & Nurohmah, 2023). The foam parameter obtained a favorability score of 75%, that means that the majority of panelists considered the foam to be quite abundant and soft., although there were still a small proportion (25%) who considered its intensity or stability to be less than optimal. This variation was likely influenced by the type and amount of oil used in the formulation as well as the presence of green mussel shell powder that may affect the texture of the foam. Nonetheless, high scores on color and shape show that the appearance of milky white soap resembles commercial soap, and the proportional and easy-to-grip shape provides comfort for daily use (Garneta et al., 2023).

Table 5. Hedonic Test Results

Formula	n	Hedonic Test				Persentage (%)
		Color	Aroma	Shape	Foam	
I	20	Like (100)	Like (100)	Like (100)	Like (75)	93,75%
II	20	Like (100)	Like (100)	Like (100)	Like (75)	93,75%
III	20	Like (100)	Like (100)	Like (100)	Like (75)	93,75%
IV	20	Like (100)	Like (100)	Like (100)	Like (75)	93,75%

In addition, the very high aroma score indicates that the green mussel shell powder does not impart a fishy or marine odor typically associated with animal ingredients, as it is successfully masked by the stable natural fragrance in the formulation. This supports that the use of natural active ingredients can be combined devoid of degrading the organoleptic quality of the product (Das et al., 2024).

Overall, the hedonic test results indicate that the solid soap made from green mussel shells has strong consumer acceptance, suggesting it could be accepted in the market as a natural antibacterial product that is attractive, safe, and convenient to use. High acceptance scores indicate that the utilization of shell-derived antibacterial agents does not

compromise consumer preference, which is a critical factor for product development and market feasibility.

CONCLUSION

This study demonstrates that solid soap formulations containing green mussel shell powder exhibit antibacterial activity against *Staphylococcus aureus*, with effectiveness increasing with concentration; the 6% formulation showed the highest inhibition. All formulations met physical quality standards and showed high consumer acceptance, indicating that green mussel shell waste is a promising sustainable antibacterial ingredient for solid soap. However, as this study was limited to in vitro testing and did not include skin irritation or long-term safety evaluation, further studies involving skin compatibility tests and formulation optimization are recommended to support broader application.

ACKNOWLEDMENT

It is recommended that future researchers use more concentration variations to determine the maximum dose that is still safe for the skin.

AUTHOR CONTRIBUTION

BAM : Primary researcher, literature search, analytical analysis, manuscript writing.
JS : Research mentor and consultant.
ES : Research mentor and consultant.

ETHICS APPROVAL

This study did not involve animal experiments or clinical trials. The hedonic (sensory) evaluation involved adult human participants who participated voluntarily. Prior to participation, all panelists were informed about the purpose of the study and provided verbal informed consent. The sensory evaluation did not collect personal identifying information, and all responses were recorded anonymously. The procedure posed no physical or psychological risk to participants and was conducted in accordance with general ethical principles for non-invasive sensory testing.

CONFLICT OF INTEREST

The authors have no competing interests related to this study.

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