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Liquid Soap with Pineapple Hump Extract and Nanoformulation Against *Staphylococcus aureus*

Minda Sari Lubis, Ziza Putri Aisyia Fauzi, Sri Harti Dewi, Zulmai Rani, Rafita Yuniarti

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Abstract: *Staphylococcus aureus* is a Gram-positive bacterium that frequently causes skin infections and can become resistant to various antibiotics. Pineapple (*Ananas comosus*) waste, particularly the hump, contains active compounds such as bromelain, saponins, and flavonoids with known antibacterial properties. This study aimed to determine the antibacterial potential of liquid soap formulated with extract and nanoextract of pineapple hump against *S. aureus*. The pineapple hump was extracted using maceration with 96% ethanol, followed by nanoparticle formulation using a high-speed homogenization method. Liquid soap was made with three formulas, one formula contains a concentration of 12.5% extract and two formulas contain 1.25% nanoextract of pineapple hump. Antibacterial activity was evaluated using the disc diffusion method, and the diameter of the inhibition zones was measured. Results showed that all tested concentrations produced antibacterial activity, with the 12.5% concentration (Formula 1) exhibiting the highest inhibition zone (23.2 mm), followed by 1.25% nanoextract nano liquid soap (18.41 mm) (Formula 3) and 1.25% nanoextract liquid soap (14.53 mm) (Formula 2). The positive control (Dettol Handwash) produced a larger inhibition zone (20.08 mm). Data analysis using one-way ANOVA revealed significant differences for each formula, with a p-value of less than 0.05. These findings indicate that nanoextract of pineapple hump formulated in liquid soap has potential as a natural antibacterial agent against *S. aureus*.

Introduction

Skin infections are a common health problem that affects individuals worldwide and rank among the top ten most prevalent diseases in Indonesia (1). Globally, skin infections contribute significantly to the burden of infectious diseases, particularly in tropical and developing countries (2). Among the many causes, bacterial skin infections are a major concern due to their recurrence, potential complications, and the rising threat of antibiotic resistance. In Indonesia, bacterial skin infections are the second most frequently reported dermatological condition, affecting both children and adults across various regions (1). *Staphylococcus aureus* is one of the primary bacterial pathogens responsible for skin infections. This Gram-positive bacterium is known to cause a wide range of infections, from mild conditions such as impetigo and folliculitis to more severe diseases, including abscesses, cellulitis, and even life-threatening sepsis. Its ability to colonize the skin and mucosal surfaces, coupled with the emergence of multidrug-resistant strains like MRSA (*Methicillin-resistant S. aureus*), makes it a significant public health threat (3). The overuse and long-term use of antibiotics and synthetic antimicrobials in skin care and treatment regimens have contributed to adverse effects such as skin irritation, allergic reactions, and microbial resistance. Therefore, there is an urgent need for safer, more sustainable, and cost-effective alternatives to conventional antibiotics (4).

One promising preventive approach is the use of soap, particularly liquid soap, to reduce the microbial load on the skin. Compared to solid soap, liquid soap is considered more hygienic and practical, as it reduces the risk of cross-contamination in communal or clinical settings. Furthermore, natural liquid soap is gaining popularity due to its biodegradability and minimal potential for side effects (2). Natural-based formulations can be enriched with plant-derived extracts that possess antibacterial properties, offering both efficacy and safety.

Pineapple (*Ananas comosus* (L.) Merr.) is one such plant with known antimicrobial potential. Interestingly, the pineapple hump, often discarded due to its hard texture and lack of sweetness, is a valuable source of bioactive compounds. Research has shown that pineapple hump extract contains secondary metabolites, including flavonoids, alkaloids, tannins, saponins, steroids, and glycosides, which exhibit antibacterial activity (3). A study demonstrated that nanoserum formulated from pineapple hump extract inhibited the growth of *S. epidermidis*, with a significant inhibition zone diameter at higher concentrations (4). The incorporation of nanoparticle-sized active ingredients into

liquid soap has been proposed as a means to enhance its effectiveness (5). Nanoparticles can increase the bioavailability and penetration of active compounds, allowing them to reach target sites more efficiently due to their small size and large surface area (6). This innovation is particularly relevant for topical applications, where barrier penetration is crucial (5).

However, to date, studies evaluating the antibacterial efficacy of liquid soap formulations containing both conventional and nano-sized pineapple hump extracts remain limited. There is a lack of direct comparative data on whether nanoformulation significantly enhances antimicrobial performance in such soap-based systems (7). Therefore, this study aims to develop and compare liquid soap formulations containing extract and nanoextract of pineapple hump, and to evaluate their antibacterial activity against *S. aureus*.

Experimental Section

Materials

The materials used in this research included Coconut Oil (Tropical®, PT. Bina Karya Prima, Indonesia), Potassium Hydroxide (Merck®, Merck KGaA, Germany), Hydroxypropyl Methylcellulose (Sigma-Aldrich®, Merck KGaA, Germany), Butylated Hydroxitoluene (Sigma-Aldrich®, Merck KGaA, Germany), Glycerin (Brataco®, PT. Brataco Chemical, Indonesia), Stearic Acid (Sigma-Aldrich®, Merck KGaA, Germany), 96% Ethanol (Brataco®, PT. Brataco Chemical, Indonesia), 0.1 N Hydrochloric Acid (Merck®, Merck KGaA, Germany), Mueller Hinton Agar (Oxoid®, Thermo Fisher Scientific, UK), Plate Count Agar (Oxoid®, Thermo Fisher Scientific, UK), 0.9% Natrium Chloride (Otsuka®, Otsuka Pharmaceutical Co., Japan), *S. aureus* ATCC 25923 (Oxoid®, Thermo Fisher Scientific, UK) and Dettol Handwash (Dettol, Reckitt Benckiser, UK).

The equipment used in this research was a Rotary Evaporator (Eyela® N-1110, Tokyo Rikakikai Co., Ltd., Japan), Micropipette (Larcksci® LP-Series, Larcksci, USA), Oven (Memmert® UN110, Memmert GmbH + Co. KG, Germany), Autoclave (B-One® 24L, B-One Medical Equipment, China), Incubator (Memmert® IN55, Memmert GmbH + Co. KG, Germany), Laminar Airflow (Biobase® BBS-V800, Biobase Biodustry Co., Ltd., China), Homogenizer (IKA® T25 digital ULTRA-TURRAX®, IKA-Werke GmbH & Co. KG, Germany), Ultrasonic Cleaner (B-One® UC-20L, B-One Medical Equipment, China), Particle Size Analyzer (PSA) (Fritsch® ANALYSETTE 22 NanoTec plus, Fritsch GmbH, Germany) and Transmission Electron Microscopy (TEM) (JEOL® JEM-1400Flash, JEOL Ltd., Japan).

Sample Collection and Preparation

The sample used was the pineapple hump, obtained fresh from a local rujak vendor in Medan, Indonesia. The hump was first washed thoroughly under running water to remove dirt and contaminants, then peeled, chopped into small pieces, and air-dried at room temperature (25–27 °C) for 2–3 days in a shaded, well-ventilated area to prevent photodegradation of active compounds. The dried material was then oven-dried at 40 °C for 12 h to remove residual moisture before being ground into a coarse powder using a laboratory blender. The powdered material was macerated in 96% ethanol for 72 h at room temperature in a closed container, kept in the dark, and stirred gently every 12 h for 15 min to enhance the extraction efficiency. After maceration, the extract was filtered, and the filtrate was concentrated using a rotary evaporator at 50 °C under reduced pressure to obtain a thick extract. The extract was stored in an amber bottle at 4 °C until further use. For nanoextract preparation, the pineapple hump extract was homogenized using a homogenizer at a speed of 1,700 rpm for 1 h at room temperature. Subsequently, the homogenized extract was placed in an ultrasonic cleaner and sonicated for 1 h to reduce particle size to the nanometer scale. The resulting nanoextracts were then analyzed for particle size using a Particle Size Analyzer (PSA). The morphology of the nanoextract was studied using Transmission Electron Microscopy (TEM).

Phytochemical Screening

Phytochemical screening was conducted to qualitatively identify the presence of key secondary metabolites in both the pineapple hump extract and its nanoformulation. The tests were performed using standard colorimetric methods, as described by (8) and further adapted by (9), which enable the detection of major phytochemical groups, including alkaloids, flavonoids, saponins, tannins, steroids, and glycosides.

Liquid Soap Preparations

Heat the coconut oil to 60 °C, then add a 10% KOH solution gradually while continuing to heat. Use a magnetic stirrer until a soap paste is formed. Then, add approximately 15 mL of distilled water and stir until the mixture is homogeneous. Add pineapple root extract and stir until the mixture is smooth and homogeneous. Add Hydroxypropyl Methylcellulose (HPMC), developed with hot distilled water, to the mixture. Then, add melted glycerin, Butylated Hydroxytoluene (BHT), and stearic acid. Then, add distilled water and stir with a magnetic stirrer until a homogeneous liquid soap is obtained (10).

The nanoextracts formulations (Formulas 2 and 3) were deliberately designed to contain a lower concentration (1.25%) compared to the conventional extract formulation (Formula 1, 12.5%). This decision was based on the unique properties of nanoparticles, which offer significant advantages in terms of delivery efficiency and bioactivity. When plant extracts are processed into nanoscale particles, the total surface area increases dramatically, thereby enhancing the interaction between active compounds and bacterial cell membranes. This allows the same or even greater biological effect to be achieved using a smaller quantity of the active substance.

Moreover, nanoparticles possess better penetration capabilities due to their small size, allowing them to more effectively reach and disrupt bacterial cells. Previous studies have reported that nanostructured delivery systems can improve solubility, stability, and antibacterial performance, even at lower doses (6, 7). Thus, in this study, we evaluated whether reducing the concentration to one-tenth of the original (1.25% vs. 12.5%) would still provide adequate antibacterial activity. In addition to efficacy considerations, formulation stability was also taken into account. Higher concentrations of nanoextracts can potentially alter the physicochemical properties of liquid soap, including viscosity, foam stability, and pH. Therefore, selecting a lower yet functionally effective concentration was intended to maintain both antibacterial performance and product quality. This approach aligns with the core principle of nanotechnology in formulation science: achieving more with less.

Table 1. Pineapple hump extract liquid soap formula.

No	Material	Formulas (gr)			
		F0	F1	F2	F3
1	Pineapple hump extract	-	12.5	-	-
2	Pineapple hump nanoextract	-	-	1,25	1,25
3	Coconut oil	28.8	28.8	28.8	28.8
4	KOH 10%	5.15	5.15	5.15	5.15
5	HPMC	0.5	0.5	0.5	0.5
6	BHT	0.02	0.02	0.02	0.02
7	Glycerin	18.75	18.75	18.75	18.75
8	Stearic acid	2	2	2	2
9	Aquadest	100	100	100	100

Description: F0 = blank, F1 = liquid soap formula contains 12.5% pineapple hump extract, F2 = liquid soap formula contains 1.25% pineapple hump nano extract, and F3 = nanoliquid soap formula contains 1.25% pineapple hump nano extract.

Nanoliquid Soap Preparations

The soap mass prepared based on the composition shown in **Table 1** was then incorporated with 1.25 g of pineapple hump nanoextract to formulate Formula 3. The mixture was homogenized for 1 h to ensure uniform dispersion of the nanoextract, followed by sonication for an additional hour to enhance nanoparticle distribution and minimize aggregation. This process yielded the final nanoliquid soap formulation labeled as Formula 3 (5).

Evaluation of Liquid and Nanoliquid Soap Preparations

This research evaluated various physicochemical and microbiological properties of the liquid and nanoliquid soap formulations. The tested parameters included:

Particle Size Measurement

The particle size of the extract, nanoextract, and final soap formulations was measured using a Particle Size Analyzer (PSA) (Fritsch® ANALYSETTE 22 NanoTec Plus). Each sample was diluted in distilled water and sonicated for 5 min to avoid aggregation. Measurements were taken in triplicate, and the average size (in nanometers) was recorded.

Organoleptic Observation

The organoleptic properties, including color, odor, and physical consistency, were assessed both visually and through olfactory evaluation. The color was observed under natural daylight, odor was assessed by a panel of three evaluators, and physical form was recorded (e.g., thick, translucent, homogeneous).

pH Measurement

pH was measured using a calibrated digital pH meter (HANNA Instruments®, USA). Approximately 5 mL of each soap formulation was placed in a clean beaker, and the pH probe was immersed for 1 minute to obtain a stable reading. All measurements were performed at room temperature (25 ± 1°C) and repeated in triplicate.

Density

Density was determined by weighing 1 mL of each formulation using a 1 mL pycnometer at room temperature. The weight was divided by the volume to obtain specific gravity in g/mL. The measurement was repeated three times for accuracy.

Free Alkali Content

Free alkali content was evaluated by titrating the soap sample against 0.1 N HCl. About 1 g of soap was dissolved in 25 mL of hot distilled water, cooled, and phenolphthalein was added as an indicator. The sample was titrated with 0.1 N HCl until the pink color disappeared. The percentage of free alkali was calculated using the standard formula.

Microbial Contamination (Total Plate Number)

Total Plate Number (TPC) testing was conducted exclusively on Formula 1, which showed the highest antibacterial activity among all formulations. The purpose of this test was to evaluate the microbial contamination level of the most promising soap formula. 1 g sample of liquid soap preparation from pineapple hump extract was serially diluted using sterile saline (0.9% NaCl) up to 10^{-3}. From each dilution, 1 mL was plated on Plate Count Agar (PCA) using the pour plate method. Plates were incubated at 37 °C for 24–48 h. Colony-forming units (CFU) were counted and expressed as CFU/g. The results were compared with the acceptable limits set by the Indonesian National Standard (SNI 16-4380-1996), which states the total plate number must be below 10^5 CFU/g.

Foam Stability

Foam stability was assessed by shaking 25 mL of the soap solution (prepared as a 1:10 dilution with distilled water) in a 100 mL graduated cylinder for 1 minute. The foam height was measured immediately and then again after 5 min had passed. Foam stability was calculated as a percentage of foam remaining after 5 min compared to the initial foam height.

Antibacterial Activity Test

The antibacterial activity of the liquid soap formulations was evaluated using the agardisc diffusion method against *S. aureus* ATCC 25923. The bacteria were reactivated in Mueller-Hinton Broth (MHB) at 37 °C for 18–24 h before testing. A suspension of *S. aureus* was adjusted to match the turbidity of a 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL). Mueller Hinton Agar (MHA) plates were prepared and evenly swabbed with the bacterial suspension using a sterile cotton swab. Sterile paper discs (6 mm in

diameter) were then impregnated with 100 µL of each soap formulation (F0–F3), dried briefly, and placed on the inoculated agar surface. In this study, Dettol® Handwash (Reckitt Benckiser, UK) was used as the positive control, and the base formulation without active ingredients (Formula 0) served as the negative control. Both controls were treated under the same experimental conditions as the test samples, including the volume of 100 µL applied per disc, and were processed in parallel throughout the entire procedure. This standardization ensured that the comparisons of antibacterial activity were reliable and attributable to the presence or absence of active ingredients in the respective formulations.. All plates were incubated at 37 °C for 24 h. After incubation, the diameter of the inhibition zones was measured in millimeters using a digital caliper. Each test was performed in triplicate, and the mean ± standard deviation was calculated. The antibacterial activity was categorized according to zone diameter as follows: weak (<10 mm), moderate (10–15 mm), strong (15–20 mm), and very strong (>20 mm) (11).

Data Analysis

The antibacterial activity data obtained in this study were statistically analyzed using one-way *Analysis of Variance* (ANOVA) to determine whether there were significant differences among the tested formulations. When a significant difference was identified ($p < 0.05$), the analysis was followed by Tukey's Honestly Significant Difference (HSD) post-hoc test to compare the mean inhibition zones between individual groups. All statistical analyses were performed using the *Statistical Package for the Social Sciences* (SPSS) version 26.0 (IBM Corp., Armonk, NY, USA).

Results and Discussion

Phytochemical Screening Result

Phytochemical screening was conducted to validate the presence of secondary metabolites in both the extract and nanoextract of pineapple hump (Ananas comosus (L.) Merr). The results revealed the presence of alkaloids, flavonoids, saponins, tannins, steroids, and glycosides in both preparations. These compounds are widely recognized for their biological activity, particularly in antimicrobial applications. Flavonoids are known for their ability to inhibit bacterial growth through multiple mechanisms, including disruption of bacterial membranes, inhibition of nucleic acid synthesis, and interference with energy metabolism. Saponins, on the other hand, act as natural surfactants and are believed to increase membrane permeability, ultimately leading to cell lysis. Alkaloids are known to bind with microbial DNA, disrupting replication processes. Tannins can inactivate microbial enzymes and precipitate proteins, while steroids and glycosides may contribute to antimicrobial activity by interfering with the stability of bacterial membranes (12, 13).

Characterization of Extract and Nanoextract

Figure 1 shows the morphology of the pineapple hump nanoextract observed at 80,000x magnification, characterized by a round shape and an approximate diameter of 50 nm. Based on **Table 2**, it can be observed that the pineapple hump nano extract has a smaller particle size than the pineapple hump extract. This is because the nanoextract was made using a homogenizer and sonicated for 1 hour. Homogenizers and ultrasonication can reduce particle size (14). The principle of a homogenizer in reducing particle size is to minimize grain size by grinding the particles, resulting in smaller particles than the original size (15). Meanwhile, the sonication method utilizes ultrasonic waves, where an ultrasonic electric generator converts an electrical signal into physical vibrations (ultrasonic waves), resulting in a powerful effect (cavitation effect) on the solution, which causes the molecules to break apart (14).

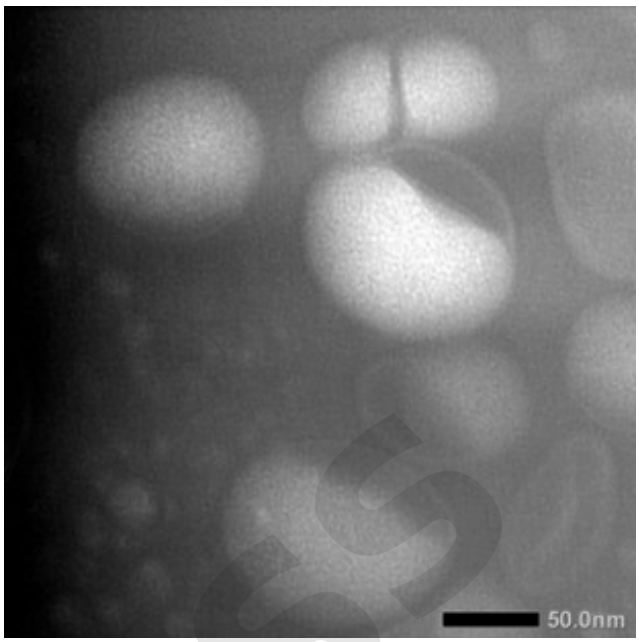

Figure 1. Results of morphological observations using TEM.

Table 2. Particle size of sonicated and non-sonicated extracts.

Sample	Particle Size
Pineapple hump extract	873 nm
Pineapple hump nanoextract	76 nm

Evaluation of Liquid Soap Extract and Nanoextract Pineapple Hump Extract

Liquid Soap Particle Size

Based on **Figure 2**, the results indicate that the smallest particle size is observed for Formula 3, specifically 452 nm in diameter. Liquid soap and liquid nano soap preparations from pineapple hump extract and nano extract did not exhibit any specific physical differences. The difference only lies in the particle size, where the particle size of Formula 3 is the smallest compared to the other formulas. This occurs because the process of making formula 3 involves a homogenizer and sonication process, each lasting 1 hour. Homogenizers and ultrasonication can reduce particle size (14).

Harsono (2021) states that nanoparticles measure between 1 and 1,000 nanometers (16). The small particle size of liquid soap can be more optimal for skin cleaning. Because the small particle size effectively lifts dirt and enters the stratum corneum, liquid soap has a maximum effect. The diameter of the nanoparticle is the most critical parameter that determines its ability to penetrate the skin. Smaller nanoparticles can passively transfer through the skin barrier and reach systemic circulation. The outermost layer of the skin, namely the stratum corneum, is practically

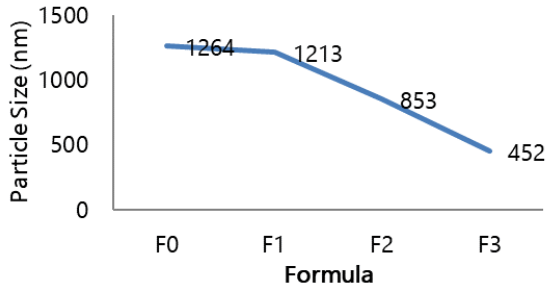

Figure 2. Liquid soap particle size chart.

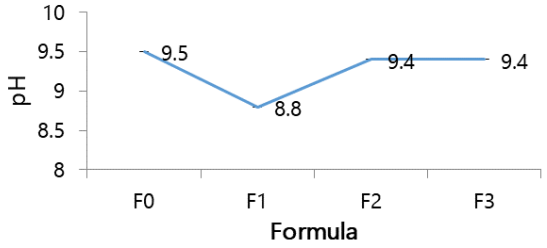

Figure 3. pH of the extract liquid soap formula.

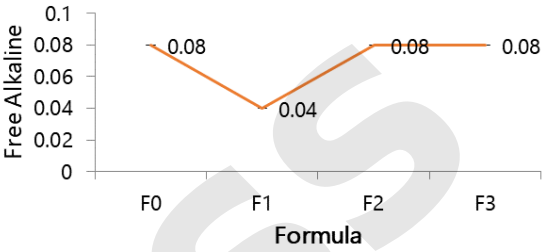

Figure 4. Alkaline content of liquid soap extract formula.

Table 3. Results of organoleptic examination of liquid soap preparations.

No	Parameter	Organoleptic			
		F0	F1	F2	F3
1.	Form	Thick	Thick	Thick	Thick
2.	Smell	Typical	Typical	Typical	Typical
3.	Color	White	Dark brown	Light brown	Light brown

impermeable to larger particles. Many studies have described a decrease in the permeability of nanoparticles through the skin with increasing particle size. The maximum ability of nanoparticles to reach deeper layers of the skin through hair follicles has been confirmed by many researchers for organic and inorganic nanoparticles (17).

Organoleptic

The organoleptic properties of the liquid soap formulations, including their texture, odor, and color, were assessed visually to evaluate the overall physical appearance and acceptability of the products. The results of this evaluation are summarized in **Table 3**. A noticeable color difference was observed among the formulations. Formula 1, which contained 12.5% pineapple hump extract, appeared dark brown. This darker hue is likely due to the higher concentration of natural phytochemicals such as polyphenols, flavonoids, and tannins, which are known to impart intense pigmentation.

In contrast, Formulas 2 and 3, both containing only 1.25% of nanoextract, displayed a lighter brown color. The reduced intensity is attributed not only to the lower concentration of the extract but also to the smaller particle size, which can influence how light interacts with the formulation. Nano-sized particles tend to scatter light differently, often resulting in a lighter or more translucent appearance. This color variation is typical in plant-based formulations and may serve as a visual indicator of the extract type and concentration. Moreover, the nanoencapsulation process may further stabilize certain phytoconstituents, subtly altering the color profile of the final product (17, 18)

pH

The pH test on liquid soap preparations is one of the key requirements for the quality of liquid soap, as it comes into direct contact with the skin. If the pH value does not match the skin's pH, it can cause problems. The pH test results are shown in **Figure 3**.

The pH values of the liquid soap formulations were found to range between 8 and 11, which aligns with the acceptable range established by the Indonesian National Standard for liquid bath soap (1) (19). This pH range is considered safe for skin application, as it helps maintain the stability and cleansing efficiency of the product without causing irritation or disrupting the skin's natural barrier (2). Among the tested formulas, Formula 1 exhibited the lowest pH value. This result is likely due to its higher concentration of pineapple hump extract (12.5%), which contains various organic acids, including citric acid, malic acid, and oxalic acid (20, 21). These acidic constituents can lower the overall pH of the formulation. A study by (22) confirmed that increasing concentrations of pineapple-derived extract can significantly reduce pH levels in topical preparations due to the acidic nature of the phytochemical content (10, 11). Maintaining an appropriate pH is crucial, as values that are too low (acidic) or too high (alkaline) may lead to skin irritation, dryness, or disruption of the skin microbiome. Therefore, pH monitoring remains an essential quality control parameter in the formulation of herbal-based soaps.

Alkaline Free

Based on **Figure 4**, the free alkali content in all tested liquid soap formulations (Formulas 0, 1, 2, and 3) met the quality requirements set by the Indonesian National Standard, which stipulates that free alkali levels in liquid soap should not exceed 0.1% to ensure safety and prevent skin irritation (1). Among the formulations, Formula 1 exhibited the lowest free alkali content. This finding corresponds with its lower pH value compared to the other formulations. In general, free alkali levels are positively correlated with pH. Higher pH values typically reflect higher concentrations of residual alkali in the soap. This relationship has been previously documented in studies analyzing the effect of alkaline components on soap pH and skin compatibility (12). Maintaining low free alkali content is essential to avoid adverse effects on the skin, such as dryness or irritation, particularly in formulations intended for frequent or prolonged use.

Density

According to SNI, the density of a liquid soap preparation

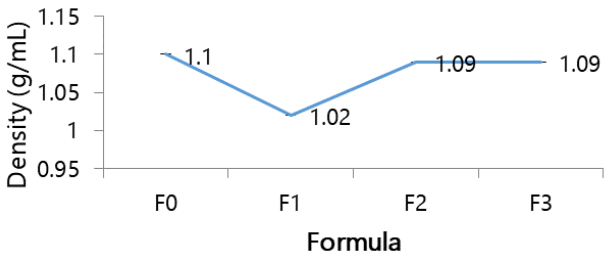

Figure 5. Specific weight of extract liquid soap formula.

ranges from 1.01 to 1.1 g/mL. **Figure 5** shows that Formula 1, which contains the highest concentration of pineapple hump extract (12.5%), had the lowest specific gravity compared to the other formulations. This finding suggests that increasing the concentration of plant extract tends to reduce the specific gravity of the liquid soap. This is consistent with previous studies, which have reported that the inclusion of plant-based additives, particularly those rich in water-soluble phytochemicals, can decrease the overall density of soap formulations (13). The observed reduction in specific gravity may be attributed to the presence of lightweight organic compounds in the extract, which alter the formulation composition and viscosity. Despite these differences, the specific gravity values of all tested formulations remained within the acceptable range, as specified by the Indonesian National Standard, which states that the specific gravity of liquid bath soap typically ranges between 1.01 and 1.10 g/mL (1).

Microbial Contamination (Total Plate Number)

The results of observations of the total plate number for liquid soap preparation from pineapple hump extract can be seen in **Table 4**. Based on the total plate count results, all liquid soap formulations containing pineapple hump extract showed microbial contamination levels that complied with the Indonesian National Standard for liquid bath soap, which sets the maximum allowable microbial load at less than 1×10^5 *colony-forming units* (CFU) per gram of product. All samples tested in this study were within this limit, indicating acceptable microbiological quality. The presence of bacterial colonies in soap formulations is often influenced by factors such as the water content, pH, preservative system, and the microbial load of raw plant materials. Higher colony counts may reflect greater microbial contamination, either from the environment during processing or from the natural microbial flora associated with plant-based ingredients (23). Therefore, maintaining good manufacturing practices and incorporating effective preservatives is essential to ensure the microbiological safety of natural-based formulations.

Foam Stability

Foam stability testing in liquid soap aims to determine the stability of the foam produced from the soap solution within 5 min. Foam stability is a critical parameter in evaluating the performance of liquid soap, as it reflects the product's ability to maintain foam over time during use. As shown in **Table 5**, Formula 1, containing 12.5% pineapple hump extract, exhibited the highest foam stability, with a mean value of 91.73 ± 0.97%. This finding suggests that the presence of pineapple extract has a positive influence on the foaming properties of the formulation. The enhanced foam stability is likely attributed to the presence of saponins, naturally occurring amphiphilic compounds found in pineapple plant material. Saponins possess both hydrophilic (water-attracting) and lipophilic (oil-attracting) components, allowing them to act as natural surfactants. When dissolved in water, saponins reduce surface tension, facilitating the generation of foam.

Furthermore, their molecular structure helps stabilize foam by forming micelles that trap air and prevent rapid collapse (24, 25). In addition to the phytochemical content, foam stability is also influenced by formulation factors, including the degree of saponification and the amount of water used in the formulation. Complete saponification of fatty acids (e.g., coconut oil in this formulation) enhances the soap's ability to form and retain foam. Moreover, water dilution can increase foam volume but may reduce its density and longevity if not optimally balanced (26). Thus, the high foam stability observed in Formula 1 likely results from the synergistic effect of high saponin content and efficient saponification.

Antibacterial Activity of Liquid Soap Preparations against *Staphylococcus aureus*

As presented in **Table 6**, the antibacterial activity of the nano liquid soap (Formula 3) showed an inhibition zone that was nearly comparable to that of Formula 1, which contains a higher concentration (12.5%) of the conventional pineapple hump extract. Interestingly, although Formula 3 contained only 1.25% of the nanoextract, approximately one-tenth of the active substance compared to Formula 1, it still demonstrated a relatively strong antibacterial effect. This could be attributed to its smaller particle size, which enhances the ability of the active compounds to interact with bacterial cell walls. Smaller particles may increase surface area and improve cellular penetration, a phenomenon supported by (27), who reported that nanoparticles can disrupt bacterial membranes more effectively due to their enhanced permeability and retention properties. The antibacterial effect of liquid soap against *S. aureus* is primarily attributed to the surfactants that disrupt bacterial membranes and facilitate the mechanical removal of microbes. When enriched with pineapple hump extract, the soap gains additional antibacterial mechanisms due to the presence of bioactive phytochemicals. Compounds such as bromelain, flavonoids, saponins, and organic acids act synergistically to degrade bacterial proteins, disrupt membranes, inhibit nucleic acid synthesis, and lower environmental pH. These combined effects enhance the soap's ability to inhibit S. aureus, offering both surface cleansing and biological antimicrobial action (28, 29).

However, in this study, Formula 1 exhibited the greatest inhibitory effect, surpassing both nano-based formulations. This finding suggests that while nanoparticle technology offers theoretical advantages in terms of delivery and bioavailability, it does not always guarantee superior biological efficacy, particularly for certain types of plant-based extracts (30) (31). One possible explanation is that the conventional extract may contain a broader spectrum of bioactive compounds in their native form, some of which might be degraded or altered during the homogenization and sonication processes used to produce nanoparticles. Moreover, the extraction and nano-sizing processes may influence the stability, solubility, or synergistic interactions among phytochemicals. In some cases, certain compounds responsible for antimicrobial activity may become less effective or even inactivated when reduced to the nanoscale (32). This finding aligns with previous studies suggesting that

the biological activity of herbal nanoparticles is highly dependent on the type of plant material and the nature of its active constituents (22) (33). In conclusion, while nanoformulations offer promising potential, this study highlights that particle size reduction does not always enhance antibacterial efficacy, especially when working with complex botanical extracts, such as pineapple hump. Further investigation into the stability and bioactivity of individual compounds during the nanoformulation process is warranted.

One-way ANOVA analysis revealed a statistically significant difference in the inhibition zone diameters among the tested liquid soap formulations ($p < 0.05$), indicating that the type and concentration of the extract influenced antibacterial efficacy against *S. aureus*. Post-hoc analysis using Tukey's HSD test showed that Formula 1 (containing 12.5% pineapple hump extract) had a significantly larger inhibition zone compared to Formulas 0 (blank), 2, and 3 (both containing 1.25% nanoextract). However, there was no statistically significant difference between Formula 1 and the positive control (commercial antibacterial soap), suggesting that the antibacterial activity of Formula 1 is comparable to that of the commercial product. Conversely, the inhibition zones of Formulas 0, 2, and 3 were significantly different from both Formula 1 and the positive control. This confirms that while the nanoextract formulations exhibited moderate to strong antibacterial activity, they were less effective than the crude extract at higher concentrations.

Conclusion

Among the tested formulations, the soap containing 12.5% crude extract (Formula 1) showed the highest inhibition zone, indicating the strongest antibacterial effect. Although the nanoextract-based formulation (Formula 3) contained only one-tenth of the active substance, it still displayed considerable inhibitory activity, suggesting that nanoparticle size may enhance bioavailability and penetration. Statistical analysis using one-way ANOVA followed by Tukey's post-hoc test confirmed significant differences among the formulations ($p < 0.05$). However, the findings also revealed that nano-sizing does not always guarantee improved antibacterial efficacy. In this case, the conventional extract outperformed its nano counterpart, potentially due to the loss or alteration of bioactive compounds during the nanoformulation process. These results suggest that while nanoparticle technology holds promise, the formulation strategy must be tailored to the specific characteristics of the plant extract. Further studies are needed to optimize nanoformulation parameters and to investigate the stability and integrity of active compounds in nanoscale preparations. This study demonstrated that liquid soap formulations containing both pineapple hump extract and nanoextract exhibit antibacterial activity against *S. aureus*.

Abbreviations

HPMC = Hydroxypropyl Methylcellulose; KOH = Potassium Hydroxide; BHT = Butylated Hydroxitoluene; HCl = Hydrochloric Acid; MHA = Mueller Hinton Agar; PCA = Plate Count Agar; SNI = Standar Nasional Indonesia; PSA = Particle Size Analyzer; TEM = Transmission Electron Microscopy; ANOVA = Analysis of Variance; SPSS = Statistical Package for the Social Sciences.

Declarations

Author Informations

Minda Sari Lubis
Corresponding Author
Affiliation: Faculty of Pharmacy, Universitas Muslim Nusantara Al-Washliyah, Medan 20147, Indonesia.
Contribution: Funding acquisition, Investigation, Supervision, Validation, Writing - Review & Editing.

Ziza Putri Aisyia Fauzi
Affiliation: Faculty of Pharmacy, Universitas Muslim Nusantara Al-Washliyah, Medan 20147, Indonesia.
Contribution: Conceptualization, Writing - Original Draft, Writing - Review & Editing.

Sri Harti Dewi
Affiliation: Faculty of Pharmacy, Universitas Muslim Nusantara Al-Washliyah, Medan 20147, Indonesia.
Contribution: Methodology, Project administration, Resources.

Zulmai Rani
Affiliation: Faculty of Pharmacy, Universitas Muslim Nusantara Al-Washliyah, Medan 20147, Indonesia.
Contribution: Conceptualization, Data Curation, Software.

Rafita Yuniarti
Affiliation: Faculty of Pharmacy, Universitas Muslim Nusantara Al-Washliyah, Medan 20147, Indonesia.
Contribution: Funding acquisition, Investigation, Supervision, Validation, Writing - Review & Editing.

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Conflict of Interest

The authors declare no conflicting interest.

Data Availability

The unpublished data is available upon request to the corresponding author.

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Not applicable.

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References

1. Erikawati D, Santosaningsih D, Santoso S. Tingginya prevalensi MRSA pada isolat klinik periode 2010-2014 di RSUD Dr. Saiful Anwar Malang, Indonesia. J Kedokt Brawijaya. 2016;29(2):149–156.

2. Hay RJ, Johns NE, Williams HC, Bolliger IW, Dellavalle RP, Margolis DJ, et al. The global burden of skin disease in 2010: an analysis of the prevalence and impact of skin conditions. J Invest Dermatol. 2014;134(6):1527–1534.

3. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler Jr VG. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev. 2015;28(3):603–661.

4. Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. Pharm Ther. 2015;40(4):277.

5. Fitri RM, Lubis MS, Dalimunthe GI, Yuniarti R. Skrining fitokimia, formulasi dan uji mutu fisik nanoserum ekstrak bonggol nanas (Ananas comosus (L.) Merr). J Pharm Sci. 2023;1346–1355.

6. Wissing SA, Müller RH. Cosmetic applications for solid lipid nanoparticles (SLN). Int J Pharm. 2003;254(1):65–68.

7. Bilal M, Iqbal HM. New insights on unique features and role of nanostructured materials in cosmetics. Cosmetics. 2020;7(2):24.

8. Harborne AJ. Phytochemical methods a guide to modern techniques of plant analysis. springer science & business media; 1998.

9. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotechnol. 2005;4(7):685–688.

10. Rafita RY, Nasution HM, Rani Z, Fahmi F. The Buas Buas Leaf Utilization of Buas Buas Leaf (Premna pubescens Blume) Ethanol Extract as Liquid Soap With Anti-Bacteria Activity. Int J Sci Technol Manag. 2022;3(3):733–743.

11. Legi AP, Edy HJ, Abdullah SS. Formulasi dan uji aktivitas antibakteri sediaan sabun cair ekstrak etanol daun sirsak (Annona muricata Linn) terhadap bakteri Staphylococcus. PHARMACON. 2021;10(3):1058–1065.

12. Tewari G, Joshi P, Tewari LM. Modern Trends in Medicinal and Aromatic Plants. Indu Book Services Pvt. Limited; 2023.

13. Chakraborty AJ, Mitra S, Tallei TE, Tareq AM, Nainu F, Cicia D, et al. Bromelain a potential bioactive compound: a comprehensive overview from a pharmacological perspective. Life. 2021;11(4):317.

14. Yunira EN, Suryani A, Dadang D, Tursiloadi S. Identifikasi Karakteristik Pengecilan Ukuran dengan Metode Sonikasi dari Formula Insektisida yang Ditambahkan Surfaktan Berbasis Sawit. J Sci Appl Technol. 2021;5(1):85–91.

15. Jusnita N, Diaz MSP. Formulasi nanoemulsi ekstrak temulawak (Curcuma xanthorrhiza Roxb) dengan metode inversi suhu. J Farm Higea. 2019;11(2):144–153.

16. Harsono H, Wardana ING, Sonief A, Darminto D. Crystallography, Impurities and Magnetic Properties of Mn-Doped ZnO Nanoparticles Prepared by Coprecipitation Method. J Nano Res. 2016;35:67–76.

17. Raszewska-Famielec M, Flieger J. Nanoparticles for topical application in the treatment of skin dysfunctions—an overview of dermo-cosmetic and dermatological products. Int J Mol Sci. 2022;23(24):15980.

18. Vaishampayan P, Rane MM. Herbal nanocosmeceuticals: A review on cosmeceutical innovation. J Cosmet Dermatol. 2022;21(11):5464–5483.

19. SNI 3532-2016 [Internet]. [cited 2025 Apr 19]. Available from: https://akses-sni.bsn.go.id/dokumen/2016/SNI%203532-2016/#p=6

20. Batista-Silva W, Nascimento VL, Medeiros DB, Nunes-Nesi A, Ribeiro DM, Zsögön A, et al. Modifications in organic acid profiles during fruit development and ripening: correlation or causation? Front Plant Sci. 2018;9:1689.

21. Techavuthiporn C, Boonyaritthongchai P, Supabvanich S. Physicochemical changes of 'Phulae'pineapple fruit treated with short-term anoxia during ambient storage. Food Chem. 2017;228:388–393.

22. Lubis MS, Asmarani A, Yuniarti R, Nasution HM. The Antibacterial Activity Of Conventional Serum And Nano Face Serum From Pineapple Stem Extract (Ananas Comosus (L.) Merr) Against Staphylococcus Epidermidis. J Eduhealth. 2024;15(02):1149–1155.

23. Sandle T. Pharmaceutical microbiology: essentials for quality assurance and quality control. Woodhead Publishing; 2015.

24. Tiwari R, Latheef SK, Ahmed I, Iqbal HM, Bule MH, Dhama K, et al. Herbal immunomodulators-a remedial panacea for designing and developing effective drugs and medicines: current scenario and future prospects. Curr Drug Metab. 2018;19(3):264–301.

25. Sultana B, Anwar F, Ashraf M. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules. 2009;14(6):2167–2180.
26. Silsia D, Susanti L, Apriantonedi R. Effects of KOH concentration on characteristics of used cooking oil liquid soap having kalamansi cittrus fragrance. J Agroindustri. 2017;7(1):11–19.

27. Islam R, Sun L, Zhang L. Biomedical applications of Chinese herb-synthesized silver nanoparticles by phytonanotechnology. Nanomaterials. 2021;11(10):2757.

28. Cushnie TT, Lamb AJ. Recent advances in understanding the antibacterial properties of flavonoids. Int J Antimicrob Agents. 2011;38(2):99–107.

29. TR Gomes M, L Oliva M, TP Lopes M, E Salas C. Plant proteinases and inhibitors: an overview of biological function and pharmacological activity. Curr Protein Pept Sci. 2011;12(5):417–436.

30. Pradhan AJ, Pukale PM, Pukale MM, Rajbar AJ, Rathod RP. Formulation and Evaluation of Herbal Soap. Int J Res Publ Rev. 2024;5(5):11322–11340.

31. Ashraf MV, Pant S, Khan MH, Shah AA, Siddiqui S, Jeridi M, et al. Phytochemicals as antimicrobials: prospecting Himalayan medicinal plants as source of alternate medicine to combat antimicrobial resistance. Pharmaceuticals. 2023;16(6):881.

32. Mohanta YK, Biswas K, Jena SK, Hashem A, Abd_Allah EF, Mohanta TK. Anti-biofilm and antibacterial activities of silver nanoparticles synthesized by the reducing activity of phytoconstituents present in the Indian medicinal plants. Front Microbiol. 2020;11:1143.

33. Majeed S, Samad M. Plant mediated synthesis of silver nanoparticles and its antibacterial effect. J Pure Appl Microbiol. 2019;13(2):1267–1272.

Additional Information

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