



Original Research Article

Comparative antimicrobial analysis of indigenous black soap variants

Received 22 April, 2022

Revised 28 May, 2022

Accepted 3 June, 2022

Published 15 September, 2022

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The *in-vitro* antimicrobial efficacy of Indigenous Black Soaps (IBS) to promote and increase the awareness of the economic viability of these skin-care products were comparatively evaluated in this study. Three (3) different locally produced black soups variants (turmeric, honey and lime) and two (2) different commercially available black soap in Nigeria (Dudu-Osun® and Zee black soap®) were analyzed against some selected microorganisms isolated from human skin. The clinical isolates were *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*. The susceptibility pattern of each of the tested organism to the different soap samples was determined using agar well diffusion method. The result obtained from this study reveals that the commercially available black soaps and the locally produced once, showed significant antimicrobial activities against the test organisms. This justifies the use of the traditional materials found in black soaps as potent antiseptics. The average inhibition zone diameter (IZD) of the locally produced black soap containing turmeric showed the highest zone of inhibition (41.1mm) and the locally produced black soap with lime (8 mm) was the least. The percentage of activity of the soaps on the test organisms showed that *Staphylococcus aureus* displayed higher susceptibility to most of the black soaps compared to *Candida albicans* and *Pseudomonas aeruginosa*. Therefore, it is recommended that locally produced soaps with turmeric and honey could be considered as viable alternatives in future topical and skin-care formulations for the treatment of infections caused by the tested organisms used in this study.

Keywords: Antimicrobial analysis, black soap variants, indigenous, honey, lime, turmeric.

INTRODUCTION

Black soap has been used by West African natives from time immemorial for treatment of skin infections, injuries and poison, beautification, regular bodily bath, relaxation, sickness and massage (Aliyu et al., 2012; Adebomi et al., 2017). It is known as *Sabulun salo* in Hausa, *Anaga* or *Alatasamina* in Ghana, *Eko-zhiko* in Nupe and *Osedudu* or *Abuwe* among the Yoruba speaking people in the South Western part of Nigeria (Getradeghana, 2000, Aliyu et al., 2012). In Nigeria, black soap may be made from either roasted plantain skins, dried waste cocoa pods or cocoa seeds as chocolate butter, palm oil and palm nut oil. Some

are also produced from a mixture of palm kernel oil and shea butter (Ikpoh et al., 2012; Olajuyigbe et al., 2017).

Staphylococcus aureus is Gram-positive, round-shaped member of the normal microbiota of the human body (Taylor and Unakal, 2022). The nose and perineum are the most common sites for *Staphylococcus aureus* colonization, which is present in over 10 - 40% of normal adults (Sakr et al., 2018).

Pseudomonas aeruginosa is a common encapsulated Gram-negative rod-shaped bacterium that can cause diseases in plant and animals including humans (Varsha,



Plate 1: Locally produced black soaps

2016). This bacterium can be found on medical equipment including catheters, causing cross infections in hospital and clinics (Litwin et al., 2021).

Candida albicans is a common opportunistic yeast that is pathogenic to immune-compromised persons (Donlan, 2001; Atriwal et al., 2021). It is a common member of the human gut flora and it is the most common fungal species isolated from biofilms either formed on (permanent) implanted medical devices or on human tissues (Cavalheiro and Teixeira, 2018; Atriwal et al., 2021).

The traditional African black soap which has in combination, water, roasted plantain skin or cocoa pods or seeds (charcoal), as well as, palm kernel oil or shea butter when put together are collectively referred to as “black soap” (Aliyu et al., 2012; Adebomi et al., 2017). Consequently, the therapeutic potential of these black soaps have become inconsequential, probably because of its manufacturing procedure, packaging and misconception about its use by the traditional herbalists and its being indigenous (Aliyu et al., 2012; Olajuyigbe et al., 2017). However, because of its ethno-therapeutic applications in the treatment of skin infections, wounds and daily intake of its leather solution mixed with other plants extracts for detoxification, it becomes essential to investigate its antibacterial potency in comparison with those of some already established medicated soaps with antibacterial properties commonly sold (Sofowora, 1982; Olajuyigbe et al., 2017). Hence, this study focuses on the antimicrobial potentials of three locally produced black soap variants and two commercially produced soaps with a view to promoting and increasing the awareness of the efficacy and economic viability of these skin-care products.

MATERIALS AND METHODS

Collection of Commercially Produced Antimicrobial Soaps

The Zee black soap® (ZBS) and Dudu-Osun soap® (DOS) were randomly purchased from local markets in Auchi, Edo

State, Nigeria.

Composition and formulation of the locally produced black soap

Three different locally produced soap samples with different concentrations and constituents used for this study were formulated as follows:

The locally produced black soap with turmeric (A) contains: 50g of African black soap (base), 20g of turmeric powder, 10g of shea butter, and 10g of orange peel powder. The locally produced black soap with honey (B) contains 50g of African black soap (base), 10g of shea butter, 10g of orange peel powder and 20ml of organic honey while the locally produced soap with lime (C) also contains 50g of African black soap (base), 10g of shea butter, 10g of orange peel powder and 20ml of lime juice. The soap samples were formulated and collected into sterile plastic packs using the guidelines described by Obi, (2014) and the soaps were labelled accordingly (Plate 1) Locally produced black soap with lime (LPBSL), Locally produced black soap with turmeric (LPBST) and Locally produced black soap with honey (LPBSH).

Sample Preparation

The samples were prepared according to the methods described by Aliyu et al. (2012) and Adebomi et al. (2017) in which 20g of each soap samples were weighed and dissolved in 250ml of distilled water (Plate 2) by continuous agitation.

The Test Microorganisms

The pure clinical isolates which included *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* used in the study were obtained from Microbiology Laboratory Auchi Polytechnic Auchi, Edo State, Nigeria and were then confirmed by inoculating them into their respective selective media thereafter, Gram staining, biochemical characterization and identification of the colonies were



Plate 2: dissolved locally produced black soaps

Table 1. Antimicrobial activity of the various indigenous black soap variants on the test organisms

Soap category	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albicans</i>
	Average zone of inhibition in millimeter (mm)		
ZBS	22	9	12
DOS	28	8	19
LPBST	41.1	10	24
LPBSL	8	4	6
LPBSH	39	7	13

Key: ZBS=Zee Black Soap®, DOS=Dudu-Osun Soap®, LPBST=Locally Produced Black Soap with Turmeric, LPBSL=Locally Produced Black Soap with Lime, LPBSH=Locally Produced Black Soap with Honey

carried out using standard methods as described by Kin et al., (2018).

Standardization of Inoculum

Each of the test organism in 24 hours old culture was aseptically inoculated into nutrient broth, incubated at 37°C and room temperature for 5 hours for yeast and bacterial growth respectively. The broth culture each was adjusted with sterile normal saline solution to 0.5 MacFarland standard before use (Kin et al., 2018).

Black soap susceptibility assay

The susceptibility of each of the test organism to the different soap samples was determined using agar well diffusion method described by Ndukwe et al. (2005). The plates were incubated at 37°C for 24 hours and a transparent ruler was used to measure the diameter of the clear zones of inhibition (mm) noticed on the plates (Cheesebrough, 2010). The test was carried out twice, and the mean of all readings was taken as the zone of inhibition in each case. The method of clinical and laboratory standard institute was adopted to compare the efficacy of the commercially produced soaps with locally produced soap samples on clinical bacterial and fungal isolates (Obi, 2014).

Statistical Analysis

The statistical package SPSS Version 22 was used to analyze the data. Analysis of variance of $P \leq 0.05$ was done to check for differences between treatment and where difference occurred, Duncan's multiple range test was used to separate the means. Frequency of susceptibility pattern of the isolates was also done using same statistical package. (Aliyu et al., 2012).

RESULTS AND DISCUSSION

The *in-vitro* antimicrobial efficacy of Indigenous Black Soaps (IBS) to promote and increase the awareness of the economic viability of these skin-care products were comparatively evaluated in this study. The results obtained from this study showed that commercially produced black soap and locally made black soap have significant antimicrobial activities against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*, although to a certain identifiable position as shown by the inhibition of growth pattern of the isolates (Table 1). This result is in agreement with the research of Varsha, (2016) who conducted a study on the antimicrobial activity of antiseptic soaps and herbal soap against selected human pathogen. His result revealed that most of the tested antiseptic and

Table 2. The mean percentage susceptibility pattern of the soaps on the test organisms

Bacterial/Fungi	Mean	Std. Error	P-value<0.05 Remark	95% Confidence Interval	
				Lower Bound	Upper Bound
<i>Staphylococcus aureus</i>	28.020	± 4.024	Very high	18.740	37.300
<i>Pseudomonas aeruginosa</i>	4.800	± 4.024	Very low	-4.480	14.080
<i>Candida albicans</i>	14.800	± 4.024	High	5.520	24.080

Key: Std. Error = standard error

Table 3. Analysis of Variance for susceptibility pattern of the soaps on the test organisms

Dependent Variable: Treatment

Variables	Sum of Squares	Df	Mean Square	F	Sig.
Soap Category	788.429	4	197.107	2.434	.132
Bacterial/Fungi	1356.561	2	678.281	8.377	.011
Error	647.779	8	80.972		
Total	2792.769	14			

R Squared = .768 (Adjusted R Squared = .594) Key: DF=degree of freedom, sig = significant

Table 4. The average susceptibility pattern of the different soap category on the test organisms

Soap category	Mean±Std Error	Sensitivity
ZBS	15.00±5.12	Moderate
DOS	19.00±5.12	High
LPBST	36.03±5.12	Very high
LPBSL	3.67±5.12	Very low
LPBSH	15.67±5.12	Moderate

Key: ZBS=Zee Black Soap®, DOS=Dudu-Osun Soap®, LPBST=Locally Produced Black Soap with Turmeric, LPBSL=Locally Produced Black Soap with Lime, LPBSH=Locally Produced Black Soap with Honey, Std.Error = Standard error

herbal soaps had antimicrobial activity against *E. coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* at a different diameter zone of inhibition. The result (Table 1) in this study also showed that generally, the black soap formulated with turmeric gave the highest zone of inhibition (41.1mm), followed by those formulated with honey (39mm), while lime black soap had the least inhibition zone diameter (IZD) of 8mm. This also agreed with the findings of Varsha, (2016), which justified that the composition of herbal soap determines its efficacy. In his study, the soap formulated with Neem showed the highest antimicrobial activity against all the tested pathogens. However, among the commercially produced black soap Dudu-Osun® was more effective against the selected test organisms than Zee black soap®.

Result of susceptibility pattern (Table 2) as shown by the diameter of the zone of inhibition by the test organism (*Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*) depicted that there was a significant ($P \leq 0.05$) difference within the IZD of the different soap samples. The percentage of activity of the soaps on the test organisms (Table 2) showed that *Staphylococcus aureus* displayed the highest sensitive to most of the soaps compared to *Pseudomonas aeruginosa* and *Candida albicans*. This difference in the susceptibility pattern between the test organisms could be as a result of

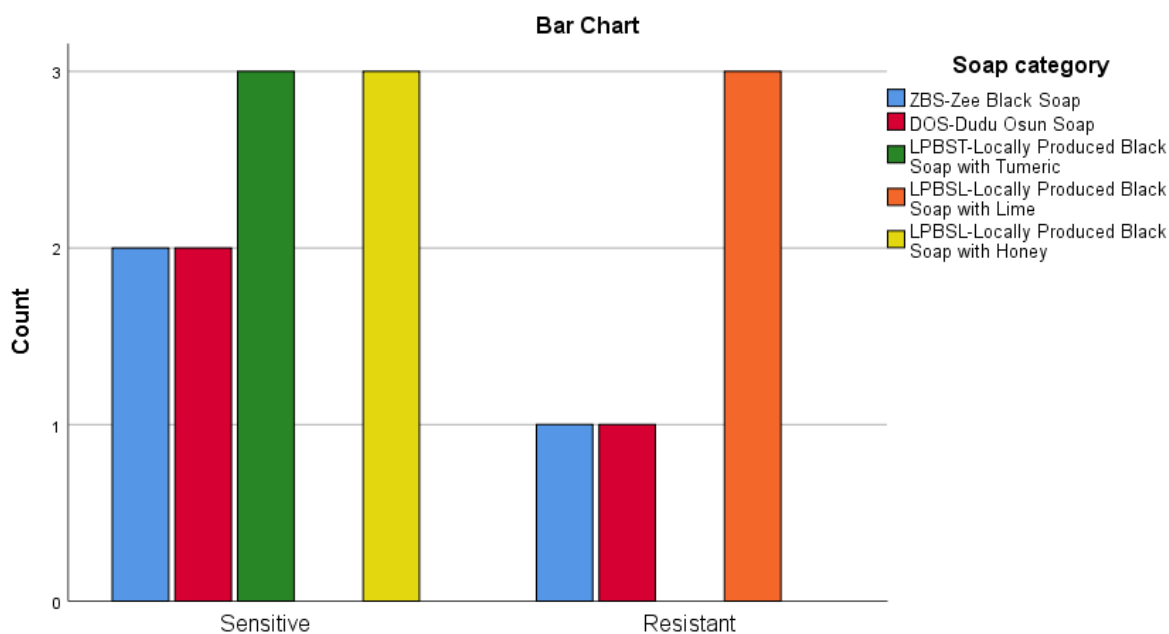
difference in the compositions of the bacteria cell wall (Kin et al., 2018) and the ability of *Pseudomonas aeruginosa* to develop resistance through the production of inactivation enzymes or restricted permeability of the outer-membrane as well as its efflux system when exposed repeatedly to antimicrobial agent (Angulu et al., 2020). This also agreed with the work of Obi, (2014) who reported that repeated use of an antimicrobial agent like soap, could reduce their efficacy and cause some organisms to develop resistance. *Pseudomonas aeruginosa* was resistant to most of the black soaps used in this study. The comparison test using Least Significant Different (LSD) confirms that *Staphylococcus aureus* sensitivity in term of soap category is high and significantly different from *Pseudomonas aeruginosa* and *Candida albicans* at varying replications.

The Table 3 shows the analysis of variance result of the significant difference in the mean of soap category at varying replications. The findings indicated that soap category is not statistically significant (sig. 0.132 > 0.05) while sensitivity to bacteria and fungi is significant (sig. 0.011 < 0.05). This implies soap sensitivity to some bacteria and fungi are different. The experiment suggests that the sensitivity of soap category (Zee Black Soap®, Dudu-Osun Soap®, Locally Produced Black Soap with Turmeric, Locally Produced Black Soap with Lime and Locally Produced Black Soap with Honey) to bacterial / fungi (*Staphylococcus*

Table 5. Comparisons of the average susceptibility pattern of the different soap category

	Soap Category	Mean	Std.Error	P-value<0.05	Remark
	ZBS	11.033	7.347	.172	Not sig.
LPBSL	DOS	7.033	7.347	.366	Not Sig.
	LPBST	22.367*	7.347	.016*	Sig. (different)
	LPBSH	10.367	7.347	.196	Not sig.

*The mean difference is significant at the 0.05 level. Key: ZBS=Zee Black Soap®, DOS=Dudu-Osun Soap®, LPBST=Locally Produced Black Soap with Turmeric, LPBSL=Locally Produced Black Soap with Lime, LPBSH=Locally Produced Black Soap with Honey, Std.Error = Standard error Std.Error = Standard error

**Figure 1:** Bar graph of sensitivity and resistivity of soap treatment category

aureus, *Pseudomonas aeruginosa* and *Candida albicans*) is explained by 0.594 revealing 59.4% total variations.

The experimental results (Table 4) of category of treated black soap (Zee Black Soap®, Dudu Osun Soap®, Locally Produced Black Soap with Turmeric, Locally Produced Black Soap with Lime and Locally Produced Black Soap with Honey) and bacterial/fungi (*Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*) sensitivity at varying replications revealed that both Zee Black Soap® and Locally Produced Black Soap with Honey have moderate sensitivity to fungi and bacteria while the Locally Produced Black Soap with Lime was less sensitivity.

Dudu Osun Soap® has high sensitivity and Locally Produced Black Soap with turmeric presents the very high sensitivity to bacterial/fungi. This implies that Locally Produced Black Soap with turmeric is better compared to the Locally Produced Black Soap with Lime that showed low sensitivity to the test bacteria and fungi.

From the table of soap comparison, (Table 5) Locally

Produced Black Soap with Lime is statistically different from other soaps (Zee Black Soap®, Dudu Osun Soap®, Locally Produced Black Soap with Turmeric and Locally Produced Black Soap with Honey) in terms of sensitivity to bacteria and fungi. However, Zee Black Soap®, Dudu Osun Soap®, Locally Produced Black Soap with turmeric and Locally Produced Black Soap with Honey have the same level of sensitivity.

Sensitivity and resistivity of soap treatment under study (Figure 1) confirms that based on the bar graph that Locally Produced Black Soap with Turmeric and Locally Produced Black Soap with Honey are most sensitive to bacteria/fungi when compared to the Zee black soap® and Dudu Osun Soap®. The Locally Produced Black Soap with Lime is most significantly resistant among other soap category.

In terms of sensitivity and resistivity of bacteria/fungi to soap treatment, the Figure 2 showed that *Staphylococcus aureus* and *Candida albicans* have higher sensitivity than *Pseudomonas aeruginosa*. However, *Pseudomonas*

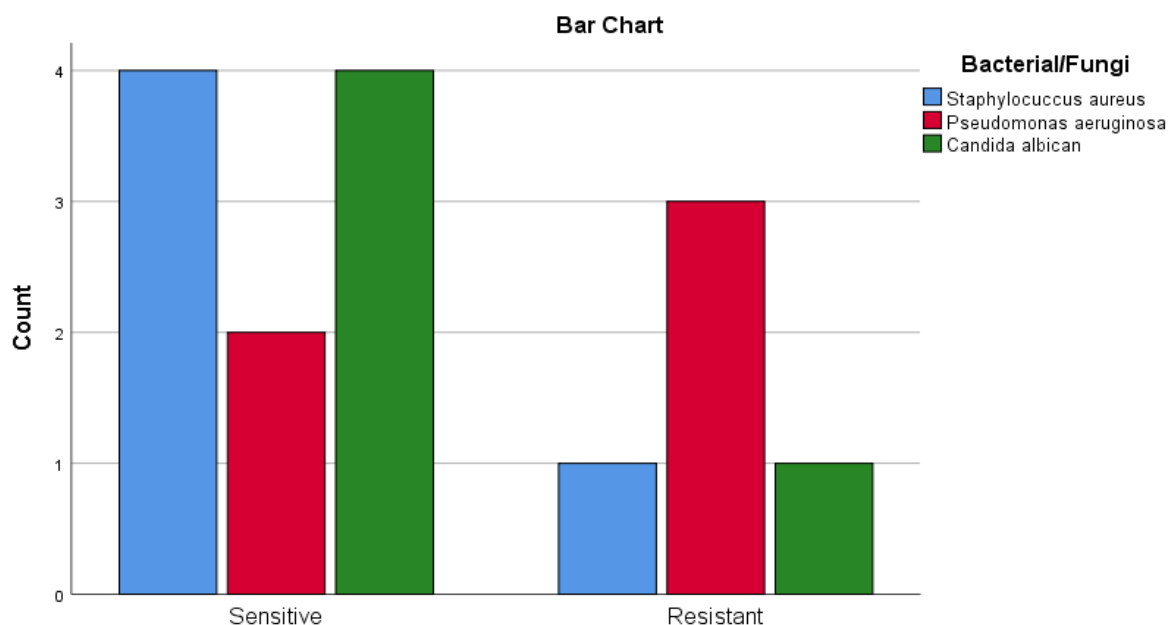


Figure 2: Bar graph of sensitivity and resistivity of test organisms (Bacterial/Fungi)

aeruginosa possess higher resistivity.

CONCLUSION

The findings of this study show that black soaps have antimicrobial activity and could be attributed to the presence of its unique bioactive constituents. However, the locally produced black soap with turmeric showed higher antimicrobial activity against the test organisms while locally produced black soap with lime was less effective on the test organisms. Therefore, it is recommended that locally produced soaps with turmeric and honey could be considered as viable alternatives in future topical and skin-care formulations for treatment of infections caused by the tested organisms used in this study. This also justify the use of traditional materials such as those found in black soap as potent antiseptic agents.

Conflict of interests

The authors declared that there is no conflict of interest regarding this manuscript.

Acknowledgement

We wish to appreciate all the cited authors and those who contributed to the success of this research work.

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