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FORMULATION AND CHARACTERIZATION OF LIQUID HAND SOAP CONTAINING *Physalis angulata* L. LEAF RESIDUAL FRACTION AS AN ANTIBACTERIAL AGENT AGAINST *Escherichia coli*

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ABSTRACT

Physalis angulata L., commonly known as Ciplukan, is an Indonesian medicinal plant rich in antibacterial bioactive compounds. This study formulated a liquid hand soap enriched with the active fraction of Ciplukan leaf extract and evaluated its physicochemical characteristics and antibacterial efficacy against *Escherichia coli*. The leaves underwent maceration and subsequent fractionation with n-hexane, ethyl acetate, and methanol. A 1000 ppm residual fraction exhibited the highest antibacterial activity (2.75 mm), and phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, saponins, and steroids. Furthermore, FTIR analysis results supported the presence of these compounds. The active fraction was incorporated into a liquid soap formulation, resulting in a product with a pale-yellow color and lemon fragrance. Physicochemical evaluation confirmed that the soap met quality standards, featuring a pH of 6.84, foam stability of 85.53%, viscosity of 14.95 cP, and free fatty acid content of 0.85%. Furthermore, antibacterial testing showed that the formulated soap yielded an inhibition zone of 21.27 mm, which was not significantly different from the negative control. These results indicate that the soap's strong antibacterial activity is primarily driven by its base, suggesting the Ciplukan fraction serves as a natural bioactive enrichment.

Keywords: antibacterial activity, *Escherichia coli*, leaf extract, liquid hand soap, *Physalis angulata* L.

INTRODUCTION

Ciplukan (*Physalis angulata* L.) has a long history as a herbal medicine, utilizing nearly all parts of the plant, including the roots, stems, leaves, and fruits (Novitasari *et al*., 2024). It is used to treat various conditions, such as malaria, asthma, hepatitis, dermatitis, rheumatism, and leukemia, due to its anticancer, antidiabetic, anti-inflammatory, antioxidant, and antibacterial properties (Fadhli *et al*., 2023). Ciplukan leaf extract contains flavonoids, tannins, alkaloids, and saponins (Nurhilaliyah *et al*., 2026). These compounds exhibit antibacterial activity against both Gram-negative and Gram-positive bacteria (Huang *et al*., 2024; Li *et al*., 2022; Li and Monje-galvan, 2023; Zhang *et al*., 2026). Specifically, a 25% extract concentration produced an inhibition zone of 16.23 mm against *Bacillus subtilis* (Delina *et al*., 2024). Another study reported that the same concentration yielded an inhibition zone of 2.67 mm against *Streptococcus pneumoniae* (Rakhman *et al*., 2025).

Escherichia coli is a notable Gram-negative bacteria responsible for infections such as urinary tract infections, diarrhea, and gastroenteritis (Abdulabbas *et al*., 2023). Unlike resident flora, *E. coli* can be found on the skin–particularly the hands–acting as a transient pathogenic microorganism (Ogundare *et al*., 2024). Hand contamination by *E. coli* among health-care workers (HCWs) has been shown to produce isolates that are 100% identical to those found in patients (Puig-Asensio *et al*., 2020). To prevent the transmission of such pathogens, sanitation movements emphasize handwashing with soap (Das *et al*., 2025). Indeed, washing hands with soap has been reported to be more effective than using hand sanitizer or ozonized water (Breidablik *et al*., 2020).

Many commercial hand soaps use triclosan or triclocarban as antibacterial agents (Halden *et al*., 2017). However, the continuous or excessive use of triclosan can lead to bacterial resistance, as well as potential side effects such as allergies and endocrine disruption (Cheng *et al*., 2024). Replacing these synthetic chemicals with natural antibacterial ingredients offers a viable solution. Consequently, this study aimed to formulate a liquid hand soap enriched with the active fraction of Ciplukan leaf extract. To obtain this fraction, the extract was fractionated using *n*-hexane and ethyl acetate to separate its bioactive compounds (Abubakar and Haque, 2020). The fraction exhibiting the largest

inhibition zone againts *E. coli* was then incorporated into the soap formulation. A liquid format was chosen for the final product due to its practicality, portability, ease of storage, durability, and potential for attractive packaging (Dixon *et al*., 2017).

MATERIALS AND METHODS

Materials

The materials used in this study included 1.5 kg fresh Ciplukan leaves collected from Petambakan, Madukara, Banjarnegara, which were subsequently dried and ground into a powder using a laboratory blender, along with methanol, ethyl acetate, and *n*-hexane as solvents for the extraction and fractionation processes. The liquid hand soap base was formulated using palm oil, potassium hydroxide (KOH), stearic acid, and distilled water, further supplemented with sodium lauryl sulfate (SLS), carboxymethyl cellulose (CMC), and fresh lemon fragrance as additives. For phytochemical screening, various reagents were employed, including concentrated HCl, Mg powder, concentrated H_2SO_4, 0.1% $FeCl_3$, and Wagner's reagent. Additionally, for the antibacterial assays, *Escherichia coli* (*E. coli*) bacteria stock, nutrient agar (NA) and nutrient broth (NB) were utilized as growth media, with triclosan and chloramphenicol serving as comparative controls, while Whatman filter paper and a 1% phenolphthalein indicator were used for filtration and analytical procedures, respectively.

Equipments

The equipment utilized for processing and preparation consisted of a laboratory blender, an IKA RV 10 Basic Vacuum rotary evaporator, magnetic stirrer, a hot plate, and a separatory funnel. Microbiological procedures and sterilization were supported by an autoclave, a Bunsen burner, an incubator, Petri dishes, test tubes, inoculation loops (ose), a Drigalski spatula, and a cork borer, with liquid handling facilitated by micropipettes. Analytical measurements and characterization were performed using a Shimadzu IR Tracer-100 FTIR spectrometer (located at the Faculty of Pharmacy, Universitas Muhammadiyah Purwokerto), an analytical balance, a titration set, a pH meter, an Ostwald viscometer, and vernier calipers, while a refrigerator was employed for the storage.

Extraction and Sequential Fractionation of Ciplukan Leaf Powder

A total of 165.66 grams of Ciplukan leaf powder was initially macerated in methanol for 7 x 24 hours with occasional stirring. The resulting filtrate was collected and concentrated using a vacuum rotary evaporator at 60 °C and 60 rpm to obtain a crude methanol extract. An aliquot of this extract (18.33 grams) was then subjected to sequential fractionation by soaking it in *n*-hexane and ethyl acetate (3 x 24 hours each). The *n*-hexane fraction, ethyl acetate fraction, and the final residual fraction were each concentrated under the same conditions until a thick consistency was achieved. The resulting fractions were then weighed (Ningsih *et al*., 2016).

Antibacterial Testing of Each Fraction

The antibacterial activity assay was conducted to determine the most potent extract or fraction of Ciplukan leaf in inhibiting bacterial growth using the agar well diffusion method (Ningsih *et al*., 2016). The test microorganism used in this study was *E. coli*. The methanol extract of Ciplukan leaf and each fraction were prepared at a concentration of 1000 ppm, respectively. Regenerated *E. coli* bacteria were inoculated using an inoculation loop into 30 mL of Nutrient Broth (NB) and incubated for 24 hours at 37 °C. A total of 5 mL aliquot of the regenerated *E. coli* culture was taken to measure its optical density (OD) at a wavelength of 620 nm. If the OD value was >1, a 50 µL volume of the culture was used; if the OD value was <1, a 100 µL volume was utilized.

Nutrient Agar medium (15 mL) was poured into Petri dishes and allowed to solidify. The *E. coli* culture was then inoculated onto the medium surface using the spread plate technique with a Drigalski spatula. Wells with a diameter of approximately 6 mm were created in the inoculated agar medium using a sterile cork borer. Each well was filled with 40 µL of 1000 ppm methanol extract or its fractions. Chloramphenicol served as positive controls, while distilled water and each respective solvent were used as the negative controls. The petri dishes containing the samples and bacteria were then incubated for 24 hours at 37 °C. The antibacterial activity was determined by measuring the diameter of the inhibition zone around each well using Vernier caliper.

Identification using Fourier Transfer Infrared Spectrometer

The identification procedure using an FTIR spectrometer was carried out by analyzing the methanol extract or fraction of Ciplukan leaf extract that showed the highest activity in inhibiting the growth of *E. coli*. This analysis was performed to identify the absorption bands of the functional

groups belonging to the chemical compounds within the sample (Ningsih *et al*., 2016).

Fitochemical Testing

Phytocemical screening was conducted to identify the secondary metabolites present in the extract or the most active methanol extract fraction of Ciplukan leaf against *E. coli*. The screening included the identification of flavonoids, steroids, tannins, saponins, and alkaloids. For flavonoid identification, the most active extract or fraction was treated with strong HCl and Mg powder, followed by vigorous shaking. A positive result was indicated by the formation of an orange solution. To identify steroids, the most active extract or fraction was applied to a drop plate and allowed to dry; subsequently, two drops of strong H_2SO_4 were added. A positive result was marked by the appearance of purple coloration.

For tannin testing, the sample was dissolved in distilled water, boiled, and filtered. The resulting filtrate was then treated with 0.1% $FeCl_3$. A positive result was indicated by the formation of a bluish-black or green color. Saponin identification was performed by adding hot water to the sample and shaking it vigorously. A positive result was characaterized by the formation of stable foam persisting for 10 minutes. Finally, alkaloid identification involved adding 1 mL of the most active extract or fraction to Wagner's reagent, followed by vigorous shaking. The presence of a brown precipitate indicated a positive result (Dubale *et al*., 2023).

Formulation of Liquid Hand Soap

The ingredients were prepared according to the concentrations specified in Table 1. To initiate saponification, 15 mL of palm oil was heated in a beaker to 80 °C, followed by the gradual addition of 8 mL of KOH solution. until a paste-like consistency was achieved. This soap paste was then diluted with distilled water and stirred until homogeneous liquid soap was formed. The additive agents were integrated into the mixture under continuous stirring. The fragrance and the most potent Ciplukan leaf extract or its active fraction were then incorporated. Finally, the solution was adjusted to a total volume of 70 mL with distilled water and transferred into a clean container (Haque *et al*., 2022).

Antibacterial Testing of Formula

The antibacterial activity assay was conducted to evaluate the efficacy of the formulated liquid hand soap in inhibiting the growth of *E. coli*. The testing was performed using the agar well diffusion method (Ningsih *et al*., 2016). The positive control consisted of the liquid hand soap base supplemented with triclosan as the active agent, while negative control was the liquid hand soap base without any antibacterial agent. The antibacterial activity testing of the liquid hand soap followed the same procedure as the antibacterial assays conducted for the extract and each respective fraction.

Characterization of Formula

The formulated liquid hand soap was characterized through organoleptic assessment, pH testing, foam height and stability tests, viscosity measurements, and the analysis of free alkali or free fatty acid content.

Table 1. Liquid hand soap ingredients composition

Ingredients	Negative control	Positive control	Formula	Function
Most active methanol extract fraction of Ciplukan leaves (1000 ppm)	-	-	7 µL	Antibacterial agent
KOH 20%	8 mL	8 mL	8 mL	Alkali source
SLS	0.28 g	0.28 g	0.28 g	Foaming agent
CMC	0.05 g	0.05 g	0.05 g	Thickener
Stearic acid	0.15 g	0.15 g	0.15 g	Foam stabilizer
Citric acid	1.3 g	1.3 g	1.3 g	pH balancer
Palm oil	15 mL	15 mL	15 mL	Triglyseride source
Fragrance	0.1 mL	0.1 mL	0.1 mL	Fragrance
Triclosan 1000 ppm	-	7 µL	-	Positive control
Distilled water	Added up to a total volume of 70 mL			Solvent

Abbreviations: SLS (Sodium Lauryl Sulfate) and CMC (Carboxymethyl Cellulose)

Organoleptic properties, including form, color, and odor, were assessed through a hedonic test involving 10 panelists using a scoring scale ranging from 1 (dislike) to 5 (strongly like). The pH level was determined by dissolving 0.5 g of the soap in 10 mL of distilled water and measuring with a pH meter to ensure compliance with the SNI 2588:2017 standard (pH 4-10). Foam height and stability were evaluated by shaking a mixture of 1 g of soap and 10 mL of distilled water for 20 seconds, with height measurements recorded at 0 and 5 minutes using a 0.1 cm scale ruler. Viscosity was measured using an Ostwald viscometer to determine flow time, while the specific gravity was calculated by the pycnometer. Finally, the free alkali or free fatty acid content was determined according to SNI 2588:2017 by heating 1 g of soap in 20 mL of 99.5% ethanol for 30 minutes, followed by titration using a 1% phenolphthalein indicator and standard KOH or HCl solutions to calculate the values as KOH or oleic acid, respectively.

$$\text{Free alkali content} = \frac{40 \; x \; V \; x \; N}{b} \text{ x } 100\% \qquad (1)$$

Description:
Free alkali is expressed in units of % mass fraction
V = Volume of HCl used in titration (mL)
N = Normality of HCl (N)
b = Sample weight (mg)
40 = Equivalent weight of KOH

$$\text{Free fatty acid content} = \frac{282 \; x \; V \; x \; N}{b} \text{ x } 100\% \qquad (2)$$

Description:
Free fatty acid is expressed in units of % mass fraction
V = Volume of KOH used in titration (mL)
N = Normality of KOH (N)
b = Sample weight (mg)
282 = Equivalent weight of oleic acid ($C_{18}H_{34}O_2$)

Data Analysis

The research data were analyzed using Analysis of Variance (ANOVA) to determine whether significant differences existed among the results of each formula. A one-way ANOVA was performed on the results of the antibacterial activity assays for the methanol extract, *n*-hexane fraction, ethyl acetate fraction, and the residual fraction of the Ciplukan leaf methanol extract. Additionally, the analysis was applied to the pH, viscosity, foam stability, free fatty acid or alkali content, and the antibacterial activity of the liquid hand soap supplemented with the most active Ciplukan leaf extract or fraction. If the ANOVA results indicated a significant difference, a post-hoc test was required. Duncan's Multiple Range Test (DMRT) was employed as the post-hoc analysis to examine the effects between treatments with high sensitivity (Satyantini *et al*., 2025).

RESULTS AND DISCUSSION

A total of 1.5 kg of fresh Ciplukan leaves were thoroughly washed, cut into small pieces, and dried away from direct sunlight. Exposure to direct sunlight can alter the leaf color from green to pale green due to the degradation of chlorophyll into pheophytin (Puspita *et al*., 2021). The dried Ciplukan leaf were then ground into a powder to reduce the particle size, thereby increasing the contact area between the sample and the solvent and facilitating the extraction process (Ningsih *et al*., 2017). The final weight of the obtained Ciplukan leaf powder was 165.66 grams, resulting in a powder yield of 11.04%. The preparation stages of the Ciplukan leaf extract are represented in Figure 1.

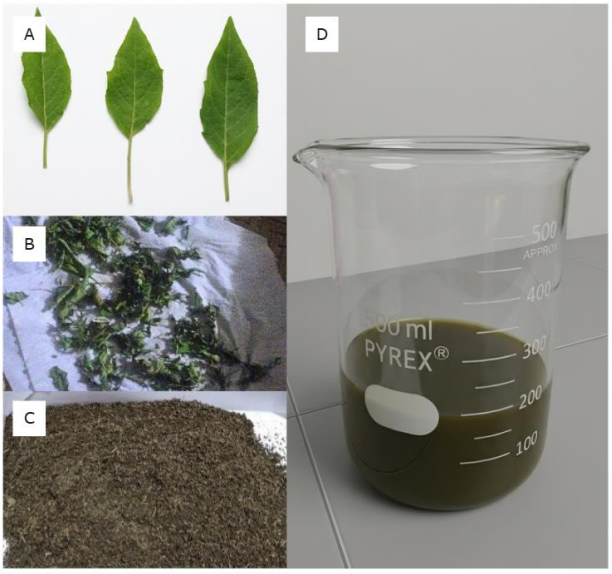

Figure 1. Stages of Ciplukan leaf extract preparation: (A) fresh Ciplukan leaves, (B) dried Ciplukan leaves, (C) Ciplukan leaf powder, and (D) result of the maceration process

Ciplukan leaf powder was extracted to isolate the bioactive compounds within the sample. Methanol was selected as the solvent due to its versatility as a universal solvent in natural product extraction (Riyadi *et al*., 2023). The extraction mechanism relies on the concentration gradient between the solute inside and outside the cell, which facilitates the outward diffusion of highly concentrated compounds (Salamah *et al*., 2017). The resulting filtrate was concentrated using a

vacuum rotary evaporator at 60 °C and 60 rpm. This temperature was maintained to prevent the thermal degradation of the chemical constituents. The final yield of the concentrated methanol extract was 16.6%. This result meets the national standard requirement for a yield of no less than 7.2% (Departemen Kesehatan RI, 2000).

A total of 18.33 grams of the methanol extract from Ciplukan leaves was taken for gradient maceration fractionation using *n*-hexane and ethyl acetate solvents. The yield values for each resulting fraction are presented in Table 2.

Table 2. The yield for each resulting fraction

Fraction	Weight (g)	Yield (%)
n-hexane	2.53	13.80
Ethyl acetate	0.25	1.36
Residual	15.55	84.83

The antibacterial activity test was conducted to determine the ability of the extract and each Ciplukan leaf extract fraction to inhibit the growth of *E. coli* bacteria. Chloramphenicol was used as a positive control because it is a broad-spectrum bacteriostatic antibiotic (Ningsih *et al*., 2016). Table 3 illustrates that 1000 ppm of extract and each fraction of the Ciplukan leaf extract possess the capability to inhibit the growth of *E. coli*.

Table 3. Antibacterial testing results of Ciplukan leaf extract and each fraction

Sample	Inhibitory zone diameter (mm)
Distilled water	0.00 ± 0.00^{a}
Methanol	0.00 ± 0.00^{a}
Methanol extract	2.28 ± 0.14^{b}
Residual fraction	2.75 ± 0.26^{b}
Ethyl acetate	0.00 ± 0.00^{a}
Ethyl acetate fraction	2.21 ± 0.03^{b}
n-hexane	0.00 ± 0.00^{a}
n-hexane fraction	2.27 ± 0.20^{b}
Chloramphenicol	25.00 ± 1.39^{c}

Notes: Data are represented as mean ± standard deviation from three replications (n = 3). Values in the same column followed by the same superscript letter are not significantly different ($p > 0.05$) according to Duncan's Multiple Range Test

The residual fraction, methanol extract, *n*-hexane fraction, and ethyl acetate fraction showed comparable antibacterial activities, with no significant differences among them ($p > 0.05$). This result indicates that the antibacterial compounds in the Ciplukan leaf samples are broadly distributed across various polarities. Although the one-way ANOVA indicated significant differences across all tested groups due to the controls, the Duncan post-hoc test confirmed that the activities of the extract and fractions were statistically equivalent. Therefore, the residual fraction was selected as the final choice due to its remarkably highest yield of 84.83%. Additionally, the activities of these samples were significantly higher than those of distilled water and methanol, which showed no inhibition, confirming that the solvents did not influence the antibacterial values. In contrast, chloramphenicol exhibited a significantly larger inhibition zone compared to all treatments. The mechanism by which chloramphenicol inhibits bacterial growth is by inhibiting the activity of the peptidyl transferase enzyme, thereby disrupting protein synthesis (Tereshchenkov *et al*., 2018).

The FTIR absorption bands obtained from the residual fraction of the Ciplukan leaf extract as represented in Figure 2 support the presence of secondary metabolites, including flavonoids, tannins, saponins, steroids, and alkaloids. The peaks observed at 921.97 cm^{-1} (weak), 975.98 cm^{-1} (medium), 1045.42 cm^{-1} (strong), and 1293.83 cm^{-1} (medium) are attributed to the stretching vibrations of the C–O (ether) group, which commonly found in the glycoside structures of saponins and flavonoids (El-keiy *et al*., 2019). The peak at 1589.34 cm^{-1} (medium), which falls within the 1450–1600 cm^{-1} range, corresponds to the aromatic C=C stretching of the aryl group, which is characteristic of the aromatic rings in flavonoids, tannins, and the heterocyclic structures of alkaloids (Jannah *et al*., 2025; Sari and Yulis, 2023). The absorptions at 2931.8 cm^{-1} (weak) and 2958.8 cm^{-1} (weak) identify the presence of aliphatic C–H sp^3 groups, representing the triterpenoid or steroid backbone typically present in saponins and steroids (Abrori *et al*., 2025). Furthermore, the broad peak at 3186.4 cm^{-1} and small intensities at 3576.02 cm^{-1} represent OH stretching vibrations, confirming the abundance of phenolic hydroxyl groups from tannins and flavonoids (Krysa *et al*., 2022). These findings support the presence of the identified metabolites, which are further corroborated by the medium intensity peaks at 1334.74 cm^{-1} that also correspond to OH bending vibrations (Al-otaibi, 2018).

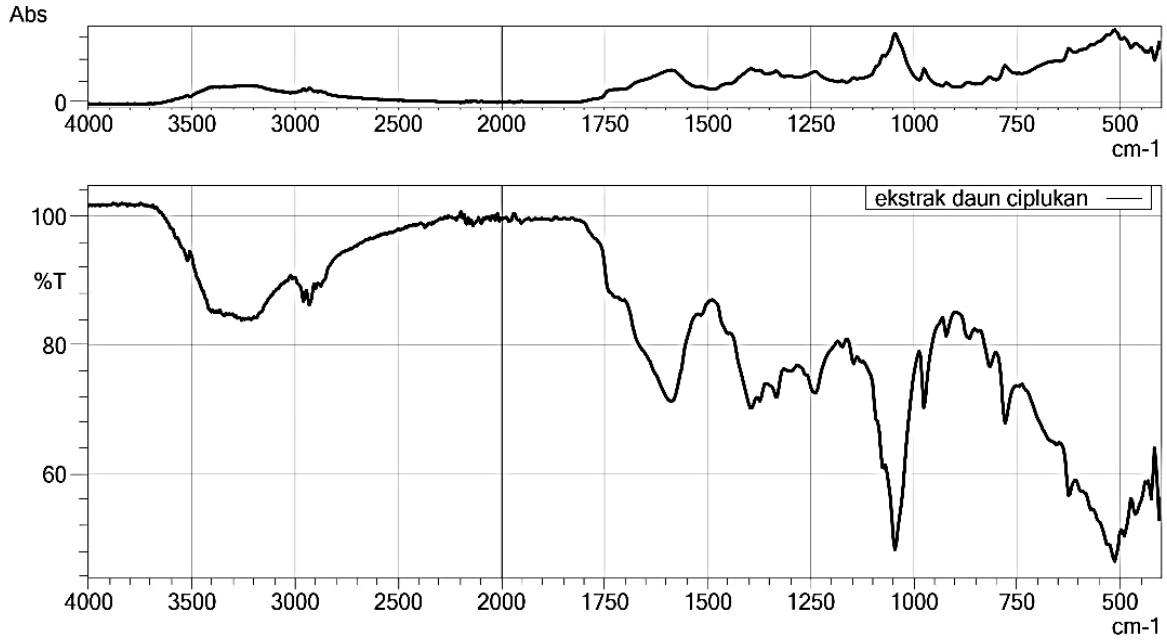

Figure 2. IR spectra of residual fraction of Ciplukan leaf methanol extract

Table 4. Phytochemical testing result of Ciplukan leaf residual fraction

Compound	Testing reagent	Indicator	Result
Flavonoid	Strong HCl and Mg powder	Orange solution	+
Steroid	Concentrated H_2SO_4	Purple color	+
Tannin	0.1% $FeCl_3$	Green-black color	+
Saponin	Distilled water	Foam formation	+
Alkaloid	Wagner's Reagent	Brown precipitate	+

Notes: Positive sin (+) shows the presence of compounds and negative sign (–) shows the absence of compounds

The phytochemical screening was also conducted on the residual fraction of the Ciplukan leaf methanol extract to identify its secondary metabolite constituents. Plants produce secondary metabolites to survive in environmental conditions that are unfavorable for their continued existence (Qaderi *et al*., 2023). The sreening parameters in this study included flavonoids, steroids, tannins, saponins, and alkaloids. The results, as presented in Table 4, indicate that the residual fraction of Ciplukan leaf extract contains all of these metabolites.

Palm oil and KOH were utilized through the semi-boiled method at 80 °C. A 20% KOH solution was added gradually to the heated oil with continuous stirring for 60 minutes until it reached the trace stage, which is characterized by the thickening of the mixture into a paste (Bakhri *et al*., 2023). This saponification reaction involves the hydrolysis of triglycerides in the oil by an alkaline base to produce alkali salts (soap) and glycerol as a byproduct (Rahman *et al*., 2026). Palm oil was selected as the fat source due to its high triglyceride content, while KOH was utilized to produce a liquid soap consistency. The resulting saponification paste was subsequently diluted with distilled water and enriched with additives, including CMC, SLS, citric acid, stearic acid, fragrance, and colorant. The mixture was allowed to stand to ensure complete reaction and achieve a stable, homogeneous liquid soap.

The antibacterial activity test was conducted to determine the ability of the liquid hand soap, enriched with the active residual fraction of Ciplukan leaf methanol extract, to inhibit the growth of *E. coli*. The method employed was the well diffusion method. Distilled water served as the negative control, while triclosan was utilized as the positive control

Table 5. Antibacterial testing result of formulated hand liquid soap

Testing parameter	Negative control	Soap + residual fraction	Positive control
[1]Inhibition zone diameter (mm)	19.81 ± 0.90[1a]	21.27 ± 2.22[1a]	30.75 ± 0.73[1b]
[2]pH	6.53 ± 0.02[2a]	6.84 ± 0.01[2b]	6.85 ± 0.01[2b]
[3]Foam stability (%)	83.94 ± 0.27[3a]	85.53 ± 0.52[3b]	87.29 ± 0.19[3c]
[4]Viscocity (cP)	13.96 ± 0.17[4a]	14,95 + 0.25[4c]	14.43 ± 0.06[4b]
[5]Free alkali (%)	0.68 ± 0.14[5a]	0.85 ± 0.14[5a]	0.94 ± 0.14[5a]
[6]Foam stability (%)	83.94 ± 0.10[6a]	85.53 ± 0.30[6b]	87.29 ± 0.20[6c]

Notes: Values are expressed as mean ± standard deviation (n = 3). Different superscript letters within the same row indicate a significant difference between treatments according to Duncan's Multiple Range Test ($P < 0.05$)

Table 5 shows that the antibacterial activity of the soap enriched with the methanol extract residual fraction was numerically higher than that of the liquid hand soap base. However, the results of the one-way ANOVA and the Duncan post-hoc test indicated no significant difference between these two treatments suggesting the baseline antibacterial strength of the formulation is well-supported by its primary ingredients. Palm oil is known to possess antibacterial activity as it contains both saturated and unsaturated fatty acids (Fauziati *et al*., 2019). Fatty acids with a carbon atom count greater than ten cause lysis of the bacterial cell protoplasm (Léguillier *et al*., 2024). Additionally, citric acid, as an organic acid, can counteract microorganisms due to its high pKa value (Manalu *et al*., 2023).

Beyond its antibacterial properties, the addition of the residual fraction significantly optimizes the soap's physical characteristics. The pH value of the enriched soap is significantly different from the negative control and aligns closely with the positive control, indicating better stability and skin compatibility. Moreover, the foam stability and viscosity of the enriched soap are significantly superior to the negative control. The improved foam stability is likely enhanced by the presence of saponins and flavonoids identified in the residual fraction, which act as natural surfactants. The higher viscosity also suggests a more robust interaction between the secondary metabolites and the soap base. While the antibacterial potency has not yet matched the positive control, these results demonstrate that the Ciplukan leaf residual fraction significantly improves the overall quality and physical stability of the liquid hand soap.

CONCLUSION

The residual fraction of *Physalis angulata* L. methanol extract demonstrated an antibacterial inhibition zone of 2.75 mm and was selected for the soap formulation due to its remarkably highest yield of 84.83%. Its integration into the liquid hand soap produced a pale-yellow, lemon-scented product with standardized physicochemical attributes, including a pH of 6.84, 85.53% foam stability, 14.95 cP viscosity, and 0.85% free alkali content. Although the final product achieved a notable antibacterial inhibition zone of 21.27 mm, this effect did not differ significantly from the negative control, indicating that the Ciplukan fraction functions as a natural bioactive additive.

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