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Formulation and Antibacterial Evaluation of Liquid Soap Containing Pineapple Peel Extract Against *Staphylococcus aureus* and *Streptococcus pyogenes*

Eliya Mursyida[1*], Frengki Molek Wirajaya[2], Olvaria Misfa[3]

[1,3)] Department of Microbiology, Faculty of Medicine, University of Abdurrab, Riau, Indonesia
[2)] Faculty of Medicine, University of Abdurrab, Riau, Indonesia

e-mail:
eliya_mursyida@univrab.ac.id

ABSTRACT

The global surge in antimicrobial resistance necessitates the exploration of novel botanical therapeutics, especially targeting prominent Gram positive pathogens like *Staphylococcus aureus* and *Streptococcus pyogenes*. Pineapple peel (*Ananas comosus* (L.) Merr.), a widespread agricultural waste product, features crucial secondary metabolites with established antimicrobial attributes. This research aimed to develop a liquid soap enriched with the ethanolic extract of pineapple peel and to rigorously assess its physicochemical criteria alongside its antibacterial performance. The raw extract was integrated into the soap bases at concentrations of 5%, 10%, and 15%. The physical optimization matrix evaluated key attributes: organoleptic stability, structural homogeneity, pH values, free alkali percentages, and specific gravity. Concurrently, the antibacterial clearance potential was verified via the agar well diffusion technique against both target microorganisms. The experimental profiles showed that all formulated batches successfully satisfied the national regulatory baselines for topical liquid soaps, yielding pH readings spanning 7,3 to 9,7, specific gravity distributions between 1,01 and 1,04g/mL, and residual free alkali levels restricted to ≤0,1%. The biological assays revealed a clear concentration-dependent expansion in the diameter of clearing zones. The 15% extract formulation unlocked the peak therapeutic output, generating distinct zone diameters of 13,30mm against *S. aureus* and 20,25mm against *S. pyogenes*. These outcomes differed significantly from the lower concentration experimental blocks ($p < 0,05$). Ultimately, these data confirm that pineapple peel matrices can be efficiently stabilized into topical soap vehicles without diminishing their baseline antimicrobial properties, highlighting the 15% prototype as a viable, bio-based antiseptic cleansing candidate.

Keywords: liquid soap formulation, pineapple peel extract, antibacterial activity, phytochemicals

Introduction

Antimicrobial resistance (AMR) continues to pose a serious global public health challenge, particularly in infections caused by Gram positive pathogens such as *Staphylococcus aureus* and *Streptococcus pyogenes* (George *et al*., 2022). *S. aureus* is widely associated with skin and soft tissue infections and is known for its biofilm-forming ability and resistance development, while *S. pyogenes* remains a significant cause of pharyngitis and invasive infections. The increasing resistance of these bacteria to conventional antibiotics highlights the urgent need for alternative antimicrobial strategies, including plant-derived agents.

Natural products have gained considerable attention as potential antibacterial sources due to their rich diversity of bioactive secondary metabolites, including flavonoids, tannins, alkaloids, and saponins (Pérez-flores *et al*., 2025). These phytochemicals exhibit multiple mechanisms of action, such as membrane disruption, enzyme inhibition, and interference with nucleic acid synthesis, reducing the likelihood of resistance development compared to single-target synthetic antibiotics.

Pineapple peel (*Ananas comosus* (L.) Merr.), an abundant agricultural by-product, represents a promising source of bioactive compounds. Previous studies have reported that pineapple peel contains phenolic compounds and flavonoids with documented antimicrobial activity (Castro *et al*., 2025). In our earlier investigation, ethanol extract of pineapple peel demonstrated concentration-dependent antibacterial activity against *S. aureus* and *S. pyogenes* (Mursyida *et al*., 2025). Although these findings confirmed its antibacterial potential, the study was limited to crude extract testing and did not address formulation development or product stability.

Transforming plant extracts into appropriate pharmaceutical dosage forms is essential to improve stability, practicality, and user acceptability. Liquid soap formulations offer advantages for topical antibacterial applications, including ease of use, uniform distribution, and enhanced patient compliance (Barel *et al*., 2014). Furthermore, surfactant systems commonly used in liquid soaps, such as sodium lauryl sulfate, can disrupt bacterial cell membranes and potentially enhance the penetration and activity of incorporated phytochemicals (Maillard and Pascoe, 2024). Therefore, formulation into a liquid soap may not only preserve but also enhance the antibacterial effectiveness of pineapple peel extract.

Based on these considerations, this study aimed to formulate ethanol extract of pineapple peel into a liquid soap preparation and to evaluate its physicochemical quality and antibacterial activity against *S. aureus* and *S. pyogenes*.

Methodology

Research Design

This study employed an in vitro experimental design using a post-test only control group approach to evaluate the physicochemical characteristics and antibacterial activity of liquid soap formulations containing pineapple peel extract.

Preparation of Simplisia and Pineapple Peel Extract

Pineapple peel was washed, cut into small pieces, and air-dried until a constant weight was obtained. The dried material was milled into powder (simplisia) and sieved to ensure uniform particle size.

Extraction was performed following the previously reported method (Mursyida *et al*., 2025). Briefly, the dried peel powder was macerated with 96% ethanol at a ratio of 1:10 (w/v) for 72 hours at room temperature with occasional stirring. The mixture was then filtered, and the filtrate was concentrated under reduced pressure using a rotary evaporator to obtain a viscous ethanol extract. The extract was stored in an airtight container at 4°C until further use.

Preparation of Pineapple Peel Extract Concentrations

The 100% ethanol extract of pineapple peel was diluted using dimethyl sulfoxide (DMSO) to obtain final concentrations of 5%, 10%, and 15%. The dilution was calculated using the standard dilution formula:

$$V_1 \times M_1 = V_2 \times M_2$$

where:

M_1 and V_1 represent the initial concentration and volume of the extract,

M_2 and V_2 represent the desired concentration and final volume, respectively.

Formulation and Preparation of Pineapple Peel Liquid Soap

The composition of the liquid soap formulations is presented in Table 1. The formulation was adapted from Rinaldi *et al*. (2021) with minor modifications to accommodate the incorporation of pineapple peel extract at different concentrations. Three formulations containing 5%, 10%, and 15% extract were prepared, along with a negative control (soap base without extract) and a positive control (commercial antiseptic soap).

Table 1. Composition of Pineapple Peel Liquid Soap

Ingredients	F1 (5%)	F2 (10%)	F3 (15%)	Negative Control	Positive Control
Pineapple peel extract	5	10	15	–	–
Olive oil	20	20	20	20	–
KOH 15%	16	16	16	16	–
Stearic acid	1	1	1	1	–
CMC	0.5	0.5	0.5	0.5	–
EDTA	1	1	1	1	–
SLS	1	1	1	1	–
Distilled water	Ad 100	Ad 100	Ad 100	Ad 100	–
Commercial antiseptic soap (Dettol®)	–	–	–	–	✓

The liquid soap was prepared using the saponification method. Olive oil was heated to approximately 50°C in a water bath. A 15% potassium hydroxide (KOH) solution was added gradually under continuous stirring until complete saponification occurred and a homogeneous soap paste was formed.

The resulting soap paste was diluted with distilled water under gentle stirring. Carboxymethyl cellulose (CMC), previously dispersed in a small amount of warm distilled water to ensure proper hydration, was incorporated to enhance viscosity and improve texture. Stearic acid was added as a consistency enhancer and stabilizer. Sodium lauryl sulfate (SLS) was then introduced as a surfactant to improve foaming and cleansing properties, followed by ethylenediaminetetraacetic acid (EDTA) as a chelating agent to enhance formulation stability by binding metal ions.

Finally, pineapple peel extract was incorporated into each formulation according to the designated concentration (5%, 10%, or 15%), and distilled water was added to adjust the final volume to 100mL. The preparation was stirred until uniform and allowed to cool to room temperature before further evaluation.

Physical Quality Evaluation of Liquid Soap Formulations

Following preparation, all liquid soap formulations were subjected to physicochemical evaluation to ensure compliance with general quality standards for topical cleansing products. The assessed parameters included organoleptic characteristics, homogeneity, pH, free alkali content, and specific gravity. These evaluations were conducted to verify formulation stability, safety, and suitability for topical application.

Organoleptic and Homogeneity Assessment

Organoleptic evaluation was performed through direct visual and sensory observation to assess color, odor, and physical consistency. An acceptable liquid soap preparation should exhibit a uniform liquid form, characteristic color, and pleasant odor without signs of instability such as phase separation or precipitation. Variations in color intensity among formulations were anticipated due to differences in extract concentration.

Homogeneity testing was carried out by placing a small amount of the formulation onto a clean glass slide and spreading it evenly. The sample was observed under adequate lighting to detect the presence of coarse particles, aggregates, or phase separation. A formulation was considered homogeneous when no visible particles or non-uniform distribution were observed, indicating proper dispersion of all components within the base system (Adjeng *et al*., 2020; Rahmawati *et al*., 2021).

pH Determination

The pH of each formulation was measured using a calibrated digital pH meter. Prior to measurement, the instrument was standardized using buffer solutions at pH 4,0 and 7,0. The electrode was immersed directly into the liquid soap sample and gently stirred until a stable reading was obtained. Measurements were performed in triplicate to ensure accuracy, and the mean value was recorded.

Liquid soap preparations generally have an acceptable pH range of 6-11 to maintain cleansing effectiveness while minimizing potential skin irritation (Rahmawati *et al*., 2021). Maintaining pH within this range is essential to ensure product safety and compatibility with the skin barrier.

Free Alkali Content

Residual alkalinity resulting from the saponification process was determined by acid–base titration. Approximately 5g of liquid soap was accurately weighed and transferred into a 250mL beaker, followed by the addition of 100mL of 96% ethanol. The mixture was gently heated in a water bath for approximately 30 minutes to ensure complete dissolution. After cooling to room temperature, a few drops of phenolphthalein indicator were added.

If a pink coloration appeared, the solution was titrated with 0,1N hydrochloric acid (HCl) until the color disappeared, indicating neutralization of free alkali. The free alkali content was calculated, and the acceptable maximum limit for liquid soap preparations was ≤0,1%. Lower free alkali values indicate a more complete saponification process and improved product safety (Rinaldi *et al*., 2021). The percentage of free alkali was calculated using the following equation:

$$\text{Free Alkali (\%)} = \frac{V \; x \; N \; x \; 0{,}0561}{W} \; X \; 100\%$$

where:
V = volume of HCl used in titration (mL)
N = normality of HCl (N)
W = weight of sample (g)
0.0561 = equivalent weight factor of KOH

Specific Gravity

Specific gravity was determined using the pycnometer method at room temperature. The clean, dry pycnometer was first weighed empty, then filled with distilled water and weighed again to obtain the reference weight. After drying, the pycnometer was filled with the liquid soap sample and reweighed.

Specific gravity was calculated by comparing the weight of the sample to the weight of distilled water under identical conditions. The acceptable specific gravity range for liquid soap is 1,01-1,10g/mL, indicating appropriate consistency and formulation stability (Rahmawati *et al*., 2021). The specific gravity was calculated using the following equation:

$$\text{Specific Gravity} = \frac{W}{W1}$$

where:
W = weight of the liquid soap sample
W_1 = weight of distilled water

Antibacterial Activity of Pineapple Peel Extract Liquid Soap

The antibacterial activity of pineapple peel extract liquid soap formulations was evaluated against *S. aureus* and *S. pyogenes* using the agar well diffusion method. Bacterial suspensions of *S. aureus* and *S. pyogenes* were prepared and adjusted to 0,5 McFarland standard turbidity. The standardized inoculum was evenly spread onto MHA plates using sterile cotton swabs and allowed to stand briefly to ensure surface absorption. Sterile wells were created in the agar medium using a cork borer. Each well was filled with 50µL of the test samples consisting of: Pineapple peel extract liquid soap at concentrations of 5%, 10%, and 15%, Liquid soap base without extract as negative control, and Commercial antiseptic soap (Dettol®) as positive control. All treatments were conducted in triplicate. The inoculated plates were incubated at 37°C for 24 hours. After incubation, inhibition zones formed around each well were observed and measured using a digital caliper (Rahmawati *et al*., 2021). The diameter of the inhibition zone was calculated using the following equation: Inhibition Zone (mm) = Total diameter of clear zone including the well - Diameter of the well (mm).

Statistical Analysis

Data were analyzed using statistical software. Normality of data distribution was assessed using the Shapiro-Wilk test. Since the data were not normally distributed, non-parametric analysis was performed using the Kruskal-Wallis test to determine overall differences among groups, followed by the Mann-Whitney post hoc test for pairwise comparisons. Statistical significance was set at $p < 0,05$.

Result and Discussion

Physical Quality Evaluation of Pineapple Peel Extract Liquid Soap

The physical evaluation demonstrated that all liquid soap formulations complied with general quality requirements for topical cleansing products. Organoleptic assessment revealed a concentration-dependent change in color intensity, ranging from

light yellow (5%) to light orange (10%) and deep orange (15%), while the negative control exhibited a pale yellowish-white appearance. The progressive color intensification reflects increasing concentrations of pineapple peel extract incorporated into the formulation, indicating uniform extract dispersion within the soap matrix.

The 10% and 15% formulations exhibited a characteristic pineapple odor, whereas the 5% formulation retained a more noticeable olive oil scent. All preparations were in liquid form, although the 5% formulation appeared slightly more viscous. This difference may be attributed to variations in extract–base interaction affecting rheological properties. Homogeneity testing confirmed that all formulations were uniformly dispersed without visible coarse particles or phase separation, indicating successful incorporation of the extract into the soap base (Table 2).

Table 2. Physical Quality Test Results

Treatment	Organoleptic			Homogeneity	pH	Free Alkali (%)	Specific Gravity (g/mL)
	Color	Odor	Form				
5%	Light yellow	Pineapple	Slightly viscous	Homogeneous	7,3	0,0897	1,04
10%	Light orange	Pineapple	Liquid	Homogeneous	9,7	0,1	1,023
15%	Deep orange	Pineapple	Liquid	Homogeneous	8,6	0,0897	1,01
Negative Control	Pale yellowish-white	Olive oil	Liquid	Homogeneous	11	0,078	1,044

The pH values of the extract-containing formulations ranged from 7,3 to 9,7, whereas the negative control showed a pH of 11. These values fall within the generally acceptable range for liquid soap preparations and are considered suitable for topical cleansing applications (Muna *et al*., 2021). The slightly higher pH observed in the negative control may be attributed to residual alkalinity from the saponification process. Variations in pH among extract containing formulations may reflect differences in phytochemical composition and their interaction with the alkaline soap base.

The free alkali content ranged from 0,0897% to 0.1%, with the negative control at 0,078%. All values complied with the maximum allowable limit (≤0.1%) established in the Indonesian National Standard for liquid soap (Adjeng *et al*., 2020). These findings indicate adequate completion of the saponification process and suggest minimal risk of skin irritation associated with residual alkali.

Specific gravity values ranged from 1,01 to 1,04g/mL, which are within the acceptable standard range for liquid soap preparations (Adjeng *et al*., 2020). A slight decrease in density was observed with increasing extract concentration, possibly due to differences in solute composition and molecular interactions within the formulation system.

Overall, the physicochemical evaluation confirms that pineapple peel extract can be successfully incorporated into a stable liquid soap formulation without adversely affecting essential quality parameters. The results demonstrate that increasing extract concentration did not compromise formulation stability, thereby supporting its suitability for further antibacterial evaluation.

Antibacterial Activity Testing of Liquid Soap

The bioassay profiles demonstrated that incorporating ethanolic pineapple peel extract into the liquid soap formulations successfully restricted the growth of both *S.*

aureus and *S. pyogenes*. A distinct dose-dependent profile was observed, wherein higher levels of the plant extract produced systematically larger clearing zones (Figure 1, Figure 2, Table 3). This concentration-driven pattern aligns tightly with previous findings on agricultural-derived bioactives used in topical sanitation matrices (Alemu *et al*., 2024).

Quantitatively, *S. aureus* exposed to the 5%, 10%, and 15% formulations produced mean zone boundaries of 9,28 ± 0,70mm, 10,21 ± 1,43mm, and 13,30 ± 1,51mm, respectively. Meanwhile, the expansion of clear zones against *S. pyogenes* reached 15,16 ± 0,62mm, 16,35 ± 1,25mm, and 20,25 ± 2.13mm across identical treatment escalations. A notable standard deviation (SD) was observed within the S. pyogenes data block, particularly in the negative control 12,00 ± 2,67mm) and the 15% formulation 20,25 ± 2,13mm).

Although all antibacterial assays were conducted using strict triplicates (triplo), this prominent replication dispersion stems primarily from the fastidious and highly sensitive biological nature of S. pyogenes. In solid agar environments, this hemolytic pathogen responds sharply to minor micro-environmental gradients, including local moisture shifts, oxygen depletion levels, and localized diffusion kinetics of the dense surfactant matrix. These factors combined can produce marginal boundary variations during the 24-hour incubation phase.

Referring to standard clinical susceptibility thresholds, zones spanning 10-20mm reflect strong antibacterial performance, whereas values intersecting or exceeding 20mm indicate very strong capabilities. Guided by this system, the 15% liquid soap candidate achieved a strong inhibitory rating against *S. aureus* and advanced to a very strong performance tier when evaluated against *S. pyogenes*.

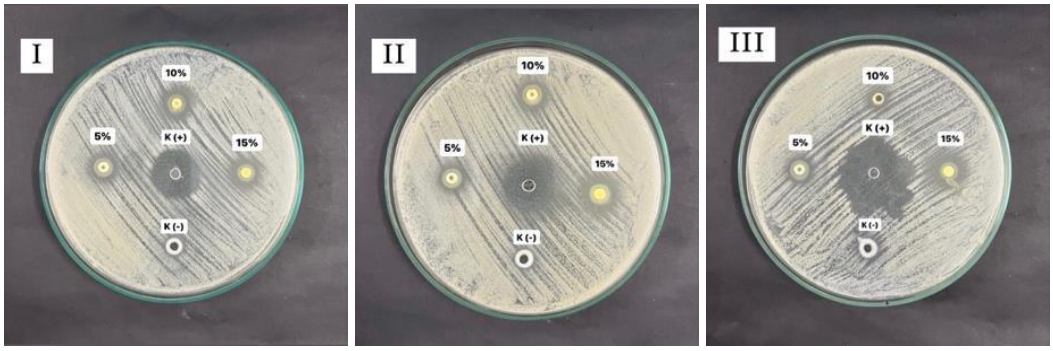

Figure 1. Inhibition zone test of liquid soap containing ethanol pineapple peel extract against *S. aureus*. Note: K (+) = X Liquid Soap, K (-) = Soap base.

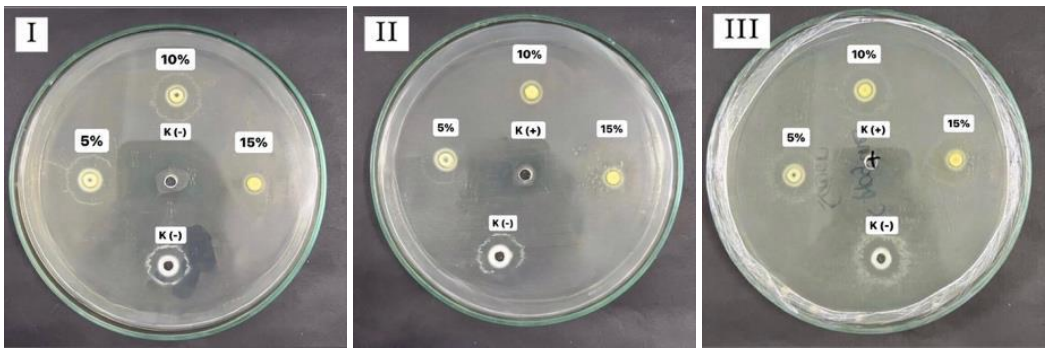

Figure 2. Inhibition zone test of liquid soap containing ethanol pineapple peel extract against *S. pyogenes*. Note: K (+) = X Liquid Soap, K (-) = Soap base.

The unadulterated soap base (negative control) displayed baseline clearing diameters of 5,56 ± 0,60mm and 12,00 ± 2,67mm against *S. aureus* and *S. pyogenes*, respectively. This highlights the inherent antimicrobial properties of the core fatty acid-surfactant framework. Anionic surfactants, such as SLS, coupled with potassium-saponified oil complexes, naturally destabilize bacterial envelopes by inducing lipid bilayer fragmentation (Sharma *et al*., 2022). Crucially, however, the significantly wider clear zones documented in the extract-supplemented soap trials confirm that the elevated potency is driven by the therapeutic action of the raw pineapple peel components rather than the soap base alone.

Table 3. Inhibition zone test results of liquid soap containing ethanol pineapple peel extract against *S. aureus* and *S. pyogenes*

Treatment	Min	Max	Mean ± SD
***Staphylococcus aureus*:**			
5% Concentration	8,60	10,00	9,28 ± 0,70
10% Concentration	9,00	11,80	10,21 ± 1,43
15% Concentration	11,60	14,50	13,30 ± 1,51
Control (+)	22,00	28,00	24,33 ± 3,21
Control (-)	5,10	6,25	5,56 ± 0,60
***Streptococcus pyogenes*:**			
5% Concentration	14,50	15,75	15,16± 0,62
10% Concentration	14,90	17,15	16,35± 1,25
15% Concentration	18,25	22,50	20,25 ± 2,13
Control (+)	23,25	27,00	24,63 ± 2,05
Control (-)	9,00	13,75	12,0 ± 2,67

To evaluate practical relevance, a comparative glance at the work of Rahmawati *et al*. (2021) shows that their 15% pineapple peel liquid soap yielded an 11,23mm inhibition zone against *S. aureus* ATCC 25923. In contrast, our corresponding 15% formulation yielded a superior clearance profile (13,30 ± 1,51mm). This performance deviation may be linked directly to differences in base saponification chemistry; our selection of an olive oil base combined with a 15% KOH framework potentially creates a molecular environment with lower steric hindrance, allowing lipid-soluble phytochemical fractions to diffuse more smoothly through the solid MHA gel network.

When compared to previous literature, Rahmawati *et al*. (2021) evaluated a liquid soap formulated with pineapple peel extract against *S. aureus* ATCC 25923 and reported an inhibition zone of 11,23mm at a 15% concentration. In comparison, our 15% formulation achieved a slightly higher zone (13,30 ± 1,51mm) against S. aureus. This discrepancy likely stems from differences in the soap base composition, specifically our use of olive oil combined with a 15% KOH saponification system, which may offer better biochemical compatibility and lower steric hindrance for the diffusion of the extract's active components through the agar matrix.

The dataset also highlights that *S. pyogenes* was vastly more vulnerable to all extract treatments compared to *S. aureus*. This susceptibility gap is deeply rooted in cell wall structural divergence. The outer surface of *S. aureus* features a heavily cross-linked peptidoglycan network backed by a pronounced capacity to organize thick, protective biofilms. These extracellular structures act as a physical barrier that restricts the

penetration of complex plant secondary metabolites (Craft *et al*., 2019; Tong *et al*., 2015). Conversely, *S. pyogenes* relies heavily on surface-anchored M proteins for environmental survival. While these macromolecular structures prevent human immune cell recognition, their outward-projecting, highly charged filamentous design can inadvertently function as electrostatic traps for amphiphilic botanical components such as saponins and polyphenols. This interaction creates high localized concentrations of active compounds on the cell surface, accelerating envelope rupture and cell lysis before a protective biofilm barrier can be organized.

The comprehensive antibacterial action observed in these liquid soaps is driven by the qualitative presence of secondary metabolites, including tannins, saponins, flavonoids, and alkaloids (Mursyida *et al*., 2025). Structurally, flavonoids induce membrane leaking and impede intracellular replication machinery (Farhadi *et al*., 2019). Saponins exert natural detergent-like forces that permeabilize cell envelopes (Podolak *et al*., 2023), which directly facilitates the entry of alkaloids to break down core peptidoglycan assembly pathways (Cushnie *et al*., 2014). It must be noted as a study limitation that quantitative profiling of these fractions (such as Total Phenolic or Total Flavonoid Content) within the final soap form was not conducted in this phase. Future quantitative verification is highly recommended to clarify precise dose-response parameters within commercial-grade cosmetic matrices.

Non-parametric evaluation via the Kruskal-Wallis test established statistically significant variances across the evaluated groups ($p < 0,05$), validating that extract dosage directly dictates the final zone size (Table 4). Subsequent Mann-Whitney pairwise analysis showed that differences between adjacent lower concentrations (5% versus 10%) did not achieve statistical significance. This implies that a definitive concentration threshold specifically transitioning to a minimum of 15% is crucial to achieve a mathematically and biochemically superior antibacterial performance.

Table 4. Kruskal-Wallis test results for the antibacterial activity of liquid soap containing ethanol pineapple peel extract against *S. aureus* and *S. pyogenes*

Treatment	P-value
***Staphylococcus aureus*:**	
5% Concentration	
10% Concentration	
15% Concentration	0,015
Control (+)	
Control (-)	
***Streptococcus pyogenes*:**	
5% Concentration	
10% Concentration	
15% Concentration	0,011
Control (+)	
Control (-)	

Table 4. Mann-Whitney test results for the inhibitory activity of liquid soap containing ethanol pineapple peel extract against *S. aureus* and *S. pyogenes*

Treatment	Concentration				
	5%	10%	15%	Control (+)	Control (+)
***Staphylococcus aureus*:**					
5% Concentration		0,275	0,05*	0,05*	0,05*
10% Concentration			0,376	0,05*	0,05*
15% Concentration				0,05*	0,05*
Control (+)					0,05*
***Streptococcus pyogenes*:**					
5% Concentration		0,275	0,05*	0,05*	0,05*
10%Concentration			0,05*	0,05*	0,05*
15% Concentration				0,05*	0,05*
Control (+)					0,05*

Explanation: * = significant difference

Conclusion

Liquid soap formulated with ethanolic pineapple peel extract satisfied basic physicochemical safety metrics and delivered significantly enhanced antibacterial performance compared to the soap base alone. The 15% formulation established the peak inhibition threshold against both Gram positive organisms, with *S. pyogenes* showing the highest sensitivity. These data validate the viability of transforming agricultural waste by-products into functional, natural-based antiseptic cleansing alternatives.

Declaration of Competing Interest

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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