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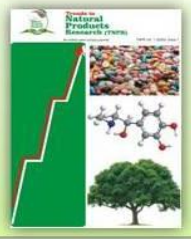

Antibacterial activities of four medicated soaps on clinical bacterial isolates

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Keywords Medicated soap, Antibacterial activity, Active antimicrobial ingredient.

Abstract: Medicated soaps contain antibacterial properties to care for the skin. This study was aimed at evaluating the antibacterial activities of four medicated soaps; Sample A, B, C and D against clinical bacterial isolates namely: *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. Antibacterial activity was carried out using both Agar disk and Agar-well diffusion techniques at concentrations of 200, 100, 50, 20, 10 mg/ml on Mueller-Hinton medium and distilled water was used as a negative control. The experiments were carried out in duplicates and the zones of inhibition recorded in millimeters. Sample A which contained Chloroxylenol (0.5 %) inhibited the growth of *Bacillus subtilis*, *S. aureus* and *E. coli*. Sample B which contained Monosulfiram (5 %) showed highest inhibitory activity against *E. coli*, while Sample C containing Triclosan (0.5 %) as active ingredient showed the highest antibacterial activity against *P. aeruginosa* at the lowest concentration used. Sample D containing Chloroxylenol (0.3 %) showed low inhibition zones compared to other soap samples and this could be due to the low concentration of the active ingredient. The Minimum inhibitory concentrations (MIC) of Sample B and C was 20 mg/ml against *P. aeruginosa* and *E. coli*, while that of Sample A was 50 mg/ml for *B. subtilis* and *S. aureus*. The minimum bactericidal concentration (MBC) Sample B and C for *P. aeruginosa* and *E. coli* was 50 mg/ml and MBC of Sample A for *B. subtilis* and *S. aureus* was 100 mg/ml. From our findings it was obvious that Sample A displayed high activity at low concentrations and therefore, can be considered as good antiseptic soaps for topical cleansing purposes. However, the concentrations of the active ingredients (Chloroxylenol) which are known to have broad spectrum activity should be properly monitored in products prepared for topical use to avoid counter effects on the users.

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INTRODUCTION

Antibacterial activity is important in the prevention of sepsis and skin infections in humans (Ike, 2016). Soaps are essential for cleaning and elimination of microorganisms, despite the fact that fats and oils make up the majority of soap ingredients, detergents are added to boost antibacterial properties (Ughamba *et al.,* 2019). Medicated soaps contain additional ingredients, usually for the treatment of skin disorders, and have germicidal substances added in a specific amount and percentages that are always stated on the soap case or leaflet that contains information on how to use the soap for various purposes (Ike, 2016). Fuls *et al.,* (2008) reported the inhibitory potential of antimicrobial and non-antimicrobial soaps in clinical instances, notwithstanding the relevance of bacteria attacking the human body in terms of health. The skin is the body's first line of defense, bacteria such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* live there and are the leading cause of skin infections (Abbas *et al.,* 2016). Scrubbing hands and body with soap is the first line of defense against bacteria and other pathogens that can cause flu, colds, skin infections, and even deadly communicable diseases (Adeyemi *et al.,* 2016).
Soaps contain antibacterial active components that also have a lowering effect on pyogenic skin infections caused by Staphylococcus aureus and other Gram-negative bacteria (Nmema *et al.,* 2017). Antibacterial soap is a type of soap that contains chemical components that are said to help destroy bacteria. Triclosan is found in the majority of antibacterial soap; however other chemical additions are also popular (Aiello *et al.,* 2007). Some scholars, as well as the US Food and Drug Administration (FDA), have questioned the effectiveness of antibacterial products (Stromerg, 2014). Carbolic soap, which included up to 5 % phenols, was the first antibacterial soap. Because of concerns regarding the safety of carbolic soap chemical components on the skin, several of these chemical components have been banned (Obog and Aluyor 2011). The most frequent antibacterial ingredients in soap are triclosan and triclocarban (McGinley, 2016). Other antibacterial chemicals commonly found in soaps include benzalkonium chloride, benzethonium chloride, chloroxylenol, and others (Alison, 2016).

Materials and Methods

Test tubes, Conical flask, Petri dish, Beakers, Bijou bottles, Test tube rack, Plastic pipette, Micropipette, Autoclave, Forceps, Bunsen burner and Gas cylinder, Cotton swab, Normal saline, Distilled water, Wire loop, Cotton wool, 70 % Ethanol, Cork borer, Forceps, Whatman number 4 filter paper, Universal container, Razor blade, 0.5 McFarland standard and black-line McFarland reference card.

Test Soaps

The medicated soaps were purchased from a standard cosmetic and pharmacy store in Ogige market Nsukka, Enugu State. The batch numbers, expiry dates, and the presence or absence of the manufacturers seal were noted.

Test Organisms

The test organisms for the study were obtained from the Medical Centre, University of Nigeria Nsukka. The Organisms were tentatively identified as *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa*. The identities of these organisms were confirmed by using colony characteristics on differential media according to standard microbiological techniques (Nmema *et al.,* 2017).

Sterilization of Materials

The glass wares used (test tubes, beaker, conical flasks and Bijou bottles) were washed in soapy water, rinsed, dried and then sterilized in the hot air oven at 180 °C for 2 h. Wire loops and needles were sterilized by heating to red hot in open gas flame. Cork borers were dipped into 70% ethanol before flaming to burn off the alcohol and then cooled beside the flame before use.

Standardization of Test Organisms

Each of the test organisms were standardized using colony suspension method (EUCAST, 2000). The organisms were inoculated on nutrient agar slants and incubated for 24 h at 37 °C so as to obtain fresh culture. A loop full of each organism were placed in different test tubes containing 5 ml of normal saline. Each Organism suspension were matched with 0.5 McFarland standards to give a resultant concentration of 1.0×10^6 cfu/mL.

Preparation of Different Concentration of Soaps

A sterilized razor blade was used to scrape flakes of each soap sample separately into sterile Petri dishes (Obi, 2014). A chemical balance was used to weigh out 2 g of each soap sample which was subsequently introduced into a conical flask containing 10 ml of sterile distilled water. This mixture was stirred with a glass rod and then placed in a water bath at 60 °C to aid dissolution of the soap. This mixture made the 200 mg/ml concentration of soap. Using the concentration formula $C_1V_1 = C_2V_2$, this was carried out by transferring 2 ml from the stock solution (200 mg/ml) to a test tube containing 2 ml of distilled water to get 100 mg/ml. This formula was used to get the following concentration 50, 20, 10 mg/ml (Nmema, 2017).

Preparation of Soap-impregnated Disks

A paper punch was used to drill holes into Whatman No. 4 filter papers in order to create paper disks. The paper disks were packed into a clean universal bottle and sterilized by autoclaving at 121 °C for 15 minutes. The sterilized disks were then placed into different test tubes containing the various concentration of soap solutions, and kept for 1 h for the disks to be saturated with soap solutions. The disks were positioned on the sides of the test tubes for 1 h to drain excess fluid. The disks were then placed in sterile Petri dishes and allowed to dry at under room temperature (28°C) The disks were then transferred into sterile universal containers and labelled respectively to each soap sample and concentration (Ughamba *et al.,* 2019).

Antimicrobial Sensitivity Test

This test is used to determine which of the bacteria isolate can be susceptible to a particular antibacterial agent (in this case the Antibacterial soaps). This method involves the use of Agar Well Diffusion Technique and Disk Agar Diffusion Method on Mueller Hinton medium using 4 medicated soaps with different concentrations.

Agar well Diffusion Method

The susceptibility of the different isolates to the different soap samples was determined using the modified Kirby–Bauer diffusion technique (Cheesbrough, 2002) by swabbing the Mueller–Hinton agar (MHA) plates with the resultant saline suspension of each organism. Wells were then bored into the agar medium with a heat sterilized 6-mm cork borer. The wells were filled with 100 µL (0.1 ml) of different concentrations (200, 100, 50, 20, 10 mg/ml and distilled water was used as control) of each soap sample prepared taking care not to allow spillage of the solutions onto the surface of the agar. The plates were allowed to stand for at least 30 min before being incubated at 37 °C for 24 h (BSAC, 2013). The determinations were done in duplicate. After 24 h of incubation, the plates were examined for zones of inhibition (Bauer *et al.,* 1966). The diameters of the inhibition zones produced by each soap sample were measured to the nearest millimeters using a transparent meter rule.

Disk agar Diffusion Method

The disk agar diffusion method as originally described by (Bauer *et al.,* 1966) was used. The standardized suspension was used to inoculate the surfaces of Mueller Hinton agar plates using sterile cotton swab. The plates were left for about 30 minutes; the disks (ranging from 200 to 10 mg/ml and also including a negative control i.e., a disk immersed in distilled water) were aseptically transferred directly unto the sensitivity plates with the aid of a sterile forceps. Within 30 minutes of application, plates were inverted, incubated at 35 °C for 24 h and then were examined for of zone of inhibition around the disk (Selvamohan and Sanhya, 2012). All data were reported as mean ± standard deviation of duplicate experiments.

Minimum Inhibitory Concentration (MIC)

MIC was determined for all the samples. The MICs of the selected soaps were determined by the Standard Macro-broth dilution method in Mueller–Hinton broth (NCCLS, 1999). The MIC was determined using five (5) for each isolate for the different concentration (200, 100, 50, 20, 10 mg/ml) containing 1 ml of each soap and 9 ml of sterile Mueller Hinton broth. A loop full of each test isolate were inoculated into Mueller Hinton Broth, 0.5 ml was transferred from the already inoculated Mueller Hinton broth to the test tubes containing the soap samples with different concentration. A tube containing Mueller Hinton Broth and four test tube containing each test isolate in Mueller Hinton Broth were used as control. All cultures were incubated at 37 °C for 24 h. The MIC was taken as the lowest soap concentration that allowed no visible growth in the Mueller–Hinton broth.

Minimum Bactericidal Concentration (MBC)

The MBC of the different soaps were determined according to the method described by Ughamba *et al.* (2019). Each culture broth (in the test tube) used in the minimum inhibitory concentration (MIC) assay that showed no growth (non-turbid) after incubation, was inoculated (streaked) onto a solid nutrient agar plate and then incubated at 37 ^{o}C for 24 h. After the incubation, the lowest concentration of the soap that showed no growth on the solid medium was established as MBC value for each soap sample. The materials from each test tube used in the minimum inhibitory concentration assay that showed no growth after incubation, were streaked onto a solid nutrient agar plate and then incubated at 37^{0}C for 24hours. The lowest concentration of the extract that showed no growth on the plate after 24 hours was taken as the minimum bactericidal concentration (Alade and Irobi, 1993).

RESULTS

Antimicrobial Active Ingredient

The four distinct medicated soaps utilized as samples in this investigation, as well as their active components (as seen on the pack), are listed in Table 1.

Table 1: Active ingredients of the medicated soap samples.

Soap Sample	Active ingredient	Category of soap
A	Chloroxylenol (0.5 %)	Medicated
B	Monosulfiram (5 %)	Medicated
C	Triclosan (0.5 %)	Medicated
D	Chloroxylenol (0.3 %)	Medicated

Antimicrobial Susceptibility Patterns of the Test Isolates.

Antimicrobial Susceptibility test was carried out using ~~f~~our ~~m~~edicated soaps against four clinical isolated bacteria. All four soaps where active against all the test isolates at varying concentrations, the lowest being 20 mg/ml.

Table 2: Antibacterial activity of soap Sample A on clinical isolates using disk agar diffusion method.

Bacterial isolates	Mean zone diameter of inhibition (mm)					
	Control	10 mg/ml	20 mg/ml	50 mg/ml	100 mg/ml	200 mg/ml
Bacillus subtilis	Nil	Nil	6.0 ± 2.8	10.0 ± 1.4	13.5 ± 2.1	17.5 ± 0.7
Escherichia coli	Nil	Nil	6.0 ± 1.4	10.5 ± 2.1	14.5 ± 2.1	18.0 ± 1.4
Pseudomonas aeruginosa	Nil	Nil	Nil	10.0 ± 1.4	14.0 ± 1.4	16.5 ± 0.7
Staphylococcus aureus	Nil	Nil	5.5 ± 0.7	10.0 ± 1.4	13.0 ± 1.4	16.5 ± 2.1

Table 3: Antibacterial activity of soap Sample B on clinical isolates using disk agar diffusion method.

Bacterial isolates	Mean zone diameter of inhibition (mm)					
	Control	10 mg/ml	20 mg/ml	50 mg/ml	100 mg/ml	200 mg/ml
Bacillus subtilis	Nil	Nil	Nil	6.5 ± 3.5	12.5 ± 3.5	15.5 ± 2.1
Escherichia coli	Nil	Nil	Nil	3.0 ± 4.2	9.0 ± 1.4	12.0 ± 1.4
Pseudomonas aeruginosa	Nil	Nil	3.5 ± 4.9	11.5 ± 2.1	15.0 ± 1.4	19.5 ± 0.7
Staphylococcus aureus	Nil	Nil	Nil	8.5 ± 0.7	14.5 ± 0.7	18.0 ± 1.4

Table 4: Antibacterial activity of soap Sample C on clinical isolates using disk agar diffusion method.

Bacterial isolates	Mean zone diameter of inhibition (mm)					
	Control	10 mg/ml	20 mg/ml	50 mg/ml	100 mg/ml	200 mg/ml
Bacillus subtilis	Nil	Nil	5.5 ± 0.7	9.5 ± 2.1	14.0 ± 1.4	16.5 ± 2.1
Escherichia coli	Nil	Nil	Nil	5.0 ± 0.0	11.0 ± 1.4	14.0 ± 1.4
Pseudomonas aeruginosa	Nil	Nil	8.5 ± 0.7	13.0 ± 0	16.5 ± 0.7	21.0 ± 1.4
Staphylococcus aureus	Nil	Nil	Nil	4.5 ± 6.3	11.5 ± 2.1	14.0 ± 1.4

Table 5: Antibacterial activity of soap Sample D on clinical isolates using disk agar diffusion method.

Bacterial isolates	Mean zone diameter of inhibition (mm)					
	Control	10 mg/ml	20 mg/ml	50 mg/ml	100 mg/ml	200 mg/ml
Bacillus subtilis	Nil	Nil	5.0 ± 1.4	8.0 ± 1.4	11.0 ± 0.0	13.5 ± 0.7
Escherichia coli	Nil	Nil	Nil	5.0 ± 1.4	9.5 ± 2.1	13.0 ± 0.0
Pseudomonas aeruginosa	Nil	Nil	Nil	4.0 ± 5.6	10.5 ± 0.7	13.5 ± 2.1
Staphylococcus aureus	Nil	Nil	Nil	4.0 ± 5.6	10.5 ± 2.1	14.5 ± 2.1

Agar-well diffusion method

The different bacterial isolates showed varying degree of susceptibility to the various concentrations of all four medicated soaps.

Table 6: Antibacterial activity of soap Sample A on clinical isolates using Agar-well diffusion method.

Bacterial isolates	Mean zone diameter of inhibition (mm)					
	Control	10 mg/ml	20 mg/ml	50 mg/ml	100 mg/ml	200 mg/ml
Bacillus subtilis	Nil	Nil	6.5 ± 0.7	8.0 ± 1.4	15.0 ± 1.4	20.5 ± 2.1
Escherichia coli	Nil	Nil	5.5 ± 0.7	11.5 ± 0.7	14.5 ± 2.1	19.0 ± 1.4
Pseudomonas aeruginosa	Nil	Nil	6.0 ± 2.8	10.5 ± 0.7	14.0 ± 1.4	19.5 ± 3.5
Staphylococcus aureus	Nil	Nil	7.0 ± 1.4	11.5 ± 2.1	17.0 ± 1.4	23.0 ± 2.8

Table 7: Antibacterial activity of soap Sample B on clinical isolates using Agar-well diffusion method.

Bacterial isolates	Mean zone diameter of inhibition (mm)					
	Control	10 mg/ml	20 mg/ml	50 mg/ml	100 mg/ml	200 mg/ml
Bacillus subtilis	Nil	Nil	4.5 ± 0.7	9.0 ± 1.4	13.5 ± 0.7	17.0 ± 0.0
Escherichia coli	Nil	Nil	8.5 ± 0.7	12.5 ± 0.7	17.0 ± 1.4	20.5 ± 2.1
Pseudomonas aeruginosa	Nil	Nil	5.5 ± 0.7	10.5 ± 2.1	16.5 ± 0.7	20.0 ± 0.0
Staphylococcus aureus	Nil	Nil	5.5 ± 0.7	8.5 ± 2.1	14.5 ± 0.7	18.0 ± 1.4

Table 8: Antibacterial activity of soap Sample C on clinical isolates using Agar-well diffusion method.

Bacterial isolates	Mean zone diameter of inhibition (mm)					
	Control	10 mg/ml	20 mg/ml	50 mg/ml	100 mg/ml	200 mg/ml
Bacillus subtilis	Nil	Nil	6.0 ± 0.0	9.5 ± 0.7	14.5 ± 0.7	18.0 ± 1.4
Escherichia coli	Nil	Nil	4.5 ± 0.7	10.0 ± 1.4	13.5 ± 2.1	17.0 ± 2.8
Pseudomonas aeruginosa	Nil	Nil	8.0 ± 0.0	13.0 ± 1.4	17.0 ± 0.0	21.5 ± 0.7
Staphylococcus aureus	Nil	Nil	5.5 ± 0.7	11.0 ± 0.0	15.0 ± 1.4	20.0 ± 1.4

Table 9: Antibacterial activity of soap Sample D on clinical isolates using Agar-well diffusion method.

Bacterial isolates	Mean zone diameter of inhibition (mm)					
	Control	10 mg/ml	20 mg/ml	50 mg/ml	100 mg/ml	200 mg/ml
Bacillus subtilis	Nil	Nil	Nil	7.0 ± 1.4	11.0 ± 1.4	15.5 ± 0.7
Escherichia coli	Nil	Nil	4.0 ± 1.4	8.5 ± 0.7	12.5 ± 0.7	17.0 ± 0.0
Pseudomonas aeruginosa	Nil	Nil	Nil	6.5 ± 2.1	10.5 ± 0.7	16.0 ± 1.4
Staphylococcus aureus	Nil	Nil	5.0 ± 1.4	8.5 ± 0.7	11.5 ± 0.7	15.0 ± 1.4

Minimum Inhibitory Concentration

The MIC values of the soaps used against the test bacterial isolates are presented in Table 10. Sample A inhibited growth of *Bacillus subtilis* and 50 mg/ml, while other soaps inhibited growth at 200 mg/ml *Staphylococcus aureus* at 200 mg/ml, 100 mg/ml (Sample D) and 100 mg/ml (Sample B and C) which made 50 mg/ml the MIC value against *Bacillus subtilis* and *Staphylococcus aureus*. Sample B and C displayed very low MIC values against *Escherichia coli* and *Pseudomonas aeruginosa* at 20 mg/ml.

Table 10: Minimum inhibitory concentration (MIC) of the medicated soaps.

Bacterial isolates	Minimum inhibitory concentration (MIC) (mg/ml)				
	Control	Sample A	Sample B	Sample C	Sample D
Bacillus subtilis	Nil	50	100	100	100
Escherichia coli	Nil	50	20	20	100
Pseudomonas aeruginosa	Nil	50	20	20	100
Staphylococcus aureus	Nil	50	100	100	100

Minimum Bactericidal Concentration

The Minimum Bactericidal Concentration (MBC) values are shown in Table 11. A 50 mg/ml was recorded as the MBC value for Sample A against *Bacillus subtilis* and *Staphylococcus aureus.* Sample B and C showed activity against *Escherichia coli* and *Pseudomonas aeruginosa* while Sample D had very high value (200 mg/ml).

Table 11: Minimum bactericidal concentration (MBC) of the medicated soaps

Bacterial isolates	Minimum Bactericidal Concentration (MBC) (mg/ml)				
	Control	Sample A	Sample B	Sample C	Sample D
Bacillus subtilis	Nil	100	200	200	200
Escherichia coli	Nil	100	50	50	200
Pseudomonas aeruginosa	Nil	100	50	50	200
Staphylococcus aureus	Nil	100	200	200	200

DISCUSSION

From table 1, it was observed that the medicated soaps tested had different active antimicrobial ingredients and these ingredients were at different concentrations. Sample A and D were composed of chloroxylenol 0.5 % and 0.3 % respectively, sample B was composed of 5 % Monosulfiram and sample C was composed of 0.5 % triclosan. These ingredients are added to many consumer products with the intent of reducing or preventing bacterial infection (Nesta *et al.*, 2014). Chloroxylenol is bactericidal against most Gram-positive bacteria but less effective against Staphylococci and Gram-negative bacteria, and often inactive against Pseudomonas species (NCBI, 2022). It is also ineffective against bacterial spores. Monosulfiram found in Sample B is mainly exploited for its scabicide effect, but it has been known to produce moderate antimicrobial activities (Mwambete and Lyombe, 2011). Triclosan is an antimicrobial agent that can either be bacteriostatic or bactericidal, depending on its formulation, it has activity against Gram-negative and Gram-positive bacteria, as well as some viruses and protists (Tanner and Eric, 2011). As seen in table 2 above, the medicated soap sample A using disk agar diffusion method showed similar activity against the organisms except *Pseudomonas aeruginosa.* Sample A composed of 0.5 % chloroxylenol was highly effective against *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus* at 20 mg/ml and *Pseudomonas aeruginosa* at 50

mg/ml. it was also observed that the results were very similar amongst all the test isolates. From table 3, using disk agar diffusion method it was observed that at concentrations below 50 mg/ml, sample B with composition of 5 % monosulfriam as its' antibacterial ingredient did not have effect against the organisms tested except *Pseudomonas aeruginosa,* which was susceptible at 20 mg/ml while other test isolates were susceptible at 50 mg/ml. it is also noticeable that *Bacillus subtilis* and *Escherichia coli* had low results compared to *Pseudomonas aeruginosa* and *Staphylococcus aureus*. From table 4 using disk agar diffusion method, it was observed that sample C which is composed of 0.5 % triclosan showed high activity against *Bacillus subtilis* and *Pseudomonas aeruginosa* at 20 mg/ml while at concentration of 50 mg/ml, *Escherichia coli* and *Staphylococcus aureus* were susceptible. It as also observed that Sample C displayed very high activity against *Pseudomonas aeruginosa* when compared to the other test isolates. From table 5, the results showed that the medicated soap (sample D) had relatively low effect against all the organisms tested using disk agar diffusion method. Sample D which is composed of 0.3% chloroxylenol was shown to have activity against *Bacillus subtilis* at 20 mg/ml. it is also observed that the inhibitory zone diameter exhibited by the medicated soap against the different organisms were slightly similar. The result displayed in Table 6 using Agar-well diffusion method, shows that all four test isolates were susceptible to the medicated soap (sample A) which contains 0.5 % chloroxylenol at concentration of 20 mg/ml, *Bacillus subtilis* and *Staphylococcus aureus* had relatively high zine of inhibition when compared to *Escherichia coli* and *Pseudomonas aeruginosa.* The result displayed by *Escherichia coli* and *Pseudomonas aeruginosa* were found to be similar, with little variations. The result displayed in Table 7 using Agar-well diffusion method shows that the medicated soap Sample B with 5% monosulfriam as its active antibacterial ingredient was highly effective against all test isolate. It was observed that *Escherichia coli* showed high zone of inhibition even at low concentration of 20 mg/ml. it was also observed that the medicated soap had low effect on *Bacillus subtilis.* The result seen in Table 8 using Agar-well diffusion method, shows that four test isolate was highly susceptible to Sample C with 0.5% Triclosan. *Pseudomonas aeruginosa* showed high inhibitory zone diameter against Sample C at 20 mg/ml. *Escherichia coli* had relatively low inhibitory zone diameter at 20 mg/ml. From Table 9, the results showed that the medicated soap sample D had relatively low effect against all the organism tested using Agar-well diffusion method when compared to other results of different samples. Sample D which is composed of 0.3 % chloroxylenol was shown to have activity against *Escherichia coli* and *Staphylococcus aureus* at 20 mg/ml, it is also observed that the inhibitory zone diameter exhibited by the soap against both organisms are slightly similar. The result revealed that at concentrations below 50 mg/ml, *Bacillus subtilis* and *Pseudomonas aeruginosa* were not susceptible to sample D. The minimum inhibitory concentration (MIC) value of the soap samples against the test bacterial isolates are presented in Table 10. The MIC value of sample A against all the test organisms was 50 mg/ml. sample B and C inhibited the growth of *Bacillus subtilis* and *Staphylococcus aureus* at a concentration of 100 mg/ml and also inhibited the growth of *Escherichia coli* and *Pseudomonas aeruginosa* at 20 mg/ml. The minimum value for sample D against all the organisms was 100 mg/ml. The minimum bactericidal concentration (MBC) values are shown in table 11. A 100 mg/ml was recorded as the MBC value for sample A against all the test organism. The MBC value of Sample B and C were 50 mg/ml against *Escherichia coli* and *Pseudomonas aeruginosa*, and also for *Bacillus subtilis* and *Staphylococcus aureus* at 200 mg/ml. the MBC value for sample D for all the test isolates was 200 mg/ml. The results of Sample A using methods (Disk agar diffusion and Agar well diffusion method) clearly showed that Sample A was more effective, having the highest zone of inhibition against *Staphylococcus aureus* and *Bacillus subtilis* even at a low concentration of 20 mg/ml (Table 2 and 6), and it was found to be comparable with previous studies (Abbas et al., 2016; Chauhari, 2016). This finding could be due to its active ~~a~~ntibacterial ingredient, Chloroxylenol, which has been known to be bactericidal against Gram positive bacteria (NCBI, 2022). As observed by Hare *et al.,* (1963), the compound chloroxylenol is rapidly lethal to a number of gram-negative and gram-positive bacteria and there has been reports of resistance of *Pseudomonas sp.* to chloroxylenol. this could be a possible reason why it was not highly effective against *Pseudomonas aeruginosa* at 20 mg/ml as compared to the other organisms tested. Sample A showed a significantly high zone of inhibition while using Disk agar diffusion method against *Escherichia coli* (Table 2), while Sample B showed the highest zone when the Agar well diffusion method was employed (Table 7). From previous studies, Sample A containing 0.5 % Chloroxylenol, and Sample B containing 5 % Monosulfiram have been shown to exhibit strong effects against *Escherichia coli* (Kahlan *et al.,* 2018; Mwambete and Lyombe, 2011; Ogba *et al.,* 2018). Monosulfiram found in Sample B is mainly exploited for its scabicidal effect, but it has been known to produce moderate antimicrobial activities (Mwambete and Lyombe, 2011). From the result, it is noticeable that the result varies as Agar well diffusion method showed more activity even at low concentration (20 mg/ml) than that of Disk agar

diffusion method. This could be due to different factors such as the ability of the prepared medium to absorb enough of the active antibacterial ingredient or the ability of the disk to diffuse when placed on the media, the size of inoculum, and the culture media which should be able to support the growth of organisms but not interfere with diffusion of the antimicrobial agent (Oladosu *et al.*, 2018) and would have led to the difference seen in the result between Sample A and B against *Escherichia coli*. This therefore shows that Agar well diffusion method is more reliable than Disk agar diffusion method (Nmema, 2017).

In the current study, Sample C being composed of 0.5 % Triclosan which is known to have activity against Gram-negative and Gram-positive bacteria, as well as some viruses and protists, showed high antibacterial activity against *Pseudomonas aeruginosa* using both Disk agar diffusion and Agar well diffusion methods (Table 4 and 8). Several studies have shown the relationship between Triclosan and antibiotic resistance, especially against *Pseudomonas aeruginosa* (Ughamba *et al.*, 2019; Olajuyigbe *et al.*, 2017). However, it is possible that Triclosan may be effective against *Pseudomonas aeruginosa*, as bioinformatics and experimental investigations have shown that the presence of the gene FabV is associated with high tolerance of Triclosan (Yuji *et al.*, 2014; Zhu *et al.*, 2010). The deletion of the FabVgene (a mutant strain) could be the reason for the result presented or the strain of *Pseudomonas aeruginosa* used in the present work which is unknown is susceptible to Triclosan. Sample D containing Chloroxylenol (0.3 %) showed low inhibition zones compared to other soap samples while using both Disk agar diffusion and Agar well diffusion method (Table 5 and 9) and this could be due to the low concentration of the active ingredient. Similar observation was noticeable in the work of Ughamba *et al.*, (2019). However, it is possible that Sample D having shown some activities on the test isolates at higher concentrations suggest that it can be effective only at higher concentrations. it was also observed that the result of the present study as compared to the work which reported that the active antimicrobial agent which is 0.3 % Chloroxylenol in the present study and 0.1 % phenoxyethanol as recorded by Ughamba *et al.*, (2019), to the inhibitory zone diameter recorded. It was noted that the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration varied for all the Soaps. The MIC of *Bacillus subtilis* and *Staphylococcus aureus* was 50 mg/ml using sample A. For *Escherichia coli* and *Pseudomonas aeruginosa*, Sample B and C had equal MIC value of 20 mg/ml. The MBC value was Sample A 100 mg/ml for *Bacillus subtilis* and *Staphylococcus aureus*. Sample B and C had equal MBC value of 50 mg/ml against *Escherichia coli* and *Pseudomonas aeruginosa*. This suggests that Sample A was highly bactericidal against *Bacillus subtilis* and *Staphylococcus aureus* (Bauer *et al.*, 1966), Samples B and C were highly bactericidal against *Escherichia coli* and *Pseudomonas aeruginosa* (Stromerg, 2014). It was observed that the MIC and MBC values for *Pseudomonas aeruginosa* varied with to the findings of Ughamba *et al.* (2019), who reported resistance of *Pseudomonas aeruginosa* to all the medicated soaps used in the work. Sample A was highly active against *Bacillus subtilis* and *Staphylococcus aureus*, even at low concentration and it had moderate/low effect on the other test isolates. Sample B and C were highly active against *Escherichia coli* and *Pseudomonas aeruginosa* respectively. Zones of inhibition were recorded for Sample A against *Escherichia coli* and *Pseudomonas aeruginosa* but were not as active as compared to Sample B and C, Sample D had relatively low effect against all test isolates as compared to other medicated soaps used.

CONCLUSION

In this ~~s~~tudy, the antibacterial activities of some selected soaps namely: Sample A, B, C and D, against clinical bacterial isolates which belong to the genera *Bacillus, Escherichia, Pseudomonas* and *Staphylococcus* were investigated. The activities were found to be concentration-dependent and also varied with type and formulations of soap. *Bacillus subtilis* and *Staphylococcus aureus* were killed by Sample A while *Escherichia coli* and *Pseudomonas aeruginosa* were killed by Sample B and C respectively. The activities of the soaps were in the order: Sample A> C > B > D.

RECOMMENDATION

Amongst all four medicated soaps, Sample A soap having displayed high activity at low concentration can be considered a good antibacterial agent for cleansing and other purposes. Sample B and C can be considered as well but should be monitored regularly. However, prolonged use of medicated soaps could lead to development of microbial resistance in future. Therefore, it is advised to change or reduce the use of medicated soaps.

CONFLICT OF INTEREST

There is no conflict of interest

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