



# Antibacterial Activities of Some Medicated and Herbal Soap against *Staphylococcus aureus* and *Escherichia coli* isolated from the skin

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## ABSTRACT

The *in vitro* antimicrobial activity of different medicated soaps labelled A and B and Herbal Soap C and D was evaluated using agar well diffusion method against *Staphylococcus aureus* and *Escherichia coli*. One hundred skin samples were obtained from the face, neck and armpit of healthy individuals by means of sterile swabs. Samples were cultured on Nutrient agar, screened using Mueller Hinton chrome agar and confirmed using standard micro-biological procedures. Results showed that a total of fifty (50) bacterial isolates were recovered. *Staphylococcus aureus* was the most prevalent (35), followed by *Escherichia coli* (15). The susceptibility of the bacterial isolates to herbal and medicated soaps were evaluated by determining their zones of inhibition using agar well diffusion method. The results indicated that both herbal soaps showed their effectiveness at different diameter of zones of inhibition. Soap B had the best activity (28.33±1.53mm mean diameter) for *E. coli* and (26.00±1.00mm mean diameter) for *S. aureus* followed by soap D (28.00±1.00mm mean diameter) for *E. coli* and (23.33±1.53 mean diameter) for *S. aureus*. Soap C also exhibited its effectiveness with (17.67±0.57mm mean diameter) for *E. coli* and (26.00±1.00mm mean diameter) for *S. aureus* while soap A had no activity against the test organisms. This study revealed that herbal soaps and medicated soaps are effective in preventing and treating skin infections caused by *S. aureus* and *E. coli*.

## INTRODUCTION

The skin is the outer covering of the body of humans. It guards the internal organs, bones and muscles (Gary and William, 2007). The Skin protects the body against disease- causing microbes and excessive loss of water (Madison, 2003; Proksch *et al.*, 2008). Protecting the skin from sun, eating nutritionally rich diet, and managing stress are good skin care practices (Mayo clinic staff, 2018). Microorganisms known as skin flora reside on the skin. (Grice *et al.*, 2009; Pappas, 2009). Skin flora prevents disease causing organisms from colonizing the surface of the skin (Cogen *et al.*, 2008).

It has been reported in literature that resident microbes have the ability to cause skin diseases; enter the blood system, and create diseases that can be life-threatening, particularly in immune-compromised individuals (Cogen *et al.*, 2008). *Staphylococcus aureus* and aerobic streptococci are the most common causative agent of skin diseases (Fung *et al.*, 2003; Gach *et al.*, 2002; Sharma *et al.*, 2001; Stulberg *et al.*, 2002). *E. coli* is also involved (Afifi *et al.*, 2008; Corredoira *et al.*, 1994; Fraser *et al.*, 2006; Rodgers *et al.*, 2000 Tourmousoglou *et al.*, 2008). Antifungal and antibacterial agent are used for the treatment of skin diseases. In developing countries, herbal remedies for skin care are prepared from

different parts of plant such as root, leaves, fruit, sap or bark (Ferro *et al.*, 2003; Kareru *et al.*, 2008; Mukherjee and Suresh 2000). They are topically applied in the form of lotion, cream, soaps, gel, sap, ointment or solvent extract, (Esimone *et al.*, 2008; Nebedum *et al.*, 2009; Semkina, 2005). Soaps are common medium for applying these medicinal plants (Ahmed *et al.*, 2005; Ajaiyeoba *et al.*, 2003; Eje *et al.*, 2009; Kareru *et al.*, 2010). Different medicated and herbal soap have been recommended in preventing and treating skin diseases. This study will provide information on the antibacterial activities of some medicated and herbal soaps.

### 3. MATERIALS AND METHOD

#### 3.1 Sampling

The soaps used were purchased from a standard supermarket in Yenagoa, Bayelsa state, Nigeria. Their batch numbers, registration numbers, and the presence or absence of manufacturer's seal were noted. A total of 100 samples were collected. Samples were obtained by collecting skin swab from the face, neck and armpit of healthy volunteers. Each swab was replaced in its package and transferred to the pharmaceutical microbiology laboratory for examination.

#### 3.2 Growth Media

Nutrient broth, Mueller Hinton agar, Mannitol salt agar, Nutrient agar, Peptone water, Eosin methylene blue agar.

#### 3.3 Preparation of Growth Media

Media were prepared under aseptic conditions according to the manufacturer's instruction and stored under appropriate condition.

#### 3.4 Isolation and Identification of Isolates

Nutrient agar plates were inoculated with skin swab samples, incubated at 37<sup>0</sup> C for 24 hours. Pure isolates were inoculated into nutrient broth and nutrient agar slants. Identification methods include growing the isolates on Eosin methylene blue agar and mannitol salt agar. The bacterial isolates were identified by morphological and biochemical methods.

##### 3.4.1 Gram Staining

A smear made on a clean glass slide was air dried and fixed over a gentle flame. Few drops of crystal violet ink were added to the fixed smear, allowed to stand for 60 seconds and rinsed with water. Few drops of Grams iodine were added, left for 60 seconds and rinsed off with water. The smear was decolorized with 70% ethanol, washed with clean water, counter stained with safranin for 1 minute, carefully rinsed with water, air dried and observed under a light microscope using x100 oil immersion objective lens. The organism which stained purple were Gram Positive Cocci while those that stained red were Gram negative.

**Table 1: Ingredients of soaps tested for antimicrobial activity**

S/N	Soap	Ingredients as listed on packages
1	A	Soap base, water, fragrance, colour, talc, silicon, emulsion, antibacterial agent, chloroxylenol 0.3% w/w, total fatty matter NLT 65% w/w
2	C	Coconut palm oil, sodium hydroxide solution, papaya extract, Q10+Vitamin C and E + AHA
3	D	Palm kernel oil, osun(camwood), cocoa pod ash, aloe vera, lemon juice, fragrance, water, pure honey, lime juice, palm bunch ash, shea butter,
4	B	Soap base, monosulfiram B.P 5%w/w, citronella oil

##### 3.4.2 Indole Test

Tryptophan broth was inoculated with overnight culture of test organism and incubated at 37 °C for 24 hours – 48 hours. 0.5ml Kovac's reagent was added to the broth culture. Pink colored ring indicated a positive result.

##### 3.4.3 Catalase Test

Bacterial isolates were placed on clean glass slide; a drop of hydrogen peroxide was added to the isolates. Production of gas bubbles indicated that the tested bacterial isolates were catalase positive.

### 3.4.4 Coagulase Test

Drops of sterile water were placed on a slide and emulsified with the test organism using a wire loop. A drop of plasma was added to the suspension, and mixed gently for about 10 seconds. The presence of agglutination showed coagulase positive.

### 3.5 Inoculum Preparation

Cooled freshly prepared nutrient broth in McCartney bottles were inoculated and incubated at 37 °C for 24 hours. Dilution of bacterial inoculum was done with sterile water to match the turbidity level adjusted to 0.5 McFarland's standard prepared.

### 3.6 In vitro antibacterial sensitivity testing

#### 3.6.1 Preparation of soaps

The soaps were scraped with sterile blade. Each soap was weighed and dissolved in appropriate milliliters of distilled water to give different concentrations of 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml, 31.25mg/ml. The soaps were dissolved such that no foam was produced to form a stock solution. 100uL of each concentration was used in the experiment. Stock solution was placed in well-sealed containers and stored in the refrigerator.

#### 3.6.2 Antimicrobial assay

The antimicrobial activity of different concentrations of the soaps was determined by agar well diffusion method. 20ml of Mueller Hinton agar was poured on petri dish and allowed to solidify. Agar surface of each plates was streaked by a sterile swab with the isolates. Agar plates were punched with a sterile 8mm cork-borer centrally and five wells at equidistance in each of the plates. 100ul of each concentration of soap was poured with micropipette into the wells. The same amount of sterile distilled water was introduced into the first well as control, the plates were allowed to dry for 30 minutes. The plates were incubated at 37°C for 48 hours. The antimicrobial activity was evaluated by measuring the diameter of zones of inhibition in mm (Abbas *et al.*, 2016).

### 3.7 Statistical analysis

The data obtained were analyzed using IBM SPSS version 23

## 4. RESULTS

The susceptibility testing for **soap B** against *S. aureus* showed that the zone of inhibition ranges from 18.00±1.00mm to 28.33±1.53mm. The highest zone of inhibition was observed at 500mg/ml(28.33±1.53mm) while the lowest zone of inhibition was observed at 31.25mg/ml (18.00±1.00mm). For *E. coli*, the zone of inhibition ranges from 19.00±1.00mm to 26.00±1.00mm. The highest zone of inhibition occurred at 500mg/ml (26.00±1.00mm) and the lowest zone of inhibition was observed at 31.25mg/ml (19.00±1.00mm). (Table 2) There was no activity for **soap A** Against *S. aureus* and *E. coli* (Table 3). The susceptibility testing for **soap D** against *S. aureus* showed that the zone of inhibition ranges from 11.00±1.00mm to 28.00±1.00mm. The highest zone of inhibition was observed at 500mg/ml(28.00±1.00mm) while the lowest zone of inhibition was observed at 31.25mg/ml (11.00±1.00mm). For *E. coli*, the zone of inhibition ranges from 19.00±1.00mm to 26.00±1.00mm.

The highest zone of inhibition occurred at 500mg/ml (23.33±1.53mm) and the lowest zone of inhibition was observed at 31.25mg/ml (15.00±1.00mm). Susceptibility testing for **soap C** against *S. aureus* showed that the zone of inhibition ranges from 7.67±2.08mm to 17.67±0.57mm. The highest zone of inhibition was observed at 500mg/ml(17.67±0.57mm) while the lowest zone of inhibition was observed at 31.25mg/ml (7.67±2.08mm). For *E. coli*, the zone of inhibition ranges from 18.00±1.00mm to 26.00±1.00mm. The highest zone of inhibition occurred at 500mg/ml (26.00±1.00mm) and the lowest zone of inhibition was observed at 31.25mg/ml (18.00±1.00mm). However, **soap D** herbal soap was more effective than **soap C** (Table 4).

**Table 2: Results of antimicrobial susceptibility using Soap B**

	500 (mg/ml)	250 (mg/ml)	125 (mg/ml)	62.50 (mg/ml)	31.25 (mg/ml)
<i>S. aureus</i>	28.33±1.53c	21.00±1.00b	19.67±1.15b	19.33±1.15b	18.00±1.00b
<i>E. coli</i>	26.00±1.00b	21.00±1.00b	20.67±1.15b	20.00±1.00b	19.00±1.00b
Control	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a

Data is zone of inhibition (mm) expressed as Mean Standard Deviation, differences in alphabetical subscript along the column indicates significant difference (Version 23, IBM SPSS). >7mm is susceptible, <7mm is resistant.

**Table 3: Results of antimicrobial susceptibility using Soap A**

	500 (mg/ml)	250 (mg/ml)	125 (mg/ml)	62.50 (mg/ml)	31.25 (mg/ml)
<i>S. aureus</i>	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
<i>E. coli</i>	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
Control	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a

Data is zone of inhibition (mm) expressed as Mean± Standard Deviation, differences in alphabetical subscript along the column indicates significant difference (Version 23, IBM SPSS). >7mm is susceptible, <7mm is resistant

**Table 4: Results of antimicrobial susceptibility using Herbal Soaps**

Test Organisms	500 (mg/ml)	250 (mg/ml)	125 (mg/ml)	62.50 (mg/ml)	31.25 (mg/ml)
Soap D <i>S. aureus</i>	28.00±1.00e	19.00±1.00c	17.00±1.00c	15.33±1.53c	11.00±1.00c
<i>E. coli</i>	23.33±1.53c	19.67±0.58c	18.00±1.00c	17.33±1.15d	15.00±1.00d
Soap C <i>S. aureus</i>	17.67±0.57b	17.00±1.00b	14.00±1.00b	13.00±1.00b	7.67±2.08b
<i>E. coli</i>	26.00±1.00d	22.67±1.52d	21.00±1.00d	19.67±0.58e	18.00±1.00e
Control	0.00±1.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a

Data is zone of inhibition (mm) expressed as Mean± Standard Deviation, differences in alphabetical subscript along the column indicates significant difference (Version 23, IBM SPSS). >7mm is susceptible, <7mm is resistant.

## 5. DISCUSSION

The present research was carried out to determine the antimicrobial efficacy of medicated soaps (A and B) and herbal soaps (C and D) against skin micro flora isolates, *S. aureus* and *E. coli*. The active ingredient of soap A is Chloroxylenols while monosulfiram is the active ingredient for soap B. Herbal soap C has papaya extract, collagen, and coconut palm oil as active ingredient while soap D has camwood, aloe Vera, cocoa pod ash, lemon juice and shea butter as active ingredient. Results obtained from the experiment revealed that most of the studied antiseptic and herbal soaps had antimicrobial properties, though to a varying degree. Except soap A which showed no activity.

Soap B emerged as the most effective medicated soap, based on the diameter of the zone of inhibition 28.33±1.53mm diameter for *S. aureus* and 26.00±1.00mm mean diameter for *E. coli*. Soap A showed no activity in the experiment carried out. This observation is different from studies by Varsha, 2016 where there was activity detected for soap with similar active ingredient of soap A against the test organism (*S. aureus* and *E. coli*). With respect to the herbal soap, C and D had appreciable antimicrobial activity. With soap D having 28.00±1.00mm diameter for *S. aureus* and 23.33±1.53mm diameter for *E. coli*. This observation corresponds to the study by Pius *et al.*, 2017 which showed activity for soap that has similar active ingredient with soap D against test organism *E. coli* with 28mm diameter zone of inhibition. Soap C had 17.67±0.57mm mean diameter for *S. aureus* and 26.00±1.00mm mean diameter for *E. coli*.

## CONCLUSION

All soaps used for the research showed antibacterial activities, except medicated soap A. Medicated soap B was the most effective followed by herbal soap D. It is recommended that soaps with active ingredients similar to soap B, be the first choice of soap to be used. To this end, medicated and herbal soaps can be used to prevent and treat infections of the skin.

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