SIAR - Global Journal of Medicine & Pharmaceutical Review (GJMPR)
www.siarpublications.org ISSN: 3122-0762
info@siarpublications.org Vol. 2 Issue 1 Jan.-March 2025

Antimicrobial Activity of Locally Made Soaps against Selected Bacteria

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Abstract

Some studies have reported the considerable antimicrobial activities of locally made soaps, prompting the investigation of the antibacterial effects of three selected locally produced soaps in this study. The antimicrobial activity of the three locally produced soaps [neem, turmeric, and African black soaps] were challenged against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, and *Escherichia coli* at different concentrations [3%, 6%, 9%, 12%, and 15%]. African black soap [44.5 mm] showed the highest diameter of zone of inhibition (DZOI) , followed by neem soap [12.75 mm] and turmeric soap [6.26 mm], which recorded the lowest DZOI. Neem soap revealed a minimum inhibitory concentration (MIC) of 120 mg/mL], and minimum bactericidal concentration (MBC)] of 125 mg/mL for *Staphylococcus aureus*. Turmeric soap exhibited a MIC of 50 mg/mL against *Klebsiella pneumoniae* and a MBC of 50 mg/mL against Escherichia coli. African black soap exhibited a MIC of 50 mg/mL] for S. aureus] with a MBC of 50 mg/mL for *Proteus mirabilis*. The results from this research support the fact that locally produced African black soaps and neem soap may be exploited as additional sources of care in tackling and preventing skin bacterial diseases.

Keywords: Locally produced soap, Antimicrobial activity, Diameter zone of inhibition

Introduction

Soaps exist in liquid, solid, semisolid or powder form and are used for the removal of dirt, stains and bad odor from our bodies or other material (Friedman *et al.,* 2013), and antibacterial soaps have the capacity of destroying 65% to 85% of skin microbes (Solanki *et al.,* 2011). The research aims to evaluate antimicrobial activity of selected locally made soaps against *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsella pneumoniae and Proteus mirabilis.* The hydrophilic head of soap readily bonds with water while the hydrophobic tail has affinity for fats and oil, and this is responsible for the preferred attachment to the lipid membrane of microbes (Jonathan *et al*; 2013).Soap was initally made by cooking a combination of animal fat and lye (wood ashes), as it stands, a lot of other substances are being included, and the Europeans were the first to use olive oil as soap in the 7th century (Mukhopadhyay, 2011).The African black soap (dudu-osun) is made with plantain skins, palm tree leaves, cocoa pods, shea tree bark which are processed to produce ash and later mixed with,coconut oil and palm oil has antimicrobial activity against some bacteria and fungi (Oleghe *et al*., 2022). Turmeric is the dried rhizome powder derived from the Curcuma longa

plant and the active ingredient is curcumin which demonstrated antimicrobial activity against bacteria and fungi via inhibition of cell division, disruption of cell wall and membrane, DNA damage and anti-biofilm activity. Neem is also known as *Azadirachta indica*, Indian lilac or dogonyaro (Nigeria) contains active ingredients in seed, bark, and leaf. The leaf extract contains nimbidin, cyclic trisulphide, cyclic tetrasulphide, and polyphenolic flavonoids, which have antibacterial, antifungal,antioxidant and anticancer properties (Lakshmi *et al.,* 2015). Some researchers have documenented the antimicrobial activities of neem soap against pathogens like *Staphylococcus aureus* (Hossain *et al*., 2013). Bacteria have higher zones of inhibition to the locally made soap than medicated soap and the activities of locally made soaps against bacteria have been linked to the metabolic components present in palm kernel oil, Neem and Shea butter which are major ingredients of the soaps. Medicated soaps mostly contain synthetics rather than these natural available bioactive components, and show mild antimicrobial effects compared to locally made soaps. Shea butter is composed of a greater UV-B absorbing triterpenes esters and fatty acids, that complement greater antimicrobial activity of locally made soaps.

Methodology

Collection of Samples

Locally made soap samples in Nigeria such as African black soap, Neen soap and Tumeric soap were purchased from Nyanya gwandara in Nasarawa state and Amassoma market in Bayelsa state and taken to the laboratory.

Test microorganisms

Isolates such as *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis and Klebsiella pneumoniae* were subjected to Gram staining and biochemical tests such as oxidase, coagulase, acid production from glucose sucrose, lactose, indole, citrate, kligler iron agar and urease tests (Cheesbrough, 2010).

Soap processing

Soap was scraped with the aid of a scapel (sterilized) to obtain 250mg, 200mg ,150mg, 100mg and 50mg, each dissolved in one milliliter of sterile distilled water (Kin *et al.,* 2018).

Soap disks preparation

6mm diameter disks were bored and wrapped in foil paper and sterilized by autoclaving and 6microliters of each of the concentrations (3%,6,%,9%, 12%, and 15%) was obtained and dispensed on the disk and allowed to dry (Kin *et al.,* 2018).

Mcfarland standard preparation

Firstly, 1% of $H_2SO_4$was prepared by adding 1ml of concentrated H_2SO_4 to 99ml of distilled water in conical flask. Also 1% of barium chloride solution was prepared in another conical flask by adding 0.5g of dehydrated Barium chloride to 50ml of distilled water. Then slowly, with constant agitation, 0.6ml of Barium chloride solution was added to 99.4ml of H_2SO_4 (Kin *et al*., 2018)

Soap preparations antimicrobial activity

Three colonies of each test organism were taken from a 24-hour culture and suspended in 5ml of peptone water in test tubes using a sterile wire loop and was incubated overnight. The test tubes were centrifuged at 3000rpm for 10minutes and the supernant was decanted. sterile physiological saline was added drop by drop and then adjusted to 0.5 McFarland Standard. 1ml of the standardized test isolates was added to the surface of the Mueller Hinton agar

evenly in the plate and excess fluids was decanted .The inoculated plates were incubated at 37°C for 20 minutes for acclimatization and growth of the inoculums. The soap disks were then applied on the inoculated plates using a sterile forcep. The plates were incubated invertedly at 37°C for 24hours. This analysis was carried out in triplicate. At the end of incubation period the diameter zones of inhibitions around each disc were measured and recorded (Cheesebrough, 2010).

Minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC and MBC were determined according to the National Committee for Clinical Standard (1999). About 50mg,100mg,150mg 200mg and 250mg each of soap was weighed and dissolved in 1ml of sterile distilled water to prepare the soap solutions. Then, 1ml each of peptone water was transferred into six test tubes. Followed by adding 1ml each of 50mg,100mg,150mg ,200mg and 250mg of the soap samples to tube one to five. 1ml of the standardized test bacteria was introduced into the test tubes except tube six which is the positive control; the tubes were incubated at 37^0C for 18 hours. The MIC was taken as the lowest concentration that prevented visible growth. All test tubes without visible growth were sub cultured on Mueller Hinton agar and incubated for forty eight hours at 37°C, then the lowest concentration where there was no growth is adopted as the MBC

Results

Gram staining and biochemical tests of test bacteria

Table 1 illustrates the Gram and biochemical test reactions of *Proteus mirabilis*, *Klebsiella Pneumoniae*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

Table 1: Gram and biochemical tests of test isolates

S/N		Gram Reaction	Catalase	Coagulate	Citrate	Indole	KIA			Oxidase	Urease
							Acid	Gas	H_2S		
1	*Proteus mirabilis*	-	+	-	+	-	R/Y	+	+	-	+
2	*Klebsiella Pneumoniae*	-	+	-	+	-	Y/Y	+	-	-	+
3	*Staphylococcus aureus*	+	+	+	-	-	-	-	-	-	-
4	*Escherichia coli*	-	+	-	-	+	Y/Y	+	-	-	+
5	*Pseudomonas aeruginosa*	-	+	-	+	-	R/R	-	-	+	-

-=Negative;+=Positive; R=Red slope or butt; Y=Yellow slope or butt

Antimicrobial activity of neen soap against test bacteria

Table 2 presents the mean ± standard deviation of the diameter zone of inhibition (DZOI) of Neen soap against different test bacteria at varying concentrations. Neen soap exhibited the

highest DZOI against *Proteus mirabilis* (12.75 ± 14.48 mm) and *Staphylococcus aureus* (4 ± 3.77 mm) at15% concentration. *Escherichia coli* and *Klebsiella pneumoniae* had the highest DZOI of 2.75 ± 5.185 mm and 2.75 ± 5.185 mm, respectively at 3% concentration, while the highest DZOI of 1.25 ± 2.357 mm for *Pseudomonas aeruginosa* was at 6% concentration

Table 2: Antimicrobial activity of neen soap against test bacteria

Concentrations	*Proteus mirabilis*	*Escherichia coli*	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Pseudomonas aeruginasa*
3%	10.25±5.66	2.75±5.185	1.75±3.30	2.75±5.185	0
6%	6.7±2.5	2±3.771	1.75±3.30	2±3.771	1.25±2.357
9%	5.74±5.75	0	1.75±3.30	0	0
12%	6.7±6.0	0	4±3.77	0	0
15%	12.75±14.48	0	4±3.77	0	0

values = mean ± standard deviation of the diameter zone of inhibition (DZOI) in millimeters

Antimicrobial activity of turmeric soap against selected bacteria

Table 3 presents the mean ± standard deviation of the diameter zone of inhibition (DZOI) of tumeric soap against different test bacteria at varying concentrations. Tumeric soap exhibited the highest DZOI against *Proteus mirabilis* (6.25 ± 0.47 mm) at 12% concentration. *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* had the highest DZOI of 6.26 ± 1.25 mm and 4 ± 4.03 mm respectively at 15% concentration. Tumeric soap exhibited the highest DZOI against *Staphylococcus aureus* (6.25±0.47 mm) and *E. coli* (1.75±3.30 mm) at 12% and 3% concentrations respectively.

Table 3: Antimicrobial activity of turmeric soap against selected bacteria

	Zone of inhibition of tumeric soap				
Concentrations	*Proteus mirabilis*	*Escherichia coli*	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*
3%	2±3.77	1.75±3.30	2±3.77	1.75±3.30	2±3.77
6%	3.75±3.56	0	1.75±3.77	0	2±3.77
9%	1.75±3.30	0	1.75±3.77	1.75±3.30	2.25±4.24
12%	1.75±3.30	0	6.25±0.47	6.26±1.25	1.75±3.56
15%	3.75±3.56	0	1.75±3.3	2.25±1.25	4±4.03

values = mean ± standard deviation of the diameter zone of inhibition (DZOI) in millimete

Antimicrobial activity of African black soap against test bacteria

Table 4 presents the mean ± standard deviation of the diameter zone of inhibition (DZOI) of African black soap against different test bacteria at varying concentrations. African black soap exhibited the highest DZOI against *Staphylococcus aureus* (20.5±1 mm) at 12% concentration. *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* had the highest DZOI of 16.75±2.05 mm and 14.5±1 mm respectively at 9% concentration. Against *Proteus mirabilis* the highest DZOI of 10.25±1.15mm, 10.30±1.18mm and 10.30±1.18mm at 9%, 12% and 15% concentrations respectively were observed. While against *E.coli* the highest DZOI recorded was 19.5±1mm at 15% concentration.

Table 4: Antimicrobial activity of African black soap against test bacteria

	Zone of inhibition of African black soap				
Concentrations	*Proteus mirabilis*	*Escherichia coli*	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*
3%	0.75±0	13.5±1.5	0	0	0
6%	1.5±0	7±0.71	0	0	0
9%	10.25±1.15	2.25±0	17.25±1.41	16.75±2.05	14.5±1
12%	10.30±1.18	3±0	20.5±1	11.25±0.0	12.75±1

15%	10.30±1.18	19.5±1	0	4±0.0	7.5±0.0

values = mean ± standard deviation of the diameter zone of inhibition (DZOI) in millimeters

Minimun inhibitory concentration and minimum bactericidal concentration of soaps against test bacteria

Table 5 depicts the Minimum Inhibitory Concentration (MIC) and minimum bacteriacidal concentration results for different types of soap against various test bacterial strains. The MIC is the lowest concentration of a substance that inhibits visible growth of a microorganism. The MIC of 250 mg/ml for Neem soap against *Staphylococcus aureus* and *Proteus mirabilis* indicates that neem soap inhibited the growth of both bacteria at this concentration. While the MIC for Neem soap against *Pseudomonas aeruginosa* and *E.coli* was 200 mg/ml . The MIC for Tumeric soap against *E.coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* was 150, 50 and 250 mg/ml respectively. The MIC for African black soap against *Staphylococcus* aureus, *Pseudomonas aeruginosa* and *E.coli* was 150, 250 and 100 mg/ml respectively. While that for both *Klebsiella pneumoniae* and *Proteus mirabilis* was 50 mg/ml. The MBC of 200 mg/ml for neem soap against *Pseudomonas aeruginosa* indicates that the neem soap was bactericidal at this concentration. The MBC for neem soap against *Staphylococcus aureus* and E.*coli* was 150 mg/ml, while the MBC for *Proteus mirabilis* was 50 mg/ml.

Table 5: Soap preparation and test bacteria minimun inhibitory concentration/ minimum bactericidal concentration

MIC NEEN SOAP				**MIC** TUMERIC			**MIC** AFRICAN BLACK SOAP			
S	PA	EC	PM	PA	EC	PA	S	PA	EC	KP
250							150			
120	200	200	250	100	50	250	50	250	100	75
MCB NEEN SOAP				**MCB** TUMERIC			**MCB** AFRICAN BLACK SOAP			
S	PA	EC	PM	PA	EC	KP	S	PA	EC	KP
125	200	150	50	200	50	200	100	200	250	150

PM

50

S= *Staphylococcus aureus*; PA=*Pseudomonas aeruginosa*; EC=*Escherichia coli*; PM=*Proteus mirabilis*; KP= *Klebsiella pneumoniae*; values represent the MIC and MCB in mg/ml

Discussion

Neen soap demonstrated the highest zone of inhibition against *Proteus mirabilis* at 3% and 15% concentration and this may likely be due to cell wall composition variation (Kin *et al*., 2018) and inactivation enzymes causing resistance and restricted permeability of the outer-membrane driven by *Pseudomonas aeruginosa* (Caleb *et al*., 2022). *Pseudomonas aeruginosa* was resistant to Neen soap in this study. As the concentration of Neem soap increases (6% to 15%), there is a trend of decreasing effectiveness against most bacterial strains, particularly *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The phenomenon of decreasing zone of inhibition as the concentration of Neem soap increases can be understood through several factors such as saturation of active Compounds, Neem soap contains active compounds such as azadirachtin, nimbin, and nimbidin, which exhibit antimicrobial properties. At lower concentrations, these compounds are more concentrated and readily available to interact with bacterial cells, leading to a larger zone of inhibition. However, as the concentration increases, the active compounds may reach saturation, meaning that all available binding sites on bacterial cells are occupied, resulting in diminished antimicrobial activity and smaller zones of inhibition. At higher concentrations, the diffusion rate may decrease due to increased viscosity of the soap solution, hindering the penetration of active compounds into the agar and limiting their reach to bacterial cells. This slower diffusion rate can contribute to reduced antimicrobial efficacy and smaller zones of inhibition at higher concentrations. Changes in soap concentration can alter the pH of the agar medium, which can influence the antimicrobial activity of Neem soap. Neem soap has been reported to exhibit optimal antimicrobial activity within a specific pH range. At higher concentrations, the pH of the agar medium may deviate from this optimal range, affecting the efficacy of the soap and resulting in smaller zones of inhibition. The zone of inhibition of Tumeric soap exhibited the highest diameter zone of inhibition against *Staphylococcus aureus* and *Klebsiella pneumoniae* at 12% concentration. For *Escherichia coli* at concentrations of 6% and above, there was no observed zone of inhibition against *Escherichia coli*, indicating that Tumeric soap is not effective against this bacterial strain at these concentrations probably due to bacteria cell wall composition variation (Kin *et al*., 2018). This suggests that Tumeric soap was inhibitory when challenged with *Staphylococcus aureus* and *Pseudomonas aeruginosa* particularly at higher concentrations. At all concentrations tested, African Black soap exhibits minimal effectiveness against *Proteus mirabilis* and a non-linear relationship between soap concentration and effectiveness against *Escherichia coli*. African Black soap showed moderate to high effectiveness against *Staphylococcus aureus* and the effectiveness increases with concentration, indicating that higher concentrations of the soap are more effective against *Staphylococcus aureus*. The effectiveness against *Klebsiella pneumoniae* is moderate to high suggesting a concentration-dependent antimicrobial activity against *Klebsiella pneumoniae*. At concentrations of 9% and 12%, African Black soap demonstrates moderate effectiveness against *Pseudomonas aeruginosa*, However, at higher concentrations (15%), the effectiveness decreases. This result is in agreement with Varsha (2016) and his result revealed that most of the tested antiseptic and herbal soaps had antimicrobial activity against *Escherichia coli*, *Bacillus subtilis Staphylococcus aureus* and *Pseudomonas aeruginosa* at a different diameter zone of inhibition. This result suggest that the antimicrobial activity of African Black soap is concentration-dependent and varies with bacterial strain, highlighting the importance of optimizing soap concentration for specific antimicrobial applications. The MIC of Neem soap against *Staphylococcus aureus* is 250 mg/ml and 120 mg/ml, indicating that these concentrations are the lowest at which the soap inhibits the visible growth of the bacteria. For *Pseudomonas aeruginosa* and *Escherichia coli*, the MIC is 200 mg/ml, while for Proteus *mirabilis* it is 250 mg/ml. The MBC of Neem soap against *Staphylococcus aureus* is 125 mg/ml, suggesting that

this concentration is the lowest at which the soap kills the bacteria rather than just inhibiting its growth. For *Pseudomonas aeruginosa* and *Escherichia coli*, the MBC is 200 mg/ml, and for *Proteus mirabilis* it is 50 mg/ml. Turmeric soap shows varying MIC against different bacterial strains. For *Pseudomonas aeruginosa* it is 100 mg/ml, for *Escherichia coli*, it is 150 mg/ml, for *Klebsiella pneumonia,* it is 50 mg/ml, and for *Proteus mirabilis*, it is 250 mg/ml. The MBC of Turmeric soap against *Pseudomonas aeruginosa* is 200 mg/ml, against *Echerichia coli* it is 50 mg/ml, and against *Klebsiella pneumoniae* it is 200 mg/ml. Unfortunately, the MBC for *Proteus mirabilis* is not provided in the given data. For African Black Soap, the MIC against *Staphylococcus aureus* ranges from 50mg/ml to 150 mg/ml, against *Pseudomonas aeruginosa* it is 250 mg/ml, against *Escherichia coli* it is 100 mg/ml, and against *Klebsiella pneumonia* it ranges from 75 mg/ml to 150 mg/ml. The MBC of African Black Soap against *Staphylococcus aureus* ranges from 100 mg/ml to 200 mg/ml, against *Pseudomonas aeruginosa* it is 200 mg/ml, against *Escherichia coli* it is 250 mg/ml, against *Klebsiella pneumoniae* it is 50 mg/ml, and against *Proteus mirabilis* it is not provided.

Conclusion

Locally made soaps exhibited inhibitory effects when challenged with test bacteria. Results suggest that each soap has its unique antimicrobial properties, with different effectiveness against various bacterial strains. The MIC and MBC values provide insights into the concentration at which these soaps inhibit bacterial growth and kill bacteria, respectively. This can therefor spark the recommendation of African black soap, Neen soap and Tumeric soap as potent antimicrobial agents for some bacteria skin infections.

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