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Antibacterial assessment of antiseptic and herbal soaps available in Sri Lanka against selected skin pathogens

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Abstract Antiseptic and herbal soaps labeled as antibacterial are widely available in Sri Lanka, yet their antimicrobial efficacy against skin pathogens is not well documented. This study aimed to evaluate the antibacterial activity of eight selected soap samples, including two widely available commercial brands, two doctor-prescribed antiseptic soaps, and four herbal soaps, against selected skin pathogens. Agar well diffusion and broth dilution assays were used to measure antibacterial activity and determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values. Test organisms included a common skin pathogen *Staphylococcus aureus* (ATCC 25923), as well as *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (ATCC 700603) known to be associated with skin infections in immunocompromised people. Among these, only *S. aureus* revealed zones of inhibition (ZOI) in the agar diffusion assay against all tested soap samples. Antiseptic soaps prescribed by physicians exhibited maximum antibacterial activity with the highest ZOI (15 ± 1.0 mm), while herbal soaps revealed comparatively less activity, with the lowest zone of (9.2 ± 0.3 mm) at the concentration of 150 mg/mL. The MIC values for *P. aeruginosa* and *K. pneumoniae* ranged from 25 to 100 mg/mL, while those for *S. aureus* ranged from 12.5 to 50 mg/mL. In broth dilution tests, all soap samples demonstrated both bacteriostatic and bactericidal activity, indicating their potential role in controlling skin infections caused by these pathogens. However, Further studies are needed to evaluate their efficacy against a broader range of microorganisms responsible for skin-related infections.

Keywords: Bacteria, Soaps, Skin infections, Zones of inhibition, Agar well diffusion, *Staphylococcus aureus*

1 Introduction

Skin infections are a common public health concern in many developing countries, especially tropical regions, due to favorable temperatures and humidity for the growth

of microbes (Igbeneghu *et al*. 2013). According to a community-based study conducted in Sri Lanka, 47.6% of people in a suburban population had skin disorders, with dermatitis and fungal infections being the most common, highlighting significant skin conditions in the region (Perera *et al*. 2000). Though less commonly reported, bacterial skin infections are nonetheless clinically significant due to their severity and increasing antibiotic resistance.

Various environmental microorganisms can come into contact with human skin, particularly in the absence of proper hygiene. Some can directly cause infections, while others become toxic when introduced into wounds or transferred via contaminated surfaces (Beumer and Kusumaningrum 2003). Among the bacteria, *Staphylococcus aureus* is a major cause of skin and soft tissue infections. When the skin barrier is compromised, it becomes invasive, although it often colonizes the skin and nasal passages. (Jones *et al*. 2007, Larru and Gerber 2014, Santos Junior *et al*. 2022). The Gram-negative bacillus *Pseudomonas aeruginosa* is known for its ability to form biofilms and its resistance to many antibiotics (Strateva and Yordanov 2009). Although it primarily affects immunocompromised individuals, it can also cause superficial infections in healthy people, such as hot tub folliculitis (Wu *et al.* 2011). *Klebsiella pneumoniae* is another opportunistic pathogen associated with hospital-acquired infections and severe skin complications in immunosuppressed individuals (Park *et al*. 2004).

The clinical importance of these organisms has raised concerns about their resistance to widely used antibiotics. Antimicrobial resistance is becoming more prevalent in Sri Lankan hospitals and communities, based on recent studies. According to a community-based study conducted among university students, extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* or Klebsiella species were found in 15% of participants, and methicillin-resistant *Staphylococcus aureus* (MRSA) colonization was detected in 4.3% of healthy individuals, highlighting the spread of resistant bacteria outside of clinical settings (Gunasekara *et al*. 2021). A 2024 surveillance study at a major teaching hospital revealed that 35.9% of *S. aureus* isolates were MRSA and 65.8% of Enterobacteriaceae isolates produced ESBLs. Additionally, carbapenem resistance was present in 7.78% of these Gram-negative isolates. Senanayake *et al*. (2024) reported that 29.48% of *Pseudomonas* species were resistant to fluoroquinolones or aminoglycosides, and 8.54% were resistant to carbapenems. These findings emphasize how crucial it is to implement efficient infection control measures and highlight the potential role of antimicrobial soaps in preventing the spread of skin infections that are resistant to treatment.

Current antimicrobial resistance trends are making it harder to treat these organisms, which raises the possibility of persistent or chronic infections. In this sense, using an effective antibacterial soap may reduce the bacterial load of the skin and lower the risk of infection. In addition, soap can significantly slow the emergence and spread of resistance by promoting personal hygiene and reducing the need for antibiotics. However, some antimicrobial agents used in personal hygiene preparations, such as triclosan, have long-term effects that have raised concerns because they may cause cross-resistance if misused (Yazdankhah *et al*. 2006).

Formulations of soaps evolved over time to include synthetic antiseptic agents and plant-derived compounds that exhibit antibacterial properties. Antiseptic soaps are usually formulated with chemical agents such as triclocarban or chlorhexidine, which are designed to kill or inhibit a broad spectrum of bacteria. On the other hand, herbal soaps are enriched with phenolic compounds, natural extracts, or essential oils that exhibit antimicrobial activity by disrupting microbial membranes and interfering with cellular functions (Ogunnowo *et al*. 2010, Ribeiro *et al*. 2015, Khosrowpour *et al*. 2019). These differences in composition would result in varied levels of effectiveness.

Both herbal and antiseptic soaps are widely accessible and promoted with antibacterial claims in Sri Lanka. While only a few preliminary studies exist (Samaraweera *et al*. 2023), there is a lack of scientific comparisons that evaluate their actual antibacterial activities against common skin pathogens. This lack of evidence affects consumer purchasing decisions, limits the ability of health professionals to make evidence-based recommendations, and may result in continued use of potentially ineffective products for preventing skin infections or reducing the spread of bacteria.

In Sri Lanka, where growing rates of antibiotic resistance underscore the necessity of efficient, evidence-based hygiene practices, assessing the antibacterial qualities of these soaps is vital. Using herbal preparations for personal hygiene is another cultural tradition in Sri Lanka. Thus, evaluation of the antibacterial qualities of these herbal soaps may offer a scientific understanding of well-known cultural customs. There is limited openly accessible scientific data available assessing these products using standard microbiological techniques, despite their widespread use and extensive antibacterial claims. Therefore, filling this evidence gap and producing locally relevant evidence on the efficacy of these hygiene products is essential for assisting the public and medical professionals in making well-informed decisions.

This study was therefore conducted to evaluate the antibacterial activity of antiseptic and herbal soaps available in Sri Lanka against selected skin pathogens. Specifically, the study aimed to assess the antibacterial effects of doctor-prescribed antiseptic soaps, commercially available antiseptic soaps, and herbal soaps against *S. aureus*, *P. aeruginosa*, and *K. pneumoniae*. In addition, the study compared their relative effectiveness using zone of inhibition, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) values.

2 Material and Methods

This study was conducted as a laboratory-based experimental study at the Biomedical Science Laboratory of Kaatsu International University (KIU), Battaramulla, Sri Lanka.

2.1 Sample selection and collection

Eight samples of soap were selected and categorized into antiseptic and herbal groups, both of which were labeled by their manufacturers as having antibacterial properties. Although the sample size is limited, this selection was designed to represent a cross-

section of products commonly available to Sri Lankan consumers, balancing resource constraints with product variety.

For the antiseptic category, the two most available commercial soaps (C1 and C2) were chosen based on documented purchase frequency and consistent availability across five major supermarkets (coded Supermarkets A–E) in the Battaramulla area. Monthly sales data collected in December 2022 confirmed that these two brands were the most frequently purchased antiseptic soaps among all observed outlets. Two prescription antiseptic soaps (D1 and D2) were also included based on direct recommendations by practicing pharmacists and dermatologists. Four brands of herbal soaps (H1–H4) were selected on the basis of packaging claims of antibacterial activity and plant ingredient content with reported or known antimicrobial activity. Two of these were bought from supermarket shelves, and the other two were purchased through a major Sri Lankan online retail platform to address regional variation in availability. Although a convenience sampling strategy was used, the selection criteria were designed to reflect commonly used and easily accessible herbal soap products for Sri Lankan consumers. This method aimed to capture typical consumer products within the limits of product availability. All soap samples were anonymized and stored under ambient conditions before analysis.

During the selection process, samples were carefully examined for their ingredients. However, as exact concentrations of active components were not disclosed by all manufacturers, the analysis was limited to publicly available information (Table 1).

Table 1: Selected soaps and their active ingredients as per label disclosure.

Category of soap	Soap sample	Listed ingredients as per product label disclosure
Most commercially available antiseptic soaps	C1	Sodium Palmate, Sodium Palm Kernelate Triclocarbon, Talc, Tetrasodium EDTA
	C2	Sodium Soap Base, Talc, Pine oil, Chloroxylenol, Sodium C14-16 Olefin Sulfonate.
Doctor-prescribed antiseptic soaps	D1	Coal tar extract, Salicylic acid, Sodium Lauryl Sarcosinate, Corbopol, Aloe vera extract
	D2	Coal tar extract, Salicylic acid, Sorbitol, Corbopol, Carpric acid, Lauric acid, Caprylic acid.
Herbal soaps	H1	Sodium Palmate, Sodium Palm Kernelate, Lemongrass oil, Lemongrass root powder
	H2	Sodium Palmate, Sodium Palm Kernelate Turmeric, *Erythrina Variegata* (Erabadu) Leaf Extract, *Cassia Fistula* (Ehela) Leaf Extract, *Indigofera Tinctoria* (Nil-Awari) Leaf Extract, *Mimusops Elengi* (Munamal)
	H3	Sodium Palmate, Sodium Palm Kernelate Eucalyptus, Neem, Lavender, Lemon, Roamary & Peppermint, Licorice, Moringa
	H4	Sodium Palmate, Sodium Palm Kernelate Peppermint, Clove, Eucalyptus, Camphor

2.2 Collection of reference bacterial strains

The reference bacterial strains *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), and *Klebsiella pneumoniae* (ATCC 700603) were collected from the Biomedical Science Laboratory of Kaatsu International University (KIU), Battaramulla, Sri Lanka. Further, the bacterial strains were incubated for 24 hours at 37°C after being separately inoculated onto nutrient agar (CM 0148) plates. The subculture of organisms onto nutrient agar was maintained at 4°C to preserve viability and prevent overgrowth during storage (Teniola *et al*. 2019).

2.3 Preparation of test samples

With the help of sterile blades, soaps were scraped, and 0.5 g, 1.0 g, and 1.5 g of each soap sample were weighed and dissolved in 10 mL of sterile distilled water separately to obtain 50 mg/mL, 100 mg/mL, and 150 mg/mL, respectively (Teniola *et al*. 2019).

2.4 Agar well diffusion method

The test organisms from an overnight culture plate incubated at 37°C were suspended in a saline solution (0.85% NaCl) and adjusted to match a turbidity of 0.5 McFarland Standard (Olajuyigbe *et al*. 2017). The standardized test organism suspension was inoculated on the surface of sterile Mueller-Hinton agar (MHA) (REF24756) plates using a sterile cotton swab (CLSI 2012). Five wells, each with a diameter of 6 mm were then punched on the agar plate using sterile pipette tips (200 µL and 100 µL). Different concentrations of soap samples were added to three of the wells using a micropipette. Other wells were filled with positive and negative controls.

Gentamicin antibiotic intravenous fluid was used as the positive control, diluted to 0.1 mg/mL. This concentration was chosen based on preliminary testing, which showed that it produced clear and consistent inhibition zones without being excessively large, thereby allowing fair comparison with the soap samples. Gentamicin was selected to use as the positive control due to its broad-spectrum activity against the Gram-positive and Gram-negative skin pathogens examined in this study, including *S. aureus*, *P. aeruginosa*, and *K. pneumoniae* (CLSI 2024 a, b). Its routine use as a reference antibiotic in agar diffusion and broth dilution susceptibility tests recommended by CLSI (M02, M07) supported its selection as a suitable standard for determining the antibacterial activity of the soap samples. Sterile distilled water was used as the negative control.

The plates were allowed to stand for 30 minutes before proceeding. Subsequently, the plates were incubated at 35 ± 2 °C for 18-24 hours. The plates were examined after incubation, and the diameter of each zone of inhibition (ZOI) was measured in millimeters. The mean ZOI value from three replicates was reported for each concentration (Abbas *et al*. 2016, Balouiri *et al.* 2016)

2.5 Quality Control and Experimental Conditions

All culture media were prepared based on the manufacturer's guidelines, and standard aseptic techniques were followed for all the microbiological procedures. All experiments were conducted in triplicate on separate days to take day-to-day variation into account and to ensure the reproducibility of results. For minimizing experimental bias, the order of testing bacterial strains and soap samples was varied across replicates. All incubations were conducted in temperature-controlled incubators at 35 ± 2°C under controlled relative humidity of approximately 35-55% to maintain stable environmental conditions throughout the experiment.

2.6 Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Minimum inhibitory concentration (MIC) for all the soap samples was determined by broth macro-dilution as per CLSI (2002) guidelines and previous studies (Obi 2014, Balouiri *et al*. 2016). The stock solution of 400 mg/mL was prepared by dissolving 4.0 g of soap in 10 mL of sterile distilled water. A two-fold serial dilution technique was selected to prepare a series of working concentrations from this original stock solution. In an aseptic setting, 1 mL of nutrient broth was poured into six Khan tubes. To tube 1, 1 mL of the 400 mg/mL soap solution was added and mixed well, resulting in a final concentration of 200 mg/mL. 1 mL was removed from this tube and poured into tube 2 with 1 mL of nutrient broth to have a 1:2 dilution (100 mg/mL). This serial dilution was performed to yield subsequent concentrations of 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL, using sterile micropipettes under aseptic conditions. All the dilution tubes were inoculated with 100 µL of the test bacterial suspension, which was diluted to correspond to a 0.5 McFarland standard. Gentamicin intravenous solution (0.1 mg/mL) was used as the positive control due to its broad spectrum of activity and CLSI-recommended availability in antimicrobial panels. Sterile distilled water was used as the negative control. All the tubes were incubated at 35 ± 2 °C for 24 hours.

The MIC was defined as the lowest concentration of soap at which no visible bacterial growth (turbidity) was observed. Each test was conducted in triplicate, and mean MIC values were recorded. To determine the minimum bactericidal concentration (MBC), one loopful from each non-turbid broth tube was streaked onto fresh nutrient agar plates and incubated at 35 ± 2 °C for 24 hours. The MBC was recorded as the lowest soap concentration at which no bacterial colonies appeared on the agar surface (Obi 2014).

2.7 Data analysis

Data collected from the agar well diffusion assay were tested for normality with the Shapiro–Wilk test and revealed a non-normal distribution. Thus, non-parametric tests

were used. The Kruskal–Wallis H test was used to assess statistically significant differences in zone of inhibition (ZOI) among different soap categories and concentrations against *S. aureus*. Where significant differences were detected, post hoc pairwise comparisons with adjustment for multiple testing were performed. Analyses were conducted using SPSS version 22. Statistical significance was set at $p < 0.05$.

3 Results

Agar well diffusion test results are shown in Table 2. Tested soap samples exhibited their antibacterial action against *S. aureus*, but with varying degrees of inhibition depending on the category and concentration of the soap. However, none of the soap samples demonstrated inhibition zones against *P. aeruginosa* or *K. pneumoniae* at any tested concentration, although they exhibited measurable MIC and MBC values in broth-based assays.

Samples D1 and D2, which belong to the category of doctor-prescribed antiseptic soaps, exhibited the maximum antibacterial action, yielding the highest Zone of Inhibition (ZOI) of 15±1.0 mm. On the other hand, most of the herbal soaps had comparatively less activity, and the lowest ZOI of 9.2±0.2 mm was found with sample H4 at 150 mg/mL. Samples C1 and C2, which are the most commercially available antiseptic soaps, also demonstrated antibacterial activity, with the highest ZOI of 12.3±0.3 mm observed for this category at a concentration of 150 mg/mL. The ZOI of the commercially available antiseptic soaps, however, was lower than that of the antiseptic soaps prescribed by physicians. Yet, none of the test samples showed any inhibition zones against *P. aeruginosa* and *K. pneumoniae* when using the agar well diffusion method.

Overall, the zone of inhibition (ZOI) values increased with increasing concentration levels across all categories, especially between 50 and 150 mg/mL. At higher concentrations, some herbal samples, such as H4, exhibited only slight inhibition (up to 9.2 ± 0.2 mm), with responses either plateauing or only slightly increasing. On the other hand, samples D1 and D2, which belonged to the doctor-prescribed soap category, displayed a stronger dose-response relationship.

Kruskal-Wallis analysis revealed statistically significant variance ($p<0.05$) in the antibacterial activity between the soap types, with doctor-prescribed antiseptic soaps exhibiting significantly higher activity than commercial and herbal soaps. No significant difference was observed between the two soaps of the same group in each type. Soap concentration also significantly influenced ZOI values ($p < 0.05$), with higher concentrations (150 mg/ml) consistently producing larger zones of inhibition.

Considering the differences in methodology and sensitivity between the selected test methods, the concentration range used with the broth-based MIC and MBC determination was extended beyond that of the agar diffusion test to ensure detection of bactericidal and inhibitory activities against selected skin pathogens.

Table 2: Zones of Inhibition of the tested soaps against the mentioned bacteria by the well-diffusion method.

Soap Category	Soap Sample	Concentration of Soap Samples	Diameters of Zone of Inhibitions (mm)		
			Staphylococcus aureus	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*
Most commercially available soap	C1	50 mg/mL	9.2 ± 0.3	-	-
		100 mg/mL	10.0 ± 0	-	-
		150 mg/mL	12.3 ± 0.3	-	-
		+ control	30.5 ± 0.9	23.7 ± 0.6	27.3 ± 0.5
		- control	-	-	-
	C2	50 mg/mL	9.0 ± 0	-	-
		100 mg/mL	10.2 ± 0.3	-	-
		150 mg/mL	12.2 ± 0.3	-	-
		+ control	30.7 ± 1.2	23.8 ± 0.2	27.8 ± 0.2
		- control	-	-	-
The Doctor Prescribed soap	D1	50 mg/mL	12.2 ± 0.3	-	-
		100 mg/mL	13.3 ± 0.3	-	-
		150 mg/mL	15.0 ± 0	-	-
		+ control	30.7 ± 1.2	23.6 ± 0.5	27.3 ± 0.5
		- control	-	-	-
	D2	50 mg/mL	12.1 ± 0.2	-	-
		100 mg/mL	13.3 ± 0.6	-	-
		150 mg/mL	15.0 ± 1	-	-
		+ control	30.7 ± 1.2	24 ± 0.8	27.5 ± 0.8
		- control	-	-	-
Herbal Soap	H1	50 mg/mL	10.3 ± 0.3	-	-
		100 mg/mL	11.3 ± 0.3	-	-
		150 mg/mL	11.7 ± 0.5	-	-
		+ control	30.5 ± 0.9	25.3 ± 0.5	27.6 ± 0.5
		- control	-	-	-
	H2	50 mg/mL	9.0 ± 0	-	-
		100 mg/mL	9.7 ± 0.3	-	-
		150 mg/mL	10.2 ± 0.3	-	-
		+ control	30.3 ± 0.6	23.6 ± 0.5	27.3 ± 0.5
		- control	-	-	-
	H3	50 mg/mL	10.2 ± 0.6	-	-
		100 mg/mL	10.8 ± 0.3	-	-
		150 mg/mL	11.2 ± 0.3	-	-
		+ control	30.0 ± 0	24 ± 0.2	27.1 ± 0.5
		- control	-	-	-
	H4	50 mg/mL	9.0 ± 0	-	-
		100 mg/mL	9.0 ± 0	-	-
		150 m/mL	9.2 ± 0.2	-	-
		+ control	30.5 ± 0.8	23.5 ± 0.7	27.8 ± 0.8
		- control	-	-	-

Based on the results shown in Table 3, all the soap samples examined were found to be bacteriostatic and bactericidal against the three skin pathogens. Against *S. aureus*,

D1 and D2 recorded the lowest MICs of 12.5 mg/mL. MIC values against *P. aeruginosa* and *K. pneumoniae* ranged from 12.5 mg/mL to 100 mg/mL, depending on the tested soap sample. Among doctor-prescribed antiseptic soaps, D1 exhibited the least effectiveness against *P. aeruginosa* and *K. pneumoniae*, with MICs and MBCs of 100 mg/mL and 200 mg/mL, respectively. Notably, D2 demonstrated lower MICs and MBCs against all the pathogens tested, showing broader activity. Herbal and commercially available antiseptic soaps particularly possessed MIC and MBC values within 25–50 mg/mL (MIC) and 50–100 mg/mL (MBC). However, H1 was found to be the most potent herbal soap sample, with MIC and MBC values of 25 mg/mL and 50 mg/mL, respectively, comparable to the most commercially available soap samples (C1 and C2), which also showed MICs of 25 mg/mL and MBCs of 50 mg/mL for all three organisms.

Table 3: MIC and MBC values of tested samples against selected bacteria species by agar-diffusion method.

Soap Category	Soap Sample	Organism	MIC (mg/mL)	MBC (mg/mL)
Most commercially available soap	C1	*Staphylococcus aureus*	25	50
		Pseudomonas aeruginosa	25	50
		Klebsiella pneumoniae	25	50
	C2	*Staphylococcus aureus*	25	50
		Pseudomonas aeruginosa	25	50
		Klebsiella pneumoniae	25	50
Doctor Prescribed soap	D1	*Staphylococcus aureus*	12.5	25
		Pseudomonas aeruginosa	100	200
		Klebsiella pneumoniae	100	200
	D2	*Staphylococcus aureus*	12.5	25
		Pseudomonas aeruginosa	12.5	25
		Klebsiella pneumoniae	25	50
Herbal Soap	H1	*Staphylococcus aureus*	25	50
		Pseudomonas aeruginosa	25	50
		Klebsiella pneumoniae	25	50
	H2	*Staphylococcus aureus*	25	50
		Pseudomonas aeruginosa	50	100
		Klebsiella pneumoniae	50	100
	H3	*Staphylococcus aureus*	50	100
		Pseudomonas aeruginosa	50	100
		Klebsiella pneumoniae	50	100
	H4	*Staphylococcus aureus*	50	100
		Pseudomonas aeruginosa	50	100
		Klebsiella pneumoniae	50	100

4. Discussion

Soaps are commonly used for hygienic purposes and serve as an important barrier to infections by reducing the microbial burden on the skin (Burton *et al.* 2011). This study evaluated the antibacterial efficacy of various antiseptic and herbal soaps available in Sri Lanka against selected skin pathogens. The qualitative (agar well diffusion) and quantitative (MIC and MBC) assays were utilized to assess their activity.

This study revealed that the antibacterial efficacy varied depending on the type of soap and the bacterial strain tested. A clear concentration-dependent pattern was observed in most tested samples, especially in the doctor-prescribed antiseptic soaps (D1 and D2). As soap concentration increased from 50 mg/mL to 150 mg/mL, the zone of inhibition (ZOI) generally increased, although this trend was especially evident in doctor-prescribed antiseptic soaps (D1 and D2), while herbal soaps showed more modest responses. Soap H1, however, demonstrated relatively strong antibacterial activity among the herbal category.

The variability in ZOI results seen in the study may be attributed to the nature and concentration of active ingredients, formulation type, or physical properties such as viscosity and density in each soap. This study did not chemically quantify active components. Therefore, such aspects are noted as possible contributing factors rather than confirmed causes. According to the prior studies, the physical characteristics of formulations can influence diffusion through solid media, affecting zone size and antimicrobial interpretation (King *et al.* 2008).

The findings were not entirely consistent when comparing the outcomes of agar diffusion tests and broth dilution assays. All of the tested samples showed strong antibacterial activity against *S. aureus* in both techniques, but they performed differently against *P. aeruginosa* and *K. pneumoniae.* Diffusion rates, interactions with agar components, microbial growth conditions, and the potential evaporation or degradation of active agents during the diffusion process are some of the variations between the two methods that could account for these discrepancies. The antibacterial compounds must diffuse through the agar in order to reach the target organisms in the agar well diffusion assay; if they degrade or evaporate during this process, there may be fewer or no inhibition zones (King *et al*. 2008). Furthermore, the higher susceptibility of Gram-positive bacteria to the agents being tested may also be related to the distinct inhibition zones observed exclusively for *S. aureus*. Additionally, the outer membrane of Gram-negative bacteria may act as a barrier, further limiting the penetration and functionality of antimicrobial compounds, potentially leading to the loss of distinct inhibition zones. Stronger inhibition of *S. aureus* compared to Gram-negative pathogens has also been reported in prior studies assessing the effectiveness of soap (Chaudhari 2016).

Despite being classified as doctor-prescribed soaps, D1 and D2 demonstrated varying levels of antibacterial activity based on MIC and MBC values, especially against Gram-negative bacteria. Among all the soaps tested, D1 had the highest MIC (100 mg/mL) and MBC (200 mg/mL) values against *P. aeruginosa* and *K. pneumoniae*, suggesting its comparatively less antibacterial activity than D2. Based on our

observation, the high density and viscosity of D1 may have affected its ability to disperse properly in the broth medium, which may have limited its diffusion and availability to reach the target organisms. Whereas this could be a contributing factor, formulation variability or the intrinsic nature of the active ingredient could also have been responsible for its reduced activity against Gram-negative bacteria.

There are several imitations that should be acknowledged in this study. The bacterial strains and soap samples used in the current study were limited in quantity to provide methodological parity to facilitate sufficient replicates to guarantee the quality of results under controlled conditions in the laboratory. While the sample size was limited, care was taken when choosing the product types to cover a wide variety of soap types commonly marketed to Sri Lankan consumers. Additionally, *Streptococcus pyogenes*, a clinically important Gram-positive bacterium and a common skin pathogen was initially considered for the testing but had to be excluded from the analysis. This decision was based on consistent observation obtained during preliminary testing, where the interaction between soap samples and blood agar resulted in clear ring-shaped zones similar to bacterial inhibition, but most likely due to lysis of red blood cells rather than true antimicrobial activity. Therefore, due to the interpretability issues resulting from the inability to differentiate between hemolysis and actual inhibition, *S. pyogenes* was eliminated from the study in order to preserve the validity of the findings.

Preliminary testing and prior research indicated that the concentrations used in this study (50–150 mg/mL) were practical for accurate pipetting and effective for detecting antibacterial action (Abbas *et al*. 2016). Since higher viscosity and density can impede accurate pipetting and efficient sample distribution, potentially compromising the accuracy of the experimental results, higher concentrations were avoided. (King *et al*. 2008).

The results of this study demonstrate that samples from both antiseptic and herbal categories exhibit differing levels of antibacterial activity against the tested skin pathogens, *S. aureus*, *K. pneumoniae*, and *P. aeruginosa*. Although this activity was not uniformly effective against all tested bacterial strains, the samples all showed susceptibility to *S. aureus*, suggesting that they might contribute to preventing infections caused by this pathogen, including common skin conditions like cellulitis, boils, and deep-seated abscesses (Ikegbunam *et al*. 2013, Larru and Gerber 2014, Santos Junior *et al*. 2022). However, it is also important to consider the long-term safety and effects of these antibacterial soaps on skin microbiota and their contribution to the emergence of microbial resistance. It has been shown through previous studies that uncontrolled or prolonged usage of triclosan or triclocarban products has been linked to the development of resistance and alteration of the skin microbiota (White and McDermott 2001, Mwambete and Lyombe, 2011). These challenges highlight the need for evidence-based use of antimicrobial cleaning products, especially in non-clinical, general settings. In this context, Soaps formulated from natural products can offer safer options for long-term application if carefully examined. Nevertheless, more studies are required to determine their long-term safety and effectiveness on a wider range of microbes that are responsible for skin infections (Kumar *et al*. 2014, Teniola

et al. 2019). Strong microbiological profiling and chemical description must be incorporated throughout further studies to facilitate the development of efficient, resistant-conscious hygiene solutions.

5. Conclusions

In the present study, both antiseptic and herbal soaps demonstrated antibacterial activity against the tested skin pathogens, with the highest susceptibility observed in *S. aureus.* The soaps exhibited both bacteriostatic and bactericidal effects, as evidenced by the MIC and MBC results, indicating their potential for managing infections caused by the tested organisms. Among the tested products, doctor-prescribed antiseptic soaps showed the strongest antibacterial activity. Most commercially available antiseptic soaps also demonstrated considerable effectiveness, though generally less than that of the doctor-prescribed formulations. Herbal soaps, while exhibiting moderate activity, still showed noteworthy inhibitory effects, particularly in selected samples, suggesting their potential as milder alternatives for routine cleansing. However, as the long-term effects of regular use were not evaluated in this study, further research is needed to determine their safety and efficacy over a prolonged period.

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