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Antimicrobial Activities of Sokoto Prepared Black Soap (Sabulun Salo) on Clinical Isolates of *Candida albicans, Staphylococcus aureus* and *Escherichia coli.*

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Abstract

In general, Sabulun salo can be any antifungal and antibacterial soap with added antimicrobial active ingredients. These chemicals kill bacteria and other microorganisms, although they are not as effective at inactivating viruses as any other soap. The antifungal and antibacterial activity of Sabulun salo was determined on *Candida albicans, Staphylococcus aureus* and *Escherichia coli* isolated from gastric and skin infections. This project aimed to determine the activity of sabulun salo on some fungal, gram-negative and gram-positive bacteria. Confirmation of isolates was performed using standard microbiological methods. Different concentrations (12.5mg/ml, 25mg/ml, 50mg/ml and 100mg/ml) of each soap were tested by disc diffusion method against *Candida albicans, Staphylococcus aureu*s and *Escherichia coli* respectively, and the zone of inhibition was recorded. Sabulun salo was found to be effective against fungal strains and some bacterial strains tested with a peak zone of inhibition of 27mm for *Staphylococcus aureus* and 20 mm for *Candida albicans* at the highest dilution used at 100mg/ml, followed by *Escherichia coli* with 10mm at 100 mg/ml. The minimum inhibitory concentration results show that Sabulun salo has a minimum inhibitory concentration of 12.5mg/ml for *Staphylococcus aureus, Escherichia coli* and *Candida albicans*, respectively, and a minimum bactericidal concentration of 25mg /ml, 12.5mg/ml and 25mg/ml corresponds to *Candida albicans, Staphylococcus aureus* and *Escherichia coli.* These sabulun salo have fungicidal and bactericidal effects, especially at high concentrations; They need to be used in high concentrations for them to be effective.

Keywords: Sabulun salo, *Candida albicans*, antibacterial, Sokoto, antifungal

Introduction

Sabulun salo is a local traditional medicated soap widely used by different tribes in Nigeria such as Hausa, Yoruba (Ose Dudu), and Nupe (Eko zhiko). It is a black soap that has been used for centuries in many African homes especially in Ghana and Nigeria. The soap is produced from a mixture of vegetable oils such as palm kernel oil and Shea butter oil (Getradeghana, 2000). The attribute of the soap includes gentleness on the skin, rich lather, protection against skin disorders (such as rashes, eczema, and scabies), treatment of skin infections (like ringworm), protection of even skin toning and smoothness of the skin (Getradeghana, 2000). The benefit of soap finally managed to appeal to the European population in the 17th century and the art of maintaining a high personal hygiene experience increased also (*Hsieh,* 2019). The main fatty acids present in palm kernel oil, a major ingredient for making *sabulun salo*, are lauric acid (C12, 48%), myristic acid (C14 16%), and oleic acid. Certain fatty acids (medium chain saturates) and their derivatives have activity on various microorganisms *(*Kabara, 2000). Monolaurin has been specifically found to have activity on potentially pathogenic microorganisms. Yang *et al.* (2003) reported the inactivation of *S. epidermis* and group β-haemolytic Gram-positive Streptococci by lipase with high monolaurin content. The lauric

acid content of the palm kernel oil has the additional beneficial function of being formed into monolaurin in human or animal bodies (Ubogu *et al*., 2006). This means that the palm kernel oil may have a higher antimicrobial effect *in vivo.*

Although the antimicrobial activity observed is low, Kabara (2000) has shown that the use of this type of inhibitory agent does not lead to the development of resistance. Shea butter is an extract from the kernel of the *Vitellaria paradox* which is found in 19 countries across the African savanna zone. Shea butter contains a high level of U.V-β ray absorbing triterpenes ester including cinnamic acid, tocopherols (vitamin. A), and phytosterols. It also contains a high percentage of unsaponifiable camp sterol, stigma sterols, beta, and alpha spino sterols (Wiesman, 2003). Shea butter is composed of five principal fatty acids: palmitic acid, stearic acid, linoleic acid, and arachidic acid. Stearic acid and oleic acid account for about 85-90% of the acids (Maranz *and* Weisman, 2004). The analysis of the kernel reveals the presence of phenolic compounds such as garlic acid, catechin, epicatechin, gallate as well as quercetin, and trans cinnamic acid (Steven *et al*., 2003).

Most of the commercially imported soaps cause different problems to humans including the development of resistant microorganisms (Ingham *et al*., 2012). Hence the need for a mild universally acceptable antimicrobial soap with minimal side effects and Sabulun salo has exhibited some of these properties (Aliyu *et al.,* 2013). Lauric acid is a major component of palm kernel oil which is an active ingredient for making *sabulun salo*. Although the antimicrobial activity shown by lauric acid is low, the use of this type of inhibitory agent does not lead to the development of microbial resistance (Karbara, 2000). This research aims to determine the antifungal and antibacterial activity of *sabulun salo*, a local traditional medicated soap, commonly used in Sokoto State Nigeria with the ultimate goal of providing scientific bases to validate its claimed antimicrobial activity.

Materials and Methods

Materials

The materials used in this project are petri-dishes, test tubes, inoculating loop, cotton wool, swab sticks, test tubes, conical flask, glass slides, Gram's reagents, hydrogen peroxide, Mannitol salt agar, Mueller Hinton Agar, Sabouraud Dextrose Agar (SDA), Nutrient agar, distilled water and reagents for gram staining and biochemical tests.

Methods

All the glass wares listed above were properly washed and sterilized in the oven at 160°C for one hour and stored at the temperature of 40°C.

Study Area

Sokoto State is located in the extreme North-Western part of Nigeria near to the confluence of the Sokoto and Rima rivers with an annual average temperature of 28°C (82.^{0}F). Sokoto in general is a very hot area. However maximum day time temperature for most of the year is generally under 40^{0}C (104.0^{0}F). The warmest months are February to May when daytime temperature can exceed 45^{0}C (113.0^{0}F). The rainy season is from June to October during which showers is a daily occurrence. There are two major seasons, wet and dry seasons. Report from 2007, National population commission indicated that the state had a population of 3.6 million (NPC, 2006). Sokoto State, shares border with the Republic of Niger to the North. It also shares boundaries with Kebbi State to the West and South and Zamfara to the South and East. The majority of people are farmers. The major language is Hausa and Fulani with minority of other Nigerian languages (Igbo, Yoruba, and so on).

A local Sokoto black soap samples usually made from locally harvested and dried plant materials such as burn grain stick, cocoa pods, plantain peels, palm tree leaves and shea butter tree bark, was collected from old market in Sokoto North towns in Sokoto State.

Collection of Clinical Isolates

Two bacterial strains *Staphylococcus aureus* and *Escherichia coli* including *Candida albicans,* were obtained from the Medical Microbiology Laboratory of Usmanu Danfodiyo University

Teaching Hospital (UDUTH) Sokoto.

Preparation of Culture Media

Nutrient agar is a basic medium. It was therefore used here on the assumption that as many organisms on the samples will grow. The medium was prepared from ready to use dehydrated powder. It contains Peptone, Lab-Lemco powder, yeast extract, sodium chloride, and agar. Nutrient agar is usually used at a concentration of 28g in every 100 L distilled water (concentration may vary depending on manufacturer). The agar was prepared as instructed by the manufacturer; 28g was weighed and dissolved in 1L of distilled water and then it was sterilized by autoclaving at 121℃ for 15 minutes. After cooling, it was then dispensed aseptically in the required amounts, 5ml in each petri dish and then stored in a cool dark place (Cheesbrough, 2006).

Identification of Isolates

Gram Staining and Microscopic Examination

A drop of normal saline was placed at the center of a clean grease free glass slide; a colony was touched with a sterilized wire loop. The growth collected was emulsified on to the drop of normal saline on a glass slide. The smear was allowed to air dry and was flamed fixed. The smear was flooded with crystal violet and allowed to stain for one minute. The stain was rinsed with clean water rapidly. Then the smear was treated with lugols iodine for one minute. The smear was rinsed with clean water and then decoulorized with acetone briefly, it was then rinsed with distilled water. The smear was counter stained with neutral red for one minute. The smear was rinsed with water. The back of the slide was cleaned with cotton wool and it was allowed to air dry. Immersion oil was applied and then viewed under microscope using X100 objectives and the gram reaction, morphology and arrangement of the organism was noted as described by (Cheesbrough, 2002).

Biochemical Tests

The following biochemical tests were used to determine the presence of *candida albicans*

Germ Tube Test:

Germ Tube Test is a screening test which is used to differentiate *Candida albicans* from other yeast. Germ tube (GT) formation was first reported by Reynolds and Braude in 1956. When Candida is grown in human or sheep serum at 37°C for 3 hours, they form a germ tubes, which can be detected with a wet KOH films as filamentous outgrowth extending from yeast cells. It is positive for *Candida albicans* and *Candida dubliniensis.* Approximately 95 – 97% of *Candida albicans* isolated develop germ tubes when incubated in a proteinaceous media.

Germ tube test to identify C. albicans.

Five hundred microlitres, 500µl (0.5ml) of human or preferably bovine or rabbit serum was pipetted into a small test tube. Using a sterile wire loop, the serum was inoculated with a yeast colony from the culture plate. The tube was then placed in a water bath at 35–37ºC for 2–3 hours. Using a Pasteur pipette, a drop of the serum yeast culture was transferred to a glass slide and covered with a cover glass. The preparation was examined using the 10 and 40 objectives with the condenser iris diaphragm closed sufficiently to give good contrast (If preferred, a drop of lactophenol cotton blue can be added to the preparation to stain the yeast cells). Sprouting yeast cells that is tube-like out- growths from the cells (known as germ tubes) were then looked for. The culture is reported as *C. albicans* isolated when sprouting yeast cells are seen (Chesbrough, 2006).

The following biochemical tests were used to determine the presence of *S. aureus*.

Coagulase Test

Slide and tube method was used (Carpenter, 1977). In the slide test, a loopful of the isolate was mixed with human plasma and allowed to stand *for* some minutes. Particles indicating agglutination were used as indication of coagulase reaction. In tube method, plasma was added into a culture of the isolate in peptone water in Bijou bottles. The bottles were incubated at 37°C *for* 24hours. A clumping/ agglutination of the plasma were used *to i*ndicate presence of coagulase.

Catalase Test

This test was used to demonstrate which of the isolates could produce the enzyme catalase that release oxygen from hydrogen peroxide. This test is usually used as an aid to differentiate

Staphylococci from *Streptococci* and to differentiate other catalase positive organisms from catalase negative. The method employed here was that described by Ochei and Kolhatkar (2010). A loopful of the pure colony was transferred into a plane, clean glass slide. The sample was then mixed with a drop of 3% v/v hydrogen peroxide. The reaction was observed immediately. Gas production indicated by the production of gas bubbles confirmed the presence of catalase.

These biochemical tests were done to determine the presence of *E. coli*.

Methyl Red Test

This test was used to detect which of the isolates could produce and maintain sufficiently a stable acid product from glucose fermentation. The test is usually used as an aid in the identification and differentiation of the *Enterobacteriaceae* (Ochei and Kolhatkar, 2010). This test was carried out as described by Shane *et al.* (2017). Tubes of buffered glucose-peptone broth were lightly inoculated with the isolates. The tubes were incubated at 37°C for not less than 48 hours. About 5 drops of the methyl red reagent were added into 5ml of the culture. The production of a bright red colour immediately on the addition of the reagent showed a positive test. Methyl red test indicator consists of 0.1g methyl Red, 300ml of 95% ethyl alcohol.

Voges -Proskeur Test (V.P. test)

This test is used to determine the capability of some organisms to produce non- acidic or neutral end products, such as acetyl methyl carbinol, from the organic acids that results from glucose metabolism. The Barrett's reagents were added to the test organism broth and left to stand for about 20 to 30 minutes. *E. coli* is negative to this test and does not show any change in colour.

Indole Test

This test was used to determine which of the isolates has the ability to split indole from tryptophan present in buffered peptone water. The test is usually used as an aid in the differentiation of Gram- negative bacteria, *Bacilli* especially those of the *Enterobacteriaceae* (Ochei and Kolhatkar, 2010). The test was carried out as described by Shane *et al.* (2017). Tubes of peptone water were inoculated with young culture of the isolates. The tubes were incubated at 37°C for 48hrs. About 4 drops of Kovac's reagent were added into 1ml of each of the culture tubes. Positive test was indicated by a red colour that occurs immediately at upper part of the test tube.

Citrate Utilization Test

This test was used to identify which of the isolates can utilize citrate as the sole source of carbon for metabolism. The- test is usually used as an aid in the differentiation of organisms in the *Enterobacteriaceae and* most other genera (Ochei and Kolhatkar, 2010). The medium used for this test was the Simon's citrate agar. Slant tubes of Simon's citrate agar were inoculated with young culture of the isolates. The inoculation was done by stabbing the medium on the tubes using sterile straight inoculating wire containing the culture. The tubes were then incubated at 37°C for about 24hours. Change in colour from green to blue after about 24hours of incubation indicated positive result.

Screening For Antimicrobial Activity

Clinical isolates *of Candida albicans, S. aureus* and *E. coli were* obtained from Usmanu Danfodiyo University Sokoto, Department of Medical Microbiology Laboratory, in Usmanu Danfodiyo Teaching Hospital (UDUTH) Sokoto. The susceptibility of the organisms to *sabulun salo* were tested and assayed using a gar-well diffusion method. The overnight culture of the test organism was suspended in saline solution (0.85% NaCl) and adjusted to match a turbidity of 0.5 McFarland standard (*Aliyu et al.,* 2009). The standardized suspension was used to inoculate the surfaces of Mueller Hinton agar plate and SDA plate (90mm in diameter) using sterile cotton swab. The stock solution of the sabulun salo was prepared by dissolving 10g of sabulun salo in 100ml sterile distilled water to form a stock solution of 100mg/ml from which Different concentrations follows 50%, 25%, 12.5%, w/v of the *sabulun salo and* it were prepared (using sterile distilled water), serial dilution. Six were made on each plate using sterilized cork borer (8mm) and 0.3 ml of each concentration were transferred in to each of the 5 wells (appropriately labelled) and distilled water into the sixth well as the negative control. The plates were incubated at 37^{O}C

for 24 hours and at room temperature for 48 hours, for the bacteria and fungus respectively. The plates were observed for zone of inhibition around the wells and the zones of inhibition were measured and recorded using transparent metre rule. The entire test was conducted in duplicate.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration was determined according to the National Committee for Clinical Standard (1999). The *sabulun salo* were dissolved in sterile distilled water and 2ml each of sterile Mueller Hinton broth and SDA broth were transferred into a set 4 of tubes and 2ml of each concentration (100%, 50%, 25%, 12.5%, w/v) of the *sabulun salo* were added to obtained final concentrations of 50%, 25%, 12.5% and 6.25%, w/v respectively. Each test organism was inoculated into the labeled tube except the control; the tubes were incubated at 37^{O}C for 18 hours for the bacteria and at room temperature for 48hours for fungus.

The MIC were taken as the lowest concentration that prevented visible growth. The minimum bactericidal concentration and minimum fungicidal concentration were determined according to the National Committee for Clinical Standard (1999). From the test tubes used in the determination of MIC, the tubes that show no visible growth were sub-cultured onto freshly prepared Mueller Hinton agar and SDA at 37^{O}C for 48 hours for the bacteria and at room temperature for 48 hours for fungus. The least concentration at which the organisms did not recover and grow were taken as the MBC.

Result

The result of the antimicrobial assay of Sabulun salo showed that the soap possesses antimicrobial activity against three of the test organisms. The pattern of inhibition varied with the soap concentration and the organisms tested. The largest zone of inhibition was observed against *S. aureus* (27mm) followed by *C. albicans* (20mm) at 100mg/ml. However, *Escherichia coli* was resistant to the soap (10mm, 11mm, 12mm, 13mm) at the concentrations of 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml (Table 1). Table 1 presents the observed antimicrobial activities of the first sample of the soap collected in terms of the zone of inhibition observed at various concentrations of the soap.

Table 1: Diameter of the zone of inhibition for sample A

S/N	Test organism	Mean diameter zone of inhibition(mm)			
		100mg/ml	50mg/ml	25mg/ml	12.5mg/ml
1	*Candida albicans*	20	17	15	12
2	*Staphylococcus aureus*	27	20	15	13
3	*Escherichia coli*	10	11	12	13

The MICs of the soap against both *S. aureus* and *C. albicans* and *E. coli* were 12.5mg/ml. The minimum bactericidal concentration (MBC) of *S. aureus* and *E. coli* was found to be 12.5mg/ml and 25mg/ml respectively while the minimum fungicidal concentration (MFC) of *C. albicans* was 25mg/ml (Table 2).

Table 2 : Minimum Inhibitory Concentration (MIC) and Minimum Lethal Conc entration (MLC)

S/N	Test organism	MIC	MBC	MFC
1	Candida *albicans*	12.5	NA	25
2	*Staphylococcus aureus*	12.5	1.25	NA
3	*Escherichia coli*	12.5	25	NA

Key:
MIC = Minimum Inhibitory Concentration
MBC = Minimum Bacteriocidal Concentration
MFC = Minimum Fungicidal Concentration
NA = Not applicable.

Discussion

Sabulun salo could be any antifungal and antibacterial soap to which antimicrobial active ingredients have been added. These chemicals kill bacteria and other microorganisms, though they are not effective in deactivating viruses just like any other kind of soaps. The antifungal and antibacterials activities of Sabulun salo was determined on *Candida albicans,* Staphylococcus aureus and Escherichia coli isolated from stomach and skin infections.

Findings obtained from this study showed that Sabulun salo is effective against *Candida albicans* and *Staphylococcus aureus* except *Escherichia coli* which it has no effect on. In this study *Staphylococcus aureus* and *Candida albicans* were sensitive to the effect of Sabulun salo while *Escherichia coli was not*. *Staphylococcus aureus* has higher zone of inhibition of 27mm compare to *Candida albicans* which has 20mm at higher concentrations of 100mg/ml.

Zones of inhibition were recorded in which *Candida albicans* has 20mm and *Staphylococcus aureus* 27mm while *Escherichia coli* has 10mm. Therefore, zone of inhibition of *Candida albicans* is similar to findings reported by Samaila *et al.* (2016) in Bauchi but *Staphylococcus aureus* differs. However, this is in contrast with the work documented by Oleghe *et al. (*2023).

The results of the minimum inhibitory concentration revealed that Sabulun salo had Minimum inhibitory concentration of 12.5mg/ml on *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* respectively, and Minimum bacteriocidal concentration were 25mg/ml,12.5mg/ml and 25mg/ml against *Candida albicans*, *Staphylococcus aureus* and *Escherichia coli* respectively. These Sabulun salo exhibited fungicidal and bacteriocidal effect especially at high concentration indicating the need to potentially use them at high concentration for effective action.

The result obtained on minimum inhibitory concentration here is similar with results recorded in Bauchi state by Samaila *et al*. (2016) and differs on minimum bacteriocidal concentrations and also similar with another findings in ABU Zaria and Akure by Adebayo *et al*. (2012) and Alabi *et al. (*2018) respectively.

Therefore, result of this study show that Sabulun salo has antimicrobial effect against *S. aureus* and *C. albicans* with a maximum zone of inhibition of 27mm and 20mm respectively. *S. aureus* and *C. albicans* have been incriminated in causing skin infections including boils, thrush, impetigo etc.; the susceptibilities of these organisms to the soap indicate the therapeutic potentials of the soap in the treatment of such diseases. The cell wall of *S. aureus* which is a gram-positive bacterium is made up of mainly peptidoglycan. Peptidoglycan is found to be distorted by long chain fatty acids that are found in palm kernel oil an active ingredient in Sabulun salo.

The activity of the soap against *S. aureus* therefore, could be attributable to the palm kernel oil present in the soap (Ugbogu, 2006; Alabi and Makinde, 2022). Ugbogu (2006) reported that palm kernel oil has an inhibitory effect on *S. aureus* and *Streptococcus sp*. The major fatty acids

in palm kernel oil used for the production of sabulun salo are lauric acid, myristic acid and oleic acid. Certain fatty acids (medium chain saturates) and their derivatives have adverse effects on various microorganisms (Peters *et al*., 2018). Monolaurin has been specifically found to have adverse effect on potentially pathogenic microorganism. Oleghe *et al.* (2022) reported the inactivation of *S. epidermidis* and group B Gram-positive Streptococcus by lipases with high monolaurin content.

The effect of long chain fatty acid may be the disruption of the fungal membrane leading to leakage of macromolecules such as nucleotide, inorganic acid or phosphorylated ammonium compound (Arora, 2004). This explains the inhibitory effect exerted by the soap against the fungus *C. albicans*.

There was no observed inhibitory effect on *E. coli* by the soap at all concentrations used. *E. coli* being Gram negative organism has little peptidoglycan in its cell wall and this may hinder the activity of the active components of the soap (fatty acids). The resistance of *E. coli* to antimicrobial agents is usually due to chromosomal mutation which lowers the permeability of the bacteria to the agents or acquisition of resistance (R) plasmids and transposons (Arora, 2004; Peters *et al*., 2018).

Therefore, the resistance showed by *E. coli* to Sabulun salo may be due to chrosomal mutation which may have resulted to lower permeability of the bacterial cell. However, Alabi and Makinde (2022) reported that palm kernel oil has inhibitory action against *E. coli* with no inhibition against *S. aureus*, *Pseudomonas aeruginosa*, *Aspergillus niger* and *C. albicans*. Although the effect of the soap was static on *C. albicans* and *S. aureus,* raising the concentration of the soap may possibly be bactericidal and also activity may be found on *E. coli*. It should be noted that increase in purity of the palm kernel oil and shea butter (used in the preparation of the soap) may have significant effect on the properties and quality of the soap.

Conclusion

The antimicrobial activity exhibited by the Sabulun salo extract against the test organisms (*S. aureus* and *C. albicans*) that are associated with various skin infections has provided scientific justification for the ethnomedicinal uses of the soap by Hausa, Yoruba and Nupe tribes in Nigeria. In general, there was inhibition against all test organisms by the soap. Therefore, Sabulun salo can serve as a potential source of broad-spectrum antibiotics. It is recommended that further studies should be conducted on Sabulun salo with a view to its industrial production employing hygienic and more standard techniques.

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