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Development and Evaluation of Antimicrobial Handwash Formulation Containing *Paederia foetida* Leaf Extract Against Skin Pathogenic Bacteria

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Abstract

Hand hygiene is an important strategy for reducing the transmission of pathogenic microorganisms. Increasing interest in plant-based antimicrobial agents has encouraged the development of topical cleansing products containing bioactive botanical extracts. *Paederia foetida* L. is a medicinal plant reported to contain flavonoids, phenolic compounds, tannins, saponins, terpenoids, and other secondary metabolites with potential antibacterial activity. However, evidence regarding the incorporation of *P. foetida* leaf extract into a liquid handwash formulation and its relationship with physicochemical characteristics and antibacterial activity remains limited. This study aimed to develop and evaluate a liquid handwash containing *P. foetida* leaf extract and to determine its physicochemical characteristics, physical stability, and antibacterial activity against *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* ATCC 12228. *P. foetida* leaves were extracted by maceration using 70% ethanol. The resulting extract was subjected to qualitative phytochemical screening and incorporated into liquid handwash formulations at concentrations of 0%, 1%, 3%, and 5% (F0, F1, F2, and F3, respectively). The formulations were evaluated for organoleptic characteristics, homogeneity, pH, viscosity, foamability, foam stability, and physical stability using six temperature cycles between 4 ± 2°C and 40 ± 2°C. Antibacterial activity was evaluated using the agar well diffusion method, and inhibition zones were measured after 24 h of incubation at 37°C. Data were analyzed using one-way analysis of variance followed by Tukey's post hoc test or the corresponding non-parametric analysis when appropriate. Extraction of 500 g of dried leaf powder yielded 68.5 g of extract, corresponding to a 13.70% extraction yield. Phytochemical screening indicated the presence of flavonoids, phenolic compounds, tannins, saponins, and terpenoids. All formulations were homogeneous and showed no visible phase separation or precipitation. Increasing extract concentration resulted in a slight decrease in pH from 5.71 ± 0.01 in F0 to 5.62 ± 0.01 in F3 and an increase in viscosity from 2,463.33 ± 15.28 to 2,923.33 ± 15.28 mPa·s. Foam stability remained above 85% in all formulations. After six temperature cycles, the formulations maintained their general physical characteristics. Antibacterial activity increased significantly with increasing extract concentration ($p < 0.001$). Against *S. aureus*, inhibition zones increased from 8.20 ± 0.10 mm in F0 to 16.23 ± 0.15 mm in F3, while against *S. epidermidis*, inhibition zones increased from 8.10 ± 0.10 mm to 16.93 ± 0.15 mm. The 5% formulation demonstrated the strongest antibacterial activity among the plant-extract formulations. *P. foetida* leaf extract can be incorporated into a liquid handwash formulation while maintaining acceptable physicochemical characteristics and physical stability. The antibacterial activity increased in a concentration-dependent manner, with the 5% formulation showing the greatest inhibition against both *S. aureus* and *S. epidermidis*. These findings support the potential of *P. foetida* leaf extract as a plant-derived antimicrobial active ingredient for handwash development. Further studies should investigate chemical standardization, minimum inhibitory and bactericidal concentrations of the finished formulation, preservative efficacy, dermal safety, long-term stability, and handwashing efficacy under practical conditions.

Keywords: Antibacterial activity; *Paederia foetida*; medicinal plant; *Staphylococcus aureus*; *Staphylococcus epidermidis*; topical formulation.

Abbreviations: American Type Culture Collection (ATCC); Analysis of Variance (ANOVA); Colony-Forming Units (CFU); Disodium Ethylenediaminetetraacetic Acid (EDTA); High-Performance Liquid Chromatography (HPLC); Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS); Methicillin-Resistant *Staphylococcus aureus* (MRSA); Minimum Bactericidal Concentration (MBC); Minimum Inhibitory Concentration (MIC); Mueller-Hinton Agar (MHA); Sodium Lauryl Ether Sulfate (SLES); Standard Deviation (SD); *Paederia foetida* L. (*P. foetida*); *Staphylococcus aureus* (*S. aureus*); *Staphylococcus epidermidis* (*S. epidermidis*); Statistical Package for the Social Sciences (SPSS).

INTRODUCTION

Hand hygiene is one of the most important and practical measures for reducing the transmission of infectious microorganisms through direct contact (Arhin et al., 2024). The hands can act as transient carriers of pathogenic bacteria and facilitate their transfer between

individuals, surfaces, and body sites. This is particularly relevant for *Staphylococcus aureus*, an important human pathogen associated with skin and soft-tissue infections and capable of forming biofilms that contribute to bacterial persistence and treatment difficulties (Boyce, 2024). Recent evidence has also demonstrated that handwashing with soap and water can substantially reduce bacterial contamination of the hands, supporting its importance as a fundamental strategy for preventing microbial transmission in both community and healthcare settings (Hilton et al., 2025).

Conventional handwashing products generally rely on surfactants to remove microorganisms mechanically from the skin surface. However, the increasing interest in natural and plant-based ingredients has encouraged research into botanical sources of antimicrobial compounds that may provide additional biological activity (Barai et al., 2025). Plant-derived secondary metabolites, including flavonoids, phenolic compounds, tannins, terpenoids, and alkaloids, have been reported to exert antibacterial effects through several mechanisms, including disruption of bacterial cell membranes, inhibition of bacterial enzymes, interference with adhesion and biofilm formation, and alteration of cellular metabolism (Cushnie and Lamb, 2011; Daglia, 2012). Therefore, medicinal plants represent a promising source of bioactive compounds for the development of topical antimicrobial preparations.

One medicinal plant with considerable pharmacological potential is *Paederia foetida* L., a member of the Rubiaceae family that is widely distributed in tropical and subtropical regions (Chettri et al., 2025; Maret et al., 2025). The plant has traditionally been used in various medicinal preparations, while phytochemical investigations have identified numerous bioactive constituents. A recent review reported that *P. foetida* contains iridoid glycosides, flavonoid glycosides, anthraquinones, terpenoids, and other secondary metabolites that may contribute to its diverse pharmacological activities, including antimicrobial, antioxidant, anti-inflammatory, and gastroprotective effects (Mishra et al., 2023). The presence of these compounds makes *P. foetida* a potential candidate for further development as a natural antimicrobial ingredient.

The antibacterial activity of *P. foetida* has been demonstrated in several experimental studies. Santajit et al. (2024) reported that ethanolic leaf extract of *P. foetida* exhibited antibacterial activity against *S. aureus*, including methicillin-resistant *S. aureus* (MRSA). The extract demonstrated minimum inhibitory concentrations of 7.81 µg/mL against *S. aureus* ATCC 25923 and 15.63 µg/mL against MRSA, while the minimum bactericidal concentration was 31.25 µg/mL. Chemical profiling using LC-MS/MS identified several flavonoid glycosides, including quercetin and kaempferol derivatives, which may contribute to the observed antimicrobial and anti-virulence effects (Santajit et al., 2024). These findings suggest that *P. foetida* leaf extract has potential as a source of antibacterial compounds targeting skin-associated pathogens.

Evidence from other studies further supports the antimicrobial potential of this plant. Savitri et al. (2024) reported that *P. foetida* leaf extract demonstrated antibacterial activity against *Escherichia coli* in vitro. More recently, Yunita et al. (2026) evaluated active fractions derived from *P. foetida* leaves against several infectious pathogenic bacteria and reported measurable antibacterial activity, including activity against *S. aureus* and *Cutibacterium acnes*. These findings are particularly relevant to topical applications because *S. aureus*, *S. epidermidis*, and *C. acnes* are commonly associated with the skin microbiota and may become opportunistic pathogens under certain conditions.

Although the antibacterial activity of *P. foetida* extract has been increasingly documented, most previous investigations have focused on crude extracts or purified fractions rather than their incorporation into a standardized topical cleansing formulation (Dwivedi et al., 2025; Santajit et al., 2025). This represents an important research gap because antimicrobial activity demonstrated by a plant extract in vitro cannot necessarily be extrapolated directly to a finished formulation. The interaction between plant-derived compounds and formulation components, including surfactants, humectants, preservatives, pH regulators, and viscosity modifiers, may influence the stability, solubility, availability, and biological activity of the active compounds.

Several studies have explored the formulation of *P. foetida* into topical preparations (Shaikh et al., 2025). A previous study reported that *P. foetida* leaf extract could be incorporated into a liquid handwash formulation and that variations in extract concentration affected several physicochemical characteristics, including organoleptic properties, foam stability, and pH (Yuliani et al., 2022). Similarly, a recent study investigating *P. foetida* leaf extract in a hand sanitizer gel reported antibacterial activity against *S. aureus* and suggested that increasing extract concentration could influence the antimicrobial response (Sari et al., 2025). However, the available evidence remains limited regarding the systematic relationship between extract concentration, physicochemical quality, formulation stability, and antimicrobial activity in a liquid handwash designed specifically for skin-associated pathogenic bacteria.

The development of a plant-based antimicrobial handwash therefore requires more than simply demonstrating antibacterial activity of the plant extract (Hilton et al., 2025). An effective formulation should possess acceptable organoleptic characteristics, homogeneity, pH appropriate for topical application, suitable viscosity, satisfactory foaming properties, and adequate physical stability. At the same time, incorporation into the formulation should preserve

sufficient antimicrobial activity against relevant bacterial targets (Lailatusholihah et al., 2026). Evaluation of these parameters is essential to determine whether the plant extract can function as a practical active ingredient rather than merely as a source of antibacterial compounds in laboratory assays.

The present study addresses this research gap by developing a liquid handwash formulation containing *P. foetida* leaf extract and evaluating its physicochemical characteristics, stability, and antibacterial activity against selected skin pathogenic bacteria. The novelty of this study lies in the integration of plant extract utilization, formulation development, physicochemical characterization, stability assessment, and antibacterial evaluation within a single experimental framework. This approach provides a more comprehensive assessment of the potential application of *P. foetida* as a natural antimicrobial active ingredient for topical hygiene products.

Therefore, this study aimed to develop and evaluate an antimicrobial handwash formulation containing *Paederia foetida* leaf extract and to determine its physicochemical properties, physical stability, and antibacterial activity against selected skin pathogenic bacteria. The findings are expected to provide scientific evidence supporting the utilization of *P. foetida* as a natural antimicrobial ingredient and to contribute to the development of plant-based hand hygiene products with potential practical applications.

MATERIALS AND METHODS

Research Design

This study was conducted as a laboratory-based experimental study to develop and evaluate a liquid handwash formulation containing *P. foetida* L. leaf extract. The study consisted of several stages, including preparation and extraction of *P. foetida* leaves, phytochemical screening of the extract, formulation of the antimicrobial handwash, physicochemical evaluation, stability testing, and determination of antibacterial activity against selected skin pathogenic bacteria. Four handwash formulations were prepared containing different concentrations of *P. foetida* leaf extract, namely 0%, 1%, 3%, and 5%. The formulation without plant extract was used as the negative control, while a commercially available antibacterial handwash containing chlorhexidine was used as the positive control. Each experimental treatment was evaluated in triplicate.

The study was conducted at the Medical Laboratory Technology Laboratory, Faculty of Health Sciences, Universitas Kadiri, Kediri, East Java, Indonesia. The laboratory was used for the preparation and extraction of *Paederia foetida* leaves, handwash formulation, physicochemical evaluation, stability testing, and antibacterial activity assays.

Plant Material and Authentication

Fresh leaves of *P. foetida* L. were collected from Kediri, East Java, Indonesia, in January 2026. The plant material was identified and authenticated at the Materia Medica Batu. The leaves were selected based on their morphological characteristics and were free from visible fungal contamination, insect damage, and physical deterioration. The collected leaves were washed with running water to remove soil and other foreign materials and subsequently rinsed with distilled water.

Preparation of *Paederia foetida* Leaf Extract

The leaves were dried at room temperature in a well-ventilated area protected from direct sunlight until a constant weight was obtained. The dried leaves were then pulverized using a laboratory grinder and sieved to obtain a relatively uniform powder. Approximately 500 g of dried leaf powder was extracted by maceration using 70% ethanol at a ratio of 1:10 (w/v). The extraction process was performed for 3 × 24 h with intermittent stirring. The extract was filtered using Whatman No. 1 filter paper, and the residue was re-extracted under the same conditions. The combined filtrates were concentrated using a rotary evaporator at a temperature not exceeding 40°C and subsequently evaporated to obtain a concentrated crude extract. The percentage extraction yield was calculated using the following equation: The obtained extract was stored in an airtight amber container at 4–8°C until further analysis and formulation.

Phytochemical Screening

Qualitative phytochemical screening was performed to identify the major classes of secondary metabolites present in the *P. foetida* leaf extract. The screening included tests for flavonoids, phenolic compounds, tannins, alkaloids, saponins, terpenoids, and steroids using standard phytochemical procedures. The presence or absence of each metabolite class was determined based on characteristic color changes, precipitate formation, or foam formation following the addition of specific reagents. The results were recorded as positive (+) or negative (−).

Preparation of Handwash Formulations

The liquid handwash formulations were prepared using a surfactant-based formulation consisting of sodium lauryl ether sulfate (SLES), cocamidopropyl betaine, glycerin, sodium chloride, disodium EDTA, phenoxyethanol, citric acid, and distilled water. *P. foetida* leaf extract was incorporated at concentrations of 1%, 3%, and 5%.

Table 1. Composition of *Paederia foetida* leaf extract handwash formulations.

Ingredient	F0 (%)	F1 (%)	F2 (%)	F3 (%)	Function
P. foetida leaf extract	0	1	3	5	Active antimicrobial ingredient
SLES	10	10	10	10	Primary surfactant
Cocamidopropyl betaine	5	5	5	5	Co-surfactant
Glycerin	3	3	3	3	Humectant
Sodium chloride	1.5	1.5	1.5	1.5	Viscosity modifier
Disodium EDTA	0.1	0.1	0.1	0.1	Chelating agent
Phenoxyethanol	0.8	0.8	0.8	0.8	Preservative
Citric acid	q.s.	q.s.	q.s.	q.s.	pH adjustment
Distilled water	ad 100	ad 100	ad 100	ad 100	Vehicle

F0 represented the handwash base without *P. foetida* extract, whereas F1, F2, and F3 contained 1%, 3%, and 5% *P. foetida* leaf extract, respectively. The handwash formulations were prepared by dissolving disodium EDTA in approximately 70% of the required amount of distilled water. Glycerin was subsequently added, followed by gradual incorporation of SLES and cocamidopropyl betaine with gentle stirring to minimize excessive foam formation. Phenoxyethanol was then incorporated into the mixture. The *P. foetida* extract was previously dispersed in a small amount of the formulation vehicle and gradually incorporated into the base. Sodium chloride was added gradually until the desired consistency was obtained. Citric acid was used to adjust the pH to approximately 5.0–6.0. The final volume was adjusted with distilled water to 100%, and the preparation was stirred gently until homogeneous. The finished formulations were transferred into clean, labeled containers and stored at room temperature before evaluation.

Organoleptic Evaluation

Organoleptic evaluation was performed by direct observation of the formulations. The evaluated parameters included color, odor, appearance, and consistency. Observations were performed immediately after formulation and during the stability study. Changes in color, odor, phase separation, precipitation, or other visible alterations were recorded.

Homogeneity Test

The homogeneity of each formulation was evaluated by placing approximately 0.5 g of handwash on a clean glass slide and observing it under adequate illumination. The formulations were considered homogeneous when no visible particles, aggregates, or phase separation were observed.

pH Measurement

The pH of each handwash formulation was measured using a calibrated digital pH meter. Before measurement, the instrument was calibrated using standard buffer solutions at pH 4.00 and 7.00. Approximately 10 g of each formulation was diluted with distilled water at a ratio of 1:10 before measurement. The pH measurement was performed in triplicate, and the results were expressed as mean ± standard deviation (SD). The measured pH values were compared with the acceptable pH range for topical cleansing preparations, particularly the physiological pH range of the skin.

Viscosity Measurement

The viscosity of the handwash formulations was determined using a Brookfield digital viscometer equipped with an appropriate spindle. Measurements were conducted at 25 ± 2°C using a rotational speed of 20 rpm. Each formulation was measured in triplicate, and viscosity was expressed in mPa·s. The obtained values were used to determine the flow characteristics and consistency of the liquid handwash formulations.

Foamability and Foam Stability

Foaming properties were evaluated using the cylinder shaking method. A volume of 10 mL of each handwash formulation was placed in a graduated cylinder containing 90 mL of distilled water. The cylinder was shaken vertically 10 times under standardized conditions, and the initial foam height was immediately recorded. Foam height was subsequently measured after 5, 10, 15, 30, and 60 min to evaluate foam stability. Foam stability was calculated using: Each measurement was performed in triplicate.

Spreadability

Because the product was developed as a liquid handwash rather than a semisolid topical preparation, spreadability was not used as a primary quality parameter. Instead, the flow characteristics were assessed through viscosity and homogeneity measurements. This approach avoids applying a semisolid-product test that is less relevant to a liquid handwash.

Physical Stability Test

The physical stability of the formulations was evaluated using an accelerated temperature cycling test. The formulations were stored alternately at 4 ± 2°C for 24 h and 40 ± 2°C for 24 h, with one cycle consisting of exposure to both temperatures. The procedure was repeated for six cycles.

Before and after the stability test, the formulations were examined for changes in:

1. color;
2. odor;
3. homogeneity;
4. phase separation;
5. pH;
6. viscosity; and
7. foam characteristics.

The stability results obtained after the cycling test were compared with the baseline measurements.

Bacterial Strains and Preparation of Bacterial Suspensions

The antibacterial activity of the formulations was evaluated against Staphylococcus aureus ATCC 25923 and Staphylococcus epidermidis ATCC 12228 as representative Gram-positive skin-associated bacteria. Bacterial cultures were obtained from Microbiology Laboratorium, Kadiri University and maintained on appropriate culture media. Fresh bacterial colonies were suspended in sterile 0.85% physiological saline and adjusted to a 0.5 McFarland standard, corresponding to approximately 1×10^8 CFU/mL. The standardized bacterial suspension was used immediately for the antimicrobial assay.

Antibacterial Activity Assay

The antibacterial activity of the *P. foetida* leaf extract and handwash formulations was evaluated using the agar well diffusion method. Mueller-Hinton Agar (MHA) plates were inoculated with the standardized bacterial suspension using a sterile cotton swab to produce a uniform bacterial lawn. Sterile wells with a diameter of 8 mm were prepared using a sterile cork borer.

A volume of 100 µL of each test preparation was introduced into the respective wells. The treatment groups consisted of:

- F0: handwash base without *P. foetida* extract;
- F1: handwash containing 1% *P. foetida* extract;
- F2: handwash containing 3% *P. foetida* extract;
- F3: handwash containing 5% *P. foetida* extract;
- Positive control: commercial antibacterial handwash containing chlorhexidine;
- Negative control: sterile distilled water.

The plates were allowed to stand for 30 min at room temperature to facilitate diffusion before incubation. The plates were subsequently incubated at 37°C for 24 h. After incubation, the diameter of the inhibition zones surrounding each well was measured in millimeters using a digital caliper. The measurement included the diameter of the well and was recorded as the total inhibition-zone diameter. Each treatment was evaluated in three independent replicates, and the antibacterial activity was expressed as mean inhibition-zone diameter ± SD.

Statistical Analysis

All experimental measurements were performed in three independent replicates and expressed as mean ± standard deviation (SD). The normality of numerical data was evaluated using the Shapiro-Wilk test, while homogeneity of variance was assessed using Levene's test. For normally distributed and homogeneous data, differences among treatment groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. For data that did not meet the assumptions of parametric analysis, the Kruskal-Wallis test followed by Dunn's post hoc test was used. A *p*-value of <0.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics version 27.0 (IBM Corp., Armonk, NY, USA).

Biosafety Considerations

All microbiological procedures were conducted in accordance with institutional biosafety procedures. Bacterial cultures were handled using appropriate aseptic techniques and personal protective equipment. Microbiological materials and contaminated waste were sterilized by autoclaving before disposal according to institutional biosafety regulations.

RESULTS AND DISCUSSION

Results

Extraction Yield and Phytochemical Characteristics

Maceration of 500 g of dried *Paederia foetida* leaf powder using 70% ethanol produced 68.5 g of concentrated extract, corresponding to an extraction yield of 13.70%. The extract appeared as a greenish-brown viscous preparation with a characteristic odor.

Table 2. Extraction characteristics and phytochemical screening of *Paederia foetida* leaf extract.

Parameter	Result
Dried leaf powder	500 g
Extraction solvent	70% ethanol
Extraction method	Maceration
Extraction duration	3 × 24 h
Concentrated extract	68.5 g
Extraction yield	13.70%
Physical appearance	Greenish-brown viscous extract
Odor	Characteristic

Table 3. Phytochemical screening of *Paederia foetida* leaf extract.

Phytochemical constituent	Result
Flavonoids	+
Phenolic compounds	+
Tannins	+
Alkaloids	−
Saponins	+
Terpenoids	+
Steroids	−

The phytochemical screening revealed the presence of flavonoids, phenolic compounds, tannins, saponins, and terpenoids, whereas alkaloids and steroids were not detected. These findings indicate that the ethanolic extract contained several classes of secondary metabolites with potential biological activity.

Organoleptic Characteristics and Homogeneity

Four handwash formulations were successfully prepared. F0 was the extract-free base, whereas F1, F2, and F3 contained 1%, 3%, and 5% *P. foetida* leaf extract, respectively.

Table 4. Organoleptic characteristics of handwash formulations.

Parameter	F0	F1	F2	F3
Extract concentration	0%	1%	3%	5%
Color	Transparent	Light green	Green	Dark green
Odor	Characteristic base	Slightly herbal	Herbal	Strong herbal
Physical form	Liquid	Liquid	Liquid	Liquid
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous
Phase separation	None	None	None	None

Increasing the concentration of *P. foetida* extract resulted in progressively darker coloration and a stronger characteristic herbal odor. Nevertheless, all formulations remained homogeneous without visible precipitation or phase separation. The observed changes in color and odor are expected because increasing the concentration of crude plant extract increases the amount of pigments and volatile or semi-volatile plant constituents incorporated into the formulation. Similar observations have previously been reported for *P. foetida*-containing liquid handwash, where variations in extract concentration influenced organoleptic characteristics, foam stability, and pH (Evy & Syarifah, 2022).

pH and Viscosity

The pH and viscosity results are presented in Table 5.

Table 5. pH and viscosity of handwash formulations.

Formulation	Extract (%)	pH (mean ± SD)	Viscosity (mPa·s, mean ± SD)
F0	0	5.71 ± 0.01	2,463.33 ± 15.28
F1	1	5.69 ± 0.01	2,593.33 ± 15.28
F2	3	5.66 ± 0.01	2,750.00 ± 10.00
F3	5	5.62 ± 0.01	2,923.33 ± 15.28

The pH values decreased slightly with increasing extract concentration, from 5.71 in F0 to 5.62 in F3. Despite this decrease, all formulations remained within the mildly acidic range intended for topical application. One-way ANOVA demonstrated a significant difference in pH among the formulations ($p < 0.001$). However, the magnitude of the difference was small, indicating that incorporation of the extract produced only a modest change in formulation acidity. Viscosity increased progressively from 2,463.33 mPa·s in F0 to 2,923.33 mPa·s in F3. One-way ANOVA showed a statistically significant difference in viscosity among formulations ($p < 0.001$).

Foamability and Foam Stability

Table 6. Foam characteristics of handwash formulations.

Formulation	Initial foam height (cm)	Foam height at 60 min (cm)	Foam stability (%)
F0	7.8 ± 0.10	6.9 ± 0.10	88.46 ± 1.62
F1	7.7 ± 0.10	6.8 ± 0.10	88.31 ± 1.45
F2	7.6 ± 0.10	6.6 ± 0.10	86.84 ± 1.43
F3	7.4 ± 0.10	6.3 ± 0.10	85.14 ± 1.54

All formulations generated stable foam. A slight reduction in foam height was observed with increasing extract concentration. Nevertheless, foam stability remained above 85% in all formulations. The decrease in foam stability at higher extract concentrations may be related to interactions between plant-derived compounds

and surfactant micelles. However, the relatively small reduction suggests that the incorporation of *P. foetida* extract did not substantially compromise the foaming characteristics of the formulation.

Physical Stability

Following six temperature cycles between 4 ± 2°C and 40 ± 2°C, no visible phase separation or precipitation was observed in any formulation.

Table 7. Physical stability of handwash formulations.

Parameter	F0	F1	F2	F3
Color	Stable	Stable	Stable	Stable
Odor	Stable	Stable	Stable	Stable
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous
Phase separation	None	None	None	None
Precipitation	None	None	None	None
Change in pH	Minimal	Minimal	Minimal	Minimal
Change in viscosity	Minimal	Minimal	Minimal	Minimal

The formulations maintained their physical appearance and homogeneity throughout the stability assessment. No evidence of phase separation or precipitation was detected.

Antibacterial Activity against *Staphylococcus aureus*

The antibacterial activity against *S. aureus* ATCC 25923 is presented in Table 8.

Table 8. Antibacterial activity of handwash formulations against *S. aureus* ATCC 25923.

Treatment	Inhibition zone (mm), mean ± SD
Negative control	8.00 ± 0.00
F0	8.20 ± 0.10
F1 (1%)	9.97 ± 0.15
F2 (3%)	12.73 ± 0.15
F3 (5%)	16.23 ± 0.15
Positive control	21.53 ± 0.15

The extract-free formulation F0 produced only a minimal inhibition zone, whereas incorporation of *P. foetida* extract increased antibacterial activity. F1, F2, and F3 produced mean inhibition zones of 9.97, 12.73, and 16.23 mm, respectively. The largest inhibition zone among the plant-based formulations was observed for F3 containing 5% extract. One-way ANOVA demonstrated a highly significant difference among treatment groups ($p < 0.001$). Post hoc Tukey analysis demonstrated that the extract-containing formulations had significantly greater antibacterial activity than F0 ($p < 0.05$). F3 also exhibited significantly greater activity than F1 and F2 ($p < 0.05$).

Antibacterial Activity against *Staphylococcus epidermidis*

Table 9. Antibacterial activity of handwash formulations against *Staphylococcus epidermidis* ATCC 12228.

Treatment	Inhibition zone (mm), mean ± SD
Negative control	8.00 ± 0.00
F0	8.10 ± 0.10
F1 (1%)	10.43 ± 0.15
F2 (3%)	13.37 ± 0.15
F3 (5%)	16.93 ± 0.15
Positive control	20.63 ± 0.15

A similar concentration-dependent response was observed against *S. epidermidis*. The inhibition zones increased from 8.10 mm in F0 to 16.93 mm in F3. One-way ANOVA demonstrated a significant difference among treatment groups ($p < 0.001$). Tukey's post hoc analysis indicated that F1, F2, and F3 exhibited significantly greater inhibition than F0 ($p < 0.05$), with F3 producing the strongest antibacterial effect.

Comparative Antibacterial Activity

Table 10. Comparison of antibacterial activity between *S. aureus* and *S. epidermidis.*

Formulation	*S. aureus* (mm)	*S. epidermidis* (mm)
F0	8.20 ± 0.10	8.10 ± 0.10
F1	9.97 ± 0.15	10.43 ± 0.15
F2	12.73 ± 0.15	13.37 ± 0.15
F3	16.23 ± 0.15	16.93 ± 0.15
Positive control	21.53 ± 0.15	20.63 ± 0.15

F3 produced inhibition zones of 16.23 mm against *S. aureus* and 16.93 mm against *S. epidermidis*. Thus, *S. epidermidis* exhibited a slightly larger inhibition zone than *S. aureus* at the highest extract concentration. The results indicate that the antibacterial effect of the formulation was not restricted to a single *Staphylococcus* species.

Statistical Summary

Table 11. Statistical analysis of antibacterial activity.

Bacterium	ANOVA F-value	*p*-value	Interpretation
S. aureus	4097.02	<0.001	Significant
S. epidermidis	3646.48	<0.001	Significant

The statistical analysis confirmed that the concentration of *P. foetida* extract significantly affected antibacterial activity against both bacterial species. The consistent concentration-response relationship strengthens the interpretation that *P. foetida* extract was responsible for the additional antibacterial activity observed in F1-F3.

Discussion

Extraction and Phytochemical Characteristics

The 70% ethanolic extraction of *P. foetida* leaves produced a 13.70% yield. The use of hydroethanolic solvent is relevant because ethanol-water mixtures can extract compounds with a relatively broad range of polarity, including phenolic and flavonoid constituents. The phytochemical screening identified flavonoids, phenolic compounds, tannins, saponins, and terpenoids. The presence of these metabolites is consistent with previous reports describing the phytochemical complexity of *P. foetida*. Mishra et al. (2023) reported that *P. foetida* contains several classes of secondary metabolites, including iridoid glycosides, flavonoids, anthraquinones, and terpenoids.

More importantly, Santajit et al. (2024) used LC-MS/MS to characterize ethanolic *P. foetida* leaf extract and identified several flavonoid glycosides, including quercetin- and kaempferol-related compounds. Their extract demonstrated antibacterial activity against *S. aureus* ATCC 25923 and MRSA, with MIC values of 7.81 and 15.63 µg/mL, respectively. The current results therefore support the hypothesis that the antibacterial activity of the handwash is associated with the phytochemical constituents of the leaf extract.

Effect of Extract Concentration on Antibacterial Activity

A clear concentration-dependent antibacterial response was observed in both bacterial species. Against *S. aureus*, the inhibition zone increased from 9.97 mm in F1 to 12.73 mm in F2 and 16.23 mm in F3. A similar pattern occurred against *S. epidermidis*, with inhibition zones increasing from 10.43 mm in F1 to 13.37 mm in F2 and 16.93 mm in F3. This concentration-response relationship suggests that increasing the amount of *P. foetida* extract increased the availability of antibacterial constituents in the formulation. The finding is consistent with the study by Santajit et al. (2024), which demonstrated direct growth inhibition of *S. aureus* by ethanolic *P. foetida* leaf extract. The same study also showed that the extract affected biofilm formation and quorum-sensing-related pathways, suggesting that the antibacterial potential of the plant may extend beyond simple growth inhibition.

A recent 2026 study by Yunita et al. further reported antibacterial activity of *P. foetida* leaf fractions against six pathogenic bacteria. Interestingly, the fraction showed lower activity than previously reported crude extract, leading the authors to suggest that synergistic interactions among multiple secondary metabolites may contribute to the antibacterial activity of the whole extract. This observation is important for the current study because the handwash uses a crude leaf extract rather than a single purified compound.

Possible Antibacterial Mechanisms

The antibacterial activity observed in the present study may involve multiple mechanisms. Flavonoids can interact with bacterial cell membranes and membrane-associated proteins and may interfere with bacterial enzymes and nucleic acid-related processes. Phenolic compounds can affect membrane integrity and protein function. Tannins may bind proteins and interfere with bacterial enzymes, while saponins can interact with membrane components. The findings of Santajit et al. (2024) provide additional mechanistic support. Their study showed morphological changes in *S. aureus* following exposure to *P. foetida* extract and demonstrated inhibition of biofilm-related and quorum-sensing-associated processes. LC-MS/MS analysis identified flavonoid glycosides as candidate bioactive constituents. However, the current study did not directly investigate bacterial membrane damage, biofilm formation, quorum sensing, or molecular targets. Therefore, these mechanisms should be regarded as plausible explanations supported by previous studies, rather than mechanisms demonstrated directly in the present experiment.

Contribution of the Formulation Base

F0 exhibited only minimal inhibition, whereas the formulations containing the extract showed progressively larger inhibition zones. This comparison is important because it indicates that the antibacterial effect was not simply attributable to the surfactant base. The difference between F0 and F1-F3 suggests that incorporation of *P. foetida* extract contributed additional antibacterial activity. Nevertheless, surfactants can themselves influence bacterial cells and facilitate microbial removal. Therefore, the antibacterial activity of the finished

product should be interpreted as the combined effect of the cleansing base and the plant-derived active ingredient. The increasing inhibition from F1 through F3 provides stronger evidence for the specific contribution of the plant extract.

Activity against *S. aureus*

S. aureus represents an important skin-associated pathogen and is particularly relevant to antimicrobial product development because of its capacity to form biofilms and its association with antimicrobial resistance. The present study demonstrated that the 5% formulation produced a 16.23 mm inhibition zone against *S. aureus*, substantially greater than the 8.20 mm observed for F0. These findings are consistent with Santajit et al. (2024), who demonstrated that ethanolic *P. foetida* leaf extract inhibited *S. aureus* ATCC 25923 and MRSA. Their reported MIC values were 7.81 µg/mL for ATCC 25923 and 15.63 µg/mL for MRSA. The present study extends this evidence by demonstrating that antibacterial activity can be retained after the extract is incorporated into a liquid handwash matrix.

Activity against *S. epidermidis*

The formulation also demonstrated antibacterial activity against *S. epidermidis*, with F3 producing an inhibition zone of 16.93 mm. Although *S. epidermidis* is commonly recognized as a member of the normal skin microbiota, it can behave as an opportunistic pathogen under certain conditions. Therefore, the activity observed against this organism is relevant to the development of topical antimicrobial preparations. The slightly greater susceptibility of *S. epidermidis* compared with *S. aureus* in the current experiment may reflect differences in cell-envelope characteristics, surface proteins, biofilm capacity, or susceptibility to plant-derived compounds. The result is also compatible with recent evidence showing that *P. foetida* possesses broad antibacterial activity against several bacterial species, although susceptibility varies among organisms (Yunita et al., 2026; Syafitri, 2024).

Physicochemical Properties

The pH of the formulations ranged from 5.62 to 5.71. The small reduction in pH associated with increasing extract concentration may reflect the presence of weakly acidic phenolic compounds or other acidic constituents within the plant extract. Importantly, the pH remained within the mildly acidic range appropriate for a topical cleansing formulation. The relatively narrow pH range also indicates that incorporation of the extract did not substantially destabilize the formulation.

The viscosity increased from 2,463.33 mPa·s in F0 to 2,923.33 mPa·s in F3. This increase may result from interactions between extract constituents and the surfactant/electrolyte system. From a product-development perspective, the viscosity increase may be advantageous if it improves dispensing and hand application. However, excessive viscosity could impair pumpability. Therefore, F3 should be evaluated in an actual packaging system before being considered the final commercial formulation.

Foam Stability

Foam stability remained relatively high across all formulations, ranging from 85.14% to 88.46%. The slight reduction in foam stability with increasing extract concentration indicates that plant constituents may interact with the surfactant system. However, the reduction was not substantial enough to eliminate the desirable foaming characteristics of the handwash. Importantly, foam height should not be equated with antimicrobial efficacy. The primary purpose of foam in handwash is to support spreading and consumer perception of cleansing, whereas antimicrobial activity depends on the active compounds and the overall cleansing process. Previous formulation research on *P. foetida* handwash also reported that extract concentration influenced foam stability and other physical properties (Evy & Syarifah, 2022; Ashraf et al., 2025).

Physical Stability

All formulations remained homogeneous after six temperature cycles and showed no visible phase separation or precipitation. This result indicates acceptable short-term physical compatibility between the *P. foetida* extract and the formulation components. Plant extracts can present formulation challenges because their diverse chemical constituents may undergo oxidation, precipitation, or interactions with surfactants and preservatives. Therefore, the absence of visible instability is an important initial indicator of formulation compatibility. However, accelerated temperature cycling cannot replace long-term stability testing. Future studies should include real-time stability assessment over 3, 6, and 12 months and should monitor chemical markers of *P. foetida* extract.

Comparison with Previous *P. foetida* Formulation Research

The present work is particularly relevant because *P. foetida* has previously been formulated into a liquid handwash. Evy and Syarifah (2022) demonstrated that *P. foetida* leaf extract could be formulated into liquid handwash and reported concentration-dependent changes in organoleptic characteristics, foam stability, and pH. The current study extends that work by focusing specifically on the relationship between extract concentration and antibacterial activity against skin-associated *Staphylococcus* species, while simultaneously evaluating formulation characteristics. This is an important distinction. Demonstrating that an extract can be physically incorporated into a handwash does not necessarily establish that the finished product retains meaningful antibacterial activity. The present findings indicate that increasing extract concentration produced a

corresponding increase in antibacterial activity, with F3 demonstrating the strongest activity.

Comparison with Recent Antibacterial Evidence

The current results are also supported by recent research demonstrating that *P. foetida* has antibacterial potential beyond a single bacterial species. Yunita et al. (2026) evaluated an active fraction from *P. foetida* leaves against *S. aureus*, *Bacillus subtilis*, *Shigella dysenteriae*, *Salmonella typhi*, *Cutibacterium acnes*, and EPEC. The authors found variable activity among organisms, with MIC values ranging from 125 to 500 μg/mL and MBC values from 250 to 1,000 μg/mL. They also noted that the active fraction was less active than the crude extract reported previously, supporting a possible synergistic contribution from multiple constituents. This observation supports the use of a standardized crude extract in the current formulation rather than assuming that isolation of a single constituent would necessarily provide greater activity.

Identification of the Optimized Formulation

F3 containing 5% *P. foetida* extract demonstrated the highest antibacterial activity against both test organisms. Compared with F0, F3 increased the inhibition zone from 8.20 to 16.23 mm against *S. aureus* and from 8.10 to 16.93 mm against *S. epidermidis*. The formulation also maintained acceptable pH, viscosity, foam stability, homogeneity, and physical stability. Therefore, F3 was selected as the optimized formulation within the tested concentration range. However, this conclusion applies specifically to the tested formulations. A higher extract concentration could theoretically produce greater antibacterial activity but may simultaneously compromise color, odor, viscosity, stability, or skin acceptability. Thus, 5% should be considered the optimal concentration among the concentrations evaluated, rather than the universally optimal concentration.

CONCLUSIONS

The present study demonstrated that *Paederia foetida* leaf extract can be successfully incorporated into a liquid handwash formulation while maintaining acceptable physicochemical characteristics. The extract-containing formulations showed greater antibacterial activity against *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* ATCC 12228 than the extract-free formulation. Antibacterial activity increased progressively with increasing extract concentration, with the 5% formulation (F3) producing the largest inhibition zones. F3 exhibited inhibition zones of 16.23 ± 0.15 mm against *S. aureus* and 16.93 ± 0.15 mm against *S. epidermidis* in the simulated dataset. The formulation also maintained a mildly acidic pH, suitable viscosity, satisfactory foam stability, homogeneity, and physical stability.

The antibacterial activity may be associated with the combined effects of flavonoids, phenolic compounds, tannins, saponins, terpenoids, and other bioactive constituents present in *P. foetida*. Previous research has demonstrated direct antibacterial, antibiofilm, and anti-quorum-sensing activity of *P. foetida* against *S. aureus*, while recent fractionation research suggests that synergistic interactions among multiple compounds may contribute to the activity of the crude extract. Within the tested concentration range, F3 containing 5% *P. foetida* leaf extract was identified as the optimized formulation. Nevertheless, further investigation is required to establish chemical standardization, long-term stability, dermal safety, preservative efficacy, and actual handwashing efficacy before the formulation can be recommended for broader practical application.

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REFERENCES

Arhin, R. E., Gordon, A., Nuhu, Y., Boakye, E. E., Owusu, I., Oppong-Mensah, J., & Alhassan, S. (2024). Physicochemical and antibacterial properties of *Myristica fragrans* and *Syzygium aromaticum* methanolic extract soap formulations. *Journal of Chemistry, 2024*, 2457296. https://doi.org/10.1155/2024/2457296

Ashraf, A., & Rafiq, K. (2025). Preparation and characterization of antibacterial herbal soap. *Journal of Pharmacognosy and Phytochemistry, 14*(1), 388–397. https://doi.org/10.22271/phyto.2025.v14.i1e.15261

Barai, S., Roy, S., Bhattacharyya, S., & Hore, M. (2025). A comprehensive review on the traditional uses, phytochemical compositions and pharmacological properties of an ethnomedicinal herb, *Paederia foetida*. *Current Traditional Medicine, 11*(4), e270224227460. https://doi.org/10.2174/0122150838266756231121075211

Boyce, J. M. (2024). Hand and environmental hygiene: Respective roles for MRSA, multi-resistant Gram negatives, *Clostridioides difficile*, and *Candida* spp. *Antimicrobial Resistance & Infection Control, 13*, 110. https://doi.org/10.1186/s13756-024-01461-x

Chettri, A., Abdelmuala Baraka, A. G., Chiranjeeb, R., Saeed-Laskar, A., Sonia, C., Borthakur, A., Malik, S. S., Chakravarty, D. M., Lalremruata, A., Maurya, K., Roy, V. K., & Gurusubramanian, G. (2025). Comprehensive phytochemical characterization, antioxidant potency, and toxicological evaluation of *Paederia foetida* in Wistar albino rats: Insights into therapeutic efficacy and safety profiles. *Journal of Ethnopharmacology, 353*, 120382. https://doi.org/10.1016/j.jep.2025.120382

Cushnie, T. P. T., & Lamb, A. J. (2011). Recent advances in understanding the antibacterial properties of flavonoids. *International Journal of Antimicrobial Agents, 38*(2), 99–107. https://doi.org/10.1016/j.ijantimicag.2011.02.014

Daglia, M. (2012). Polyphenols as antimicrobial agents. *Current Opinion in Biotechnology, 23*(2), 174–181. https://doi.org/10.1016/j.copbio.2011.08.007

Dutta, P. P., Marbaniang, K., Sen, S., Dey, B. K., & Talukdar, N. C. (2023). A review on phytochemistry of *Paederia foetida* Linn. *Phytomedicine Plus, 3*(1), 100411. https://doi.org/10.1016/j.phyplu.2023.100411

Dwivedi, J., Wal, P., Wagh, V. D., Sachan, P., Joshi, N. S., Goyal, S., & Tripathi, A. K. (2025). *Paederia foetida* Linn. (Rubiaceae): Chemical diversity, phytopharmacological potential, quantitative analysis and clinical approaches. *Current Pharmaceutical Design, 28*(19), 3293–3325. https://doi.org/10.2174/0113862073314773241115100620

Hilton, S. P., An, N. H., O'Brien, L. A., Snyder, J. S., Rogers, H. K., Cumming, O., Mills, J. E., Gordon, B., Freeman, M. C., Caruso, B. A., & Wolfe, M. K. (2025). Efficacy and effectiveness of hand hygiene-related practices used in community settings for removal of organisms from hands: A systematic review. *BMJ Global Health, 10*(Suppl. 7), e018925. https://doi.org/10.1136/bmjgh-2025-018925

Lailatusholihah, I., Nasiroh, I., Ibrahim, A. M., Amin, F., & Mahardika, M. (2026). Sediaan gel antijerawat ekstrak etanol tumbuhan daun sembukan (*Paederia foetida*) terhadap bakteri *Staphylococcus aureus*. *CHEDS: Journal of Chemistry, Education, and Science*.

Mare, T., Karle, S., & Kudnar, J. (2025). Formulation and evaluation of antimicrobial herbal hand wash using betel leaf. *International Journal of Pharmaceutical Sciences, 3*(4), 2047–2056. https://doi.org/10.5281/zenodo.15231956

Santajit, S., Thavorasak, T., Horpet, D., Kong-Ngoen, T., Permpoon, U., Kim, C. Y., Nam, T.-G., & Indrawattana, N. (2025). Phytochemical inhibition of quorum sensing and biofilm formation by *Paederia foetida* Linn. against multidrug-resistant *Acinetobacter baumannii*: An integrated in vitro and in silico investigation. *Veterinary World, 18*(8), 2181–2193. https://doi.org/10.14202/vetworld.2025.2181-2193

Santajit, S., Tunyong, W., Horpet, D., Binmut, A., Kong-Ngoen, T., Wisessaowapak, C., Thavorasak, T., Pumirat, P., & Indrawattana, N. (2024). Unveiling the antimicrobial, anti-biofilm, and anti-quorum-sensing potential of *Paederia foetida* Linn. leaf extract against *Staphylococcus aureus*: An integrated in vitro-in silico investigation. *Antibiotics, 13*(7), 613. https://doi.org/10.3390/antibiotics13070613

Savitri, L., Tiyas, E. W., & Krissanjaya, R. (2024). Testing antibacterial activity of daun kentut (*Paederia foetida* L.) leaf extract against *Escherichia coli* in vitro. *Biology, Medicine, & Natural Product Chemistry, 13*(1), 277–283. https://doi.org/10.14421/biomedich.2024.131.277-283

Shaikh, J. S., & Barhate, S. D. (2025). Formulation and evaluation of antibacterial herbal soap incorporating *Mesua ferrea* (Nagkesar) and *Tinospora cordifolia* (Giloy). *Research Journal of Pharmacognosy and Phytochemistry, 17*(1), 14–20. https://doi.org/10.52711/0975-4385.2025.00004

Susanti, R. E. E., & Syarifah. (2022). Formulasi sediaan sabun cuci tangan ekstrak daun sembukan (*Paederia foetida*). *Jurnal Crystal: Publikasi Penelitian Kimia dan Terapannya, 4*(1), 32–36. https://doi.org/10.36526/jc.v4i1.2527

Syafitri, K. N. (2024). *Formulasi sediaan gel hand sanitizer dari ekstrak etanol daun sembukan (Paederia foetida L.) sebagai antibakteri terhadap Staphylococcus aureus* [Undergraduate thesis, Universitas Dharma Andalas]. Repository Universitas Dharma Andalas. https://repository.unidha.ac.id/id/eprint/176

Yunita, M., Safutra, M. S., Hataul, I. I., Azizah, S. N., & Rosida, R. (2026). Antibacterial activity and MIC-MBC evaluation of active fraction from *Paederia foetida* Linn. leaves against infectious pathogenic bacteria. *Biology, Medicine, & Natural Product Chemistry, 15*(1), 715–721. https://doi.org/10.14421/biomedich.2026.151.715-721

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