Evaluating the Antimicrobial Effects of Medicated Soaps and Black Soaps (Organic) Against Microorganisms Isolated from the Skin

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Abstract: Soaps are recognized primarily as cleaning agents due to their antibacterial properties. The antibacterial efficacy of four distinct soaps, specifically a beauty soap, medicated soap, black soap, and local soap, was examined. Standard microbiological procedures, were employed for the identification of the isolates. The disc diffusion assay was employed to investigate the antibacterial efficacy of the soap samples against the isolates at various concentrations: 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml. The bacteria were identified as *Escherichia coli*, *Staphylococcus* sp., and *Bacillus* sp. At a concentration of 200 mg/ml, local soap inhibited the growth of all isolates, with the maximum inhibition measuring 31 mm and the minimum inhibition measuring 8.0 mm. At a concentration of 100 mg/ml, black soap and local soap exhibited significant inhibition against all isolates, with the zone of inhibition decreasing as the concentration of the soap samples decreased. Beauty soap shown antibacterial activity at concentrations of 100 mg/ml and 50 mg/ml. The minimum inhibitory concentrations (MIC) for the isolates varied between 12.5 mg/ml and 25 mg/ml, whereas the minimum bactericidal concentrations (MBC) for black soap and local soap were determined to be 50 mg/ml and 25 mg/ml, respectively, against the isolates. Black soap and local soap, exhibiting superior efficacy at low concentrations, may be regarded as effective antibacterial agents for cleansing purposes. The evaluation of antimicrobial efficacy for all four soaps indicated that black soap and Local soap had superior effectiveness against all isolates in comparison to medicated and beauty soap.

***Keywords**: Medicated, Soap, Organic, Microorganism, Skin.*

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I. INTRODUCTION

Soap is produced by combining salts and oils derived from either plant or animal sources. Typically, soaps are salts formed by the saponification of free fatty acids present in fats and oils with alkaline agents. The incorporation of supplementary components into these salts of free fatty acids or soap bases results in the production of several types of soaps (Chaudhary, 2016). Soaps are vital for cleansing and eradicating pathogens. Although fats and oils are the primary components of soap, some detergents are used to enhance the antibacterial efficacy of soaps (Bhat et al., 2011). Washing hands with soap and water is a well-recognized activity for maintaining personal hygiene.

Microorganisms inhabit diverse settings, such as soil, water, wastewater, stagnant water, and the human body. Pathogenic bacteria are particularly significant concerning public health (Ori, 2014). Fuls (2018) revealed that both

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antibacterial and non-antibacterial soaps had inhibitory capacity in clinical studies, highlighting their significance in health, alongside germs that affect the human body. Proper handwashing with soap can diminish the prevalence of *Propioni bacterium* acnes and lower the incidence of secondary infections in acne-prone skin, as well as limit the spread of infectious illnesses in healthcare settings more efficiently.

Normal microbial flora, or microbiota, refers to the assemblage of microorganisms residing on the skin and mucosal surfaces of healthy persons. Research indicates that normal flora, referred to as normal microbiota, functions as a primary defense against microbial infections, assists in digestion, facilitates toxin destruction, and helps to the development of the immune system (Saba, 2019). Modifications in the standard microbiota, or the activation of inflammation by commensals, might result in conditions such as Inflammatory Bowel Disease (Tachibana, 2016).

The skin's natural flora varies in density and composition based on anatomical location. The composition is influenced by several factors, including genetic predisposition, gender, age, environmental circumstances, stress, nutritional status, and dietary practices. Human skin has degenerative alterations due to regular exposure to environmental conditions and the influence of microbes. The typical flora inhabits numerous regions of the body, including the skin, with the specific microbial composition of the skin varying by anatomical location. Moist regions, such as the axillae and groin, exhibit elevated and unique bacterial proliferation compared to dry locations. The predominant bacteria on the skin are Gram-positive, catalase-positive cocci belonging to the genera *Staphylococcus* and *Micrococcus*. It is more prevalent among those who harbour *S. aureus* in their nasal passages (Grice & Sergie, 2011). Additional sites of bacterial flora within the body include the oral cavity and upper respiratory tract, which are typically inhabited by a more diverse array of microorganisms, including Streptococci. Notably, alpha-hemolytic Streptococci, commonly known as "viridans Streptococci," are particularly prevalent in the mouth. These encompass *S. mutans*, *S. sanguis*, and *S. mitis*. *Streptococcus mutans*, in particular, plays a pivotal role in the development of dental plaque and caries (Grice & Sergie, 2011).

Black soap serves several functions, including applications for hair, face, and body, and is recognized for its anti-aging characteristics. It effectively diminishes fine lines, wrinkles, dark spots, and other imperfections. The organic components in black soap are efficacious in cleansing and deodorizing the skin. Many individuals like it since it does not inhibit the proliferation of resistant germs. Medicated soaps significantly eliminate dirt and compromise cytoplasmic membranes to eradicate germs (Desbois & Smith, 2010). Medicated soaps include many antimicrobial agents that exert diverse mechanisms of action on different skin microorganisms. This study seeks to identify microorganisms commonly present in the human skin and compare the effectiveness of medicated soaps and locally prepared black soaps on isolates derived from the skin.

II. MATERIALS AND METHODS

➢ *Sample Collection*

A sterile swab was utilized to get samples from the skin. The specimens were obtained from the hand, armpit, face and legs. Local and medicinal soaps were acquired at Relief and Douglas markets in Owerri, Imo State, Nigeria. The batch numbers, expiration dates, and the presence or absence of manufacturer seals were recorded.

Table 1: Assayed Soaps and their Active Ingredients as per Label Disclosure

Category	Active ingredients
Medicated	Chloroxylenol (0.12 %)
Beauty soap	Glycerin, Tetra sodium EDTA, and Vitamin E Acetate (0.3 %)
Black soap	Osun (Cam wood), palm kernel oil, Cocoa pod ash, Aloe vera, lime and Lemon, glycerine, pure honey, shea butter (0.1 %
Local soap	Polish (Sulphur)

➢ *Isolation of Bacteria*

The method employed by Njoku et al. (2016) was utilized for the isolation of the bacteria. The swab sticks were immersed in four (4 ml) milliliters of sterile 0.85 % w/v normal saline contained in test tubes. An aliquot of 0.1 ml was introduced into sterile nutrient agar plates employing the spread plate technique. The plates were incubation for a duration of 24 h at a temperature of 37°C. Colonies that emerged on the agar plates were subcultured.

➢ *Purification of the Isolates*

Colonies that develop on the agar plates were subsequently subcultured onto freshly prepared nutrient agar plates. The plates were incubated at 37°C for 24 h. Colonies that emerged following incubation were selected and transferred into freshly prepared nutrient agar slants.

➢ *Identification of the Isolates*

After purification of isolates, the isolates were inoculated onto nutrient agar plates. The characterization and identification of the isolates were conducted through morphological, microscopic, and biochemical testing. Gram's stain examination and several biochemical tests were conducted for the identification of bacterial isolates utilizing ABIS online (Bacterial identification software) online software (Sorescu & Stoica, 2021).

➢ *Preparation of Soap Extract*

A sterile blade was employed to scrape each of the soaps. Two grams (2g) of the black soap and local soap, along with

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one gram (1g) of medicated soap and beauty soap, were accurately measured and placed in a 100 ml conical flask. Ten (10) ml of 10 % DMSO (dimethyl sulfoxide) was incorporated into the soap formulation. The extract was stored in a refrigerated flask for 15 h to ensure the soap was fully dissolved.

➢ *Inoculum Standardization*

The inoculum was standardized using McFarland standard according to Lonsway (2021)

➢ *Antimicrobial Assay*

The method of Kingsley *et al.* (2019) was adopted for this assays.

➢ *Agar Well Diffusion Assay*

Wells were created in sterile Muller Hinton agar plates using a sterile core borer of 6 mm diameter. The spread plate method was used to inoculate 0.1 ml of a standardized bacterial suspension onto freshly prepared Muller Hinton agar plates using a 100 µl micropipette. The inoculum was allowed to absorb onto the agar plates for a brief duration. The soap extract was added to the wells at concentrations of 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml. The plates were incubated for 24 h at 37°C. Zones of inhibition were quantified in millimeters using a ruler.

➢ *Minimum Inhibitory Concentration Determination (MIC)*

Only soap extracts demonstrating inhibitory activity had their minimum inhibitory concentrations (MICs) determined. The minimum inhibitory concentrations (MICs) of the selected soaps were assessed using the standard macro broth dilution method in nutrient broth within test tubes. To achieve a two-fold dilution, 0.1 ml of the standardized bacterial inoculum was introduced into the tubes containing the extract and nutrient broth. The stock concentrations were 200 mg/ml for black soap and local soap, and 100 mg/ml for the beauty soap and medicated soap. A control was prepared using only the soap extracts and nutrient broth. The tubes underwent incubation for 24 h at room temperature. The minimum inhibitory concentration (MIC) was identified as the lowest concentration of soap that inhibited visible growth in the test tube.

➢ *Minimum Bactericidal Concentration Determination*

The minimum bactericidal concentrations (MBC) assay was conducted by inoculating fresh nutrient agar plates with a loopful of culture obtained from each broth culture that exhibited no growth in the MIC tubes. The plates were maintained at ambient temperature for 24 h. After the incubation time, the minimum inhibitory concentration of the soap that suppressed bacterial growth on the solid medium was determined as the MBC values for each soap sample.

III. DATA ANALYSIS

Data was analyzed using ANOVA.

IV. RESULTS

The isolates were identified as *Escherichia coli, Staphylococcus sp* and *Bacillus sp.* The antimicrobial zones of inhibition (measured in millimeters) of the isolates on the various kinds of soaps are shown in Figures 1 through Figure 4. The majority of the soaps that have been tested have antimicrobial effects on the test organisms. The findings demonstrated that the test organisms were susceptible to various concentrations of the soap samples. The result from the study showed that the Zones of inhibition significantly increases as the concentration of the soap increases.

Black soap was found to be the most effective soap when the efficacy of the soaps was compared using the disc agar diffusion method, while medicated soap does not have a zone of inhibition at different concentrations, the zone of inhibition of other soaps increased as the concentration increased. Local soap was also effective against the pathogens. Black soap was observed to have the highest zone of inhibition against *E. coli*, ranging from 15 mm to 42 mm at different concentrations. At concentration levels of 100 and 50 mg/ml, the beauty Soap also demonstrated a negligible antibacterial activity against *Staphylococcus* sp. and *Bacillus* sp. Only a 9.0 mm zone of inhibition was measured by the medicated soap against *E. coli* at 100 mg/ml. Additionally, it was noted that even at the lowest concentration of 12.5mg/ml, no zone of inhibition was observed against the organisms.

According to the study's findings, the test isolates were mostly resistant to medicated soaps while being most susceptible to black soap and local soaps, which showed large zones of inhibition.

Positive correlations were found (P 0.05) in the analysis of variance for the means of antibacterial activities among the soaps. The concentrations used for the study varied significantly, with 200 mg/ml (30, 42, 40, 22, 35, 33, 35, and 36 mm) having more zones of inhibition than other concentrations and being more effective.

Table 3 showed the MIC values of the various soaps against the test bacterial isolates. At concentrations of 200 mg/mL, 100 mg/mL, 50 mg/mL, and 25 mg/mL, local soap and black soap inhibited the growth of the organisms, designating 25 mg/mL as the MIC value. Medicated soap only had an inhibitory effect against *E. coli*, which had an MIC value of 12.5 mg/mL, whereas the beauty soap had an MIC value of 12.5 mg/mL for all isolates.

Table 4 displays the MBC values of the various soaps against the test bacterial isolates. Local soap and black soap inhibited the organisms from growing at the lowest concentration, which was 50 mg/ml and 25 mg/ml. All isolates except *S. aureus* showed an MBC value of 25 mg/ml when treated with the beauty soap.

Table 2: Comparison of the zones of inhibition (mm) of soaps used against the four clinical isolates

Test Isolates	**Conc.(mg/mL)**	**Beauty**	**Medicated**	**Black soap**	**Local soap**
E.coli	200	-	-	30	42
	100	8	9	27	31
	50	0	0	20	27
	25	0	0	18	15
	12.5	0	0	-	-
	10% DMSO	0	0	0	0
Staph. Sp	200	-	-	35	33
	100	15	0	27	17
	50	13	0	20	14
	25	9	0	15	10
	12.5	0	0	-	-
	10% DMSO	0	0	0	0
Bacillus sp	200	-	-	35	36
	100	15	0	20	21
	50	11	0	15	16
	25	0	0	10	14
	12.5	0	0	-	-
	10% DMSO	0	0	0	0

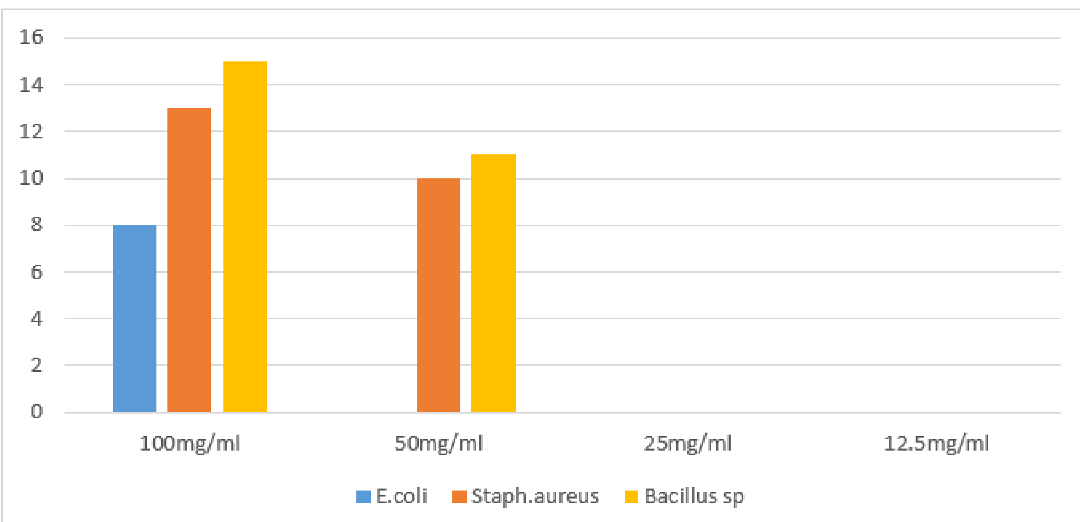

Fig 1: Inhibitory Action of the Beauty Soap Against the Isolates at Different Concentrations

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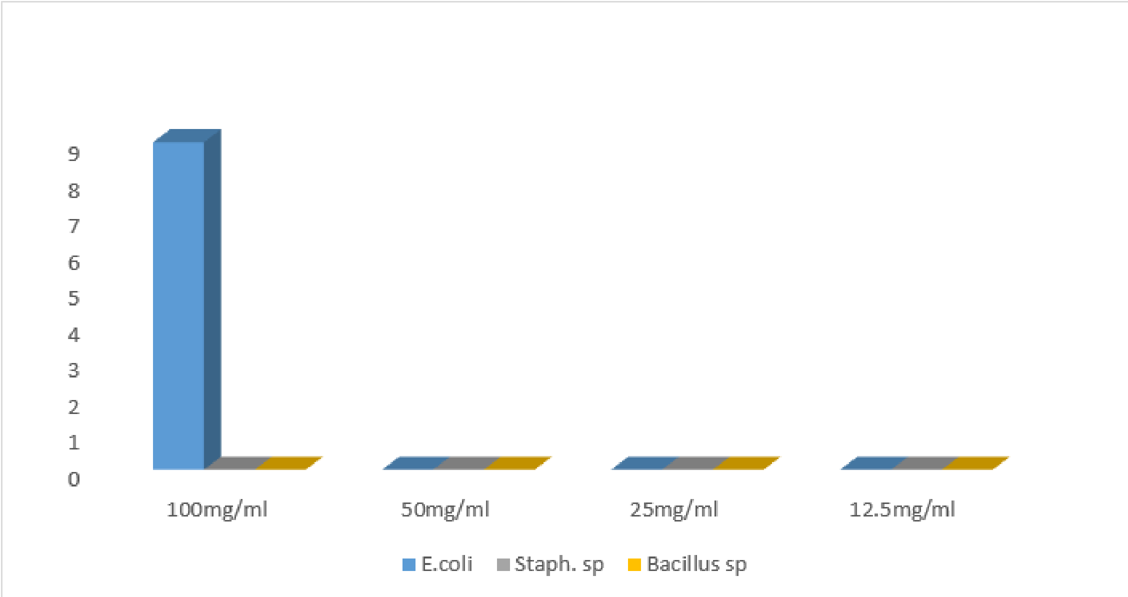

Fig 2: Inhibitory Action of Medicated Soap Against the Isolates at Different Concentrations

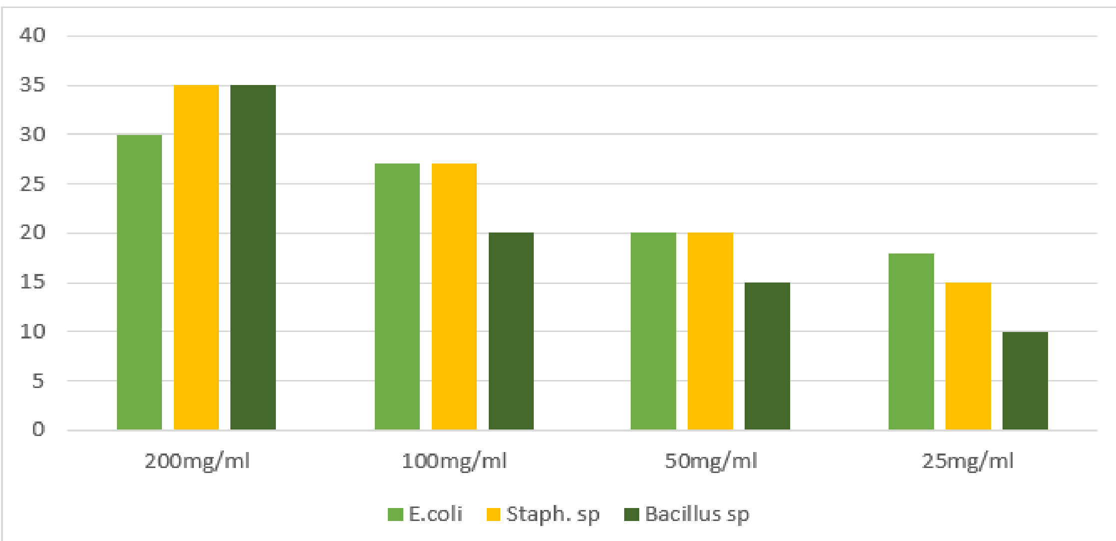

Fig 3: Inhibitory Action of Black Soap Against the Isolates at Different Concentrations

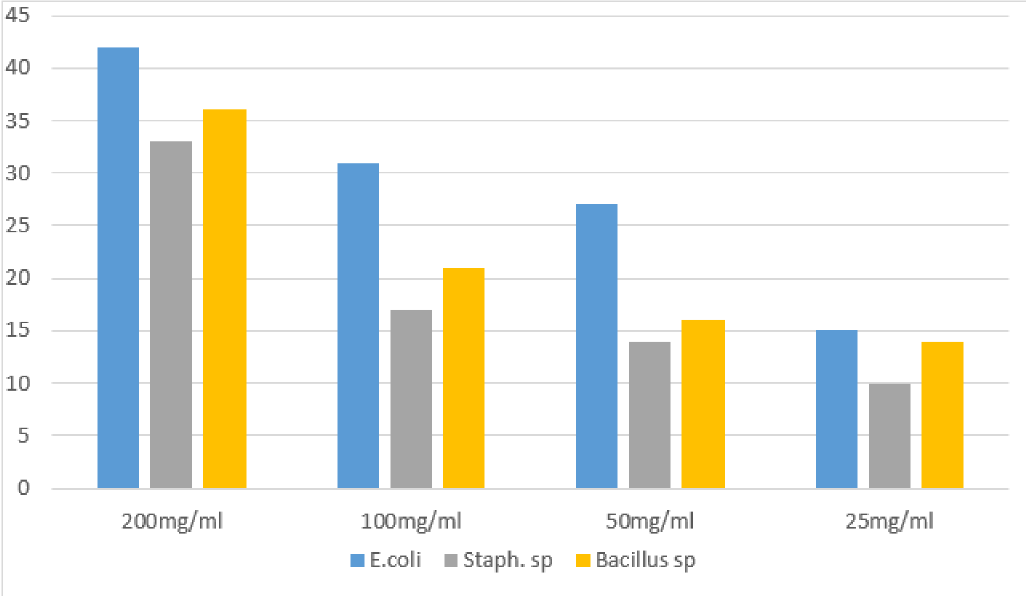

Fig 4: Inhibitory Action of Local Soap Against the Isolates at Different Concentrations

Table 3: Minimum Inhibitory Concentrations of Soap Samples Against the Isolates

Soaps samples	**Test organisms**	**MIC (mg/ml)**
Local Soap	*E. coli* *Staph. sp* *Bacillus sp*	25 25 25
Black soap	*E. coli* *Staph. sp* *Bacillus sp*	25 25 25
Beauty soap	*E. coli* *Staph. sp* *Bacillus sp*	12.5 12.5 12.5
Medicated soap	*E. coli*	12.5

Table 4: Minimum Bactericidal Concentrations of Soap Samples Against the Isolates

Soaps samples	**Test organisms**	**MBC (mg/ml)**	
Local Soap	*E. coli* *Staph sp* *Bacillus sp*	50 25 50	
Black soap	*E. coli* *Staph sp* *Bacillus sp*	50 25 50	

Soaps samples	Test organisms	MBC (mg/ml)	
Beauty soap	*E. coli*	25	
	Staph sp	25	
	Bacillus sp	25	
Medicated soap	*E. coli*	25	

V. DISCUSSION

Generally, surfaces are cleaned and germs eliminated using soaps. Each person's skin reacts differently to different soaps since the human skin is the main location of soap exposure. It is important to remember that different soaps have different antibacterial properties depending on the bacteria present. The various concentrations tested on the various organisms recovered from human skin demonstrate that the soaps have antibacterial properties that prevented the microbes from growing.

The results of the investigation demonstrated that the majority of the soaps examined had antibacterial action, albeit to differing degrees, as seen by the suppression of the growth patterns of the isolates. When the effectiveness of the soaps was compared using the agar well diffusion method, black soap was shown to be the most effective. Local soap was more effective against *E. coli* and had an average zone of inhibition of 21.4 mm. Black soap exhibited the largest average zone of inhibition (23.3 mm) and was more efficient against *Staphylococcus* sp. than any other examined bacterial isolate. Oladosu et al. (2018) discovered that locally produced soaps exhibited a 25 mm zone of inhibition on *Staphylococcus* sp. and other pathogenic organisms, which was in line with these findings. They disagreed, although, with the results of Riaz et al. (2019), who discovered a significantly smaller zone of inhibition (8.0 mm) in their investigation of the antibacterial activity of soaps against frequently encountered microorganisms. Furthermore, the medicated soap showed marginally higher levels of antimicrobial activity against the isolates, which is in line with Obi (2014) findings on the antibacterial properties of certain medicated soaps against specific human diseases. With the exception of a little inhibition against *E. coli* at high concentrations, the isolates were largely able to withstand any effect from medicated soap. The findings aligned with Anyiam and Obulie (2016) research on microbes recovered from Madonna students' skin. The pattern of the data is consistent with previous research by Oladosu et al. (2018), who showed that the activities of antimicrobial agents are dependent on concentration and that the activities of some antimicrobials have been found to decrease with concentration.

The results of the MIC showed that both local soap and black saop had enhanced MICs of 25 mg/ml against *E. coli*. For *E. coli*, the MBC for both soaps was 50 mg/ml. This amount was higher than the 12.5 mg/ml in medicated soap and beauty soap. This conclusion is consistent with the study conducted by Ikegbunam et al. (2013) and suggests that higher concentrations of this soap are needed to either kill or restrict the development of isolates.

This study clearly showed that most soaps significantly suppressed Gram's positive bacteria like *Staphylococcus* sp., but they had little effect on Gram's negative bacteria like *E. coli*. According to Rama et al. (2011), this finding may be explained by the fact that most of the compounds exhibit efficacy against gram-positive bacteria because of modifications in the cell walls' composition. The promise expressed on the soap labels that they possessed antibacterial action was supported by the majority of the tested soaps' good findings, especially in this area. Out of all the soaps used, Black soap has the most potent antibacterial qualities. This may be explained by the presence of antimicrobial phytochemicals such as alkaloids, tannins, flavonoids, cyanogenic glycosides, and saponins (Nnema, 2017). The experiment's results are in line with those of Omobuwajo et al. (2011), who found that black soap derived from Cassia senna effectively combats a number of harmful germs on human skin. Skin illnesses including boils, thrush, and impetigo have been related to the growth of *Staphylococcus* and *Bacillus* species. The significant sensitivity of these organisms to black soap and local soap suggests that the soaps have therapeutic potential for treating these conditions. Furthermore, it has been noted that the long chain fatty acid in palm kernel oil, which constitutes a substantial amount of black soap and local soap, deforms the peptidoglycan layer of Gram-positive bacteria (*Staphylococcus* sp.) (Ekwenye & Ijeomah, 2015). This explains why *Staphylococcus* sp. exhibited the largest zone of inhibition when compared to the other microorganisms that were analyzed. Palm kernel oil, which is used to create these soaps, has three major fatty acids: lauric acid, myristic acid, and oleic acid. Membrane disruption may result from the effects of long-chain fatty acids (Selvamohan & Sandhya, 2012). This explains why the soap has an inhibiting impact on the organisms. Local soap and black soap both showed strong inhibitory effects on *E. coli*, but the effect of the beauty soap was weaker than that of *Staphylococcus* sp. The activity of the soap's active components is diminished by *E. coli*, a Gram-negative bacterium with a thin peptidoglycan coating. However, the enhanced purity of the shea butter and palm kernel oil (used in the making of the soap) may have a major influence on the characteristics and quality

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of the soap used. Most of the resistance of *E. coli* to the beauty soap may be due to chromosomal mutation. It reduces the bacteria's susceptibility to agents or the acquisition of transposons and resistance (R) plasmids (Feroze et al., 2014).

Both natural and artificial ingredients used in the composition of the beauty soap and medicated soaps have the capacity to inhibit the growth of some bacteria. The suppression of the isolates' growth patterns indicates their varying ability to resist the antimicrobial effect of the soaps. These differences may instead be the consequence of differences in the kind and structures of the bacterial cell wall, which is consistent with the findings of Obi (2014), as the bacterial cell wall is the final target of any antimicrobial medication or disinfectant. Based on the active component that goes into making it, every soap is different. Chloroxylenol, glycerin, and triclosan were identified as the active antimicrobial compounds in the medicated and antiseptic soaps used in this investigation (Table 1). This leads to the conclusion that other elements, such the concentration of the active component and other additions, may alter the antibacterial capabilities, therefore evaluating a soap's antimicrobial efficiency only based on its active agents may not be sufficient. The study of Geraldo et al. (2018) supports this, showing that a mixture of several active natural compounds is better than triclosan alone. Traditional black soaps were typically the most effective when compared to the other two types of soap that were utilized.

VI. CONCLUSION

This study uncovered differences in the effectiveness of various soaps used to address the tested microbial isolates. In comparison to other soaps across various categories, black soap and Local Soaps exhibited the most significant antibacterial activity. In the context of hand washing, these soaps serve as effective agents in preventing skin infections and inhibiting the transmission of skin pathogens. These can also be utilized during treatment to cleanse the skin when a bruise, cut, or wound occurs, as well as in cases of eczema, lesions, and other microbial infections. Nonetheless, consistent application of these soaps could lead to the development of microbial resistance, in addition to causing skin dryness and increasing vulnerability to pathogenic microorganisms by eliminating both beneficial and harmful microbes present on the skin. Evidence from scientific studies indicates that the soap is utilized in traditional medicine by numerous tribes in Nigeria and beyond, due to its antibacterial properties that combat test organisms associated with various skin conditions. This investigation has provided evidence for the efficacy of herbal, medicated, and black soaps in addressing skin infections.

RECOMMENDATION

The soap tests conducted in the research shown differing levels of efficacy against the test isolates. Consequently, it is recommended that black soap and local soaps be considered for the treatment of skin infections. These soaps are also more economical than other antiseptic and therapeutic soaps. The public should be apprised that the alternative brand of soap utilized in this study is suitable for handwashing and surface disinfection, as not all medicated soaps exhibit efficacy against all bacterial strains.

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- *Declaration of Competing Interest*

The authors declare no known competing financial interests or personal relationships that could potentially influence the reported work.

- *Authors Contribution*

Nlemolisa O. R., conducted laboratory investigations, wrote, corrected, and reviewed the paper, Kemka, U.N., Gaius-Mbalisi, V.K., Odonye, P. E., Ikeme, C.O. and Nwokorie, R. C. contributed to the writing and review of the manuscript. While Madubugo, F.C. assisted in conducting laboratory investigations.

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