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Research article

Evaluation of moisturizing property and antimicrobial activity of alcohol-based hand sanitizer formulations using coconut oil as a moisturizing agent against *Staphylococcus aureus* and *Escherichia coli*

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Abstract

During COVID-19 outbreak, alcohol-based hand sanitizer (ABHS) has been widely used for hand cleaning and removing pathogens including human pathogens and zoonosis pathogens. High concentration of alcohol induces dehydrated skin in the users. Therefore, the objective of this study was to investigate moisturizing property and antimicrobial activity of alcohol-based hand sanitizer formulations using coconut oil as a moisturizing agent against pathogens including *Escherichia coli* and *Staphylococcus aureus*. The properties including antimicrobial activity, stability of the ABHS, and satisfaction levels of the coconut oil with two existing formulations by WHO and the Ministry of Public Health of Thailand (MOPH) were determined. The formulation containing the coconut oil demonstrated antibacterial activity against both *E. coli* and *S. aureus* with the minimal inhibitory concentration (MIC) of 8.75% V/V as same as MOPH. However, the WHO-modified formulation has the highest antimicrobial activity with the MIC value of 2.19% V/V. The stability result of 3 ABHSs showed that the preserved had the same efficacy compared to that of the fresh formulations. Hand washing with coconut formulation produced an average score of 7.19±1.71 on color, 7.06±1.56 on appearance, 5.82±2.10 on odor, 6.77±1.68 on moisture, 6.88±1.42 on overall acceptance out of 9. However, the sensory analysis of these three formulations results showed no significant difference in all parameters. The data suggest that moisturizing agents tested in our study do not affect the efficacy of ethanol. The coconut oil formulation is another good option for people searching for an effective hand sanitizer for germs protection and moisturizing.

Keywords: Antimicrobial activity, Coconut oil, *Escherichia coli*, Moisturizing agents, *Staphylococcus aureus*

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INTRODUCTION

An outbreak of antibiotic resistance of human and zoonotic pathogens has raised concerns worldwide. *Staphylococcus aureus* are well-known as major causes of skin infection in human and animals. Furthermore, *S. aureus* has been considered as zoonotic bacteria that can be transmitted from the infected animals to humans (Stein, 2009). *Escherichia coli* is a commensal bacterium found in the intestinal tract in humans and animals. However, some groups of this bacteria are classified as zoonosis pathogens that cause fatal diseases in both the human (Mare et al., 2021). In addition, *E. coli* is considered as an indicator microorganism in food sanitation.

Several strategies have been used to prevent the pathogenic microorganism transmission and infection, especially during coronavirus outbreaks (van Doremalen et al., 2020). Hands are needed to be clean to reduce the chance of getting the disease through multiple routes, such as nose, mouth, and others (Jing et al., 2020). Hand sanitizer is one of the widely used tools during the COVID-19 outbreak as it effectively kills many microorganisms, safe, and available in markets. However, with high demand, their price is markedly increased. In general, hand sanitizers are classified into alcohol-based hand sanitizers (ABHS) and alcohol-free sanitizers (Jing et al., 2020). ABHS is composed of one or more types of alcohol with or without moisturizing agents and excipients. ABHS was recommended by the Centers for Disease Control and Prevention (CDC) because it has a broad spectrum of germ destruction (Jing et al., 2020). In principle, ABHS works by the alcohol activity dissolving the lipid within the membranes of germs (Jing et al., 2020). The alcohol solution is involved in protein denaturation, transcription, and translation inhibition (McDonnell and Russell, 1999; Haft et al., 2014). The concentration of alcohol between 60% and 90% is suitable for killing bacteria (Morton, 1950). In viruses, the target of alcohol-based hand sanitizers is mostly the viral lipid envelope or capsid protein (Kampf and Kramer, 2004; Kampf, 2018). Since overuse of the ABHS can lead to dry and cracked skin in the long-term user (World Health Organization, 2009; Jin et al., 2020), moisturizing or gelling agents were added to their formulation. The formulations recommended by the World Health Organization (WHO) are widely accepted and recognized by researchers (World Health Organization, 2010; Suchomel et al., 2017). The main constituent for moisturizing purpose in the formulation is glycerol which effectively protects the hand skin from dryness and dermatitis.

In 2019, ABHS formulation was developed by the Department of Medical Sciences, Ministry of Public Health of Thailand (MOPH). Their idea has been distributed to people through multiple social media. The formula consists of a carbomer as a gel-forming agent. Among the emollients, carbomers or carbopol synthesized in ethyl acetate are generally applied in the pharmaceutical and cosmetics industries (Berardi et al., 2020). Carbomer are synthetic polyacrylic acid crosslinked with allyl pentaerythritol or allyl sucrose. Since forming as 3D cross-linked microgels, carbomers do not entirely dissolve as they can be visible in water after neutralizing to form a gel or semisolid (Qiu et al., 2017; Lubrizol Corporation, 2021). The neutralizing agent, such as triethanolamine, was added to provide the neutralized pH (pH 6.5-7.5) to reach the maximum viscosity of the solution (Lubrizol Corporation, 2021).

Coconut oil has been used for multiple purposes: cooking, pharmaceutical, cosmetics, and infant foods (Dia, et al., 2005; Krishna et al., 2010). It contains more than 91% of saturated fatty acids, which are medium-chain fatty acids (MCFA) (C8:0-C12:0), more than 51%. Coconut oil has some specific medicinal properties, including antiviral, antibacterial, antiplaque, antiprotozoal, antifungal, anti-oxidant activity, anti-obesity effects, anti-inflammatory, and skin moisturizing properties (German and Dillard, 2004; Mu and Hoy, 2004; Marina, et al., 2009; Krishna, et al., 2010; Arora, et al., 2011; Varma et al., 2019). Because of their various benefits, coconut oil is popular to be applied in multiple products. In this study, the homemade ABHS also includes coconut oil as one of the constituents in the formulation. Therefore, the objective of this study was to investigate moisturizing property and antimicrobial activity of alcohol-based hand sanitizer formulations using coconut oil as a moisturizing agent against pathogens including *E. coli* and *S. aureus*. Furthermore, the properties including stability of the ABHS, and satisfaction levels of the coconut oil were determined.

MATERIALS AND METHODS

Coconut oil preparation

Fresh coconut meat, locally grown in Nakhon Si Thammarat, Thailand, was scratched and mixed with water at 65°C. The mixture was then pressed and fermented for 48 hours in an anaerobic condition. The oil layer was collected and slightly heated to remove the remaining moisture. The coconut oil was finally collected to a sterile bottle and stored at room temperature until use (Abbas, 2017).

Hand sanitizer preparation

The composition of three hand sanitizer formulations was summarized in Table 1 and Figure 1. Formulation 1 was prepared according to the standard guideline of WHO (World Health Organization, 2010) with slight modification to reach 70% alcohol. Formulation 2 was prepared and modified according to the MOPH with carbomer 940 and triethanolamine as gelling agents. Formulation 3, as developed in this study, was composed of ethanol and coconut oil as a natural moisturizing agent. All reagents used to prepare alcohol hand sanitizer were analytical grade (VWR Chemicals BDH, Pennsylvania, USA).

Table 1 The ingredients of three kinds of hand sanitizer

Reagent for formulation 1 (200 ml)	Reagent for formulation 2 (200 ml)	Reagent for formulation 3 (200 ml)
95% Ethanol 140 ml	95% Ethanol 140 ml	95% Ethanol 140 ml
3% Hydrogen peroxide 8.34 ml	Carbopol 940 1 gram	Coconut oil 6 ml
98% Glycerol 2.90 ml	Triethanolamine 0.7 gram	Sterile distilled water up to 200 ml
Sterile distilled water up to 200 ml	98% Glycerol 1.5 ml	
	Sterile distilled water 56 ml	

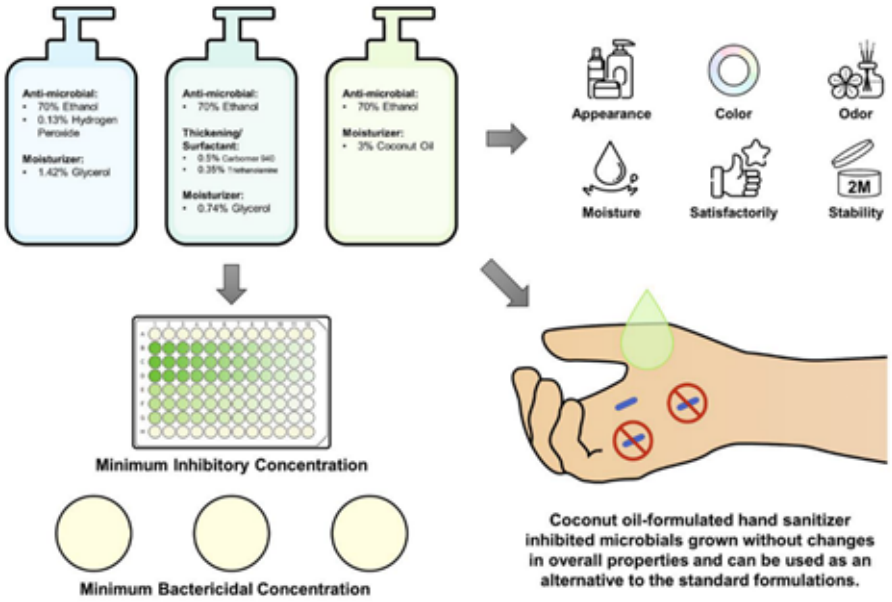

Figure 1 Schematic diagram of study procedures.

Study subjects

A total of sixty adult volunteers were recruited in this study. In this study, they were equally divided into three groups. Groups 1, 2, and 3 were treated with ABHS formulation 1, 2, and 3, respectively. Before testing, rubbing hands were required for all volunteers to equally distribute microorganisms/ bacteria on both hands. The left hand of each volunteer was not tested with any hand sanitizers and was used as a baseline bacterial number. A sterile cotton swab was used to examine the number of bacteria on hand to wipe the left-hand palm area, then smeared on a nutrient agar plate. The right hand was treated with a particular ABHS formulation mentioned above as described herein. To evaluate the disinfectant effect of hand sanitizer, 1 ml of ABHS was applied onto the right hand, spread over the palm by a sterile cotton ball, and air-dried for a minute. The right-hand palm was then wiped with the sterile cotton swab and smeared over the nutrient agar. The plate was later incubated at 37ºC for 18 hours. The number of bacterial colonies was quantified and compared with untreated hand. The results were compared among the three formulations. The before and after treatment.The data was analyzed by paired t-test and a one-way ANOVA.

Minimal inhibitory concentration (MIC)

Staphylococcus aureus ATCC 25923 and *Escherichia coli* ATCC 25922 were grown and diluted with 0.85% normal saline to 0.5 McFarland standard turbidity. The bacteria were diluted to 1:200 with normal saline for MIC assays. Subsequently, 100 µL of Mueller Hinton broth (HI Media Laboratories, Mumbai, India) were added to a 96-well microtiter plate. A series of 2-folded-dilutions of 100 µL of each type of hand sanitizing agent was performed. Finally, 100 µL of diluted bacteria were added to give final ethanol concentrations of 17.5%, 8.75%, 4.375%, 2.1875%, and 1.0937 % (V/V). The well with the mixture of bacteria and 50 µL of tetracycline (512 µg/mL) was used as a positive control. These assays were typically performed in triplicate for each formulation of hand sanitizer. Then, the microtiter plate was incubated at 37°C for 24 hours. The MIC of each substance was determined.

Minimal bactericidal concentration (MBC)

An aliquot of 20 µL from each well of all non-turbid bacterial supernatant from MIC was pipetted and dropped on the surface of Mueller Hinton agar (MHA) plate. The MHA plates were incubated at 37°C for 24 hours. MBC defines as the lowest concentration of the hand sanitizer agent required for removing or killing bacteria as appeared with no growth. The 70% ethanol (without a moisturizing agent) was used as the internal control in the MIC, and MBC techniques.

Stability test

The commercialized alcohol packages available in the market are generally kept in the clear press pump plastic bottles (about 300 mL in each bottle), while its usage period is usually within two months. In this study, all three formulas of ABHS stability were kept in fresh and preserved condition at room temperature for two months. In the stability test, the antimicrobial activities of these three ABHS formulas were tested in new and preserved conditions using MIC and MBC techniques. Then, the antimicrobial activities of preserved were compared to the fresh formulations to verify their efficacy.

Sensory analysis

Sensory analysis of the three ABHS formulations was assessed by 96 volunteers with the inclusion criteria on the following: 1. Some of these volunteers might experience using ABHS daily and 2. These volunteers participated with no history of a wound on their hands, skin diseases, and alcohol allergy. All participants gave informed consent before the study.

All ABHS formulations were placed on clear press pump plastic bottles with a different number. Before testing each formulation, all participants must keep their hands cleaned and dried. The ABHSs were later evaluated in color, texture, odor, taste, and overall acceptance. The satisfaction assessment form is the 9-point hedonic scale. The panelists were requested to score their responses on a 9-point scale when a scale including 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like or dislike, 6 = like slightly, 7 = like moderately, 8 = like very much 9 =like extremely. The data were analyzed by a one-way analysis of variance followed by LSD post hoc test to determine differences between each ABHS formulation at a significance level of 5%. (Chamsai et al., 2010; Garrido et al., 2015).

Ethical approval

This study was approved by the Human Research Ethics Committee of Walailak University, Nakorn Si Thammarat, Thailand as followed a reference number: WU-EC-AL-2-449-63.

Statistical analysis

All data were recorded, edited, and entered using the standard SPSS statistics version 25 (IBM, New York, USA). In this study MIC and MBC experiments were operated in triplicate. All data were then demonstrated as mean±standard deviation (SD). The data experiment with the hands of the volunteers was analyzed by paired t-test and a one-way ANOVA. At the same time, the data on sensory analysis were analyzed by a one-way ANOVA. A P-value was less than 0.05 is considered as a significant difference.

RESULTS

ABHS formulations

We were successfully producing three types of hand sanitizers; the WHO recommendation formulation using glycerol as moisturizing agents, MOPH recommendation formulation using carbomer as a gelling agent, and a conventional/homemade formulation using coconut oil as moisturizing agent. In this study, we further investigated the antimicrobial activities for each type of these three hand sanitizers.

Anti-bacterial activity of ABHS

Changing bacterial numbers on the hand before and after applying each ABHS formulation were shown in Figure 2. The statistical analysis showed that the bacterial contamination was significantly reduced after using any 3 ABHS of formulations. The untreated group of formulation 1, 2, 3 showed an average bacterial colony of 53.44, 236.8, and 202.7, respectively. The treated group of formulation 1,2,3 showed an average bacterial colony of 6.67, 5.55, and 3.72, respectively. However, the bacterial number of the treated group was not significantly different among the three formulations.

MIC and MBC of ABHS

MIC shown in Tables 2 revealed that the minimum inhibitory percentage of residual alcohol in each formulation was different. The effect of the WHO formulation on the growth of *E. coli* was inhibited in the presence of 2.1875% ethanol. The MBC of this formulation against *E. coli* is 4.375 % ethanol. This formulation has MIC and MBC against *S. aureus* at 2.1875 % and 8.75 %, respectively. This formulation showed the lowest MIC and MBC values against both *S. aureus* and *E. coli*, suggesting that the WHO formulation has the highest antimicrobial activity against both Gram-positive and negative bacteria. The MOPH and coconut formulations demonstrated the same MIC and MBC values with 70 % alcohol without moisturizing agents against the presence of *E. coli* at 8.75 % and 17.5 % and *S. aureus* at 8.75 % and without efficiency. The MIC and MBC show no difference in the fresh vs preserved ABHS formulations, as shown in Table 1.

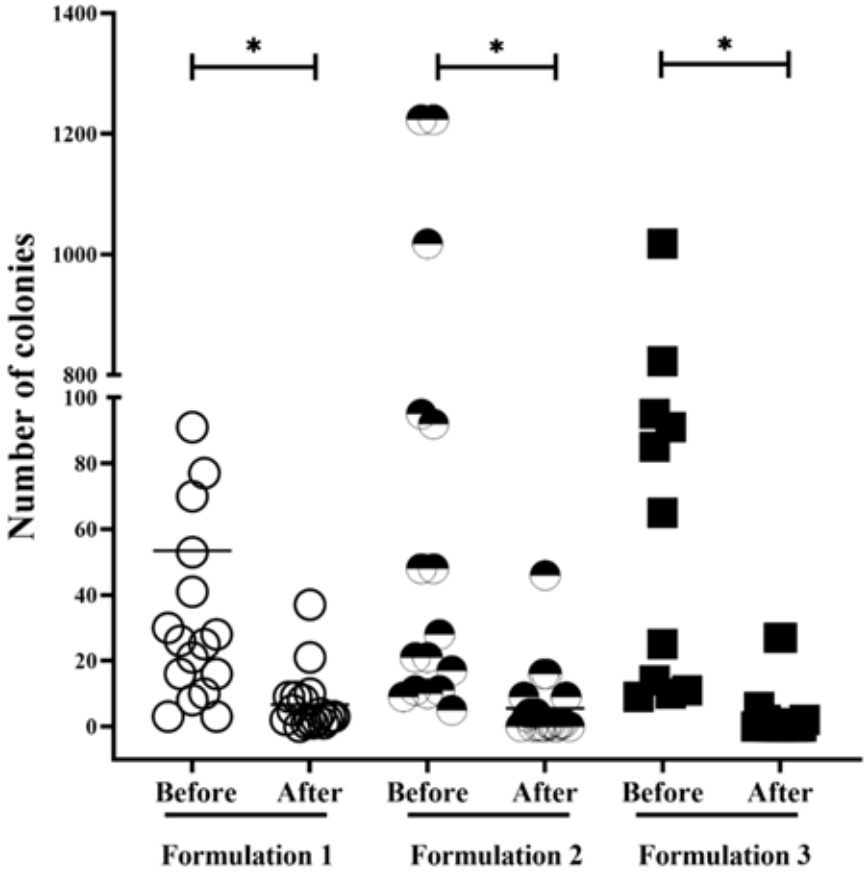

Figure 2 Number of bacterial colonies in the dot plot. Left hand (untreated/before) and right hand (treated/after treatment) with ABHS formulation. One dot represents the number of bacterial colonies from 1 volunteer. The average number is represented with a dashed line. *, $p < 0.05$.

Stability of ABHS

MIC and MBC techniques results of all formulations demonstrated that the antimicrobial activity of fresh ABHS versus preserved ABHS was not different. This result indicates that the clear press pump plastic bottle can maintain the effectiveness of the three formulations, as evidenced by the alcohol that was kept for two months show the same effect as the fresh alcohol.

Sensory analysis of ABHS

The three ABHS formulations showed a similar level of ethanol concentration, but their moisturizing substance was different. The results of the sensory assessment of these formulations by the 96 panelists were summarized in Figure 3. The panelists assessed these three formulations regarding color, appearance, odor, moisture, and overall acceptance. The test reflects the humidity of the skin and the satisfaction of the users. The results showed that handwashing with WHO formulation produced an average score of 7.53±1.73 on color, 7.16±1.68 on appearance, 5.39±2.15 on odor, 6.58±1.80 on moisture, 6.77±1.48 on overall acceptance. Handwashing with MOPH formulation produced an average score of 7.46±1.51 on color, 7.32±1.52 on appearance,

Table 2 Antibacterial activities of fresh alcohol-based hand sanitizer against *S. aureus* and *E. coli* using the broth microdilution technique.

ABHS	*E. coli*		*S. aureus*	
	MIC Ethanol (%)	MBC Ethanol (%)	MIC Ethanol (%)	MBC Ethanol (%)
Formulation (Fresh)				
Formulation 1	2.19	4.38	2.19	8.75
Formulation 2	8.75	17.50	8.75	>35%
Formulation 3	8.75	17.50	8.75	>35%
70 % alcohol	8.75	17.50	8.75	>35%
Formulation (Preserved)				
Formulation 1	2.19	4.38	4.38	8.75
Formulation 2	8.75	17.50	8.75	>35%
Formulation 3	8.75	17.50	8.75	>35%
70 % alcohol	8.75	17.50	8.75	>35%

5.56±2.10 on odor, 6.67±1.73 on moisture, 6.99±1.17 on overall acceptance. Moreover, hand washing with coconut formulation produced an average score of 7.19±1.71 on color, 7.06±1.56 on appearance, 5.82±2.10 on odor, 6.77±1.68 on moisture, 6.88±1.42 on overall acceptance. The sensory analysis of these three formulations results showed no significant difference in all parameters, i.e., color, appearance, odor, moisture, and overall acceptance.

DISCUSSION

ABHS composition contains moisturizers to prevent hand dry when it is often used. Two to five percent of glycerol and propylene glycol are the first and second most common for moisturizing purposes in cosmetic products (Flick, 1989; Barel et al., 2014). A recent study showed that glycerol inhibited the bactericidal efficacy of an isopropanol-based hand rub. It suggested that other moisturizing agents should be screened for their ability to maintain antimicrobial efficacy (Suchomel et al., 2017). Coconut oil is another moisturizing agent used in cosmetic and pharmaceutical productions. In addition, it has antimicrobial activity (Ogbolu et al., 2007). This study demonstrated that ABHS using coconut oil as a moisturizing agent was an effective formulation. MIC and MBC techniques were performed in parallel to measure the antimicrobial activity of the three formulations of ABHS toward two different bacterial species, *S. aureus* and *E. coli*, zoonosis pathogens. Both are generally found on the human skin surface and are a notable health care concern (Chojnacki et al., 2021). The MBC result showed that *E. coli* was more susceptible to all tested formulations than *S. aureus*, as indicated by antimicrobial effects. The first formulation or WHO recommended ABHS demonstrated the lowest MIC and MBC values, which suggested the highest antimicrobial activity compared to other formulations regardless of bacterial species. The WHO formulation contained 3% of hydrogen peroxide as an active additive

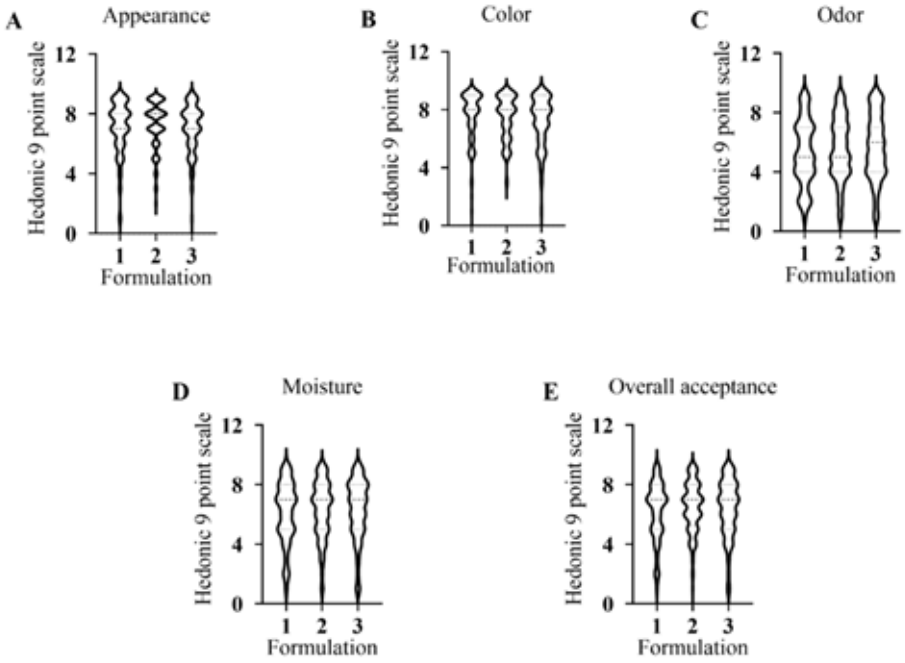

Figure 3 Violin plots of density data at different values of ABHS sensory test. Each formulation was evaluated in terms of appearance (A), color (B), odor (C), moisture (D), and overall acceptance (E). Formulations 1, 2, and 3 are WHO, MOPH, and the coconut oil-supplemented formulation, respectively. A p-value less than 0.05 ($p < 0.05$) was considered statistically significant.

agent to increase the antimicrobial activity of ethanol (Clifford and Repine, 1982; Suchomel et al., 2017). Another two formulations, the MOPH and coconut oil formulations, and the 70 % ethanol as an internal control, demonstrated the similarity of MIC and MBC against *E. coli* and *S. aureus*. These results showed that 3% of coconut oil and carbomer gelling agent, which are substances that help keep hands moisturized, did not contribute to the inhibitory effect of ethanol. Coconut oil has been reported to have antimicrobial activity (Ogbolu et al., 2007). However, a low percentage of the coconut oil did not increase the inhibitory effect of ethanol on formulation 3. In addition, some studies reported that the active agent in the liquid modes significantly showed better antibacterial activity than gel formulation. Our finding also showed no differential effect of three ABHSs formulations against bacterial contamination of the hands. The stability test results suggest that the clear press pump plastic bottle could keep the effectiveness of the three formulations, as evidenced by the alcohol that was kept for two months show the same effect as the fresh alcohol.

Moreover, the user acceptability in the sensory test showed that coconut oil formulation did not alter the human sensory when compared with the two standards formulation. This study demonstrated that coconut oil could act as an emollient other than glycerol, propylene glycol, or gelling agent. Due to its antibacterial activity, moisturizing property, long-lasting stability, easy preparation, and no toxicity, incorporating coconut oil as a humectant can be another option to implement in ABHS formulation and provide a comparable efficacy as a standard formulation.

CONCLUSION

This study added coconut oil into 70 % ethanol and compared the antimicrobial activities and stability with the two standard formulations. Using MIC and MBC techniques, our results suggested that the WHO formulation has more antibacterial activities than the coconut oil and the carbomer gel formulations. The coconut oil formulation has antimicrobial activity, stability, and moisturizing effect comparable to the MOPH formula. Therefore, it is another good option for people searching for an effective hand sanitizer regarding germs protection and moisturization.

Furthermore, all formulations showed the same efficacy to maintain the effectiveness of 70 % ethanol for another two months. Moreover, users' perceptions of these three formulations were shown no difference. Therefore, this study strongly recommends the cheap and effective coconut oil formulation among people, in general, to use as their hand sanitizer as one of the preventive measures against air-borne outbreak diseases like COVID-19. However, further studies need to be performed to demonstrate the long-term use of the coconut oil formulation, which can be locally made, cheap safe and more beneficial for people.

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AUTHOR CONTRIBUTIONS

SK, PK and WM: Conceived and designed the experiments. SK, PP, YC, NK, OS, and PK: Performed the experiments, analyzed, and interpreted the data. PK and WM: Wrote the manuscript. All authors read and approved the final manuscript.

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