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COMPARISON OF ANTIMICROBIAL ACTIVITY OF LOCALLY PRODUCED SOAPS AND CONVENTIONAL MEDICATED SOAPS ON BACTERIAL ISOLATES FROMSKIN AND WOUND

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ABSTRACT

Antimicrobial activity of Locally made and Conventionalcustomized medicated soaps sold and widely used by the residents of Port Harcourt Metropolis. Rivers State, Nigeria was examined against some clinical bacterial isolates from skin surface and wound. Clinical isolates used were *Staphylococcus aureus* and *Pseudomonas aeruginosa*. the results showed that the test organisms were sensitive to the soap samples at different concentrations. As the concentration increased the zones of inhibition significantly increased. The results obtained showthat among the conventional customized medicated soaps, the highest effectiveness was carried out by Dettol (25mm) at 500mg/ml and Safeguard (24mm) at 500mg/ml against *S. aureus* and *P. aeruginosa*.In the category of locally made soaps, the red soap (LPRS) was discovered to be more effective on all the isolates (*S. aureus*,11.5mm and *P. aeruginosa*, 16.5mm) at 500mg/ml, as compared to locally produced black soap (LPBS) with this effectiveness *S. aureus*,8.5mm and *P. aeruginosa*, 12mm. The percentage of activity of the soaps on the test organisms showed that *S. aureus* displayed higher resistant to most of the soaps compared to *P. aeruginosa*. The Minimum Inhibitory Concentration (MIC) of the soaps with efficacy was found to be 125mg/ml on *S. aureus* and 250mg/ml on *P. aeruginosa* while the Minimum Bacteriocidal Concentration (MBC) was 250mg/ml for all theisolates.

KEYWORDS: Locally made soaps, Conventional customized medicated soaps, Antimicrobial activity, Minimum Inhibitory Concentration, Minimum Bacteriocidal Concentration, and Test organisms.

INTRODUCTION

Antimicrobial activity of any substance is described as it ability to either kill bacteria or inhibit the growth of bacteria. Antimicrobial activity is important when considering the human body in regard to preventing diseases and skin infections.[12] Soap may be defined as a chemical compound resulting from the interaction of fatty acids, oils and salt. [10]Soaps and other cleaning agents are extensively used for a very long time for different cleaning purposes.[18]For generations it has been thought that washing hands with soap and water is a measure of one's personal hygiene. Microorganisms such as bacteria and fungi are generally believed to be ubiquitous, that is, they are found everywhere in places like soil, water, air, sewage and on human body and hence of great importance with reference to health.[13] Soaps play a very important role both in cleaning and killing bacteria. To enhance their antibacterial activities some active ingredients are added to soap.[25]

Soaps with antibacterial properties have been reported to remove 65% to 85% of bacteria from human skin.[20] A huge number of chemical compounds are present that have the ability to stop the growth of bacteria and can kill them. These compounds are very large in number possibly 10,000 of which 1000 are being used in hospitals and homes. These chemical compounds exist in the form of solids, liquids and gases. Quite a number of chemicals are implicated to decrease or destroy microbes. Significant groups include halogens, phenols, soaps, detergents, ammonia compounds, alcohols, heavy metals, acids and certain extraordinary compounds. [16]In ethnomedicine, described as total combination of knowledge, practice and belief incorporating plants, animals and minerals based medicine in treating, preventing or get rid of physical, mental or social disease and which may depend largely on past experience passed down from generation to generation either verbally or in writing.[28] The use of soaps as vehicles for the application of medicinal plants for external use and in the treatment of skin diseases havebeen reported.[2,3,4] Locally manufactured soaps have some antimicrobial properties.[17]Over the years, the traditionally or locally manufacturedsoap, otherwise known as "African black soap" has been used, in Ghana and Nigeria, to help relieve acne, oily skin, clear blemishes and various other

skin issues. Black soap has been employed to get rid of skin rashes, ringworm, measles and body odours and for treating many infections caused by microorganisms as well as for exfoliating and deep cleansing.[30]The locally or traditionally produced medicated soap is widely used by many tribes in Nigeria, such as Igbo (Nichaojie), Hausa (Sabulusalo), and Yoruba (Oṣedudu).[1] This locally produced soap is made from a mixture of vegetable oils such as palm kernel oil and shea butter which make the soaps to have the acclaimed antimicrobial properties recognized in the traditional African households.[11] Apart from the materials outlined before there are other items that are added. They are dried herbs, flowers and stems which are used as the soap base. Due to the constituents of the locally made soap, this soap is referred to as herbal soap. Herbs are the natural products that are recognized to play a significant role in the treatment of almost all diseases and skin problems because of their high medicinal value, affordability, availability and ability.[26,27] The attribute of the soap includes mildness on the skin, rich lather, protection against skin disorders (including rashes, eczema, scabies), treatment of skin infection (such as ringworm), protection of even skin toning and smoothness of the skin.[11] Fus,[12] reported the inhibitory potential of antimicrobial and non-antimicrobial soaps in clinical cases. Toshima[29] indicated that soaps that have antimicrobial properties may remove more bacteria than the soap that lackembellishment.

The purpose of this research therefore was to determine the antimicrobial activity of different conventional medicated and locally produced soaps available in some local markets in Port Harcourt metropolis against daily encountered skin infecting human bacteria.

MATERIALS AND METHODS

Study Area

The study was conducted in the Rivers State University in Port Harcourt metropolis. Rivers State is in the South-South Geopolitical zone of Nigeria.

Collection of antimicrobial agents and preparation

The soaps were randomly purchased from two (2) local markets in Port Harcourt. The conventional customized medicated soaps and their designations are: Delta (MS. C), Dettol (MS.D), Tetmosol (MS.A), Rainbow (MS.B), and safeguard (MS. E) certified by the Nigerian National Agency for Food and Drug Administration and Control (NAFDAC) were obtained from commercial vendors. A total of 2 different locally produced soap samples (Black and Red) were purchased and collected into sterile polythene packs using the guidelines described by Olajuyigbe [23] and the locally made soaps are designated LPBS (locally produced black soap) and LPRS (locally produced red soap) respectively. For the conventional customized medicated soap samples, the batch numbers, expiry dates and the presence or absence of the manufacturers seal were noted.

Sample preparation

The methods described in [1,6] were adopted for sample preparation in which sterile distilled water was used as diluents. With the help of a sterile sharp knife soaps were scraped into pieces in weights 62.5mg, 125mg, 250mg, and 500mg for each soap sample and dissolved in 1ml of sterile distilled water inbijoubottles separately.

Isolation of test Microorganisms

The test microorganisms *Staphylococcus aureus and Pseudomonas aeruginosa* used in the study were isolated in the laboratory from wound and skin swabs collected from some students of Rivers State University and residents of Rukpokwu Community, Port-Harcourt Metropolis. The samples were collected using sterile swab sticks by swabbing the points of collection with the sterile cotton swab sticks after which they were transferred into the swab stick containers. The samples collected with swab sticks were then used to inoculate already prepared Mannitol Salt Agar (MSA) and Centrimide which are selective media for *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively, the plates were then incubated at 37°C for 24 hours. From the cultured selective media platesdiscrete colonies were sub-cultured on freshly prepared Nutrient agar. After the incubation the colonies counts were done. Also from the Nutrient agar plates biochemical characterization and identification of the test organisms were also carried out using the Bergey‟s Manual of Systematic Bacteriology.

Preservation of Isolates

The identified bacterial isolates were preserved on Nutrient Agar Slants and stored at 4°C as described by.[9]

Standardization of Inoculum and Agar well diffusion method

Overnight cultures were kept ready for anti-microbial activity. Each of the isolate was standardized using colony suspension method.[8] The test organisms from Nutrient agar plates, incubated at 37^{o}C for 24 hours were suspended in saline solution (0.85% Nacl). The density of each isolate suspension was matched with 0.5 McFarland standards to give a resultant concentration of 1.0×10^{6} cfu/ml (Biomerus, France).[22] The susceptibility of the test organisms to the different soap samples concentrations was assayed using agar-well diffusion method.[19] Twenty millilitre (20ml) of freshly prepared Mueller-Hinton agar was poured on each petri dish plates of same size and allowed to solidify. The agar plates were punched with a sterile Cork borerof6mm size, 4 equally spaced holes were bored in the agar plate with a fifth hole in the center of the plate and the agar plugs were discarded using a sterile needle. The already prepared Mueller–Hinton agar (Oxoids UK) bored plate was flooded with the resultant saline suspension of each test organism in well labelled sterile bijou bottles and excess decanted. About 2-3 drops of the different concentrations (62.5, 125, 250 and 500mg/ml) of the soap samples already prepared were dispensed into the bored holes using sterile Pasteur pipette while the well at

the center was filled with an equal volume of sterile distilled water to serve as control.[5] The plates were allowed to standby for 30minutes. The plates were incubated at 37°C for 24 hours in an upright position. They were then examined for zones of inhibition which indicated the degree of susceptibility or resistance of the test organism to the antibacterial agent. The method of Clinical and Laboratory Standards Institute [21] was adopted to compare the efficacy of the conventional customized medicated soaps with locally produced soap samples on clinical bacterial isolates. [21]

The test was carried out twice, and the mean of all readings was taken as the zone of inhibition in each case. A transparent ruler was used to measure the diameter of the clear zones of inhibition (mm) noticed on the plates [9] The minimum inhibitory concentration (MIC) of the soap samples was determined by the tube dilution method of Junaid.[14] The dilutions that showed no turbidity for the MIC were sub- cultured on freshly prepared Mueller-Hinton agar plates, well labelled for each test organism and soap sample. The lowest concentration which the test organisms could not recover and grow after the transfer to the freshly prepared Mueller-Hinton agar plate was noted as the minimum bactericidal concentration (MBC).[14]

Statistical Analysis

The statistical package SPSS version 22 was used to analyze the data. Analysis of variance at P≤ 0.05 was done to check for differences between treatment and where difference occurred, Duncan's multiple range test was used to separate the means. Frequency of susceptibility pattern of the isolates was also done using same statistical package.[5]

RESULTS AND DISCUSSION

Soaps are employed mainly for washing or bathing with the aim of removing dirts and microorganisms present on the skin surface. Although, among several industrially produced and locally made soaps that are available around us individual preference determines the choice of a soap but it is advised that whichever brand of soap that an individual chooses to use, the soap should be such that will not affect the sensitive skin and it should beeffective against potential disease causing microbes present on skin.[31] The results obtained from this study showed that conventional customized medicated and locally produced soaps have antimicrobial activity against *S. aureus* and *P. aeruginosa*, although to a certain identifiable position as showed by the inhibition of the growth pattern of the isolates. Results of susceptibility pattern as shown by the diameter of the zone of inhibition by the test organisms (*S. aureus* and *P. aeruginosa*) depicted that there was a significant ($P \leq 0.05$) increase with an increase in the concentration of the soaps (figures 1 & 2). The results obtained showed that among the conventional customized medicated soaps, the highest effectiveness was carried out by Dettol (25mm) at 500mg/ml and Safeguard (24mm) at 500mg/ml against *S. aureus* and *P. aeruginosa*. This result is in agreement with the research conducted by Varsha "Studies on antimicrobial activity of antiseptic soaps and herbal soaps against selected human pathogens"[31]In the category of locally made soaps, the red soap (LPRS) was discovered to be more effective on all the isolates (*S. aureus*,11.5mm and *P. aeruginosa*, 16.5mm) at 500mg/ml, as compared to locally produced black soap (LPBS) with this effectiveness *S. aureus*,8.5mm and *P. aeruginosa*, 12mm. The percentage of activity of the soaps on the test organisms showed that *S. aureus* displayed higher resistant to most of the soaps compared to *P. aeruginosa* as presented in table 1 The Minimum Inhibitory Concentration (MIC) of the soaps with efficacy was found to be 125mg/ml on *S. aureus* and 250mg/ml on *P. aeruginosa* while the Minimum Bacteriocidal Concentration (MBC) was 250mg/ml for all the isolates as represented in table 2.

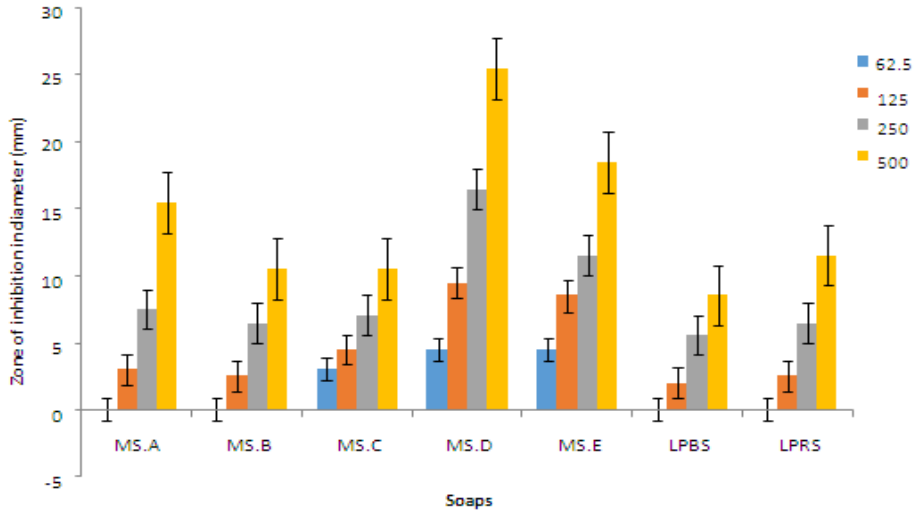

Figure 1: Showing Analyzed Zones of Inhibition for Isolated *Staphylococcus aureus* at Different Concentrations.

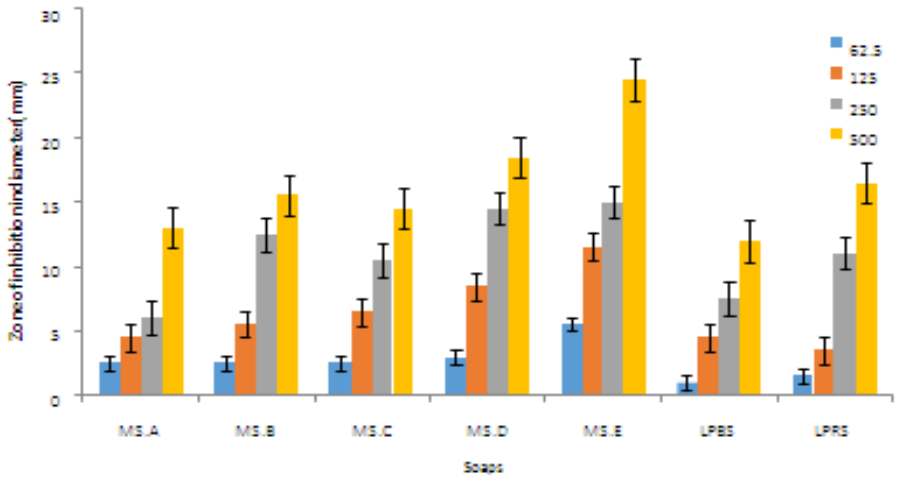

Figure 2: Showing Analyzed Zones of Inhibition for Isolated *Pseudomonas aeruginosa* at Different Concentrations.

Keys: MS. A: Tetmosol soap, MS.B: Rainbow soap; MS. C: Delta soap; MS.D: Dettol soap; MS.E: Safeguard soap; **LPBS:** locally produced black soap, LPRS: Locally produced redsoap.

Table 1: Showing the percentage of activity of the soaps on the test organisms.

Soap	Staphylococcus aureus			Pseudomonas aeruginosa		
	R	I	S	R	I	S
MS.A	75	25	0	75	25	0
MS.B	75	25	0	50	50	0
MS.C	75	25	0	50	50	0
MS.D	50	0	50	50	25	25
MS.E	50	25	25	25	50	25
LPBS	100	0	0	75	25	0
LPRS	75	25	0	50	25	25

Keys: MS. A: Tetmosol soap, MS.B: Rainbow soap; MS. C: Delta soap; MS.D: Dettol soap; MS.E: Safeguardsoap; LPBS: locally produced black soap, LPRS: Locally produced red soap, S: Sensitive (≤ 16.00 mm and above), I: Intermediate (at ≤ 11.00 - 15.00 mm), R- Resistant (at ≤ 10.00 mm), there is significant difference at p≤0.05 level of significance.

Table 2: MIC and MBC of the Soaps Against the Test Organisms.

S/N.	Test organisms	Concentration (mg/ml)	
		MIC	MBC
1	*S. aureus*	125	250
2	***P. aeruginosa***	250	250

The MIC and MBC are specifically for Dettol and Safeguard soaps as other samples did not show significant sterility

Keys: MIC = Minimum Inhibitory Concentration; MBC= Minimum Bacteriocidal Concentration.

CONCLUSION AND RECOMMENDATION

The soaps tested in the present research depicted varied levels of activity against *S. aureus* and *P. aeruginosa*. Hence, Dettol followed by Safeguard among other soaps showed higher antibacterial activity while in the category of locally produced soaps; the locally produced red soap (LPRS) depicted higher activity. Therefore, it is recommended, that in case of skin infections associated with the type of test organisms used in this work, Dettol and Safeguard soaps can be considered for treatment. Also, to prevent transmission of skin opportunistic pathogens from one person to another through contact. The soaps can be used for washing hands and bathing.

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