ANTIBACTERIAL ACTIVITIES OF ANTISEPTICS AND NON-ANTISEPTIC SOAP ON Staphylococcus aureus and Staphylococcus epidermidis

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ABSTRACT

Antibacterial activity of soap is defined as the ability to either destroy or inhibit the growth of microorganisms. Antibacterial effect of Antiseptic and Non-anticeptic soap against *Staphylococcus aureus* and *Staphylococcus epidermidis* was evaluated. A number of (3) three samples were collected using swab stick from different parts of the body namely ear, nasal and skin using aseptic method to collect the samples. Swabs were streaked on appropriate agar and incubation temperature was done at 37ºC for 24 hours. Agar well diffusion techniques were used. The result of the study showed the highest zone of inhibition in Antiseptic soap (Tura) with 1 and 2mg concentration and inhibition of 1.8 and 2.0mm respectively for *Staphylococcus aureus* while *Staphylococcus epidermidis* revealed the highest zone of inhibition in Antiseptic soap (Safeguard) with 1 and 2mg concentration and inhibition of 1.9 and 1.9mm respectively. Conclusively, Antiseptic soaps Tura and Safeguard based on the findings of this work could be used during medication in cleaning the skin during time of hurt, cut or eczema lesions and other bacterial infection sites, as control for pathogens through bacteriostatic or bactericidal activities. It is therefore recommended that research should be carried out using bioassay guided fractionation to identify, isolate and characterize the bioactive components of the soap.

INTRODUCTION

Antibacterial activity is defined as the ability of a substance to either destroy or inhibit the growth of microorganisms. This can be called as either cidal or static effects respectively. This is significant with respects to the human body in preventing sepsis and skin infections (Higaki *et al.,* 2000). Soap cleanses because molecules are attracted to the fatty part of the anions of the soap solution and are pulled off by dirty surface into water. Antiseptic soaps contain additional ingredients, usually for the treatment of skin disorders (Eckburg *et al.,* 2005).

Antiseptic soaps have germicidal substances like, irgasan, trichlorocarbanlide, (TCC) etc, incorporated into them to enhance their antibacterial activity (Friedman and Wolf, 1996). These germicidal substances are added in a specific amount and their percentages are always stated on the soap case or leaflet which contains the information on how to use the soap for various purposes. Normal microflora is found on the surface of all human skin (Prescott *et al.,* 2008).

The normal skin bacterial flora in human is composed of three major groups of gram-positive bacteria: the coliform bacteria, the micrococci and the staphylococci with only a minor component of gram-negative bacilli (Nobel, 1998). This is because the skin is a comparatively dry habitat, with available water as the major factor controlling growth. Occlusion of the skin is a potent way to increase the number of bacteria on the skin (Breuer *et al.,* 2002).

Staphylococcus species, though a common cause of human infections are found as non-pathogenic microorganisms in human samples. *Staphylococcus aureus* is the most important member of this group (Diekema *et al.,* 2001) and has been associated with different clinical conditions and syndromes (Javid *et al.,* 2006). In Ikegbunam *et al.,* (2013), *Staphylococcus aureus* is an opportunistic pathogen affecting both immunocompetent and immunocompromised individuals frequently resulting in high morbidity and complications which constitutes problems to health. It is a gram-positive, non-spore forming cocci bacterium that is a member of the firmicutes, which are found as normal human microbiota of the skin and nasal cavity. It is the most frequently encountered bacterial species in hospitals (Emmerson, 2004).

The major reservoirs of *Staphylococcus aureus* in hospitals are colonized in infected patients and hospitals workers (Javid *et al.,* 2006). Carriers of *Staphylococcus aureus* and *Staphylococcus epidermidis* are at risk of developing endogenous infections or transmitting infections to health care workers and patients. Its disease manifestation ranges from minor skin infections to life threatening diseases such as folliculitis, furuncle (boil), dermitis (eczema) carbuncle, ulcers, pneumonia sepsis and wound infections. *Staphylococcus aureus* may also cause food poisoning, scalded-skin syndrome and toxic shock syndrome (TSS) through production of toxins.

Statement of the Problems

The human skin is the largest organ in the body, forming the outer surface of the entire body and acts to keep the internal tissues free from infection. It does this by forming a physically protective water proof layer that blocks the entry of bacteria, viruses, fungi and parasites (Grice *et al.,* 2008). Every person has a different complement of friendly bacteria on their skin surface and there can be as many as 180 different species growing there. These include: Staphylococcus *epidermidis, Staph. hominis, Staph. aureus, Micrococcus luteus, Arcanobacterium haemolyticum* and *Propionibacterium acnes.* Other commensals are part of the Corynebacterium group, the Brevibacterium species and the Dermabacter group (Lambers *et al.,* 2009). Transient bacteria may be deposited on the skin surface from environmental sources and cause skin infections. Examples of such bacteria are Pseudomonas aeruginosa (Fluit *et al.,* 2001) and *Staphylococcus aureus* (Higaki *et al.,* 2000).

Some friendly bacteria species are known to normally cover the human skin and are known as the normal flora of the skin. This normal flora protects the skin by covering all the spaces thereby preventing other harmful bacteria species from growing on the body. Wound is defined as a break in integrity of the skin or discontinuity of the skin as a result of breakage (Al-saimary *et al.,* 2013). Wound healing or restoration of skin continuity, a biological process can be accomplished by regeneration, cell proliferation and collage production which can be encouraged by washing the wound surface and other infected skin lesions like atopic dermatitis especially

with antiseptic soap which due to its content of phenolic compounds help in keeping off organisms like Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa away from the sites (Al-saimary *et al.,* 2013). This research is aimed at evaluating the antimicrobial effects of antiseptic and non-antiseptic soap against *Staphylococcus aureus* and *Staphylococcus epidermitis.*

The main objectives of this research include the following:

1. To determine the minimum inhibitory concentration of medicated soap on *Staphylococcus aureus* and *Staphylococcus epidermitis* at different concentration.
2. To determine the minimum inhibitory concentration of non-septic soap on *Staphylococcus aureus* and *Staphylococcus epidermitis* at different concentration.
3. To compare the inhibitory effects of the two extracts

Materials and Methods

Materials:

Nutrient Agar, MSA Mannitol salt Agar, Glass Slide, weighing balance, filter paper, measuring cylinder, conical flask, cotton wool, Bunsen burner, autoclave, hot air oven, petridishes, incubator wire loop, microscope, oil immersion, staining rack, distilled water, swab stick.

Gram stain reagent

Crystal violet, Lugos iodine, alcohol acetone safranin and distilled water.

Biochemical test reagent

Catalase test reagent

Hydrogen peroxide

Coagulase test reagent

Blood plasma.

Sample Collection

A number of (3) three samples were collected using swab stick from different part of the body namely ear, nasal and skin using aseptic method.

Media Preparation

All the media prepared were according to manufacture's instruction. The media used were nutrient Agar. 7grams in to 250mls of distilled water and Mannitol salt Agar (MSA) 27.75grams in to 250ml of distilled water. The media were heat to dissolved for homogeneous mixture, and autoclaved at 121^0c for 15 minute; it was allowed to cool down for at least 30-35^0c before pouring.

Pouring

The media was poured in to a sterilized Petri-dish and allowed to solidified.

Sterility Test

The solidified media was incubated at 37^0c for 24hrs without any sample on it, in other to confirm the sterility of the media.

Innoculation

The sterilized media was inoculated with the swab sample using streak plate method and incubate at 37^0c for 24hrs.

Gram staining

The twenty four hours cultures were observed with different colonies. A sterilized grease free glass slide was used to drop a normal saline, a sterilized wire loop was used to pick a colony and smear with the normal saline, the smear was heat fixed by passing through flame three to four times, and was allowed to air dry.

Crystal violet was used to cover the smear for 2 minutes, it was washed with distilled water, lugos iodine was added for 2 minute and washed with distilled water, alcohol acetate was used to decolorize and washed with distilled water, immediately safranin was used to counter stain the smear and allowed for 1 minute and washed with distilled water, the slide was allowed for air dry. A drop of oil immersion was placed on the stained slide and viewed using 100x objective lens.

Biochemical Tests

Coagulase test

The slide method of Cheesbrough, (2005) was used. A drop of saline on two separate spots was placed on a grease-free slide. Then a speck of growth of the test organism was picked with sterile wire loop and emulsified to form a smear. To one spot, a drop of plasma was added, while to the other a drop of saline was added. The treated mixtures were mixed thoroughly by rocking. Coagulation was an indication of positive test to slide. The presence of clotting indicates positive test for *Staphylococcus aureus*. This test was based on the capability of test organism to produce coagulase enzyme which causes the coagulation of human plasma.

Catalase test

The slide method test of Cheesbrough, (2005) was adopted. This was carried out to determine the ability of the test organism to produce catalase enzyme and degrade hydrogen peroxide (H_2O_2). A drop of 3% hydrogen peroxide was place on a clean glass slide. A speck of growth of each isolate was collected from the medium using wire loop and was emulsified in the drop. A positive test was indicated by the appearance of bubbles of gas (oxygen) indicating free oxygen liberation and used to differentiate between pathogenic and non-pathogenic *Staphylococcus.*

Sub-culture from Nutrient Agar to Mannitol Salt Agar (MSA)

A sterilized wire loop was used to pick a colony from twenty four hours culture and transfer into mannitol salt Agar and incubated at 37^0c for 24hrs.

Sensitivity Testing

A sterilize nutrient Agar media was poured into a sterilize petridishes and allowed to solidify, the (20) plates were labeled with two different isolate, *Staphylococcus aureus* ten plates and *Staphylococcus epidermidis* ten plates.

Mean while each of the ten plates were divided in to five i.e. five for the anti-septics soap and five for non antiseptic soap per each isolate. And also each plates were labeled with four different concentration as follows: original 1^{10}, 5^1, 5^2, and 5^3 respectively.

Transfer of the pure isolate

The twenty plates were smeared with pure isolate of *Staphylococcus aureus* and *Staphylococcus epidermidis* with ten plates each.

Using Agar well diffusion method the media was cork bored using cork borer.

Serial Dilution

Ten test tubes were arrange and labeled with the names of the soaps five antiseptics and five non antiseptics, one gram 1g of each soap was weighed and dissolved in 10ml of distilled water differently in to separate container, it was shaken to dissolve completely.

1g ⟶ 10ml of distilled water serve as original concentration, each of the original concentration were labeled with three different test tubes with 5ml of distilled water each and also indicated on the test tubes 5^1, 5^2, and 5^3. From the original concentration 1ml was pipetted and transfered in to the 1^{st} test tube labelled 5^1 and 1ml was pipette from 1^{st} to the 2^{nd} and from 2^{nd} to the 3^{rd} test tube respectively.

All the ten different type of soaps were diluted into four different concentrations including the original concentration.

Transfer Of the Concentration in To Well

Each of the dilution were transferred in to appropriate labelled soap, and concentration on the plates respectively, it was incubated at 37^0C for 24hrs in other to observe the zone of inhibition.

Results

The biochemical analysis and colony description for the identification of *Staphylococcus aureus* and *Staphylococcus epidermidis* was shown in Table 1. Catalase and coagulase tests were positive for *Staphylococcus aureus* while coagulase was negative for *Staphylococcus epidermidis* as well as catalase being positive for *Staphylococcus epidermidis*.

Table 1: Biochemical Analysis of *Staphylococcus aureus* and *Staphylococcus epidemidis*

Sample	**Media**	**Culture**	**Gram reaction**	**Biochemical tests**			**Bacterial identified**
				Cata	Coa	Hae	
A	NA/MSA	Yielded growth of colony with yellow glittering surface	Gram +	+	+	β	*Staphylococcus aureus*
B	NA/MSA	Yielded growth of colony with yellow glittering surface	Gram +	+	-	ϫ	*Staphylococcus epidermidis*

Key:
NA: Nutrient Agar
MSA: Mannitol Salt Agar
Coa: Coagulase
Cata: Catalase
Hae: Haemolysis

Table 2: Antibacterial activity of Antiseptic and Non Antiseptic soap on *Staphylococcus aureus*

Isolate	Antiseptics soap		Non Antiseptic soap		Control (Antibiotics)	
	Conc (mg)	**Inhibition (mm)**	**Conc (mg)**	**Inhibition (mm)**	**Antibiotics (mg)**	**Inhibition (mm)**
Staphylococcus aureus	Dudu Osun		Imperal		Ofloxacin	3.8
	1	0.0	1	1.0	Cefraxone	4.6
	2	0.0	2	0.0	Amoxacilin	2.6
	3	0.0	3	0.0		
Staphylococcus aureus	Dettol		UMC		Ofloxacin	3.8
	1	1.2	1	0.0	Cefraxone	4.6
	2	0.0	2	0.0	Amoxacilin	2.6
	3	0.0	3	0.0		
Staphylococcus aureus	Tura		Joy		Ofloxacin	3.8
	1	1.8	1	0.0	Cefraxone	4.6
	2	2.0	2	0.0	Amoxacilin	2.6
	3	0.0	3	0.0		
Staphylococcus aureus	Safeguard		EVA		Ofloxacin	3.8
	1	1.5	1	0.0	Cefraxone	4.6
	2	0.0	2	0.0	Amoxacilin	2.6
	3	0.0	3	0.0		
Staphylococcus aureus	Tetmosol		Visita B		Ofloxacin	3.8
	1	1.0	1	0.0	Cefraxone	4.6
	2	1.6	2	0.0	Amoxacilin	2.6
	3	0.0	3	0.0		

Table 2 showed the zone of inhibition of *Staphylococcus aureus* using different concentrations of the antiseptic and non antiseptic soap. The results showed that *Staphylococcus aureus* was sensitive to Dettol at concentration of 1mg with inhibition of 1.2mm, while Tura was also sensitive at concentration of 1 and 2mg with inhibition of 1.8 and 2.0mm respectively. Similarly, *Staphylococcus aureus* was sensitive to antiseptic soap Safeguard at concentration of 1mg with inhibition of 1.5mm while Tetmosol was sensitive at concentration of 1 and 2mg with inhibition of 1.0 and 1.6mm respectively. Non antiseptic soap Imperal leather was sensitive at concentration of 1mg with inhibition of 1.0mm. However, Antiseptic soaps Dudu Osun and Non antiseptic soap such as UMC, Joy, EVA and Visita B were all resistant to the test organisms at varied concentration. The highest zone of inhibition was recorded in Antiseptic soap (Tura) with 1 and 2mg concentration and inhibition of 1.8 and 2.0mm respectively. The controls were all sensitive to *Staphylococcus aureus* at all concentration.

Table 3: Antibacterial activity of Antiseptic and Non Antiseptic soap on *Staphylococcus epidermidis*

Isolate	**Antiseptics soap**		**Non Antiseptic soap**		**Control (Antibiotics)**	
	Conc (mg)	Inhibition (mm)	Conc (mg)	Inhibition (mm)	Antibiotics (mg)	Inhibition (mm)
Staphylococcus epidemidis	Dudu Osun		UMC		Ofloxacin	3.2
	1	1.4	1	1.0	Cefraxone	3.8
	2	0.0	2	0.0	Amoxacilin	2.2
	3	0.0	3	0.0		
Staphylococcus epidemidis	Safeguard		Joy		Ofloxacin	3.2
	1	1.9	1	0.0	Cefraxone	3.8
	2	1.9	2	0.0	Amoxacilin	2.2
	3	0.0	3	0.0		
Staphylococcus epidemidis	Tura		Imperal		Ofloxacin	3.2
	1	0.0	1	0.0	Cefraxone	3.8
	2	0.0	2	0.0	Amoxacilin	2.2
	3	0.0	3	0.0		
Staphylococcus epidemidis	Dettol		Vista B		Ofloxacin	3.2
	1	0.0	1	0.0	Cefraxone	3.8
	2	0.0	2	0.0	Amoxacilin	2.2
	3	0.0	3	0.0		
Staphylococcus epidemidis	Tetmosol		Eva		Ofloxacin	3.2
	1	0.0	1	0.0	Cefraxone	3.8
	2	0.0	2	0.0	Amoxacilin	2.2
	3	0.0	3	0.0		

Table 3 showed the zone of inhibition of *Staphylococcus epidermidis* using different concentrations of the antiseptic and non antiseptic soap. The results showed that *Staphylococcus Epidermidis* was sensitive to Dudu Osun at concentration of 1mg with inhibition of 1.4mm, while Safeguard was sensitive at concentration of 1 and 2mg with inhibition of 1.9 and 1.9mm respectively. The antiseptic soap Tura, Detol and Tetmosol were all resisted by *Staphylococcus epidermidis* at all concentration. Similarly, *Staphylococcus epidermidis* was sensitive to the non antiseptic soap UMC at concentration of 1mg and inhibition of 1.0mm. While others Joy, Imperal Leather, Vista B and Eva soap were all resisted at varying concentration to the tests organisms. The highest zone of inhibition was recorded in Antiseptic soap (Safeguard) with 1 and 2mg concentration and inhibition of 1.9 and 1.9mm respectively. The controls were all sensitive to *Staphylococcus epidermidis* at all concentration.

Discussion

The result of the study showed the highest zone of inhibition in Antiseptic soap (Tura) with 1 and 2mg concentration and inhibition of 1.8 and 2.0mm respectively for *Staphylococcus aureus* while *Staphylococcus epidermidis* revealed the highest zone of inhibition in Antiseptic soap (Safeguard) with 1 and 2mg concentration and inhibition of 1.9 and 1.9mm respectively. Friedman and Wolf, (1996) opined that Antiseptic soaps have germicidal substances like, irgasan, trichlorocarbanlide, (TCC) etc, incorporated into them to enhance their antibacterial activity. The best in antibacterial activity of all the soaps used is Tura and Safeguard exhibiting maximum zone of inhibition for *Staphylococcus aureus* and *Staphylococcus epidermidis* respectively. This could be attributed to its unique formulation of duo combination of irgasan and potassium mercuric iodide. Non antiseptic soap Imperal Leather and UMC primarily are used for its scabicide effect; however, it exhibited moderate antibacterial activity which is attributed to monosulfiram within its formulation.

It is well known that antiseptic soap could be any cleaning soaps to which antimicrobial active ingredients (AAIs) have been added. These chemicals kill bacteria and other microorganisms, though they are not effective at deactivating viruses just like any other kind of soaps. Soaps are intended for reduction of the inoculum sizes of both pathogenic and non-pathogenic microorganisms; the latter include the normal flora. It is routine practice to wash hands prior to eating, after examining a patient and before surgery, in order to remove some potentially harmful transient flora as well as reduce a number of resident floras, which might cause opportunistic infections (Nobel,1998).

The results of the study showed that antiseptic soap Tura and Safeguard, displayed varying degrees of inhibition on the test organisms they 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. The results obtained in this study are in agreement with the work of Ike, (2016) in Aba, Abia State Nigeria who reported that *Staphylococcus aureus* isolated and subcultured were sensitive to the three different antiseptic soaps.

Tura and Safeguard soaps have demonstrated satisfactory antimicrobial effect, particularly in the antibacterial activity. From the results of the study it is shown that there is variability in antimicrobial activity and it is due to difference in antimicrobial active ingredients (AAI) contents, and type of formulations.

Conclusion

Conclusively, Antiseptic soaps; Tura and Safeguard based on the findings of this work could be used during medication in cleaning the skin during time of hurt, cut or eczema lesions and other bacterial infection sites, as control for pathogens through bacteristatic or bactericidal activities.

Recommendations

From the results obtained in this study, it is recommended that:

1. Experiments should be carried out at higher concentrations assess their activity on Multi Drug Resistance Staphylococcus
2. Research should be carried out using bioassay guided fractionation to identify, isolate and characterize the bioactive components of the soap.
3. The ministry of health should make it mandatory that all soap products be subjected to scientific verifications before being used on the skin.

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