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Effectiveness of *Ketepeng Cina* (*Cassia alata* Linn.) Leaf Powder as an Active Ingredient in Antiseptic Liquid Soap

Encik Eko Rifkowaty[1]*, Adha Panca Wardanu[1], Kendri Wahyuningsih[2]
[1]Department of Agriculture and Business, Ketapang State Polytechnic, Ketapang 78813, Indonesia.
[2]Indonesia Center for Agricultural Development and Modernization of Postharvest, Ministry of Agriculture, Bogor, Indonesia.

Corresponding author*
encik.rifkowaty@gmail.com

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Abstract

This study aimed to evaluate the effectiveness of *Cassia alata* Linn. (*ketepeng Cina*) leaf powder as an active ingredient in virgin coconut oil (VCO)-based antiseptic liquid soap. A Completely Randomized Design (CRD) was employed with three treatments consisting of 2 g, 4 g, and 6 g of leaf powder (b/v). The evaluated parameters included total flavonoid content, phytochemical screening, functional group analysis using FTIR, LC-MS, pH, specific gravity, viscosity, free alkali, foam stability, unsaponifiable fraction, and inhibition zones against *Escherichia coli* and *Malassezia furfur*. The results indicated that increasing concentrations of *ketepeng* leaf powder positively correlated with total flavonoid levels and significantly enhanced the inhibition zone against *E. coli*, but showed no notable activity against *M. furfur*. Phytochemical analysis confirmed the presence of flavonoids and steroids in the powder, while liquid soap formulations contained flavonoids and saponins. FTIR analysis further verified flavonoid compounds, whereas LC-MS identified lauric Acid (m/z 274) and quercetin (m/z 303), contributing to antimicrobial activity. Statistical analysis (ANOVA, $\alpha = 0.05$) showed no significant effect of leaf powder addition on pH, free alkali, unsaponifiable fraction, viscosity, specific gravity, or foam stability. The formulated liquid soap complied with the Indonesian National Standard (SNI 06-4085-1996) for pH and free alkali, ensuring safety for consumer use. Overall, *ketepeng cina* leaf powder demonstrated potential as a natural antimicrobial agent in herbal antiseptic liquid soap formulations.

Keywords: Antimicrobial activity; Antiseptic Liquid Soap; *Cassia alata* Linn; *Ketepeng Cina*; Natural Ingredients.

INTRODUCTION

Infections caused by bacteria and fungi greatly influence skin health. Common skin diseases encountered in the community include tinea versicolor, ringworm, and scabies. One preventive measure against skin infections is the use of antiseptic soap. Unlike regular soap, antiseptic soap contains active antiseptic agents capable of inactivating or inhibiting the growth of microorganisms (Lompo et al., 2023; Ferdian et al., 2023). Previous studies have demonstrated that antiseptic soap, such as *Shigella flexneri* and *Escherichia coli*, effectively reduces pathogenic contamination on hands (Stevens, 2003).

One of the plant-based sources of antiseptic agents is *Cassia alata* Linn., commonly known as *ketepeng cina*. Its fresh leaves have traditionally been applied directly to infected skin areas in specific communities to alleviate skin disorders. *C. alata* leaves contain secondary metabolites, including alkaloids, saponins, flavonoids, tannins, and anthraquinones (Lumbessy et al., 2013). These compounds exhibit antibacterial activity against Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa (Ekwenye & Okorie, 2010). Furthermore, several active constituents of *C. alata* possess properties similar to ketoconazole, an antifungal drug used to treat fungal skin infections (Kurniawan et al., 2012). These findings strengthen the potential of *C. alata* as a promising candidate for herbal antiseptic soap formulations.

Nevertheless, previous research has predominantly focused on herbal extracts, whereas the use of leaf powder in liquid soap remains limited. Related studies have only reported the application of *Centella asiatica* powder in solid soap, which yielded promising results (Nuryati & Lestari, 2021). Using leaf powder is comparatively more straightforward and economical than extracts, making it more feasible for implementation in small- and medium-scale industries.

Based on this background, the present study evaluated the effectiveness of *C. alata* leaf powder as an active ingredient in liquid antiseptic soap. The evaluation encompassed antibacterial activity against *Escherichia coli* and antifungal activity against *Malassezia furfur*, phytochemical and active compound analysis, as well as quality assessment of liquid soap in accordance with the Indonesian National Standard (SNI 06-4085-1996). This study is expected to contribute to developing safe,

effective, and locally applicable herbal liquid antiseptic soap products in Indonesia.

MATERIALS AND METHODS

Materials

Fresh leaves of *Cassia alata* Linn. were obtained from healthy plants. Virgin Coconut Oil (VCO, 50 g) was the primary oil phase. The chemicals employed included potassium hydroxide (KOH, Merck), sodium chloride (NaCl, Merck), citric Acid, glycerin (Brataco), distilled water, carboxymethyl cellulose (CMC), granulated sugar (Gulaku brand), and 70% ethanol (Merck). All reagents used were of pro-analysis grade.

Preparation of Cassia alata Leaf Powder

Leaves were divided into five parts from the stem base and dried using a cabinet dryer at 40 °C for 19 hours. The dried leaves were then ground using a blender and sieved through a 100-mesh sieve to obtain uniformly sized Cassia alata leaf powder.

Liquid Soap Formulation

The liquid soap was formulated by heating 50 g of VCO at 70 °C. A KOH solution was prepared by dissolving 12.5 g of KOH in 9 mL of distilled water, which was then added to the heated oil and stirred until saponification occurred. Subsequently, 7.5 g of sugar dissolved in 10 mL of distilled water was added, followed by 0.2 g NaCl, 0.2 g citric acid, 6.5 g glycerin, 0.5 g CMC, 16 g ethanol, and 30 mL of distilled water. The mixture was stirred until homogeneous. After cooling, *Cassia alata* leaf powder was incorporated at 2 g, 4 g, and 6 g, respectively, and stirred until homogeneous to obtain liquid soap. The formulation of liquid soap treatments with the addition of Cassia alata leaf powder is presented in Table 1.

Table 1. Formulation of Liquid Soap with the Addition of Chinese Senna Leaf Powder.

Materials	Sample		
	***Liquid Soap Formulation*-1 (LSF-01)**	***Liquid Soap Formulation*-2 (LSF-02)**	***Liquid Soap Formulation*-3 (LSF-03)**
VCO (g)	50	50	50
KOH (g) in 9 mL aqudest	12,5	12,5	12,5
Sugar (g) in 10 mL aqueous solution	7,5	7,5	7,5
Sodium Chloride (g)	0,2	0,2	0,2
Citrat Acid (g)	0,2	0,2	0,2
Glyserin (g)	6,5	6,5	6,5
CMC (g)	0,5	0,5	0,5
Etanol (g)	16	16	16
Aquadest (mL)	30	30	30
Ketepeng leaf powder (g)	2	4	6

Note: LSF-01 = Liquid soap with 2 grams added; LSF-02 = Liquid soap with 4 grams added; LSF-03 = Liquid soap with 6 grams added.

The liquid soap produced was subsequently analyzed to determine its active components and evaluate its antimicrobial inhibitory effectiveness. The testing parameters included:

Total Flavonoid, Phytochemical, and Fourier-transform Infrared Spectroscopy (FTIR).

The analyses of total flavonoid content, phytochemical profile, and Fourier-transform infrared spectroscopy (FTIR) were carried out at the Laboratory of Chemical Applications and Services, Universitas Padjadjaran, Jatinangor.

Liquid Chromatography-Mass Spectrometry (LC-MS).

LC-MS analysis was performed using an LC-MS-2050 instrument with an in-house method at Shimadzu Lab Solutions, PT. Ditek Jaya, West Jakarta.

The physicochemical quality assessment of the liquid soap, in accordance with the Indonesian National Standard (SNI) 06-4085-1996 (Badan Standardisasi Nasional, 1996), comprised the evaluation of free alkali content, pH value, and specific gravity, as follows:

pH.

One gram of liquid soap was dissolved in 10 mL of distilled water. If necessary, heating was applied to accelerate the dissolution process. The pH of the solution was then measured using a pH meter immersed in the solution, and the observed pH value was carefully recorded.

Free Alkali

Ten grams of liquid soap were dissolved in 50 mL of hot alcohol, and 2–3 drops of phenolphthalein indicator were added. The mixture was refluxed for approximately 30 minutes. After cooling, the solution was titrated with 0.1 N KOH until a pink color was formed. The titration volume was recorded, and the free alkali content was calculated using the standard formula.

$$\text{Free alkali} = \frac{Vx0.05611x\ N}{W}$$

Description:
V = Titration volume
0.05611 = KOH equivalent weight
W = Sample weight (gram)

Unsaponification Fraction
The Determination of unsaponifiable fat content was done using the same solution to determine free fatty acids. This solution was added with 5 ml of 0.5 N alcoholic KOH (dissolved in alcohol). The mixture was then heated over a water bath and refluxed using a vertical condenser for 1 hour. After that, it was cooled to 70 °C and titrated with 0.5 N alcoholic HCl (HCl dissolved in alcohol) until the phenolphthalein indicator's red color disappeared.

$$Unsaponification\ fraction = \frac{V1 - V2 \times 0.0561}{0.2580 \times W} \times 100\%$$

Description:
N = Normality of HCl
VI = blank titration volume (ml)
V2 = sample titration volume (ml)
W = sample weight (gram)
0,0561 = KOH equivalent weight
0,2580 = average saponification number of coconut oil

Specific Gravity
The mass of the empty pycnometer (a) is measured. Distilled water and liquid soap are then carefully added to the pycnometer using a dropper. The pycnometer is sealed and placed in a temperature-controlled environment until it reaches 25°C. Subsequently, the mass of the pycnometer containing water (b) and the mass of the pycnometer containing liquid soap (c) are meticulously recorded.

$$Specific\ gravity\ (g/ml) = \frac{c - a}{b - a}$$

Description:
a = empty pycnometer weight
b = pycnometer + water weight
c = pycnometer + liquid soap weight

Foam Stability
A sample weighing 1 gram was dissolved in 9 mL of water and transferred to a reaction tube. Then, the mixture was shaken using a vortex for 30 seconds. This process resulted in foam formation, and the foam's height was measured. The foam sample was left undisturbed for one hour, after which its height was measured again. To ascertain the stability of the foam, the following formula may be utilized:

$$\text{Foam Stability (\%)} = \frac{\text{Final foam height (mm)}}{\text{Initial foam height (mm)}} \text{x } 100\ \%$$

Bacteria Inhibition Zone Test
Microbe Rejuvenation Test
NA Media was weighed 5 g and dissolved in aquadest to 250 ml. Sterilization was performed until the temperature reached 121ºC. Keep it still for 15 minutes, then the media was put into reaction tubes to form titled NA. After the solid titled NA was obtained from the *Eschericia coli* ATCC 25922 strain, as much as one inoculating loop was used, and they were inoculated in the titled NA media. The tested bacteria were incubated for 24 hours at 37ºC and they could be used as the tested bacteria.

Making a test bacterial suspension
Test bacteria resulting from the rejuvenation process went through the suspension process using a NaCl 0.9% solution, which was put into the respective reaction tubes. After that, it was mixed with a sterilized NA medium and immunized.

Escherichia coli inhibition zone test
NA media was turned into a solid form in a petri dish. After being solid, prepare a bacterial suspension test. Dip a stick with cotton at the end of the bacteria suspension test, then swab vertically and horizontally on the NA surface, which is solidified until all surfaces are covered. Leave it until all surfaces are dry. Meanwhile, each transparent soap sample was melted on a hot plate. Soak the disc paper for 1 hour in the soap sample, which was melted, and then dry the disc paper. After all the NA surfaces dried, the disc paper, which was soaked in the transparent soap, was put on the NA surface using a pin set. It was then incubated at 37ºC for 24-48 hours. The petri dish, which was incubated for 24-48 hours, was then observed and measured to obtain its inhibition diameter.

Fungi Inhibition Zone Test
Preparation of Microbial Suspension
Saline Solution Preparation: A 0.9% sodium chloride (NaCl) solution was prepared by dissolving 0.9 grams of NaCl in 100 milliliters of distilled water. While Microbial Suspension was transferred, 9 milliliters of the prepared 0.9% NaCl solution was transferred into a test tube. Add one packet of Malassezia mushrooms to the NaCl solution test tube. Then, homogenize the mixture thoroughly until a 10-1 dilution is achieved. Perform serial dilutions (10-2, 10-3, etc.) until a solution with an absorbance of 0.08-0.13 at a wavelength of 625 nm is obtained. Note: The dilution solution with an absorbance within the specified range (0.08-0.13) is used for the inhibition zone assay.

Preparation of Sabouraud Dextrose Agar (SDA) media
Dissolve 65 grams of SDA in 1 liter of distilled water. Heat the mixture on a hot plate while stirring until it boils. Subsequently, plug the opening of the Erlenmeyer flask with cotton. Subject the mixture to sterilization at a temperature of 121 °C for 15 minutes.

Malassezia furfur inhibition zone test
Homogenize 1 mL of mushroom suspension into 10 mL of SDA. Pour the mixture into a Petri dish and allow it to solidify. Divide the Petri dish into four equal sections using a permanent marker on the bottom of the dish. Create a well with a 6 mm diameter using a blue tip. Place the test samples, negative controls, and positive controls into the wells, ensuring that the samples do not extend beyond the well boundaries. Use 0.9% NaCl as the negative control and ketoconazole as the positive control. Cover the Petri dish and sterilize it using a Bunsen burner. Incubate the Petri dish at 28°C for 5-7 days. Measure the diameter of the inhibition zone for each sample.

Experimental Design and Statistical Analysis
The study employed a Completely Randomized Design (CRD) with three treatments corresponding to adding 2 g, 4 g, and 6 g of *ketepeng* leaf powder, each replicated three times. Data were analyzed using analysis of variance (ANOVA) at a 5% significance level. When significant differences were observed, a Least Significant Difference (LSD) post hoc test was conducted ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Total Flavonoid Content

The total flavonoid content in the liquid soap formulations enriched with *Cassia alata* leaf powder was relatively low, ranging from 0.03% to 0.12% (Table 2). In comparison, Angelina et al. (2021) reported that the *C. alata* leaf simplicia contained a total flavonoid content of 15.74%.

Table 2. Total flavonoid content of Cassia alata leaf powder and liquid soap formulations.

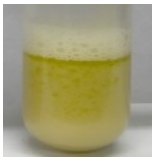
No	Treatment	Total Flavonoid (%)
1	*C. alata* Leaf Powder	2.16
2	Liquid Soap Formulation-1 (LSF-01)	0.03
3	Liquid Soap Formulation-2 (LSF-02)	0.07
4	Liquid Soap Formulation-3 (LSF-03)	0.12

The total flavonoid levels in the liquid soap formulations were markedly lower than those in the *C. alata* leaf simplicia. This reduction is presumably influenced by the processing stages, particularly drying and soap formulation. The leaves were dried at 40 °C for 19 hours before being processed into powder, which may have resulted in the degradation of bioactive compounds or partial loss of flavonoids. Magdalena and Kusnadi (2015) demonstrated that elevated temperatures could lead to significant thermal degradation and decreased flavonoid content in plant extracts. Therefore, although the liquid soap formulations still contained flavonoids, their concentrations were substantially lower than those of fresh simplicia. This highlights the need to optimize production processes to preserve flavonoid content as much as possible to support the liquid soap's biological activities.

Phytochemical Analysis

Phytochemical analysis was conducted as a preliminary test to identify the presence of secondary metabolites in Cassia alata leaf powder and its liquid soap formulations. The results (Table 3) revealed that the leaf powder contained flavonoids and steroids. Meanwhile, all three liquid soap formulations supplemented with Cassia alata leaf powder consistently demonstrated the presence of flavonoids and saponins.

Table 3. Phytochemical compounds in *C. alata* leaf powder and *C. alata* -based liquid soap.

No	Phytochemicals	Reagent	Treatments				Changes with Reagent
			Leaf Powder	LSF-01	LSF-02	LSF-03	
1	Phenolics	5% $FeCl_3$	-	-	-	-	No precipitate formed
2	Tannins	1% $FeCl_3$	-	-	-	-	No precipitate formed
3	Alkaloids	Wagner's reagent	-	-	-	-	No precipitate formed
	Triterpenoids	Concentrated H_2SO_4	-	-	-	-	No precipitate formed
4	Steroids	Concentrated H_2SO_4	+	-	-	-	
5	Flavonoids	a.Concentrated HCl + Mg	-	-	-	-	
		b. 2 N H_2SO_4	+	+	+	+	
		c.10% NaOH	+	+	+	+	
6	Saponins	2 N HCl	-	+	+	+	

Flavonoids are widely distributed secondary metabolites in plants, known for their antioxidant and antimicrobial activities. As polyphenolic compounds, flavonoids are free-radical scavengers (Lukman et al., 2019). The flavonoid phenolic groups allow interactions with microbial proteins, leading to protein denaturation. This process alters bacterial cell wall structure, reducing cellular functionality (Mufti et al., 2017). At low concentrations, phenolic compounds may induce cytoplasmic membrane leakage and the loss of essential metabolites in bacterial enzymatic systems. In contrast, at higher concentrations, they can cause irreversible damage to the cytoplasmic membrane and cellular proteins (Nugraha et al., 2019). These findings highlight the contribution of flavonoids to the antimicrobial activity of the liquid soap formulations.

In addition to flavonoids, the liquid soap also exhibited the presence of saponins (Table 3). Saponins are high-molecular-weight glycosides of a sugar moiety (hexose) and a non-sugar aglycone, typically a triterpene or steroid. Saponins in the liquid soap may result from forming glycosidic compounds between the steroidal components of the Cassia alata leaf powder and sugar groups in the soap formulation. Saponins possess a range of biological activities, including antimicrobial properties, which—together with flavonoids—are likely to provide synergistic effects on the bioactivity of the formulated liquid soap.

Fourier-Transform Infrared Spectroscopy (FTIR)

The liquid soap formulation was developed using virgin coconut oil (VCO) as the triglyceride source and potassium hydroxide (KOH) as the alkali, producing surfactants through saponification. *Ketepeng* leaf powder was incorporated as an antibacterial agent at concentrations of 2, 4, and 6 g, leveraging the phenolic, flavonoid, and alkaloid compounds contained within. FTIR analysis was performed to confirm saponification and the presence of bioactive compounds. As shown in Figure 1, the FTIR spectra of *ketepeng* leaf powder (Figure 1a) and liquid soap with different concentrations of *ketepeng* leaf powder (Figure 1b) displayed broad absorption bands within 3200–3600 cm^{-1}, indicating strong O–H absorption. The absorption peaks for liquid soap were observed at 3365.04 cm^{-1} (2 g), 3343.85 cm^{-1} (4 g), and 3341.85 cm^{-1} (6 g). High-intensity absorption bands were also detected at 2923.98 cm^{-1}, 1652.04 cm^{-1}, 1550.83 cm^{-1}, 1448.38 cm^{-1}, and 1077.23 cm^{-1}. In

addition, carbonyl (C=O) absorption peaks were identified at 1740.22 cm^{-1}, 1646.83 cm^{-1}, and 1644.94 cm^{-1}, while weak absorption bands appeared at 2135.23 cm^{-1}, 2128.43 cm^{-1}, and 2124.04 cm^{-1} for soaps with 2, 4, and 6 g of ketepeng leaf powder, respectively.

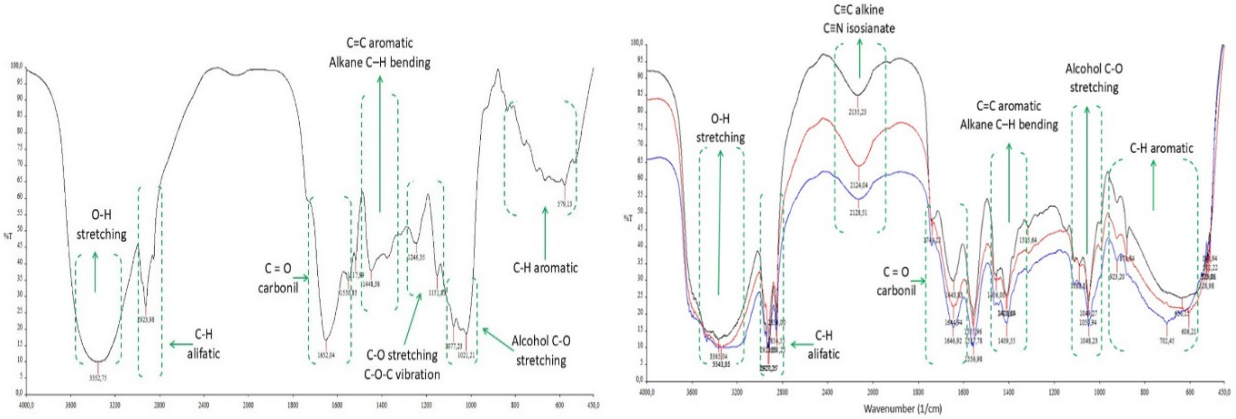


Figure 1. FTIR spectra of ketepeng leaf powder (a) and liquid soap of ketepeng leaf (b).

The FTIR spectra confirmed successful saponification and the retention of bioactive components from ketepeng leaf powder within the liquid soap matrix. The broad O–H absorption bands (3200–3600 cm^{-1}) correspond to hydroxyl groups from compounds such as water, glycerol, and flavonoids. The slight shifts in O–H stretching absorption peaks (3365.04–3341.85 cm^{-1}) indicate the incorporation of flavonoid compounds into the soap formulation. Salimi et al. (2022) similarly reported that *ketepeng* leaf extract exhibited O–H absorption at 3410.47 cm^{-1}, aromatic C–H absorption at 661.16 cm^{-1}, and C=O stretching at 1695.36 cm^{-1}, confirming the presence of flavonoids and related compounds.

The absorption bands at 2923.98, 1652.04, 1550.83, 1448.38, and 1077.23 cm^{-1} further support the successful encapsulation of active compounds, as no significant shifts were observed from the flavonoid spectrum. Meanwhile, the C=O absorption peaks at 1740.22–1644.94 cm^{-1} reflect the transformation of triglycerides into carboxylate salts, a hallmark of saponification. This is consistent with Basu et al., who reported similar carbonyl stretching peaks in soap formulations. Furthermore, the weak absorption bands at 2135.23–2124.04 cm^{-1} suggest the presence of alkyne (C≡C) groups, which may be associated with saponin compounds generated during the saponification process.

Liquid Chromatography–Mass Spectrometry (LC–MS)

LC–MS analysis was performed to identify bioactive compounds in the powdered leaves of *Cassia alata* (*ketepeng cina*) and its liquid soap formulation. The mobile phase employed was acetonitrile (ACN), which possesses high purity, low UV absorbance, and nonpolar characteristics. ACN was selected due to its ability to enhance detection sensitivity through ion adduct formation, thereby improving the signal-to-noise ratio (Violi et al., 2021). The LC–MS chromatogram of *Cassia alata* leaf powder (Figure 2) revealed a diversity of secondary metabolites, including flavonoids (kaempferol, quercetin, myricetin), anthraquinones (emodin, aloe-emodin, chrysophanol), tannins, and saponins (Udin et al., 2023). These compounds were detected at retention times ranging from 9.150 to 15.539 minutes, with m/z values of 303, 359, 362, 390, and 475, indicating the presence of polar polyphenolic compounds with complex aromatic structures. A detailed identification of these compounds is presented in Table 4.

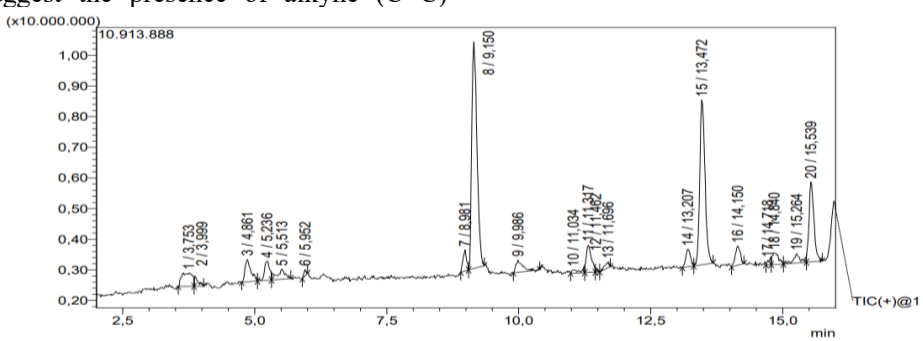


Figure 2. Typical LC–MS chromatogram of *ketepeng* leaf powder.

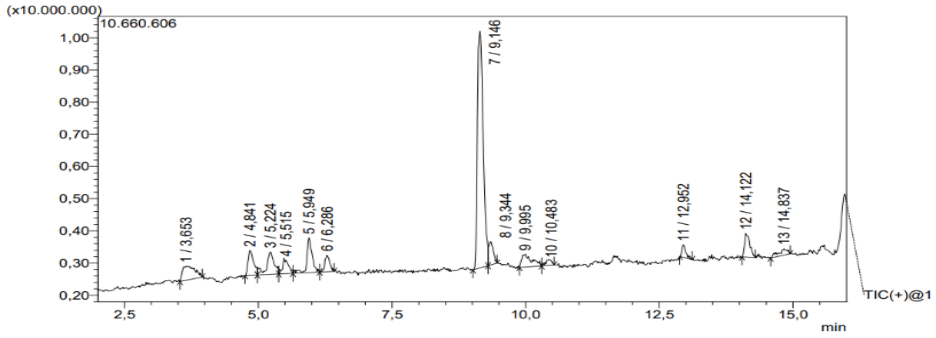


Figure 3. Typical LC–MS chromatogram of liquid soap of *ketepeng* cina leaf.

Table 4. Compounds identified in *C. alata* leaf powder based on LC–MS analysis.

Compound	Retention Time	m/z	Reference
Quercetin (Parent compound):			
m-Hydroxyphenylaceticacid	9,986 - 11,696	151	Prasain et al., (2004); Kaliawan & Danardono, (2023)
Chrysophanol	15,539	253	Zhao et al., (2013)
Myricetin	9,15	274	Ruslin et al., (2022
Quercetin ($C_{15}H_{11}O_7$)	5,513	303	Saptarini et al., (2024); Ruslin et al., (2022)
Dimethyl galloyl hexoside	5,952	359	Li & Seeram, (2018)
Oleuropein aglycon	3,753	362	Ruslin et al., (2022)
Oleuropein aglycon	3,999	362	Ruslin et al., (2022)
Polydatin	14,15	390	Sharma et al., (2018)
Aloe-emodin-sinapoyl	4,861	475	Zhao et al., (2013)
Aloe-emodin-sinapoyl	5,236	475	Zhao et al., (2013)

In contrast, the LC–MS chromatogram of liquid soap with *ketepeng* leaf powder (Figure 3) showed earlier retention times (3.653–6.258 min), corresponding to compounds derived mainly from virgin coconut oil (VCO), including saturated fatty acids (lauric, capric, caprylic acids), monoglycerides, and diglycerides. The dominant m/z signal of lauric Acid (m/z 274) was consistently detected as a primary antibacterial agent. The overlapping peaks of lauric Acid (m/z 274) and quercetin (m/z 303) further confirmed the coexistence of fatty acids and flavonoids in the soap formulation. Compound identification for liquid soap is presented in Table 5.

Table 5. Identified compounds in the liquid soap of the *ketepeng cina* leaf.

Compound	Retention Time	m/z	Reference
Quercetin (Parent compound)	10,483	151	Prasain et al., (2004); Kaliawan & Danardono, (2023)
Myricetin	9,146	274	Ruslin et al., (2022)
Quercetin ($C_{15}H_{11}O_7$)	5,515	303	Saptarini et al., (2024)
Ricinoleic	9,995	343	Ayorinde et al., (2000)
Dimethyl galloyl hexoside	5,949	359	Li & Seeram, (2018)
Oleuropein aglycon	3,653	362	Ruslin et al., (2022)
Polydatin	14,122	390	Sharma et al., (2018)
Chrysophanol-8-O-β-D-glucopyranoside	6,286	416	Sharma et al., (2018)
Aloe-emodin-sinapoyl	4,841	475	Zhao et al., (2013)
Aloe-emodin-sinapoyl	5,224	475	Zhao et al., (2013)

The LC–MS analysis confirmed the richness of *C. alata* leaf powder in flavonoids and anthraquinones, such as quercetin, myricetin, chrysophanol, and aloe-emodin. These polyphenolic compounds are well-documented for their antioxidant, antifungal, and radical scavenging properties (Losso, 2022; Ruslin et al., 2022; Saptarini et al., 2024). The detection of oleuropein aglycon and dimethyl galloyl hexoside further supports the multifunctional potential of the extract, as these compounds are associated with antioxidant and anti-inflammatory effects (Li & Seeram, 2018). In liquid soap formulations, the coexistence of quercetin (m/z 303) and lauric Acid (m/z 274) highlights the synergistic contribution of plant-derived phenolics and VCO-derived

fatty acids. Lauric Acid primarily disrupts microbial membranes, while flavonoids and anthraquinones provide antioxidant and antifungal defense mechanisms (Ismiyati et al., 2023). The overlap of aromatic and aliphatic peaks in the chromatograms indicates the successful integration of bioactive compounds into the soap matrix, thereby enhancing its antimicrobial functionality

pH

Soap is the product of a chemical reaction between a weak acid and a base, thus exhibiting alkaline properties when dissolved in water. Typically, soap has a pH range between 9.0 and 10.8. A pH value exceeding 10.8 is generally attributed to incomplete hydrolysis during saponification. This study's findings indicate that adding *Cassia alata* leaf powder to liquid soap did not significantly affect pH. Liquid soap formulations with varying concentrations of leaf powder (2 g, 4 g, and 6 g) consistently exhibited a pH value of 9.0 (Table 6). This value falls within the standard pH range for liquid soap as specified in SNI 06-4085-1996.

Table 6. Quality Parameters of Liquid Soap with Variations in *C. alata* Leaf Powder Addition.

Treatment	pH	*Free Alkali (%)*	*Unsaponification fraction (%)*	*Specific Gravity (g/ml)*	*Viscosity (cp)*	*Foam Stability (%)*
Liquid Soap Formulation-1 (LSF-01)	9	0.0017^{a}±0.00023	2.3864±0.74	1.0374±0.003	147±43.20	27.94±5.09
Liquid Soap Formulation-2 (LSF-02)	9	0.0026^{b}±0.00028	1.5917±1.04	1.0431±0.004	213±40.82	28.49±6.32
Liquid Soap Formulation-3 (LSF-03)	9	0.0033^{c}±0.00017	1.1586±0.64	1.0479±0.006	263±61.28	28.98±4.22

The consistent pH value of 9.0 across all treatments suggests that incorporating Cassia alata leaf powder does not alter the alkaline properties of liquid soap. This pH range is considered safe and compliant with the quality standards of liquid soap established by SNI 06-4085-1996, ensuring its suitability for topical application. Hardian et al. (2014) state that soaps with pH values exceeding the permissible standard may cause skin irritation, including itching, lesions, exfoliation, and dryness. Therefore, the liquid soap formulations produced in this study do not pose risks of irritation associated with pH deviations. These results further imply that the active compounds in *C. alata* leaf powder can be incorporated into liquid soap formulations without compromising pH stability.

Free Alkali

Incomplete hydrolysis during saponification can result in free alkali, which refers to unreacted base remaining in the soap due to excess oil or alkali addition (Widyasanti et al., 2017). According to the Indonesian National Standard (SNI 06-4085-1996), the maximum allowable level of free alkali in liquid soap is 0.1%. The results revealed that the free alkali content in liquid soap formulations with *ketepeng cina* leaf powder remained well below this limit, indicating safety and no risk of irritation or skin dryness (Table 6). Statistical analysis using LSD (0.05) showed that the three treatments were significantly different, with increasing concentrations of *ketepeng* leaf powder corresponding to higher free alkali values.

The increased free alkali with higher concentrations of *ketepeng* leaf powder is closely associated with the higher flavonoid content in the formulations. The total flavonoid test positively correlated with the quantity of leaf powder added. Flavonoids are polyphenolic compounds with strong electron-donating properties, allowing them to transfer electrons to free radicals (Yuhernita & Juniarti, 2011; Vo et al., 2019). According to Lewis's acid–base theory, a base is a substance possessing a lone pair of electrons that can be donated to another substance. This explains why flavonoid compounds increase alkalinity or free alkali content when incorporated into liquid soap. Therefore, higher concentrations of ketepeng cina leaf powder lead to an elevated free alkali content in liquid soap, although the values remain within safe limits as established by SNI standards.

Unsaponifiable Fraction

The unsaponifiable fraction refers to free fatty acids that do not react with alkali to form soap (Anggraini, Ismanto, & Dahlia, 2015). Statistical analysis using LSD (0.05) indicated that the three treatments were not significantly different. As shown in Table 5, the addition of ketepeng cina leaf powder reduced the unsaponifiable fraction in liquid soap formulations. The reduction in unsaponifiable fraction suggests that the saponification process was more complete in the presence of *ketepeng* leaf powder. According to Cotte et al. (2006), the saponification process is enhanced under alkaline conditions. *Ketepeng cina* leaf powder contains flavonoids and saponins, both polyphenolic compounds with electron-donating properties. Their presence creates a more alkaline environment in the liquid soap, which facilitates the hydrolysis of triglycerides into soap. Therefore, increasing the concentration of *ketepeng* leaf powder promotes a more efficient saponification process, as evidenced by the lower unsaponifiable fraction. This indicates that fewer unreacted fatty acid residues remain in the soap, improving product quality.

Specific Gravity

The specific gravity of liquid soap with the addition of *ketepeng cina* leaf powder complied with the Indonesian National Standard (SNI 06-4085-1996), which specifies a range of 1.01–1.10 g/mL. As shown in Table 5, the specific gravity increased with higher concentrations of ketepeng leaf powder, although the differences among treatments were not statistically significant. The raw materials incorporated in the formulation determine the specific gravity of soap. According to Hutauruk et al. (2020) and Widyasanti et al. (2017), a higher amount of added raw material increases specific gravity. In this study, the observed increase was attributed to the presence of *ketepeng* leaf powder, which can absorb and retain water, thereby reducing the liquid phase of the soap. Moreover, a positive correlation was observed between specific gravity and viscosity. Incorporating water-soluble secondary metabolites, such as flavonoids, can increase the viscosity of liquid soap formulations (Kabera et al., 2014). Thus, the increase in specific gravity in liquid soap with *ketepeng* leaf powder aligns with the concurrent rise in viscosity values.

Antimicrobial Activity of Liquid Soap

An inhibition zone assay was conducted to evaluate the antimicrobial activity of liquid soap formulated with the addition of *C alata* leaf powder. The results demonstrated that the liquid soap inhibited the growth of Escherichia coli ATCC 25922, although the inhibition zones were relatively small, ranging from 3 to 4 mm (Table 7). A positive correlation was observed between the increasing concentration of C. alata leaf powder and the inhibition zone diameter, albeit with weak strength. Conversely, against the fungus *Malassezia furfur*, the liquid soap exhibited no antifungal activity. This was evidenced by the absence of a clear inhibition zone around the wells and contamination in the surrounding area.

Table 7. Inhibitory Activity of Cassia alata Leaf Liquid Soap Against Microorganisms.

Treatment	Inhibition Zone against *Escherichia coli* (mm)	Inhibition Zone against *Malassezia furfur* (mm)
Liquid Soap Formulation-1 (LSF-01)	3.1 ± 0.14	–
Liquid Soap Formulation-2 (LSF-02)	3.4 ± 0.17	–
Liquid Soap Formulation-3 (LSF-03)	4.0 ± 0.82	–

The ability of the liquid soap to inhibit E. coli growth is attributed to the presence of flavonoids and saponins identified in the phytochemical analysis. Flavonoids, such as quercetin, bind to the GyrB subunit of DNA gyrase in E. coli, thereby inhibiting ATPase function and reducing cellular energy production (Cushnie & Lamb, 2005). This finding is consistent with Ernilasari et al. (2021), who reported that flavonoids and terpenoids possess strong antimicrobial potential. However, the relatively low flavonoid content in the liquid soap (0.03–0.12%) contributed to its limited antibacterial activity. Moreover, the effectiveness of flavonoids is generally higher against Gram-positive bacteria than Gram-negative strains (Ernilasari et al., 2021; Magdalena & Kusnadi, 2015). This is due to structural differences in the cell wall, as Gram-negative bacteria possess a thick lipopolysaccharide layer that impedes the penetration of polar compounds such as flavonoids.

In contrast, the test results against M. furfur indicated no antifungal activity. According to Sanjaya et al. (2021), the genus Malassezia possesses a complex cell wall with helical multilayered lipid structures and NOD-like receptors (NLRs), which recognize and repel foreign substances. This structure protects against antifungal agents, including bioactive compounds from *C. alata* leaf powder. Similarly, Lompo et al. (2023) reported that antiseptic and hand hygiene products are often ineffective against fungal or specific bacterial contaminants due to the limited penetration of active compounds.

Overall, liquid soap formulated with C. alata leaf powder demonstrates potential as a natural antiseptic, yet its activity remains limited. While it inhibited E. coli to a small extent, no activity was observed against M. furfur. Optimization of the formulation is therefore necessary, either by increasing the concentration of active ingredients or combining them with other antimicrobial agents, to enhance their biological efficacy.

CONCLUSION

This study evaluated the potential of *Cassia alata* Linn. leaf powder as an active ingredient in antiseptic liquid soap, with particular emphasis on antimicrobial efficacy and the physicochemical quality of the product. Regarding antimicrobial activity, the formulated liquid soap exhibited limited antibacterial activity against *Escherichia coli*, with inhibition zones of 3–4 mm, and showed no antifungal activity against *Malassezia furfur*. These findings suggest that although bioactive compounds such as flavonoids and saponins were incorporated into the formulation, the relatively low total flavonoid content (0.03–0.12%) restricted its antimicrobial performance. LC–MS analysis confirmed the presence of lauric Acid (m/z 274) from virgin coconut oil and quercetin (m/z 303) from *C. alata* leaf

powder, both known for their antimicrobial properties; however, their synergistic effects were not fully realized in the present formulation.

Regarding physicochemical properties, adding *C. alata* leaf powder did not significantly affect key quality parameters, including pH, viscosity, specific gravity, foam stability, free alkali, and unsaponifiable matter. All parameters complied with the Indonesian National Standard (SNI 06-4085-1996), ensuring the liquid soap's safety and suitability for consumer use. FTIR analysis further confirmed the presence of flavonoids, while phytochemical screening validated the incorporation of flavonoids and saponins into the formulation. Accordingly, liquid soap formulated with Cassia alata leaf powder can be considered a safe, standard-compliant product with moderate antimicrobial potential. Although the current formulation demonstrated weak activity against E. coli and no effect against M. furfur, it represents a promising prototype of herbal antiseptic soap. Further optimization—enhancing active metabolite concentrations, improving processing methods, or combining with other antimicrobial agents—is necessary to strengthen this natural antiseptic liquid soap's antimicrobial effectiveness and functional value.

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