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COMPARATIVE STUDY ON THE ANTIMICROBIAL ACTIVITY OF SOME MEDICATED AND HERBAL SOAPS AGAINST STAPHYLOCOCCUS AUREUS ISOLATED FROM SKIN OF FEMALE STUDENTS OF CHUKWUEMEKA ODUMEGWU OJUKWU UNIVERSITY ANAMBRA STATE

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ABSTRACT

The study was carried out to compare the antimicrobial activity of some medicated soaps and Herbal soap against *Staphylococcus aureus* isolated from skin of female students of Chukwuemeka Odumegwu Ojukwu University Anambra state University. Nineteen different skin samples were collected with sterile swab stick and inoculated into Mannitol salt agar using spread plate methods and incubated for 24 hours at 37^0C., *Staphylococcus aureus* were isolated from eight Skin samples. The isolated *Staphylococcus aureus* was sub-cultured into agar slant and was identified by biochemical test. Using disc diffusion method, the antimicrobial activity was carried out. From the result of the study, the herbal soap HAI showed high antimicrobial activity on all the *Staphylococcus aureus* isolates as it inhibited all the isolates in the range of 16-19mm, four *Staphylococcus aureus* isolates resisted sample HAII, while four other isolates were inhibited by HAII in the range of (13-18mm). Sample HAIII showed inhibition on only two strains of *Staphylococcus aureus* in the range (15-16mm) as other six isolate resisted the soap. The medicated soap showed that MAI and MAII soap was effective on all the isolate of *Staphylococcus aureus* in the range of (16-25mm). MAIII soap was effective on six isolate of *Staphylococcus aureus* in the range of (12-15mm) as other two isolate resisted the MAIII soap. Comparatively the medicated soaps have satisfactory antibacterial activity on the *Staphylococcus aureus* isolates than the Herbal soaps. In view of the findings of this study it recommended that medicated soaps should be in use by people who are on treatment on skin diseases traced to be caused by *Staphylococcus aureus* as they showed better antimicrobial activity on unlike the Herbal soap studied.

Keyword: Herbal, Medicated, *Staphylococcus aureus*, antimicrobial, Skin

INTRODUCTION

Soap are generally known to be formulations of either chemical or plant extracts used in combination with water produces foam and lather used in scrubbing contact surfaces, environment, and human body to remove stain, grease, eliminate microorganism and also to maintain good personal hygiene. Soaps can come in liquid, or solid state depending on the agent or ingredient used in the formulations.

Soaps are used to remove dirt, including dust, microorganisms, stains and bad smells in order to maintain health, beauty and remove bad odor from the body or inanimate objects, including clothes (Ikegbunam *et al*., 2013). Chemically soaps are the combination of fats, oils (of animal or vegetable origin) and Salt (Friedman and Wolf, 2016)

Herbal Soaps are often formulated with only plant extracts or combination of different plant extracts. Herbal soaps which in trado-medical setting are being applied in treatment of diseases of mostly skin origins in mostly African setting. The Herbal soap preparation is often regarded as herbal drugs due to its naMAIIl ability to have antibacterial & antifungal

agents which are mainly constituents of the part of plants such as like leaves, stem, roots & fruits used in the treatment of disease or to achieve optimum health (Kareru et al., 2010).

Today, in South western Nigeria the Yoruba's are known for their innovative production of various soaps of plant composition which are strongly implored in traditional medicine used in eradicating various skin diseases like Eczma, Danddruff, ring worm and skin rash although they are basically traced to be caused by fungi. Some of these soaps today have also gain the heart of the African market due to its extensive remark gained by the users.

The medicated soaps today in general are basically soaps having antimicrobial strength also known as an antiseptic or medicated soap. An antibacterial soap can remove 65% to 85% of bacteria from human skin (Solanki, 2011). Medicated soaps to a large extend remove dirt and disrupt cytoplasmic membrane to kill microorganisms (Tachibana, 1976). Any medicated soap is often formulated by the use of synthetic antimicrobial compounds which have the ability of destroying or inhibiting the growth of microorganisms. Today, in the world at large due to the continues microbial attack to man, human rely on these medicated soap to obtain 70% protection against microorganisms. Just at 90% sanitation could not just be achieved using soap without antimicrobial activity. During and after the Covid 19 era humans rely, mainly on medicated soap to achieve 90% sanitation as the case may be. Adeyemi et al. (2016) reported It also works against enveloped virus like human immunodeficiency virus (HIV). Several antimicrobial substances are found in medicated soaps and they have various mode of action on various skin microflora (Adeyemi et al., 2016)

The normal human skin harbors microorganisms that can be grouped into transient and resident flora (Tachibana, 1976). The human skin is the basic or the focal point which these soaps are exposed on without prior understanding of the effect of the soap on the respective skin applied on.

Staphylococcus aureus are basically gram positive bacterial known also as "golden staph", is a gram-positive coccus belonging to the class Bacilli, order Bacillales, family *Staphylococcaceae* and genus *Staphylococcus* (Masalha et al., 2001). It is a facultative anaerobe often positive for catalase and nitrate reduction and is coagulase variable i.e. maybe cogulase positive or negative (Matthews et al., 1997)

The bacterium is non-motile, non-spore forming and appearing as bunch of grapes and they are always in cluster, microscopically (Gulzar and Zehra, 2018). On Mannitol salt agar it shows large, round, golden-yellow colonies on the yellow zone of the media. Majority of the bacteria skin infection are caused by *Staphylococcus aureus*, fungi, or *Streptococcus* spp (Moran et al., 2006).

The pathogenic process of *Staphylococcus aureus* infection is often linked to five stages which involves colonization of the site, local infection, systematic dissemination or sepsis, metastatic infections and toxinosis (Arumugam *et al*., 2016). They have strong affinity in the colonization of both mammals and rodent and in reverse cases they can easily be transferred among both species. *Staphylococcal* infections are zoonotic in nature (Gulzar and Zehra, 2018).

The bacteria can spread from person to person by direct contact and also with the contaminated objects (such mobile phones, telephones, door handles, tap faucets, computer keyboards and mouse, knife, currency, medical equipments, etc) (Gulzar and Zehra, 2018). There is even a possibility of transmission by inhalation of infected droplets which are dispersed at the time of sneezing or coughing (Lary et al., 2016). Due to numerous cases of *Staphylococcus* infection and its alarming resistance to common antibiotics, these have led to these research to compare the antimicrobial activities of some medicated and herbal soaps against *Staphylococcus aureus* isolated from skin of female students of Chukwuemeka Odumegwu Ojukwu university Anambra state

MATERIALS AND METHODS

Sample Collection

Nineteen Skin samples (Hand and Armpit) was collected from Female student of Chukwuemeka Odumegwu Ojukwu University, Anambra state. The skin samples were collected with a sterile swab stick. All samples were aseptically handled. And the medicated soap and Herbal used were purchased from Uli market and coded as Herbal HAI, HAII an HAIII while Medicated was coded MAI, MAII and MAIII.

Soap studied and ingredients used in their formulations

HAI: palm kernerl oil, cocoa pod ash, palm buch ash, shea butter,lime juice, honey, whole leaf aloe vera, canwood powder, perfume, lemon juice and water.

HAII: native black soap base, aqua, palm kernel oil, Raw shea butter rich in vitamin, cocoa pod, palm bunch ash solution (botanic potash), native honey, canwood(osun) fragrance and aloe vera.

HAIII: No ingredient Listed

MAI: Soap base, Monosulfiran B.P 5%W/W, Citronella oil, NLT 70% and TFA.

MAII: Soap base, 0.28% Triclosan, Vitamin E, Allantoin, Glycerine, EDTA, Soduim silicate, perfume, colour, minimum total fatty matter70%

MAIII: Soap Base, Perfume, Pine Oil, TCC, TiO_2, Water Glycerin, EDTA, BHT, EHDP, and Colorant

Sample preparation

Collected soap material (MAI, MAII, MAII and HAI, HAII, HAII) were crushed, using a pestle and mortar, to provide a greater surface area. After crushing, the soap material was dissolved in autoclaved sterilized water (121°C, 15 psi).

Soap sample preparation

1g of crushed soap was dissolved in 10 mL of the sterile water, the solution was swirled to mix it well (swirling was gentle to avoid foam formation). Solutions were labeled accordingly for antibacterial activity.

Media used in the Isolation

Nutrient agar, Mannitol salt agar, Nutrient both, Muller Hinton agar. All the media was prepared to their manufacturer's instructions. It was autoclaved at 121^{o}C for 15 minutes.

Isolation of Bacteria

All the skin samples were collected with sterile swab stick and inoculated into Manitol salt agar and incubated for 24 hours at 37^{0}C. The samples that shows golden yellow colonies after 24hrs' incubation was subjected to sub-culturing. The isolates were sub-cultured into Nutrient agar and incubated for 24 hours at 37^{o}C.

Identification of the Isolates

Gram Staining

A firm smear of the isolates was prepared by picking a small portion of microbial growth from the plates with the aid of a sterile wire loop into a drop of sterile distilled water on a clean grease free glass slide and after making the smear, the slides was heat-fixed by carefully passing over a Bunsen burner flame. The heat-fixed smear was stained with crystal violet for 60 seconds and was washed off gently with water and drained. The sample/slide was rinsed with ethanol for 30 seconds and was rinsed off with water and drained. The slide was then counter stained with safranin for 60 seconds (1 minute) and was washed off with water, the slides was air dried. Immersion oil was dropped on the smears and examined under oil immersion objective of the microscope of x100 magnification. The shape and arrangement of the cells was recoded. Gram positive bacteria stained purple while Gram negative bacteria stained pink.

Biochemical Test

The test carried out includes catalase, Oxidase, Motility, citrate, Hydrogen sulphide test, Urease and Indole test according to (Cheesbrough 2019)

Catalase Test

Catalase is an enzyme found in most bacteria. It catalyzes the breakdown of hydrogen peroxide with the release of free oxygen and water. The test is used to determine whether a bacterial can produce the catalase enzyme. A loop full of 24 hours old culture of each isolate was put on a clean slide. A drop of 3% hydrogen peroxide was added to it. The production of bubbles shows the presence of catalase enzyme.

Oxidase Test (Filter Paper Method)

This is used to assays for the presence of cytochromo oxidase, an enzyme sometimes called indolephenol oxidase. A piece filter paper was placed inside a Petri dish and 3 (three) drops of freshly prepared oxidase reagent was added in the filter paper. A sterile glass rod was used to remove a colony of the test organisms from a culture plate and smeared on the filter paper. Blue coloration within 5-10 seconds indicates oxidase positive

Motility Test

This is a test for detecting motility of microorganism. A small amount of pure culture was inoculated into a labeled tube by means of stab inoculation in sim medium. It was incubated at 37^{0}c for 24 hours. Growth appearance by the presence of diffuse growth away from the line of inoculation indicates motile or positive motility test.

Citrate Test

Simmon Citrate agar is used to determine the microorganism that uses citrate as its sole source of carbon. The wire loop was flamed and used to pick a small portion of microbial growth from the plate

and inoculated deep into the bijour bottle that contain citrate agar which is green before inoculation. It was incubated at 37^0c for 24 hours. Blue coloration after incubation shows positive citrate.

Hydrogen Sulphide Test

This is used to detect hydrogen sulphide gas produced by an organism. A small amount of pure culture was inoculated into labeled tube by means of stab inoculation in sim medium it was incubated at 37^0c for 24-48 hours. Black precipitate formed on the medium indicates positive hydrogen sulphide.

Urease Test

It is used to demonstrate which organism tat produces the enzyme urease which split forming ammonia. A loop full of the microbial growth from the plate was collected with the aid of sterile wire loop and incubated at 37^0C for 24 hours. A change from yellow to red after, incubation confirmed the presence of urease and shows its positive.

Indole Test

This test is used to screen for the ability of an organism to degrade the amino acid tryptophan and produced indole. The tube of tryptophan broth was inoculated with a small amount of pure culture and it was incubated at 37^0C for 24 to 48 hours. A kovac reagent was added directly to the tube to test for indole production. A positive indole test was indicated by the formation of pink or read colour in the reagent layer on top of the medium within seconds of adding the reagent.

Antimicrobial assay

The antimicrobial activity was performed by agar diffusion method. The bacteria strains were grown in nutrient broth. Muller Hinton agar was the media used to study the bacteria susceptibility. The broth cultures were grown for 24 hours and serially diluted in the same broth (sterilized at 121^0C for 15 minutes) to 10^{-3}. The 24 hrs. broth culture contains approximately 3.0 X 10^8 Cfu/ml, (1.0 McFarland standard) of *Staphylococcus aureus,* as determined by plate count method. Sterile swab stick was used to inoculate the media by dipping it in the diluted culture and spreading all the surfaces of the agar plate. Sterile paper disc about 10mm diameter was soaked with the extract and allowed to dry for some minutes. This was placed on the surface of inoculated agar plates. The plates were then incubated for 24hours at 37^oC. After incubation, the diameter zone of inhibition was measured and recorded using millimeter rule. The soap with the highest zone of inhibition was chosen for subsequent experimental work.

RESULTS

In this present study, out of Nineteen different samples analyzed, eight samples showed the presence of *Staphylococcus aureus.*

Identification of the Isolates

Representative of the bacterial strains were subjected to biochemical test.

Table 1: Isolates and their Biochemical test.

Biochemical test / Isolates	Gram staining	Catalase test	Oxidase test	Motility test	Citrate test	
Isolates 1	+	+	-	-	+	-
Isolates 2	+	+	-	-	+	-
Isolates 3	+	+	-	-	+	-
Isolates 4	+	+	-	-	+	-
Isolates 5	+	+	-	-	+	-
Isolates 6	+	+	-	-	+	-
Isolates 7	+	+	-	-	+	-
Isolates 8	+	+	-	-	+	-

Key: + = indicates positive result
- = indicates Negative result.

Table 2 Antibacterial Susceptibility Test
Table 2: Diameter Zone of Inhibition of Test medicated soap and Herbal soaps against different Isolates of *Staphylococcus aureus* strains

		Soap in Discs Isolates (mm)							
Category of soap	**Conc 0.1mg/g**	***Staph 1***	***Staph 2***	***Staph 3***	***Staph 4***	***Staph 5***	***Staph 6***	***Staph 7***	***Staph 8***
Herbal soaps	**HAI**	19	15	17	16	18	16	17	18
	HAII	18	15	Nil	Nil	16	13	Nil	Nil
	HAIII	15	16	Nil	Nil	Nil	Nil	Nil	Nil
Medicated soaps	**MAI**	25	20	25	20	25	16	15	18
	MAII	15	12	25	15	21	18	21	17
	MAIII	12	15	15	15	12	Nil	12	Nil

Key: The Nil indicates no inhibition

Discussion

In this study, eight strains of *Staphylococcus aureus* were isolated from skin of student of Chukwuemeka Oduemegwu Ojukwu University. The justify, that *Staphylococcus aureus* are normal flora of the skin. This justified the findings of Izabela et al., (2015) where in their research successfully isolated *Staphylococcus aureus* from skin and Nasal cavity using skin swab.

Results obtained from the study revealed that most of the studied medicated soaps have higher antimicrobial activity, though to varying degrees as indicated by the inhibition of the growth pattern of the isolates when compared to the herbal soaps as the varied levels of effectiveness by soaps were observed against the isolated skin flora pathogens.

The herbal soap HAI showed high antimicrobial activity on all the *Staphylococcus aureus* isolates as it inhibited all the isolates in the range of 16-19mm, four *Staphylococcus aureus* isolates resisted HAII, while four isolates were inhibited by HAII in the range of 13-18mm. HAIII showed inhibition on only two strains of *Staphylococcus aureus* in the range 15-16mm as other six isolate resisted the soap.

The reasons for the antibacterial activity of these herbal soaps could be traced or link to the present of plant biotic substance and phytochemicals which possess antibacterial activity. While the level of antibacterial resistance observed in the herbal soaps could be due to varying concentrations of the plant biotic ingredient and nature of plant ingredient used. Ike, (2016) also reported the active ingredient in the soap is what distinguishes them in their antimicrobial strength.

The findings of these study also agrees with the work by Varsha (2016) that different herbal soaps studied and produced with Neem showed highest antimicrobial activity against all pathogen studied as exhibited the antibacterial activity with zone of inhibitions of 10.2 mm for *S. aureus*. This level of inhibition could be traced to the presence of *Aloe vere* and Neem leaf in the soap ingredient.

The result of the antibacterial activity study of the medicated soap showed that MAI soap was effective on all the isolate of *Staphylococcus aureus* in the range of (16-25mm). in similar manner MAII soap was effective on all isolate of *Staphylococcus aureus* in the range of (15-21mm). MAIII soap was effective on six isolate of *Staphylococcus aureus* in the range of (12-15mm) as other two isolate resisted the MAIII soap. The result of these study is similar to the finding of Imarenezor *et al*, (2020) which reported that *Staphylococcus aureus* was susceptible to the medicated soap studied at various concentration.

Comparatively the medicated soap showed good antimicrobial activity when compared to herbal soaps. As most medicated soaps have satisfactory antibacterial activity on the *Staphylococcus* isolates. These could be traced to the active ingredients and synthetic chemical compounds used in the formulation of these soaps. The reason for the higher zones of inhibition observed on the side the medicated soap may be attributed to the higher concentration of the antibacterial ingredients used in the soap formulation. The resistance observed on the aspect of MAIII soap by two isolate in an indication of gradual increasing bacterial resistance which is of great research importance as the continuous use of MAIII may be discourage in near future if, the formulation is not improve as the particular strains of *Staphylococcus aureus* are beginning to resist them.

CONCLUSION

From the study, it is clear that medicated soap such as MAI, MAII and MAIII showed better antimicrobial activity when compared to the herbal soap HAIII and HAI. Hence MAIII need to be

improved on as *Staphylococcus aureus* strains are gradually resisting the active antibacterial agent used in formulating the soap.

RECOMMENDATION

In view of the findings of this study it recommended that medicated soaps should be in use by people who are on treatment on skin diseases traced to be caused by *Staphylococcus aureus* as they showed better antimicrobial activity on unlike the Herbal soap studied.

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