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Comparative Studies in the Release of Sodium and Potassium Ions by Indigenous Black Soaps from some Selected Skin Pathogens

¹K.A. Oyeniran, ¹M.K. Oladunmoye and ²H.O. Aladeselu

¹Department of Microbiology, Federal University of Technology, Akure, P.M.B. 704, Akure, 342052, Nigeria

²Department of Biochemistry, Federal University of Technology, Akure, P.M.B. 704, Akure, 342052, Nigeria

Corresponding Author: K.A. Oyeniran, Department of Microbiology, Federal University of Technology, Akure, P.M.B. 704, Akure, 342052, Nigeria

ABSTRACT

The indigenous black soap possesses antimicrobial activities with varying mechanisms of action. In the present study, the comparative release of sodium and potassium ions from clinical isolates: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Trichophyton rubrum* and *Candida albicans* by three different indigenous black soaps were investigated by flame photometry method. The black soaps were subjected to qualitative and quantitative screening for phytochemical using standard methods. Sodium ion was leaked to a value of 833 ppm for *S. aureus* while potassium ion to a value of 20 ppm for *T. rubrum*. Qualitative and quantitative screening for phytochemicals showed saponin was the highest with values between 20.50-33.31 mg g⁻¹ while tannin the lowest between 1.05-2.05 mg g⁻¹ across the black soap samples. This study has posited that the antimicrobial activities of the black soap may be attributed to its phytochemicals and that the soap has lytic effect on the pathogens.

Key words: Black soap, phytochemicals, sodium ions, skin pathogens, intracellular-ions

INTRODUCTION

Antimicrobial activity of the indigenous black has been well established as a result its acceptance and wide usage among the people of West Africa from time immemorial. Indigenous black soap commonly called “black soap” is a viable ingredient in traditional medicine preparations where it serves as excipient (Oladunmoye and Oyeniran, 2015). The black soap is gentle and mild on the skin it prevents bumps and ensures smooth and supple skin. Aliyu *et al.* (2012) opined that it possesses antimicrobial activities specifically against Gram positive microorganism by its ability to release partially saponified oil that disrupts the cell wall of the organism. Folklore medicine in Nigeria cannot be mentioned specifically in the context of skin associated therapy without referencing the indigenous black as it majorly serves as a medium of conveying traditional concoction (Anyakoha, 2011). Black soap is mild and so can be applied to hair, face and body. Its anti-aging properties can reduce fine lines and wrinkles promoting elasticity and youthful look (Ikpo *et al.*, 2012). Dark spots and blemishes are readily extirpated by the cleansing and deodorizing action of its natural ingredients. Black soap does not promote antimicrobial resistance because it contains several bioactive compounds that work in synergy; thus many people prefer its natural scent to the synthetic soaps (Okeke, 2009). Black soap is also a natural source of vitamins A, E and iron which help to rejuvenate the skin. Mechanisms of action of an antimicrobial agent vary from one another. Most medicinal plants for instance prevent cell wall and protein

synthesis, interfere with nucleic acid biosynthesis and disrupt cytoplasmic membrane with eventual loss of cellular integrity (Mailard, 2002). There has been dearth of information regarding the mechanisms of action of the indigenous black soap and its ability to release sodium and potassium ions from the cells of microorganisms. It is therefore necessary to investigate this plausible mechanism of its antimicrobial action.

MATERIALS AND METHODS

Collection of test organisms and black soap

Purified clinical isolates: *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Candida albicans* and *Trichophyton rubrum* were collected from the University College Hospital (UCH), Ibadan. Necessary biochemical test were conducted to confirm their identities and were maintained at 4°C for subsequent usage. Plain indigenous black soaps were bought from market (A). Indigenous black soaps were also prepared by saponification and fortified separately with brown (B) and white (C) eggshell powder, effectively at 20 g/250 mL of the molten soap.

Preparation of standard inoculum: Test bacteria were previously cultured in nutrient broth and incubated at 37°C for 18 h. Yeast was cultured in potato dextrose broth and incubated at 35°C for 48 h after which they were suspended in saline solution (0.85% NaCl) and adjusted with the aid of a spectrophotometer (Unico1100RS) to match a turbidity of 0.5 McFarland standards at wavelength of 540 nm according to the method described by CLSI (2010). The dermatophyte was standardized by flooding the surface of the Petri-plates with filamentous fungus in confluence growth with 10 mL of sterile distilled water. The 0.5 mL containing approximately 2.4×10^6 cells mL⁻¹ was used.

Determination of mechanism of action of indigenous black soap: A 0.5 mL each of the standardized organism was added to 5 mL of the prepared Mueller Hinton and Sabaraud dextrose broth to which 0.5 mL (150 mg) of the black soap solution was added. Control was soap and broth only without organism. This was done in order to eliminate the possibility of the black soap being naturally high in sodium ions interfering with the results. All tubes were incubated for 18 and 72 h for bacteria, yeast and the mould, respectively. The solution was centrifuged at 7000 rpm and 3 mL of the supernatant was diluted with 50 mL sterile distilled water and analyzed using a flame photometer (Sherwood 360) at 589 and 766 nm for sodium and potassium ion leakage respectively. The above process was also repeated for the selected commercial creams as appropriate.

Calculation:

$$\text{Sodium (Na) or potassium (K) (ppm)} = \frac{\text{Mean absorbance} \times V}{W}$$

where, V is the volume of digest and W is the weight of sample.

Qualitative screening of indigenous black soaps for phytochemicals: All reagents were analytical grade. Analyses were carried out according to the methods described by AOAC (2007).

Alkaloid: The 0.5 g of the sample was stirred 5 mL of 1% aqueous HCl on a steam water bath, 1 mL of the filtrate was treated with a few drops of Dragendorff reagent, blue black turbidity was taken as preliminary evidence for the presence of alkaloid.

Saponin: The 0.5 g of sample was shaken with distilled water in a test tube frothing which persist on warming was taken as preliminary evidence for the presence of saponin.

Tannin: Exactly 0.5 g of sample was stirred with 100 mL of distilled water, filtered and ferric chloride reagent was added to the filtrate a blue black green or blue green precipitate was taken as evidence for presence of tannin.

Phlobatannin: Deposition of red precipitate when 0.5 g of the soap was boiled with 1% aqueous HCl was taken as evidence for the presence of phlobatannin.

Flavonoid: A 0.5 g of the black soap was stirred with 20 mL of dilute ammonia solution a yellow colouration was observed, the disappearance of the yellow colour after the addition of 1 mL conc. H_2SO_4 indicate the presence of flavonoid.

Terpenoid: A 0.5 g of the sample was mixed with 20 mL of chloroform and filtered 3 mL of concentrated H_2SO_4 was added to the filtrate to form a layer. A reddish brown colour at the interface was observed which indicate the presence of terpenoid.

Cardiac glycosides: The 2.0 g of sample was dissolved in pyridine and a few drops of 2% sodium nitroprusside with few drops of 20% NaOH were added. A deep red colouration which faded to a brownish yellow indicates the presence of cardenolides.

Quantitative screening of the indigenous black soaps for phytochemicals: All reagents were analytical grade. Analyses were carried out according to standard methods and are acknowledged by means of reference.

Determination of saponin content: The spectrophotometric method of Brunner (1994) was used for saponin determination. A 2 g of sample was weighed into a 250 mL beaker and 100 mL of isobutyl alcohol or (But-2-ol) was added. Shaker was used to shake the mixture for 5 h to ensure uniform mixing. The mixture was filtered with Whatman No. 1 filter paper into 100 mL beaker containing 20 mL of 40% saturated solution of magnesium carbonate (MgCO_3). The mixture obtained was filtered again using Whatman No. 1 filter paper to obtain a clean colourless solution. One milliliter of the colourless solution was taken into 50 mL volumetric flask using pipette and 2 mL of 5% iron (III) chloride (FeCl_3) solution was added and made up to the mark with distilled water. It was allowed to stand for 30 min for the colour to develop. The absorbance was read against the blank at 380 nm.

Determination of flavonoid content: The total flavonoid content was determined using a slightly modified method reported by Meda *et al.* (2005). A 0.5 mL of appropriately diluted sample was mixed with 0.5 mL methanol, 50 μL 10% AlCl_3 , 50 μL 1 M potassium acetate and 1.4 mL water and allowed to incubate at room temperature for 30 min. The absorbance of the reaction mixture

was subsequently measured at 415 nm and the total flavonoid content was subsequently calculated. The non flavonoid polyphenols were taken as the difference between the total phenol and total flavonoid content.

Determination of tannin content: About 1.0 g of sample was weighed into a 50 mL sample bottle. The 10 mL of 70% aqueous acetone was added and properly covered. The bottle were put in an ice bath shaker and shaken for 2 h at 30°C. Each solution was then centrifuged and the supernatant stored in ice. About 0.2 mL of each solution was pipetted into the test tube and 0.8 mL of distilled water was added. Standard tannin acid solutions were prepared from a 0.5 mg mL⁻¹ of the stock and the solution made up to 1 mL with distilled water. The 0.5 mL of Folin-ciocateau reagent was added to both sample and standard followed by 2.5 mL of 20% Na₂CO₃ the solution were then vortexed and allowed to incubate for 40 min at room temperature, its absorbance was read at 725 nm against a reagent blank concentration of the same solution from a standard tannic acid curve that was prepared (Makkar and Goodchild, 1996).

Determination of terpenoid content: A 2.0 g of soap sample was soaked in 50 mL of 95% ethanol for 24 h. The extract was filtered and the filtrate extracted with petroleum ether (60-80°C) and concentrated to dryness. The dried ether extract was treated as total terpenoids (Ferguson, 1956).

Determination of cardiac glycosides content: The procedure described by Sofowora (1993) was used for the determination of cardiac glycosides. The 10 mL of the soap water extract was pipetted into a 250 mL conical flask. The 50 mL chloroform was added and shaken on vortex mixer for 1 h. The mixture was filtered into 100 mL conical flask. The 10 mL of pyridine and 2 mL of 29% sodium nitroprusside were added and shaken thoroughly for 10 min. The 3 mL of 20% NaOH was added to develop a brownish colour. Glycosides standard (Digitoxin), concentrations which ranged from 0-50 mg mL⁻¹ were prepared from stock solution and the absorbance was read at 510 nm.

RESULTS

Sodium and potassium ions released: Sodium and potassium ions were leaked by the black soaps from the cell of the organisms as illustrated in Fig. 1. Sodium ion was leaked to a high value of 833 ppm for *S. aureus* while potassium ion to the very lowest value of 20 ppm for *Trichophyton rubrum*. For the selected commercial creams, sodium ions were leaked to a value of 400 ppm by Acneaway as well as Funbact A (Fig. 2). Ions leakage from *T. rubum* was generally lower across boards.

Qualitative phytochemical profile of indigenous black soaps: Qualitative phytochemical screening has shown that black soaps contain saponin, tannin, flavonoid, terpenoid and cardiac glycosides. Alkaloid and phlobatannin were absent (Table 1).

Quantitative phytochemical profile of indigenous black soaps: The quantitative analysis of the black soaps for phytochemicals revealed variation in the compositions. The occurrence levels are reported in Table 2. Saponin has the highest occurrence and ranged between 20.0-33.31 mg g⁻¹ in the studied samples. Tannin has the lowest occurrence with values ranging between 1.0-2.05 mg g⁻¹ in the samples. The values were not significantly different except for black soap fortified with white eggshell powder.

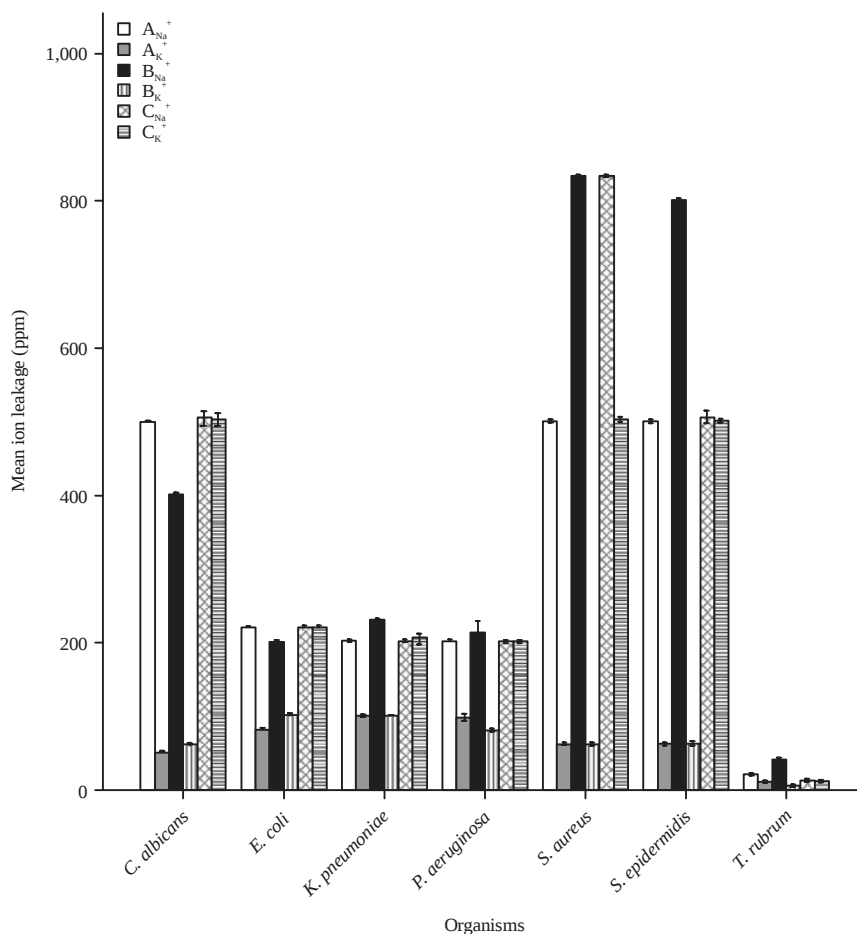


Fig. 1: Comparative sodium and potassium ions leakage from test organisms by indigenous black soaps. A: Black soap market sample, B: Black soap fortified with brown eggshell powder, C: Black soap fortified with white eggshell powder, Na⁺: Sodium ions, K⁺: Potassium ions

Table 1: Qualitative phytochemical profile of indigenous black soaps

Phytochemicals	A	B	C
Alkaloid	-	-	-
Saponin	+	+	+
Phlobatannin	-	-	-
Flavonoid	+	+	+
Tannin	+	+	+
Terpenoid	+	+	+
Cardiac glycosides	+	+	+

A: Black soap market sample, B: Black soap fortified with brown eggshell powder, C: Black soap fortified with white eggshell powder, +: Present, -: Absent

DISCUSSION

The mechanism of action of indigenous soap investigated by flame assay of the broth culture for sodium and potassium ions showed that ions were leaked in various proportions as shown in the results. This can only mean that the black soap believably induced antibacterial effects through the leakage of intracellular materials which is demonstrated in its membrane damaging action. The indigenous black even in the crude state were able to compete favourably with purified

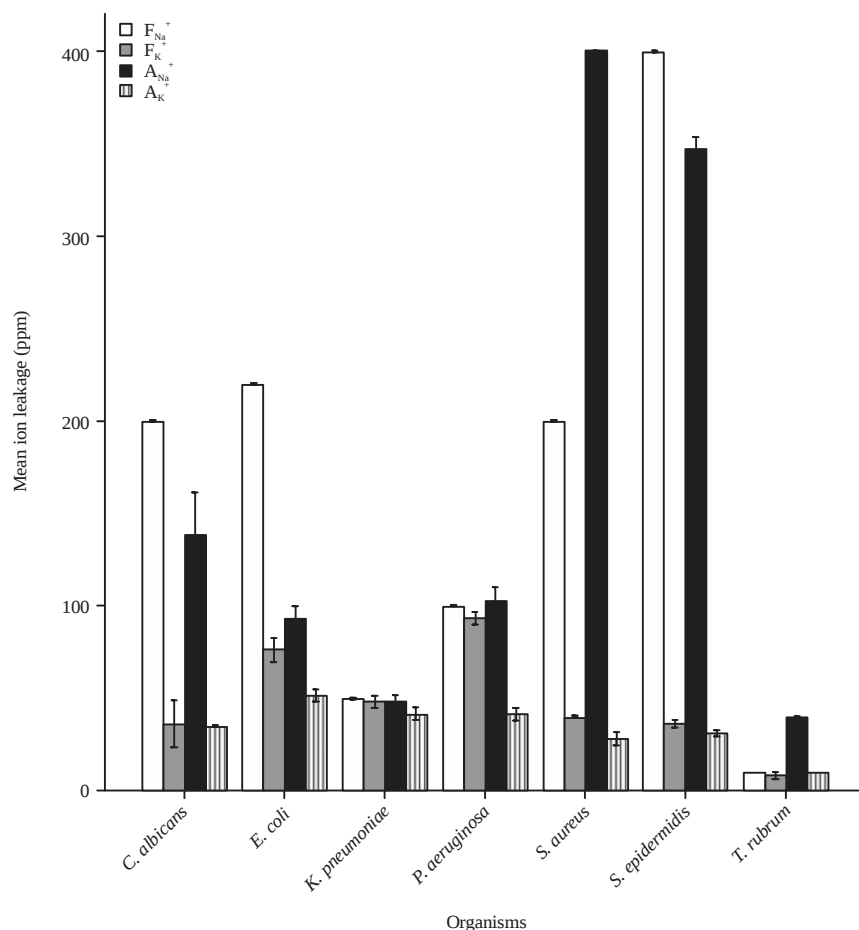


Fig. 2: Comparative sodium and potassium ions leakage from test organisms by selected commercial creams. F: Funbact A, A: Acneaway, Na⁺: Sodium ions, K⁺: Potassium ions

Table 2: Quantitative phytochemical compositions of indigenous black soaps

Phytochemicals (mg g ⁻¹)	A	B	C
Saponin	20.50±0.92 ^c	28.36±0.23 ^c	33.31±0.82 ^b
Flavonoid	3.50±0.89 ^c	4.33±0.00 ^c	3.83±0.89 ^b
Tannin	1.05±0.03 ^a	2.05±0.02 ^a	2.05±0.03 ^a
Terpenoid	9.96±0.84 ^c	9.09±0.72 ^c	6.66±0.04 ^a
Cardiac glycosides	7.36±1.31 ^c	5.72±1.91 ^b	8.79±2.81 ^a

Values represent Mean±Standard Deviation of triplicate readings. Superscripts with the same alphabets in a row are not significantly different (p>0.05). A: Black soap market sample, B: Black soap fortified with brown eggshell powder, C: Black soap fortified with white eggshell powder

selected commercial creams. This is at equivalence with past findings of Aliyu *et al.* (2012) and Adebajo *et al.* (2004) that antimicrobial activities of the black soap on bacterial and yeasts like *C. albicans* is due to its oil contents with disruptive actions on cell wall. Oladunmoye *et al.* (2007) has also submitted that ethanolic extract of *Cassia occidentalis* a plant also had lytic effects on pathogenic microorganisms as intracellular ions were leaked from their cells. This readily explains that the indigenous black soaps may as well have similar mechanism of action to medicinal plants. Antifungal action of the black soap as well as the commercial creams on *T. rubrum* may not be lytic in nature as evident in the lower levels of leaked ions; this could be explained in terms of other

mechanisms like the inhibition of ergosterol found in the fungal cell membrane by interfering with cytochrome P450 dependent demethylation of lanosterol a common precursor for cholesterol and ergosterol in humans and fungi respectively (Jawetz *et al.*, 2010).

Phytochemicals are compounds evolved by plants for their own defenses, beside other biological functions are tailored to minimize utilization of nutrients like proteins, vitamins and minerals to the maximum (Felix and Mello, 2000; Shanthakumari *et al.*, 2008). Saponin recorded the highest level of occurrence in the black soaps basically because it is a major component of soaps as a result of saponification and variations observed for instance, the black soap fortified with white eggshell powder had highest saponin followed by the black soap fortified with brown eggshell powder could be predicated on the interactions of compounds at fortification level.

Previous submission of Oladunmoye *et al.* (2009) posited that saponin possesses haemolytic, antimicrobial, insecticidal and molluscicidal properties because of the presence of a lipid-soluble aglycone and water soluble sugar chain (s) in their structure (amphiphilic nature). Recent evidence shows that saponins possess hypocholesterolemic, immunostimulatory and anticarcinogenic properties (Gemedé and Ratta, 2014).

CONCLUSION

Antimicrobial activities of the indigenous black soap could be attributed to the presence of these phytochemicals. The indigenous black soap has lytic effect on the pathogens.

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