Malaysian Applied Biology
https://doi.org/10.55230/mabjournal.v55i3.3908

Research Article

A Laboratory Framework for Evaluating Antimicrobial Activity in Herbal Soaps: A Model System Using Neem and Turmeric Formulations

Nur Izzati Husna Hashim[1], Norsyuhada Alias[1*]

[1]*Department of Biomedical Science, Kulliyyah of Allied Health Sciences, International Islamic University Malaysia (IIUM), 25200 Kuantan, Pahang.*

[*]*Corresponding author: norsyuhada_alias@iium.edu.my*

ABSTRACT

The rising concern of skin infections caused by common microbes like *Staphylococcus aureus* and *Candida albicans* exacerbated by increased antimicrobial resistance from overuse of conventional drugs has increased the public's interest in natural and herbal-based products. While commercial medicated soaps have extensive studies on their antimicrobial activities, existing research on herbal soaps predominantly focuses on plant extracts rather than finished soap formulations, leaving a gap in understanding the true efficacy of herbal-based soaps against clinically relevant pathogens. Additionally, variations in preparation methods and the absence of standardised testing protocols complicate the comparison and validation of results across different studies. The study aims to evaluate the antimicrobial efficacy of herbal-based soaps using standardised laboratory procedures. A broth microdilution assay was conducted, followed by subculture confirmation to determine the minimum inhibitory concentrations (MICs), minimum bactericidal (MBC) and fungicidal (MFC) concentrations. Time-kill assay assessed concentration- and time-dependent effects at MBC/MFC levels through serial dilutions and colony-forming units (CFU) enumeration. The MIC and MBC values for *Staphylococcus aureus* were 53.33 mg/mL and 80 mg/mL, respectively, with time-kill analysis showing bacterial reduction within 30 s and complete elimination by 3 min. *Candida albicans* exhibited higher MIC and MFC values of 120 mg/mL, with complete inhibition observed at 4 min. Overall, the low MIC values and rapid microbial inhibition highlight the influence of soap concentration and exposure time on its potency. Overall, this investigation not only demonstrated the microbial reduction capability of the herbal soap but also contributed valuable methodological insights. By providing a useful testing framework, it paves the way for ongoing improvements in product formulation and ensures that consistent efficacy standards can be maintained. This contributes to the broader field of natural product development and supports the increasing interest in plant-based antimicrobial agents as safe and effective alternatives to conventional chemicals.

Key words: Antimicrobial, broth microdilution, herbal soaps, minimum inhibitory concentration, time-kill assay

Article History

Accepted: 10 August 2026
First version online: 21 September 2026

Cite This Article:
Hashim, N.I.H. & Alias, N. 2026. A laboratory framework for evaluating antimicrobial activity in herbal soaps: a model system using neem and turmeric formulations. Malaysian Applied Biology, 55(3): 79-85. https://doi.org/10.55230/mabjournal.v55i3.3908

INTRODUCTION

The skin is exposed to a lot of harmful stimuli and naturally harbours many types of microbiota such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Candida albicans* and *Mycobacterium* spp. among others (Upula *et al*, 2021). Microbiota often assist in maintaining the equilibrium of skin's health, but when skin defences are compromised, they can transform into opportunistic pathogens causing infections (Yeroushalmi *et al.*, 2020). *Staphylococcus aureus* is known as the leading cause of most skin infections, followed by *Streptococcus pyogenes* and even *Candida* species, which cause superficial fungal infections (Smith *et al.*, 2020). Infections occur once pathogens infiltrate the body through a wound and proliferate by colonising tissues rich in nutrients.

Globally, the incidence of fungal and bacterial skin diseases is rising, influenced by both increased international travel and an increase in the population of immunocompromised individuals (Yeroushalmi *et al.*, 2020). The infection is often treated with antibiotics or antifungal medication. The high reliance on antimicrobial drugs can be the cause of drug resistance, which is a major concern in global healthcare. In Malaysia alone, there is an escalating trend of antimicrobial resistance due to overuse and misuse of antibiotics for the past 5 years (World Health Organisation, 2022). This issue calls for alternative treatments that can work against these infection-causing pathogens without leading to more emerging drug-resistant strains.

Additionally, natural-based products have been popularised for many years now, and this is causing more consumers to seek out natural products with potent ingredients to treat skin infections due

to undesirable side effects from conventional medications. Herbal soaps are not typically known as medicated soaps, but they often contain specific herbs selected for their medicinal properties, which can help in skin healing, nourishment and hydration. Moreover, the natural ingredients also have a lower risk of causing negative side effects and antimicrobial resistance compared to chemically infused medicated soaps (Seetha Devi *et al.*, 2021). This makes them a much safer option for daily use and suitable preventive skincare.

Despite their popularity, herbal soaps are seldom recommended in clinical settings due to the limited availability of laboratory data supporting their antimicrobial claims. Existing research predominantly focused on plant extracts rather than finished soap formulations, often using in-house herbal soap preparations that emphasise focus more on ingredient safety and effects on dry skin (Das *et al.*, 2023; Shaibu & Orivri, 2025). Antimicrobial testing is commonly limited to well diffusion assays, leaving a gap in fully understanding the true efficacy of herbal-based soaps against clinically relevant pathogens. Additionally, variations in preparation methods and the absence of standardised testing protocols complicate the comparison and validation of results across different studies. Establishing a reliable and reproducible laboratory framework by incorporating methods such as broth microdilution for determining minimum inhibitory concentrations (MICs), as well as time-kill assays (TKA), would enable consistent evaluation of antimicrobial performance across various herbal soap formulations.

Neem (*Azadirachta indica*) and turmeric (*Curcuma longa*) are well-documented for their potent antimicrobial, anti-inflammatory and antioxidant properties and are often incorporated into herbal soaps. Neem exhibits strong antiseptic activity against bacteria such as *Staphylococcus aureus* and *Propionibacterium acnes*, with extracts from neem bark showing particularly high effectiveness in inhibiting bacterial growth (Mohammed *et al.*, 2022). Turmeric, on the other hand, contributes to skin disease management by inhibiting bacterial growth through its structural characteristics and by producing antioxidant compounds (Kasprzak-Drozd *et al.*, 2024). Furthermore, the combination of neem and turmeric has demonstrated synergistic antimicrobial effects against several bacterial strains (Dhinakaran *et al.*, 2017). A commercial soap containing neem and turmeric was selected in this study to serve as a model for assessing the antimicrobial activity of herbal-based formulations. Commercial products offer the advantage of being manufactured under standardised conditions, ensuring reproducibility and reflecting real-world consumer use. The product's ingredients, based on manufacturer information and supporting literature, are safe for topical use and free from harsh chemicals such as alcohol and triclosan. Key active ingredients with potential antimicrobial effects include neem seed oil, turmeric root oil, and lemon peel oil, while other additives such as emulsifiers, solvents, chelating agents, fragrances, and pigments function primarily as formulation stabilisers. This natural composition enhances the product's value as an eco-friendly alternative to synthetic soaps, fulfilling consumer demand for gentle yet therapeutic skincare solutions.

The primary aim of this study is to evaluate the antimicrobial efficacy of herbal-based soaps using standardised laboratory procedures. A neem and turmeric-infused soap was selected as a model formulation to determine its antibacterial and antifungal activities against *Staphylococcus aureus* and *Candida albicans*. The study specifically aimed to:

- Determine the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of the herbal soap using the broth microdilution method.
- Assess its time-dependent antimicrobial activity using time-kill assays (TKA).

The findings are expected to establish a reproducible methodological framework for evaluating other herbal soap formulations and provide insights into their potential application as natural antimicrobial agents for infection prevention and skin health maintenance.

MATERIALS AND METHODS

Soap selection

The soap was selected based on the herbal products as the key ingredients that are known to possess antibacterial and antifungal properties and are available in the local market. The expiry date of the purchased product was taken into consideration. The study focuses on this single soap to allow a detailed evaluation of its antimicrobial efficacy and to establish a standardised testing protocol. Limiting the scope ensures consistent results and a clearer interpretation of the specific active ingredients.

Microorganism selection and confirmation of isolates

The cultures of *Staphylococcus aureus* (ATCC 25923) and *Candida albicans* (wild strains) were revived from glycerol stocks obtained from the Microbiology Laboratory, Department of Biomedical Science, IIUM. The isolates were selected based on their characteristics as opportunistic pathogens capable of causing skin infections. The confirmation of isolates was done through morphological identification on agar plates and microscopic examination using Gram staining.

Sample preparation

Bacterial and fungal colony isolates were inoculated into Mueller-Hinton and Sabouraud dextrose broth, respectively. The suspension was adjusted to 0.5 McFarland standard turbidity using the McFarland tube densitometer (BioSan), in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute, 2024).

The soap suspension was prepared at a concentration of 120 mg/mL as the stock solution by dissolving 6 grams of soap in 50 mL of sterile distilled water. This mixture was placed on a warm plate set to a temperature between 60℃ and 70℃ and continuously stirred with a magnetic stirrer to facilitate the dissolution process.

Bacterial broth microdilution assay

This procedure was adapted from Mbouche *et al.* (2022), with suitable modifications. Each designated well of a 96-well microplate was filled with 100 µL of Mueller-Hinton broth. The stock solution of soap suspension was added to the first well and serially diluted by transferring 66.7 µL from one well to the next and adding 33.3 µL of broth to maintain a final volume of 100 µL per well. Each test well was then inoculated with 100 µL of *Staphylococcus aureus* suspension. Control wells included mixtures of ciprofloxacin HCL (Biobasic) and *Staphylococcus aureus* added through a series of 2-fold serial dilutions, Mueller-Hinton broth and *Staphylococcus aureus* soap suspension only and Mueller-Hinton broth only. The microplate was incubated at 37℃ for 24 hr. Following incubation, 50 µL of resazurin was added to each well to assess bacterial growth. The plates were then incubated at room temperature for an additional 3 to 5 hr before results were recorded. Each test was conducted in triplicate across three sets.

Fungal broth microdilution assay

A similar protocol was applied for this assay while using Sabouraud dextrose broth as the medium and fluconazole suspension (Pharmaniaga) as a negative control. The 96-well microplate was incubated at 37℃ for 48 hr. Resazurin suspension was also added following incubation to assist the visualisation of fungal growth through the colour changes in the wells. Each test was conducted in triplicate across three sets.

Determination of minimum bactericidal and fungicidal concentrations

Samples from the wells without microbial growth as indicated by resazurin dye were sub-cultured onto respective agars. They were incubated at 37℃, bacteria cultures for 24 hr and fungal cultures for 48 hr. The presence or absence of bacterial and fungal growth was observed after the incubation period. The MBC and MFC were determined as the lowest concentrations showing no growth or a maximum of five colonies upon subculture.

Time-kill assay

This test was carried out using a modified procedure by Alrashidi *et al.* (2021). Concentrations equal to MBC and MFC of the soap suspension were prepared as the test solution, and sterile distilled water was prepared as a negative control for this test. In a sterile 1.5 mL vial, a 100 µL aliquot of the tested microorganism was added to 900 µL of soap suspension. The contents of the vial were mixed thoroughly using a micropipette. Subsequently, the mixture was incubated at specific time points (0.2, 0.5, 1, 2, 3, 4 & 5 min). After each time point, 100 µL aliquots of the mixture were transferred to 900 µL of sterile saline to halt the antimicrobial action. The neutralised mixture was further diluted in 1:10 serial dilutions of medium broth twice, to reach a total dilution factor of 10^{-3} for easier colony counting.

From the dilution, 100 µL was transferred to nutrient or Sabouraud dextrose agar plates and incubated under aerobic conditions at 37℃ (24 hr for bacterial and 48 hr for fungal cultures). Following incubation, the surviving colonies on each plate were counted. The concentration of viable cells was recorded as the colony-forming units (CFUs) for each time point by calculating with this formula:

$$\text{CFUs} = \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Volume of culture plated (mL)}}$$

Equation 1

Data analysis

The collected data were tabulated, and the graphs of incubation period against viable cell counts were generated using the Microsoft Excel program to illustrate the effect of the soap on *Staphylococcus aureus* and *Candida albicans* growth inhibition over time. Descriptive analysis was conducted to determine the MIC, MBC and MFC values with graphical presentation of the time-kill curve.

RESULTS AND DISCUSSION

Confirmation of isolates

The *Staphylococcus aureus* isolates were confirmed through microscopic examination with Gram staining, showing purple-stained colonies with cocci in grape-like clusters. *Candida albicans* isolates were determined through microscopic examination via simple stain using crystal violet, revealing clear morphology of oval yeast cells with budding, a clear indication of *Candida* strain (Binkhamis & Haldane, 2018).

Antibacterial analysis

The antibacterial evaluation of the soap was measured through the value of MIC and MBC. MIC is determined as the lowest concentration of an antibacterial agent able to completely inhibit the growth of the tested organism (Kowalska-Krochmal & Dudek-Wicher, 2021). The bacterial broth microdilution assay revealed bacterial growth through the colour changes of the well from blue to pink. Through decreasing serial dilutions, the first three soap concentrations (120 mg/mL, 80 mg/mL & 53.33 mg/mL) revealed blue colour in each well, indicating no bacterial growth. From the fourth well onward, pink colour changes were observed, indicating the presence of bacterial growth. The pattern was consistent across all triplicates done in the test. Table 1 summarises the growth indication of *Staphylococcus aureus* in this test, with the third dilution, 53.33 mg/mL, recorded as its MIC value. Previous studies have shown that various commercial antiseptic soaps have achieved MIC values against the same species ranging from 12.5 to 50 mg/mL (Nwankwo *et al.*, 2022; Raji *et al.*, 2024). In comparison, the MIC value of this soap is notably higher, indicating that it requires a greater concentration to inhibit bacterial growth effectively. For further confirmation of the absence of bacterial growth, every blue coloured well was sub-cultured onto nutrient agar.

Based on the sub-cultured results, abundant bacterial growth was detected on agar with 53.33 mg/mL soap concentration. Despite the blue appearance of the well in the previous test and its being identified as the MIC value, it is possible that soap concentrations less than 80 mg/mL do not effectively inhibit *Staphylococcus aureus* growth. The bacterial metabolic activity may be too low to be detected by the resazurin dye. As a result, MBC is determined at 80 mg/mL as the lowest concentration of soap suspension needed to effectively inhibit the growth of *Staphylococcus aureus*, which was then applied in a time-kill assay to assess its effect with time exposure.

Antifungal analysis

The same methods were applied to assess the antifungal effect of this product. Through the fungal broth microdilution assay, blue coloured wells were observed in three soap concentrations (120 mg/mL, 80 mg/mL & 53.33 mg/mL) to indicate absence of fungal growth. However, for the wells with 80 mg/mL and 53.33 mg/mL soap concentration, cloudiness of the mixture in the wells was observed, suggesting possible fungal growth. Meanwhile, the other test wells of lower soap concentrations all exhibited pink colour changes, indicating clear signs of *Candida albicans* growth. This pattern was consistent across the triplicates as tabulated in Table 2, determining the 120 mg/mL soap concentration as the MIC value. Upon observation of sub-cultured plates, no agar plates showed complete fungal inhibition with zero colony growth at any soap concentration tested. However, at 120 mg/mL soap concentration, fungal colony counts were consistently below five per plate. This reduction is sufficient to determine this concentration as the MFC, as it demonstrates effective antifungal activity by significantly reducing fungal growth at this level. Hence, 120 mg/mL soap suspension was determined as both the MIC and MFC based on the marked reduction in fungal colony recovery following subculture, indicating potent antifungal activity at this concentration. These findings suggest that the soap's efficiency is significantly concentration-dependent, with higher concentrations required to achieve fungicidal activity. It also indicates that eliminating fungal growth generally requires a higher concentration than that needed to control bacterial growth.

Time-kill assay analysis

Time-kill assays are usually done to quantitatively assess the bactericidal activity of antimicrobial agents against specific bacterial strains by measuring viability at various time intervals, determining the rate of microbial inhibition over time at minimum concentrations, and distinguishing between microbicidal and microbiostatic effects (Alshareef, 2021). It provides insights into the pharmacodynamics of the agents and helps understand the possible therapeutic effect on humans. Conducting this test allows for the examination of both concentration- and time-dependent microbicidal activities. This study specifically measured the soap's antimicrobial kinetics against *Staphylococcus aureus* and *Candida albicans* at their respective MBC and fungal MIC values, quantifying the onset and duration of action through colony counts.

Table 3 presented the number of bacterial and fungal colony counts on agar plates and their CFU values, with the graphical presentation of exposure time against viable microbial cells in Figure 1. Time-kill data were analysed descriptively and are presented as mean ± SD of three technical replicates. As independent biological replication was insufficient for robust inferential statistical analysis, no

hypothesis testing was performed, and time-kill findings should be interpreted as preliminary.

Figure 1 visualised the microbial viability of *Staphylococcus aureus* and *Candida albicans* over 5-min exposure to the soap. The distilled water control for both species showed no growth inhibition throughout the entire duration, confirming microbial viability in the absence of active agents. Upon exposure to the 80 mg/mL soap suspension, *Staphylococcus aureus* exhibited a progressive growth inhibition from 6.05 ± 0.03 to 4.62 ± 0.28 CFU/mL between 0.2 and 1 min. It then dropped sharply to 1.33 ± 2.31 CFU/mL at 2 min,

Table 1. Growth indication of *Staphylococcus aureus*.

			Replicate 1	Replicate 2	Replicate 3
Test wells	Soap Concentration (mg/mL)	120.0	X	X	X
		80.0	X	X	X
		53.3	X	X	X
		35.6	✓	✓	✓
		23.7	✓	✓	✓
		15.8	✓	✓	✓
		10.5	✓	✓	✓
		7.0	✓	✓	✓
		4.7	✓	✓	✓
		3.1	✓	✓	✓
		2.1	✓	✓	✓
		1.4	✓	✓	✓
Control wells	*S. aureus* + Mueller-Hinton broth		✓	✓	✓
	S. aureus + Mueller-Hinton broth + Ciprofloxacin suspension		X	X	X
	120 mg/mL soap suspension only		X	X	X
	Mueller-Hinton broth only		X	X	X

X = no bacterial growth; ✓ = bacterial growth present.

Table 2. Growth indication of *Candida albicans*.

			Replicate 1	Replicate 2	Replicate 3
Test wells	Soap Concentration (mg/mL)	120.0	X	X	X
		80.0	✓	✓	✓
		53.3	✓	✓	✓
		35.6	✓	✓	✓
		23.7	✓	✓	✓
		15.8	✓	✓	✓
		10.5	✓	✓	✓
		7.0	✓	✓	✓
		4.7	✓	✓	✓
		3.1	✓	✓	✓
		2.1	✓	✓	✓
		1.4	✓	✓	✓
Control wells	*C. albicans* + Sabouraud dextrose broth		✓	✓	✓
	C. albicans + Sabouraud dextrose broth + fluconazole suspension		X	X	X
	120 mg/mL soap suspension only		X	X	X
	Sabouraud dextrose broth only		X	X	X

X = no fungal growth; ✓ = fungal growth present.

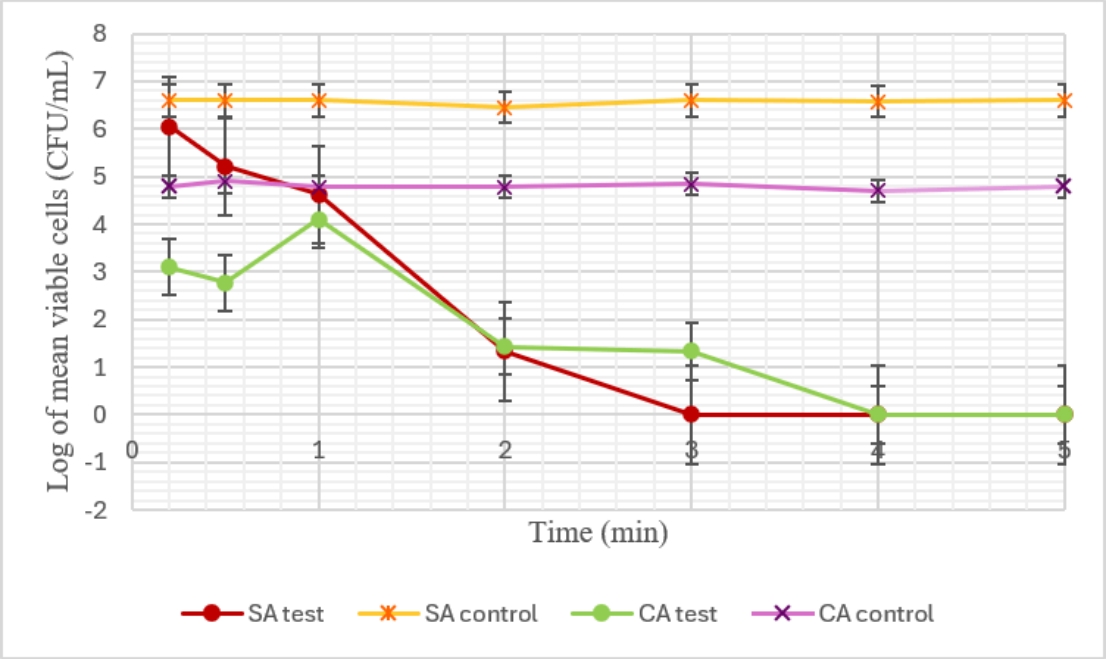

Fig. 1. Time-kill curve showing the viability of *Staphylococcus aureus* and *Candida albicans* over different incubation times when exposed to soap concentrations equivalent to their respective MBC and MFC values, compared with distilled water controls. SA test = *Staphylococcus aureus* tested with 80 mg/mL of soap suspension; SA control = *Staphylococcus aureus* tested with distilled water; CA test = *Candida albicans* tested with 120 mg/mL of soap suspension; CA control = *Candida albicans* tested with distilled water. Data are presented as mean ± SD (n=3 technical replicates).

followed by complete inhibition at 3 min and sustained through the end of the timeline, demonstrating the soap's rapid antibacterial effect to achieve complete inhibition of *Staphylococcus aureus* within three min of exposure.

Meanwhile, the soap demonstrated a moderate inhibitory effect against *Candida albicans*. Initially, the fungal population decreased slightly from 3.10 ± 2.69 to 2.77 ± 2.40 CFU/mL for 0.5 min, then a sharp surge of colony growth was observed at 1 min before progressively declining to complete inhibition by 4 min. The early fluctuation could be due to the short 30-s interval, which may have been insufficient for complete neutralisation and for the product to exert its full antifungal effect, leading to a transient increase in fungal colonies. This non-linear pattern may also be associated with biological or experimental variability and has been reported in some studies as paradoxical growth, a phenomenon where microorganisms temporarily proliferate at higher drug concentrations despite being susceptible at lower concentrations (Franconi & Lupetti, 2023). However, the present findings are insufficient to confirm this phenomenon. Since time-kill analysis evaluates the continuous interaction between the soap and fungal cells over time, it focuses on the overall trajectory rather than specific, isolated time points. Overall, the complete reduction of *Candida albicans* across the 5-min exposure demonstrates the soap's antifungal efficacy. While the observed non-linear killing pattern requires cautious interpretation, the overall trend serves as a reliable indicator of activity, suggesting that prolonged exposure was needed for the soap to exert its full fungicidal effect.

According to the Centres for Disease Control and Prevention (2024) and the World Health Organisation (2009), it is recommended to spend at least 20 s of handwashing in everyday settings, and up to 1 to 3 min in surgery preparation or healthcare settings for effective microbial reduction. An observational study also confirmed the optimal handwashing duration to be around 30 s with proper technique (Shi *et al.*, 2023). These reports and guidelines on proper hand-washing technique and duration support the results of this study, which showed a marked reduction of bacterial and fungal growth from 0.2 min to 0.5 min (between 10 to 30 s). Thus, soap exposure of at least 20 s may contribute to reducing microbial load, suggesting its potential application in daily hygiene practices.

The impact of the assays on antibacterial and antifungal results

In-depth analysis of broth microdilution assay and TKA revealed that the tested product possesses greater antibacterial activity than antifungal activity as indicated by the lower MIC value against *Staphylococcus aureus* compared to *Candida albicans* and by faster inhibition of bacterial growth. The consistent decrease in bacterial colony counts in TKA reflects a predictable antibacterial effect, while the slightly irregular pattern observed in fungal inhibition suggests more complex interactions between the soap and fungal cells. This variation may be attributed to differences in the pathogens' characteristics. The bacterial cell wall is mainly composed of peptidoglycan, while the fungal cell wall is made up of glucans. Peptidoglycan is a non-complex structure that is more susceptible to damage when exposed to an antimicrobial agent, as compared to glucans (Cardoso *et al.*, 2023). Supporting this theory, higher MIC values were observed against *Candida albicans* in powdered neem extract, indicating a lesser effect compared with *Staphylococcus aureus* (Rebekah *et al.*, 2022). Moreover, studies have consistently shown that several commercial products exhibit stronger antibacterial than antifungal activity. For instance, Kaliyadan *et al.* (2014) reported that CAREX antibacterial soap and Palmolive hygiene hand-wash significantly reduced the populations of multiple bacterial species including *Staphylococcus aureus* but had minimal effect on *Candida albicans*, suggesting that these soaps are more effective against bacteria than fungi. Despite these differences, the MIC values for both species remain relatively low and within close range, indicating a strong antimicrobial property of this commercial herbal soap.

Through these assays, the antibacterial and antifungal effect of the soap can be observed clearly and measured quantitatively, giving precise results of the product's efficiency. Each method has the capability to detect differential effects between bacteria and fungi, providing a comprehensive evaluation of the product's antimicro-

Table 3. Colony growth of *Staphylococcus aureus* and *Candida albicans* at different exposure times.

Exposure time (min)	*S. aureus* Soap suspension (80 mg/mL)	*S. aureus* Distilled water (control)	*C. albicans* Soap suspension (120 mg/mL)	*C. albicans* Distilled water (control)
0.2	6.05 ± 0.03	6.60	3.10 ± 2.69	4.78
0.5	5.21 ± 0.19	6.60	2.77 ± 2.40	4.90
1.0	4.62 ± 0.28	6.60	4.10 ± 0.17	4.78
2.0	1.33 ± 2.31	6.45	1.43 ± 2.48	4.78
3.0	0	6.60	1.33 ± 2.31	4.85
4.0	0	6.57	0	4.70
5.0	0	6.60	0	4.78

Values are expressed as mean ± SD (log CFU/mL), n=3 technical replicates.

bial spectrum. It also highlights how varying concentration and exposure time systematically influenced the antimicrobial activity observed, demonstrating the sensitivity and practical applicability of the assays. This directly reflects the objective of the test to evaluate the antimicrobial efficacy of herbal-based soaps using standardised laboratory procedures.

Potential for standard reference in microbial testing

In any microbial testing, it is crucial to have a reliable, reproducible, and standardised method for evaluating antimicrobial activity. As of now, there is a lack of uniformity across studies evaluating the antimicrobial activity of herbal soaps. A standardised method may facilitate comparison across studies and improve reproducibility of antimicrobial claims, promoting more high-quality research.

The methods presented in this study, which tested varying concentrations and exposure times against both bacteria and fungi, provide quantitative measures relevant for routine antimicrobial efficacy evaluation. The results obtained can be readily applied in everyday settings. The serial dilution in the broth microdilution assay mimics the natural dilution of soaps with water during use, which ensures realistic results relevant to skin exposure rather than undiluted laboratory conditions. Furthermore, testing multiple time points in TKA estimates how long soap typically remains on the skin during washing. It reveals the rate and efficiency of the soap in reducing microbial load within a practical handwashing duration. General handwashing lasts 20 to 30 s but longer contact time or repeated exposure may be necessary for complete microbial inhibition. This knowledge supports public health recommendations and underscores the importance of considering both dilution and exposure time to maximise antimicrobial efficacy in routine hand hygiene. The combined methodological approach may provide a useful framework for evaluating antimicrobial activity in formulated herbal soap products.

Studies have applied the broth microdilution technique to determine MICs and MBCs, followed by time-kill kinetics, to evaluate the antimicrobial properties of diverse products, including an alcohol-free hand sanitiser against foodborne pathogens and plant extracts, effectively demonstrating their antimicrobial efficacy (Appiah *et al.*, 2017; Adusei *et al.*, 2019; Bayer *et al.*, 2023). These established assays have been the standard methods in drug development research to define safe and effective therapeutic doses for novel compounds, with CLSI guidelines providing standardised protocols for MIC determination across various microbial species. Their proven reliability in pharmaceutical testing extends to soap formulations. Bar soap samples can be readily prepared by weighing and dissolving in distilled water to achieve the desired concentration. D'Arezzo *et al.* (2012) and Farzana *et al.* (2011) have also tested liquid formulations using the broth microdilution technique. Recent literature rarely combines both assays for antimicrobial evaluation on soaps, focusing instead on validation for essential oils or general antimicrobials, with time-kill applied to soap-like products. Thus, this study addresses this gap by combining both assays to evaluate a commercial herbal soap formulation, an approach infrequently reported in prior research.

CONCLUSION

This study demonstrated the antimicrobial activity of the neem and turmeric-infused herbal soap against two common microorganisms, *Staphylococcus aureus* and *Candida albicans*. The MIC value for *Staphylococcus aureus* was 53.33 mg/mL with MBC at 80 mg/mL, while *Candida albicans* recorded both MIC and MFC at 120 mg/mL soap concentration. Both isolates exhibited antimicrobial responses within 30 s to 1 min, with complete inhibition at 3 and 4 min, respectively. Overall, the tested soap demonstrated measurable antibacterial and antifungal activity, with its antimicrobial effects influenced by both soap concentration and exposure time. The findings suggest that both the concentration and the duration of exposure are important factors influencing its ability to reduce microbial growth.

These results are consistent with current hygiene guidelines and support the potential application of the tested formulation in daily hygiene routines in healthcare or community settings. Furthermore, the study employed established quantitative antimicrobial assays that may provide a useful methodological basis for future evaluation of herbal-based antimicrobial formulations. The combined use of these assays may facilitate more systematic assessment of antimicrobial activity in future research, product development and quality control studies involving herbal-based formulations.

Overall, this investigation not only demonstrated the microbial reduction capability of the herbal soap but also contributed valuable methodological insights. By providing a useful testing framework, it paves the way for ongoing improvements in product formulation and ensures that consistent efficacy standards can be maintained. This contributes to the broader field of natural product development and supports the increasing interest in plant-based antimicrobial agents as safe and effective alternatives to conventional chemicals.

ACKNOWLEDGEMENTS

The authors have declared that no grants were involved to support this work.

ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Adusei, E.B.A., Adosraku, R.K., Oppong-Kyekyeku, J., Amengor, C.D.K. & Jibira, Y. 2019. Resistance modulation action, time-kill kinetics assay, and inhibition of biofilm formation effects of plumbagin from *Plumbago zeylanica* Linn. Journal of Tropical Medicine, 2019(1): 1-8. https://doi.org/10.1155/2019/1250645

Alrashidi, A., Jafar, M., Higgins, N., Mulligan, C., Varricchio, C., Moseley, R., Celiksoy, V., Houston, D.M.J. & Heard, C.M. 2021. A time-kill assay study on the synergistic bactericidal activity of pomegranate rind extract and Zn(II) against methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Biomolecules, 11(12): 1889. https://doi.org/10.3390/biom11121889

Alshareef, F. 2021. Protocol to evaluate antibacterial activity MIC, FIC and time kill method. Acta Scientific Microbiology, 4(5): 2-6. https://doi.org/10.31080/ASMI.2021.04.0825

Appiah, T., Boakye, Y.D. & Agyare, C. 2017. Antimicrobial activities and time-kill kinetics of extracts of selected Ghanaian mushrooms. Evidence-Based Complementary and Alternative Medicine, 2017: 4534350. https://doi.org/10.1155/2017/4534350

Bayer, G., Shayganpour, A. & Bayer, I.S. 2023. Efficacy of a new alcohol-free organic acid-based hand sanitizer against foodborne pathogens. Toxics, 11(11): 938. https://doi.org/10.3390/toxics11110938

Binkhamis, K. & Haldane, D. 2018. An observation of altered morphology of *Candida albicans* in vivo. Clinical Microbiology Newsletter, 40(19): 162-164. https://doi.org/10.1016/j.clinmicnews.2018.02.006

Cardoso, B.E.M., Medeiros, A.A.A., Martins, M.L., Araujo de Sousa, A.L., Alves, N.Dos S., Veras, L.M.C., Araújo, A.R. & Pinto, A.S.B. 2023. Antibacterial and antifungal activity of curcumin and methylene blue. Revista Ciência Plural, 13(4). https://doi.org/10.21876/rcshci.v13i4.1454

Centers for Disease Control and Prevention. 2024. Handwashing facts. CDC. Available at: https://www.cdc.gov/clean-hands/data-research/facts-stats/index.html (Accessed: 25 December 2024).

Clinical and Laboratory Standards Institute. 2024. M100 Performance Standards for Antimicrobial Susceptibility Testing. 34th Ed. CLSI supplement M100.

D'Arezzo, S., Lanini, S., Puro, V., Ippolito, G. & Visca, P. 2012. High-level tolerance to triclosan may play a role in *Pseudomonas aeruginosa* antibiotic resistance in immunocompromised hosts: evidence from outbreak investigation. BMC Research Notes, 5: 43. https://doi.org/10.1186/1756-0500-5-43

Das, D.A., Sherin, F., Mathew, S. & R, S. 2023. Design and characterisation of herbal soap for the treatment of acne and dry skin: factorial design approach. Saudi Journal of Biomedical Research, 8(8). https://doi.org/10.36348/sjbr.2023.v08i08.001

Dhinakaran, M., Sundarasen, S. & Arunraj, A. 2017. Detailed study on the synergistic effect of neem extract loaded with curcumin in wound healing using textile substrate. International Research Journal of Pharmacy. https://doi.org/10.7897/2230-8407.087126

Farzana, K., Batool, S., Ismail, T., Asad, M.H.H.B., Rasool, F., Khiljee, S. & Murtaza, G. 2011. Comparative bactericidal activity of various soaps against gram-positive and gram-negative bacteria. Scientific Research and Essays. https://doi.org/10.5897/SRE11.009

Franconi, I. & Lupetti, A. 2023. *In vitro* susceptibility tests in the context of antifungal resistance: Beyond minimum inhibitory concentration in *Candida* spp. Journal of Fungi, 9(12): 1188. https://doi.org/10.3390/jof9121188

Kaliyadan, F., Aboulmagd, E. & Amin, T. 2014. Antimicrobial activity of commercial "antibacterial" handwashes and soaps. Indian Dermatology Online Journal, 5(3): 344. https://doi.org/10.4103/2229-5178.137799

Kasprzak-Drozd, K., Niziński, P., Hawrył, A., Gancarz, M., Hawrył, D., Oliwa, W., Pałka, M., Markowska, J. & Oniszczuk, A. 2024. Potential of curcumin in the management of skin diseases. International Journal of Molecular Sciences, 25(7): 3617. https://doi.org/10.3390/ijms25073617

Kowalska-Krochmal, B. & Dudek-Wicher, R. 2021. The minimum inhibitory concentration of antibiotics: methods, interpretation, clinical relevance. Pathogens, 10(2): 165. https://doi.org/10.3390/pathogens10020165

Mbouche, J.V., Ngamga, F.H.N., Ngangoum, E.S. & Djikeng, F.T. 2022. Evaluation of antibacterial and antifungal activities of a medicinal soap made from *Zingiber officinale* and *Syzygium aromaticum* essential oils. Journal of Advances in Biology & Biotechnology. https://doi.org/10.9734/jalsi/2022/v25i530307

Mohammed, U.F., Danmallan, A. & Ahmed, M.H. 2022. Production and investigation of the antiseptic properties of soaps made from the barks, seeds and leaves extracts of neem tree. European Journal of Medicinal Plants. https://doi.org/10.9734/ejmp/2022/v33i130445

Nwankwo, I.U., Edwards, K.C., Itaman, V.O., Udensi, C.G. & Unah, O.G. 2022. Antibacterial activities of medicated and antiseptic soaps on *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolated from wound infections. American Journal of Life Sciences. https://doi.org/10.32861/ajls.83.39.45

Raji, M., Garba, I., Umar, I., Ayuba, A. & Adisa-Raji, N. 2024. Antibacterial activities of different brands of antiseptic soaps on *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolated from wound infections. Journal of Pharmaceutical and Allied Sciences, 21(1): 4049-4057.

Rebekah, R., Saravana, D.S., Nivethigaa, B. & Rajeshkumar, S. 2022. In vitro antimicrobial and cytotoxic activity of neem and Kirata herbal formulation mediated silver nanoparticles. Bioinformation, 18(10). https://doi.org/10.6026/973206300181069

Seetha Devi, A., Sivani, D.V., Anusha, D., Sarath, G. & Sultana, S.M. 2021. Formulation and evaluation of antimicrobial herbal soap. International Journal of Pharmaceutical Sciences Review and Research. https://doi.org/10.47583/ijpsrr.2021.v71i02.019

Shaibu, M. & Orivri, F.O. 2025. Formulation and assessment of the antimicrobial properties of herbal soap made with bioactive neem plant *Azadirachta indica*. International Journal of Pharmaceutical Research and Applications. https://doi.org/10.35629/4494-1004913918

Shi, C., O'Donoghue, M., Yang, L., Tsang, H., Chen, J., Zou, J., Qin, J., Mak, Y.W., Pittet, D., Xie, Y.J., Lai, T., Li, C. & Cao, J. 2023. Factors associated with hand washing effectiveness: an institutional ethnographic study. Antimicrobial Resistance & Infection Control, 12. https://doi.org/10.1186/s13756-023-01293-1

Smith, R., Russo, J., Fiegel, J. & Brogden, N. 2020. Antibiotic delivery strategies to treat skin infections when innate antimicrobial defense fails. Antibiotics, 9(2): 56. https://doi.org/10.3390/antibiotics9020056

Upula, S.A., Bassey, E.E. & Ije, U. 2021. Antiseptics soaps and body cleansing agents and its effects on the normal flora of the human skin. World Journal of Pharmaceutical and Medical Research, 7(4): 28-34.

World Health Organization. 2009. WHO Guidelines on Hand Hygiene in Health Care. World Health Organization.

World Health Organization. 2022. Antimicrobial Resistance TrACSS Malaysia 2022 Country Profile. World Health Organization.

Yeroushalmi, S., Shirazi, J.Y. & Friedman, A. 2020. New developments in bacterial, viral, and fungal cutaneous infections. Current Dermatology Reports, 9(2): 152-165. https://doi.org/10.1007/s13671-020-00295-1