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Original article

Preparation and evaluation of benzalkonium chloride hand sanitizer as a potential alternative for alcohol-based hand gels

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ABSTRACT

Hand hygiene is one of the effective measures for reducing the transmission of infections. Alcohol-based hand sanitizers containing ethanol or isopropanol are considered efficient alternatives to handwashing with water and soap. Despite being effective against a broad-spectrum of microbes, fining an effective alternative to the alcohol-based hand sanitizers became a necessity owning to the limitations associated with their use, such as skin dryness, irritant contact dermatitis, and intoxication upon their accidental ingestion. Furthermore, in certain circumstances when the demand for alcohol exceeds the supply, like in the current COVID19 pandemic, formulating an effective non-alcoholic hand sanitizer would be a potential solution. Therefore, in this study, a non-alcoholic hand sanitizer containing benzalkonium chloride (BKC) as an active ingredient was prepared and evaluated as a less irritant and more persistent hand sanitizer gel. The hand gel was characterized by pH, viscosity, and spreadability. Results showed that this product has low viscosity, high spreadability and pH of 6.3, which is less likely to cause skin irritation. The antibacterial assessment (zone of inhibition) of the BKC-based hand sanitizer demonstrated antibacterial activities against nine out of eleven gram-positive and gram-negative bacterial strains, while the acceptability study on ten participants showed no signs of skin irritation nor redness upon its application. Consequently, this non-alcoholic based hand sanitizer is suggested as a potential alternative to alcohol-based hand gels.

1. Introduction

The emerge of the novel coronavirus disease (COVID19) that is also known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been a serious challenge to public health worldwide (Zhu et al., 2020). The number of global deaths has increased exponentially since its emergence in December 2019, reaching more than 3.5 million as of May 30, 2021 (JHU, 2021; Worldometer, 2021). This virus can be transmitted from one person to another by inhaling on an individual's infected respiratory droplets through coughing, talking, or sneezing (Chu et al., 2020; Morawska et al., 2020; Wiersinga et al., 2020). Moreover, this virus is also transmissible via direct contact with contaminated surfaces or objects, in which the virus can persist for hours or even days (Carraturo et al., 2020; Kampf et al., 2020; Morawska et al., 2020). To contain the transmission of the infection, precautions should be taken, including physical distancing, wearing facial masks, and disinfecting the hands and surfaces frequently (CDC, 2020a; MOH, 2020; WHO, 2021).

Hand hygiene is one of the effective and simple measures to reduce the transmission of infections, including COVID19, as hands can be easily contaminated through direct contact with infected droplets (CDC, 2020b). Handwashing with water and soap for at least 20–30 s or the use of alcohol-based hand sanitizer (ABHS), as an alternative option, are recommended by the World Health Organization (WHO) (WHO, 2020). Cleaning with ABHS is also recommended by the Centre for Disease Control and Prevention (CDC)

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over the handwashing with water and soap, since it is easily accessible, especially for healthcare providers at point-of-care (CDC, 2020c).

Due to the increased demand for hand sanitizers caused by the COVID-19 outbreak, the WHO has issued a guideline for hand sanitizers local production (WHO, 2010). Hand sanitizers can be classified to ABHS and non-alcohol-based hand sanitizers (NABHS). The active ingredient of ABHS hand sanitizers is generally consist of either ethyl alcohol (in 70% to 80% v/v) or isopropyl alcohol (in 70% to 75% v/v). However, the NABHS may contain surfactants including quaternary ammonium compounds, in particular benzalkonium chloride (BKC) (CDC, 2020c; US FDA, 2019). ABHS has a broad-spectrum activity against bacteria, fungi, and enveloped viruses, including herpes simplex virus, and most recently, SARS-CoV-2 (Jing et al., 2020).

Quaternary ammonium compounds, such as BKC, benzethonium chloride, and cetyl peridium chloride, are cationic surfactants that exhibit antimicrobial activities through their adsorption onto the cytoplasmic membrane of the microbes, hence, damaging the cell membrane structural integrity (CDC, 2002; Jing et al., 2020). Therefore, they could inactivate gram-positive bacteria and lipophilic viruses. Among the quaternary ammonium compounds, BKC is the most commonly used active ingredient of NABHS (Golin et al., 2020). It has a long history of application as a preservative in the food industry, surface disinfectant and antiseptic, as well as hand sanitizer in healthcare settings. It also has been used as an antimicrobial for almost nine decades owing to its broad-spectrum antimicrobial activity against some bacteria, viruses, and fungi (Pereira and Tagkopoulos, 2019). It is important to test the activity of hand sanitizers against bacteria and novel pathogens like the most recent SARS-CoV-2 virus. This virus belongs to the family of Coronaviridae, which has a lipid envelope surrounding the virus, mostly referred to as lipophilic or enveloped virus (Gorbalenya et al., 2020). This envelope protects the virus from the host immune system. The antimicrobial activity of ABHS is related to their ability to dissolve the lipid membrane and denature the protein of the microbes. Similarly, BKC could also disrupt the lipid viral envelope (Bondurant et al., 2020; Jing et al., 2020).

A limited number of recent studies have tested the effectiveness of BKC-based disinfectant against SARS CoV-2 (Chin et al., 2020; Ogilvie et al., 2021). BKC was used at a concentration of 0.2%, as a surface disinfectant and at a concentration of 0.13%, as commercially available hand sanitizing wipes, such as Qimei Hand Sanitizing Wipes (Zhejiang Qimei Commodity Co., Ltd). Both these concentrations are effective against SARS-CoV-2, in which they rapidly inactivate the virus within 15 s of exposure (Ogilvie et al., 2021). It was also reported that BKC at a concentration of 0.1% could inactivate the virus on surfaces within 5 min (Chin et al., 2020). Another BKC-based hand sanitizer product commercially available is DAB Hand Sanitizer (Three Kings Corporation, Corinth, MS). This product contains BKC at a concentration of 0.12%, which can reduce the pathogenic *Staphylococci* from the hands of health care workers. Hence, it helps to prevent hospital-acquired infections (Bondurant et al., 2020). Additionally, both the Environmental Protection Agency (EPA) of the United States and a monograph from Health Canada approved the use of BKC as a disinfectant against SARS-CoV-2. The latter classified BKC as a non-prescribed drug, and the recommended disinfectant concentration is 0.1 to 0.15% (EPA, 2020; Health Canada, 2020).

Despite the widespread use of ABHS, it has some potential adverse health effects on humans. These include skin dryness and irritant contact dermatitis. The presence of these side effects are probably due to the ability of alcohol to denature skin proteins and to remove natural lipids on the skin that generally act as a protection layer (WHO, 2009). On the other hand, BKC is less irritant to the skin but still can cause dermatitis (Golin et al., 2020; Kampf et al., 2020). This might be resolved by formulating BKC sanitizer using moisturizers (Bondurant et al., 2020). Regarding the persistence of the antimicrobial activity of hand sanitizer, ethanol, at different concentrations, can evaporate rapidly from the skin surface; whereas, BKC could remain on the skin for more extended time (i.e., until the product dries) (Bondurant et al., 2019; Suchomel and Rotter, 2011). Besides, ABHS can be toxic when ingested by children, even at a minimal amount, compared to BKC-based hand sanitizers (Bondurant et al., 2020).

In this study, a developed BKC-based hand sanitizer formulation was evaluated as an alternative to ABHS to reduce the spread of pathogens during the pandemic. The product contains BKC at 0.1% w/w, as the active ingredient, along with more 'consumer-pl easant' components, as inactive ingredients, including glycerin, propylene glycol, diazolidinyl urea, Carbopol® 980, and lemon fragrance to improve the moisturizing effect of this hand sanitizer.

2. Materials and methods

2.1. Materials

BKC and glycerin were purchased from Sigma-Aldrich (USA) and Pharmaceuticals marketing Co. Ltd (PHMCO, KSA), respectively. Propylene glycol and diazolidinyl urea were bought from Tokyo Chemical Industry TCI (Japan). Carbopol® 980 (Polyacrylic acid polymer) was obtained from The Lubrizol Corporation (USA). Sodium hydroxide and the lemon fragrance were purchased from Merck (USA) and Flavour World Ltd (UK), respectively. Purified distilled water was generated through Milli Q, Millipore (USA). Three commercially available hand sanitizers C1 (alcohol denat-based hand sanitizer, international company), C2 (70% ethanol-based hand sanitizer, international company), C3 (70% ethanol-based hand sanitizer, local company) were obtained from the local market.

2.2. Methods

2.2.1. Preparation of the hand sanitizer

The non-alcoholic hand sanitizer was formulated as listed in Table 1. The formulation was prepared by direct mixing a solution consisting of BKC, glycerin, propylene glycol, diazolidinyl urea, and lemon fragrance in 50% v/v water, to be mixed with the Carbopol® solution that contains the remaining of the water. The pH of the resulted gel was adjusted by adding sodium hydroxide 0.1 M dropwise until reaching to pH 6.3. The final gel was eventually mixed at 100 RPM for 45 min at ambient temperature, to ensure the homogeneity of the final hand gel product.

2.2.2. Physicochemical characterization of BKC hand sanitizer

2.2.2.1. pH evaluation. A digital pH meter (METTLER TOLEDO pH meter, USA) was used to measure the pH and evaluate the neutralization of the hand sanitizer upon its formulation. pH

Table 1
Composition of the prepared non-alcoholic hand sanitizer.

Ingredient	Concentration % (w/w)
BKC	0.10
Glycerin	2.00
Propylene glycol	2.00
Carbopol® 980	0.20
Diazolidinyl urea	0.15
Lemon fragrance	0.50
Sodium hydroxide	To adjust pH to 6.3
Purified Water	qs. to 100%

measurements were performed in triplicate, and the results were recorded as mean ± standard deviation (SD).

2.2.2.2. Spreadability measurement. The spreadability of the formulated hand sanitizer was measure accordingly to (Al-Suwayeh et al., 2014; Bachhav and Patravale, 2009). Briefly, the spreadability was determined by spreading 0.5 gm of the gel over a pre-marked 2 cm in diameter transparent glass plate, over which a second glass plate was applied. A half kilogram weight was placed on top of the upper glass for 5 min, after which the excess of the gel was removed from the edges. The diameter due to the spreading of the gel was noted and the following equation was used to calculate the percentage of spreadability:

$$\%\ spread\ by\ area\ = \frac{A2}{A1} \times\ 100 \quad (1)$$

Where *A1* is the initial area before the gel spreading (2 cm) and *A2* is the final area after spreading.

The measurements were performed in triplicate, and the results were recorded as mean ± SD.

2.2.2.3. Viscosity measurement. The rheological property of the prepared hand sanitizer was studied at 25 °C via TCV 300 viscometer (Cambridge applied laboratories viscometer, TX, USA) using a piston with a range of 1–10 centipoise (cP). One mL of the formulated hand sanitizer was placed in the measuring compartment then data was obtained after capping the compartment for 60 s. Measurements were performed in triplicate, and the results were recorded as mean ± SD.

2.2.3. In vitro assessment of BKC hand sanitizer

2.2.3.1. Antibacterial zone of inhibition study. The gram-positive and gram-negative bacteria were either obtained from the American Type Culture Collection (ATCC) as reference bacteria or isolated clinically or environmentally to evaluate the antibacterial effect of the prepared hand sanitizer. The bacterial strains that were tested included; *Acinetobacter baumannii* (*A. baumannii*) BAA 747, two strains of *Escherichia coli* (*E. coli*) ATCC 25,922 and a clinical isolate 1060, *Klebsiella pneumoniae* (*K. pneumoniae*) BAA 1705, two strains of *Pseudomonas aeruginosa* (*P. aeruginosa*) BAA 1744 and ATCC 27853, *Staphylococcus aureus* (*S. aureus*) ATCC 29213, *Staphylococcus epidermidis* (*S. epidermidis*) clinical isolate 5029, *Staphylococcus hominis* (*S. hominis*) clinical isolate 5028, *Staphylococcus haemolyticus* (*S. haemolyticus*) clinical isolate 5034, and *Micrococcus luteus* (*M. luteus*) environmental isolate SB 115. Bacterial inoculums were prepared in Mueller-Hinton broth by measuring 0.5 McFarland, as previously reported (Aburayan et al., 2020).

The antibacterial activity of the prepared hand sanitizer was evaluated by the disc diffusion microbiological assay against three commercially available products as positive controls. A final concentration of $1x10^6$ colony-forming-unit/mL (CFU/mL) inoculum was equally distributed on the agar plates' surface. The sterile disc was immersed in the hand sanitizer gel, before it was dried and placed on the agar plate. All plates were incubated at 37 °C overnight. The zone of inhibition diameters around the discs were recorded in millimeters (mm), and the results were recorded as mean ± SD of three replicates.

2.2.3.2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) study. The MIC and MBC of the prepared BKC-based hand sanitizer were determined against four representative bacteria, *E. coli* ATCC 25922, *P. aeruginosa* BAA 1744, *S. aureus* ATCC 29,213 and *S. haemolyticus* ATCC 5034, in which their sensitivity to the prepared hand sanitizer was proven in the zone of inhibition test. A serial dilution of the hand sanitizer, in a range of 50% to 0.02% v/v, was prepared by diluting the hand gel in Mueller–Hinton broth containing a final inoculum of 1×10^6 CFU/mL, and then was added to a 96-well plate. One line of the 96-well plate that contained only bacteria was used as bacterial growth control, and all 96-well plates were incubated overnight at 37 °C with a continuous shaking speed of 120 RPM, following (Aburayan et al., 2020). The MIC was measured at an absorbance of 600 nm using CytationTM 3 Cell Imaging Multi-Mode Reader (BioTek Instruments, Winooski, VT, USA). Additionally, the MBC was assessed using the three lowest concentrations resulted in the MIC test, to be subcultured on Mueller–Hinton agar plates, and incubated overnight at 37 °C, according to (Otokunefor and Princewill, 2017).

2.2.3.3. Organoleptic characterization and skin irritation study. After receiving the ethical approval from King Abdulaziz City for Science and Technology institutional review board (IRB) no. 20009, the prepared hand sanitizer was inspected and rated in terms of appearance, color, odor, texture, irritation or burning sensation, and redness, according to ten participants. After explaining the purpose of the study and all possible side effects, each participant was asked to sign a consent form. The study was carried out by applying 1 mL of the prepared hand sanitizer on each participant's hands, then allowed to stand for 5 min after rubbing their hands for few seconds. At the end, a questionnaire was provided to all participants to record the outcomes of both the organoleptic characterization and skin irritation studies. All ten participants had no previous signs of dermal irritation nor trauma.

2.2.4. Statistical analysis

The data were presented as mean ± SD calculated using OriginPro 2016 software (OriginLab Corporation, Massachusetts, USA).

3. Results and discussion

3.1. Physicochemical characterization of BKC hand sanitizers

3.1.1. pH evaluation

The hand sanitizer was successfully prepared, in which three reproducible gels were obtained on three separate set-ups. The average pH of the three prepared hand sanitizers was 6.3 ± 0.1, indicating that the formulation would not cause any skin irritation and might be suitable for the dermatological application. The pH evaluation of the prepared hand sanitizer is an essential part of studying any topically applied pharmaceutical preparation, as it should be within the range of 4 to 7 to avoid any skin irritation or inflammation (Ali and Yosipovitch, 2013; Lambers et al., 2006). This finding was in consistent with Rahmasari *et al*., who were able to formulate a non-irritant hand sanitizer gel with a pH of 6.5 to 6.7 (Rahmasari et al., 2020). In addition, a slightly higher pH from 7 is also reported to be safe for human skin (i.e. no skin irritation nor signs of erythema), which was reported in (Al-Suwayeh et al., 2014; Surini et al., 2018) studies on lornoxicam topical gel and Salam bark extract gel, respectively.

3.1.2. Spreadability measurement

Spreadability is another fundamental property of any topical formulation, not only from consumers' perspective but also the formulation itself, as it defines the product's ability to exert the intended effect (Garg et al., 2002; Al-Suwayeh et al., 2014). Accordingly, the spreadability of the BKC-based hand sanitizer was determined to assess the gel's distribution upon application on the skin. It is worth noting that the gel spreadability can be inversely affected by the viscosity, i.e. lower viscosity contributes to higher spreadability and vice versa (Garg et al., 2002). The method used

to assess the gel spreadability relies on the "slip & drag" properties of the formulated gel. The spreadability of the prepared hand sanitizer was calculated to be 456% ± 19%. Such value is approximately 4.5 times the initial pre-marked area and is associated with less spreading time and ease of spreadability.

3.1.3. Viscosity measurement

Viscosity is also an essential feature that requires consideration during optimizing the preparation of any topical formulation, which can inversely correlate with spreadability (Rahmasari et al., 2020). The viscosity was assessed to determine the thickness of the prepared hand sanitizer, and it was measured as 6.6 cP ± 1.7 cP, which is slightly higher than the viscosity of the water (0.89 cP at 25 °C) (Korson et al., 1969). This result suggested that the formulated hand gel has low viscosity and hence, high spreadability %. This finding was also consistent with previous studies (Al-Suwayeh et al., 2014; Rahmasari et al., 2020).

3.2. In vitro assessment of BKC hand sanitizer

3.2.1. Antibacterial zone of inhibition study

The antibacterial efficacy of the prepared hand sanitizer was evaluated against eleven gram-positive and gram-negative bacterial strains. The antimicrobial effect was assessed against three commercially available hand sanitizers (C1, C2, and C3). The diameters of well-defined zones of inhibition (i.e. areas of no growth) were recorded, as shown in Tables 2 and 3. The zone of inhibition diameters of the prepared hand gel showed that nine out of eleven bacteria were inhibited with higher efficiency than the commercial products, as illustrated in Figs. 1 and 2. All market available hand sanitizers exhibited antibacterial activities against gram-negative bacterial strains only, with the exception of C3 (70% ethanol-based hand sanitizer, local company) that showed effectiveness against some gram-positive bacteria. This lack of antibacterial activity against gram-positive bacteria raises a concern, as all the commercially available products are ABHSs, which are known for their antibacterial efficiency against several bacteria (Jain et al., 2016). This finding will require further investigation, as it is suspected that due to high demand for alcohol, the quality of the commercial products would probably deteriorate.

As demonstrated in Fig. 1, the BKC hand sanitizer was able to inhibit the growth of gram-positive and gram-negative bacterial strains. It was previously reported that BKC has antimicrobial efficacy against gram-positive bacteria, fungi, and viruses (Pereira and Tagkopoulos, 2019). Nevertheless, lack of effectiveness of BKC against gram-negative bacteria was also reported in multiple studies (Kim et al., 2018; Tandukar et al., 2013). Diazolidinyl urea is a heterocyclic formaldehyde-releasing compound known to be used as an antimicrobial preservative in many cosmetic formulations and personal care products (Maier et al., 2009). At concentrations ranging from 0.1 to 0.5%, diazolidinyl urea has the ability to inhibit a broader range of bacteria (Hectorne and Fransway, 1994). Accordingly, diazolidinyl urea was combined with BKC to inhibit a broader range of bacterial strains and to ensure a longer shelf-life and stability of the water-containing hand sanitizer. Therefore, the bactericidal effect of the prepared hand sanitizer was furtherly assessed by determining the MIC and MBC against representative bacterial strains.

3.2.2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) study

The MIC and MBC were evaluated in order to assess the bactericidal effect of the prepared hand sanitizer, which considered as an important commercial feature of hand sanitizers (Golin et al., 2020). The results of MIC and MBC are shown in Table 4. The MIC and MBC values were consistent against the tested bacteria, which were measured as 2% v/v, 0.05% v/v and $\leq$ 0.02% v/v against *P. aeruginosa*, *S. aureus* and *S. haemolyticus*, respectively, except for *E. coli* in which the MIC and MBC were measured differently as 0.05% v/v and 0.1% v/v, respectively. Generally, the prepared BKC-based hand sanitizer proved to be very efficient, as the highest MBC was determined to be as low as 2% v/v of the hand sanitizer (i.e. diluted to 2%). The MBC against more bacterial strains is required for further evaluation of this hand gel product.

It was reported that BKC can exert its biocidal activity through disrupting the cellular membrane of the targeted microbes at a very low concentration of 0.12% (Bondurant et al., 2019; Dyer et al., 1998). It was also demonstrated in Fazlara and Ekhtelat study that *Listeria monocytogenes, S. aureus* and *E. coli* are sensitive to BKC; with MIC and MBC equivalent to 30 and 35 mg/L, respectively against the Listeria strain and 40 and 45 mg/L, respectively against both *S. aureus* and *E. coli* (Fazlara and Ekhtelat, 2012). Furthermore, Bondurant et al. have reported that BKC-based hand sanitizer exhibited a significant reduction in the total bacterial colony counts of *S. aureus* compared to 70% ethanol-based hand sanitizer on fingertips of 40 health care workers (Bondurant et al., 2019). Consequently, the prepared hand sanitizer was furtherly assessed by a skin irritation study on 10 participants along with an organoleptic test to evaluate its acceptability as a hand gel product.

3.2.3. Organoleptic characterization and skin irritation study

The organoleptic and skin irritation studies were carried out to evaluate the quality of the prepared hand sanitizer through its physical appearance and lack of any skin conditions (i.e., skin irritation and signs of skin erythema) that may occur upon its application. A summary of the organoleptic and skin irritation results is shown in Table 5. Both studies were conducted on ten participants: eight males and two females with an age range of 24 to 61 years old. There was no allergic history reported among the participants, however, six out of ten suffered from dry skin conditions. The hand gel successfully fit the following criteria: acceptable odor, easy to pour and spread, comfortable and moisturizing feeling when rubbing the hands, rapid dry and no stickiness feeling after applica-

Table 2
The zone of inhibition diameters of the prepared hand sanitizer compared to three commercially available hand sanitizers (C1, C2, and C3) against gram-negative bacterial strains. The prepared hand sanitizer exhibited antibacterial activity against four gram-negative strains out of six, while C1, C2, and C3 were effective against 3, 4, 5 g-negative strains, respectively. Data represent the mean ± SD.

Formulation	A. baumannii BAA 747	E. coli ATCC 25,922	E. coli 1060	K. pneumoniae BAA 1705	P. aeruginosa BAA 1744	P. aeruginosa ATCC 27,853
Prepared formula	0	14 ± 2	5 ± 1	5 ± 2	10 ± 0	0
C1	0	10 ± 1	0	15 ± 1	10 ± 1	0
C2	0	11 ± 1	15 ± 0	12 ± 1	15 ± 1	0
C3	0	10 ± 1	9 ± 0	8 ± 1	9 ± 1	12 ± 1

Table 3
The zone of inhibition diameters of the prepared hand sanitizer compared to three commercially available hand sanitizers (C1, C2, and C3) against gram-positive bacterial strains. The prepared hand sanitizer exhibited antibacterial activity against all gram-positive strains, which was more effective than the controls. Data represent the mean ± SD.

Formulation	S. aureus ATCC 29,213	S. epidermidis 5029	S. hominis 5028	S. haemolyticus ATCC 5034	M. luteus SB115
Prepared formula	19 ± 1	3 ± 1	18 ± 2	15 ± 1	19 ± 2
C1	0	0	0	0	10 ± 1
C2	0	0	0	0	0
C3	0	0	15 ± 1	10 ± 1	10 ± 1

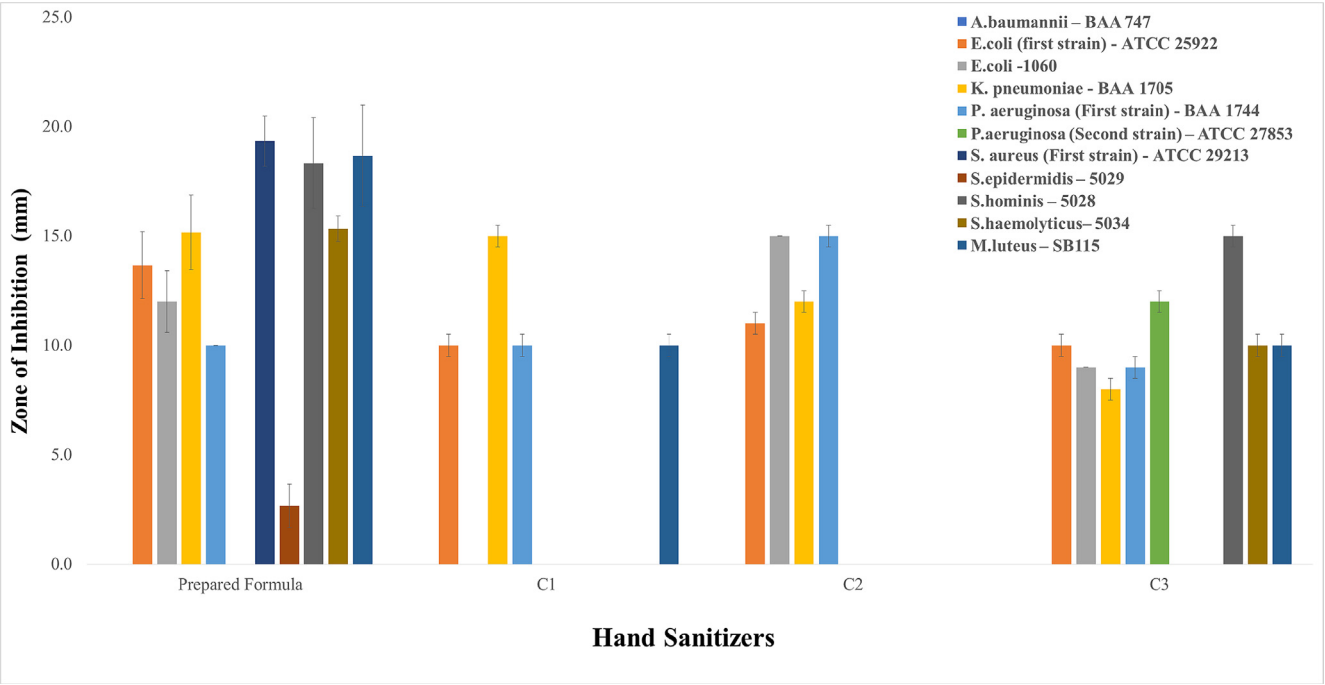

Fig. 1. The zone of inhibition diameters of the prepared hand sanitizer compared to three commercially available hand sanitizers (C1, C2, and C3) against eleven gram-negative and gram-positive bacteria (n = 3). It was demonstrated that the prepared hand sanitizer was able to inhibit nine out of eleven bacterial strains. In comparison, C1 and C2 were able to inhibit four bacterial strains each, and C3 was a more effective commercial product with an inhibition ability of eight out of eleven bacterial strains.

tion, in addition to good antibacterial activity and lack of any irritation and skin erythema.

All participants reported that the prepared hand sanitizer was a homogenous white-colored milk-like solution with a citrus (or detergent) odor. The drying time of the product was less than a minute for all participants, except for two who reported a drying time after 4 min. It is probably due to an extended moisturizing feeling that remained for approximately 5 min according to four participants. The hand gel was easy to apply and spread upon rubbing both hands with no coarse particles feeling after its spreading, which was due to the homogeneity of the formulation. There was also no reported phase separation (i.e. oil and water separate layers) of the hand sanitizer before its application on participant's hands; however, a stability test is required to evaluate the quality of the prepared hand sanitizer on storage.

Most importantly, there were no signs of skin irritation nor redness reported among all participants. Overall, nine out of ten demonstrated their satisfaction of the prepared hand sanitizer, except one who was not satisfied with the feeling (i.e., texture) of the hand gel. All of these results were consistent with other hand sanitizers of previous studies (Rahmasari et al., 2020; Surini et al., 2018).

4. Conclusion

The emerging of COVID-19 pandemic has in turn underlined the importance of hand hygiene, which became one of the most effective measures to slow the spreading of this viral infection. NABHSs were suggested to be utilized as potential alternatives to ABHSs, in order to cover the supply shortage in circumstances that the global demand on alcohol has enhanced massively. Herein, a BKC-based hand sanitizer was successfully formulated, which demonstrated an overall satisfied organoleptic and rheological properties, pH, and antibacterial activity. The prepared hand sanitizer exhibited an antibacterial activity against nine out of eleven gram-positive and gram-negative bacterial strains. In addition, this alcohol-free hand sanitizer showed a bactericidal effect on four representative bacterial strains. Although this BKC-based formulation was not tested on viruses, BKC was reported to be an effective disinfectant during the current COVID-19 pandemic, owing to its SARS-CoV-2 viral inactivation ability (Chin et al., 2020; Ogilvie et al., 2021). The exposure time of the hand sanitizer against bacteria, product assessment before and after application (by taking swabs from individuals' hands), and the stability of the hand gel would require further investigation. Additionally, more antimicrobial efficiency assessment against a broad range of bacteria, fungi, and viruses

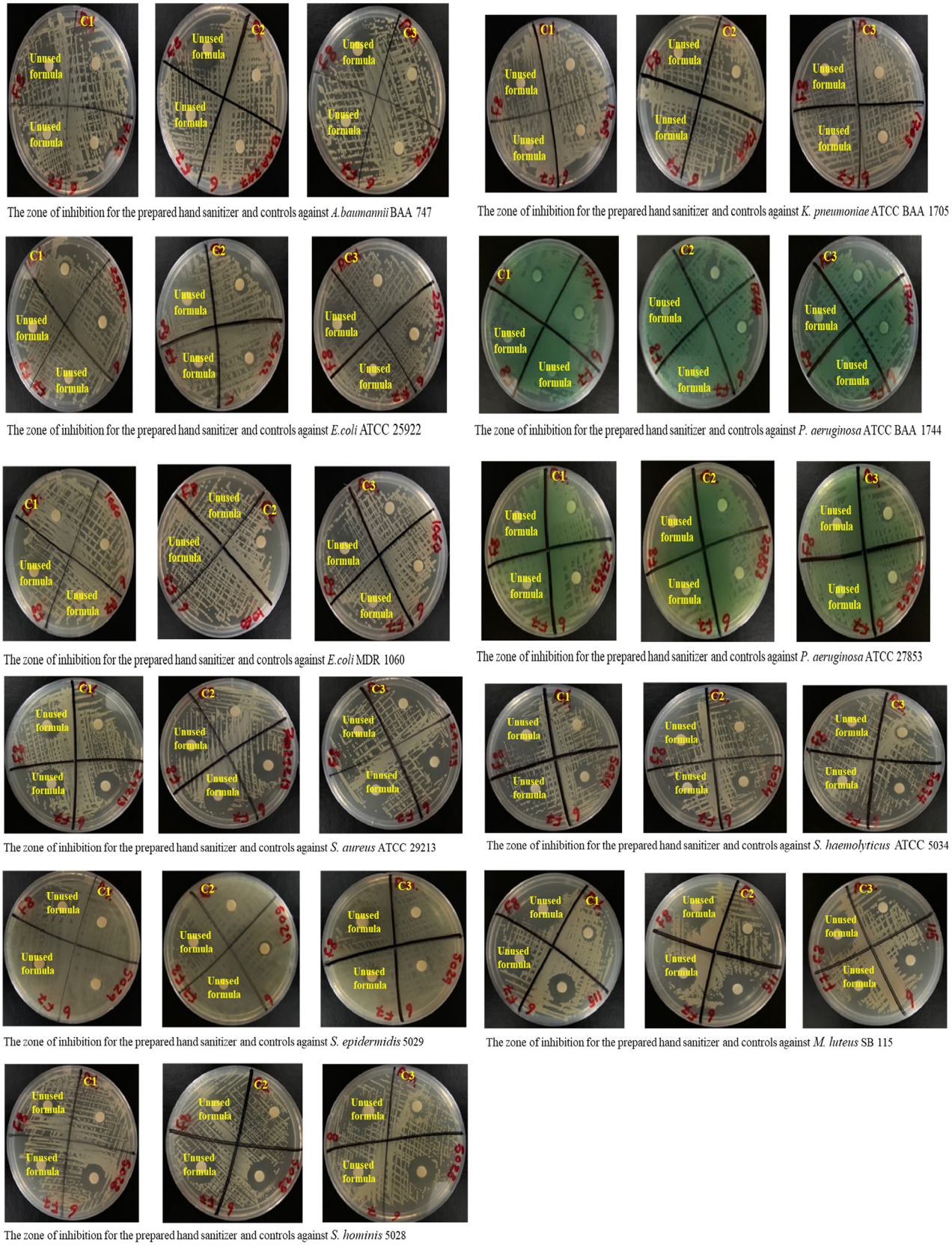

Fig. 2. The zone of inhibition diameters of the prepared hand sanitizer compared to three commercially available hand sanitizers (C1, C2, and C3) against eleven gram-negative and gram-positive bacteria. The prepared hand sanitizer was able to inhibit nine out of eleven bacterial strains. In comparison, C1 and C2 were able to inhibit four bacterial strains each, and C3 was a more effective commercial product with an inhibition ability of eight out of eleven bacterial strains. Nine refers to the prepared hand sanitizer. Two unused formulations were tested on the same plates.

Table 4
MIC and MBC of the prepared BKC-based hand sanitizer against gram-negative (E. coli ATCC 25922, P. aeruginosa BAA 1744) and gram-positive (S. aureus ATCC 29,213 and S. haemolyticus ATCC 5034) bacteria. The MIC and MBC of the prepared hand sanitizer were consistent for the tested bacteria, which were measured as 2% v/v, 0.05% v/v and ≤ 0.02% v/v against P. aeruginosa, S. aureus and S. haemolyticus, respectively, except against E. coli, in which the MIC and MBC were measured differently as 0.05% v/v and 0.1% v/v, respectively.

	E. coli (ATCC 25922)		P. aeruginosa BAA 1744		S. aureus ATCC 29,213		S. haemolyticus ATCC 5034	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Prepared formula	0.05%	0.1%	2%	2%	0.05%	0.05%	≤ 0.02%	≤ 0.02%

Table 5
Summary of the organoleptic properties and skin compatibility of the prepared hand sanitizer. The studies were conducted on ten participants (n = 10).

Participants information	
Gender	Eight males- two females
Age range	34 to 61 years
Organoleptic property	
Appearance	White, milk-like solution
Odor	Citrus, detergent
Drying time	Less than minute (after 4 min in two participants)
Skin irritation study	
Skin redness	Not reported
Irritation	Not reported

may also be needed, in which a comparison study of this hand sanitizer with several commercial available ABHSs that hold known concentrations of ethanol or isopropanol (ranging from 60% to 95% v/v) is recommended.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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