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Efficacy of Different Herbal and Chemical-Based Hand Sanitizers to Control the Microbial Growth on the Hands of Bank Cashiers

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ABSTRACT

Hand hygiene plays a vital role in reducing the transmission of infectious diseases, principally in occupations involving the regular handling of contaminated objects, such as currency notes. **Objectives:** To estimate the effectiveness of three classes of hand sanitizers, including commercial non-alcoholic, alcoholic, and lab-prepared herbal formulations, for reducing microbial growth from the hands of bank cashiers in Lahore. **Methods:** This experimental study evaluated 32 cashiers aged 25–40 years for sanitizer efficacy. Samples were gathered from both hands before sanitizer application, after 25 seconds, mid-sampling 1 minute, and post-sampling after 1 hour. **Results:** The findings verified clear modifications among sanitizer types. Alcoholic sanitizers, mainly containing ethanol or isopropyl alcohol, were the most effective, attaining up to an 87% decrease in CFU count. In some alcoholic sanitizers, inhibition was 99.9%. Lab-prepared herbal sanitizers, including constituents such as aloe vera, neem, alum, and basil, reduced bacterial growth by up to 61%. The non-alcoholic sanitizers have also shown activity against bacterial growth and reduced CFU by up to 48%. The bacteria isolated by biochemical isolation were *Staphylococcus aureus* and *Escherichia coli*. A two-way ANOVA with a post-hoc Tukey's test revealed significant decreases in CFU (colony-forming units) at $p<0.0001$ among all categories and proved alcoholic sanitizers to be the strongest antimicrobial agents. **Conclusions:** This result shows that alcoholic hand sanitizers are highly effective against hand-borne microbes in a real-life bank environment, suggesting their use as an integral component of hand hygiene interventions for cashiers.

INTRODUCTION

Hands are the primary mode of transmission of microbes and infections. Hand hygiene is therefore the most important measure to avoid the transmission of harmful germs and prevent infections. Hand hygiene is the single most important, simplest, and least expensive means of preventing nosocomial infection [1]. The microbes may be removed through washing practices and are largely recommended by the use of hand sanitizers. Hand sanitizers are well-adapted to the skin, work by stripping the outer layer of oil on the skin, removing skin bacteria too, and their use is an important way to break bacterial transmission [2]. Currently, different types of hand sanitizers, cleansers, or disinfectants are available on the market in various forms, such as gels, quick-drying materials, foams, and wipes, which are sometimes mixed with moisturizing lotion. These sanitizers can be generally divided into alcohol-based (ABHS) and non-alcohol-based formulations, which comprise chemical antiseptics and

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plant-based herbal preparations. Generally, hand sanitizers are available as alcohol and non-alcohol-based cleansers, and their use in liquid, foam, gel, and cosmetic forms is common [3]. Of the 62% alcohol-based disinfectants commonly in use, most of them contain 60 to 85% alcohol. Among alcoholic sanitizers, ethyl alcohol and isopropyl alcohol are the common active ingredients, while propylene glycol, glycerin, and polyacrylic acid are some inactive ingredients. One of the most important transmission vectors in society is infected currency. Banknotes can harbor and transmit pathogens, including multidrug-resistant bacteria such as *Staphylococcus aureus* and *Escherichia coli* [4]. The group of occupations with a high risk of contracting and spreading infections in this way includes bank cashiers, who handle hundreds of notes every day. Effective hand hygiene is, therefore, an important occupational health measure for this population [5]. The COVID-19 epidemic led to an unprecedented reliance on alcohol-based hand sanitizers (ABHS) [6, 7]. However, concerns such as skin irritation, dryness, and the emergence of alcohol-resistant strains have necessitated the use of alternate sanitizers [8]. This has led to a desire to utilize not just chemical non-alcoholic sanitizers (e.g., chlorhexidine or benzalkonium chloride), but also natural herbal treatments comprising antibacterial plant extracts such as neem, aloe vera, and basil. Although the effectiveness of ABHS is widely documented, there is a lack of direct comparisons of herbal and chemical non-alcoholic sanitizer performance and practicality in a high-risk, real-world setting such as a bank [9, 10]. Different types of delivery systems are also formulated, for instance, rubs, foams, or wipes [11]. This study hypothesized that, although chemical non-alcoholic sanitizers would be significantly effective, some herbal sanitizers prepared in the laboratory would have similar antimicrobial potential, providing a feasible and skin-friendly choice for regular use in this workplace setting.

This study aimed to evaluate and compare the efficacy of herbal and chemical-based hand sanitizers in reducing the microbial load of bank cashiers' hands.

METHODS

The materials used in the experiment include different commercialized alcoholic hand sanitizers, namely Bkovit, Lifebuoy, Handzer Safex, Anvil by adore, Handzer, Anzo, Antizer, E.P.C Hand Sanitizer Gel, Meiji Cool & Cool, Dentox, and different non-alcoholic hand sanitizers, namely Germitoll, Hi-clean, and some conventional antibiotics and pharmaceutical medicines (Zeecin Azithromycin U.S.P., Saftin, GSK, Tineazol, Fortum, Augmentin) were additionally included in the testing protocol to offer a more comprehensive comparison analysis of antimicrobial efficacy. Plant extracts, namely Neem leaves extract Azadirachta indica, Fresh Aloe vera extracts, Basil leaves extract Ocimum basilicum, Camphor, Pink and white alum. The laboratory equipment was Himedia nutrient agar, Himedia blood agar, Himedia MacConkey agar, Himedia nutrient broth, aluminum foil, and an incubator set at 37 degrees Celsius. The composition of alcohol sanitizers and non-alcoholic sanitizers was prepared in lab sanitizers (Table 1).

Table 1: Chemical Composition of Different Alcoholic Sanitizers and Non-Alcoholic Sanitizers

Sr. No.	Brand Name	Chemical Compositions
Alcohol Sanitizers		
1	Bkovit	Alcohol 80%, Glycerol 1.45%, Hydrogen peroxide 0.125%
2	Lifebuoy	Alcohol, water, glycerin, acrylates, alkyl acrylate cross polymer, triethanolamine, perfume, panthenol, Lonicera japonica flower extract, Alpha-isomethyl ionone, benzyl salicylate, citronellol, Hexylcinnamal, Limonene, linalool
3	SafEx	Alcohol hand rub, alkoholisches, Handedes in fektonsmittel Gel hydroalcoolique pour la disinfection des mains
4	Handzer	Active ingredients: Isopropyl alcohol 75%
5	Anvil by adore	Demineralized water, Carbomer, ethyl alcohol, Vitamin E, PPG, Perfume, Triethanolamine
6	Anzo	IPA 65-75%, alcohol 71-80%
7	Antizer	Ethyl alcohol 80%, Glycerol 1.45%, Hydrogen peroxide 0.125%
8	E.P.C Hand Sanitizer Gel	Ethanol 80%, Glycerol 1.45%, Hydrogen peroxide 0.125%
9	Meiji Cool and Cool	Aqua, Glycerine, Propylene Glycol, Alcohol, Carbopol, Ultrez, De- De-Panthenol, Aloe Vera Gel, Fragrance
10	Zeecin Azithromycin U. S. P	Ethanol 80%, Hydrogen peroxide 0.125%, Glycerol 1.45%
11	Dentox	Alcohol Denat, Aquawater, Propylene Glycol, Perfume Fragrance, Linalool

The composition of non-alcoholic sanitizers was prepared in lab sanitizers (Table 2).

Table 2: Chemical Composition of Different Non-Alcoholic Sanitizers

Sr. No.	Brand Name	Chemical Compositions
1	Saftin	Loratadine 10mg Tablets and 5mg Syrup with Compliments
2	Germitoll	Active ingredients: Germitol Chloride 0.1%
3	GSK	Compliments
4	Tineazol	Iron polymaltose, Folic acid
5	Fortum	Ceftazidime
6	Augmentin	Co-amoxiclav
7	Hi-clean	Cationic surfactant, Nonionic surfactant, Amphoteric Surfactant, Polybiguanide

Hand-made sanitizers were prepared in lab sanitizers (Table 3).

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Table 3: Composition of Lab-Prepared Sanitizers

Sr. No.	Sanitizer Name	Standardized Composition and Preparation
1	Lemon Oil Sanitizer	Isopropyl Alcohol (20% v/v), Aloe Vera Gel (60% v/v), Lemon Oil (1.5% v/v), Eucalyptus and Lavender Oils (0.2% v/v). Prepared by mixing.
2	Herbal Sanitizer	Aqueous extract from Neem leaves (40g/250ml), Aloe Vera pulp (24% v/v), Basil leaves (10 leaves/250ml), Camphor (2% w/v), Pink and White Alum (2% w/v each). The aqueous extract was boiled, filtered, and mixed with other components.
3	Camphor Sanitizer	Camphor (3.8% w/v) in distilled water, with essential oil (0.4% v/v). Prepared by dissolution and mixing.
4	Pink Alum Sanitizer	Pink Alum (2% w/v) in distilled water. Prepared by dissolution.

The experimental study was conducted to assess the effectiveness of different hand sanitizers by collecting samples from participants before and after sanitizer application. This study was designed for the comparison of different hand sanitizers and hand cleaning of bank cashiers, and the results are described in descriptive and inferential ways. The study followed ethical guidelines. The experimental work was carried out at the Conservation Biology Lab, Institute of Zoology, University of the Punjab, Lahore, and the samples were collected from the different banks of Lahore. Working days of banks are Monday to Friday, and mostly selected banks have 3 to 4 cashiers (32 cashiers in total). This study employed an intensive, repeated-measures design where each participant provided multiple samples (pre-, mid-, and post-application), generating a robust dataset for statistical analysis. The sample size of 32 participants was primarily determined by feasibility and the targeted recruitment from a specific high-risk occupational group. The study was conducted from 8 March to 22 June 2022. The participants were included voluntarily, and their consent was taken. These participants were provided with brief details of the research study and its wider implications on society. A total of 32 cashiers (24 male, between the age ranges of 25-40, and 8 females, between the age ranges of 25-30) were tested with different hand sanitizers from randomly selected banks. To ensure an impartial distribution, participants were randomly assigned to one of three sanitizer test groups using a random selection technique. The working hours of most participants were 8-10 hours per day, and they washed their hands after 1-2 hours during work. Mostly, participants wash their hands early in the morning when they come from home and during work hours when they go to the washroom or eat something with soap. The samples were taken in three steps, and the amount of sanitizer used was 3ml as per the WHO standard 2019 [12]. The samples were taken on nutrient agar plates. For every sanitizer, control sampling was also done. First of all, the pre-readings were taken of bank cashiers before sanitizing both their hands. Then three types of sanitizers were taken: alcoholic, non-alcoholic, and herbal. In the first group, the alcoholic sanitizers were applied on both hands, and after 25 seconds, and then after 1 minute, for pre- and mid samples were taken. Then, after 1 hour, post samples were taken. In one second, group, the non-alcoholic sanitizers were applied on both hands, and after 25 seconds, and then 1 minute, pre- and mid samples were taken. Then, after 1 hour, post samples were taken. In one third group, the herbal sanitizers were applied after 25 seconds, and then after 1 minute, for pre- and mid samples were taken. Then, after 1 hour, post samples were taken. Then sample plates were incubated for 24 hours at 37°C. After this, the morphological characteristics of bacteria were identified on solid media, and at the end, the Colony count [13]. Then, blood agar was used to differentiate bacteria based on their hemolytic properties, and MacConkey agar was used to isolate the Gram-negative bacteria and differentiate them based on lactose fermentation. Petri plates were packed with the help of tape and covered them by using aluminum foil. After the packaging of Petri plates, these plates were taken to the Bank for sample collection. The pre-samples of bank cashiers were taken before sanitizing their hands. They were divided into three groups. The hands of the first group were sanitized with alcoholic sanitizers and the hands of the second group were sanitized with non-alcoholic sanitizers, and the hands of the third group were sanitized with herbal sanitizers. After 25 seconds, the mid samples were taken of all groups by applying their hands on Petri dishes, and after 1 hour, the post samples were taken of all groups by applying their hands on Petri dishes. The data were analyzed by counting the bacterial colonies from Petri plates after incubation [24]. The statistical analysis was done using OriginPro2022. The number of bacterial colonies (CFU) was log-transformed, and normality and homogeneity of standard errors were verified by the Shapiro-Wilk and Levene tests, respectively. All the comparisons, such as the overall efficacy of the three types of sanitizers (alcoholic, non-alcoholic, and herbal), were done with two-way Analysis of Variance (ANOVA). The comparison between right and left hands was done with ANOVA, and the post-hoc Tukey's (HSD) was used for pairwise comparison. A p-value of less than 0.0001 was considered statistically significant.

RESULTS

(A) Lifebuoy (94.2% ± 0.1%), Meiji Cool and Cool (86.4% ± 16.1%), Antizer (83.9% ± 12.5%), Handzer (80.3% ± 22.6%), and E.P.C Gel (74.0% ± 19.7%) are the most effective groups, according to the post-hoc analysis (Fig. 3a). (B) Despite variations, alcoholic sanitizers resulted in a highly

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significant decrease in the overall bacterial load ($p<0.0001$). (B) The sanitizers had a significant main effect on the right hand ($F(10,66)=16296$, $p=0.0001$) and the left hand ($F(10,66) = 13770$, $p=0.0001$), according to the two-way ANOVA. Additionally, both hands showed a highly significant difference in application time (before vs. after) (Right: $F(2,66) = 9308$, $p<0.0001$; Left: $F(2,66) = 2949$, $p<0.0001$). (Figure 1).

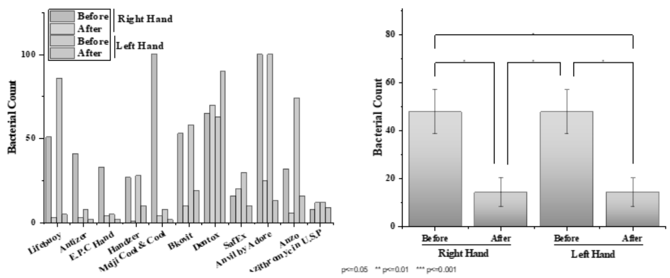
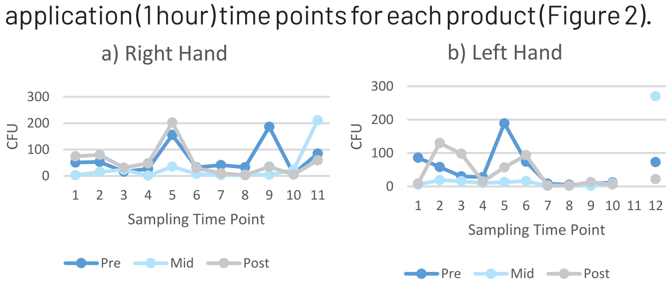


Figure 1: (A) The Efficacy of Different Alcoholic Hand and (B) Sanitizers on Participants

Changes in bacterial load on the (A) right hand and (B) left hand, following application of various alcoholic hand sanitizers. The graph depicts Colony Forming Units (CFU) at pre-application (baseline), mid-application, and post-application (1 hour) time points for each product. The efficacy of alcoholic sanitizers followed a distinct temporal pattern: a sharp, significant reduction in bacterial colonies at the mid-application point (25 seconds) was observed for most products, confirming their rapid antimicrobial action. However, this was followed by notable bacterial regrowth on many hands at the post-application point (1 hour), indicating limited residual efficacy. Changes in bacterial load on the (C) right hand and (D) left-hand following application of various non-alcoholic hand sanitizers. The graph depicts Colony Forming Units (CFU) at pre-application (baseline), mid-application, and post-application (1 hour) time points for each product. Changes in bacterial load (E) on the right hand and (F) left hand following application of various lab-prepared hand sanitizers. The graph depicts Colony Forming Units (CFU) at pre-application (baseline), mid-application, and post-application (1 hour) time points for each product (Figure 2).



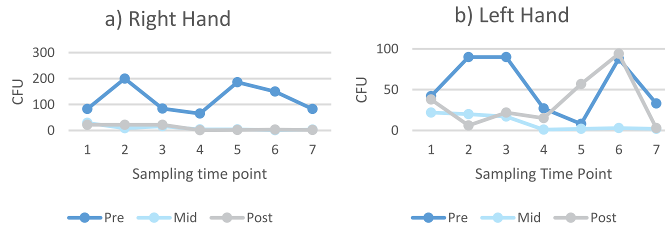


Figure 2: Bacterial Load in Alcoholic Hand Sanitizers (A, B), in Non-Alcoholic Hand Sanitizers (C, D), and in Lab-Prepared Hand Sanitizers (E, F)

A paired comparison plot with Tukey's test between the right and left hands of the participant before and after the utilization of alcoholic hand sanitizers. On the right and left hands, there was a significant difference between the efficacy of hand sanitizers before and after use. The $p<0.0001$ are indicated with asterisks (*). The means and standard errors are depicted by bars for each treatment. The main effect was significant in both product (Right: $F(6,42) = 3084$, $p<0.0001$; Left: $F(6,42) = 3084$, $p<0.0001$) and application time (Right: $F(2,42) = 82502$, $p<0.0001$; Left: $F(2,42) = 82502$, $p<0.0001$), according to the non-alcoholic analysis. The group made up of the pharmaceutical antibiotics Fortum (86.4% +16.1) and Tineazol (95.1% +1.8) and the antiseptic Hi-Clean (95.2% +1.8) showed statistically better, high, and consistent efficacy (Fig. 5a). In the right hand, augmentin's efficacy was likewise high (99.3% decrease), but the left hand's initial confluent development made the result impossible to measure. Saftin, on the other hand, demonstrated significantly lower efficiency (56.4% ± 12.4%). Following the treatment, the bacterial load was observed to have significantly decreased ($p<0.0001$). Non-alcoholic agents showed a sharp initial reduction at mid-sampling, but their efficacy diverged significantly at post-sampling, with Hi-clean and the antibiotics Tineazol and Augmentin maintaining low counts, while others like Saftin and Fortum showed substantial bacterial regrowth (Figure 3).

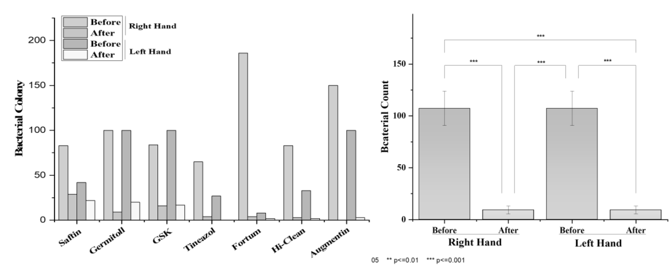


Figure 3: (A): Changes in Bacterial Load on the Right Hand and (B) on the Left-Hand Following Application of Various Non-Alcoholic Hand Sanitizers

The analysis of ten lab-prepared sanitizers revealed significant main effects on formula and application time ($p<0.0001$ in all hands). The Pink Alum Sanitizer (93.0% ± 8.7%) and Eucalyptus oil sanitizer (50.0% ± 23.6%) showed a high and consistent level of efficiency, according to the

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WHO requirement (A). Following application, the category's overall bacterial load reduction was found to be considerable (p<0.0001) (B). The efficacy of lab-prepared sanitizers was highly variable across time points: while Pink Alum and WHO formulations showed a sharp reduction at mid-sampling that was sustained at post-sampling, others, like Lavender Oil, showed increased growth at mid- and post-sampling compared to pre-application baselines (Figure 4). A paired comparison plot with Tukey's test between the participant's right and left hands before and after using alcoholic hand sanitizers. On the right and left hands, there was a significant difference between the efficacy of hand sanitizers before and after use. The p-values (p<0.0001) are indicated with asterisks (*). The means and standard errors are depicted by bars for each treatment.

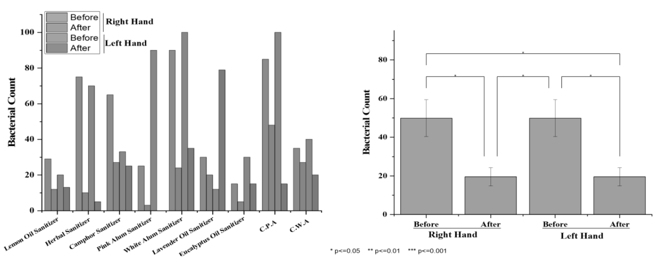

Figure 4: (A) The Efficacy of Different Lab-Made Hand Sanitizers on Participants, and (B) The Statistical Analysis of the Efficacy of Alcoholic Hand Sanitizers

Results after testing the effect of different types of hand sanitizers on bacterial colonies showed that Alcoholic sanitizers were the most potent, inhibiting the growth of all the bacteria, while Non-alcoholic sanitizers were found to be less effective on bacterial colonies (A). Lab-prepared sanitizers showed similar efficacy to the alcoholic ones. Alcoholic sanitizers showed sensitivity against many bacteria with 87% efficacy, non-alcoholic sanitizers showed 48% efficacy, and lab-prepared sanitizers showed 61% efficacy. Their susceptibility to bacterial species is shown as Alcoholic Sanitizers > Lab-prepared Sanitizers > Non-alcoholic Sanitizers. On the right and left hands, there was a significant difference between the efficacy of hand sanitizers before and after use. A paired comparison plot with Tukey's test between the participant's right and left hands before and after using alcoholic hand sanitizers (B). The p-values (p<0.0001) are indicated with asterisks (*). The means and standard errors are depicted by bars for each treatment. (Figure 5).

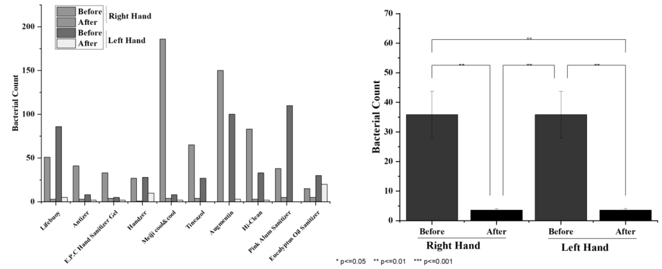

Figure 5: (A) Three Categories of Hand Sanitizers and (B) A Paired Comparison Plot with Tukey's Test

DISCUSSION

The main goal of this study was to evaluate the efficacy of various hand sanitizers in controlling the growth of microbes on the hands of bank cashiers. Interestingly, the results showed distinct differences in the effectiveness of these sanitizers. According to the findings, all three sanitizers reveal contrasting effectiveness differences. The alcoholic sanitizers were the most effective solution in controlling the growth and reducing the bacterial colonies on the hands of bank cashiers. Our central finding confirms the superior performance of alcoholic formulations, with products like Lifebuoy and Meiji Cool and Cool achieving up to 99.9% bacterial reduction, and is supported by [15]. The significant reduction in Colony Forming Units (CFUs) post-application (p<0.0001) underscores their reliability for fast and effective hand hygiene in such environments. The effectiveness of alcoholic sanitizers was mainly due to the presence of active ingredients like ethanol and isopropyl alcohol that have proven antimicrobial properties [16-18]. Alcohol disintegrates the cell membrane in a cell and denatures the protein, thereby effectively reducing the activity of the microorganism [19, 20]. Only due to which the alcoholic sanitizers were able to achieve a 99.9% kill rate for bacteria [21]. Researchers have already reviewed and demonstrated how effective alcohol-based sanitizers were in breaking the protein material in the cell wall of enveloped viruses like SARS-COV-2 [21]. These findings resonate with the previous studies that showed the superior efficacy of alcoholic sanitizers in different scenarios, either healthcare or community usage [16, 22]. Although non-alcoholic sanitizers were effective to a certain extent but not potent enough, as they demonstrated lower efficacy than their alcoholic counterparts. Among non-alcoholic sanitizers, Hi-Clean (95.2% ± 1.8%) showed the highest efficacy and demonstrates that effective non-alcoholic chemical antiseptics do exist and can be highly effective when properly formulated [23]. The control of bacterial colonies was significant but not as fine as previously, which shows that further improvements can be made with the formulations. The divergent post-application efficacy among non-alcoholic agents, where dedicated antiseptics

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like Hi-clean performed consistently while some antibiotics failed to sustain effect, highlights that initial microbial reduction does not guarantee residual protection and underscores the unsuitability of pharmaceuticals for hand hygiene. The ingredients like Co-amoxiclav and folic acid in the non-alcoholic sanitizers indicate the presence of anti-microbial properties [24]. However, we can conclude from their results that they are better suited for environments with a low risk of microbial transmission. Surprisingly, Lab-made herbal sanitizers exhibited a great range of potency towards microbial colonies, thereby decreasing their growth as compared to the alcoholic sanitizers. These solutions contain active ingredients of pink alum and eucalyptus oil, which were more effective in reducing bacterial count than non-alcoholic sanitizers. According to WHO guidelines, statistical analysis revealed significant main effects ($p<0.0001$) of the Pink Alum Sanitizer (93.0% ± 8.7%) due to its antimicrobial property of alum (potassium aluminum sulfate) [25], which is statistically comparable to the alcoholic sanitizers. Eucalyptus Oil Sanitizer showed a significant reduction in bacterial growth in addition to its moderate efficacy (50.0% ± 23.6%). The Aloe vera extracts have previously been studied to exhibit a great number of antimicrobial properties [26, 27]. The presence of Aloe vera and other oil derivatives in herbal sanitizers effectively eliminates bacteria as they are active compounds found in plants [28]. For instance, the neem contains an active compound called azadirachtin that has both antimicrobial and anti-fungal properties [29, 30]. They are biologically active materials but require greater exposure time to show their effectiveness. The volume of sanitizer used (mostly 3 ml) and the time for sanitizer exposure also play a key role in killing these pathogens. Environmental factors like heat and humidity also disrupt the effective ability of sanitizers [15]. The inference from these findings is significant as this study can control microbial infection in high-risk environments, such as banks, using the herbal sanitizers, too. According to the results, this study can conclude that the herbal sanitizers can be used as a viable alternative to the alcoholic derivatives, particularly for individual's sensitive to alcohol-based products. In the future, further development on its formulations, standardization, and quality assurance can lead to maximum usage in hospitals, clinics, and banks. The herbal sanitizers would be cost-effective than the alcoholic sanitizers, too, due to which they will be more accessible to the general public than alcohol-based sanitizers. The purpose of this study was to provide a better option for hand hygiene. WHO has made guidelines on how to wash your hands properly and which are the best products to wash your hands [31]. After the 2020 covid pandemic, the need for hand hygiene has increased. WHO provided a detailed, thorough analysis of which ingredients are best to kill these pathogens [11]. By following the WHO guidelines on hand hygiene [32], one can contribute further to herbal sanitizers [1]. The current study has proved the potency of our lab-prepared sanitizer. Further research and development can lead to improved hand hygiene practices, thereby reducing the ratio of transmission of viral diseases.

CONCLUSIONS

According to this study, alcoholic hand sanitizers are the best at quickly reducing the microbial load on bank cashiers' hands. However, it also shows great potential in lab-prepared herbal sanitizers, where certain alum compositions are just as effective as some commercial ones. The findings support the continued use of alcohol-based hand sanitizers as the primary approach to hand hygiene in high-risk environments. However, as the field advances, standardized herbal formulations will be accessible as practical alternatives for those who are sensitive to alcohol-based products or in areas where commercial sanitizers are difficult to find.

Authors Contribution

Conceptualization: BNK
Methodology: ZM, BNK, MHUR, UEB
Formal analysis: BNK, MHUR, HM, AN
Writing review and editing: RZ, HM, AN

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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