Original Research Article

Assessment of Skin Condition and Efficacy of Liquid and Cream Hand Sanitizers

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ABSTRACT

Hand sanitizer has become a popular alternative to traditional hand washing due to its ease of use, convenience, and effectiveness. Most hand sanitizers on the market are formulated with high concentrations of alcohol and lack of moisturizing agents, which can cause skin problems by altering the skin barrier's integrity and function with repeated use. To counteract these drying and irritating effects, a moisturizing hand sanitizer cream was developed. This study aims to assess the moisturizing efficacy of the developed cream and compare skin condition and antimicrobial effectiveness after using two different forms of hand sanitizer. The *in vivo* study involved comparing skin conditions after two weeks of using the developed moisturizing hand sanitizer cream and a commercial hand sanitizer liquid. Biophysical parameters such as trans epidermal water loss (TEWL), skin hydration, and elasticity were measured. Fifteen volunteers completed the study. Results showed a significant decrease in TEWL for the hand sanitizer liquid, but a gradual decrease for the cream. Skin hydration significantly decreased with the liquid but increased with the cream. Additionally, skin elasticity significantly improved with the cream compared to the liquid. Microbiological analysis indicated some contamination, but the hand sanitizer cream generally was more effective in reducing bacterial and fungal counts compared to the liquid sanitizer. In conclusion, these findings suggest that the moisturizing hand sanitizer cream not only supports skin health but also provides effective antimicrobial protection, highlighting its potential as a beneficial alternative to traditional hand sanitizers.

Keywords: Moisturizing hand sanitizer, skin hydration, biophysical parameters, antimicrobial efficacy, skin barrier integrity.

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1.0 Introduction

The frequency of the use of hand hygiene products increased during the coronavirus disease 2019 (COVID-19) pandemic. According to the World Health Organization (WHO), this disease can be transmitted directly by contact with infected people, or indirectly by contact with the surfaces in the immediate environment or with the tools used by the infected individual. As people constantly touch their nose, mouth, and eyes, maintaining proper hand hygiene practices helps to prevent the virus from entering the body through the hands (1). Hand hygiene has been practiced for ages, especially in the healthcare settings to prevent health care-associated infections (HCAIs). WHO stated that hand hygiene improvement programs can help to prevent infection occurring while providing health care up to 50% (2). This is because the majority of HCAIs are spread through the direct contact of healthcare workers, making hand hygiene is a crucial step in breaking the infection's chain (3). A study stated that hygiene compliance among doctors and nurses in a hospital in Finland was linked to a reduction in the occurrence of HCAIs (4). Another study showed that multidrug-resistant organism (MDRO) such as methicillin-resistant *Staphylococcus aureus* (MRSA) contamination in patient hands is prevalent and is associated with contamination of high-touch room surfaces (5), thus contributing to the spread of MDRO.

There are various types of hand hygiene products currently available in the market such as soaps, alcohol-based hand sanitizers, antiseptic hand wipes and antiseptic agents (6). Numerous studies have compared those products with respect to their effectiveness. Generally, hand washing with soap and water is recommended over the application of hand sanitizers. It is stated that pesticides and heavy metal such as lead may not be removed with the application of hand sanitizer, meanwhile hand washing is effective in removing all those harmful chemicals. However, the use of hand sanitizers is advised when the soap and water is unavailable to stop the spreading of COVID-19 and other infectious viruses. This is because hand sanitizers are available widely in all places and it is easily accessible in any situation where hand washing with soap is not practical.

The main categories of hand sanitizers include the alcohol-based hand sanitizer (ABHS) and non-alcohol-based hand sanitizer (NABHS). In the formulation of ABHS, it may contain one type of alcohol or a combination of alcohols, excipients, and humectants (7). The examples of alcohol used are ethanol, isopropyl alcohol, and *n*-propanol (8). Jing *et al*. (2020) (7) stated that at least 60% of alcohol is needed for it to exert the desired microbicidal activity. On the other hand, the antimicrobial effects of NABHS are achieved by using chemicals with antiseptic properties (7). Among the list of non-alcohol compounds that are commonly used, quaternary ammonium compounds such as benzalkonium chloride (BKC) are the most widely used active ingredients in the formulation of NABHS (8). It is because hand sanitizer with BKC does not irritate the skin, is odorless and does not cause damage to the surfaces. BKC is usually used in a low concentration of 0.1-2%, hence making the hand sanitizer relatively non-toxic (9).

Quaternary ammonium compounds act by adhering to the cytoplasmic membrane, resulting in the leakage of the components, thus inhibiting the growth of the microbes on the living tissue (7). They are more effective against Gram-positive bacteria and enveloped viruses, with a weaker activity against Gram-negative bacteria, fungi and mycobacteria. The advantage of NABHS over ABHS is that the NABHS is less concerning in terms of their flammability and abuse potential (10). However, the study also stated that despite being less skin-friendly than NABHS, ABHS are more common in medical settings due to their affordability and effectiveness in preventing the spread of infectious diseases.

Currently, different formulations were developed to deliver the function of the hand sanitizer, which includes gels, creams, spray and foams (7). However, repeated use of the hand hygiene products can cause depletion of the lipid barrier, resulting from lipid-emulsifying detergents and lipid-dissolving alcohols (11). The impaired skin barrier due to the depletion of stratum corneum's lipid barrier and protein denaturation will show an increase in trans epidermal water loss (TEWL) and epidermal penetration of irritants and allergens (12). Consequently, inflammatory response is propagated, leading to hand dermatitis. Application of moisturizer after the usage of hand hygiene products is important to prevent skin problems. However, a study stated that only 22.1% of health care workers that applied moisturizers despite washing their hands for more than 10 times daily during COVID-19 outbreak in China (13).

To solve this problem, the emollient-based hand sanitizers with excellent microbicidal activity, while also enhancing skin quality are being developed (1). Moisturizing hand sanitizer cream is one of the hand sanitizers formulated to give moisturizing effect while effectively killing the microbes. However, there are limited studies that compare the skin condition between the different dosage forms of hand sanitizer. Other than that, there is lack *in vivo* study on the efficacy of the developed moisturizing hand sanitizer cream. Therefore, this study will provide new insights into the efficacy of the hand sanitizer cream in moisturizing the skin. Besides, the findings will also provide an information on the effectiveness of the moisturizing hand sanitizer cream in exerting its microbicidal activity. This is important so that the hand sanitizer cream can helps protect the society against harmful microorganisms while maintaining a healthy skin condition. Additionally, it will be helpful for the potential researchers to use this finding as a starting point to do more research in comparing the effectiveness of different dosage form of hand sanitizers and their effects on the skin condition. The objective of this study is to assess the moisturizing efficacy for the developed moisturizing hand sanitizer cream, to compare skin condition after the application of two different form of hand sanitizer and to assess the antimicrobial effectiveness between two different formulations of hand sanitizer.

Additionally, this study is a continuation from previous study that successfully formulated a stable and effective non-alcohol-based hand sanitizer cream. The previous study developed and evaluated alcohol-based and non-alcohol-based hand sanitizer cream formulations to address skin irritation caused by traditional sanitizers. Stability tests, including homogeneity, pH, droplet size, zeta potential, and microbial stability, were conducted over a preliminary accelerated study. All formulations were stable, with non-alcohol-based F3 (benzalkonium chloride) demonstrating the highest zeta potential and best antimicrobial activity against various bacteria, outperforming the alcohol-based variants. The research concluded that non-alcohol-based formulations, especially F3, were superior in both stability and efficacy (14). Since it was developed with the combination of various moisturizing agent, hypothetically it will offer protection to the skin and minimizing skin irritation from the active ingredient. Therefore, the present study will confirm it by conducting human study to assess the effectiveness of moisturizing hand sanitizer cream in term of killing the microbes and moisturizing the skin. We hypothesized that the moisturizing hand sanitizer cream can give moisturizing effect on the hands while effectively kills the microbes when it is used daily.

2.0 Materials and methods

2.1 Ethical approval

This study was approved by the Research Ethics Committee of Faculty of Pharmacy, Universiti Teknologi MARA (UiTM) with the ethics approval number of REC/08/2023 (UG/FB/4). The participants were informed of the objectives and the procedures that will be done in the study. Informed consent form was signed to ensure confidentiality and voluntary participation.

2.2 Participants

Fifteen volunteers (5 males, 10 females) participated with a mean age of 22.9 years old (22-24 years old). Exclusion criteria included skin problems or known hypersensitivities to product ingredients. Volunteers signed informed consent forms and underwent a patch test 48 hours before the study to detect allergic contact dermatitis.

2.3 Test products

The study used a commercial hand sanitizer liquid containing BKC as active ingredient purchased from local shop and a developed moisturizing hand sanitizer cream (see Table 1 for formulation). The commercial hand sanitizer liquid contained several ingredients other than BKC which include water, glycerin, *Aloe barbadensis* leaf extract, chlorphenesin, phenoxyethanol and fragrance.

2.4 Instrumental skin assessment

Adapted from Tarka et al. (2019) (15), Azizi & Azad (2016) (16), and Kelchen *et al*. (2018) (17) assessments were conducted in a controlled environment (20-22°C, 40-60% relative humidity). Volunteers were acclimatized for 20 minutes before measurements on 0 week (baseline), 1 week and 2 weeks. The baseline reading was taken before the application of the samples and the readings were taken after 1 week and 2 weeks of the sample applications. Each volunteer applied 0.2 g of both products daily on marked ventral forearm areas (2 cm x 2 cm squares) as illustrated in Figure 1 for two weeks. The first area is for the application and assessment of the moisturizing hand sanitizer cream, the second area is for the commercial hand-sanitizer and the third one is the control area where there is no sample application in the area.

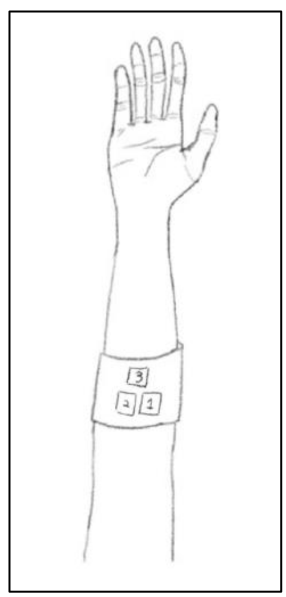

Figure 1: Location of measurement sites

Table 1: Formulation of developed moisturizing hand sanitizer cream

Ingredient	Maximum allowed concentration (%)	Functions	Percentage used (%)
Benzalkonium chloride	0.1	Active compound	0.1
Purified water	100	Solvent	64.9
Glycerin	79.2	Humectant	12.5
Olivem®	5	Emulsifier	5
Unrefined cocoa butter	37	Emollient/ Occlusive	16
Phenoxyethanol	1	Preservatives	1
Melon	2	Fragrance	0.5

2.4.1 Trans epidermal water loss (TEWL)

The amount of water lost from within the body by diffusion across the stratum corneum is measured by TEWL using Tewameter TM 300 (Courage & Khazaka, Cologne, Germany) (18). TM 300 is an open chamber type which is based on the Fick's law of diffusion (19). TEWL was expressed in $g/m^2/h$, with lower values indicating better skin hydration.

2.4.2 Skin hydration

Evaluation of skin hydration was done using Corneometer CM 825 (Courage & Khazaka, Cologne, Germany). The measurement principle of this corneometer is based on capacitance, where the instrument depends on the physical feature of water which has comparatively high dielectric constant that would affect the capacitor's capacitance (20). The shift in the dielectric constant is measured as the skin surface hydration affects the precision capacitor in the instrument (21). The measurements were taken by placing the skin hydration probe on the measurement sites for approximately one second (22). Arbitrary units (AU) were used to express the hydration values, with higher values indicating higher skin hydration levels (23).

2.4.3 Skin elasticity

The elasticity of the skin was assessed using the Cutometer MPA 580 (Courage & Khazaka, Cologne, Germany) (24). The principle of this instrument is based on the suction of a probe with negative pressure to the skin, hence causing the skin to be drawn into the aperture of the probe (25). The suction time for each of the measurement was for a period of two seconds and followed by two seconds of relaxation time before taking another measurement. The overall skin elasticity ("R2") values were taken and expressed in AU.

2.5 Microbiological evaluation

This method is adapted from Montero-vilchez *et al*. (2022) (26). Microbial sample was taken upon the volunteers' arrival (26). They were instructed to not wash their hands 1 hour before coming to the assessment (27). The volunteers were instructed to apply the product sample on their hands. After 1-2 minutes (28), the microbiological sample was obtained by direct application of the fingertips in a petri dish with culture medium. The right hand was placed in tryptic soy agar for the evaluation of bacteria, while evaluation of fungi was done by placing the left hand in Sabouraud dextrose agar. The petri dish was incubated (bacteria: 72 hours, fungi: 96 hours) and the number of colonies per plate was counted after 72 or 96 hours of incubation. The differences between number of colonies per plate at the baseline and after the application of hand-sanitizer was used to assess the microbial load.

2.6 Statistical analysis

Analyses were conducted using SPSS (version 24.0). Normality was assessed with Shapiro-Wilk test. Paired sample T-tests were used for TEWL, hydration, elasticity, and microbial evaluation before and after using both sanitizers. Significance was set at $P < 0.05$.

3.0 Results

3.1 Instrumental skin assessment

Changes in skin barrier function parameters for liquid and cream hand sanitizers are shown in Figure 2. The mean TEWL values decreased from 0 week to after 1 week and 2 weeks for both hand sanitizers. For the control area, there was a significant reduction in TEWL after 2 weeks ($p < 0.05$). The cream hand sanitizer showed no significant reduction in TEWL between 0 week and after 1 week and 2 weeks ($p > 0.05$). However, the liquid hand sanitizer exhibited a significant reduction in TEWL after 2 weeks ($p = 0.000274$).

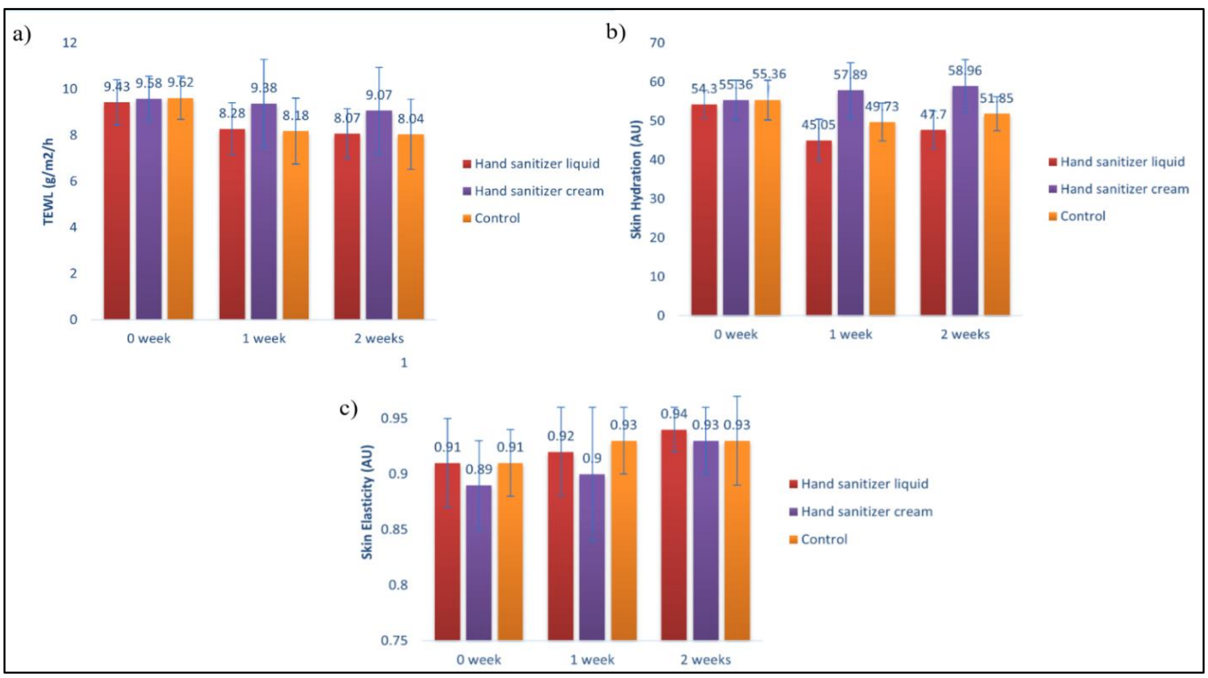

Figure 2: Mean values of skin barrier function parameters of hand sanitizer; a) TEWL, b) Skin hydration, c) Skin elasticity ($n = 3$)

For skin hydration, the mean values from 0 week to after 1 week and 2 weeks are reported as follows: for the control, hydration decreased initially and then slightly increased, while for the liquid hand sanitizer, a significant drop occurred after 1 week, followed by a notable increase by the following week (2 weeks) ($p < 0.05$). The overall decrease in hydration from 0 week to 2 weeks for the liquid hand sanitizer was significant ($p = 0.000012$). The cream hand sanitizer showed a gradual increase in hydration over 2 weeks, with a significant improvement noted between baseline and after 2 weeks ($p = 0.047$). A significant difference in hydration was also observed for the control area between 0 week and after 2 weeks ($p < 0.05$). The mean skin elasticity values increased from baseline to after one and two weeks for both hand sanitizers. The values showed a slight but steady improvement over time for the control, liquid, and cream hand sanitizers. A significant difference was observed for the control area between baseline and after two weeks ($p < 0.05$). Both the liquid and cream hand sanitizers demonstrated a significant increase in skin elasticity after two weeks, with p-values of 0.005 and 0.000009, respectively.

3.2 Microbiological evaluation

The microbiological evaluation presented in Table 2 reveals that both the liquid and cream-based hand sanitizers were effective in reducing bacterial and fungal colonies. However, the cream-based hand sanitizer showed a more pronounced reduction, particularly for both bacteria ($p = 0.002$) and fungi ($p = 0.000001$), compared to the liquid hand sanitizer (bacteria, $p = 0.017$; fungi, $p = 0.000041$). Furthermore, the variability between participants in terms of baseline microbial load and post-application reduction can be attributed to individual skin characteristics, baseline microbial flora, and environmental factors. For example, participants 4, 9, 12, and 15 had higher microbial loads initially, yet both sanitizers effectively reduced colony counts. Nonetheless, the cream formulation generally achieved a more significant reduction,

The presence of "cannot be counted" (CBC) values in the dataset, likely due to contamination of agar plates, should be noted as a limitation but does not detract from the overall conclusion. These CBC values should be addressed as outliers and acknowledged in the discussion for transparency

Table 2: The number of colonies before and after the application of hand sanitizers

Participants	Hand Sanitizer Liquid				Hand Sanitizer Cream			
	Bacteria		Fungi		Bacteria		Fungi	
	Before	After	Before	After	Before	After	Before	After
1	CBC	6	CBC	3	14	6	10	5
2	14	7	48	17	35	19	22	13
3	CBC	4	6	3	CBC	16	17	10
4	CBC	CBC	45	21	63	18	42	CBC
5	32	15	40	14	CBC	23	23	17
6	20	5	29	11	25	13	18	9
7	53	25	38	21	35	18	14	9
8	CBC	31	23	12	47	23	12	5
9	CBC	19	25	12	CBC	CBC	15	9
10	24	CBC	12	5	32	19	7	2
11	11	3	8	4	13	7	19	7
12	39	CBC	48	13	CBC	29	27	13
13	31	CBC	39	7	53	31	35	19
14	CBC	9	19	6	CBC	16	12	5
15	41	CBC	73	31	31	CBC	12	7
p- value	0.017		0.000041		0.002		0.000001	

***CBC: Cannot be counted**

4.0 Discussion

In this study, liquid and cream hand sanitizers were treated on the forearm of 15 healthy volunteers. The assessment of skin barrier impairments was done by measuring the biophysical parameters to evaluate the effectiveness of hand sanitizer cream in moisturizing the skin compared to the hand sanitizer liquid. The efficacy of the cream in exerting its antimicrobial effects was also assessed to ensure that the hand sanitizer cream can help user to improve hand hygiene compliance.

4.1 Instrumental skin assessment

The primary role of the skin is to serve as a barrier against harmful bacteria and environments. Therefore, any alteration to the physiology of the hand increases the risk of microorganisms penetrating the skin. Healthy skin minimizes excessive water loss to the environment, which correlates with a small TEWL value (17). Changes in TEWL values are suggestive of epidermal barrier function deficiencies, and elevations of baseline by two to three times have been observed following exposure to irritating substances (29). The decrease in TEWL was significant for the hand sanitizer liquid, meanwhile there was no significant reduction in TEWL observed from the baseline and after 2 weeks for the hand sanitizer cream. This indicates that the cream does not disrupt the skin barrier function (17).

As for skin moisture, liquid hand sanitizer showed the decrease in skin hydration value from baseline to after two weeks and it was significant. On the other hand, there was a significant increase in skin hydration value from baseline to after two weeks for the hand sanitizer cream. The humectants or natural moisturizing components in the cream provide occlusive qualities of the lipids to prevent trans epidermal water loss and restores the intercellular lipid bilayers' ability to absorb, retain, and redistribute water, hence increasing the stratum corneum water content and promote skin hydration (30).

In the discussion of skin hydration and TEWL, the interaction between humectants and occlusives is crucial to understanding the observed results. Humectants, such as glycerin and *Aloe barbadensis* leaf extract in the liquid hand sanitizer, are compounds that attract moisture from the surrounding environment or the deeper layers of the skin (epidermis) to the surface of the skin. However, when used in the absence of adequate occlusives, which form a protective barrier to prevent water evaporation, humectants can paradoxically draw moisture from the deeper layers of the skin, potentially leading to dehydration of the skin’s outer layers (31). This can explain why the liquid hand sanitizer, which lacks strong occlusive agents, shows a reduction in skin hydration despite reducing TEWL.

In contrast, the moisturizing cream hand sanitizer contains both humectants (glycerin) and occlusive agents (unrefined cocoa butter and Olivem®). These occlusives form a barrier on the skin surface, preventing the moisture drawn up by humectants from evaporating. This synergistic action between humectants and occlusives ensures that the skin retains moisture, leading to the observed significant improvement in skin hydration with the cream hand sanitizer (31). Thus, the difference in skin hydration between the two products can be attributed to the presence of occlusive agents in the cream, which prevent moisture loss from the skin surface, ensuring that the moisture attracted by humectants stays in the skin, whereas the liquid hand sanitizer lacks these occlusive agents, leading to moisture evaporation after initial hydration. This aligns with the concept that humectants should ideally be combined with occlusives to provide balanced moisturization and prevent potential drying of the skin's outer layers.

Skin elasticity assessment is crucial in both cosmetics and dermatology, and it has been experimentally proven to increase with hydration levels (19). In this study, there was a significant increase in skin elasticity between baseline and after two weeks for both liquid and cream hand sanitizers. However, the increase was more significant in hand sanitizer cream compared to liquid. The significant improvement in both skin hydration and elasticity after two weeks of using the hand sanitizer cream can be attributed to its formulation. The cream contains glycerin and cocoa butter, both known for their excellent moisturizing properties. Glycerin acts as a humectant, drawing moisture into the skin, while cocoa butter serves as an occlusive agent, preventing moisture loss (31). This dual action likely contributed to the enhanced hydration levels, which in turn supported skin elasticity, as hydrated skin tends to be more elastic and resilient. Interestingly, despite the decrease in hydration, skin elasticity improved after two weeks of using the liquid sanitizer. This could indicate that while hydration is vital for overall skin health, elasticity may also be influenced by other factors such as collagen production or the skin's structural integrity. The liquid's formulation such as *Aloe barbadensis* leaf extract could stimulate some degree of skin repair or adaptation over time, leading to improved elasticity even in the face of reduced hydration levels (32).

The difference for the control area between the baseline and after two weeks was significant for every parameter ($p < 0.05$). First, this result may be due to the error made during the measurements. In order to limit variability, it is also advised that all TEWL and skin hydration measurements be performed by the same individual, so this factor also may influence the result as the measurements were made by different person at certain times. Besides, variable TEWL values have been documented when it is measured at different places on the same anatomical position. It has been reported that TEWL value can be higher and varied when the position is closer to the wrist and elbow on the volar forearm (33). In this study, although not much, the measurement areas for the hand sanitizer cream and liquid were closer to the elbow than the control area, which may also influence the overall results.

4.2 Microbiological evaluation

The microbiological evaluation presented in Table 2 indicates that both the liquid and cream hand sanitizers effectively reduced the number of bacterial and fungal colonies, as both formulations contain BKC, a quaternary ammonium compound known for its ability to disrupt microbial cell membranes, cause cell lysis, and provide sustained antimicrobial activity (34). However, despite containing the same active ingredient, there was a noticeable difference in the reduction of colonies between the two formulations, with the cream hand sanitizer performing better overall.

The difference in efficacy between the two formulations can be attributed to the formulation base rather than the active ingredient itself. The cream, being a more viscous formulation, likely allowed for better retention on the skin surface, creating a more prolonged contact period with microbes. This could enhance the ability of BKC to exert its sustained antimicrobial action. Cream formulations generally adhere better to the skin, forming a protective layer that can continuously release the active ingredient over time (35). The moisturizing properties of the cream may also have helped improve the skin's condition, reducing the likelihood of cracks or dryness that could harbour bacteria, which supports a more effective microbial reduction (36).

On the other hand, the liquid sanitizer, while containing the same BKC, may have had a shorter contact time with the skin due to its low viscosity (37). The liquid formulation might not adhere as well to the skin, leading to a reduced overall microbial reduction compared to the cream. Furthermore, the presence of moulds and contaminants in some plates, indicated by the CBC designation, suggests environmental contamination that could have impacted the microbial counts on some plates. This is common in microbiological evaluations and can affect the reliability of the results, particularly when aseptic techniques are compromised (38).

5.0 Conclusion

This study offers essential insights into the impact of two hand sanitizer formulations on transepidermal water loss, skin hydration, and elasticity. The hand sanitizer cream showed notable enhancements in skin hydration and elasticity after two weeks of use, indicating its efficacy in improving moisture retention and resilience. The combination of humectant and occlusive agents, glycerin and cocoa butter, presumably facilitated these favourable results by reducing moisture loss and enhancing skin barrier integrity. In contrast, although the hand sanitizer liquid exhibited a large decline in skin hydration during the research duration, it also revealed a significant decrease in TEWL. This indicates that the liquid formulation may confer certain protective benefits to the epidermal barrier, although the reduction in moisture levels. The noted enhancement in skin elasticity with the liquid sanitizer suggests that elements beyond hydration may affect skin resilience, possibly including the promotion of collagen synthesis or other adaptive mechanisms. These findings underscore the need of assessing transepidermal water loss, skin hydration, and elasticity when evaluating the effects of hand sanitizers on skin health. Although both solutions effectively reduce microbes, their varying impacts on skin condition underscore the necessity for meticulous selection according to individual skin requirements. Future study should investigate supplementary moisturizing compounds that can augment the hydrating qualities of liquid sanitizers while maintaining their antibacterial effectiveness. This study emphasises the need for hand hygiene solutions that safeguard against infections while preserving skin health.

Authorship contribution statement

NSZ: Skin evaluation, microbiological study, data analysis, original authoring. **SS**: Ethics application, visualisation, and methodology. **NMJ**: Skin assessment study training and assistance. **MIZ**: Oversee skin evaluation, microbiological study, methodology, data analysis, paper review, and editing.

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Conflict of Interest

The authors declared that they have no conflicts of interest to disclose.

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