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EVALUATION OF ANTIMICROBIAL POTENTIAL OF LOCALLY FORMULATED SOAP CONTAINING NEEM (*AZADIRACHTA INDICA*) AND TURMERIC (*CURCUMA LONGA*) POWDERS

Sadia Bibi Jakhrani[1]*, Abdul Samad[1], Imran Ahmed[1], Sidra Siraj[1], Hafsa Noor[1], Zafar Ahmed[1], Babar Hilal Ahmad Abbasi[1], Yousaf Hassan[1], Sangeen Izat Ali[1]

[1]Center for Advance Studies in Vaccinology & Biotechnology (CASVAB), University of Balochistan, Quetta, Pakistan

***Corresponding Author:** Sadia Bibi Jakhrani. E. mail: sadiajakhrani99@gmail.com

Abstract

Background: *The increasing prevalence of antibiotic-resistant pathogens and consumer preference for natural products has renewed interest in plant-based antimicrobial formulations. This study was undertaken to formulate soaps incorporating neem (Azadirachta indica) and turmeric (Curcuma longa) powders and to evaluate their antibacterial efficacy against Staphylococcus aureus and Escherichia coli, in comparison with selected commercial soaps.*

Methods: *Antimicrobial activity was assessed using three complementary methods: total viable count (TVC) following simulated hand-washing, disc diffusion assay, and broth microdilution for minimum inhibitory concentration (MIC) determination. The MICs of the formulated soaps were determined via two-fold serial dilutions ranging from 100 to 1.56 µL/200 µL.*

Results: *In the TVC assay, neem soap achieved a 90% reduction in microbial load post-handwash, whereas turmeric soap and Dettol soap each showed a 70% reduction, followed by Lifebuoy (50%) and Capri (30%). In disc diffusion testing, neem soap produced the largest inhibition zones (20 mm against both S. aureus and E. coli). Turmeric soap exhibited moderate activity (14 mm and 10 mm, respectively), while among commercial soaps, Dettol showed the highest zones (17 mm against S. aureus and 13 mm against E. coli), followed by Lifebuoy (12 mm and 11 mm) and Capri (10 mm and 9 mm). The MIC of neem soap was 12.5 µL/200 µL against S. aureus and 25 µL/200 µL against E. coli; for turmeric soap, MIC values were 25 µL/200 µL and 50 µL/200 µL, respectively.*

Conclusion: *The formulated neem and turmeric soaps demonstrated potent, dose-dependent antibacterial activity, with neem soap outperforming all commercial products tested. These findings suggest that herbal-based soaps represent a promising, cost-effective alternative to conventional antimicrobial soaps for personal hygiene and infection control.*

Keywords: *Disc diffusion assay, E. coli, MIC, Neem, Saponification, S. aureus. Turmeric, Vaible count test*

INTRODUCTION

Daily cleansing and personal hygiene is necessary practice in life. The process of cleansing is a basic purpose of hygiene has been now brought forward as an activity of grooming in order to enhance the body well-being (1). Different cleansing agents have been using around the globe since ancient time, among these cleansing agents, soaps, detergents and hand washes are the most common. As the skin is known as first line of defense and most of the skin infections caused by bacteria and fungi that live on the skin, like *S. aureus* and *P. aeruginosa* (2).

Plants contain a wide range of phytochemicals, including alkaloids, tannins, terpenoids, flavonoids, essential oils and phenolic compounds. Herbal soaps provide a natural alternative to conventional soaps and are formulated using plant-based ingredients. Many medicinal plants used in herbal soaps possess antimicrobial, antioxidant and anti-inflammatory properties which maintain healthy skin and may treat skin related problem and diseases. Therefore, the incorporation of medicinal plants into soap formulations has gained considerable attention as potential natural alternative to commercial soaps (3).

A variety of chemical compounds are usually present in the soap that has the potential to control and kill the population of microorganisms. A large number of these compounds are used in hospitals and

homes as a disinfectant to kill a variety of bacteria and viruses. These chemical compounds are existed in the form of solids and liquids (4). According to several assessments of people, the antibacterial components present in the soaps are effective against different microbes and prevent us from a number of disease causing pathogens (5).

The largest organ of the human body is the skin; the outer layer of the skin protects the internal tissues from infections. To keep bacteria, viruses, fungi, and parasites away, skin provides a physical barrier and water-proof covering (6). Additionally, various herbal extracts and plant-derived oils are incorporated in soap manufacturing; the biochemical compounds present in coconut oil are more suitable in saponification compared to coconuts a whole. The coconut oil contains 46% lauric acid content that makes it suitable for the skin moisturization. It would be ideal to use coconut oil as a primary component in natural liquid soap manufacturing (7).

The fats and oils are used in soap manufacturing are discussed in detail, along with role of alkali bases/salts and various additives. Before soap production, the fats and oils undergo several pretreatment processes like degumming, bleaching, deodorization, fractionation, and hydrogenation to remove impurities and achieve desired physiochemical properties of soap making (8).

During the saponification, triglycerides (fatty acid esters) present in fats and oils react with strong alkali such as sodium hydroxide (NaOH) to produced soap and glycerol. The soap formed is initially dispersed as a colloidal system, which contribute to its cleansing properties (9).

As the primary means of preventing infections transmission in communities and healthcare facilities, hand washing with soaps and disinfectants are unavoidable (10). Basically, soaps are sodium or potassium salts of stearic acids or other fatty acids. The fatty acid composition of oil determines the soap's hardness, lather formation, cleansing properties, stability, and potential antimicrobial activity. Therefore, the choice of oil is important in determining the quality and functional properties of soaps (11).

MATERIALS AND METHODS

STUDY SETTING

The experimental work was conducted at the Bacteriology Laboratory of the Centre of Advanced Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan, Quetta.

PREPARATION OF MEDICATED SOAPS

Two medicated soaps were formulated using the cold-process saponification method. The first soap incorporated neem (*Azadirachta indica*) powder as the active antibacterial agent, while the second contained turmeric (*Curcuma longa*) powder. The base formulation for both soaps comprised 85 mL of coconut oil, 10 mL of castor seed oil, 16 g of sodium hydroxide, 36 mL of distilled water, and 2 mg of sodium lauryl sulfate as a foaming agent. For the neem soap, 3 g of neem powder was added, whereas the turmeric soap received 3 g of turmeric powder.

To prepare the soaps, sodium hydroxide was dissolved in distilled water to form a lye solution and allowed to cool to room temperature. Concurrently, the coconut oil and castor seed oil were combined and gently warmed. The cooled lye solution was gradually incorporated into the oil mixture with continuous stirring until the emulsion reached a pudding-like consistency (trace). Sodium lauryl sulfate and the respective medicinal powder (neem or turmeric) were then added and thoroughly mixed. The final mixtures were poured into sterile molds and allowed to harden for 24 hours (neem soap) or 48 hours (turmeric soap) to complete saponification. The solidified soaps were removed, inspected for quality, and subsequently evaluated for antibacterial activity (12, 13).

EVALUATION OF ANTIBACTERIAL ACTIVITY

TOTAL VIABLE COUNT (TVC) ASSAY

The antibacterial efficacy of the manufactured soaps was compared with that of commercial soaps using the Total Viable Count method. Hand rinse samples were collected from volunteers using sterile swabs before and after hand washing. Each swab was immersed in sterile saline, vortexed, and the resulting

suspension was plated onto nutrient agar. Plates were incubated at 37 °C for 24 hours, and bacterial colonies were enumerated to determine the reduction in bacterial load post-washing (14). To differentiate the bactericidal effect from mechanical removal, a water-only washing control was included. Standardized conditions were maintained throughout the procedure, including a 20-second hand washing duration, 2 g of soap, 10–15 seconds of active rubbing, water temperature of 25–27 °C, and immediate post-wash sampling to minimize variability (15). Hand washing procedures followed the World Health Organization (WHO) guidelines for hand hygiene.

DISC DIFFUSION ASSAY

The antimicrobial activity of the manufactured soaps was assessed against *Staphylococcus aureus* and *Escherichia coli* using the disc diffusion method. Bacterial suspensions were prepared and adjusted to a 0.5 McFarland standard. Nutrient agar plates were inoculated with each bacterial strain using sterile cotton swabs to create a confluent lawn. The manufactured soaps were fragmented, dissolved in sterile distilled water, and sterile filter paper discs were saturated with the resulting soap solutions. Discs were placed onto the inoculated agar plates using sterile forceps and incubated at 37 °C for 24 hours. Following incubation, zones of inhibition were measured in millimeters using a ruler to evaluate antibacterial activity (16).

MINIMUM INHIBITORY CONCENTRATION (MIC) DETERMINATION

The broth dilution method was employed to determine the minimum inhibitory concentrations (MIC) of the manufactured soaps. Stock solutions of each soap were prepared and serially two-fold diluted to achieve concentrations ranging from 100 to 1.56 µL/200 µL. Each well of a microtiter plate received 100 µL of nutrient broth, 100 µL of bacterial culture, and the corresponding soap dilution. For each bacterial strain, seven wells with varying soap concentrations were tested. A negative control (broth without bacterial inoculum) was included to verify sterility, and a positive control (bacterial inoculum without soap) was used to confirm bacterial growth. The plates were covered and incubated at 37 °C for 24 hours. After incubation, wells were examined for turbidity using a magnifying mirror to determine the MIC. The experiment was performed in triplicate (17).

STATISTICAL ANALYSIS

Data were analyzed using descriptive statistics and are presented as observed values, including zone of inhibition diameters (mm), percentage reduction in bacterial counts, and MIC values. Inferential statistical tests, such as t-tests or ANOVA, were not applied due to the absence of experimental replication.

RESULTS

The neem- and turmeric-based soaps formulated in this study demonstrated superior physicochemical properties relative to commercially available local soaps, including enhanced texture, lathering capacity, and skin compatibility. Both formulations exhibited a pleasant fragrance, smooth and homogeneous consistency, and effective cleansing action without inducing dryness or irritation. The pH of the soaps remained within the physiologically acceptable range for topical application, indicative of a well-controlled saponification process.

The antibacterial activity of the formulated soaps was notably robust, effectively inhibiting the growth of a broad spectrum of bacteria, including common cutaneous pathogens. This enhanced antimicrobial property ensures prolonged protection against infections, thereby offering superior hygienic benefits compared to standard commercial soaps. Furthermore, even with frequent use, the soaps preserved the skin's natural barrier function, preventing irritation and excessive transepidermal water loss.

Fig. 1 (a) and (b) presents the prepared turmeric soap bars within the mold and following demolding, respectively.

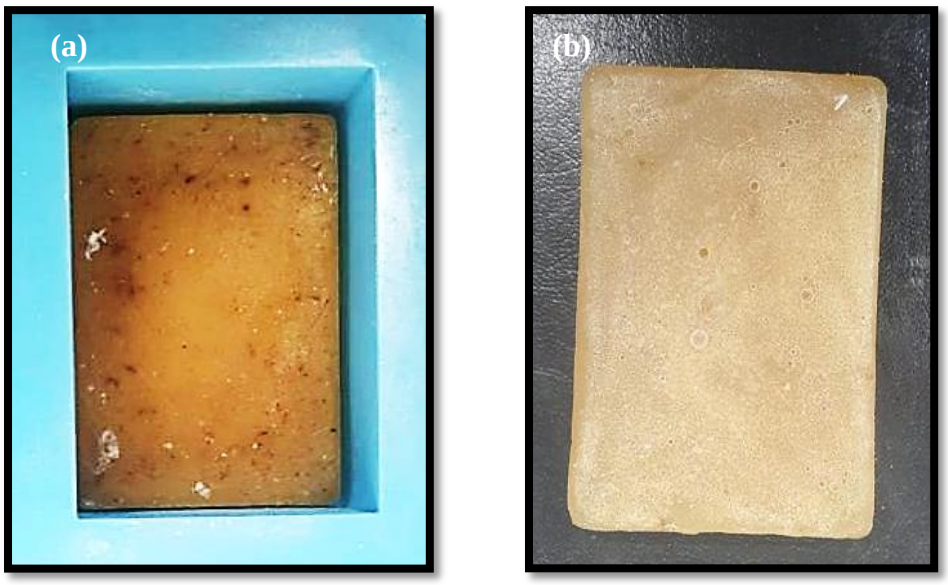

Fig. 1 (a). Turmeric soap in mould; **(b)**. Turmeric soap out of mould

COMPARISON OF ANTIBACTERIAL ACTIVITY OF MANUFACTURED SOAPS WITH COMMERCIAL SOAPS BY TOTAL VIABLE COUNT (TVC)

The antibacterial efficacy of the manufactured soaps was quantitatively assessed by determining the percentage reduction in colony-forming units (CFUs) on hands following washing. The neem-based soap (Soap 1) effected a substantial 90% reduction in bacterial load, while the turmeric-based soap (Soap 2) demonstrated a 70% reduction compared to pre-wash counts. In contrast, untreated control samples exhibited no appreciable change in CFU counts, confirming that the observed antibacterial effect was attributable to the active herbal components of the soaps rather than to mechanical removal alone. These findings indicate that both formulated soaps possess significant antibacterial activity, with the neem formulation displaying superior efficacy against the resident microbial flora on hands.

COMPARISON OF ANTIMICROBIAL ACTIVITY OF MANUFACTURED SOAPS WITH COMMERCIAL SOAPS BY DISC DIFFUSION METHOD

The antimicrobial efficacy of the prepared soaps (incorporating neem and turmeric powder) and selected commercial soaps was evaluated against *Staphylococcus aureus* and *Escherichia coli* using the disc diffusion method. The zones of inhibition (ZOI) were meticulously measured and recorded. Among the formulated soaps, the neem-based soap demonstrated potent activity, producing a ZOI of 20 mm against *S. aureus* and 22 mm against *E. coli*. In contrast, the turmeric-based soap exhibited moderate to low activity, with inhibition zones of 14 mm and 10 mm against *S. aureus* and *E. coli*, respectively. Regarding the commercial products, soap 1 showed the highest antibacterial activity, followed by soap 2, which displayed intermediate efficacy, while soap 3 exhibited the least activity against both tested bacterial strains. Overall, the neem soap outperformed all other soaps against *E. coli* and was second only to soap 1 against *S. aureus*, whereas the turmeric soap consistently showed the lowest inhibition among all tested formulations (Fig. 2).

The commercial soaps were also used to evaluate the antimicrobial activity against *S. aureus* and *E. coli* and their antibacterial activity were also compared with the manufactured soaps (neem and turmeric). Among the three different soaps; soap 1 exhibited high antimicrobial activity against *S. aureus* that produced 17 mm inhibition zone while 13 mm inhibition zone was produced against *E. coli*. The second soap; soap 2, was less effective than soap 1, it produced 12 mm inhibition zone against *S. aureus* while 11 mm against *E. coli*.

Lastly, soap 3 produced 10 mm inhibition zone against *S. aureus*, whereas a 9 mm inhibition zone was observed against *E. coli*. These results indicated that soap 3 exhibited relatively lower antibacterial activity than other tested soaps. Overall, the manufactured neem and turmeric soaps demonstrated considerable antimicrobial potential against tested microbes, although neem soap showed the greatest

activity among the tested soaps shown in table 5. All the antibacterial results were interpreted according to CLSI and Eukast guidelines (Fig. 3).

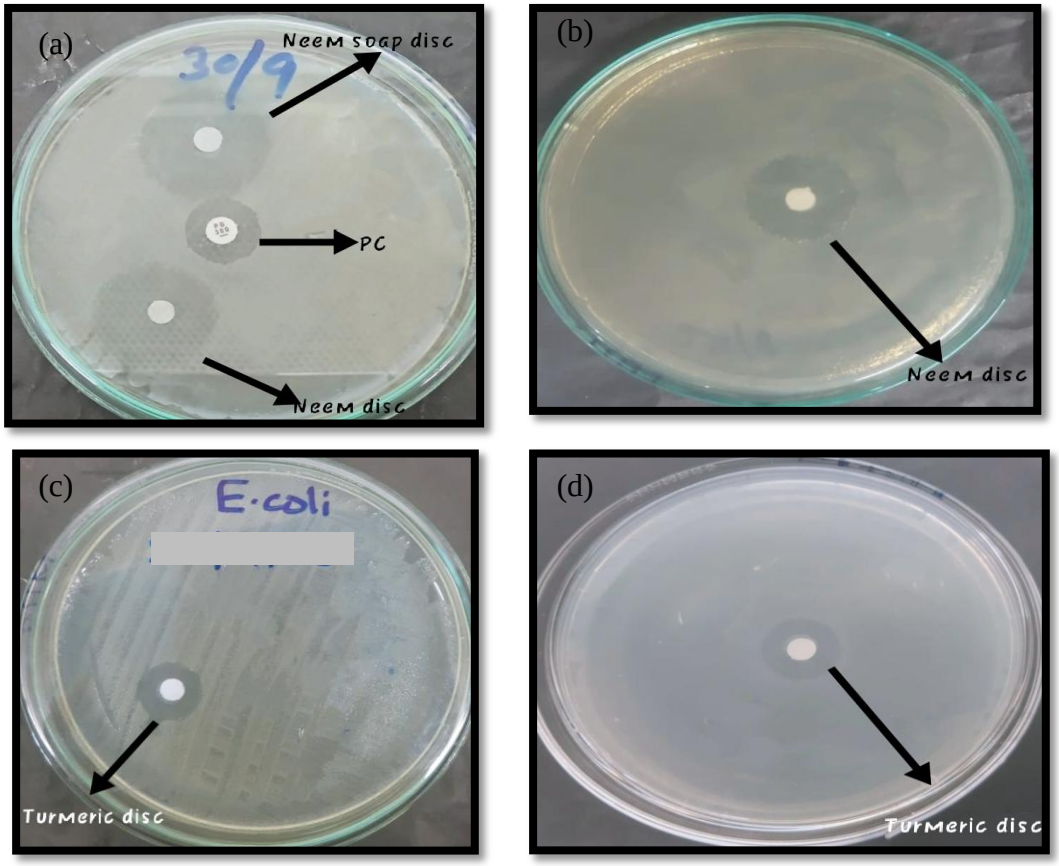

Fig. 2 (a). Inhibition zone (20 mm) using neem soap against *S. aureus;* **(b).** Inhibition zone (22 mm) using neem soap against *E. coli;* **(c).** Inhibition zone (10 mm) using soap (Turmeric) against *E. coli;* **(d).** Inhibition zone (14 mm) using turmeric soap against *S. aureus*

Fig. 3 (a). Shows antibacterial activity using the commercial soaps (1, 2, and 3) by forming the zones of inhibition against *S. aureus;* ***(b).*** Antibacterial effect (1, 2, and 3) against *E. coli*

BROTH DILUTION METHOD (MIC) FOR MANUFACTURED SOAPS

The MIC of the soap (neem and turmeric) was determined using the broth dilution method in order to evaluate the antibacterial activity against *S. aureus* and *E. coli.* The MIC against *S. aureus* was evaluated at 12.5µl/200µl of neem soap solution, indicating that this concentration of the soap was sufficient to inhibit the visible bacterial growth under experimental conditions. The MIC value of soap was also determined against *E. coli* and recorded at 25µl/200µl. This finding indicates that the soap has high antimicrobial potential and can inhibit the bacterial growth at relatively low concentrations. The experiment was performed in duplicate.

Similarly, the antimicrobial activity of turmeric soap was also determined through MIC using from the stock soap solution (100 to 1.56µl/200µl). The MIC value of soap against *S. aureus* was evaluated at

25µl/200ml. The same concentration of soap solution was also used against *E. coli* and the MIC was recorded at 50µl/200µl. The exhibited antibacterial activity of turmeric soap was very effective against *E. coli.*

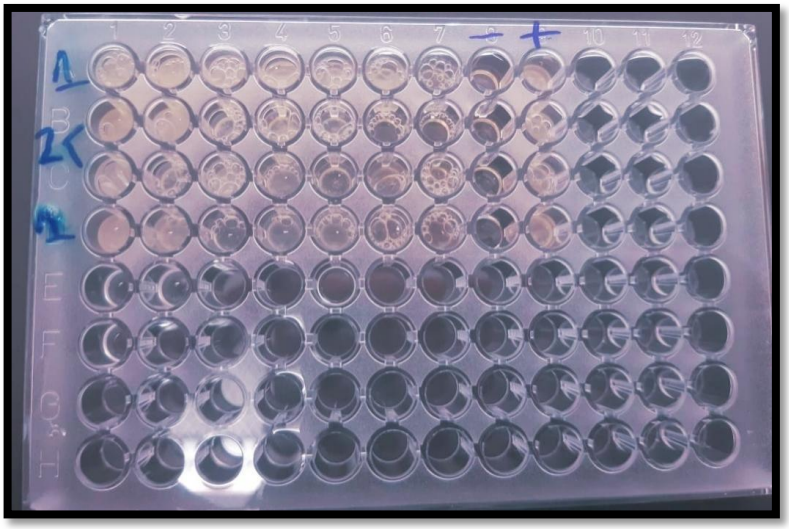

Fig. 4. MIC value of neem and turmeric soaps with positive and negative control

The Fig. 4 shows the micro-titer plate that displays the results of an experiment to determine the MIC of two different lab manufactured soaps. The micro-titer plate also contains the negative and positive control in wells. Furthermore, Fig. 5 (a-d) is showing the growth of microbes recovered from the wells of micro-titer plate in which bacterial growth was inhibited. The small number of bacterial colonies on agar plates indicates that the tested soap concentrations inhibited bacterial growth (MIC) but did not completely eliminate viable microorganism.

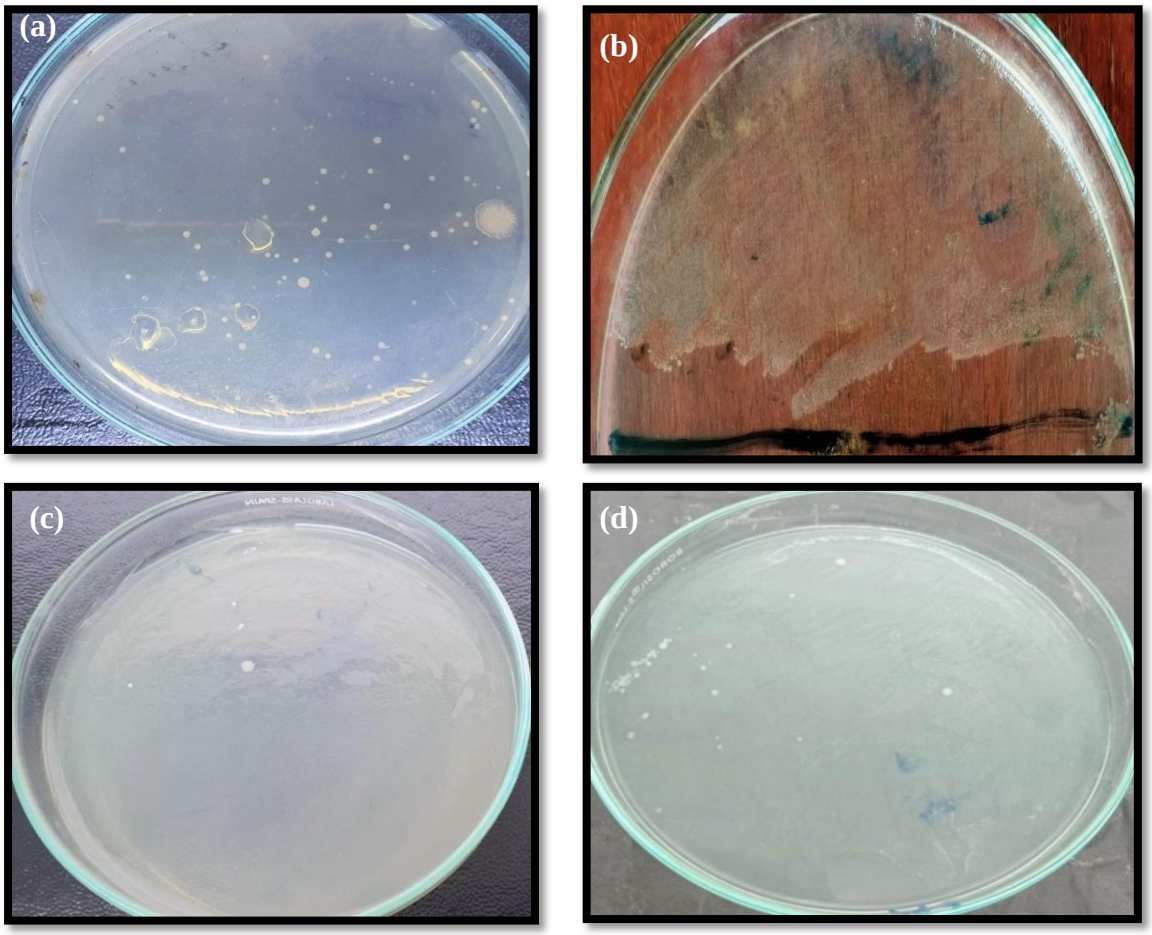

Fig. 5 (a). MIC value 12.5 µl for *S. aureus* (neem soap); **(b).** MIC value 25 µl for *E. coli* (neem soap); **(c).** MIC value 25 µl for *S. aureus* (turmeric); **(d).** MIC value 50 µl for *E. coli* (turmeric)

DISCUSSION

The term *saponification* is derived from the Latin word *saponins*, meaning soap, which remains an essential agent for achieving hygiene through cleaning and washing. Medicated soaps can be formulated using various oils and herbal extracts that exhibit inhibitory or bactericidal activity against a wide range of microorganisms (18). For instance, extracts from *Terminalia catappa* leaves have been incorporated into soap formulations and demonstrated significant antibacterial activity against *S. epidermidis*, *Escherichia coli*, and *Staphylococcus aureus* (19). In alignment with these findings, the present study evaluated the antibacterial potential of soaps fortified with powdered *Azadirachta indica* (neem) and *Curcuma longa* (turmeric). Both plant powders were readily incorporated during the saponification process and exhibited strong antimicrobial activity against the tested bacterial strains.

The choice of oils is critical in soap manufacturing, as they influence both the physicochemical properties and the antimicrobial efficacy of the final product. Coconut oil, widely used in the Pakistani soap industry, was selected for its excellent moisturizing properties and lathering characteristics. Castor oil was additionally included due to its well-documented role in enhancing cream formation and foam stability during saponification. Previous research on *Sesamum indicum* (sesame) oil recognized for its high-quality lipid content has demonstrated its utility in soap production, particularly when blended with *Jatropha* seed oil, which is rich in palmitic acid and imparts water resistance and durability to the soap (20). The combination of coconut and castor oils in the current formulation proved effective, yielding satisfactory foam quality and consistency.

The antibacterial activity of the manufactured soaps was quantitatively assessed using the minimum inhibitory concentration (MIC) method. Nwankwo *et al.* (2025) reported MIC values of 6.25 mg/mL for crusader soap against *S. aureus* and 25 mg/mL for black soap against *E. coli* (21). In comparison, the neem-based soap (Soap 1) in the present study exhibited an MIC of 12.5 µL/200 µL against *S. aureus*, while the turmeric-based soap (Soap 2) demonstrated an MIC of 25 µL/200 µL against *E. coli*. These findings corroborate earlier reports and underscore the potent antimicrobial potential of neem and turmeric when incorporated into soap matrices. Furthermore, the significant reduction in total viable counts observed after hand washing with the manufactured soaps, relative to pre-wash counts, confirms their practical efficacy in reducing bacterial load on skin.

The antimicrobial activity of neem is attributed to its rich phytochemical profile, which includes azadirachtin, nimbidin, nimbin, quercetin, and gedunin. These bioactive compounds exert their effects by disrupting bacterial cell membranes and interfering with essential cellular functions, thereby inhibiting both Gram-positive and Gram-negative bacteria, including *S. aureus* and *E. coli* (24). Similarly, the antibacterial properties of turmeric are primarily associated with curcumin, a polyphenolic compound, alongside other bioactive constituents such as demethoxycurcumin, bisdemethoxycurcumin, and various essential oils. These compounds act through multiple mechanisms, including membrane disruption, inhibition of protein and nucleic acid synthesis, and suppression of biofilm formation (26). Curcumin typically constitutes 2% to 5% of the dry weight of turmeric powder, though its concentration can vary depending on plant origin and environmental conditions (27). In the present study, whole-plant powders were used rather than purified extracts, which may have introduced variability in active constituent concentrations. However, the formulation was standardized by weight (3 g per soap batch) to ensure reproducibility.

One limitation of the current study is the use of sodium lauryl sulfate (SLS) as a foaming agent. While SLS is effective in enhancing foamability and cleaning efficiency, prolonged or repeated exposure may compromise the skin barrier and cause irritation, particularly in individuals with sensitive skin (23). Future formulations could benefit from the substitution of SLS with milder, skin-compatible surfactants such as sodium lauroyl sarcosinate, sodium cocoyl isethionate (SCI), sodium cocoyl glutamate, decyl glucoside, or coco glucoside. These alternatives offer comparable foaming properties with reduced irritancy potential. Additionally, future research should employ High-Performance Liquid Chromatography (HPLC) or other quantitative analytical techniques to precisely characterize and standardize the bioactive

phytochemical content in the plant powders used, thereby enhancing reproducibility and facilitating dose-response correlation.

In conclusion, the neem- and turmeric-based soaps developed in this study demonstrated substantial antibacterial activity against clinically relevant pathogens, supporting their potential use as effective herbal antimicrobial agents for hand hygiene. Optimization of the formulation using milder surfactants and rigorous phytochemical standardization could further improve their safety, efficacy, and commercial viability.

.CONCLUSION

This study successfully demonstrated the development of effective hand-washing soaps fortified with herbal extracts exhibiting significant antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. Total viable count assays confirmed a substantial reduction in microbial load on hands post-washing, indicating robust cleansing efficacy. Comparative analysis with commercially available soaps revealed that the formulated soaps possessed superior or comparable antimicrobial activity against the tested pathogens. These findings suggest that neem- and turmeric-based soaps hold promise as preventive agents against common skin infections and as effective tools for reducing microbial transmission through routine hand hygiene.

Nevertheless, this investigation was limited to evaluating antibacterial activity against only two bacterial species. Future research should expand the antimicrobial spectrum to include a broader range of clinically significant pathogens. Additionally, comprehensive studies on product stability, skin compatibility, and safety profiles are warranted. Optimization of the formulation using milder, skin-friendly surfactants and standardization of bioactive phytochemical content through advanced analytical techniques would further enhance product quality. Ultimately, well-designed clinical trials are essential to validate the real-world efficacy and safety of these herbal soaps in diverse populations.

Conflict of interest:

The authors report no conflicts of interest

Author s' contributions:

SBJ & AS Designed the study and drafted the manuscript; SBJ, IA, SS & HN Performed the experimental work and data collection; ZA, BHAA & YH Conducted data analysis and interpretation. SIA contributed to the revision and final approval of the manuscript.

Declaration of generative AI-Assisted Tools:

No AI-assisted tools were used.

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