

Optimization of a Green Detergent (PepClean) Formulated from Pepper Washing Effluent and Lime Leaf Extract (*Citrus aurantifolia*) for Sustainable Textile Sanitation

Nadia Meliaputri Anaqiya¹ , Athanasya Novena Bualangi¹ , Faisal^{1*} ,
Fuad Gani² 

¹ Yayasan Pendidikan Sorowako Singkole Junior High School, East Luwu Regency, South Sulawesi 92983, Indonesia

² Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Hasanuddin, Makassar, South Sulawesi 90245, Indonesia

*Corresponding Author e-Mail: faisal@yps.sch.id



Article History:

Submitted:

December 10th, 2025

Accepted:

August 10th, 2026

Available online:

August 26th, 2026

Published:

September 20th, 2026

Keywords:

Antibacterial efficacy.,
green detergent.,
piperine.,
triterpenoid saponins.

ABSTRACT

The downstream processing of pepper agricultural products generates massive organic wastewater, posing severe eutrophication risks. However, this agricultural effluent is fundamentally rich in piperine, a potent bioactive alkaloid. Applying a sustainable circular economy framework, this study investigates the revalorization of pepper wastewater combined with triterpenoid saponins from lime leaves (*Citrus aurantifolia*) to synthesize an eco-friendly liquid textile detergent. A Completely Randomized Design structured the methodology across three formulations: 100 ml lime extract (P1), 100 ml pepper wastewater (P2), and a 1:1 synergistic binary mixture (P3). Physicochemical quality was evaluated against the SNI 4075-1:2017 standard. Statistical analyses via One-Way ANOVA and Tukey HSD testing conclusively demonstrated the superior functional profile of P3. The optimized combination exhibited a stoichiometric pH equilibrium of 7.74, a micellar foam displacement of 20 millimeters, and stability with zero physical sedimentation over a 28-day observation. Detergency efficacy confirmed maximal detergency power, recording an absorbance of 0.075. Furthermore, antimicrobial testing revealed profound in-vitro antibacterial efficacy, yielding a 13.83 mm inhibition zone against *Escherichia coli*. The data support a mechanistic synergism where saponins destabilize bacterial membranes, facilitating piperine penetration. Consequently, this optimized botanical formulation emerges as a highly viable, biodegradable alternative to synthetic commercial petrochemical surfactants.

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How to cite this article:

Anaqiya, N. M., Bualangi, A. N., Faisal, & Gani, F. (2026). Optimization of a Green Detergent (PepClean) Formulated from Pepper Washing Effluent and Lime Leaf Extract (*Citrus aurantifolia*) for Sustainable Textile Sanitation. *Rumphius Pattimura Biological Journal*, 8(2), 093-103. <https://doi.org/10.30598/rumphiusv8i2p093-103>

Journal Homepage: <https://ojs3.unpatti.ac.id/index.php/rumphius>

Journal E-mail: rumphiusbiojournal@gmail.com ; rumphius.journal@mail.unpatti.ac.id

Research Article: [Open Access](#)

INTRODUCTION

Indonesia stands as a leading global exporter of raw spices, a position that cements its importance in worldwide culinary and pharmaceutical networks. In South Sulawesi, specifically within the East Luwu Regency, pepper cultivation spans nearly 4,239.8 hectares, yielding an impressive 24,544 tons of high-grade pepper each year (Riadi et al., 2023). However, the economic success of this agricultural sector comes at a steep environmental cost. During post-harvest processing, the traditional soaking and decortication steps require massive amounts of water to separate the pepper pericarps. Factories and farmers often discharge this nutrient-dense washing water directly into local rivers and municipal drains without prior treatment. This continuous influx of organic matter drastically lowers dissolved oxygen levels and spikes the Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD), causing significant ecological harm and creating aquatic dead zones (Nabilah et al., 2022).

From the perspective of a sustainable circular economy, however, this problematic wastewater is actually an untapped resource. A true biological circular economy seeks to eliminate waste entirely by capturing and repurposing complex biological byproducts into valuable new commodities (Jicheng Shu et al., 2022). Previous phytochemical analyses have shown that pepper washing water retains substantial concentrations of piperine ($C_{17}H_{19}NO_3$) (Gorgani et al., 2017). While primarily known for giving black pepper its characteristic pungency, piperine is also a highly potent, broad-spectrum antimicrobial agent (Tiwari et al., 2020). When properly extracted, piperine can physically disrupt the structural integrity of microbial cell membranes, allowing it to penetrate the cytosol and shut down vital protein synthesis pathways (Riyanto, 2020). By capturing this agricultural runoff before it pollutes local waterways, we can safely revalorize its piperine content as an active antibacterial ingredient for secondary commercial products.

This shift towards botanical cleaning solutions is increasingly urgent given the ecological damage caused by conventional laundry detergents. Today's household sanitation market relies heavily on petrochemical-derived synthetic surfactants, particularly Sodium Lauryl Sulfate (SLS) and Linear Alkylbenzene Sulfonate (LAS) (Maretta & Helmy, 2015). Although excellent at removing dirt, these non-biodegradable chemicals persist in aquatic ecosystems, driving severe eutrophication. The buildup of reactive nitrogen and phosphorus triggers dense algal blooms that ultimately suffocate freshwater flora and fauna (Singh, 2018). Furthermore, the chemical residue left behind on washed textiles frequently causes severe allergic contact dermatitis and chronic skin inflammation in sensitive individuals (Oktavriana et al., 2022). As a result, consumers are increasingly demanding biodegradable, plant-based detergents that effectively clean fabrics and eliminate pathogens without compromising human health or the environment.

To bridge the gap between agricultural waste management and sustainable consumer goods, this study introduces 'PepClean', a novel botanical detergent. We hypothesize that combining piperine-rich pepper washing water with a concentrated organic extract from lime leaves (*Citrus aurantifolia*) will create a powerful synergistic cleaning agent. Lime foliage was specifically chosen due to its naturally high concentration of triterpenoid saponins, a class of highly effective biosurfactants (Minarno, 2016). Saponins possess an amphiphilic molecular architecture, featuring both a hydrophobic triterpene backbone and a hydrophilic carbohydrate moiety (Mustiqawati & Yolandari, 2023). This dual nature allows them to rapidly lower the surface tension of water, successfully emulsifying and encapsulating stubborn, non-polar lipid stains on textile fibers (Wahyuni et al., 2014).

Beyond basic dirt removal, we anticipate a secondary biomolecular synergy between the piperine alkaloids and saponin biosurfactants (Hayunanda et al., 2025). Pharmacological models indicate that amphiphilic saponins aggressively interact with bacterial cell walls, tearing temporary holes in the lipid bilayer and greatly increasing cellular porosity (Nur et al., 2025). We theorize that these structural breaches will act as open channels, allowing piperine to flood into the cell much faster than it could alone (Riyanto, 2020). Once inside, the piperine irreversibly halts internal metabolism, leading to rapid cell death (Tiwari et al., 2020). Because this specific two-part botanical synergy has never been quantified in liquid laundry formulations, our laboratory investigation aims to engineer the optimal ratio of these extracts. We will rigorously evaluate the resulting mixtures against the physicochemical stability, foaming, and antibacterial thresholds set by the Indonesian National Standard (SNI).

MATERIALS AND METHOD

Experimental Research Design

Strictly controlled laboratory experiment was conducted to isolate and measure the synergistic interactions between the two botanical ingredients. The study utilized a single-factor Completely Randomized Design (CRD). The volumetric ratio of the botanical extracts within a standardized liquid detergent base was manipulated as the independent variable, and the subsequent effects on physicochemical stability, foam generation, spectrophotometric detergency, and in-vitro antibacterial potency were measured.

Extraction and Sample Preparation

To protect the heat-sensitive secondary metabolites, we followed precise extraction protocols. First, we submerged one kilogram of freshly harvested, unrefined pepper in two liters of heated distilled water. This soaking phase lasted for six hours, maximizing the amount of residual piperine pulled into the aqueous solution (Riyanto, 2020). We then filtered the liquid through a fine mesh to clear out any suspended solid particles. To ensure the final product was microbiologically safe without thermally destroying the alkaloids, the filtered pepper washing water was pasteurized at exactly 70 °C for 15 minutes.

In a parallel process, we extracted triterpenoid saponins from *Citrus aurantifolia* leaves using a highly efficient solvent maceration technique. After drying and mechanically pulverizing the fresh lime leaves to expand their reactive surface area, we submerged the powder in 96% analytical-grade ethanol at a strict 1:10 solid-to-solvent mass ratio (Ibrahim et al., 2018). After letting the mixture macerate for 48 hours to fully dissolve the saponins, we decanted the ethanolic solution. We then used a rotary vacuum evaporator set to a gentle 40 °C to evaporate the solvent, leaving behind a thick, dark-green botanical extract rich in active saponins, essential oils, and flavonoids (Sari et al., 2024).

Formulation

A precisely calibrated series of test formulations was engineered to evaluate the differential impact of the active ingredients. To ensure that any variations in cleaning efficacy were solely attributable to the botanical additives, a uniform baseline detergent matrix was established across all experimental units. This foundational matrix consisted of Glycerin, functioning as a humectant and solvent to maintain phase stability, meticulously balanced with Tween 80 acting as the primary non-ionic surfactant and emulsifier to provide baseline mechanical cleaning action. The formulations were finalized by incorporating Aquadest serving as the universal solvent to achieve the total functional base volume (Yuliyanti et al., 2019).

Table 1. PepClean Formulation (Pepper Washing Water and Lime Leaf Extract)

Ingredient Name	P3 (50% Lime Leaf Extract and 50% Pepper Washing Water)		
	P1 (100% Lime Leaf Extract)	P2 (100% Pepper Washing Water)	
Pepper Washing Water	0 ml	100 ml	50 ml
Lime Leaf Extract	100 ml	0 ml	50 ml
Glycerin	10 ml	10 ml	10 ml
Aquadest	50 ml	50 ml	50 ml
Tween 80	5 ml	5 ml	5 ml

Physicochemical Evaluation Parameters

All formulations were rigorously tested against the Indonesian National Standard (SNI) 4075-1:2017 parameters for liquid laundry detergents. Initial testing involved basic organoleptic profiling, recording the

fluid's odor, color, and visual phase homogeneity to gauge its aesthetic viability for consumer application. The relative alkalinity of each batch was measured at room temperature utilizing a digitally calibrated pH meter (Berlianti et al., 2025).

To test foam capacity, a key mechanical factor for suspending dirt a standard volumetric displacement method was employed. A 1 ml aliquot of each detergent was injected into a sealed, graduated cylinder filled with 10 ml of distilled water, agitated vigorously for exactly 20 seconds, and the absolute height of the resulting foam column was immediately recorded in millimeters (Hutauruk et al., 2020). The longitudinal kinetic and physical stability of the colloidal suspensions were empirically monitored over a 28-day incubation period under fluctuating ambient conditions. This extended observation allowed for the precise calculation of phase separation velocity and absolute sedimentation volume ratio to determine if the liquids separated over time (Wijaya & Lina, 2021).

Detergency power was evaluated utilizing a spectrophotometric assay. Standardized white cotton fabric swatches of identical dimensions were uniformly saturated with commercial sweet soy sauce, allowed to cure, and then washed in the respective detergent mixtures. Following the wash cycle, the residual wash water's optical turbidity was analyzed with a UV-Vis spectrophotometer set to 450 nm. Higher absorbance readings at this wavelength correlate directly with a higher concentration of organic stain particles successfully lifted from the fabric and dispersed into the water (Akhmad, 2017).

Antimicrobial Test

A high-quality detergent must also eliminate biological pathogens. The antibacterial activity profile of the formulations was evaluated against pathogenic *Escherichia coli* utilizing the widely accepted Kirby-Bauer agar disc diffusion methodology (Rahayu, 2024). After swabbing pure *E. coli* cultures onto sterile Nutrient Agar plates to form a uniform bacterial lawn, sterile filter paper discs each impregnated with equal volumes of P1, P2, P3, K+, or K- were aseptically placed onto the agar. The plates were incubated aerobically at 37 °C for 24 hours, after which the zones of bacterial growth inhibition surrounding the discs were meticulously measured across multiple axes utilizing vernier calipers (Putri, 2021).

Data Analysis

To guarantee the reliability of the findings, all quantitative datasets were subjected to rigorous statistical processing in IBM SPSS Statistics Version 26.0. Initially, a Shapiro-Wilk assessment was conducted to confirm the data was normally distributed, followed by Levene's test to verify the homogeneity of variance across the experimental groups. Once these assumptions were cleared, the data arrays were analyzed utilizing a One-Way Analysis of Variance (ANOVA) configured to a strict 95% confidence interval ($\alpha = 0.05$). Whenever the ANOVA indicated statistical significance, a post-hoc Tukey Honestly Significant Difference (HSD) test was systematically executed to pinpoint exactly which formulations differed from one another.

RESULTS AND DISCUSSION

Organoleptic Characterization

Because sensory appeal heavily influences consumer acceptance, organoleptic evaluation is the first major hurdle for any household product. Visual and olfactory assessments revealed that each detergent's profile was distinctly shaped by its botanical additives. The organoleptic test results are shown in Table 2.

Table 2. Organoleptic Test Results

Formula	Form	Color	Odor
P1	Liquid	Cloudy white	Non-pungent (typical of lime leaves)
P2	Liquid	Brownish-yellow	Non-pungent (typical of pepper)
P3	Liquid	Cloudy yellow	Non-pungent

Formulation P1, containing pure lime leaf extract, presented as a cloudy white liquid. This opacity was caused by microscopic plant particles suspended in the fluid, and it carried a fresh, mild citrus scent, proving

that the low-temperature evaporation successfully preserved the plant's volatile essential oils (Ibrahim et al., 2018).

In contrast, formulation P2 was a striking brownish-yellow, a color directly imparted by the polyphenols and natural pigments found in the pepper washing water. Its aroma was distinctly peppery but surprisingly mild, showing that the dilution process successfully neutralized the harsh pungency usually associated with concentrated piperine (Gorgani et al., 2017).

Most importantly, the binary P3 formulation achieved an excellent aesthetic balance. It formed a highly uniform, cloudy-yellow suspension. By blending the two extracts, the dominating sensory traits of each individual plant were effectively neutralized, leaving behind a stable liquid with a very mild, non-offensive odor. This physical stability was likely driven by a complex biomolecular interaction where the flavonoids and essential oils from the lime leaves helped stabilize the piperine alkaloids. Consequently, the P3 mixture easily met the strict organoleptic standards set by the SNI, which demand that liquid cleaners remain homogenous and free of volatile, unpleasant smells (Yuliyanti et al., 2019).

pH Test

Detergent's pH is a vital safety metric. If a formula is too harsh, it can strip natural lipids from the user's skin during hand washing and degrade delicate textile polymers. According to SNI 8770:2019 and SNI 4075-1:2017, household cleaning fluids must maintain a safe operational pH between 6 and 11 (Berlianti et al., 2025).

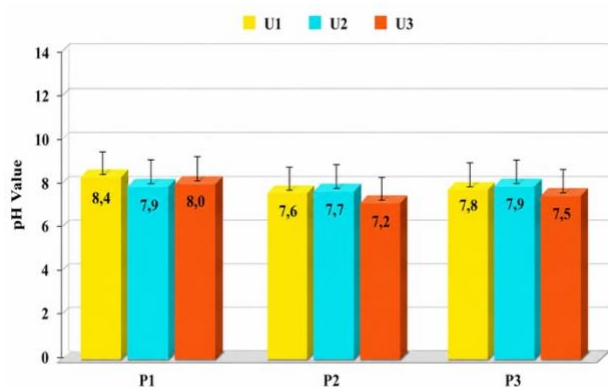


Figure 1. pH Test Result

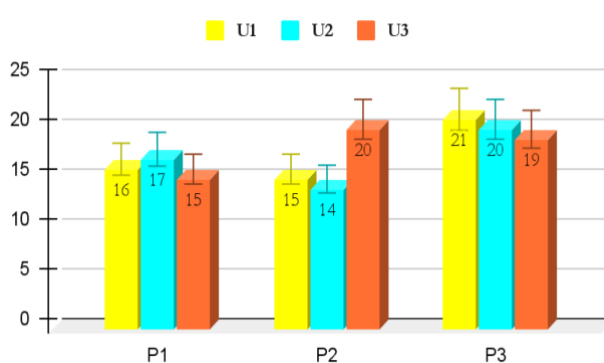


Figure 2. Foam Height Test Result

As shown in **Figure 1**, all test units comfortably met these national safety benchmarks, ranging from slightly alkaline to nearly neutral. Influenced by the botanical salts in its extraction solvent, the lime-based P1 formulation was the most alkaline, averaging 8.10 ± 0.26 . The pepper washing water formula (P2) sat closer to true neutral at 7.50 ± 0.26 , reflecting the highly aqueous nature of the agricultural effluent.

Interestingly, the P3 combination proved to be the most consistent, maintaining a highly stable mean pH of 7.74 ± 0.20 with the narrowest standard deviation. This specific chemical buffering effect is theorized to stem from complex acid-base interactions between the lime extract's mildly acidic organics and the weakly basic nitrogenous structure of the piperine. A One-Way ANOVA test yielded a significance value of 0.064 ($p > 0.05$), confirming that the minor pH variations between the three formulas are not statistically significant and fall well within the margins of experimental error. Ultimately, all three blends are chemically appropriate and dermatologically safe for consumer use (Berlianti et al., 2025).

Foam Height Test Results

While an abundance of foam does not automatically equal better cleaning power, it plays a massive role in physically lifting detached soil particles away from fabrics. It also heavily dictates whether everyday consumers perceive the product as highly effective. The data in **Figure 2** clearly shows that formulation P3 outperformed the isolated botanical bases, generating an impressive average foam column of 20.00 mm. Statistical testing via One-Way ANOVA confirmed this difference was highly significant ($p = 0.001$). Follow-up Tukey HSD tests verified that P3's foaming capacity was statistically superior to both the P1 lime extract ($p = 0.006$) and the P2 pepper effluent ($p = 0.006$).

This pronounced elevation in foaming architecture is not anomalous; rather, it is directly attributable to the biomolecular action of the triterpenoid saponins operating in a synergistic environment. When the lime leaf biosurfactants interact with the residual organic matter from the pepper washing water, the interfacial surface tension of the aqueous medium is synergistically lowered to a far greater extent than either component can achieve in isolation (Wahyuni et al., 2014). By rapidly decreasing surface tension, the mixture stabilizes the delicate liquid-gas boundary of the foam bubbles. This fortified barrier slows down the rate at which liquid drains from the bubble walls due to gravity, preventing the bubbles from coalescing prematurely and maintaining a thick foam head that traps insoluble dirt (Hutauruk et al., 2020).

Sedimentation and Colloidal Redispersion

When formulating detergents with raw, unfiltered plant extracts, maintaining absolute kinetic stability over long periods on a store shelf remains a profound challenge. If the organic particles separate and settle at the bottom of the bottle, both the aesthetic appeal and the uniform distribution of active ingredients are severely compromised (Roessiana et al., 2014).

Table 3. Longitudinal Sedimentation Volume (F) and Redispersion Metrics over a 28-Day Observation

Formula	Sedimentation Volume Ratio (F)	Agitation Effort Required	Redispersion Efficiency %
P1	0.02	1 Mechanical Shake	100%
P2	0.03	1 Mechanical Shake	100%
P3	0.00	None Required	100%

During a rigorous four-week observation period designed to mimic long-term storage, formulas P1 and P2 experienced minor phase separation, recording sedimentation volumes of 0.02 and 0.03 (Akhmad, 2017). Although these settled floccules were highly responsive instantly redispersing into the solvent with just a single gentle shake the very presence of sediment points to slight thermodynamic instability.

Formulation P3, however, achieved perfect kinetic stability. It registered a flawless zero sedimentation volume across the entire 28-day test, requiring absolutely no agitation to maintain complete visual and chemical homogeneity. This remarkable stability suggests that integrating the two extracts creates a complex, sterically hindering matrix. Plant mucilage, suspended essential oils, and the underlying surfactant base work together to build a highly structured fluid rheology that keeps microscopic particles permanently suspended against the pull of gravity (Wijaya & Lina, 2021). Furthermore, adding Tween 80 as a non-ionic emulsifier to the base matrix greatly reduced interfacial tension, ensuring that the 50 ml lime and 50 ml pepper mixture remained a permanently stable colloidal dispersion (Wahyuni et al., 2014).

Detergency Efficacy

A detergent's primary function is to chemically detach and permanently disperse complex organic stains from woven fibers. In the spectrophotometric assay, detergency power correlates directly with the optical turbidity of the leftover wash water. High absorbance values indicate a large concentration of the sweet soy sauce stain was successfully lifted off the fabric and kept securely suspended in the solvent, preventing redeposition.

Table 4. Detergency Efficacy Assessed via Optical Turbidity (Absorbance at 450 nm)

Formula	Repetition 1	Repetition 2	Repetition 3	Average Absorbance ± Standard Deviation
P1	0.032	0.054	0.042	0.043 ± 0.011
P2	0.062	0.042	0.056	0.053 ± 0.010
P3	0.066	0.084	0.075	0.075 ± 0.009

The empirical data processed in Table 4 confirms that formulation P3 was the most potent cleaner, reaching a peak average optical absorbance of 0.075 ± 0.009 . Advanced ANOVA testing uncovered a highly significant difference in basic cleaning capability across the sample groups ($p = 0.021$). Subsequent Tukey HSD post-hoc comparisons then confirmed that the P3 mixture possessed statistically superior soil-lifting power compared directly to the lime-only P1 formula ($p = 0.019$).

This major enhancement in cleaning efficiency is driven by the high concentration of amphiphilic saponins derived from the lime extract. Once introduced into the wash water, these natural biosurfactants immediately migrate to the fluid interfaces and quickly achieve their Critical Micelle Concentration (CMC) (Minarno, 2016). After crossing this threshold, the saponin molecules spontaneously self-assemble into structural spheres called micelles. The hydrophobic cores point inward, trapping and encapsulating the non-polar, greasy organic residues of the soy sauce stain (Mursal et al., 2023). Simultaneously, the hydrophilic exteriors point outward, interacting favorably with the surrounding bulk water to pull the encapsulated stain away from the textile fibers and suspend it securely in the liquid phase, thereby guaranteeing a highly efficient, deep clean (Yuliyanti et al., 2019).

In-vitro Antimicrobial Testing

In the paradigm of modern sanitation, the capacity to eradicate biological pathogens is just as critical as removing visible dirt. The dynamic inhibitory capabilities of the formulations were rigorously challenged against *Escherichia coli*, a widely accepted Gram-negative indicator organism utilized to identify fecal contamination and general pathogenic presence (Rahayu, 2024).

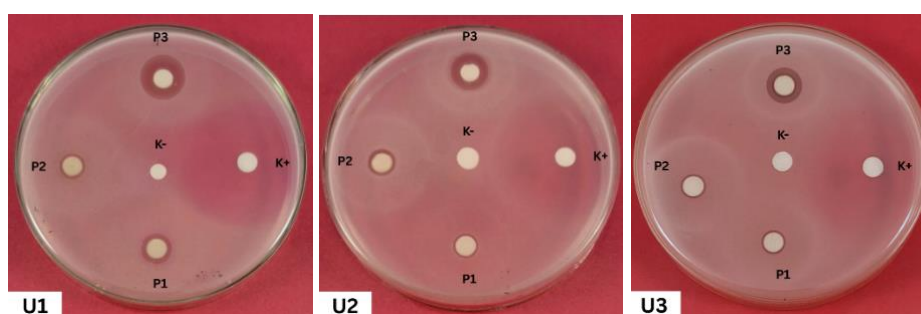


Figure 3. In-Vitro Antibacterial Inhibition Zone Diameters against *Escherichia coli*

Table 5. In-Vitro Antibacterial Inhibition Zone Diameters against *Escherichia coli*

Formula	Repetition 1 (mm)	Repetition 2 (mm)	Repetition 3 (mm)	Average Diameter $\pm$ SD (mm)	Antimicrobial Interpretation
P1	7.88	9.60	7.88	8.45 ± 0.99	Moderate Activity
P2	9.30	8.53	7.88	8.57 ± 0.71	Moderate Activity
P3	16.35	13.05	12.08	13.83 ± 2.24	Strong Activity
K+	28.90	31.20	25.88	28.66 ± 2.67	Very Strong Activity
K-	0.00	0.00	0.00	0.00	No Detectable Activity

The comprehensive microbiological data detailed in Table 5 reveals a massive, non-linear synergistic amplification in antibacterial power. When tested in isolation, the P1 and P2 formulas produced moderate, statistically indistinguishable cellular inhibition zones averaging 8.45 mm and 8.57 mm. However, when combined into the P3 architecture, the mixture generated a robust, highly significant clearance zone averaging 13.83 mm, categorically elevating its classification to a potent, strong antibacterial agent. ANOVA testing proved a definitive variance across the experimental groups ($p < 0.001$), and Tukey HSD post-hoc analysis unequivocally confirmed that P3 was statistically superior to both of the isolated botanical extracts ($p < 0.05$).

This highly magnified efficacy deeply substantiates the core biomolecular hypothesis of this research. The intricate mechanism relies on a sequential, two-tiered attack against the bacterial pathogen. Initially, the botanical saponins assault the dense lipopolysaccharide (LPS) layer protecting the Gram-negative *E. coli* cell wall (Mulangsri et al., 2019). The saponins emulsify these structural lipids, severely destabilizing the membrane and forcing the cell wall to become highly porous (Hayunanda et al., 2025). This chemically induced breach essentially removes the primary defensive armor, creating a clear transport pathway for a massive intracellular influx of piperine alkaloids from the pepper washing water (Tiwari et al., 2020). Once trapped inside the cytosol in high concentrations, the piperine aggressively binds to internal cell structures, disrupting ribosomal protein synthesis and halting crucial enzymatic processes, which ultimately triggers rapid, irreversible bacterial cell death (Riyanto, 2020). This elegant molecular synergy validates the remarkable potential of thoughtfully integrating these specific agricultural byproducts.

CONCLUSION

By strategically integrating pepper washing water with concentrated lime leaf extract (*Citrus aurantifolia*), a profound, statistically significant synergistic enhancement was achieved across all essential functional and structural metrics required for a premium liquid detergent. The P3 formulation (comprising 50 ml of pepper washing water and 50 ml of lime extract integrated into a glycerin and Tween 80 base) mathematically and functionally outperformed the isolated botanical extracts. It exhibited stability with zero sedimentation over a continuous 28-day longitudinal study, demonstrated superior organic stain dispersion with a peak spectrophotometric absorbance of 0.075, and showed highly robust antibacterial activity by generating a remarkable 13.83 mm inhibition zone against *Escherichia coli*.

Utilizing these precise 50 ml botanical ratios, the P3 formulation proves to be an optimal, highly effective, and eco-friendly textile sanitation alternative. It successfully satisfies and exceeds the strict national safety benchmarks stipulated within SNI 4075-1:2017, maintaining a skin-safe pH equilibrium of 7.74 while simultaneously generating a stable 20 mm foam displacement column. By intelligently repurposing an environmentally hazardous agricultural effluent, this formulation effectively closes the loop in pepper processing waste management, demonstrating a highly scalable, economically practical execution of the circular economy paradigm within the modern biochemical manufacturing sector.

AUTHORS CONTRIBUTION

N. M. Anaqiya, A. N. Bualangi, and Faisal conceptualized the study, performed the experiments, analyzed the data, and drafted the manuscript. F. Gani supervised the data analysis and overall research, verified statistical accuracy, critically revised the manuscript, and validated compliance with physicochemical testing standards.

ACKNOWLEDGMENTS

Profound and sincere appreciation is extended to the principal, management board, and academic faculty of SMP YPS Singkole for the unwavering provision of vital infrastructural support, financial backing, and for facilitating. Furthermore, the dedicated technical staff at the Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Hasanuddin, are highly commended. The invaluable provision of advanced laboratory instrumentation, sterile environments, and rigorous analytical guidance throughout the complex microbiological susceptibility testing phases was instrumental to the success and validation of this study.

CONFLICT OF INTEREST

The authors definitively declare that there are no financial, personal, or professional conflicts of interest associated with this research.

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