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Comparative Efficacy of a Locally Made Soap (*Sabulun Salo*) with Some Conventional Antiseptic Soaps in Checking Bacterial Growth Isolated from Human Skin

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Abstract:

Four different types of antiseptic soaps [Dettol, Delta, Tura and Sabulnsalo (a locally made Soap)] were bought from Gashu'a central market and tested in the Biology Laboratory of Umar Suleiman College of Education Gashu'a. Against bacteria isolated from the skin swab obtained from students of Umar Suleiman College of Education Gashu'a. The data collected was on the zone of bacterial growth inhibition which was measured in millimeter (mm). The data collected were analyzed using Analysis of variance (ANOVA) using the Analytical software 2011 statistics version 8.0 (SXW) and the difference between the means of zone of inhibition of the test soaps against bacterial growth were separated using the least significance difference (LSD) at 1% level of probability ($P < 0.01$). The results showed that all the antiseptic soaps used significantly ($P < 0.01$) inhibited the growth of *Staphylococcus aureus* and *Streptobacilli* species Isolated from the human skin when compared to the untreated (control). The cost-consequence ratio also shows that Sabulun Salo has a relatively high cost-effective potential. However, on the basis of efficacy, the results pointed out that Tura Soap was the most effective followed by Delta soap, then Dettol soap and lastly, Sabulunsalo. Learning that the antiseptic body washes checks skin infection, it is therefore, imperative that government regulates the price of these soaps so that everybody can afford it and most importantly, encourage our local technology in this aspect.

Keywords: Locally made soap (Sabulunsalo), antiseptic soaps and bacterial growth

1. Introduction

Bacteria are the smallest of all the groups of microscopic organisms (this description is appropriate because the bacteria and not the Viruses are the simplest group of organisms that have true cellular nature). Bacteria are described as being ubiquitous, that is they are found everywhere; water basins, soils, living and dead bodies of other organisms, food stuffs and swage (Fatubarin, 2002). A few bacteria cause diseases to animal and plants, a large number lead independent lives and are harmless, while many are directly beneficial to man. The diseases causing ones are referred to as pathogens. The ability of pathogenic bacteria to harm its host depends on its ability to proliferate in the host body and the production of toxins which damage or kill the host cells (Fawole and Oso, 1995). Fortunately for man, some other bacteria also produce certain other chemical called antibiotics which can neutralize the deleterious effect of some pathogenic bacteria (Sylvia 2006). Certain bacteria maintain an initial symbiotic relationship with the human body although this can shift to parasitism depending on the circumstances (Kavanaugh, 2009).

The common microbial flora of skin includes *Corynebacteria*, micrococci, coliform bacteria, staphylococci species, hemolytic *Streptococci* and in the moist regions, fungi including yeasts (Johnson, et al, 2002). The skin bacteria are securely entrenched in the sweat glands, sebaceous glands and folds of the skin such that scrubbing with water and soap cannot totally remove them (Chaudhari, 2016). The need for efficient health care and advancement in technology, made it possible for certain chemical substances to be incorporated into soap to confer them with antiseptic property (Pashak, Grysiuk and Bulyk, 2006).

Antimicrobial activity of any substance is defined as its ability to either kill bacteria or inhibit the growth of bacteria (Food and Drug Administration 2005). Antimicrobial activity is significant with respect to the human body in preventing diseases and skin infections (Aiello, Larson and Levy 2007). Soaps are the disinfectants required in daily practices for hygienic point. Soaps are cleaning agents, which may be liquid, solid, semisolid or powders. 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 (Raiz, Ahmad and Hasnain 2009). Antiseptic soaps are incorporated with specified amount of germicidal substances in addition to the ordinary soap base in order to increase their antibacterial activity. These antiseptic substances impart ability for the soap to kill germs even after it has been used as residual antiseptic substances remain on the skin. It is proved experimentally that antibacterial soaps kill the bacteria at a specific concentration; they also have bacteriostatic activity and can inhibit the growth of bacteria (Muanya 2006). The

soap should have good ingredients which have the ability to kill bacteria but not to damage body tissues. Number of bacteria including Gram Positive and Gram negative are deposited from the environment on the surface of skin and causes skin infection. Examples of these bacteria include *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* (Pashak, Grysiuk and Bulyk, 2006).

Bacteria are necessary to maintain optimum environments in animal and plant bodies and in environmental systems (Cheesbrough, 2001). However, even beneficial species, if they are reproducing at an uncontrolled rate, are potentially harmful or destructive to their environment (Fluit, Schmitz and Verhoef, 2001). In addition, several species of bacteria and fungi are known to be pathogenic, that is, to cause disease in animals and plants. Their growth must be controlled. An antimicrobial agent is a chemical that either inhibits or kills microbes. Therefore, this study determined the efficiency of a locally made soap (*Sabulunsalo*) compared with some commonly prescribed antiseptic soaps against bacteria isolated from human skin.

Soaps are only mildly microbicidal. Their use aids in the mechanical removal of microorganisms by breaking up the oily film on the skin (emulsification) and reducing the surface tension of water so it spreads and penetrates more readily. Some cosmetic Soap contains added antiseptics to increase antimicrobial activity. Soaps play an important role in removing and killing bacteria. Although fats and oils are general ingredient of soaps but some detergents are added to enhance the antibacterial activities of soaps (Riaz, Ahmad and Hasnain, 2009).

According to Chaudhari, 2016 antibacterial soaps can remove 65 to 85% bacteria from human skin. Bacteria are very diverse and present everywhere such as in soil, water, sewage, standing water and even in human body. Bacteria's that attacks on human body is of great importance with reference to health (Johnson et al., 2002). Transient bacteria are deposited on the skin surface from environmental sources and causes skin infections. Examples of such bacteria are *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Fluit et al., 2001). The importance of hand washing is more crucial when it is associated to health care workers because of possible cross contamination of bacteria that may be pathogenic or opportunistic (Cheesbrough, 2001). Studies have shown that soaps containing antimicrobial active ingredients remove more bacteria as compared to plain soap (Aiello, Larson, and Levy, 2007). To investigate the antibacterial efficiency of different brands of soaps, we isolate bacteria from different environments and human skin. Identification of bacteria was done by biochemical tests (Cheesbrough, 2001) and by using analytical profile index. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against these bacteria were determined. Identification of bacterial species that are most resistant to the antibacterial soaps of daily use was made. The present studies were aimed to determine the bactericidal activity/efficacy of conventional antiseptic as well as local antiseptic soaps and to determine, the efficiency of a locally made soap (*Sabulunsalo*) compare with some commonly prescribed antiseptic soaps against bacteria isolated from human skin.

2. Material and Method

2.1. Materials Used

The Materials used include; Autoclave, Electro Thermal Incubator, Hot air Oven, Electro thermostatic water bath, Weighing balance, Microscope (Electric type), Glass slides, Wire loops, Petri dishes, Swab sticks, Spatulas, Filter papers, Cotton wool, Conical flasks (different sizes), Bunsen burner, Boiling test-tubes, Tripod stands, Wire gauze, Vacutainer tubes, Ethylene Diamine Tetra acetic Acid (EDTA), Staining racks, Refrigerators, Other are; Nutrient broth (powder), Nutrient agar (powder), Four(4) types of antiseptic soaps (Tura, Delta, Dettol and a locally made soap *Sabulunsalo*), Grams staining reagents, Biochemical analysis reagents, and other accessories.

2.2. Sample Collection

Four types of antiseptic soaps (Delta, Tura, Dettol and *Sabulunsalo*) were bought from Gashua central market. Similarly, ten skin swabs were obtained from six male and four female students of USCOEGA by using sterilized swab sticks. The swab sticks were then inoculated directly on a well dry blood agar figures then incubated at 37°C for 24 hours. The growth from each plate was sub-culture on a fresh-dried blood agar by using streaked plate method. The figures were labeled and incubate at 37°C for 24 hours. Growth from various streaked blood agar were used to obtain a pure culture of the bacteria and store in a refrigerator.

2.2.1. Preparation of Media Culture-Figures

2.2.1.1. Nutrient Agar Plate

Aseptically about 7.0g of nutrient agar powder was weighed by using weighing balance and transferred into a conical flask. It was dissolved in 250ml of distilled water and stirred until it dissolved completely. Then, it was sterilized in the Autoclave at 121°C for 15 minutes. Hence, it was allowed to cool down to about 45°C, and then aseptically it was poured into well dried sterilized petri dishes and allowed solidify.

2.2.1.2. Nutrient Broth

Aseptically, about 3.5g of nutrient broth powder was weighed on a weighing balance and dissolved in 250ml distilled water in a conical flask. It was the sterilized in the Autoclave at 121°C for 15 minutes and allowed to cool and stored in refrigerator.

2.2.1.3. Blood Agar Plate

About 7.0g of nutrient agar powder was aseptically weighed and dissolved in 250ml distilled water in a conical flask. It was then sterilized by Autoclave at 121°C for 15 minutes and allowed to cool to about 45°C. Aseptically about 12.0ml of Sheep blood was added and stirred very well until it forms a homogeneous solution. It was then poured into well dried sterilized petri dishes and allowed to solidify then store in a refrigerator.

2.3. Preparation for Soap Solutions

Four (4) sterilized boiling test-tubes were labeled A, B, C, and D (where A stand for Dettol soap, B for Delta, C for Tura soap and D for *Sabulunsalo*). About 15ml of distilled water was measured and poured into each labeled test-tube and aseptically, 0.25g of bacteriological agar powder was weighed and placed into test-tubes containing the distilled water, 0.5g of each of the soap type was added into the corresponding test tubes and then shaken very well and autoclaved at 121°C for 15 minutes.

2.4. Procedure for Testing Soap Samples

The procedure employed for determining the antimicrobial activity of the test soaps were the ditch (well) method and the disc method.

Several appropriate size (diameter) of filter paper was obtained by using punch labeled A, B, C, and D and packed into different paper and sterilized in Oven at 160°C for 1hour. Aseptically, these discs were placed on four different watch glasses. The test-tubes containing prepared soap solutions were placed in water bath for 20minutes in order to dissolve the agar. After melting, the soap solution was then poured on to the corresponding watch glass and allowed to dry in oven.

The ditch (well) was prepared by using a well dried nutrient agar plate; four (4) wells (ditches) were cut on the agar media. The ditches are then labeled from underneath the figures, with the names of the test soaps samples (that is A, B, C and D) aseptically, melted soap solution was then poured into corresponding ditches. The figures were then allowed to stay at room temperature to solidify.

Aseptically, about 15ml of sterilized nutrient broth was poured in each (2) dried sterilized test-tube and inoculated with pure culture (that is inoculums from sub-cultured blood agar plate) using sterilized wire loop and labeled appropriately and then incubated at 37°C for 24 hours. A loopful of the over-night broth cultured was transferred to the center of ditch figures by using sterilized wire loop and carpet method of inoculation was made with the help of sterilized swab Stick and then inoculated at 37°C for 24 hrs. The procedure of carpet method of inoculation was applied on well dried plain nutrient agar figures. Aseptically a disc of each test soap was placed at a choose point with a control disc labeled O at the center, which contained only nutrient agar then incubated at 37°C for 24 hrs.

2.4.1. Tests Carried Out

2.4.1.1. Gram Staining

A drop of sterilized distilled water was placed at the center of clean glass slide, by using sterilized wire loop; a colony from the sub-culture blood agar was emulsified (thin smear). Dried in the air and fixed by gently passing over Bunsen flame 3 times. Then the slide was placed on a Staining rack. The film was stained with methyl violet for 30 seconds. The stain was poured off holding the slide at angle down-ward. The film was treated with iodine solution and allowed to stain for 60 seconds. The slide was washed with water and counter-stained with neutral red for 60 seconds. The slide was washed with water and allowed to air dried. It was examined under the electric microscope using oil immersion objective.

2.4.1.2. Coagulase Test

The colony of the bacteria on the streaked blood agar figures emulsified on a clean slide with normal saline, a drop of plasma was added and placed in incubator at 37 c for 3 hrs.

3. Result and Discussion

Treatment	Mean* Diameter of zone of inhibition (mm) for the test Soaps against <i>Staph. aureus</i>	Mean* Diameter of zone of inhibition (mm) for the test Soaps against <i>Streptobacilli species</i>
Control	0.00d	0.00d
Dettol Soap	3.00b	2.00c
Delta soap	3.60ab	3.00b
Tura soap	4.63a	4.50a
Local soap (<i>sabulunsalo</i>)	2.40c	1.80c
Mean	2.73	2.26
S.E	0.32	0.07
I.S.D.	1.54	0.33

Table 1: Mean Diameter of Zone of Inhibition for the Test Organisms by the Test Soaps [Ditch (Well) Method]
Values with Identical Letter(S) On the Same Column Are Not Significantly Different at 1% Level of Probability
* Mean of Three (3) Replicate

Table 1 shows the mean diameter of zone of inhibition for *Staphylococcus aureus* and Gram-positive rods in chain (*Streptobacilli species*) by the test soaps using ditch method. The result showed that all the soaps inhibited bacterial growth isolated from human skin significantly $P < 0.01$ when compared to the control. However, the result revealed that Dettol, Delta and *Sabulunsalo* are not significantly ($P \leq 0.01$) different from Dettol and *Sabulunsalo*. Delta and Tura were also not significantly ($P \leq 0.01$) different from each other in their efficacy (inhibiting bacterial growth) against *Staphylococcus aureus* for *Streptobacilli species* all the soaps tested were effective ($P \leq 0.01$) in checking the bacterial growth. However, Dettol and *Sabulun Salo* were not significantly different from each other in this respect. It is however noted that, Tura Soap proved to be the most effective followed by Delta soap and the local soap (*SabulunSalo*): the least effective. Plate's 1a and b showed the zone of growth inhibition of *Staphylococcus aureus* and *Streptobacilli species* respectively by the test soaps.

Figure 1: The Zone of Growth Inhibition of *Staphylococcus Aureus* Ditch Method

Figure 2: The Zone of Growth Inhibition of Streptobacilli Species Ditch Method

Treatment	Mean* Diameter of zone of Inhibition (mm) for the test Soaps against <i>Staph. aureus</i>	Mean* Diameter of zone of inhibition (mm) for the test Soaps against <i>Streptobacilli species</i>
Control	0.00d	0.00d
Dettol Soap	2.30c	2.20c
Delta soap	3.73b	3.40b
Tura soap	5.40a	4.80a
Local soap (<i>sabulunsalo</i>)	2.10c	2.00c
Mean	2.70	2.48
S.E	0.06	0.05
I.S.D.	0.28	0.25

Table 2: Mean Diameter of Zone of Inhibition for the Test Organisms by the Test Soaps (Disc Method)

Values with Identical Letter(S) on the Same Column Are Not Significantly Different at 1% Level of Probability
* Mean of Three (3) Replicates

Table 2 shows the mean diameter of zone of inhibition for *Staphylococcus aureus* and *Streptobacilli species* by the test-soaps using disc method. The result shows that all the Soaps tested were significantly ($p \leq 0.01$) effective in inhibiting bacterial growth isolated from human Skin when compared to control. It also revealed that, Tura was more effective followed by Delta and the Dettol and *Sabulunsalo* against both *Staphylococcus aureus* and *Streptobacilli species*. However, Tura soap proved to be significantly ($p \leq 0.01$) more effective than delta soap in checking the growth of *Staphylococcus aureus* and *Streptobacilli species*. The result also shows that, even though, Dettol soap was more effective than the local soap, they are not significantly ($p \leq 0.01$) different from each other in inhibiting the growth of *Staphylococcus aureus* and *Streptobacilli species*. Figures 2a and b showed the zone of growth inhibition of *Staphylococcus aureus* and *Streptobacilli species* respectively by the test soaps.

Figure 3: The Zone of Growth Inhibition of *Staphylococcus Aureus* Disc Method

Figure 4: The Zone of Growth Inhibition of *Streptobacilli* species Disc Method

Soap	Price per unit*(₹/g)	Average zone of Inhibition of test organisms	Cost-consequence Ratio
Dettol	2.14	2.38	0.90
Delta	1.18	3.43	0.34
Tura	2.00	4.83	0.41
Sabulunsalo	0.88	2.08	0.42

Table 3: Cost-Efficiency Analysis of Test Soaps Vis-À-Vis Zone of Growth *Staphylococcus Aureus* and *Streptobacilli* Species
Unit* Refers to Igram

The cost-consequence ratio as derived from the cost-efficiency analysis presented in Table 3 shows that it is more cost-effective to use Dettol soap followed by the locally made soap (*Sabulunsalo*) while Delta proved to be the least cost-effective.

4. Discussion

The research study revealed that, antiseptic soaps tested were effective in inhibiting growth of bacteria isolated from human skin. This finding agrees with Food and Drug Administration 2005, Aiello, Larson and Levy 2007 and Chaudhari, 2016; who reported that regular use of antiseptic soaps reduce body odor by preventing bacterial decomposition of organic material in sweat due to their growth and multiplication activities. Also the research showed

that Tura soap was found to be the most effective against *Staphylococcus aureus* and *Streptobacilli species* isolated from human skin followed by Delta soap then Dettol and lastly *Sabulunsalo* by considering their mean diameter zone of inhibition as shown in table 1 and 2. This may be attributed to the fact that, the active ingredients of Tura soap are; Tricloson, Allontoci, Vitamin E. Sodium Tallowate, sodium palm kernelate, Aqua perfume, I 12940 (pigment Red 5), (I 77260 (carbon Black), CI 74160 (Pigment Blue 15). For Delta soap active ingredients are; sodium palmate, sodium palm kernelate, stabilizer, Trichlorocarbanilide aqua, perfume, salauvant, CI 77891. While for Dettol soap active ingredients are; soap base, water glycerin, menthol, color, fragrance, Antibacterial/Deodorant agents, trichlococarbanilide 0.6% w/w and, Sabulunsalo is made from the following materials; potash, lemon, palm oil and trona. Johnson, et al, 2002 and Riaz, Ahmad and Hasnain 2009, reported that, the number of bacteria on skin depends on the concentration of antibacterial agents present on the antiseptic soap used. In view of this, Dettol soap and *Sabulunsalo* may be more effective if their active ingredients are reviewed.

In addition to that, the two methods (Ditch and Disc) employed for testing the antimicrobial activities of these antiseptic soaps were effective for the bacterial isolates at 1% level of probability ($p < 0.01$) compared to the control.

5. Conclusion

Four different types of antiseptic soaps (Dettol, Delta, Tura and *Sabulunsalo*) were tested against two species of bacterial isolates obtained from the skin swabs of some students of Umar Suleiman College of Education, Gashu'a. It was found out that, although Tura soap has a wider zone of inhibition the other soaps tested were also significantly ($p \leq 0.01$) against bacteria from human skin when compared with the control. The cost-effective analysis shows that Dettol > *Sabulunsalo* > Tura > Delta in that order. There is need for more work to be done with the aim of determining active ingredients of the locally made soap (*Sabulunsalo*) with the view to improving it to be able to compare with other antiseptic soaps in the Market.

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Appendix

Source	DF	SS	MS	F	P
Plates	2	0.7093	0.3569		
Treatments	4	36.0427	9.01067	28.64	0.0001
Error	8	2.5173	0.31467		
Total	14	39.2693			

Table 3: ANOVA of Mean Diameter Zone of Inhibition (Mm) for the Test Soaps Against *Staphylococcus Aureus* (Ditch Method)
Grand Means 2.7267 CV 2.057

Source	DF	SS	MS	F	P
Figures	2	0.3240	0.16200		
Treatments	4	32.8560	8.21400	566.48	0.000
Error	8	2.1160	0.01450		
Total	14	33.2960			

Table 4: ANOVA of Mean Diameter Zone of Inhibition (Mm) for the Test Soaps against *Streptobacilli Species* (Ditch Method)
Grand Mean 2.2600 CV 5.33

Source	DF	SS	MS	F	P
Figures	2	0.1853	0.0927		
Treatments	4	48.5027	12.1257	1192.69	0.0000
Error	8	0.0813	0.0102		
Total	14	48.7693			

Table 5: ANOVA of Mean Diameter Zone of Inhibition (Mm) for the Test Soaps Against *Staphylococcus Aureus* (Disc Method)
Grand Mean 2.7067 CV 3.3

Source	DF	SS	MS	F	P
Figures	2	0.1960	0.09800		
Treatments	4	38.0640	9.51600	1189.50	0.000
Error	8	0.0640	0.00800		
Total	14	38.3240			

Table 6: ANOVA of Mean Diameter Zone of Inhibition (Mm) for the Test Soaps against *Streptobacilli Species* (Disc Method)
Grand Mean 2.4800 CV 3.61

S/No.	Soap	Quantity (g)	Amount (N)
1	Dettol	70	150
2	Delta	85	100
3	Tura	75	150
4	Sabulunsalo	8	7

Table 7: Price of Soaps in Relation to Specific Quantities

Active Ingredients of Test Soap

- A (Dettol)-Ingredients; Soap Base, Water, Glycerin, Menthol, Color, Fragrance, Antibacterial/Deodorant Agent; Trichlorocarbonilide 0.6% W/W
- B (Delta)-Ingredients; Sodium Palmate, Sodium Palmkernelate, Stabilizers,

- Trichlorocarbonilide, Aqua, Perfume, Colorant C.I. 7791
- C (Tura)-Ingredients triclosan, Allantain, Vitamin E, Sodium Tallowate, Sodium
- Palmkernlate, Aqua, Perfume, Cliza40 (Pigment Red 5), CI77266
- (Carbon Black), CI74160 (Pigment Blue 15).
- D. [*Sabulunsalo*: (Local Soap)]; Sabulunsalo was prepared from: Potash 'Toka',
- Lemon, Palm oil, Trona (Kaanwa).