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EFFICACY OF HAND SANITIZER GELS FORMULATED WITH MEDICINAL PLANT EXTRACTS AGAINST HAND-BORNE PATHOGENS

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ABSTRACT

Background and objective: Hand sanitizer is considered as a practical alternative to traditional handwashing when soap and water are not available. This study aimed to determine the optimal concentration of various medicinal plant extracts for microbial inhibition and to evaluate the effectiveness of hand sanitizer gels formulated with these extracts.

Methodology: A Total of five medicinal plants, including: *Citrus hystrix* DC, *Azadirachta indica*, *Curcuma longa* L., *Senna siamea* (Lam.) H.S. Irwin & Barneby, and *Terminalia catappa* L. were extracted using 80% ethanol. The selected medicinal plant was applied for hand sanitizer gels formulation and antimicrobial activity was conducted using agar well diffusion assay against microbial isolated from hand swabs. In addition, sensory evaluation of the hand sanitizer gels was tested.

Main Result: The results indicated that *Terminalia catappa* L. leaf extract was the most effective, significantly exhibited a zone of inhibition (1.4423 cm), followed by *Senna siamea* (Lam.) H.S. Irwin & Barneby with 1.1883 cm and *Azadirachta indica* (0.9350 cm). In contrast, *Citrus hystrix* DC showed the lowest efficacy (0.1230 cm). The Minimum Inhibitory Concentration (MIC) for A. indica, *S. siamea*, and *T.catappa* inhibited at 12.5 mg/ml, while *C. longa* and *C. hystrix* inhibited at higher concentration (25 mg/ml). The optimized hand sanitizer gel formulated with 16 mL of *T. catappa* extract (F3) demonstrated a high microbial reduction rate of 93.19%, which was comparable to commercial positive controls. In sensory evaluations involving 20 panels tester using a 9-point Hedonic scale, the overall acceptance score was 5.41, indicating a neutral to moderate preference. Among the sensory attributes, gel spreadability gained the highest preference (5.78), whereas color received the lowest (4.89) due to the natural amber hue of the herbal extracts.

Conclusion: *Terminalia catappa* L. leaf extract showed the most potent antimicrobial activity among the five Lao medicinal plant extracts, achieving a significant inhibition zone of 1.4423 cm. The optimized hand sanitizer formulation, mix with 16 mL of this extract (F3), demonstrated a remarkable microbial reduction rate of 93.19%. This Inhibiting performance is comparable to commercial alcohol-based controls, proving the effectiveness of herbal based hand sanitizer gel against hand-borne pathogens.

Keywords: Medicinal plants, *Terminalia catappa* L., Ethanolic extracts, Hand sanitizer gel, Antimicrobial activity.

1 INTRODUCTION

Hands serve as the primary vehicle for transmitting pathogens and infections. Maintaining hand hygiene is the most crucial approach for preventing the harmful germs and reducing healthcare-associated infections (Ganguly et al.,2022). To enhance the efficacy of infection control, the World Health Organization (WHO) supports for the use of alcohol-based hand sanitizer. This recommendation is based on their demonstrated ability to protect a broad spectrum of microbial activity (Jing et al., 2020).

Hand sanitizer is considered as an practical alternative to traditional handwashing when soap and water are not available. It is waterless hygiene method that maintains comparable efficacy in neutralizing pathogens (Rahmasari et al., 2020). Due to hand sanitizers plays a vital role for maintaining hygiene, many commercial formulations contain chemicals which shows some side effect on human's skin and environment. Thus, herbal hand sanitizers become more popular since they provide a natural, organic alternative to traditional chemical-based product (Verma et al., 2023).

Medicinal plants consisted with various active compounds that serve as potent substitutes for conventional treatments, herbal drug achieve a valuable function against common bacterial infection (Raj & Manjunath,2014). Various studies have been conducted and investigated the plant compounds and its extracts on antimicrobial activity (Chanda et al 2011). Therefore, medicinal plants are valuable essential resources in health management, serve as a cornerstone of modern therapy (Parekh & Chanda, 2009). Furthermore, they provide an exceptional renewable resource for the discovery of potential novel pharmaceuticals as their constituents have ample structural and biological Diversity (as reviewed in Romha et al., 2018). Utilizing natural resource is not only significant for their efficacy but also offers a cost-effective approach with fewer adverse health effects compared to synthetic chemicals (Mounika et al., 2017). In Laos, medicinal plants have been recognizing a long history of traditional use within the country's healthcare system (Sydara et al., 2014), this includes many functional plants with anti-microbial activity (Elkington et al., 2009). However, the development and application of medicinal plant-based hand sanitizer gel remain quite limited in the region. Thus, it is important to explore the potential of local Lao local medicinal plants in the formulation of nature hand sanitizer. This study aimed to develop and evaluate an effective herbal hand sanitizer gel using *Terminalia catappa* L. providing sustainable, and locally alternative for hand hygiene.

2 MATERIAL AND METHODS

2.1 Materials

- **Laboratory Equipment**: Erlenmeyer flasks, Duran bottles , graduated cylinders, 0.2 micro sterile filter, paper, test tubes, a rotary evaporator, an incubator, an autoclave, a Laminar Flow clean bench, a spectrophotometer, a hot plate with a magnetic stirrer, aluminum foil, round-bottom flasks, beakers, a hot air oven, pipettes, Petri dishes, inoculating loops, funnels, centrifuge tubes, a thermometer, a water bath, a pH meter, a light microscope, glass slides, a staining set, and alcohol burners.
- **Chemicals and Media**: Reagents included 80% Ethanol, 80% Methanol, Nutrient Broth (NB), Barium Chloride Dihydrate (99%), Sulfuric Acid (98%), Dimethyl sulfoxide (DMSO 99.5%), and Nystatin.
- **Microbial Use:** Bacteria was collected using hand swabs from individuals without using alcohol-based sanitizers.
- **Medicinal plant samples**: A Total Five medicinal plants were prepared: *Citrus hystrix* DC, *Azadirachta indica*, *Curcuma longa* L., *Senna siamea* (Lam.) H.S. Irwin & Barneby, and *Terminalia catappa* L.

2.2 Plant Extraction

Five medicinal plants were cleaned, dried at 60°C for 2 days using Oven dry and ground into powder by using grinder (Tilburg-Holland, Model: 8010E, Voltage: 220-240V). Extraction was conducted by mixing 3g of powder with 80 mL of 80% Ethanol, then stirred for 3 hours, centrifuged at 2,000 RPM for 15 minutes. The concentrated sample was prepared using a rotary evaporator at 50°C. The crude extracts were kept at 4 °C until further use.

2.3 Antimicrobial activity

Well Diffusion Assay was used for antibacterial activity of plant extracts and hand gel formulations. While, five medicinal plant extracts were assayed for anti-microbial activity, the best performance of plant extract on anti-microbial activity was further applied as hand gel formulations and test for antimicrobial activity capacity again.

2.4 Preparation of Culture Media (Nutrient Agar)

Nutrient agar (NA) medium was prepared by combining nutrient broth (NB) at a concentration of 3.9g/300 ml and agar powder at 4.5g/300 ml. The components were dissolved in distilled water and mixed thoroughly. The medium was well mixed and sterilized at 121°C for 15 minutes using an autoclave. After sterilization, the medium flasks were transferred to a laminar air flow cabinet and exposed to UV light for 20-25 minutes, poured into sterile petri dishes and allowed to cool until solidified. Once the agar had completely gelled, the plates were ready for microbial inoculation and further experimental procedures.

2.5 Microbial culture

Microbial samples were obtained from human hands using the swabbing technique. A sterile cotton swab was rubbed thoroughly across the palm and interdigital spaces (between fingers) which were not pre-sterilized with 75-90% ethanol before sampling. The collected swabs were transferred into test tubes containing Nutrient Broth, incubated at 37°C for 24 hours. Spread plate method was employed to obtain pure cultures. The procedure was conducted within a sterile laminar air flow cabinet. A triangle-shaped glass spreader (L-spreader) was used for inoculation. The spreader was sterilized by dipping it in 95% ethanol and flaming it until sterile, then allowed to cool before using. A small volume of the bacterial suspension was spread evenly across the agar surface to isolate and separate individual cells. Bacterial suspension used for the experiment was standardized to approximately 1.5×10^8 colony forming unit (CFU)/ml as the final concentration.

2.6 Inhibition test

Aseptic Techniques and Preparation All experimental procedures were strictly conducted under aseptic conditions within a Laminar Flow Clean Bench, which had been pre-sterilized via UV irradiation. Essential materials, including culture plates, a 90% ethanol lamp, bacterial cultures, and relevant laboratory equipment, were placed inside the cabinet. Hands were thoroughly disinfected with alcohol before conducting experimental procedures. Antimicrobial activity of the extract samples was evaluated using the Agar Well Diffusion Assay (Cheesebrough, 2000). The extracts were dissolved in dimethyl sulfoxide (DMSO) to make 100 mg/mL of concentration, filtered through a 0.45 µm membrane filter for sterilization. Total of five samples (T1, T2, T3, T4, and T5) were prepared, distilled water was negative control, and positive control. The standardized bacterial suspension 1.5×10^8 colony forming unit (CFU)/ml was inoculated onto the surface of the agar medium using pour plate technique. When the agar surface is dried (approximately 5 minutes), seven wells was made using a sterile well cutter, each 5 mm in diameter. Then a volume of 0.1 mL of each extract and the controls were pipetted into the wells prepared. All treatments were performed in triplicate. The plates were then inverted and incubated at 37°C for 18-24 hours. Then, the inhibition zone was measured using ImageJ software.

Clear zone (cm)= (Diameter of welland clear zone) - (Diameter of well)

$$\text{Inhibition zone} = \frac{\text{Diameter of clear zone}}{\text{Diameter of Positive control}}$$

2.7 Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the extracts was determined following The European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines using the broth dilution and agar well diffusion methods. Total 11 test tubes containing Nutrient Broth (NB) and 1empty sterile tube were prepared. Dissolve the extract in a suitable solvent to achieve a stock concentration. This is added to one tube containing NB and the empty control tube. Two-fold Serial Dilution of the extract were prepared in the NB tubes to obtain a range of concentrations: 300, 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, and 0.78 mg/ml, respectively. Then microbial suspension (1.5×10^8 colony forming unit (CFU)/ml) was innoculated to Mueller-Hinton agar using pour plate technique. Once the medium was solidified, wells with a diameter of 5 mm were created using a sterile well cutter. A volume of 0.1 mL of each prepared concentration was pipetted into the designated wells. Conducting incubation and MIC determination, the plates were incubated at 37°C for 24 hours. The MIC was identified and recorded as the lowest concentration of the herbal extracts that visually inhibited the growth of the test microorganism (indicated by the presence of a clear inhibition zone).

2.8 Hand Gel Formulation

2.8.1 Preparation of plant Extract

The extract process was performed using an Erlenmeyer flask (250 mL). 3 g of *Terminalia catappa* L. leaves powder was mixed with 80 mL of 80% Ethanol. The mixture was placed on a magnetic stirrer (hot plate) and agitated thoroughly using a magnetic bar at room temperature for 2 hours to ensure continuous extraction. The solution was centrifuged at 2,000 RPM for 15 minutes. The supernatant was carefully collected and stored for further experiment for hand gel formulation.

2.8.2 Optimization of Base Gel Formulation

Followed with Surini et al., 2018 protocol with modification. The base gel was prepared by using Carbopol as the gelling agent and Triethanolamine (TEA) as the neutralizing agent to adjust the viscosity. Additional additives include glycerin, propylene glycol, and distilled water, formulated according to the ratios specified in the table 1

Table 1: Components of the Base Gel Formulation

Component	Formula A	Formula B	Formula C
Carbopol	0.3g	0.6g	0.9g
Triethanolamine	QS	QS	QS
Glycerin	8g	8g	8g
Propylene glycol	27g	27g	27g
Distilled water	200g	200g	200g

2.9 Evaluation of hand sanitizer gel containing *Terminalia catappa* L. extract

Followed with Guntero et al.,2017, Surini et al., 2018. Following the selection of the optimal base gel formulation, the next stage involved the development of the hand sanitizer gel incorporated with herbal extracts. In this phase, the *Terminalia catappa* L. leaf extract was added at varying concentrations to evaluate its efficacy and stability, as detailed in Table 2.

Table 2: Formulation of Hand Sanitizer Gel with Herbal Extracts

Component	Formulation 1	Formulation 2	Formulation 3
Terminalia catappa L.extract	8 mL	12 mL	16 mL
Citronella Essential Oil (*Cymbopogon nardus*)	0.1 mL	0.1 mL	0.1 mL
Carbopol	0.6g	0.6g	0.6g
Triethanolamine	QS	QS	QS
Glycerin	8g	8g	8g
Propylene glycol	27g	27g	27g
Distilled water	200g	200g	200g

2.10 Antimicrobial Efficacy Testing of the Hand Sanitizer Gel

The antimicrobial activity of the formulated hand sanitizer gel was evaluated using the spread plate method and a hand-sampling technique. The experimental procedure was conducted as mentioned in 2.3 (Anti bacteria assay) according to a standardized procedure. Sampling of bacterial samples was collected from the fingertips and palms of participants who had not yet applied the sanitizer then the samples were inoculated directly onto the prepared nutrient agar plates. Then the hand sterilize gel was applied to fingers and palms. After the gel had dried, microbial samples were collected again using the same method to assess the reduction in microbial load. Following incubation, the number of Colony Forming Units (CFU) was observed and compared between the pre-application and post-application plates to determine the percentage of microbial reduction.

2.11 Sensory Evaluation and Consumer Acceptance Test

The sensory characteristics and consumer acceptance of each hand sanitizer formulation was adapted from Saryanti & Zulfa, (2017) using a 9-point Hedonic Scale, where a score of 1 represented "dislike extremely" and 9 represented "like extremely". Total of 20 panels with no prior history of skin allergies or dermatological conditions were participated. An approximate of 0.5 g gel was applied to the participant's palm, allowed to remain for 1 minute. Sensory attributes including: Overall acceptance, spreadability, gel texture, viscosity, color, and Fragrance.

2.12 Statistical Analysis

All data were analyzed using Sirichai Statistics software (Version 6.07). The significant difference was performed at a 95% confidence level ($p < 0.05$).

3 RESULTS AND DISCUSSION

3.1 Antimicrobial Efficacy of Plant Extracts

Analysis of Inhibition zone diameters and relative inhibition of the five herbal extracts (using ethanol as the solvent) revealed significant antimicrobial activity. Among treatments, *Terminalia catappa* L. (T5) extract exhibited the highest inhibition zone at 1.4423 cm, followed by *Senna siamea* (Lam.) H.S. Irwin & Barneby (T4) at 1.1883 cm, *Azadirachta indica (T2)* at 0.9350 cm, and *Curcuma longa* L. (T3=0.4497 cm). The lowest inhibitory activity was observed in the *Citrus hystrix* DC (T1) extract, with a diameter of 0.1230 cm. Notably, the positive control demonstrated the highest overall inhibitory efficacy with a diameter of 2.7197 cm.

The investigation of five herbal extracts against hand-borne pathogens revealed that the ethanol extract of *Terminalia catappa* L.(T5) exhibited the highest inhibitory activity with a relative ratio of 0.6164. This was followed by *Senna siamea* (Lam.) H.S. Irwin & Barneby (T4, 0.5423), *Azadirachta indica* (T2, 0.4656), *Curcuma longa* L. (T3, 0.3171), and *Citrus hystrix* DC (T1, 0.2185). These results indicate a moderate level of efficacy, with the top-performing extracts achieving over 50% inhibitory potential compared to the positive control (Standard Antibiotic), which maintained the highest baseline of 1.000 (Table 3).

Table 3: Inhibition zone and inhibition rate

Sample	Control		Herbal extracts					SEM	P-value
	C1	C2	T1	T2	T3	T4	T5		
Inhibition Zone (cm)	0.222^{ef}	2.7197^{a}	0.1230^{f}	0.9350^{d}	0.4497^{e}	1.1883^{c}	1.4423^{b}	0.07	0.000
Inhibition rate	0.2475^{ef}	1.000^{a}	0.2185^{f}	0.4656^{d}	0.3171^{e}	0.5423^{c}	0.6164^{b}	0.02	0.000

P-value<0.05 indicates a statistically significant difference at a 95% confidence level. Letter a, b, c, d, e, ef, f indicates statistically significant differences between the samples. **T1:** *Citrus hystrix* DC ; **T2**: *Azadirachta indica*; **T3**: *Curcuma longa* L.; **T4**: *Senna siamea* (Lam.) H.S. Irwin & Barneby; **T5:** *Terminalia catappa* L.; **C1** = Negative control (distilled water); **C2** = Positive control. 80% Ethanol as solvent.

3.2 Minimum Inhibitory Concentration (MIC) of the Five Herbal Extracts

Table 4: Minimum Inhibitory Concentration (MIC) of the Five Herbal Extracts

Herbal extracts	Concentrations (mg/ml)									
	300	200	100	50	25	12.5	6.25	3.12	1.56	0.78
T1	-	-	-	-	-	+	+	+	+	+
T2	-	-	-	-	-	-	+	+	+	+
T3	-	-	-	-	-	+	+	+	+	+
T4	-	-	-	-	-	-	+	+	+	+
T5	-	-	-	-	-	-	+	+	+	+

(-): None detected, (+): Detected; **T1:** *Citrus hystrix* DC ; **T2**: *Azadirachta indica*; **T3**: *Curcuma longa* L.; **T4**: *Senna siamea* (Lam.) H.S. Irwin & Barneby; **T5:** *Terminalia catappa* L.

The Minimum Inhibitory Concentration (MIC) was determined to identify the lowest concentration of each ethanol-based herbal extract capable of inhibiting the visible growth of the tested microorganisms. Based on the experimental

results, the MIC values for the five herbal extracts are as follows: *Citrus hystrix* DC (T1) and *Curcuma longa* L. (T3): Both extracts demonstrated an inhibitory effect at a minimum concentration of 25 mg/ml. While *Azadirachta indica* (T2), *Senna siamea* (Lam.) H.S. Irwin & Barneby (T4), and *Terminalia catappa* L. (T5): These extracts exhibited higher potency, effectively inhibiting bacterial growth at a lower concentration of 12.5 mg/ml.

3.3 Base Gel Formulation

The optimization of the base gel, primarily using Carbopol as the gelling agent and Triethanolamine (TEA) as the neutralizing agent, yielded the following results: An amount of 0.6 g of carbopol was found to provide the most suitable physical characteristics. The result demonstrated optimal viscosity, consistency, and spreadability, closely comparable the texture and quality of commercially available hand sanitizer products. Consequently, this specific ratio was selected as the standard base for all subsequent treatments. Following the stabilization of the base gel, the herbal extracts and essential oils were incorporated at varying concentrations to further evaluate the gel's functional and physicochemical properties, as detailed in Table 5.

Table 5 Basic Components of Hand Sanitizer Base Gel

Composition	Quality
Carbopol	0.6g
Triethanolamine	QS
Glycerin	8g
Propylene glycol	27g
Distilled water	200g

3.4 Antimicrobial Efficacy of Hand Sanitizer Gel Formulated with *Terminalia catappa L.* Extract

Following the optimization of the base gel, the herbal hand sanitizer was developed by incorporating varying volumes of *Terminalia catappa* L. leaf extract. The formulations were prepared based on the *Terminalia catappa* L. extract concentrations: 8 mL (F1), 12 mL (F2), and 16 mL (F3). To evaluate their efficacy, a commercialized gel was utilized as the positive control, while a base gel without any extract served as the negative control. The results demonstrated a dose-dependent manners between extract and its antimicrobial activity. F3 (16 mL extract) Exhibited the highest antimicrobial efficacy with 93.19% of reduction rate. Meanwhile, F2 (12 mL extract) Showed a significant reduction rate of 89.86%, F1 (8 mL extract) Displayed the lowest inhibitory effect among the formulations. Notably, the Inhibition performance of F3 was found to be comparable to the positive control, suggesting that higher concentrations of *Terminalia catappa* L. extract can effectively suppress microbial growth to a level similar to commercial products.

Table 6: Antimicrobial Efficacy of the Formulated Hand Sanitizer Gels with *Terminalia catappa L.* extract

No	Examination	C1	C2	Hand Sanitizer Gel Formulations			SEM	P-value
				F1	F2	F3		
1	Beforehand sterilize (%)	100	100	100	100	100		
2	After hand sterilize (%)	1.93 a	60.56 a	25.28 b	10.21 b	6.79 b	5.59	0.0003
3	Antimicrobial Efficacy (%)	97.77 a	39.59 b	74.79 a	89.86 a	93.19 a	5.64	0.0003

P-value<0.05 indicates a statistically significant difference at a 95% confidence level.; Letter a, b, c, d, e, ef, f indicates statistically significant differences between the experimental treatment.; Positive control (C1): Commercial Gel; Negative control (C2): Hand Gel without *Terminalia catappa* L. extract

3.5 Sensory Evaluation formulated hand sanitizer

The sensory evaluation and consumer acceptance of the formulated hand sanitizer gels were assessed using a 9-point Hedonic Scale by a panel of 20 volunteers. Among the evaluated sensory attributes, spreadability received the highest average score of 5.78, followed by fragrance at 5.66. Regarding the overall preference across the four treatments, F1 obtained the highest average score of 5.53, closely followed by F0 (Commercial product as a control)) with a score of 5.48. In general, the scores fall within the "Neither Like nor Dislike" to "Like Slightly" range, indicating a neutral to moderate level of acceptance among consumers. Color of the gel may be one significant factor affecting preference since most consumers are traditionally familiar with commercial hand sanitizers that are clear, blue, or green. In contrast, the

herbal formulations in this study exhibited an amber or orange-yellow hue derived naturally from the plant extracts. This unfamiliar appearance likely influenced the participants' perception and preference, resulting in lower scores for color compared to other attributes.

Table 7: Sensory Evaluation of the Hand Sanitizer Gels

No	Attributes	Hand Sanitizer Gels Formulations				Average
		F0	F1	F2	F3	
1	Overall acceptance	5.15	5.8	5	5.9	5.46
2	Color	6.5	4.3	4.2	4.55	4.89
3	Fragrance	5.25	5.75	5.95	5.7	5.66
4	Spreadability	5.6	6	5.9	5.65	5.78
5	Gel texture	5.3	6	5.4	5.35	5.5
6	Viscosity	5.05	5.45	5	5.2	5.18
Total		5.48	5.53	5.24	5.39	5.41

In this study, Although the antimicrobial potency of these natural extracts did not surpass the commercial positive control, this study provides concrete empirical evidence and a foundation for developing novel antimicrobial formulations. The efficacy among the plants can be attributed to their unique chemical compositions and bioactive profiles. It is well-established that certain plants are more effective against fungi, while others show the ability targeting to bacteria or viruses specifically. Furthermore, the extraction method and solvent selection play a critical role in pharmaceutical potency. Different parts of the plant used (e.g., bark vs. leaves), the state of the raw material (fresh vs. dried), and the solvent type (Ethanol, Methanol, Water, or decoction) significantly influence the yield of bioactive compounds at the investigation approach. Our findings align with previous literature by Hemeg et al. (2020) who demonstrated that ethanol extracts from various plants, such as *Psidium guajava* L. and *Olea europaea* L. effectively inhibited microorganisms, including Gram-positive (S. aureus, B. cereus) and Gram-negative bacteria (*E. coli*, *Salmonella*), at concentrations ranging from 10% to 100%. Similarly, Muthulakshmi & Neelanarayanan. (2021) reported that methanol extracts of *Terminalia catappa* L. showed significant ability for inhibition against *Klebsiella pneumoniae* and various *Aspergillus* fungal species.

A simply but unique aspect of this study was the use of direct hand-swab samples rather than standardized laboratory strains. These methods may provide more practical assessment of the extracts when the bacteria strain assessment is limited. In terms of the minimum inhibitory concentration (MIC), *Terminalia catappa* L, *Senna siamea* (Lam.) H.S. Irwin & Barneby, and *Azadirachta indica* displayed superior potency with an MIC of 12.5 mg/mL, while *Citrus hystrix* DC and *Curcuma longa* L. required a higher concentration of 25 mg/mL. These results highlighted the potential of these herbal extracts as viable natural alternatives for microbial suppression at low concentrations.

The utilization of medicinal plants as active ingredients in hand sanitizers is highly significant, as these plants contain complex bioactive compounds with potent antimicrobial properties. In this study, application of *Terminalia catappa* L. extract in hand sanitizer gels demonstrated a clear correlation between concentration and antimicrobial efficacy.

Our findings are consistent with the research conducted by Allyn et al. (2018), who investigated the utilization of brown *Terminalia catappa* L. leaf extracts against fungi and bacteria, revealed that increasing the concentration of the extract (using 95% ethanol as a solvent) significantly expanded the zone of inhibition. Similarly, our results suggested that a higher volume of the extract enhance antimicrobial performance, even reaching levels comparable to commercial standard gels.

Since we use ethanol as solvent for extraction, it is important to consider the synergistic effect of the solvent. The Ethyl alcohol used during the extraction process likely contributed to the overall antimicrobial efficacy, as alcohol is a well-known agent for denaturing microbial proteins. Formulated Hand Sanitizer Gels without *Terminalia catappa* L. extract (negative control) alone exhibited an antimicrobial efficacy of 39.59%, indicating a baseline cleansing effect of the gel. Additional of the *Terminalia catappa* L. extract in formulation F3 significantly enhanced the performance, achieving a microbial reduction rate of 93.19%. The increasing clearly highlights the potent antimicrobial contribution of the *Terminalia catappa* L. extract within the hand sanitizer formulation. Furthermore, alternative extraction methods were also conducted in other study. Rahmasari et al. (2020) conducted a similar study using aqueous extracts obtained from

boiling fresh *Anredera cordifolia* leaves to formulate an herbal hand sanitizer, subsequently comparing its effectiveness with a positive control. This highlights that while ethanol is an efficient solvent, the specific bioactive constituents of the plants remain promise as primary drivers of microbial inhibition.

4 CONCLUSION

This study concludes that *Terminalia catappa* L. leaf extract showed the most potent antimicrobial activity among the five Lao medicinal plant extracts, achieving a significant inhibition zone of 1.4423 cm. The optimized hand sanitizer formulation, mix with 16 mL of this extract (F3), demonstrated a remarkable microbial reduction rate of 93.19%. This Inhibiting performance is comparable to commercial alcohol-based controls, proving the effectiveness of herbal based hand sanitizer gel against hand-borne pathogens. Sensory evaluations indicated that while the natural amber color was less preferred than clear commercial products, the gel's spreadability received positive feedback. These finding suggested good evidence that local Lao medicinal plants are served as a sustainable, cost-effective, and renewable resource for pharmaceutical development. Furthermore, utilizing natural plant constituents offers a safer alternative to synthetic chemicals that may be harsh on the skin. This research supports the integration of traditional knowledge using medicinal plants into modern hygiene solutions to enhance public health in the region. However, further investigation to improve the quality and standard of the product also need to be investigated.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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REFERENCES

[1] Allyn, O. Q., Kusumawati, E., & Nugroho, R. A. (2018). Antimicrobial activity of Terminalia catappa brown leaf extracts against Staphylococcus aureus ATCC 25923 and Pseudomonas aeruginosa ATCC 27853. F1000Research, 7.

[2] Chanda, S., Rakholiya, K., & Nair, R. (2011). Antimicrobial activity of Terminalia catappa L. leaf extracts against some clinically important pathogenic microbial strains. Chinese Medicine, 2(4), 171.

[3] Elkington, B. G., Southavong, B., Sydara, K., Souliya, O., Vanthanouvong, M., Nettavong, K., ... & Soejarto, D. D. (2009). Biological evaluation of plants of Laos used in the treatment of tuberculosis in Lao traditional medicine. Pharmaceutical biology, 47(1), 26-33.

[4] Ganguly, D., Banerjee, M., Chakraborty, A., Das, H., Sekh, A., Arif, M. D., ... & Sahoo, D. K. (2022). Preparation and Evaluation of Polyherbal Handwash of Different Herbal Sources. Neuroquantology, 20(11), 8113-8123.

[5] Guntero, V. A., Kneeteman, M. N., & Mancini, P. M. E. (2017). Preparation of Gel Alcohol Flavored with Essential Oils. An Employ of Laboratory Techniques in the Organic Chemistry Study.

[6] Hemeg, H. A., Moussa, I. M., Ibrahim, S., Dawoud, T. M., Alhaji, J. H., Mubarak, A. S., ... & Marouf, S. A. (2020). Antimicrobial effect of different herbal plant extracts against different microbial population. Saudi Journal of Biological Sciences, 27(12), 3221-3227.

[7] Jing, J. L. J., Pei Yi, T., Bose, R. J., McCarthy, J. R., Tharmalingam, N., & Madheswaran, T. (2020). Hand sanitizers: a review on formulation aspects, adverse effects, and regulations. International journal of environmental research and public health, 17(9), 3326.

[8] Muthulakshmi, G., & Neelanarayanan, P. (2021). Evaluation of Antimicrobial Activities of Terminalia catappa Leaves' Extracts against Bacterial and Fungal Pathogens. Indian Journal of Natural Sciences, 11(64), 976-997.

[9] Mounika, A., Vijayanand, P., & Jyothi, V. (2017). Formulation and evaluation of poly herbal hand wash gel containing essential oils Standardization of Inoculums. International Journal of Pharmacy and Analytical Research, 6(4), 645-653.

[10] Parekh, J., & Chanda, S. (2009). Antibacterial and phytochemical studies on twelve species of Indian medicinal plants.

[11] Rahmasari, D., Hendradi, E., & Chasanah, U. (2020). Formulation and evaluation of hand sanitizer gel containing infused of binahong leaf (Anredera cordifolia) as antibacterial preparation. Farmasains: Jurnal Farmasi dan Ilmu Kesehatan, 5(1), 23-30.

[12] Raj, H. D. P., & Manjunath, N. H. (2014). Antimicrobial activity of some common Indian medicinal plants against some selective human pathogen.

[13] Romha, G., Admasu, B., Hiwot Gebrekidan, T., Aleme, H., & Gebru, G. (2018). Antibacterial activities of five medicinal plants in Ethiopia against some human and animal pathogens. Evidence‐Based Complementary and Alternative Medicine, 2018(1), 2950758.

[14] Saryanti, D., & Zulfa, I. N. M. (2017). Optimization carbopol and glycerol as basis of hand gel antiseptics extract ethanol Ceremai leaf (Phyllantus Acidus (L.) Skeels) with Simplex Lattice design. JPSCR: Journal of Pharmaceutical Science and Clinical Research, 2(1), 35-43.

[15] Surini, S., Amirtha, N. I., & Lestari, D. C. (2018). Formulation and effectiveness of a hand sanitizer gel produced using salam bark extract. Int. J. Appl. Pharm, 10, 216-220.

[16] Sydara, K., Xayvue, M., Souliya, O., Elkington, B. G., & Soejarto, D. D. (2014). Inventory of medicinal plants of the Lao People's Democratic Republic: A mini review. J Med Plants Res, 8(43), 1262-1274.

[17] Verma, J., Mishra, R., Mazumdar, A., Singh, R., & El-Gendy, N. S. (2023). Development and Evaluation of an Eco‐Friendly Hand Sanitizer Formulation Valorized from Fruit Peels. International Journal of Biomaterials, 2023(1), 2516233.