Indonesian Journal of Medical and Pharmaceutical Science

RESEARCH ARTICLE Open Access

A Natural Alternative: The Development and Testing of Herbal-Based Hand Sanitizers with Antibacterial Properties

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ABSTRACT

Background: Hand hygiene plays a crucial role in preventing the spread of infections, making effective hand sanitizers essential. However, concerns about skin irritation and microbial resistance associated with synthetic ingredients have increased the demand for natural, plant-based alternatives. This study aimed to formulate and evaluate plant-based hand sanitizers containing ethanol, lemongrass essential oil, and aloe vera gel for their organoleptic properties, pH, and antibacterial activity. **Methods:** The prepared formulations exhibited acceptable organoleptic characteristics, with variations in odor and texture based on essential oil and aloe vera concentrations. **Results:** The pH values of all formulations were within the skin-friendly range, ensuring safe application. Antibacterial testing against *Escherichia coli* demonstrated that formulations with higher ethanol and lemongrass oil concentrations exhibited the largest inhibition zone (13,67±0,58 mm), while aloe vera contributed to skin-conditioning properties. Although the formulations were effective, none surpassed the inhibition zone of the positive control. **Conclusion:** This study suggests that the prepared natural hand sanitizer gel with Aloe vera and lemongrass essential oil can be a potential alternative to enhance the antibacterial efficacy of hand sanitizers while maintaining user comfort.

Keywords: Aloe vera; *Antibacterial activity; Ethanol; Hand Sanitizer; Lemongrass essential oil*

INTRODUCTION

Hand hygiene is a fundamental public health measure in preventing the transmission of infectious diseases, particularly those caused by bacterial pathogens [1]. According to the World Health Organization [WHO], proper hand hygiene can reduce the incidence of respiratory and diarrheal infections by up to 50%, making it an essential practice in both healthcare and community settings [2]. The widespread use of hand sanitizers has become increasingly important, especially during global health crises such as the COVID-19 pandemic. While alcohol-based hand sanitizers are widely recognized for their effectiveness, prolonged use may lead to adverse effects, including skin irritation, allergic reactions, and environmental concerns related to alcohol production and disposal. These challenges have led to growing interest in natural, plant-based alternatives that offer both antimicrobial efficacy and skin-friendly properties [2,3].

Aloe vera and Cymbopogon citratus [lemongrass] are well-known medicinal plants with broad therapeutic applications, including antibacterial, anti-inflammatory, and skin-soothing effects [4]. Aloe vera contains bioactive compounds such as anthraquinones, flavonoids, and polysaccharides, which contribute to its antimicrobial activity while also providing hydration and protection to the skin [3,4]. Similarly, Cymbopogon citratus is rich in essential oils, particularly citral, which exhibits strong antibacterial properties against common pathogens such as Staphylococcu aureus and

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Received: 16 June 2025
Accepted: 12 August 2025
Published online: 28 August 2025
https://doi.org/10.30659/ijmps.v4i2.378

Escherichia coli [5,6]. Given their complementary properties, the combination of Aloe vera and Cymbopogon citratus in a hand sanitizer formulation has the potential to offer a natural, effective, and environmentally sustainable alternative to conventional alcohol-based hand sanitizers.

Additionally, gel-based hand sanitizers offer several advantages over other forms, such as liquid [spray] or foam [4,5]. One of the key benefits of gel formulations is their ability to form a protective layer on the skin, providing longer-lasting protection compared to other hand sanitizer types [6]. The retention time of gel-based sanitizers is greater than that of liquid and foam variants, while also offering a more favourable moisturizing effect and better adherence to the skin. Therefore, in this study, the gel form was chosen as the most suitable formulation for the preparation of natural-based hand sanitizer [7]. his study investigates the effectiveness of the formulated hand sanitizer compared to conventional alcohol-based sanitizers. It also examines the optimal concentration of *Aloe vera* gel and *Cymbopogon citratus* essential oil required to achieve maximum antibacterial efficacy. Additionally, the research evaluates the impact of the formulated hand sanitizer on skin hydration and irritation in comparison to conventional hand sanitizers. By addressing these aspects, this study contributes to the growing field of plant-based antimicrobial formulations, offering potential benefits for public health, personal care, and environmental sustainability.

METHODS

Preparation of Aloe Vera Gel and Cymbopogon Citratus Essentials Oil

Fresh Aloe vera leaves were thoroughly washed, peeled, and the inner gel was collected. The gel was homogenized and filtered to obtain a clear extract and stored at 4°C until use. In the other hand, Lemongrass [*Cymbopogon citratus]* essentials oil was purchased from local market in Semarang, Central Java, Indonesia.

Hand Sanitizer Formulation

The hand sanitizer was prepared by mixing *Aloe vera* gel, *Cymbopogon citratus* [lemongrass] essential oil, ethanol [70%], and distilled water in varying concentrations, as shown in Table 1. Initially, the Aloe vera gel was combined with the essential oil and mixed thoroughly to ensure even distribution. Ethanol was then gradually added while stirring continuously to enhance homogeneity. Distilled water was incorporated to achieve the desired consistency, followed by thorough mixing for uniformity. The final formulation was transferred into sterile containers, sealed, and stored in a cool, dry place. This preparation method ensures the effective blending of antibacterial agents while maintaining the moisturizing properties of the formulation.

Table 1. Hand sanitizer formulation

Ingredients	Function	F1	F2	F3
Aloe vera gel	Antibacterial agent, Moisturizer	35 mL	30 mL	25 mL
Lemon grass essential oils	Antibacterial agent, essence	100 µL	200 µL	300 µL
Ethanol 70%	Antibacterial agent, solvent	15 mL	20 mL	25 mL
Distilled water	Solvent	Ad to 100 mL	Ad to 100 mL	Ad to 100 mL
Total		100 mL	100 mL	100 mL

Antibacterial Activity Testing

The antibacterial efficacy of the formulated hand sanitizer was evaluated using the agar well diffusion method against Escherichia coli. A 100 µL suspension of Escherichia coli [adjusted to 0.5 McFarland standard] was spread evenly on Mueller-Hinton agar plates [2]. Wells of 6 mm diameter were created and filled with 100 µL of the hand sanitizer formulations. Ethanol-based hand sanitizer commercially available brand "Dettol" served as the positive control, while distilled water was used as the negative control. The plates were incubated at 37°C for 24 hours, and the zone of inhibition [mm] was measured using a calliper. All experiments were performed in triplicate.

Organoleptic and pH Testing

The organoleptic testing of the hand sanitizer formulation was conducted to evaluate its sensory characteristics, including appearance, color, odor, texture, evaporation time, and after-feel, as shown in Table 2 [1]. A panel of 22 trained or semi-trained assessors participated in the study, with exclusion criteria set for individuals with allergies or sensitivities to alcohol or fragrance. The testing was conducted in a controlled environment [22–25°C, 40–60% humidity] to ensure consistency. Each panelist was provided with coded samples for blind testing. Before evaluation, they washed their hands and allowed them to dry for 10 minutes. A standardized volume of 1 mL of hand sanitizer was applied to the palm and rubbed for 10 seconds. Panelists assessed appearance and color under neutral lighting, odor before and after application, and texture based on viscosity and spread ability. The time taken for complete evaporation was recorded, and after-feel characteristics such as stickiness, dryness, or residue were evaluated 30 seconds post-application. Each parameter was rated on a 5-point hedonic scale [1 = very poor, 5 = excellent]. The collected data were analyzed using descriptive statistics [mean ± SD], and differences between formulations were assessed using ANOVA with a significance level of $p < 0.05$.

The pH test was conducted using a universal pH stick, with the pH color compared to the standard color range. pH measurement was also performed using a Laqua pH meter, which was first calibrated with standard buffer solutions of pH 4.00 and 7.00 before measuring the gel's pH. The measurement was carried out by immersing the pH electrode into the gel. For topical formulations, the pH is expected to match the skin's pH [1,2].

Table 2. Organoleptic Scoring

Parameter	Score 1 [Very Poor]	Score 2 [Poor]	Score 3 [Moderate]	Score 4 [Good]	Score 5 [Excellent]
Appearance & Color	Cloudy, visible separation, inconsistent color	Slightly cloudy, minor separation	Uniform but dull appearance	Clear, uniform, slight gloss	Completely clear, homogenous, bright
Odor	Strong unpleasant/off-odor, overpowering	Unbalanced, slight off-odor, strong alcohol scent	Balanced but weak fragrance, alcohol still	Pleasant, well-blended fragrance with mild alcohol	Very pleasant, no off-odor, fragrance lasts well

	alcohol		dominant	presence	
Texture	Very thick/thin, difficult to spread	Slightly too thick/thin, moderate spreading	Acceptable viscosity, requires effort to spread	Good consistency, spreads easily	Ideal viscosity, smooth, effortless spreading

RESULTS

Antibacterial activity

The antibacterial activity of the hand sanitizer formulations against *Escherichia coli* demonstrated a positive correlation between the concentration of antibacterial agents and the inhibition zone diameter. Among the three formulations (as shown in Table 3 and Figure 1), F3 exhibited the highest antibacterial efficacy with an inhibition zone of 13.67 ± 0.58 mm, followed by F2 [11.33 ± 2.08 mm] and F1 [9.50 ± 1.32 mm]. Statistical analysis revealed that the inhibition zone of F3 was significantly larger than F1 ($p < 0.01$) and F2 ($p < 0.05$). However, none of the formulations surpassed the positive control [17.33 ± 1.53 mm], with the difference between F3 and the positive control remaining statistically significant ($p < 0.01$). These findings suggest that, while effective, the formulations may require further optimization to achieve antibacterial potency comparable to ethanol-based commercial products

Table 3. Diameter Of Inhibition Zone Of Formulated Hand Sanitizer Against *E.coli*

Sample	Inhibition Zone (mm)
F1	9,50±1,32
F2	11,33±2,08
F3	13,67±0,58
Positive Control	17,33±1,53

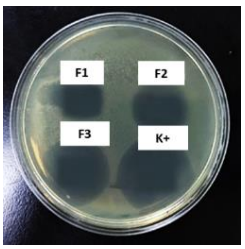

Figure 1. Antibacterial activity of Formulated Hand sanitizer against *E.coli.*

Organoleptic and pH evaluation

The organoleptic and pH evaluation of the hand sanitizer formulations [F1, F2, and F3] showed variations in appearance, odor, texture, and pH (Table 4). The appearance and color of all three formulations were rated highly, with F2 and F3 receiving the highest scores [4.00 ± 0.82 and 4.00 ± 0.93, respectively], while F1 had a slightly lower score [3.91 ± 0.81].

Table 4. Organoleptic and pH

Parameters	F1	F2	F3	Positive Control
Appearance & Color	3,91±0,81	4,00±0,82	4,00±0,93	4,12±0,62
Odor	3,36±0,95	3,45±0,80	4,00±1,02	3,76±0,91
Texture	2,82±1,05	3,23±1,07	3,86±1,08	3,97±0,56
pH	6,20±0,2	6,20±0,2	6,10±0,3	6,00±0,1

Statistical analysis showed no significant difference among the three formulations ($p > 0.05$). The odor assessment indicated that F3 had the highest acceptability [4.00 ± 1.02], followed by F2 [3.45 ± 0.80] and F1 [3.36 ± 0.95]. Statistical analysis also showed no significant difference among the three formulations ($p > 0.05$). In terms of texture, F3 had the highest rating [3.86 ± 1.08], while F1 had the lowest [2.82 ± 1.05]. Furthermore, the pH values of all formulations were within an acceptable range for skin application, with F1 and F2 having a pH of

6.2 ± 0.2 and 6.2 ± 0.1, respectively, while F3 had a slightly lower pH of 6.1 ± 0.1. The difference in pH among the formulations was not statistically significant ($p > 0.05$).

DISCUSSION

The antibacterial activity of the hand sanitizer formulations showed a direct correlation between the concentration of active ingredients and microbial inhibition [9,10]. The formulation with the highest concentration of ethanol and lemongrass essential oil exhibited the greatest antibacterial effect, while the formulation with the lowest concentration showed the least inhibition. Lemongrass essential oil, another key ingredient in the formulations, is known for its antibacterial properties, primarily due to its high citral content. Citral has been reported to disrupt bacterial cell walls and inhibit essential enzymatic functions, leading to bacterial cell death [10]. Research has shown that essential oils, including lemongrass, exhibit significant antimicrobial activity against *Escherichia coli* and other Gram-negative bacteria [2,9]. The increase in inhibition observed in the formulation with the highest lemongrass oil concentration aligns with these findings, further validating the role of essential oils in enhancing antibacterial efficacy. F3 showed the largest inhibition zone among the tested formulations, but its activity was still lower than the ethanol-based positive control. This may be due to several reasons. Ethanol diffuses quickly in agar and kills bacteria rapidly by protein denaturation, while the main active compound of lemongrass oil (citral) is hydrophobic and diffuses more slowly [11,12]. In addition, lemongrass oil is volatile and may evaporate during incubation, reducing its effective concentration. The gel matrix may also trap some of the oil, limiting its release [13]. Finally, the outer membrane of *E. coli* provides extra protection, making it less sensitive and requiring higher concentrations for inhibition [14].

Aloe vera gel was incorporated into the formulations primarily for its moisturizing and skin-soothing properties, but its contribution to antibacterial activity should not be overlooked. Aloe vera contains bioactive compounds such as anthraquinones and saponins, which have been reported to exhibit antimicrobial effects [15,16]. Studies have shown that aloe vera can inhibit the growth of various bacterial species, including *E. coli*, by disrupting bacterial membranes and interfering with cellular metabolism [17,18]. However, its antibacterial activity is generally weaker compared to ethanol and essential oils. In this study, the formulation with the highest aloe vera content showed the lowest antibacterial activity, which may suggest a dilution effect, where a lower concentration of ethanol and lemongrass oil reduced overall microbial inhibition.

The organoleptic evaluation revealed that formulations with higher essential oil content were more preferred in terms of odor and texture, likely due to the aromatic and emulsifying properties of lemongrass oil [19]. However, the variation in texture scores suggests that higher concentrations of ethanol may contribute to differences in formulation consistency, which could impact user preference. Despite these differences, all formulations maintained a pH range suitable for skin application, ensuring their compatibility and minimizing irritation risk.

While the study demonstrates a clear relationship between active ingredient concentration and antibacterial effectiveness, this study has some limitations. Future research should explore the long-term stability of these

formulations, their efficacy against a broader spectrum of microorganisms, and potential synergies between ethanol and plant-based antimicrobials. Comparisons with commercially available hand sanitizers could also provide further insights into their practical application in infection control.

CONCLUSIONS

This study shows that the natural hand sanitizer gel with *Aloe vera* and lemongrass essential oil has good antibacterial potential while keeping user comfort. Formulation F3, with the highest concentration, produced the largest inhibition zone, making it the most promising option. These results suggest that this formulation could be developed as a natural and safe alternative to conventional hand sanitizers.

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