



Innovative Ecoenzyme-Based Liquid Hand Soap from Fruit and Vegetable Waste Using Sugar and Palm Sugar: A Sustainable Antibacterial Solution Against Gram-Positive and Gram-Negative Bacteria Supporting SDGs and National Health Initiatives

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Abstract: Indonesia's abundant fruit/vegetable waste threatens the environment yet fuels sustainable hygiene innovation. The waste will be fermented anaerobically with sugar and palm sugar. The study will also assess the soap's efficacy against *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative). To achieve this, we produced an eco-enzyme by anaerobic fermentation. We then incorporated 5–10% of this enzyme directly into a pre-heated soap base. A hybrid hot-cold process was used: first, hot mixing ensured homogeneity; next, cold stabilisation was applied. pH testing and agar well diffusion assays followed. Results: Dose-response assays yielded significant ($p < 0.05$) increases in inhibition zone size; palm sugar at 10% excelled (35.3 mm *E. coli*, 23.6 mm *S. aureus*), surpassing commercial products. ANOVA followed by Tukey's post hoc ($\alpha = 0.05$) showed that sugar variants (23.9 mm *E. coli*, 22.3 mm *S. aureus*) outperformed controls. pH: F0 8.83, F1 5.26, F3 4.79. Discussion: Remarkably, the exceptionally broad 35.3 mm *E. coli* zone reveals a groundbreaking novelty: unprecedented Gram-negative hyper susceptibility contrary to LPS resistance norms uniquely triggered by eco-enzyme organic acids synergising with palm sugar gallotannins to breach outer membranes. These zero-waste breakthroughs advance SDGs 3 and 12 by pioneering bio-circular antimicrobials.

Keywords: Antibacterial hand soap; Circular economy; Eco-enzyme; Fermentation bioactives; Gram-negative disruption

Introduction

Regular handwashing with soap is widely recognised as one of the most effective public health practices for preventing infectious diseases. Proper hand hygiene can reduce the transmission of pathogenic microorganisms and has been estimated to prevent approximately 40% of deaths associated with diarrhoeal

diseases, making it a key indicator of clean and healthy living behaviour. Soap works by removing dirt, organic matter, and microbes from the skin surface through the action of surfactants that break down oils and allow contaminants to be rinsed away with water. Despite these benefits, many commercially available antiseptic soaps rely on synthetic antimicrobial agents, such as triclosan, and surfactants, such as sodium lauryl sulfate

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(SLS). Triclosan has been widely used because of its broad-spectrum antimicrobial properties; however, studies have shown that this compound is persistent in aquatic environments and can accumulate in water systems after being washed down household drains. Environmental exposure to triclosan has been associated with endocrine disruption in aquatic organisms and may contribute to the development of bacterial resistance to antimicrobial agents (Halden et al., 2017). Similarly, sodium lauryl sulfate, which is commonly used to produce foam and improve cleansing efficiency, may cause skin irritation and dryness in sensitive individuals and has been reported to accumulate in the environment under certain conditions (Bondi et al., 2015). Commercial Triclosan/SLS soaps risk resistance and pollution (Bondi et al., 2015; Kumar et al., 2024). These concerns highlight the need for safer and more environmentally friendly alternatives to conventional antiseptic soaps.

One promising alternative is the development of liquid soaps derived from eco-enzymes produced through the fermentation of fruit and vegetable waste (Cowan, 1999; Gupta & Kumar, 2021). Eco-enzymes are liquids resulting from the fermentation of organic waste with sugar and water, containing enzymes and bioactive compounds that function as natural antibacterials (Ramanda et al., 2025). One important application of eco-enzymes is as an active ingredient in liquid soap, which can inhibit the growth of both Gram-positive and Gram-negative bacteria, thereby supporting public health initiatives and SDGs objectives.

Eco enzymes are liquids produced by fermenting fruit and vegetable waste with granulated sugar or palm sugar, containing bioactive enzymes such as protease, amylase, and lipase, alongside practical antimicrobial components. This fermentation process not only reduces waste but also yields multifunctional products that benefit households and public health. Eco enzymes have been proven to possess broad-spectrum antibacterial activity owing to their content of acetic acid, alcohol, tannins, flavonoids, and saponins. These compounds are effective in inhibiting the growth of both Gram-positive and Gram-negative bacteria (Mavani et al., 2020; Hendri et al., 2023). Fermented unripe papaya (*Carica papaya*) peel extract yields an eco-enzyme high in papain, demonstrating strong antibacterial activity against *Enterococcus faecalis* (Bhardwaj et al., 2012). Recent studies indicate that the minimum inhibitory concentration (MIC) of eco-enzymes ranges from 3.1 to 25 mg/mL against bacteria responsible for skin and foodborne infections (Mavani et al., 2020). Furthermore, the use of granulated and palm sugar as carbon sources for fermentation has been shown to enhance the quality and quantity of active metabolites in eco-enzymes (Sulistyah et al., 2022).

Liquid soap formulated with surfactants and additional eco-enzymes represents an innovative solution for hand hygiene. Scientifically, this liquid soap has been demonstrated to improve cleaning efficacy and exhibits a synergistic effect in inactivating microorganisms (Klimaszewska et al., 2022; Vidalia et al., 2023). Surfactants in liquid soap enhance the mixing and penetration of cleaning agents, ensuring optimal removal of pathogens from the skin, whilst eco-enzymes offer natural antimicrobial support that is both biodegradable and environmentally friendly (Klimaszewska et al., 2022; Khan, 2025). Gram-positive and Gram-negative bacteria exhibit distinct characteristics, underscoring the need to evaluate the effectiveness of eco-enzymes on both (Sulfa et al., 2024). Whilst eco-enzymes inhibit Gram+/- bacteria, no studies explore sugar vs palm sugar substrates in hot-cold processed soaps.

Staphylococcus aureus is a Gram-positive bacterium characterised by spherical cells (cocci) arranged in grape-like clusters, and it is catalase-positive and coagulase-positive with distinctive golden-yellow colonies. Although it commonly colonises human skin and mucosal surfaces as part of the normal microbiota, it can become an opportunistic pathogen causing various infections, including those caused by Methicillin-Resistant *Staphylococcus aureus* (MRSA), skin and soft tissue infections, pneumonia, and sepsis (Dewi et al., 2024; Esposito et al., 2023). In contrast, *Escherichia coli* is a Gram-negative, rod-shaped bacterium that normally inhabits the human gastrointestinal tract but may cause disease such as urinary tract infections (UTIs) and diarrheal illness. Its pathogenicity is partly related to lipopolysaccharide (LPS) in the outer membrane, which contributes to virulence and can enhance resistance to certain antibiotics (Faja et al., 2025). Eco-enzymes fermented organic liquids derived from fruit and vegetable waste have been reported to inhibit the growth of both bacteria at a dilution ratio of 1:10 (Khan et al., 2018). Their antibacterial activity stems from bioactive compounds such as tannins, which disrupt lipopolysaccharides through hydrogen bonding, and saponins, which act as natural surfactants interacting with the lipid bilayer of cell membranes increasing permeability and causing lysis (Villanueva et al., 2023; Shiri et al., 2025).

In addition, eco-enzymes contain acetic acid and enzymes such as lipase and amylase, which can enhance antibacterial activity, making them a potential, safe, and environmentally friendly natural disinfectant (Permatananda et al., 2023). The utilization of eco-enzymes as an active ingredient in liquid soap not only supports organic waste management but also provides an innovative solution to tackling infections caused by

both Gram-positive and Gram-negative bacteria, including *S. aureus* and *E. coli*.

The habit of washing hands with soap is crucial for preventing infectious diseases and serves as a principal indicator of clean living behavior. Nonetheless, the development of liquid soap based on eco-enzymes derived from fruit and vegetable waste, using sugar and palm sugar substrates, remains rarely reported, especially for broad-spectrum antibacterial applications. Therefore, this study aims to develop eco-enzyme liquid soap formulations using hot and cold processing techniques and to evaluate their antibacterial activity against representative pathogens, including *Staphylococcus aureus* and *Escherichia coli*. By utilising organic waste as a raw material, this approach supports sustainable waste management while promoting the production of environmentally friendly hygiene products. This initiative is expected to make a tangible contribution to organic waste management, support the national "Asta Cita" and SDGs agenda, and produce sustainable, value-added, and healthy cleaning products. The development of liquid soap based on eco-enzymes derived from fruit and vegetable waste, using sugar and palm sugar substrates, represents a strategic step towards supporting public health, sustainable waste management, and achieving the SDGs and Asta Cita. This innovation is anticipated to deliver an effective, safe, and environmentally friendly cleaning product capable of addressing future challenges posed by bacterial resistance.

Method

The apparatus employed in this study included a beaker, a measuring cylinder, test tubes, a test tube rack, an electric stove, a watch glass, a dropper pipette, a spatula, magnetic stirrer, and labelling paper. The principal materials comprised eco-enzyme liquid

derived from fruit and vegetable peels, palm sugar, sugar, water, Sodium Laureth Sulfate (SLES), Nipagin, glycerine, EDTA, nutrient agar, *S. aureus*, *E. coli* nutrient broth, sterile gauze, aluminium foil, and commercially available liquid soap as a positive control.

Research Stages

Ecoenzyme Production

The eco-enzyme was prepared by fermenting fruit and vegetable waste with palm sugar and water as alternative substrates, in a 3:1:10 (w/v/v) ratio. The mixture was placed in a tightly sealed anaerobic container and fermented at room temperature (28–32°C) for 3 months. The resulting filtrate was collected by filtration through a calico filter cloth and stored in sterile (Umarudin et al., 2024; Vidalia et al., 2023). The palm sugar-based eco enzyme (Figure 1A) yielded a dark brown, highly liquid with a strong fruity-acidic aroma, resulting from Maillard reactions driven by the high molasses content of palm sugar. In comparison, the white sugar-based eco enzyme (Figure 1B) produced a light yellow, less viscous fluid with a milder sweet-fermented scent (Figure 1). The phytochemical screening involved the detection of flavonoids, saponins, and tannins, based on the methods of Marbun et al. (2024), Rasmi et al. (2023), and Kumaradewi et al. (2021).

Table 1. Formulation of liquid hand soap (modified from Umarudin et al. (2024))

Material	Formula (w/v)		
	F1	F2	F0
Eco-enzyme Concentration	5	10	-
Sodium Laureth Sulfate (SLES)	4	4	4
Nipagin	0,1	0,1	0,1
Glycerin	5	5	5
EDTA	0,1	0,1	0,1
Water	add 100ml	add 100 ml	add 100ml

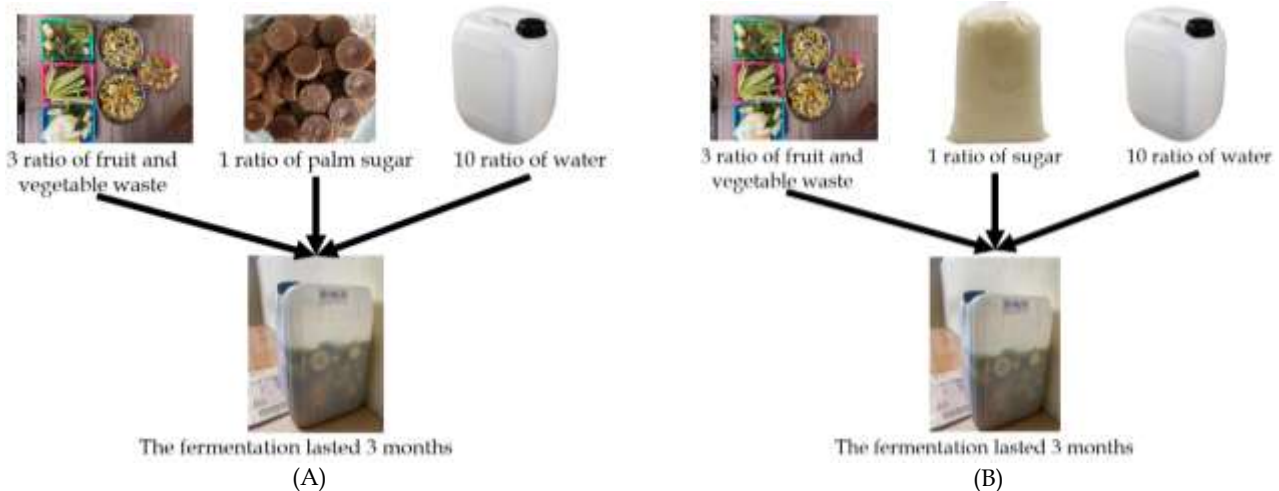


Figure 1. (A) Ecoenzyme Production fruit and vegetable waste with palm sugar and (B) Ecoenzyme Production fruit and vegetable waste with sugar

Stage I: Sodium Laureth Sulfate (SLES), Nipagin (methylparaben), and EDTA were first prepared in a clean beaker. Nipagin was pre-dissolved either in a small amount of hot distilled water (>80 °C) or in a suitable solvent to ensure complete dissolution. Afterwards, distilled water was gradually added to the beaker while stirring gently with a magnetic stirrer at 200–300 rpm for approximately 10–15 minutes at room temperature. This step was carried out carefully to obtain a uniform solution and to minimise excessive foam formation. Stage II: Glycerin was then added to the mixture as a humectant, and the mixture was stirred gently until a homogeneous solution was obtained. Subsequently, the eco-enzyme was incorporated at the predetermined concentration. The mixture was stirred continuously at 200–300 rpm for 5–10 minutes until all components were evenly distributed and a clear liquid formed. The final hand soap formulation was then poured into clean hand soap dispenser bottles and allowed to stabilise for approximately 24 hours before use (Umarudin et al., 2024).

Preparation of Test Microorganisms

A sterile inoculating loop was used to collect the test bacteria, which were then suspended in a tube containing 2 mL of 0.9% NaCl solution until the turbidity matched that of the McFarland standard. This procedure was applied to all bacterial strains under examination (Khairi et al., 2023).

Muller Hinton Agar Treatment Stage

A total of 11.4 g of Mueller-Hinton Agar (MHA) powder was dissolved in 300 mL of distilled water (scaled to prepare exactly 20 Petri dishes at 15 mL per dish). The medium was thoroughly mixed until it appeared transparently yellow, indicating complete dissolution, then autoclaved at 121°C for 15 minutes. Approximately 15 mL of molten MHA was poured into each sterile Petri dish. Once the agar had solidified, a sterile cotton swab was dipped into the standardised bacterial suspension (0.5 McFarland standard), excess fluid was expressed, and the bacteria were evenly streaked across the agar surface using the streak plate method to form a uniform lawn (Sander, 2012; Padzil et al., 2021; Sianipar et al., 2025). Six-mm diameter wells were then punched into the agar using a sterile cork borer. Each Petri dish contained four wells filled with 30 µL of the respective samples: negative control (base shope), positive control (commercially branded antibacterial soap), sugar eco-enzyme hand soap at 5% (w/v) concentration (F1), and palm sugar eco-enzyme hand soap at 10% (w/v) concentration (F2). The same protocol was followed for MHA plates inoculated with *E. coli* and *S. aureus*. Plates were incubated at 37°C for 24 hours. The diameter of the inhibition zones was

measured in two perpendicular directions and averaged to assess antibacterial activity (Triadi et al., 2022; Sianipar et al., 2025).

Data Processing

The collected data were analyzed using analysis of variance (ANOVA) according to a Completely Randomized Design (CRD) for eco-enzymes made with palm sugar and sugar, as well as for the bacterial species *E. coli* and *S. aureus*. Before further analysis, the data were tested for normality and homogeneity. Subsequent statistical evaluation included ANOVA, followed by Tukey post hoc test when appropriate.

Result and Discussion

The eco-enzymes employed in this study, derived from fruit and vegetable waste as illustrated in Figure 2, were produced using both white sugar and palm sugar as fermentation substrates.

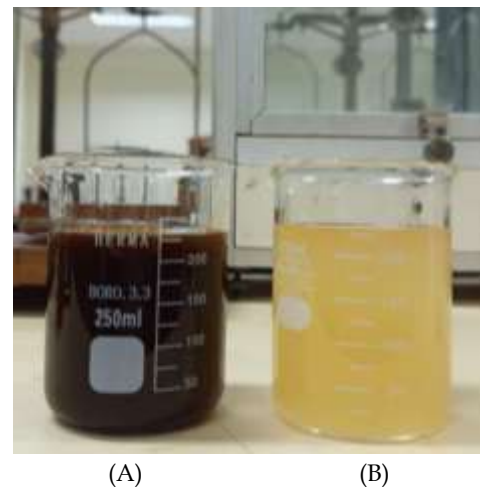


Figure 2. (A) Palm sugar eco-enzyme and (B) Sugar eco-enzyme

The eco-enzyme produced from palm sugar (Figure 2A) exhibited a dark brown colour and a strong fruity-acidic odour. These characteristics indicate the formation of Maillard reaction products resulting from the high molasses content in palm sugar. In contrast, the eco-enzyme produced from sugar (Figure 2B) appeared light yellow and emitted a milder sweet-fermented aroma (Figure 2). These morphological and colour differences reflect the substrate-specific fermentation profile palm sugar fermentation produces a darker colour due to caramelisation reactions, whereas sugar fermentation results in a clearer, lighter-coloured solution. These observable characteristics are typical of successful anaerobic fermentation processes, reflecting the Maillard reactions and microbial metabolism of fruit

and vegetable waste substrates over the 3-month maturation period.

These bio-fermented extracts served as the primary active agents in the liquid hand soap formulations, leveraging their natural antimicrobial properties for practical hygiene applications. To evaluate their chemical composition, qualitative phytochemical screening was conducted, a standard preliminary analysis aimed at identifying key secondary metabolites responsible for biological activity. The results, summarised in Table 2 below, confirm the presence of tannins and saponins in eco-enzymes from both sugar variants, with no detection of other compounds such as flavonoids in this context.

Table 2. Phytochemical screening results for eco-enzymes

Test	Sugar Eco-Enzyme	Palm Sugar Eco-Enzyme	Inference
Tannins	+	+	Present
Saponins	+	+	Present

Note: (+) = Positive; (-) = Negative.

As shown in Table 2, both eco-enzyme variants from fruit and vegetable waste exhibited positive responses to tannins and saponins, compounds well documented for their antibacterial potential through membrane disruption and protein precipitation. These findings prompted their incorporation into hand soap formulations (F1 and F2), where they function alongside conventional excipients, liquid hand soap Figure 3.



F2 F1 F0
(A)



F1 F2 F0
(B)

Figure 3. (A) Palm sugar eco-enzyme hand soap and (B) Sugar eco-enzyme hand soap

Liquid hand soaps play a vital role in hand hygiene, with robust evidence demonstrating their efficacy in eliminating bacteria and curbing the transmission of infectious diseases such as diarrhoea – a leading cause of childhood morbidity globally (WHO, 2021). In our formulations, F1 and F2 contained ecoenzyme as the active ingredient, while the control (K-) lacked it; auxiliary components included Texapon (4%), Nipagin (0.1%), glycerin (5%), EDTA (0.1%), and distilled water as solvent. Texapon, or sodium lauryl sulfate (SLS), influences foam height and density by reducing surface tension, with the 4% concentration optimising lather without compromising stability (Umarudin et al., 2024). Nipagin (methylparaben) acts as a preservative at 0.1% – well below the maximum allowable 0.4–0.8% in liquid soaps – ensuring microbial shelf-life (BPOM, 2017). Glycerin provides humectant effects, enhancing

skin moisturisation and product quality (Loden, 2005), while EDTA, at 0.1%, exhibits supplementary antimicrobial action by sequestering divalent cations essential for bacterial enzymes (Russell, 2003). This synergistic blend yields an eco-friendly, effective hand soap suitable for community-scale production.

The following presents the results of antibacterial assays against the Gram-positive bacterium *S. aureus* and the Gram-negative bacterium *E. coli*. Table 3 summarizes the inhibition zone diameters (in mm) obtained from the agar well diffusion method for eco-enzyme-based liquid hand soaps derived from fruit and vegetable waste, tested at varying concentrations against both pathogens. These findings demonstrate a clear dose-dependent antibacterial efficacy, with superior performance against *E. coli*.

Table 3. Antibacterial activity of eco-enzyme liquid hand soap from fruit and vegetable waste against *S. aureus* and *E. coli*

Treatment	Average inhibition zone (mm)			
	Antibacterial activity of sugar eco-enzyme hand soap against <i>S. aureus</i>	Antibacterial activity of palm sugar eco-enzyme hand soap against <i>S. aureus</i>	Antibacterial activity of sugar eco-enzyme hand soap against <i>E. coli</i>	Antibacterial activity of palm sugar eco-enzyme hand soap against <i>E. coli</i>
-	7.8 ^a	9.5 ^a	9.9 ^a	17 ^a
+	19.5 ^b	24.4 ^b	22.2 ^b	20.7 ^a
5%	21 ^b	23.2 ^b	22.5 ^b	28.6 ^b
10%	22.3 ^c	23.6 ^b	23.9 ^b	35.3 ^c



Figure 4. The inhibition zone formed in bacteria: (A) Sugar eco-enzyme hand soap, (B) Palm sugar eco-enzyme hand soap against *S. aureus*, (C) Sugar eco-enzyme hand soap, and (D) Palm sugar eco-enzyme hand soap against *E. coli*

Table 3 illustrates Tukey's test on *S. aureus* confirmed statistically significant differences among treatments. The negative control produced the smallest inhibition zone and differed significantly from the positive control at 5% and 10%. The positive control and 5% concentration did not differ significantly from one another ($p > 0.05$), indicating comparable antibacterial activity. However, both treatments exhibited significantly lower inhibition compared with the 10% concentration ($p < 0.05$). These findings demonstrate a dose-dependent increase in antibacterial efficacy, with the 10% concentration showing the highest inhibitory activity. Tukey's analysis of *E. coli* revealed striking differences among treatments. The negative and positive controls did not differ significantly from one another ($p > 0.05$), indicating comparable inhibitory activity; however, both were significantly inferior to the sugar eco-enzyme treatments ($p < 0.05$). The sugar eco-enzyme at 5% and 10% concentrations produced inhibition zones significantly larger than either control ($p < 0.05$). Furthermore, the 10% concentration exhibited a significantly greater inhibitory effect than the 5% concentration ($p < 0.05$), confirming a dose-dependent enhancement of antibacterial efficacy.

Tukey's post hoc test on *Staphylococcus aureus* showed that the negative control had the smallest mean inhibition zone and differed significantly from all treatment groups. Meanwhile, the positive control, along with both palm sugar eco-enzyme concentrations (5% and 10%), showed no significant differences, indicating equivalent efficacy among these three groups.

These findings demonstrate that varying the palm sugar eco-enzyme concentration within the tested range had no meaningful impact on enhancing antibacterial activity against *S. aureus*. Consequently, the eco-enzyme soap was statistically comparable in effectiveness to the commercial soap, without any dose-dependent improvement. Tukey's test revealed highly significant differences among treatments in the *E. coli* assay. The negative control produced the smallest inhibition zone and differed significantly from all other treatment groups. The positive control exhibited relatively low inhibitory activity and did not differ significantly from the negative control. Subsequently, the 5% palm sugar eco-enzyme produced a significantly larger inhibition zone than both the negative and positive controls, yet remained significantly lower than the 10% concentration. Thus, the 10% concentration demonstrated the highest inhibitory and bactericidal activity, indicating a dose-dependent increase in efficacy.

Ecoenzymes from fruit and vegetable waste ferment sugars to produce organic acids, ethanol compound, enzymes, and secondary metabolites such as tannins and saponins, which are key to their antibacterial action. These compounds disrupt bacterial physiology, supporting the observation of greater inhibition of *E. coli* compared to *S. aureus*, as the outer membrane of Gram-negative bacteria is more permeable to phenolics during acid fermentation.

The innovation to use the different type of sugar as the medium for ecoenzyme impacted to the different

ethanol and acetic acid compound as final product of the fermentation. Palm sugar reported as the best alternative carbon source in ecoenzyme after the molase that produce the acetic acid content (Rahman et al., 2025). However, sugar have ability to enhance the stability of organic acid content (Hanifa et al., 2021). This acetic acid is well known as the antibacterial agent (inhibit the *E. coli*, *S. aureus*, *P. vulgaris*, and *P. aeruginosa*), less toxic disinfectant, and nonvolatile compound (Muazzam et al., 2025; Fraise et al., 2013). It is so promising component agent for further application as hand soap.

Eco-enzyme-based liquid hand soaps formulated from fruit and vegetable waste demonstrate promising antibacterial properties against both *S. aureus* (Gram-positive) and *E. coli* (Gram-negative), corroborated by prior research and the present findings (Czerkas et al., 2024; Ramadani et al., 2022). These soaps benefit from a complex matrix of bioactive compounds generated during anaerobic fermentation, including organic acids (e.g., acetic and lactic acids), flavonoids, tannins, and saponins, which collectively disrupt microbial growth through multifaceted natural antibacterial mechanisms.

As evidenced in Figure 4, palm sugar-based hand soap produced substantially larger inhibition zones than sugar-based variants, attributable to elevated phenolic content from molasses precursors, which enhanced tannin-saponin synergy during extended fermentation. Post-hoc ANOVA (Tukey, $\alpha = 0.05$) confirmed the dose-response trend, with the 10% sugar eco-enzyme formulation achieving *E. coli* inhibition zones of 23.9 mm and *S. aureus* zones of 22.3 mm. In contrast, the 10% palm sugar eco-enzyme soap yielded markedly larger zones of inhibition against *E. coli* (35.3 mm) and *S. aureus* (23.6 mm), both surpassing the commercial hand soap benchmark. These results underscore the superior efficacy of eco-enzyme formulations, particularly against Gram-negative *E. coli* and Gram-positive *S. aureus*, driven by enhanced tannin-saponin interactions that disrupt outer membrane integrity.

This is likely due to differences in the structure and composition of the cell wall between *Escherichia coli* and *Staphylococcus aureus*. *E. coli* is a Gram-negative bacterium with a relatively thinner peptidoglycan layer in its cell wall (Marbun et al., 2024). In the outer membrane of the *E. coli* cell wall, porin proteins act as channels for the entry and exit of active compounds. Consequently, the active components in the liquid eco enzyme soap derived from fruit and vegetable waste can more readily penetrate *E. coli* cells and inhibit intracellular enzyme activity, resulting in greater cell damage than in *S. aureus* in line with the research results in Table 3.

This aligns with tannins' established ability to disrupt the lipopolysaccharide (LPS) layer in the outer membrane of *E. coli*. Tannins, polyphenolics

upregulated during palm sugar fermentation, exert antibacterial effects by binding bacterial adhesins and enzymes through hydrogen bonding, precipitating proteins, and elevating membrane permeability—effects particularly pronounced in *E. coli* (Tomiyama et al., 2016; Panduwati et al., 2025). Saponins are likely to induce leakage of intracellular components, thereby inhibiting bacterial growth. The antibacterial activity of saponins is thought to occur by damaging the bacterial cell membrane, resulting from increased membrane permeability induced by their interaction with the microbial cell wall (Panduwati et al., 2025).

Tannins, abundant polyphenolic metabolites in fermented waste, bind to bacterial proteins through hydrogen bonds and hydrophobic interactions, forming irreversible complexes that denature enzymes and increase membrane permeability. In this study, the tannin content of ecoenzymes inhibited *E. coli* more than *S. aureus*, which has a thicker peptidoglycan wall, by agglutinating cytoplasmic proteins, causing leakage and lysis (Mikhailova, 2025; Arisurya et al., 2026).

Saponins and glycosides, detected in all variants, act as surfactant-like compounds that can interact with lipopolysaccharide (LPS) in the outer membrane of *E. coli*, potentially perturbing membrane integrity and increasing leakage of alkaline phosphatase. This interaction may contribute to the exceptionally large inhibition zone observed for *E. coli* (35.3 mm), which appears as an unusually strong and anomalous response compared with typical Gram-negative susceptibility patterns, where the LPS-rich outer membrane usually confers greater resistance to phenolic compounds. In this case, the data suggest that saponins may transiently increase outer-membrane permeability in *E. coli*, thereby facilitating the access of tannins and other hydrophobic constituents to the cell envelope. In contrast, the thicker, more rigid peptidoglycan-rich wall of *S. aureus* appears to limit penetration of these compounds, which may partially explain the smaller inhibition zones in this Gram-positive species (Dharmayani et al., 2023; Yeni et al., 2025). The findings of this study are consistent with existing literature, indicating that tannins and saponins possess antibacterial properties. The main strength of this research lies in the utilisation of fruit and vegetable waste as a raw material for eco-enzyme production, combined with sugar and palm sugar, and formulated into a liquid hand-wash product that has not been widely explored for its ability to inhibit the growth of *S. aureus* and *E. coli*. This study contributes new insight into the antibacterial potential of eco-enzyme-based liquid hand soaps, which could be further developed to support the Sustainable Development Goals (SDGs) agenda. The results position eco-enzyme hand soaps as promising alternatives that align with SDG 3 (Good

Health and Well-Being) and SDG 12 (Responsible Consumption and Production). With a skin-compatible pH, these formulations effectively target enteric pathogens commonly found in tropical regions such as Indonesia.

The significance of this research indicates that the palm-sugar-based variant demonstrated superior performance compared to the sugar variant, likely due to higher gallotannin content, enhanced synergy between bioactive compounds and commercial surfactants, and the relatively lower reliance on synthetic detergents, despite some variability in inhibition zone diameters. This suggests that eco-enzyme preparations based on palm sugar may offer greater sustainable potential. Furthermore, this research has important implications for promoting clean, healthy living behaviours, particularly in the context of handwashing with eco-enzyme-containing liquid hand soap as an environmentally friendly hygiene intervention.

Conclusion

This study demonstrates that liquid hand soaps formulated with eco-enzymes derived from fruit and vegetable waste, fermented using either sugar and palm sugar, exhibit antibacterial activity against both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) pathogens. The eco-enzymes are characterised by their distinctive brownish (palm sugar) or yellowish (sugar) appearance and fermented smell, and contain bioactive tannins and saponins that contribute to dose-dependent inhibition zones. At 10% concentration, the palm-sugar-based eco-enzyme generated an inhibition zone of up to 35.3 mm against *E. coli*, which is far beyond the conventional “very strong” benchmark (>20 mm) and was statistically significant compared with the positive control. This unusually high activity against *E. coli* is likely driven by the high concentration of organic acids produced during palm-sugar fermentation, which may transiently enhance outer membrane permeability despite the intrinsic protective role of the LPS-rich Gram-negative envelope. In contrast, the same formulation showed strong but comparatively lower activity against *S. aureus*, with a maximum inhibition zone of 23.6 mm, indicating that the eco-enzyme hand soap is highly effective against Gram-negative pathogens (*E. coli*) while still maintaining strong antibacterial activity against Gram-positive (*S. aureus*). No significant dose-dependent enhancement was observed for *S. aureus* within the tested concentrations, and the palm-sugar eco-enzyme at 5% and 10% did not differ significantly from the commercial soap. For *E. coli*, however, both 5% and 10% eco-enzyme treatments produced significantly

larger inhibition zones than the controls (negative and positive), and the 10% concentration was significantly more effective than the 5% concentration, confirming a dose-dependent increase in antibacterial efficacy.

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Author Contributions

Conceptualization, U. and R.R.; methodology, visualization, N.M.U.; software, analysis, S.A.W.; validation, supervision, S.E.A., U., and R.R.; formal investigation, resources, A.H.R.; data curation, writing—original draft preparation, project administration, funding acquisition, U.; writing—review and editing, S.A.W.

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Conflicts of Interest

The authors declare no conflict of interest.

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