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ORIGINAL RESEARCH

Determination of Antibacterial Efficacy of Different Soaps Found in the Local Market against Common Pathogenic Bacteria

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ABSTRACT

Objective: This study aimed to evaluate the antibacterial efficacy of different type of soaps against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus* species.

Methods: A laboratory-based experimental study was conducted from February 2024 to July 2024. Eight commonly used soaps (Herbal, Non-medicated, Medicated, Liquid and Laundry) were randomly collected from the local market of Kathmandu valley. The bacterial cultures used for test were standardized to 0.5McFarland. The soap solutions were prepared by dissolving each soap in sterile distilled water (100mg/ml), and two-fold dilution made for MIC testing. Antibacterial activity was assessed by Agar Well Diffusion method using Mueller-Hinton Agar. Wells were filled with 1ml of each prepared soap solution and incubated at 37°C for 24 hours. Zones of Inhibition were measured, and MIC was determined as the lowest dilution showing no visible growth.

Results: Non-medicated soaps showed mean ZOI of 16.7mm (NM1 brand being effective with mean ZOI of 21 mm) and medicated soap showed mean ZOI of 16.1mm. Handwash showed ZOI range between 16-19mm with mean of 17.3mm. Herbal soap has a strong 17mm of mean ZOI with range of 8-23mm. The brand NM1 soap showed most effective against *E. coli* (20mm) and least against *S. aureus* (15mm) with MIC value 1.56mg/ml. Herbal soap showed maximum effect against *E. coli* (20mm) with MIC value 6.25mg/ml and of 3.125mg/ml against *S. aureus*. Other soaps showed MIC in the range from 3.125mg/ml to 50mg/ml.

Conclusion: Non-medicated soap showed the strongest antibacterial activity against *Bacillus* and *E. coli*, while Herbal soap was most effective against *S. aureus*. Difference in efficacy among soaps are attributed to variation in their antimicrobial compound. The finding challenges the idea that medicated soaps are always more effective than non-medicated ones. Soap formulation should prioritize proven antimicrobial efficacy while remaining affordable for widespread use.

Keywords: Soap, Antibacterial efficacy, Zone of inhibition, MIC, Pathogenic bacteria

INTRODUCTION

Soap is the cleaning agent that is available in various forms like bars, liquid and powders. It is the chemical product that has been used by the human since ancient time (Achaw & Danso-Boateng, 2021). Soap is the product made from the mixture of fat, water and alkali such as sodium hydroxide or potassium hydroxide (Jariah et al., 2021). As a common household item, soap has been utilized for thousands of years, with the earlier evidences of and hygiene is one of the most effective measures for preventing the transmission of infectious diseases (Kagan et al., 2002). Soap plays a key role in handwashing because its surfactant properties help remove dirt, oils, and microorganisms from the skin surface (Salager, 2002.). When soap molecules interact with water, they form

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micelles that trap and detach microbes, thereby reducing the risk of infection. In addition to basic cleansing action, some commercial soaps contain antimicrobial agents or herbal extracts that may enhance their ability to inhibit pathogenic bacteria (Collett et al., 2023).

Hand hygiene is considered one of the most effective and affordable public-health measures for reducing the transmission of infectious diseases (Singh et al., 2020). Hands frequently come in contact with contaminated surfaces and serve as a major vehicle for the spread of pathogenic microorganisms. Proper handwashing with soap significantly lowers the burden of enteric and respiratory pathogens by removing transient flora from the skin surface (Caioni et al., 2023). Soap remains the most widely used cleansing agent because of its ability to emulsify oils, dirt, and microorganisms (Huang et al., 2014).

From a theoretical standpoint, the cleansing ability of soap is due to the amphiphilic nature of surfactant molecules produced through the saponification of fats and alkali (Dunn, 2010). These molecules form micelles, which encapsulate lipids and microbes, allowing their removal from the skin during rinsing. In addition to basic surfactants, commercial soaps often incorporate antimicrobial agents, essential oils, herbal extracts, or antiseptic compounds that may enhance the inhibition of bacterial growth (Chaudhary et al., 2020). The presence, concentration, and stability of these additives play an important role in determining the antibacterial efficacy of different soap formulations

Pathogenic organisms such as *E. coli*, *S. aureus*, and *Bacillus* spp are commonly associated with community and environmental contamination (Hoang et al., 2021). These organisms are frequently found on hands, household surfaces, and everyday items, and can cause gastrointestinal, respiratory, and skin infections (Kagan et al., 2002). These organisms represent different structural categories: Gram-negative rods, Gram-positive cocci, and spore-forming bacilli that makes them useful indicators of the broad antimicrobial spectrum of soaps (Srain et al., 2021). Several studies have shown that the inhibitory activity of soaps varies greatly depending on pH, fatty acid composition, active antimicrobial ingredients, and the manufacturing process (Matta et al., 2022). The effectiveness of soap in reducing microbial load depends on its formulation, pH, concentration of active ingredients, and the presence of antimicrobial compounds. Studies have shown that different commercial soaps vary widely in their antibacterial activity, making comparative evaluation important for public health (Bin Abdulrahman et al., 2019).

In Nepal, a wide range of commercial, herbal, antiseptic, and medicated soaps are available, but there is limited scientific information validating their antibacterial claims (Chaudhary et al., 2020). Evaluating the antibacterial activity of commonly used soaps is important for guiding consumer choice, ensuring product quality, and supporting hygiene-related public-health recommendations. Therefore, the present study investigated the antibacterial efficacy of selected commercial soaps available in the Kathmandu Valley against common pathogenic bacteria using standard microbiological techniques.

METHODS

Research type, Study site and duration

This laboratory based experimental study was conducted in the Microbiology Laboratory of Kist college of Management, from February 2024 to July 2024.

Sample type and sampling

A total of eight commercially available soaps of different categories were collected randomly from local markets inside Kathmandu valley using convenient sampling method. All soaps were unused, stored at room temperature and tested within their expiry period.

Inclusion and Exclusion criteria

The soap sample that were purchased from different local shops of Kathmandu valley inside ring road with labeled manufacturing date, expiry date and presence of manufacturer's seal and unused were included in the study. While those soap with broken seal, no labeling of date of manufacture, expiry and used were excluded.

Preparation of selected soaps for Antimicrobial testing

The obtained soaps were taken out aseptically and were scrapped by using a sterile blade. After scrapping, 1gm of each soap sample was weighed and dissolved into 10ml of sterile water to prepare a stock solution of 100mg/ml concentration. Three bacterial species: *E. coli*, *S. aureus* and *Bacillus* spp were selected for evaluating antibacterial activity. These organisms were chosen because they commonly represent Gram negative rods, Gram positive cocci and spore-forming bacilli, respectively. Pure culture was maintained on Nutrient Agar and sub-cultured for 24 hours at 37°C before testing. The bacterial suspension was standardized to 0.5McFarland turbidity. Antibacterial activity was determined using Mueller–Hinton Agar (MHA) and the agar well diffusion method. The MHA plates were lawn-cultured with standardized bacterial inoculum using sterile cotton swabs. Wells of 4 mm diameter were punched aseptically by borer, and 1ml of each soap solution was added to the wells. Plates were allowed to diffuse for 30 minutes at room temperature and were incubated at 37°C for 24 hours. Zones of inhibition were measured in millimeters. The antibacterial efficacy of each soap extract against common pathogenic organisms was compared.

Determination of MIC

The prepared soap solutions with highest antibacterial efficacy was diluted up to seven times (1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128) for the purpose of calculating MIC which was determined by examining the zone of inhibition using several dilutions. Proper sterilized plates, utensils, and equipment are used. Data were recorded manually, and descriptive analysis was performed. Results were expressed as mean zone diameters and MIC values.

RESULTS

All tested soaps exhibited variable inhibitory effects, and the results demonstrated marked differences according to soap formulation and bacterial species. The antibacterial activity of different soap showed that NM1 and medicated soaps were most effective overall. Handwash, laundry soap, and MS2 brand demonstrated moderate antibacterial activity, while NM2, and NM3 were generally less effective. Herbal soap has a strong but selective activity, being highly effective against *E. coli* and *S. aureus* but weak against *Bacillus* spp. For all soaps, higher concentration produced larger zones of inhibition, indicating strong antibacterial effects. NM1 soap showed increasing effectiveness with concentration, being most effective against *E. coli* and least against *S. aureus*. Herbal soap showed concentration dependent activity, with maximum effect against *E. coli*. NM2 and NM3 exhibited higher antibacterial activity particularly against *E. coli*. Handwash became effective with strong effect on *S. aureus*. Medicated soap shows strong activity against *E. coli*, weak against *S. aureus* and least effective on *Bacillus* spp.

Antibacterial efficacy of working solution of different soaps against selected pathogens

Table 1 represents the antibacterial activity of different soaps based on their zone of inhibition (ZOI) against *Bacillus* spp, *S. aureus*, and *E. coli*. Among all, NM1 showed the highest mean ZOI of 21 mm, and MS2 also had strong inhibition mean of 18 mm that follows the activity of HW (17.3 mm ZOI), laundry soap (15.6mm) and MS1 (14.3mm) show moderate activity. NM2 and NM3 brand (14.6 mm) were lesser effective which followed the brand MS1 which had least activity of 14.3mm mean ZOI. The MS2 brand soap showed highest effectivity against *E. coli* with ZOI 25 mm, NM1 was almost equally effective with ZOI 24 mm; while against *S. aureus*, the brand HS was highly effective with ZOI 23 mm and NM1 showed effectivity of 19mm ZOI against *Bacillus* species.

Table 1: Antibacterial efficacy of different soap against selected pathogens

S.N.	Soap	Concentration	Zone of inhibition (ZOI) in mm			Mean ZOI
			Bacillus spp	*S. aureus*	*E. coli*	
1	NM1	100 mg/ml	19	20	24	21
2	NM2		12	13	19	14.6
3	NM3		12	12	20	14.6
4	LS		14	17	16	15.6
5	HW		17	19	16	17.3
6	HS		8	23	20	17
7	Control (CO)		0	0	0	0
8	MS1		10	15	18	14.3
9	MS2		15	14	25	18

NM= Non-medicated soap (NM1= Pears, NM2= Lifebuoy, NM3=Lux, MS= Medicated soap(MS1=Dettol, MS2= Acnoshine), LS = Laundry Soap (Dhoni), HW= Handwash (Savlon), HS= Herbal Soap (Okhati)

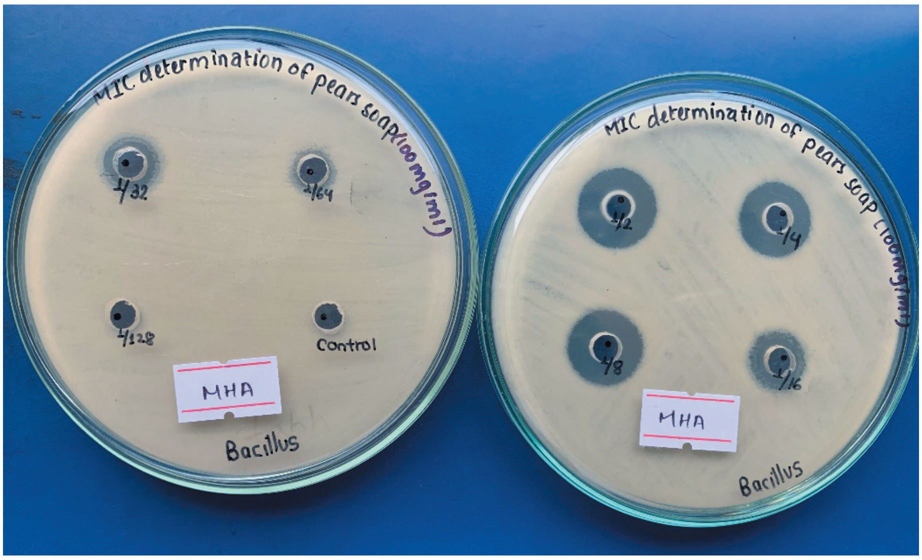

Figure 1: Showing MIC determination of NM1 brand soap against *Bacillus* spp (Right to left dilutions 1:2 to 1:128 MIC= 1.56mg/ml at 1:64 dilution)

Minimum Inhibitory Concentration of Non-medicated Soap (NM1, NM2 and NM3) brand against selected pathogens

Table 2 displayed data on antibacterial properties of Non-medicated soap which showed no antibacterial activity at the most diluted concentration (1:128). Inhibition began at 1:64 for all three bacteria, making the minimum inhibitory concentration for NM1 to be 1.56mg/ml. Likewise, NM2 had MIC against *Bacillus* spp to be 25mg/ml, and 12.5mg/ml against *S. aureus* and *E. coli*. Similarly, NM3 brand showed no antibacterial activity against *Bacillus* spp or *S. aureus* at any dilution. However, it was effective against *E. coli*, with inhibition starting at 1:8 (12mm) denoting the MIC to be 12.5mg/ml against it. As the concentration increased toward 1:2, the ZOI increased across all bacteria. *E. coli* showed the largest ZOI (20mm), making it most susceptible. *Bacillus* spp showed moderate inhibition (17mm), while *S. aureus* had the smallest inhibition zone (15mm) and was least susceptible. Overall, NM1 soap was most effective at higher concentration and showed strongest activity against *E. coli*.

Table 2: Minimum Inhibitory Concentration of Non-medicated Soap (NM1, NM2 and NM3) brand against Common Pathogenic Bacteria

Sample	Concentration	Zone of inhibition (ZOI) in mm		
		Bacillus spp	*S. aureus*	*E. coli*
NM1	1:128	0	0	0
	1:64	9	8	12
	1:32	11	10	14
	1:16	12	11	17
	1:8	14	12	18
	1:4	16	13	19
	1:2	17	15	20
NM2	1:128	0	0	0
	1:64	0	0	0
	1:32	0	0	0
	1:16	0	0	0
	1:8	0	10	12
	1:4	10	12	15
	1:2	11	13	17

Sample	Concentration	Zone of inhibition (ZOI) in mm		
		Bacillus **spp**	***S. aureus***	***E. coli***
	1:128	0	0	0
	1:64	0	0	0
	1:32	0	0	0
NM3	1:16	0	0	0
	1:8	0	0	12
	1:4	0	0	15
	1:2	0	0	16

Minimum Inhibitory Concentration of Herbal soap (HS) and Hand Wash (HW) against selected pathogens

Table 3 showed the antibacterial properties of Herbal soap (HS) that showed no antibacterial activity at the most diluted concentration (1:128). Inhibition began at 1:16 for *Bacillus* spp, and *E. coli*, and at 1:32 for *S. aureus*, indicating the minimum inhibitory concentration to be 6.25mg/ml for *E. coli* and *Bacillus* spp but 3.125 mg/ml for *S. aureus*. As the concentration increased up to 1:2, the ZOI increased for all bacteria. *E. coli* showed the largest ZOI (up to 20mm), making it the most susceptible. *Bacillus* spp show moderate inhibition (up to 18mm), while *S. aureus* showed the smallest ZOI (16mm) and was the least susceptible. For antibacterial properties of Handwash (HW), it showed no antibacterial activity at high dilutions (1:128 to 1:8). Inhibition began only at 1:4, with ZOIs of 10mm against *Bacillus* spp, 9mm for *S. aureus*, and 12mm for *E. coli* making 25mg/ml as MIC value. At the strongest concentration (1:2), the ZOI increased to 12mm, 17mm and 14mm respectively. The handwash was therefore effective only at higher concentrations (1:4 and 1:2), showing the greatest activity at 1:2. *S. aureus* was the most sensitive, while *Bacillus* spp and *E. coli* showed moderate susceptibility.

Table 3: Minimum Inhibitory Concentration of Herbal soap (HS) and Hand Wash (HW) against common pathogenic bacteria

Sample	Concentration	Zone of inhibition (ZOI) in mm		
		Bacillus spp	***S. aureus***	***E. coli***
	1:128	0	0	0
	1:64	0	0	0
	1:32	0	9	0
HS	1:16	12	11	13
	1:8	12	3	18
	1:4	16	14	19
	1:2	18	16	20
	1:128	0	0	0
	1:64	0	0	0
	1:32	0	0	0
HW	1:16	0	0	0
	1:8	0	0	0
	1:4	10	9	12
	1:2	12	17	14

Minimum Inhibitory Concentration of medicated soap against common pathogenic bacteria

Table 4 represent the antibacterial activity of medicated soap (MS1 and MS2) against *Bacillus* spp, *S. aureus*, *E. coli*, that showed no antibacterial activity against *Bacillus* spp at any dilution. For *S. aureus*, inhibition of MS2 brand began only at 1:8 (5mm) and increased slightly to 7mm at 1:2, showing the weaker effectiveness of MIC being 12.5mg/ml. For *E. coli*, activity started at 1:16 (7mm) and increased steadily to 11mm at 1:2, making MIC value 6.25mg/ml showing the strongest response among the three bacteria. But for MS1 brand, the MIC against *S. aureus* was 50mg/ml and against *E. coli* was 25mg/ml. Overall, medicated soap was most effective against *E. coli*, weakly effectively against *S. aureus*, and ineffective against *Bacillus* spp.

Table 4: Minimum Inhibitory Concentration of medicated soap against common pathogenic bacteria

Sample	Concentration	Zone of inhibition (ZOI) in mm		
		Bacillus spp	*S. aureus*	*E. coli*
MS1	1:128	0	0	0
	1:64	0	0	0
	1:32	0	0	0
	1:16	0	0	0
	1:8	0	0	0
	1:4	0	0	8
	1:2	0	7	9
MS2	1:128	0	0	0
	1:64	0	0	0
	1:32	0	0	0
	1:16	0	0	7
	1:8	0	5	9
	1:4	0	6	10
	1:2	0	7	11

DISCUSSION

Soaps are commonly used for cleaning skin and removing germs, mainly by disrupting the microbial cell membrane and protein (Schaffner et al., 2018). Although people choose soaps based on preference, an effective soap should be able to fight disease-causing bacteria on the skin (Odoyo et al., 2021; Schaffner et al., 2018). According to table 1, Non-medicated soap 1 had shown higher effectiveness with mean ZOI of 21mm, conversely, Medicated soap brand 2 had mean ZOI of 18mm that followed other Herbal soap, Handwash and Laundry soap (Range of mean ZOI:14.3-17.3mm).

Generally, the medicated soaps had higher antibacterial activity due to its antimicrobial ingredients like triclosan, chlorhexidine, povidine iodine and Sulphur that help in killing the bacteria or inhibit their growth by disrupting cell membranes, denaturating proteins or sometimes inhibiting fatty acid synthesis and altering metabolic pathways (Madigan et al., 2018). The result is also consistent with the study done in Nigeria, where different medicated soaps have shown greater activity towards common pathogens like *S. aureus* and *E. coli*. (Jesumirhewe &Timothy, 2024). However, it cannot also be neglected that the brand NM1 with the ingredients like thymol, terpinol, sodium palmitate and sodium cocoate (Fotsing & Kezetas, 2020) had shown antibacterial activity against tested bacterial pathogens in vitro. It may have correspondence to high pH and release of free fatty acids like lauric acid and palmitic acid for disrupting the bacterial membrane.

Non-medicated soaps and handwash are very useful in dealing with microbes since they act using strong physical abrasion which does not require any antimicrobial chemical. The surfactants in soap dissolve skin oils, dirt, and organic residues where microbes adhere on them, then they can be removed off the skin (Madigan et al., 2018). Oils and contaminants being surrounded by the soap molecules create so-called micelles that capture bacteria, fungi, and viruses and then are washed off with water (CDC, 2020). Moreover, soap has the potential to interfere with the lipid shell of most viruses including influenza and coronaviruses rendering them inactive (WHO, 2009).

The mechanical friction of washing hands also increases the microbial flora to a greater extent, as it removes living beings hidden in the folds of skin and by removing the nails. Since non-medicated soaps do not kill microbes, but only eliminate them, they cause less antimicrobial resistance, and can be used daily (Ananthanarayan and Paniker, 2017). As such, handwashing and non-medicated soap is among the most effective and evidence-based ways of infection prevention.

In another hand, the herbal soaps tend to be antibacterial as the products contain plant-based ingredients like neem, tulsi, turmeric and essential oils, which all contain natural antibacterials. Such phytochemicals as azadirachtin in neem, curcumin in turmeric, and eugenol in tulsi have the ability to inhibit the synthesis of cell walls, disrupt cell membranes, or disrupt the metabolic processes, and they thus reduce the survival of bacteria on the skin (Madigan et al., 2018). Secondly, the surfactant activity of soap aids in erasing dirt and microorganisms and this makes the herbal soaps have a combined chemical and physical microbial control mechanism.

Research on the use of herbal preparations has always demonstrated a great effect of antibacterial activities in comparison to controls which are not treated, and the efficacy of plant-based soaps is confirmed in daily practices of hygiene (WHO, 2009). This is in consistent with the result of this study which demonstrated the mean ZOI of 17mm against selected pathogens. The Medicated soap brand 2 showed no effectiveness against *Bacillus* spp, weak activity against *S. aureus* (MIC= 12.5mg/ml), and moderate effectiveness against *E. coli* (MIC=6.25 mg/ml) as the concentration increased (Kutol, 2019) that is higher in comparison to MIC of 1.56mg/ml of Non-medicated soap brand 1. These findings challenge the belief that medicated soaps are always superior, highlighting the need for choosing soaps based on proven antimicrobial performance rather than marketing claims (Lawan & Idris, 2021).

Overall the study emphasizes that different soaps work differently against specific bacteria, further research could help develop formulations with broader antibacterial coverage(Hoang et al., 2021). Using effective antimicrobial soaps can help reduce the risk of infections, especially in places where hygiene is critical, such as hospitals, kitchens, and public areas (Kampf & Kramer, 2004). Most soaps in this study worked best against *E. coli*, likely because its Gram-negative, lipid- rich outer membrane is easily disrupted by soap molecules. In contrast, *S. aureus* and *Bacillus* spp, being gram positive, have thick peptidoglycan layers that provide greater resistance. This explains why *E. coli* was more sensitive to soap action, while the other two bacterial species showed comparatively lower susceptibility (Matta et al., 2022).

CONCLUSION

Non-medicated soap showed the strongest antibacterial activity against *Bacillus*, while Herbal soap was most effective against *S. aureus* and Medicated soap was effective against *E. coli.* Other samples including the liquid hand wash (HW) and laundry soap exhibited comparatively lower inhibition, likely due to difference in their antibacterial components, although hand wash still performed moderately well, that is ascribed to better lathering and mechanical removal of microbes. These findings challenge the assumption that medicated soaps are always superior, highlighting the need to choose soaps based on validated microbial efficacy rather than marketing claims. Difference in efficacy among soaps are attributed to variation in their antimicrobial compound. Handwash also demonstrated notable activity, likely due to better lathering and mechanical removal of microbes. The finding challenges the idea that medicated soaps are always more effective than non-medicated ones. Soap formulation should prioritize proven antimicrobial efficacy while remaining affordable for widespread use.

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CONFLICT OF INTEREST

The authors declared no conflict of interests.

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