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Antibacterial Activity of Soaps Indigenously Made in Gombe Metropolis, Nigeria

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Abstract

As part of Federal Government policy on Small Medium Enterprises in Nigeria, a lot of small scale businesses have sprung up including soap making industries using indigenous contents. The ability of indigenously manufactured soaps to remove germs and dirt is paramount. An *in vitro* evaluation of antibacterial activity of twelve randomly collected indigenously made soaps in Gombe metropolis, Nigeria was conducted using agar well diffusion method against strains of reference microbes viz; *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis and Klebsiella pneumonia* being human skin bacteria, followed by time kill kinetic assay to determine the pharmaco-dynamics of active soaps against susceptible test organisms. The results obtained show that six of the soaps exhibited antibacterial activity with varying degree of zones of inhibition. *S. aureus* was the most susceptible amongst the organisms while *E. coli and P. aeruginosa* were the least susceptible microbes. The time kill kinetic assay shows that the bactericidal effect of the soaps is dose and contact time dependent as the susceptible organisms were eliminated after 8 h exposure. The antibacterial activities exhibited by these soaps suggest them as potential candidate in bio-prospecting for antibacterial.

Keywords: Liquid Soaps, Microorganisms, Solid Soaps, Time Kill Kinetics, MIC

1. Introduction

Soaps are cleaning agents, which may be liquid, solid or semisolid. Soaps are used to remove dirt, including dust, microorganisms, stains and bad smells in order to maintain health, beauty and remove bad odour from the body or inanimate object, including clothes. Soap may be defined as a chemical compound resulting from the interaction of fatty acids, oils and salt[8,9].

Cleansing agents have been used around us for a long time and among them soap, liquid hand-wash, detergent, etc., are noteworthy. Antibacterial soaps have been used to improve personal hygiene for generations. The antibacterial soaps can clean and remove 65% to 85% bacteria from human skin[18]. Bacteria are very sundry and diverse and can be found in water, soil, sewage, on human body and are of great importance with reference to health[20]. In the year 1961, the U.S Public Health Service Recommendation mentioned that personnel should wash their hands with soap for one to two minutes before and after client contact. Hand washing is very important and crucial when it is related to health care workers because of possible and probable cross contaminating of bacteria that may be pathogenic or opportunistic[21]. Hygiene of hands and prevention of infection through the use of antibacterial liquid hand-wash has been well recognized. There are many and a large number of chemical compounds that have the potential to inhibit the growth, contamination and metabolism of microorganisms or kill them. The quantity and number of chemicals are vast and probably at least 10,000 and among them 1,000 chemicals are generally and commonly used in hospitals and homes[14]. The important and significant groups of chemicals that help to destroy microorganisms are phenols, soaps, detergents, ammonia compounds,

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chlorine, alcohols, heavy metals, acids etc[14]. Antisepsis, sanitization, disinfection, decontamination, sterilization and so on are a few terms that tell the process of cleaning by any cleansing agents. Various and several cleansing agents are available in the markets that are found in various forms and in different formulations. Trichlorocarbanilide, triclosan and P-chloroin- xylenol (PCMX/Chloroxylenol) are the mostly used antibacterial in medicated soaps. Actually, they are present only at preservation level unless the product is properly marked as antibacterial, antiseptic or germicidal[14]. Washing, scrubbing our body or hands with soaps is the first line of defense against bacteria and other pathogens that can affect us with flu, skin infection and even deadly communicable diseases[11]. Usually, most of the people believe that an antimicrobial portion of soaps is effective at preventing communicable diseases. It is to be noted that many researchers have reported that high use of antimicrobial chemicals can have the reverse effect of spreading diseases and infections instead of preventing them[20]. Antimicrobial resistance and rendering an individual more vulnerable to more microbial attack can also result due to over utilization of antibacterial chemicals[25]. High use of these agents can give rise to drug resistant microorganisms in the future. Hence, the current study was undertaken to study the antibacterial activity of 12 different indigenously made soaps.

2. Method and Procedure

2.1 Study Area

The study was carried out in Gombe, the capital of Gombe state, Nigeria. It is located in the center of north eastern part of Nigerian on Latitude 9"30' and 12"30'N, Longitude 8"5' and 11"45'E. With a land area of 20,265 square kilometers and a population of about 2.4 million. The state is situated right within the expensive Savannah region and has 11 Local Government Areas. It comprises of many tribal or ethnic groups among which are Hausa, Tangale, Terawa, Waja, Kumo, Fulani, Kanuri, Bolewa, Jukun, Pero/Shonge, Tula, Cham, Lunguda, Dadiya, Banbuka, etc. and Hausa is the common language of the people.

2.2 The Sample Collection

Twelve different indigenously made soaps (9 solids and 3 liquids) and one commercially available soap (control) was purchased in Gombe old market Gombe State (Table 1).

2.3 The Test Microorganisms

American type culture collection of *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Klebsiella pneumonia* and *Pseudomonas aeruginosa* were sourced from National Institute for Pharmaceutical Research and Development, Abuja, Nigeria. The clinical samples were authenticated by Gram staining and biochemical tests[6].

Table 1. Indigenously manufactured soaps in Gombe metropolis and their ingredients

S.No.	Names	Denotation	Ingredients
1	Solid soap	S1	Not indicated
2	Solid soap	S2	Soap base, fragrance, aqua and color.
3	Solid soap	S3	100% vegetable
4	Solid Soap	S4	Not indicated
5	Solid soap	S5	Tallow beached, palm oil, sodium silicate, soda ash and perfume.
6	Solid soap	S6	100% herbs
7	Solid Soap	S7	100% herbs
8	Liquid soap	S8	Not indicated
9	Liquid soap	S9	Nitrosol, caustic soda, soda ash, sulphonic acid, foaming agent, sodium lauret sulphide, formalin, perfume, color
10	Solid soap	S10	Herbs
11	Liquid soap	S11	Nitrosol, caustic soda, soda ash, sulphonic acid, foaming agent, sodium lauret sulphide, formalin, perfume, color
12	Liquid soap	S12	Nitrosol, caustic soda, soda ash, sulphonic acid, foaming agent, sodium lauret sulphide, formalin, perfume, color
13	Medicated soap	Control	Not indicated

2.4 Sample Dissolution

A portion of the solid soaps were weighed and dissolved in appropriate sterile distilled water to give different concentrations of stock solutions from 400 mg/mL to 6.25 mg/mL, the samples were dissolved in such a way that no foam was produced to form the stock solution. These stock solutions were stored in a well-sealed container and refrigerated until further use[22].

2.5 Preparation of Solid Soap Samples

A sterile blade was used to scrap the portion of the solid soaps. Each of the soaps was weighed and dissolved in appropriate milliliters of distilled water to give different concentration of stock solutions from 400, 100, 50, 25, 12.5 and 6.25 mg/mL respectively. The samples were dissolved in such a way that no foam was produced to form the stock solution. These stock solutions were stored in stored in a well-sealed containers and refrigerated until further use[22].

2.6 Preparation of Stock Solution of Liquid Soap Sample

Two fold dilutions of the liquid hand-wash soaps was prepared to give a stock solution of 2^{-1}.

2.7 Antimicrobial Susceptibility Test

Antimicrobial susceptibility of the soaps was carried out by agar diffusion technique[7]. Molten Muller Hinton agar was inoculated with 100 µL of standardized test organisms and holes were bored equidistantly with a sterile cork borer of 6 mm in diameter. The bottom was sealed with a drop of agar and filled with different concentrations of the soap solutions. Control plates of a medicated soap, organism viability and media sterility were set up. The plates were incubated at 37°C for 18-24 hrs[4]. Post incubation plates were observed for zone of inhibition around the wells, measured and recorded using transparent meter rule.

2.8 Minimum Inhibitory Concentration Determination

MIC was determined for only samples that showed inhibitory activity according to the CLSI[7]. The soap samples were dissolved in a sterile normal saline and 4ml each of sterile nutrient broth were transferred in to set of 4 test tubes and 4 mL of each concentration (100 mg/mL, 50 mg/mL, 25 mg/mL and 12.5mg/mL) of the soaps were added to obtain final concentrations of 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL respectively. Similarly, (350 mg/mL, 300 mg/mL, 250 mg/mL and 150 mg/mL) concentrations, 4 mL each were also added in 4 different test tubes containing 4 mL of nutrient broth to obtain a final concentration of 175 mg/mL, 150 mg/mL, 125 mg/mL and 75 mg/mL for the soaps that began activity at 200 mg/mL. The tubes were inoculated with 50 µL of each test organism and incubated at 37° C for 18-24 hrs. The MIC was taken as the lowest concentration that prevented the visible growth of the test organisms.

2.9 Minimum Bactericidal Determination

The MBC was determined by collecting one milliliter from the tubes used to determine MIC and sub-cultured on to freshly prepared Mueller Hinton agar. The plates were incubated at 37°C for 24 hours. The least concentration at which the organisms did not recover or grow was taken as the MBC[6].

2.10 Time Kill Kinetics Antibacterial Study

In vitro time kill kinetic antibacterial of the most active indigenously manufactured soap (S10) was carried to determine the pharmacodynamics of active soaps against susceptible test organisms[5]. Microbial population at the initiation and completion was determined by spectrophotometric and plate count methods at interval of 2 h. Fifty milliliters of the soap at 2 times its MBC concentrations were mixed with equal volume of Mueller Hinton broth to obtain final concentrations equal to the MBC and inoculated with 100 µL of standardized test organisms as described above. The optical density of each dilution was recorded on uv/spectrophotometer at 540 nm (Jenway, 6405) at initiation time (0 h) and every 2 h for 12 h. For surviving organism count, an aliquot of each dilution (1 mL) was transferred and plated on 20 mL tryptic soy agar at interval of 2 h. The experiment was carried out in duplicate and results were recorded at 18-24 hours of post incubation[18]. The percentage reduction and log reduction from initial microbial population for each time point was calculated to express the change (reduction or increase) of the microbial

population relative to a starting inoculum. The change was determined as follows[5];

$$\% \text{ Reduction} = \frac{initial\ count - count\ at\ x\ interval}{innitial\ count} \times 100$$

The log reduction was calculated as follows: Log_{10} (initial count) - Log_{10} (x time interval) = Log_{10} reduction

3. Results

The antimicrobial activity of some indigenously made soaps marketed in Gombe metropolis determined by agar well diffusion method showed that the soaps have varying degree of activity against the test organisms. Out of the twelve samples screened, only six samples (S1, S5, S6, S7, S8, and S10) showed antibacterial effect against the organisms (Table 2). The zones of inhibition ranged from 4-18 mm but not as effective as the control sample. The MIC and MBC of active indigenously manufactured soaps are shown in Table 3. Time kill kinetics antibacterial study of most active locally made soap (S10) against test organisms and the control soap are shown in Table 4 and 5 respectively.

Table 2. Antibacterial activity of active indigenously manufactured soaps by disc diffusion technique

Samples	Test organisms	Zones of inhibition (mm)/concentrations (mg/mL)			
		200	100	50	25
S1	S. aureus	10	9	7	Na
	E. coli	Na	Na	Na	Na
	P. aeruginosa	9	9	9	Na
	B. subtilis	10	8	6	Na
	k. pneumonae	11	9	7	Na
S5	S. aureus	5	Na	Na	Na
	E. coli	6	Na	Na	Na
	P. aeruginosa	8	6	Na	Na
	B. subtilis	6	Na	Na	Na
	k. pneumonae	Na	Na	Na	Na
S6	S. aureus	8	6	Na	Na
	E. coli	8	4	Na	Na
	P. aeruginosa	11	Na	Na	Na
	B. subtilis	7	7	5	Na
	k. pneumonae	7	7	5	Na
S7	S. aureus	9	7	5	4
	E. coli	9	Na	Na	Na
	P. aeruginosa	8	8	6	4
	B. subtilis	8	7	5	4
	k. pneumonae	7	6	4	4
S8	S. aureus	12	9	7	5
	E. coli	13	10	7	5
	P. aeruginosa	10	7	6	4
	B. subtilis	13	9	6	4
	k. pneumonae	9	7	4	Na
S10	S. aureus	18	12	6	5
	E. coli	12	10	6	4
	P. aeruginosa	12	9	6	4
	B. subtilis	11	9	6	4
	k. pneumonae	12	10	5	4
Control	S. aureus	24	18	14	11
	E. coli	16	14	10	7
	P. aeruginosa	14	12	9	7
	B. subtilis	18	15	13	9
	k. pneumonae	18	15	13	9

Note: Na = No activity

This study unlike an MBC/MIC assay, allows the determination of the speedy bactericidal activity of the soaps[2]. The soaps exhibited bactericidal effect at their MBC concentration against all the test bacteria. The number of surviving microorganisms in the soaps was determined by plate count method at sampling time and enumerated. A significant decrease ($p<0.05$) in population of test organisms was observed at each interval.

Table 3. Determination of MIC and MBC of active indigenously manufactured soaps

Samples	Test Organisms	Concentrations (mg/mL)	
		MIC	MBC
S1	S. aureus	50	100
	E. coli	Na	Na
	P. aeruginosa	50	100
	B. subtilis	50	100
	k. pneumonae	50	100
S5	S. aureus	150	175
	E. coli	150	175
	P. aeruginosa	150	175
	B. subtilis	150	175
	k. pneumonae	Na	Na
S6	S. aureus	75	75
	E. coli	75	100
	P. aeruginosa	75	125
	B. subtilis	50	75
	k. pneumonae	50	50
S7	S. aureus	25	50
	E. coli	Na	Na
	P. aeruginosa	25	50
	B. subtilis	25	50
	k. pneumonae	25	50
S8	S. aureus	12.25	25
	E. coli	12.25	25
	P. aeruginosa	25	50
	B. subtilis	25	50
	k. pneumonae	25	50
S10	S. aureus	12.25	25
	E. coli	12.25	25
	P. aeruginosa	25	50
	B. subtilis	12.25	25
	k. pneumonae	12.25	25
Control	S. aureus	6-25	12.5
	E. coli	12.5	25
	P. aeruginosa	12.5	25
	B. subtilis	6.25	12.5
	k. pneumonae	6.25	12.5

Note: Na= No activity

4. Discussion

Results of this investigation revealed that most of the assayed indigenously made soaps have antibacterial activity, though at varying degree as indicated by the inhibition of the growth pattern of the isolates. S10, a sample containing Palm kernel was found to be the most effective with largest zones of inhibition against *S. aureus* (18 mm), *Escherichia coli, P. aeruginosa and K. pneumonia* (12 mm). This finding is similar to previous findings[3,24] that soaps from Palm kernel source possess antibacterial effect against *S. aureus and Streptococcus* sp. S50 has the least antibacterial effect with zones of inhibition ranging from 5-8 mm against *S. aureus, E. coli, P. aeruginosa, and B. subtilis* respectively. Although, there is no clinical breakpoints threshold values for herbal medicines/recipes, this activity is insignificant by CSLI standard[7]. The activity of the indigenously made soaps is not as significant ($p<0.05$) as the activity of the control medicated soap ($p>0.05$) with zones of inhibition ranging from 14-24 mm against all the test organisms at a lower concentration of 6.25 mg/mL. The MIC and MBC of all the indigenously made soaps shows that a higher concentration is required to have any significant effect on the test organisms. These test organisms are of body normal flora, dirty wears, utensils, wound infections and table tops, thus, adequate concentration is required to ensure cleanliness. Pseudomonas aeruginosa is notably notorious for its resistance to most antimicrobial agents[23].

Considering the components of few of the indigenously produced soaps (S1, S5, S6, S7, S8, S10) in Table 1, their activity has shown to be dose dependent with effective antimicrobial properties. The soaps also demonstrated to be infection specific as they are mostly active against Gram positive organisms. Total lack of activity or minute activity against Gram negative organisms could be as a result of the impermeable nature of the Gram negative cell to most antimicrobials[11] and more importantly the antimicrobial principles in the soaps tested. The differences in the zones of inhibition produced by the different soaps having the same constituents suggest that there are differences in the quantity of each ingredient in each of the soap. The quantity of each of these ingredients could however not be ascertained since the manufacturers did not

Table 4. Time kill kinetics antibacterial study of most active indigenously made soap (S10) against test organisms

Sample	Organisms	Time(h)	Population	% reduction	% log reduction
S10	E. coli	0	65	Na	Na
		2	42	35.4	0.19
		4	29	30.9	0.16
		6	9	68.9	0.51
		8	0	100	0.51
		10	0	100	0.51
		12	0	100	0.51
	S. aureus	0	38	Na	Na
		2	24	36.8	0.19
		4	13	45.8	0.27
		6	6	53.8	0.34
		8	0	100	0.34
		10	0	100	0.34
		12	0	100	0.34
	P, neumonae	0	47	Na	Na
		2	23	51.0	0.31
		4	10	56.5	0.36
		6	4	60.0	0.39
		8	0	100	0.39
		10	0	100	0.39
		12	0	100	0.39
	B. subtilis	0	40	Na	Na
		2	23	42.5	0.24
		4	17	26.1	0.13
		6	8	52.9	0.33
		8	0	100	0.33
		10	0	100	0.33
		12	0	100	0.33
	K. neumonae	0	38	Na	Na
		2	21	44.7	0.26
		4	14	33.3	0.18
		6	6	78.6	0.67
		8	0	100	0.67
		10	0	100	0.67
		12	0	100	0.67

Note: Na = Not applicable

disclose this on their labels. Poor packaging of the indigenously manufactured soaps could increase the risk of exposure of the products to environmental microbial contamination especially fungi which may render the soap samples less active in the treatment of skin infections. Sabulun salo, an indigenous soap corresponding to S10 has been reported to have antibacterial activity against *E. coli, S. aureus, B. subtilis*[1].

Majority of the assayed soaps have demonstrated satisfactory effect, particularly the antibacterial activity as compared to the control. This is due to differences in the active antibacterial ingredients and type of formulations used[15].

Time-kill kinetics of antibacterial study has been used to investigate numerous antimicrobial agents and they are also often used as the basis for *in vitro* investigations

Table 5. Time kill kinetics antibacterial study of control soap

Sample	Organisms	Time(h)	Population	% reduction	% log reduction
Control soap	E. coli	0	41	Na	Na
		2	19	53.0	0.33
		4	8	57.8	0.38
		6	0	100	0.38
		8	0	100	0.38
		10	0	100	0.38
		12	0	100	0.38
	S. aureus	0	38	Na	Na
		2	18	52.6	0.32
		4	4	77.7	0.65
		6	0	100	0.65
		8	0	100	0.65
		10	0	100	0.65
		12	0	100	0.65
	P. aeruginosa	0	42	Na	Na
		2	24	42.8	0.24
		4	12	50.0	0.30
		6	0	100	0.30
		8	0	100	0.30
		10	0	100	0.30
		12	0	100	0.30
	B. subtilis	0	34	Na	Na
		2	24	29.4	0.30
		4	16	33.3	0.16
		6	0	100	0.16
		8	0	100	0.16
		10	0	100	0.16
		12	0	100	0.16
	K. pneumonae	0	32	Na	Na
		2	16	50.0	0.30
		4	11	31.0	0.16
		6	0	100	0.16
		8	0	100	0.16
		10	0	100	0.16
		12	0	100	0.16

Note: Na = Not applicable

for pharmacodynamics of drug interaction[16]. The time kill kinetic antibacterial assay of the most active soap gave variable kinetics against susceptible bacteria tested as seen in Table 4. The soaps demonstrated both bacteriostatic and bactericidal effects as it shows a concentration-dependent effect. The bactericidal concentration of the soap and the control plummeted against *P. aeruginosa* and *E.* coli, is not surprising as Pseudomonas species have been reported to be resistant to many antimicrobial agents. A significant decrease in the population of the organisms with increase contact time was observed**.** A complete elimination of *E. coli, S. aureus, P. aeruginosa, B. subtilis* and *K. pneumonia* was achieved after 8 h exposure but not as significant as the control soap that exhibited complete elimination of test organisms at 6 h contact (Table 5).

Conclusively, the trend of cidal activities is time and dose dependent. At higher concentration and longer duration of contact (8 h), more bacteria were eliminated. Inhibitory levels of the soaps could be bacteriostatic and bactericidal independent of Gram position of test organisms. This study revealed that the soaps were rapidly bactericidal at higher concentrations achieving complete elimination of test organisms after 8 h exposure. The antibacterial activities exhibited by these soaps suggest them as potential candidate in bio-prospecting for antibacterial. The isolation and identification of the active principles of the soaps will be a step forward in medication discovery.

5. Conflict of interest:

There is no conflict of interest among the authors.

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