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Antimicrobial activity of anticeptic, herbal and beauty soaps on clinical isolates (Escherichia *coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumonia*)

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Abstract
The aim of the present research work is to compare the efficacy of various branded soaps against skin infecting human pathogenic bacteria such as *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis and Escherichia coli using agar disc diffusion method. Various microbes are deposited on the surface of skin from the dust present in external environment which causes infection.* Identification of bacterial strains was done by standard microbiological techniques. Soap samples were prepared into different concentrations of; 50mg/ml, 100mg/ml, 150mg/ml, 200mg/ml, and 250mg/ml soap concentrations were subjected to the different bacteria strains, using agar disc diffusion method to know how sensitive the organisms are to the soap concentrations. The zones of clearance increase with increase in concentration. Dettol and Safeguard were found to be more effective, amongst the antiseptic soap, while Extract and Zee, was found to more effective amongst the herbal soaps. Determination of minimal inhibitory concentration (MIC) activity of the microorganisms was performed. Antiseptic soaps showed better MIC in comparison with herbal and beauty soaps. The most resistant bacteria to all the soaps are *Klebsiella pneumonia* and *Pseudomonas aeruginosa*. It is obvious that antiseptic soaps have the antibacterial agents that can either kill or inhibit the bacterial cells. It might be possible that some bacterial strains become resistant which leads to their survival even at high concentrations of soaps. Dettol and Safeguard soaps are encouraged to be used in Hospitals, and in homes.

Keywords: Escherichia *coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia*

Introduction

Soaps are amongst those agents which have antimicrobial active against microorganisms, especially those found on the skin. Soap is common cleansing agent well known to everyone. Many authors defined soap in different ways. (Warra et al, 2013) [1], regarded it as any cleaning agent, manufactured in granules, bars, flakes, or liquid form, obtained from by reacting salt of sodium or potassium of various fatty acids that are of natural origin (salt of non-volatile fatty acids). Soaps are produced for varieties of purpose ranging from washing, bathing, and medication. They remove dirt, including dust, microorganisms, stains and bad smells in order to maintain health, beauty and remove bad odor from the human body and inanimate objects, including clothes (Ikegbunam *et al*., 2013) [2].

Soaps are either non-antimicrobial soaps or antimicrobial soap, also known as an antiseptic or medicated soap. An antibacterial soap can remove 65% to 85% of bacteria from human skin (Varsha, 2016) [3]. The cleansing action of the soap is due to the negative ions on the hydrocarbon chain attached to the carboxylic group of the fatty acids.

In addition to basic raw materials in soap, other substances are added to the composition in order to improve its application. For examples soap made for medicinal purposes, other antiseptic or herbal ingredients are added to it to produce antiseptic or herbal soaps (Antezana *et al*., 2015) [4]. Beauty soaps are produced to feature attractive fragrances, and give freshness to skin, though contain only little antimicrobial activity.

This is significant with respects to the human body in preventing sepsis and skin infections (Higaki *et. al*., 2000) [11], and also brightening, adding freshness and nice fragrance to the body. The human skin has some microorganisms that inhabit it (normal flora) that don't cause negative effect on it. Examples of these bacteria include *Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginosa* (Higaki *et al*., 2001). Any of the type of soaps can then be used to help balance the microorganisms that remain on skin. For those who often expose them self to infectious microorganisms, can make use antiseptic or herbal soaps, which will help them maintain their body micro flora, while those who are not often exposed to those infectious microorganisms can make use of beauty soaps which do not have much antimicrobial activity, so as not to reduce the normal skin flora to the degree that they can no longer protect the skin from even much more infectious microorganisms. The attribute of the soap includes gentleness on the skin, rich lather, protection against skin disorders (including rashes, eczema, scabies), treatment of skin infection (such as ring worm), protection or even skin toning and

smoothness of the skin (Getradeghana, 2000) [6]. The soap should have good ingredients which have the ability to kill bacteria but not to damage body tissues.

Several antimicrobial substances are found in antiseptics and herbal soap and they have various mode of action on various skin microflora. The Nigerian market is flooded with different types of beauty soaps, herbal soaps and antiseptic soaps for the treatment of a variety of skin infections but most of them have not been evaluated for their antimicrobial activity. The assessment of these soaps for their antimicrobial properties will give information on the activity of these soaps.

This study was carried out to assess the antimicrobial properties of locally available market beauty soaps, herbal soaps and antiseptic soaps, against common pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa, Klebsiella pneumonia,* and *Escherichia coli.*

Materials and Methods

This study was carried out in Department of Microbiology, General Microbiology Laboratory.

Collection of Samples

Different types of herbal, beauty and antiseptic soap samples, which are commonly used by humans, were purchased from standard supermarkets in Port Harcourt River States. The content and expiry dates of all soaps were noted. These samples were purchased in their original packages and taken to the laboratory.

Soaps Used

Antiseptic soaps

Safeguard, Salvon, Delta, and Dettol.

Herbal soaps

Zee, Dudu osu, Extract and Unik.

Beauty soaps

Lux and Joy.

Isolation of Test Organism

Staphylococcus aureus, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumonia* were isolated and employed in the investigation. Different selective/ differential media were used for the isolation of these organisms which include; Cetrimide, Eosin methylene blue, MacConkey, and Mannitol salt agar. Cetrimide agar is the selective medium for isolating Pseudomonas aeruginosa, showing green pigment. On MacConkey agar, *Klebsiella pneumonia* colony appeared large, mucoid, and pink in color. On Eosin methylene blue agar, *Escherichia coli* grows with a metallic green sheen. On Mannitol salt agar, *Staphylococcus aureus* grow, showing yellow colonies with yellow zones. These different colonies of organisms where further sub cultured on nutrient agar, and further identification process were done for confirmation. Identification of bacteria was done by using different biochemical tests. These tests were based on the gram stain reaction of bacterial strains. Tests includes: oxidase test, catalase test, motility test, coagulase test, urease test, acid production from glucose sucrose, and lactose, coagulase test, indole test, triple sugar iron reactions, methyl red test and Voges Proskauer test, Cheesbrough, (2005) [5].

Preparation of Soap Samples

The soaps were treated in the laboratory in the following ways. A sterile blade was used to scrap each soap, obtaining different weight of; 50mg, 100mg, 150mg, 200mg and 250mg, which were introduced in to sterile test tubes containing 1ml of distilled water. Test tubes were well labeled, with the name if the appropriate soap sample, and concentrations.

Preparation of DISKS and impregnation with soap

Disks of diameter 6.5mm were bored in the laboratory. The disks were then wrapped in foil paper and sterilized in a hot air oven at 150^{o}C for 1hour. The disks were then soaked in the different soap solutions for a period of 1 hour to ensure that the disks were fully saturated. With the aid of sterile needle, the disc was picked and placed on the plates containing Mueller Hinton agar which has been smeared with the different isolated organisms. The plates were then incubated at 37C for 18-24 hours inverted position.

Antimicrobial Susceptibility Testing of Test Organisms to Soap Suspensions

Disks Agar Diffusion Methods

The standard agar diffusion method recommended by Clinical Laboratory Standard Institute (2006) was employed. Distinct colonies of each test organism were taken from a 24-hour agar culture and were suspended in10ml of saline solution (0.85% NaCl) in test tubes using a sterile loop. The suspension was thoroughly mixed and then adjusted to 0.5 McFarland Standard. The suspension was applied to the surface of the Mueller Hinton agar in the plate using sterile swab sticks. The inoculated plates were incubated at 37°C for 20 minutes for acclimatization and growth of the inoculums. The soap discs were then applied on the inoculated plates. The

plates were incubated at 37°Cfor18–24hours. At the end of incubation period the diameter of zones of inhibition around each disc was measure experiments were recorded.

Preparation of 0.5 Mac Farland Standard

1% solution of Barium Chloride (BaCl) was prepared by dissolving 0.1g of BaCl in 9.9ml distilled water in a conical flask. In a separate conical flask, 1% solution of Sulphuric acid(H2SO4) was prepared by dissolvingc0.1g of H2SO4 in 9.9ml distilled water. Then, 9.95ml of the 1% H2SO4 solution was mixed with 0.5ml of the 1% BaCl solution in a test tube and shook thoroughly. The solution was used as a standard in order to attain the same concentration of inoculums by comparing and adjusting the turbidity of the bacterial suspensions by the addition of sterile physiological saline or inoculum to match the turbidity of the standard.

Statistical Analysis

The data obtained from this study were analyzed statistically using Analysis of Variance (ANOVA).

Results and Discussions

Among the antiseptic soaps, Dettol and Safeguard, were the most effective, in the inhibition of the bacteria strains, while amongst the herbal soaps, Extract and zee, were the most effective in inhibiting the growth of the bacteria, but the beauty soaps have mild effect *Staphylococcus aureus*, but little or no effect on *Escherichia coli, Klebsiella pneumonia,* and *Pseudomonas aeruginosa*. MIC for the different soaps was also determined.

Dettol's minimum inhibitory concentration (MIC) for *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, and, Pseudomonas aeruginosa*, are 100mg/ml, 150mg/ml, 100mg/ml, and 150mg/ml respectively. From the study, *Staphylococcus aureus* and *Escherichia coli*, are found to be more susceptible to Dettol soap when compared to *Klebsiella pneumonia* and *Pseudomonas aeruginosa*.

Safeguard MIC for *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli*, and, *Pseudomonas aeruginosa*, are 100mg/ml, 200mg/ml, 150mg/ml, and 150mg/ml respectively. Safe guard having almost if not the same effect as Dettol on these isolates, have great effect on the four bacteria stains, but was most inhibitory to *Staphylococcus aureus*, and least to *Klebsiella pneumonia*.

Delta's MIC for *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli*, and, *Pseudomonas aeruginosa,* are 150mg/ml, 200mg/ml, 150mg/ml, and 150mg/ml respectively. Delta soap also has great inhibitory effects on these isolates, ranking third behind Dettol and safeguard, though with mild effects on *Klebsiella pneumonia*.

Salvon's MIC for *Staphylococcus aureus, Klesiella pneumonia, Escherichia coli,* and, *Pseudomonas aeruginosa,* are 150mg/ml, 200mg/ml, 200mg/ml, and 200mg/ml respectively. Salvon and Delta soaps have almost the same inhibitory effects on these isolates, but were most effective on *Staphylococcus aureus*.

Extract MIC for *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, and, Pseudomonas aeruginosa*, are 200mg/ml, 250mg/ml, 200mg/ml, and 250mg/ml respectively. Extract is a herbal soap with great antimicrobial component, but not when compared with the other antiseptic soap. It is the most effective amongst the other herbal soaps used for this experiment.

Dudu Osun's MIC for *Staphylococcus aureus, Escherichia coli*, and, *Pseudomonas aeruginosa,* are 200mg/ml, 250mg/ml, and 250mg/ml respectively. Dudu Osun also has antimicrobial ingredients in it, but mildly. It has effect on *Klebsiella pneumonia*, but a little effective on the other Microbes.

Unik's MIC for *Staphylococcus aureus* is 200mg/ml, but there was no effect on *Klebsiella pneumonia, Escherichia coli,* and, *Pseudomonas aeruginosa*. Unik became the least effective amongst the herbal soap but more effective than the beauty soaps.

Zee's MIC for *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli,* and, *Pseudomonas aeruginosa,* are 200mg/ml, 250mg/ml, 250mg/ml, and 250mg/ml respectively. Zee has almost if not the same antimicrobial effects on these isolates.

The beauty soaps, Lux and Joy do not have MIC in these concentrations, though they were mildly effective against *Staphylococcus aureus*.

Table 1: Confirmation of identities of test microorganisms

Test/ Microscopy	***Staphylococcus aureus***	***Escherichia coli***	***Klebsiella pneumonia***	***Pseudomonas aeruginosa***
Shape	Cocci	Rod	Rod	Rod
Gram stain	+	-	-	-
Catalase	+	+	+	+
Oxidase	-	-	-	+
Coagulate	+	ND	Nd	-
Citrate	+	-	+	+
Indole	-	+	-	-
Methyl Red (MR)	+	+	-	-
Voges Proskauer (VP)	+	-	+	-
Ureasee	+	-	+	-
Glucose	+	+	+	-

Lactose	+	+	+	-
Sucrose	+	+	+	-
Motility	-	+	-	+

Table 2: Diameter of Zone of Inhibition (mm) o*n Staphylococcus aureus* by various soaps

	Antiseptic soap				Herbal soap				Beauty soap	
Concentration mg/ml	**Dettol**	**Safe guard**	**Delta**	**Salvon**	**Extract**	**Dudu Osun**	**Unik**	**Zee**	**Lux**	**Joy**
50	11	10	8	8	-	-	-	-	-	-
100	14	12	11	11	8	8	8	8	-	-
150	17	16	14	13	10	10	10	10	-	-
200	20	19	16	16	12	12	12	12	8	7
250	24	21	18	18	14	14	14	14	10	9

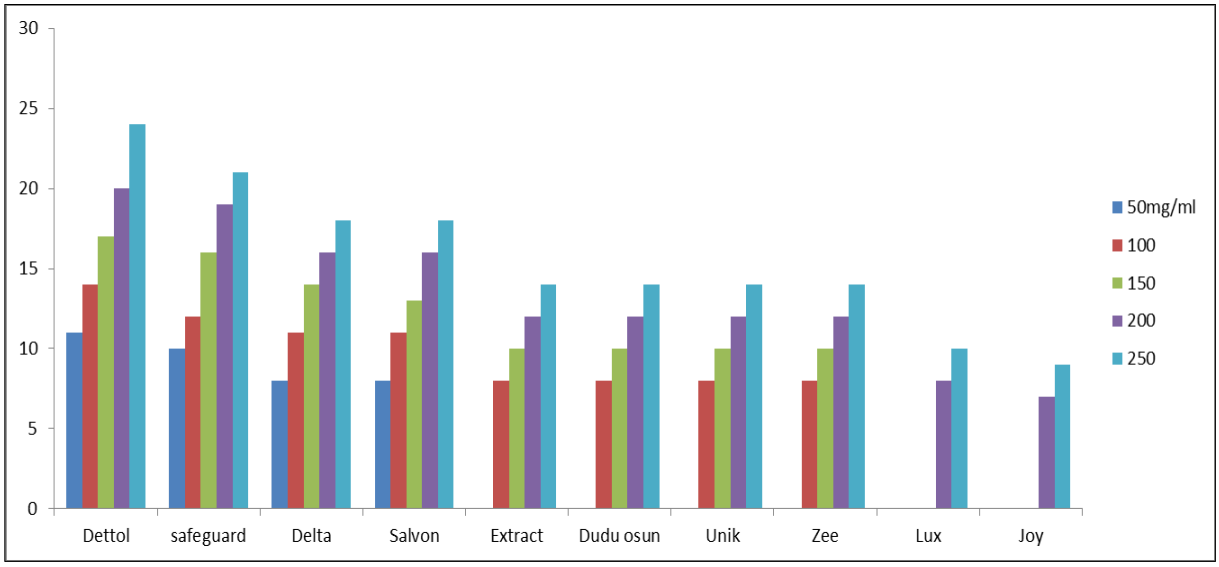

Fig 1: Zone of Inhibition (mm) o*n Staphylococcus aureus* by increasing concentrations of different soap

Table 3: Diameter of Zone of Inhibition (mm) on *Klebsiella pneumonia* by various soaps

	Antiseptic soap				Herbal soap				Beauty soap	
Concentration mg/ml	**Dettol**	**Safe guard**	**Delta**	**Salvon**	**Extract**	**Dudu Osun**	**Unik**	**Zee**	**Lux**	**Joy**
50	8	7	7	7	-	-	-	-	-	-
100	11	9	8	8	7	-	-	-	-	-
150	13	10	10	10	9	-	-	8	-	-
200	15	12	12	12	10	8	8	10	-	-
250	17	15	15	14	12	10	10	12	8	7

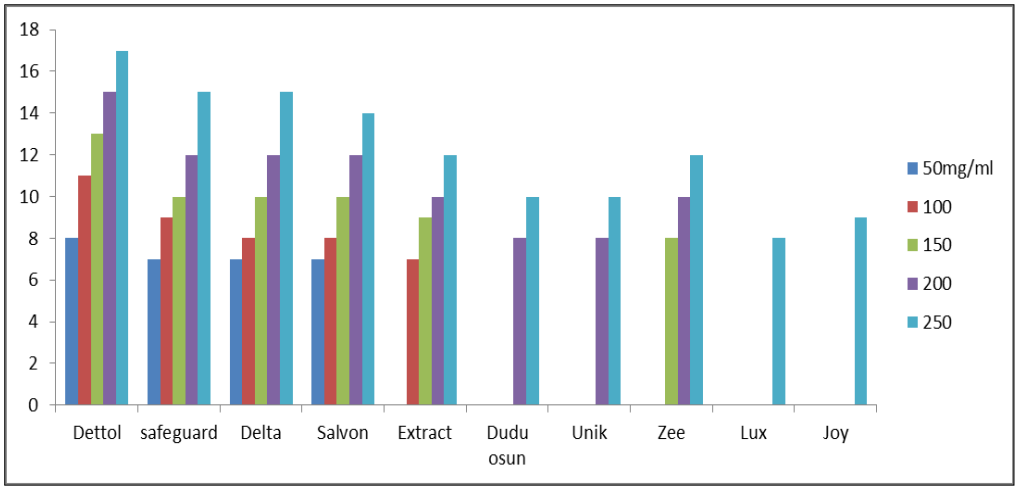

Fig 2: Zone of Inhibition (mm) on *Klebsiella pneumonia* by increasing concentrations of different soap

Table 4: Diameter of Zone of Inhibition (mm) on *Escherichia coli* by various soaps

	Antiseptic soap				Herbal soap				Beauty soap	
Concentration mg/ml	**Dettol**	**Safe guard**	**Delta**	**Salvon**	**Extract**	**Dudu Osun**	**Unik**	**Zee**	**Lux**	**Joy**
50	9	8	8	7	7	-	-	-	-	-
100	12	11	10	8	9	-	-	-	-	-
150	15	14	13	11	10	8	-	8	-	-
200	17	16	15	14	12	10	8	10	-	-
250	20	18	17	16	14	12	10	12	8	8

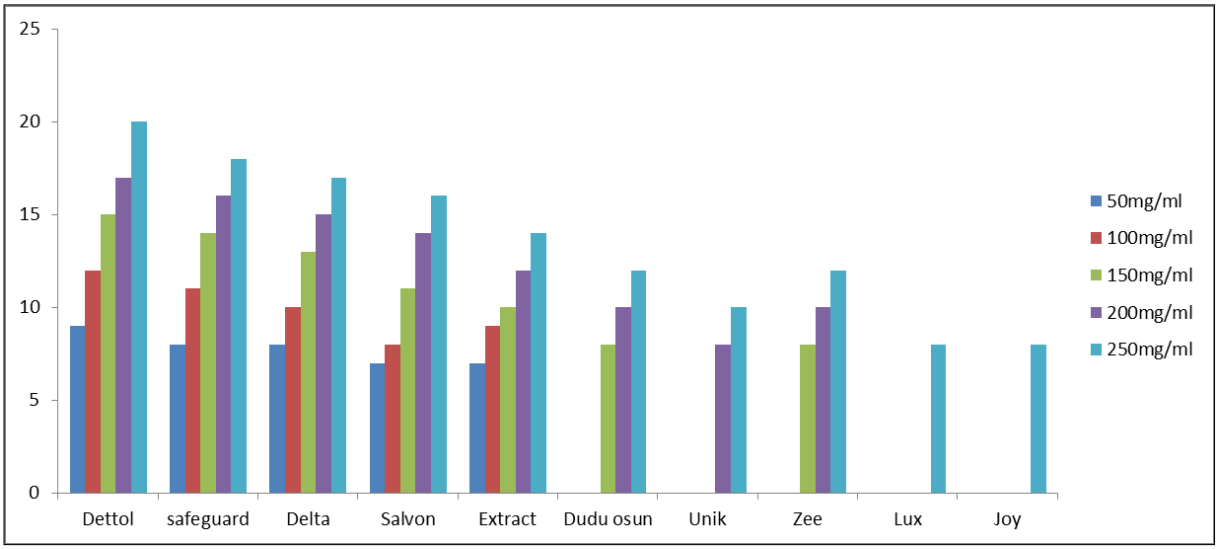

Fig 3: Zone of Inhibition (mm) on Escherichia coli by increasing concentrations of different soap

Table 5: Diameter of Zone of Inhibition (mm) on *Pseudomonas aeruginosa* by various soaps

	Antiseptic soap				Herbal soap				Beauty soap	
Concentration mg/ml	**Dettol**	**Safe guard**	**Delta**	**Salvon**	**Extract**	**Dudu Osun**	**Unik**	**Zee**	**Lux**	**Joy**
50	8	8	7	7	-	-	-	-	-	-
100	10	10	8	8	-	8	-	-	-	-
150	13	13	10	10	-	10	8	8	-	-
200	16	15	14	13	8	12	10	10	7	-
250	18	17	16	16	10	14	12	12	9	7

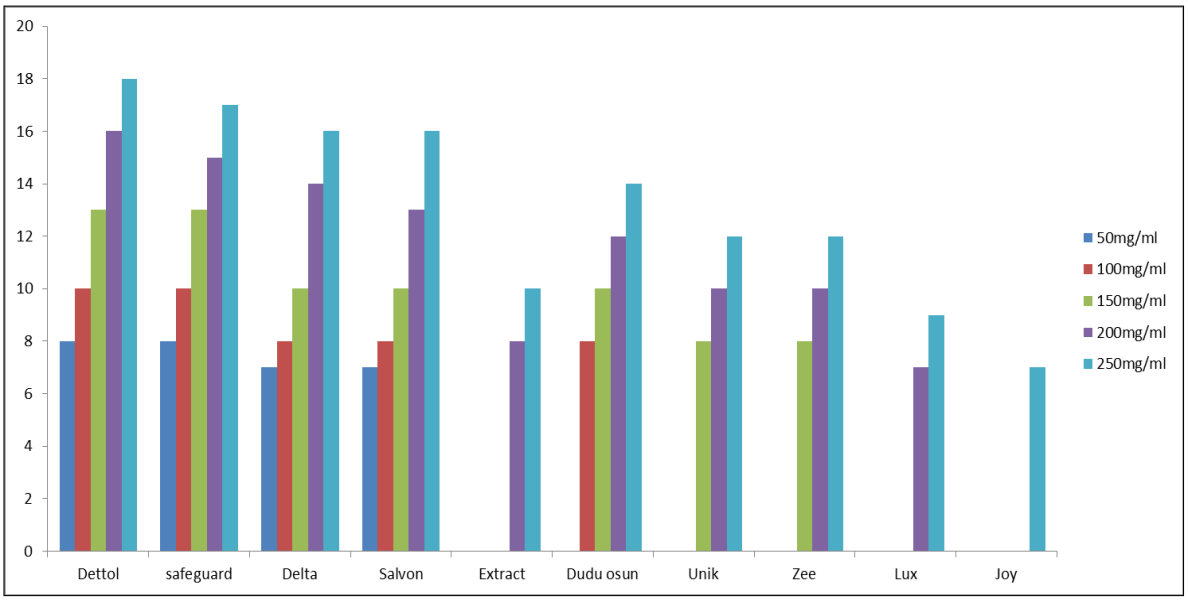

Fig 4: Zone of Inhibition (mm) on *Pseudomonas aeruginosa* by increasing concentrations of different soaps

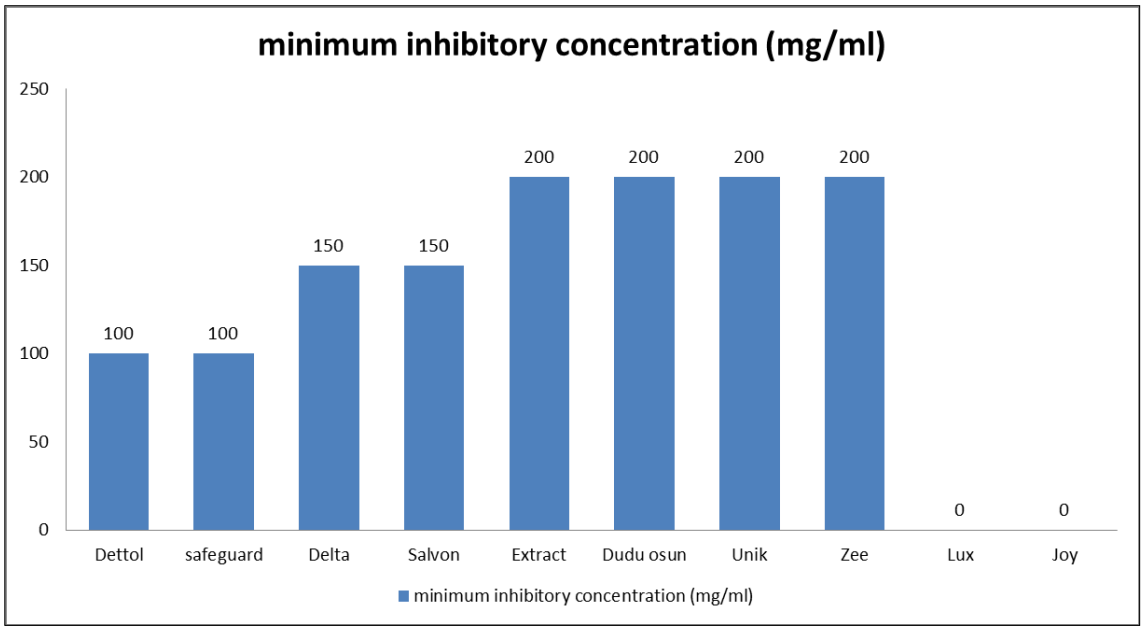

Fig 5: MIC of different soaps on *Staphylococcus aureus*

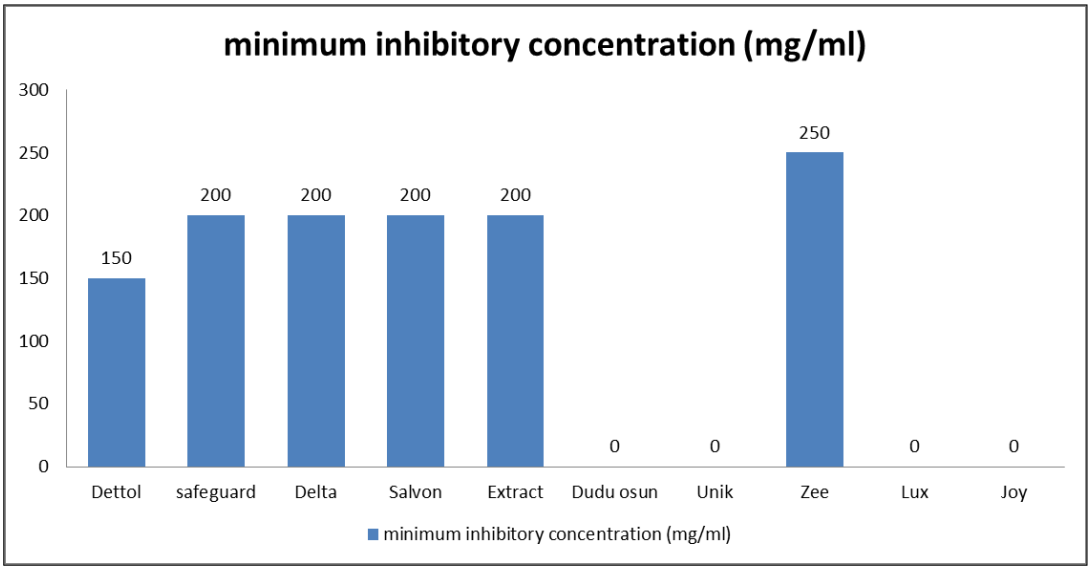

Fig 6: MIC of different soaps on *Klebsiella pneumonia*

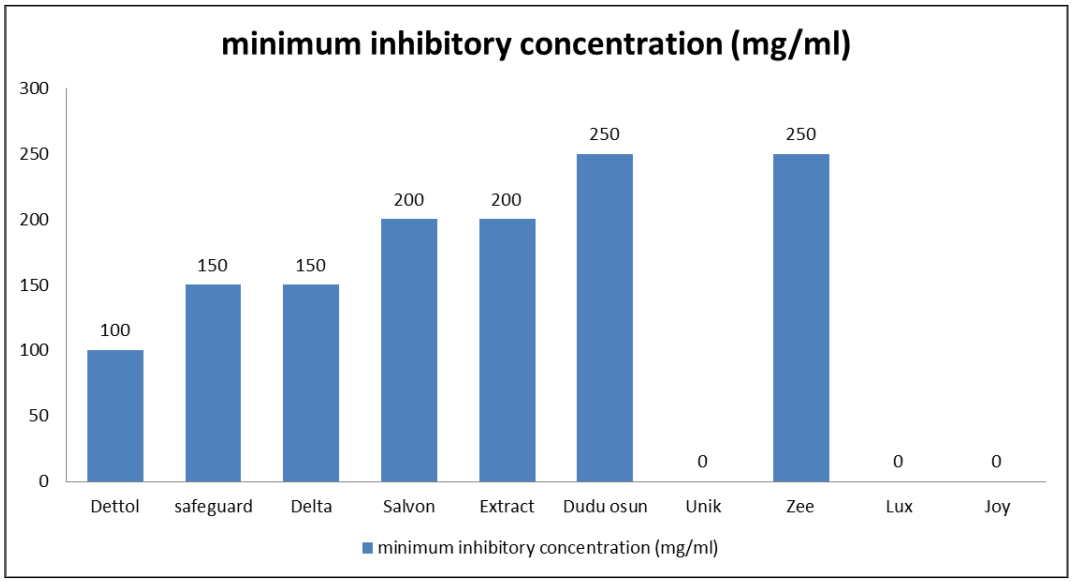

Fig 7: MIC of different soaps on *Escherichia coli*

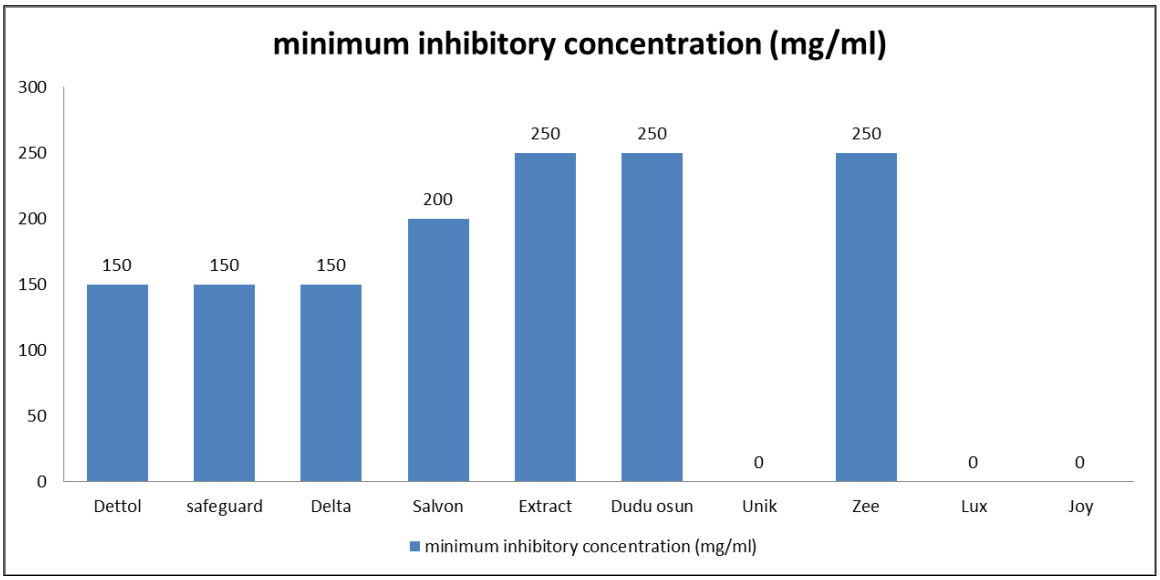

Fig 8: MIC of different soaps on Pseudomonas aeruginosa

Discussion

Results of the study showed that most of the assayed medicated soaps have antibacterial activity, though to varying degrees as indicated by the inhibition of the growth pattern of the different isolate .and also the present study also suggested that the choice of soap should be that which does not affect the facial tissues as well as effective against disease causing bacteria in a small amount. In the determination of the efficacy of the different soap samples, it was found out that the effect of the soaps increases as the concentration of the soaps increase. Results revealed that the different soap samples assayed, displayed varying degrees of inhibition on the test organisms. Antiseptic soaps contain different concentrations of antimicrobial active ingredients (AAIs). The antimicrobial active ingredients (AAIs) included irgasan, trichlorocarbanlide (TCC), mercuric iodide, monosulfiram, and trichloroxylenol which are considered manufacturer dependent. When the efficacy of the antiseptic soaps was assayed using the disc agar diffusion method, Dettol was found to be most effective against the test organism having the highest zone of inhibition (24mm), followed by Safeguard with zone of inhibition (21mm), and amongst the herbal soaps, Extract inhibited more organisms than the others. The results obtained in this study are in agreement with the work of (Obi, 2014) [8] on antibacterial activities of some medicated soaps on selected human pathogens. The assayed antiseptic soaps have demonstrated satisfactory antimicrobial effect, particularly in the antibacterial activity. Probably, the observed variability in antimicrobial activity is due to differences in AAI contents, and type of formulations. Other studies have found that soaps containing AAIs to remove more bacteria than simply washing with beauty soap and water (Obi, 2014). This study has also proved that the use of beauty soap in cleaning wounds and other skin infections as inappropriate as susceptibility tests reveals. It was clearly seen from this study that Gram positive bacterium (S. aureus) was inhibited by all the soaps used in comparison to the Gram-negative bacteria (*E. coli; P. aeruginosa; and K. pneumoniae*). The inhibition of the growth pattern of the isolates indicates the varying abilities of the organism to resist the antimicrobial effect of the soaps. However, these variations could be due to differences in the nature and structures of the bacterial cell wall since it is the ultimate target of any antimicrobial agent or disinfectant which agrees with the research by (Obi et al), Ike (2016) which was also observed by (Rama et al), who explained that triclosan exhibits particular activity against gram positive bacteria due to differences in the cell wall composition. Results of susceptibility pattern as shown by the diameter of the zone of inhibition by the test organisms (*S. aureus and P. aeruginosa, K. pneumoniae and E. coli)* depicted that there was a significant increase with an increase in the concentration of the soaps, which is in agreement with the research conducted by (Varsha et al) on studies of antimicrobial activity of antiseptic soaps and herbal soaps against selected human pathogens. Beauty soaps contain some natural and plant extracted ingredients in their composition which have the ability to inhibit the growth of gram-positive bacteria. The active ingredient in the soap is what distinguishes each soap from another. However, some other medicated soaps which are not Dettol nor Safeguard, with different active agent did not show good results. It can be concluded that the active agents alone may not be sufficient to judge the antimicrobial efficacy of a soap, as other factors such as concentration of active ingredient and other additives might influence the antimicrobial properties. This is in agreement with the work of (Geraldo et al) which shows that combination of benzenethonium chloride, polyhexamethylene biguanide, and farnesol is superior to the use of triclosan alone. Changes seen in the microbial cell structure under the microscope revealed that the activity of the soaps majorly affects the disruption of cell wall and cell membrane. Most of these values correspond to past research work. Abbas et all (2016) working on Safeguard and Dettol against Escherichia coli, had almost similar results as this research work, with safeguard of concentration, 50mg/ml 100mg/ml, and 150mg/ml, having zones of inhibition of 9mm, 9.9mm, and 12mm respectively. Which is in line with this research work, with same concentrations having zones of inhibition of 8mm, 11mm, and 14mm respectively. For

Dettol, using the same concentrations, had 10.8mm, 15.7mm and 19.7mm respectively, almost corresponding with this work with zone of inhibition; 9ml, 12ml, and 15ml respectively. Varsh et al (2016) worked with Dettol and Salvon against Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus, using concentration of 250mg/ml, had result quite similar with this research work, with Dettol having zones of inhibitions of 18mm 16mm and 31mm when subjected against Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus respectively which is close to the zones 20mm, 18mm, and 24mm respectively gotten in this research work. That of Salvon, using same concentration and isolates, Varsh et al(2016) got zone of 12mm, 10, mm, and 12mm for the respective isolates, compared to 16mm, 16mm and 18mm gotten from this research. Saba Riaz et al (2009), working on the minimum inhibitory concentration MIC) of safeguard on Staphylococcus aureus, Pseudomonas aeruginosa, and Klebsiella pneumoniae, had 125 mg/ml, 250mg/ml and 250mg/ml respectively as the MIC. In comparison with this research work with results; 100mg/ml, 150mg/ml and 200/ml respectively, it is found to be almost the same. Little differences in the results when compared to past research work, could probably be, because of differences in the strains of the microorganisms used. In general, antiseptic soaps were most effective followed by herbal soaps which are more effective than beauty soaps. Analysis of variance for the Means of antibacterial activities among the soaps revealed positive correlations ($P<0.05$) it was observed that significant differences exist among the different concentrations used for the study with 250mg /ml (having more zones of inhibition than other concentrations: 200 mg/ml 150 mg/ml, 100 mg/ml and 50 mg/ml for Dettol Soap against S. aureus and for other medicated soaps.

Soaps are cleaning agents routinely used for cleaning purposes and removing germs. Soaps and detergents disrupt the microbial cell membrane and disrupt cells proteins. Soaps tested in the present research work showed varied levels of activity against the pathogenic microbes tested. Hence Dettol followed by Safeguard among other soaps showed antibacterial activity and can be used to prevent skin infections and transmission of skin pathogens when used in hand washing. However, prolonged used of these soaps could lead to development of microbial resistance and allergic reactions to skin. The main aim of this study was to identify the antimicrobial activity of antiseptic soaps, herbal soaps and beauty soaps. Antiseptics soaps possess active germicidal chemical agent, it kills pathogen but its prolonged use should be avoided. As herbal soaps revealed similar antimicrobial activity like that of antiseptic soaps, but they are moderately effective. For serious skin infections, antiseptic soaps are recommended, but to keep the body normal flora in balance herbal soaps are recommended. The soap is routine requirement of life, but it should have good active ingredients or plant extracted material which has the ability to kill bacteria but not to harm body tissues when used constantly.

Recommendation

This area of research requires attention of scientists and people from soap industry, because quality of soaps is very important as they are the need of every home. The soap should have good ingredients which have the ability to kill bacteria but not to damage body tissues. Health care workers should use soaps according to criteria of Health and Hygiene. In this way many immuno-compromised or low immunity patients can be protected from transfer of pathogenic or opportunistic pathogens.

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