Comparative quality, efficacy, heavy metal content and safety of selected african black soaps for skincare

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ABSTRACT

Background: There is an upsurge in the utilization of African black soaps. Hence, there is a need for evaluation of their quality and safety.

Objectives: The aim of this study was to evaluate the quality, efficacy, heavy metal content and safety of selected African black soaps.

Methods: The physicochemical properties of twelve black soaps were evaluated. Microbial load was analyzed and anti-microbial activity was evaluated. Heavy metal content was analyzed using atomic absorption spectrophotometer, environmental toxicity was analyzed using *Allium cepa* test, and skin irritation tests were also evaluated.

Results: The black soaps showed fair physicochemical properties. The soaps showed the absence of microbial colonies. All the soap samples showed antimicrobial efficacy against all the organisms tested except sample UPS2 which exhibited no anti-microbial activity against *Pseudomonas aeruginosa*. The study revealed the presence of heavy metals (Pb, Cd, As, Hg and Cr^{3+}) which were within acceptable limits; none of the soaps posed a carcinogenic or non-carcinogenic risk. The Allium cepa test showed that African black soaps tested retarded onion root growth. The skin irritation test (wash-off test) showed that the post-wash pH was between 4.95- 5.76. There was an increase in the post-wash trans-epidermal water loss in 87.5 % of the subjects and decrease in post-wash sebum content in 62.5 % of the subjects.

Conclusion: This study has shown that there is a great prospect in the utilization of black soaps; however, there is a need for regulation of raw materials, standardization of manufacturing processes and proper disposal of black soaps in order to safeguard public health.

Keywords: African black soap, physicochemical, antimicrobial, *Allium cepa* test, skin irritation.

Comparaison de la qualité, de l'efficacité, de la teneur en métaux lourds et de la sécurité de savons noirs africains sélectionnés pour les soins de la peau

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RÉSUMÉ

Contexte: L'usage des savons noirs africains est en recrudescence. L'évaluation de leur qualité et leur sureté s'avère donc nécessaire.

Objectifs: Le but de cette étude était d'évaluer la qualité, l'efficacité, la teneur en métaux lourds et la sureté des savons noirs africains sélectionnés.

Méthodes: Les propriétés physicochimiques de douze savons noirs ont été évaluées. La charge microbienne a été analysée et l'activité antimicrobienne a été évaluée. La teneur en métaux lourds a été analysée à l'aide d'un spectrophotomètre d'absorption atomique, la toxicité environnementale a été analysée à l'aide du test *Allium cepa* et des tests d'irritation cutanée ont également été évalués.

Résultats: Les savons noirs ont montré des propriétés physico-chimiques considérables. Les savons ont montré l'absence de colonies microbiennes. Tous les échantillons de savon ont démontré une efficacité antimicrobienne contre tous les organismes testés, à l'exception de l'échantillon UPS2 qui n'a présenté aucune activité antimicrobienne contre *Pseudomonas aeruginosa.* L'étude a révélé la présence de métaux lourds (Pb, Cd, As, Hg et Cr^{3+}) qui se situaient dans des limites acceptables ; aucun des savons ne présentait de risque cancérigène ou non cancérigène. Le test *Allium cepa* a montré que les savons noirs africains testés retardaient la croissance des racines d'oignon. Le test d'irritation cutanée (test de lavage) a montré que le pH après lavage se situait entre 4,95 et 5,76. On a constaté une augmentation de la perte d'eau trans-épidermique après lavage chez 87,5 % des sujets et une diminution de la teneur en sébum après lavage chez 62,5 % des sujets.

Conclusion: Cette étude a montré l'existence de grandes perspectives dans l'utilisation des savons noirs ; cependant, il est nécessaire de réglementer les matières premières, de normaliser les processus de fabrication et d'éliminer correctement les savons noirs afin de protéger la santé publique.

Mots clés: Savon noir africain, physicochimique, antimicrobien, test *Allium cepa*, irritation cutanée

INTRODUCTION

African black soaps are soaps made from ash-derived alkali which are obtained from plant-based materials, mainly agricultural wastes and oil extracted from herbs without any cosmetic-enhancing benefits, although they may contain natural fragrances and/or organic ingredients included as additives.[1] The raw materials for manufacturing these soaps are readily available, inexpensive, and generated locally from Africa. African black soaps are largely employed for their cleansing and medicinal properties.[2] These soaps are also used as bases for formulations of many herbal recipes meant for the management of skin infections.[3] They are believed to possess antimicrobial, exfoliating, anti-acne, scar-fading, and skin-toning activities among other medicinal properties. There is an increase in the demand for African black soaps, this might be due to the fact that they are prepared from natural ingredients.[1]

Production of black soaps is like the general soap making process. Saponification which is the process of soap formulation, involves reaction between a base and fatty acid to form glycerol and soap molecules.[4] During black soap production, one of the basic ingredients- sodium or potassium hydroxide (lye) is obtained by burning, mixing and leaching of agricultural wastes.[5] The lye used in general soap making is obtained through chemical reactions while the lye obtained in black soap production is from natural origin.[6] Palm kernel oil or palm oil are majorly used as the source of fatty acids. The black soaps produced in South-Western Nigeria contain either wood ash, cocoa pod ash and palm kernel oil with or without fillers.[5] Different local black soap producers use different ingredients, but they undergo a similar production process.[7,8] After, the saponification process, the final stage in soap production is the value-addition stage which involves addition of extra ingredients or fillers. Fillers are usually added to make black soap look bigger and affordable to the customers. Variability in composition of black soaps can be achieved by adding some extra ingredients to the black soap and hence, the soap can be used for some specific purposes.[9] There are different formulations of African black soaps that are in use ranging from the ones that are produced by rural dwellers that are dispensed by wrapping them in polyethene bags, properly packaged ones but not registered by the regulatory authorities and brands that are packaged by manufacturers and registered by the regulatory authorities. In recent times, African black soap has been industrially modified into better and more acceptable forms with different trade names. These soaps contain different additives for different purposes like egg yolk, egg white, honey and Aloe vera, which helps in moisturizing the skin; turmeric which softens and smoothens the skin; coconut oil for hydration; neem oil as an antibacterial and rose oil as fragrance agent.[10]

The qualities of black soaps are determined by ingredients used in their formulations as well as the formulation process employed, hence the phytochemical, physiochemical, moisturizing, medicinal, safety profiles and other properties of the soaps are dependent on the method of preparation and ingredients of the soaps.[1]

The antimicrobial, physiochemical and phytochemical properties of black soaps suggest that they will have advantageous health effects on the skin.[1] However, there is still limited data on the heavy metals content of these black soaps, the effects on the skin as well as their environmental impact assessment. There is a need for establishing guidelines on the formulation of these black soaps to ensure safe use by the populace. Hence, this study was aimed at evaluating the quality, efficacy, heavy metal content and safety of selected African black soaps used for skincare.

METHODS

Sample collection

Twelve samples of commonly used African black soaps (ABS) were randomly selected from some major markets in the three senatorial districts (Lagos East, Lagos West and Lagos Central) in Lagos State, Nigeria between March and April 2022 (Table 1). Three major categories of ABS used in the study were raw and unpackaged (locally made ABS, dispensed in wrappers without any product information); packaged and unregistered (refined and packaged but not registered); and packaged and officially registered (refined and packaged ABS that has the approval of National Agency for Food and Drug Administration and Control (NAFDAC), the regulatory body for foods and drugs in Nigeria.

Ethics approval and consent to participate

Ethical approval was obtained from the Human Research and Ethics Committee of Lagos University Teaching Hospital, Idi-araba Lagos, Nigeria with Health Research Committee assigned No. ADM/DSCST/HREC/APP/S187. All the human volunteers gave a verbal and written informed consent.

Table 1: The African black soap codes and product information

Sample Category	Sample Code	Product Information
Raw and unpackaged black soap	REI	Locally wrapped soap from Lagos East Ikorodu LGA
	RES	Locally wrapped soap from Lagos East Shomolu LGA
	RWA	Locally wrapped soap from Lagos West Agege LGA
	RWM	Locally wrapped soap from Lagos West Mushin LGA
	RCI	Locally wrapped soap from Lagos Central Lagos Island LGA
	RCS	Locally wrapped soap from Lagos Central Surulere LGA
Packaged and unregistered black soap	UPS1	Packaged unregistered soap from Lagos East
	UPS2	Packaged unregistered soap from Lagos West
	UPS3	Packaged unregistered soap from Lagos Central Lagos
Packaged and NAFDAC registered black soap	NBS1	NAFDAC registered soap from Lagos East
	NBS2	NAFDAC registered soap from Lagos West
	NBS3	NAFDAC registered soap from Lagos Central

NAFDAC = National Agency for Food and Drug Administration and Control, LGA= Local

Phytochemical screening

The phytochemical screening was evaluated according to the procedure employed by a previous research. Phenolic acids, steroids, saponins, alkaloids, flavonoids and tannins were screened.[11]

Evaluation of physicochemical properties

The physicochemical properties carried out in the study included moisture content, free caustic alkali (FCA), water insoluble matter, alcohol insoluble matter, chloride content, total fatty matter (TFM), foam height and pH.

Moisture content

A known mass (1 g) of each sample was weighed and dried on a previously weighed paper in the oven at 100 ^{o}C to a consistent weight.[12] The relationship in Eq. 1 was used to determine the moisture content.

$$\textit{Moisture content (\%)} = \frac{(W2-W1)}{W} \times 100 \qquad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

Where, W2 = weight of dried paper + soap sample (g), W1 = weight of dried paper + dried soap sample (g) W = weight of soap sample used (g).

Free caustic alkali

FCA was carried out according to a previous method with some modifications.12 0.25 g of each sample was weighed and dissolved in 25 mL of distilled water. Activated charcoal was added and boiled on a hot plate for 30 min at 100 ^{o}C. The heated mixture was filtered and the beaker rinsed with distilled water. The filtrate was made up to 100 mL mark. Afterwards, 20 mL of the filtrate was titrated against 0.25 M nitric acid. A blank titration was also carried out by titrating 20 mL of distilled water against 0.25 M nitric acid. FCA content was calculated using Eq. 2.

$$\text{Free caustic alkali \%} \quad \frac{(V)\,(N)\,(F)}{W} \times 100 \qquad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

Where, V = volume of alkali solution (mL), N= normality of alkali solution, F = factor of 4.7 for potassium oxide (K_2O) and W = sample weight (grams).

Water insoluble matter

The procedure described by Oyekunle et al., was adopted.[12] Each soap sample (3.5 g) was dissolved in 50 mL hot distilled water and the resulting solution was filtered through a filter paper that had been dried to a constant weight (W_1) at 103 ^{o}C. The percentage of matter insoluble in water, using the formula in Eq. 3 was determined after drying the filter paper and residue in the oven to a constant weight (W_2).

$$\text{Matters insoluble in water \%} = \frac{(W2-W1)}{W} \times 100 \qquad \ldots\ldots\ldots\ldots\ldots\ldots \quad (3)$$

Where W_2 = weight of dried filter paper + dried residue (grams), W_1 = weight of dried filter paper (grams) and W = weight of soap sample used (grams).

Alcohol insoluble matter

The procedure described by a previous research work was adopted.[12] Each soap sample (3.5 g) was dissolved in 50 mL hot ethanol and the resulting solution was filtered through a filter paper that had been dried to a constant weight (W_1) at 103 ^{o}C. The percentage of matter insoluble in alcohol, using the formula in Eq. 4 was determined after drying the filter paper and residue in the oven to a constant weight (W_2).

$$\text{Matters insoluble in alcohol \%} = \frac{(W2-W1)}{W} \times 100 \qquad \ldots\ldots\ldots\ldots\ldots\ldots \quad (4)$$

Where W_2 = weight of dried filter paper + dried residue (grams), W_1 = weight of dried filter paper (grams) and W = weight of soap sample used (grams).

Chloride content

The chloride content analysis was carried out as previously described.[12] According to this method, 0.5 g of soap sample was dissolved in 100 mL of water and filtered using a filter paper. Thereafter, 0.5 mL of concentrated nitric acid (HNO_3) was added to the filtrate and 0.1 N Silver nitrate ($AgNO_3$) solution was added slowly with constant stirring until no precipitate further forms. The determination was carried out under subdued light. The precipitate was heated to about 85 ^{o}C and a few drops of $AgNO_3$ solution was added. The beaker was allowed to stand for 1 h. The precipitate was then filtered using a properly dried filter paper with known mass. The mass of chloride was determined using the relationship in Eq. 5

$$\text{Chloride content \%} = \frac{(A-B)xNx7.46}{W} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (5)$$

Where A is volume of silver nitrate solution required by the sample (mL), B is volume of silver nitrate solution required by the blank solution (mL), N is normality of silver nitrate, and W is weight of sample (grams).

Total fatty matter

The total fatty matter was derived using a previous method[13] with some modifications. The total fatty matter was obtained using the formula in Eq (6).

$$\text{Total fatty matter (\%)} = \frac{[100 - \text{(mositure content+matter insoluble in alcohol)}]}{1.085} \quad \ldots\ldots\ldots\ldots \quad (6)$$

Foam height

Foam height was evaluated as previously described.[14] Approximately 0.5 grams of black soap was dispersed in 25 mL distilled water then, transferred into 100 mL measuring cylinder; volume was made up to 50 mL with distilled water. The measuring cylinder was given 25 strokes and made to stand till aqueous volume measured up to 50 mL. Foam height above the aqueous volume was measured.

pH

The method for determining pH was evaluated as described by a previous article.[12] Briefly, 0.5 g of the soap samples was dissolved in 10 mL distilled water to make a 5% w/v soap solution. A calibrated pH meter (pHep®, HANNA USA) was used to measure the pH of each soap sample in triplicates and the mean was computed.

Microbial load

The microbial load analysis was carried out using pour plate method according to a previous study[15] with some modifications.

Antimicrobial activity

The antimicrobial activity of the soap samples at concentrations of 250 mg/mL and 500 mg/mL were determined using agar diffusion method.[16] Triclosan was used as positive control at concentrations values of 0.050 mg/mL, 0.025 mg/mL, 0.0125 mg/mL and 0.00625 mg/mL. The assay organisms include clinical strains of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans*. They were obtained from the laboratory stock cultures of Pharmaceutical Microbiology Laboratory, College of Medicine, University of Lagos.

Determination of heavy metal content of black soaps

The black soap samples were analyzed and screened for the presence of heavy metals such as Mercury (Hg), Arsenic (As), Cadmium (Cd), Lead (Pb) and Chromium III (Cr^{3+}) using the atomic absorption spectrometer (SOLAAR S series, Thermo Electron Corporation, Cambridge UK), Each sample (2 g) was placed in a conical flask and 40 mL of Aqua Regia (a mixture of hydrochloric and nitric acid in a ratio of 3:1 by volume) was added. The sample was pre-digested for 5 h after covering the tube. Thereafter, the mixture was heated to 110 ^{o}C on a hot plate for 1 h and made to cool to room temperature. Distilled water (5 mL) was used for reconstitution and it was then filtered through a filter paper (Whatman No. 1) and made up to 25 mL again with distilled water. This was stored in a small, pre-treated plastic container and labelled for trace metal profiling. Preparation of blank was done by following the entire analytical procedure but omitting the soap samples. The concentrations of five heavy metals in the sample solutions were analysed using the atomic absorption spectrophotometer.[12]

Health risk assessment of black soaps

Non-carcinogenic health risk assessment

Non-cancer health risk assessment may be expressed as Margin of safety (MOS) and hazard index of the heavy metals present in the black soaps.[17] The MOS can be calculated by dividing the No Observed Adverse Effect Level (NOAEL) of the sample by its systemic exposure dose (SED) as shown in Eq. 7:[18]

$$MOS \; \frac{NOAEL}{SED} \quad \ldots\ldots \quad (7)$$

The No Observed Adverse Effect Level (NOAEL) refers to the maximum dose for no observable adverse or toxicological effects on humans and animals and is expressed as mg/kg body weight /day. The expression in Eq. (8) were used to get the NOAEL values of the heavy metals:[19]

$$NOAEL = RfD_{oral} \times UF \times MF \quad \ldots\ldots \quad (8)$$

Where RfD*oral* is the reference oral daily dose. The RfD_{oral} values for Pb, Cd, As, Hg and Cr^{3+} are 4×10^{-3},[20] 5×10^{-4},[21] 3×10^{-4},[22] 3×10^{-4},[23] and 1.5 mg/kg/day[24] respectively; UF= the uncertainty factor (100); and MF= the modifying factor (1). The minimum acceptable safe level for skin product of human is estimated to have a MOS value of 100. In this study, we assumed 50% bioaccessibility (as the midpoint scenario) and 100% bioaccessibility (as the worst-case scenario) of the heavy metals being investigated for evaluating the safety of these soaps.

The expression in Eq. (9) was used to evaluate SED.[12]

$$\text{SED (mg/kg/day)} = \frac{CsxAA \text{ x } SSAxF \text{ x } RFxBF}{BW} \times 100^{-3} \quad \ldots\ldots \quad (9)$$

Where Cs = the concentration of metal (µg/g) in the soap; AA = the daily quantity of soap used (20 grams); SSA= the exposed skin surface area with the soap (17500 cm^2); F= the average application frequency (1.43/day); RF= retention factor (0.01) is a factor used to correct the wash and dilution effect from using the products (shower gel, shampoo, etc.) on wet skin or hair;[18] BF= the bioaccessibility factor; the unit conversion factor is 10^{-3}; and BW= the average body weight of humans (60 kg).

The Hazard index (HI) is used for non-carcinogenic risk assessment of exposure to skin care products. HI (Eq. 11) is the sum of hazard quotients (HQ) (Eq. 10) for all heavy metals evaluated. A hazard quotient is the ratio of the systemic exposure dose to the reference dose.[17]

$$HQ = \frac{SED}{RFD} \quad \ldots\ldots \quad (10)$$

$$HI = \sum HQ = HQ_{Pb} + HQ_{Cd} + HQ_{As} + HQ_{Hg} + HQ_{Cr} \quad \ldots\ldots \quad (11)$$

If HQ and HI values < 1, the exposed local population (consumers) is said to be safe; if the HQ and HI values < 1, then the product is considered as not safe for human health.[25]

Carcinogenic health risk assessment

Lifetime Cancer Risk (LCR) (Eq. 12) is carcinogenic health risk assessment for estimating a person's lifetime risk of developing cancer as a result of using a product. Lifetime cancer risk is usually investigated for only carcinogenic heavy metals.

$$LCR = CSF \text{ X } SED \quad \ldots\ldots \quad (12)$$

where, CSF represents the cancer slope factor $(mg/kg/d)^{-1}$ and it approximates the cancer risk per unit intake dose of an agent that can cause cancer over an average lifetime. The reported CSF for Pb, Cd and As are 0.0085, 6.7, and 1.5 $(mg/kg/d)^{-1}$, respectively.17,26 Hg and Cr^{3+} do not have a CSF because they are not considered to be carcinogenic heavy metals.[27,28]

The total lifetime cancer risk TLCR (Eq 13) is the sum of the lifetime cancer risks for all heavy metals. The permissible limits for a carcinogenic element are considered to be 1×10^{-4} to 1×10^{-6}.[25]

$$TLCR = \sum LCR = LCR_{Pb} + LCR_{Cd} + LCR_{As} \quad \ldots\ldots \quad (13)$$

Evaluation of environmental toxicity (*Allium cepa* test)
A method described previously was utilized with some modifications.[29] Average-sized onion bulbs of the purple variety (36-50 mm diameter) obtained from Ojuwoye market, Mushin, Lagos, Nigeria were sun-dried for 10 days before the start of the experiment. In order to allow for the growth of new roots, the outer dead scales of the bulbs were removed in such a way that the root primordia were not damaged. The bulbs were seeded in universal bottles using distilled water and allowed to produce roots in a dark environment at room temperature for 24 hours. Viable, healthy looking bulbs were then selected for the experiment and treated in three replicates with three different concentrations (0.1%, 0.5% and 1.0%) of three selected black soaps (one from each category of ABS -raw and unpackaged, packaged and unregistered, packaged and NAFDAC registered) as tests samples, 1 % sodium lauryl sulphate (SLS) as positive control and distilled water as negative control at room temperature for 72 h. Afterwards, the length of the whole root from both experimental and control sets (i.e. lengths of the three longest roots from each bulb) were measured. Taking root lengths of negative control group as 100 %, the relative root growth for the test concentrations of the different black soap samples were calculated.

Evaluation of safety and toxicological profile

Skin irritation potential (wash-off test)
Eight individuals with healthy skin participated in the study. The determination of the effects of the black soaps on skin surface pH, trans-epidermal water loss (TEWL) and sebum level was carried out according to the method described in a previous study.[30] The soaps were applied to the volar forearm after wetting the arm and left on for 3 min, after which the arm was rinsed generously with distilled water. The multiprobe adapter Cutometer® (Dual MPA 580 Courage+Khazaka electronic GmbH Mathias-Brüggen-Str. 91 50829 Köln, Germany) was used to measure the pH (pH meter), trans-epidermal water loss (Tewameter), and sebum level of the skin (Sebumeter) pre- and post-wash by applying the probe on the skin surface.

Statistical analysis
The evaluation of physicochemical parameters, antimicrobial analysis and *Allium cepa* test were done in triplicates and expressed as mean ± standard deviation (SD). Statistical difference between the test samples / positive control and negative control values of *Allium cepa* test was determined by unpaired t-test using Microsoft Excel 2013. A p-value $p<0.001$ was considered significant, where data were expressed as means ± standard deviation. Statistical difference between the pre and post-test values of the skin irritation test was determined by paired t-test. A p-value $p<0.05$ was considered significant, where data were expressed as means ± standard deviation.

RESULTS

Qualitative phytochemical screening
All the black soap samples contained phenolic acids, steroids and saponins. However, alkaloids, flavonoids and tannins were absent.

Physicochemical properties
Results of physicochemical properties of all the three categories of black soaps determined in triplicates are presented in Figure 1.

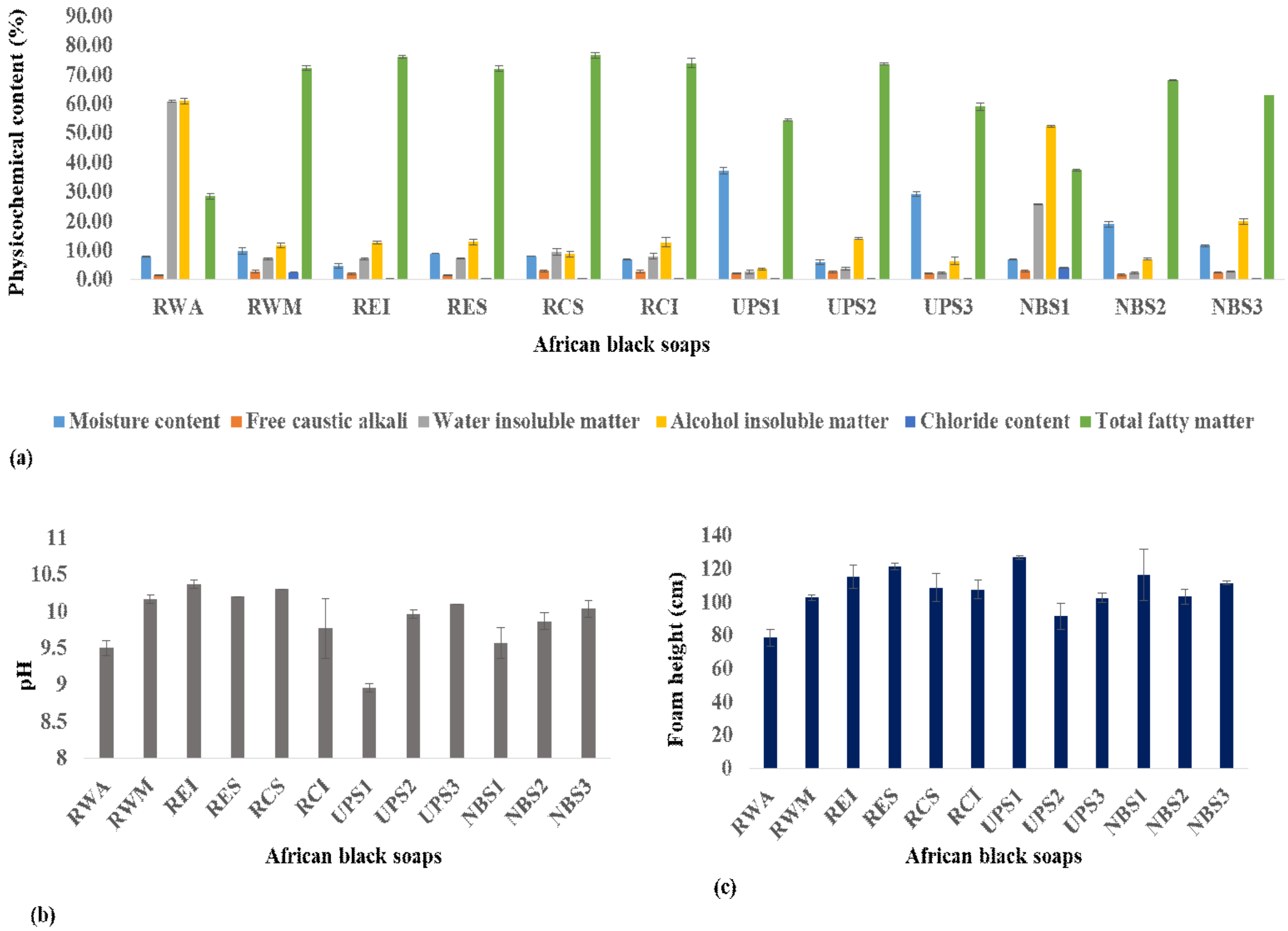

Fig. 1: Physicochemical properties of African black soaps. (a) Moisture content, free caustic alkali, water insoluble matter, alcohol insoluble matter, chloride content, total fatty matter. (b) Foam height (c) pH; RWA, RWM, REI, RES, RCS and RCI are raw African black soaps, UPS1, UPS2 and UPS3 are unregistered African black soaps, NBS1, NBS2 and NBS3 are NAFDAC registered African black soaps. Data were expressed as mean ± standard deviation with n = 3

Microbial load

The black soaps were screened for total microbial load. The bacteria colony forming units per gram (cfu/g) and fungal spore forming units per gram (sfu/g) obtained for the black soap samples at 1/10 and 1/100 dilution showed absence of bacteria or fungi.

Antimicrobial activity

Table 2 shows the inhibitory zone diameter of the black soaps on test organisms at concentrations of 250 and 500 mg/mL with the control standard triclosan being in concentrations of 6.25, 12.5, 25 and 50 µg/mL.

Table 2: Comparative antimicrobial activities of black soaps

Sample	Concentrations (mg/mL)	Inhibitory Zone Diameter (mm)			
		S.A	E.C	P.A	C.A
RWA	250	18.17±0.24	18.17±0.24	13.67±0.24	22.17±0.24
	500	20.17±0.24	20.17±0.24	15.67±0.24	25.50±0.41
RWM	250	15.17±0.24	15.17±0.24	15.33±0.47	16.17±0.24
	500	18.3 3±0.27	18.17±0.24	18.17±0.24	18.17±0.24
REI	250	14.67±0.58	16.83±0.29	15.17±0.29	17.50±0.50
	500	17.17±0.29	18.83±0.29	18.17±0.29	19.67±0.58
RES	250	17.83±0.24	17.67±0.47	14.83±0.24	21.83±0.24
	500	21.17±0.24	22.17±0.24	17.83±0.24	26.33±0.24
RCS	250	16.33±0.29	17.17±0.29	18.33±0.29	20.33±0.58
	500	19.17±0.58	19.33±0.58	19.50±0.50	24.67±0.58
RCI	250	18.33±0.29	16.83±0.29	13.17±0.29	22.33±0.58
	500	20.17±0.29	18.83±0.29	15.17±0.29	26.17±0.29
UPS1	250	19.67±0.58	15.67±0.58	0.00	20.17±0.29
	500	21.83± 0.29	18.17±0.29	13.17±0.29	25.17±0.29
UPS2	250	19.83±0.29	12.83±0.29	0.00	0.00
	500	22.00±0.50	15.17±0.29	0.00	25.17±0.29
UPS3	250	20.17±0.29	22.67±0.58	0.00	22.17±0.29
	500	24.67±0.58	25.17±0.29	19.50±0.50	24.83±0.29
NBS1	250	20.17±0.29	13.17±0.29	17.83±0.29	27.00±0.50
	500	24.67±0.58	15.17±0.29	20.17±0.29	30.33±0.29
NBS2	250	20.17±0.29	26.67±0.58	13.17±0.29	22.67±0.58
	500	24.67±0.58	25.17±0.29	17.17±0.29	25.17±0.29
NBS3	250	19.38±0.24	13.17±0.24	15.17±0.24	22.33±0.24
	500	22.67±0.47	15.17±0.24	18.17±0.24	28.17±0.24
TCS	0.00625	13.83±0.29	0.00	17.17±0.29	23.33±0.58
	0.0125	16.17±0.29	12.67±0.29	19.33±0.58	27.67±0.58
	0.025	17.83±0.76	14.83±0.29	21.33±0.58	31.67±0.58
	0.050	19.67±0.24	17.83±0.29	24.00±0.50	36.50±0.50

mg/mL- milligrams per millilitre, S.A-*Staphylococcus aureus*, E.C-*Escherichia coli*, P.A-*Pseudomonas aeruginosa*, C.A-*Candida albican*, TCS-*Triclosan control*; RWA, RWM, REI, RES, RCS and RCI are raw African black soaps, UPS1, UPS2 and UPS3 are unregistered African black soaps, NBS1, NBS2 and NBS3 are NAFDAC registered African black soaps, Data were expressed as mean ± standard deviation with n = 3.

Heavy metals analysis

The concentrations of heavy metals (Lead- Pb, Cadmium- Cd, Arsenic- As, Mercury - Hg and Chromium III- Cr^{3+}) determined in the black soap samples is shown in Table 3, where 50 % and 100 % bioaccessibility to the metals were used as the midpoint case scenario and worst-case scenario respectively. Metal concentrations at 100 % bioaccessibility ranged from 0.0250 to 0.0805 μg/g for Lead, 0 to 0.0975 μg/g for Cadmium, 0.00165 to 0.102 μg/g for Arsenic, 0.0005 to 0.016 μg/g for Mercury, and 0.0 to 0.045 μg/g for Chromium while metal concentrations at 50 % bioaccessibility were half of the values for 100 % bioaccessibility. Table 4 showed the systemic exposure dose (SED) for metals at 50 % and 100 % bioaccessibility in the studied soaps. SED at 100 % bioaccessibility ranged from 2.09E-06 to 6.72E-06 mg/kg body weight /day for Pb, 0 to 8.13E-06 mg/kg body weight/day for Cd, 1.38E-07 to 8.51E-06 mg/kg body weight/day for As, 4.17E-08 to 1.33E-06 mg/kg body weight/day for Hg, and 0 to 3.75E-06 mg/kg body weight/day for Cr, while SED values for 50 % bioaccessibility were half of the values for 100 % bioaccessibility. Table 5 showed the margin of safety (MOS) values for metals at 50 % and 100 % bioaccessibility in the studied soaps. MOS at 100% bioaccessibility ranged from 5.96E+04 to 1.92E+05 for Pb, 0 to 5.99E+05 for Cd, 3.53E+03 to 2.18E+05 for As, 2.25E+04 to 7.19E+05 for Hg, and 0 to 8.99E+08 for Cr, while MOS values for 50 % bioaccessibility were double of the values for 100 % bioaccessibility. The hazard quotients and hazard indices (Table 6) were all less than 1. Lifetime cancer risk (LCR) for all carcinogenic metals tested at 50 % and 100 % bioaccessibility were evaluated and reported in table 7. LCR at 100 % bioaccessibility ranged from 1.77E-08 to 5.71E-08 for Pb, 0 to 5.45E-05 for Cd, 2.06E-07 to 1.28E-05 for As, while LCR values for 50 % bioaccessibility were half of the values for 100 % bioaccessibility. The Total Lifetime Cancer Risks at 50 % and 100% bioaccessibility were also shown in table 7.

Table 3: Heavy metal concentrations of black soaps at 50% and 100% bioaccessibility

Sample	Heavy metals									
	Pb (µg/g)		Cd (µg/g)		As (µg/g)		Hg (µg/g)		Cr (µg/g)	
	50%	100%	50%	100%	50%	100%	50%	100%	50%	100%
RWA	4.03E -02	8.05E -02	0.00E+00	0.00E+00	2.20E -02	4.40E -02	2.25E -03	4.50E -03	0.00E+00	0.00E+00
RWM	3.28E -02	6.55E -02	5.00E -04	1.00E -03	1.43E -02	2.85E -02	2.75E -03	5.50E -03	0.00E+00	0.00E+00
REI	3.03E -02	6.05E -02	4.88E -02	9.75E -02	8.50E -03	1.70E -02	8.00E -03	1.60E -02	2.20E -02	4.40E -02
RES	2.23E -02	4.45E -02	3.75E -03	7.50E -03	4.75E -03	9.50E -03	3.00E -03	6.00E -03	2.25E -02	4.50E -02
RCS	2.38E -02	4.75E -02	7.25E -03	1.45E -02	8.25E -03	1.65E -02	3.75E -03	7.50E -03	0.00E+00	0.00E+00
RCI	3.80E -02	7.60E -02	2.20E -02	4.40E -02	5.10E -02	1.02E -01	2.50E -03	5.00E -03	0.00E+00	0.00E+00
UPS1	1.28E -02	2.55E -02	7.50E -04	1.50E -03	8.25E -04	1.65E -03	5.00E -04	1.00E -03	1.75E -03	3.50E -03
UPS2	1.40E -02	2.80E -02	0.00E+00	0.00E+00	8.50E -03	1.70E -02	1.00E -03	2.00E -03	1.75E -03	3.50E -03
UPS3	1.25E -02	2.50E -02	0.00E+00	0.00E+00	1.15E -02	2.30E -02	1.50E -03	3.00E -03	1.00E -03	2.00E -03
NBS1	1.93E -02	3.85E -02	2.10E -02	4.20E -02	2.90E -02	5.80E -02	2.75E -03	5.50E -03	1.20E -02	2.40E -02
NBS2	2.05E -02	4.10E -02	0.00E+00	0.00E+00	2.85E -02	5.70E -02	2.00E -03	4.00E -03	7.25E -03	1.45E -02
NBS3	1.30E -02	2.60E -02	0.00E+00	0.00E+00	2.65E -02	5.30E -02	2.50E -04	5.00E -04	3.00E -03	6.00E -03

Pb- Lead, Cd- Cadmium, As- Arsenic, Hg- Mercury, Cr- Chromium; RWA, RWM, REI, RES, RCS and RCI are raw African black soaps, UPS1, UPS2 and UPS3 are unregistered African black soaps, NBS1, NBS2 and NBS3 are NAFDAC registered African black soaps

Table 4: Systemic exposure dose (SED) values of heavy metals at 50% and 100% bioaccessibility

Sample	Heavy metals									
	Pb		Cd		As		Hg		Cr	
	50% SED	100% SED	50% SED	100% SED	50% SED	100% SED	50% SED	100% SED	50% SED	100% SED
RWA	3.36E - 06	6.72E - 06	0.00E+00	0.00E+00	1.84E - 06	3.67E - 06	1.88E - 07	3.75E - 07	0.00E+00	0.00E+00
RWM	2.73E - 06	5.46E - 06	4.17E - 08	8.34E - 08	1.19E - 06	2.38E - 06	2.29E - 07	4.59E - 07	0.00E+00	0.00E+00
REI	2.52E - 06	5.05E - 06	4.07E - 06	8.13E - 06	7.09E - 07	1.42E - 06	6.67E - 07	1.33E - 06	1.84E - 06	3.67E - 06
RES	1.86E - 06	3.71E - 06	3.13E - 07	6.26E - 07	3.96E - 07	7.92E - 07	2.50E - 07	5.01E - 07	1.88E - 06	3.75E - 06
RCS	1.98E - 06	3.96E - 06	6.05E - 07	1.21E - 06	6.88E - 07	1.38E - 06	3.13E - 07	6.26E - 07	0.00E+00	0.00E+00
RCI	3.17E - 06	6.34E - 06	1.84E - 06	3.67E - 06	4.25E - 06	8.51E - 06	2.09E - 07	4.17E - 07	0.00E+00	0.00E+00
UPS1	1.06E - 06	2.13E - 06	6.26E - 08	1.25E - 07	6.88E - 08	1.38E - 07	4.17E - 08	8.34E - 08	1.46E - 07	2.92E - 07
UPS2	1.17E - 06	2.34E - 06	0.00E+00	0.00E+00	7.09E - 07	1.42E - 06	8.34E - 08	1.67E - 07	1.46E - 07	2.92E - 07
UPS3	1.04E - 06	2.09E - 06	0.00E+00	0.00E+00	9.59E - 07	1.92E - 06	1.25E - 07	2.50E - 07	8.34E - 08	1.67E - 07
NBS1	1.61E - 06	3.21E - 06	1.75E - 06	3.50E - 06	2.42E - 06	4.84E - 06	2.29E - 07	4.59E - 07	1.00E - 06	2.00E - 06
NBS2	1.71E - 06	3.42E - 06	0.00E+00	0.00E+00	2.38E - 06	4.75E - 06	1.67E - 07	3.34E - 07	6.05E - 07	1.21E - 06
NBS3	1.08E - 06	2.17E - 06	0.00E+00	0.00E+00	2.21E - 06	4.42E - 06	2.09E - 08	4.17E - 08	2.50E - 07	5.01E - 07

SED- systemic exposure dose (in mg/kg body weight/day); Pb- Lead, Cd- Cadmium, As- Arsenic, Hg- Mercury, Cr- Chromium; RWA, RWM, REI, RES, RCS and RCI are raw African black soaps, UPS1, UPS2 and UPS3 are unregistered African black soaps, NBS1, NBS2 and NBS3 are NAFDAC registered African black soaps

Table 5: Margin of safety index of heavy metals at 50% and 100% bioaccessibility

Sample	Heavy metals									
	Pb		Cd		As		Hg		Cr	
	50% MOS	100% MOS	50% MOS	100% MOS	50% MOS	100% MOS	50% MOS	100% MOS	50% MOS	100% MOS
RWA	1.19E+05	5.96E+04	0.00E+00	0.00E+00	1.63E+04	8.17E+03	1.60E+05	7.99E+04	0.00E+00	0.00E+00
RWM	1.46E+05	7.32E+04	1.20E+06	5.99E+05	2.52E+04	1.26E+04	1.31E+05	6.54E+04	0.00E+00	0.00E+00
REI	1.59E+05	7.93E+04	1.23E+04	6.15E+03	4.23E+04	2.12E+04	4.50E+04	2.25E+04	8.17E+07	4.09E+07
RES	2.16E+05	1.08E+05	1.60E+05	7.99E+04	7.57E+04	3.79E+04	1.20E+05	5.99E+04	7.99E+07	4.00E+07
RCS	2.02E+05	1.01E+05	8.27E+04	4.13E+04	4.36E+04	2.18E+04	9.59E+04	4.80E+04	0.00E+00	0.00E+00
RCI	1.26E+05	6.31E+04	2.72E+04	1.36E+04	7.05E+03	3.53E+03	1.44E+05	7.19E+04	0.00E+00	0.00E+00
UPS1	3.76E+05	1.88E+05	7.99E+05	4.00E+05	4.36E+05	2.18E+05	7.19E+05	3.60E+05	1.03E+09	5.14E+08
UPS2	3.43E+05	1.71E+05	0.00E+00	0.00E+00	4.23E+04	2.12E+04	3.60E+05	1.80E+05	1.03E+09	5.14E+08
UPS3	3.84E+05	1.92E+05	0.00E+00	0.00E+00	3.13E+04	1.56E+04	2.40E+05	1.20E+05	1.80E+09	8.99E+08
NBS1	2.49E+05	1.25E+05	2.85E+04	1.43E+04	1.24E+04	6.20E+03	1.31E+05	6.54E+04	1.50E+08	7.49E+07
NBS2	2.34E+05	1.17E+05	0.00E+00	0.00E+00	1.26E+04	6.31E+03	1.80E+05	8.99E+04	2.48E+08	1.24E+08
NBS3	3.69E+05	1.84E+05	0.00E+00	0.00E+00	1.36E+04	6.79E+03	1.44E+06	7.19E+05	5.99E+08	3.00E+08

MOS- margin of safety; Pb- Lead, Cd- Cadmium, As- Arsenic, Hg- Mercury, Cr- Chromium; RWA, RWM, REI, RES, RCS and RCI are raw African black soaps, UPS1, UPS2 and UPS3 are unregistered African black soaps, NBS1, NBS2 and NBS3 are NAFDAC registered African black soaps

Table 6: Hazard quotients and indices of heavy metals in black soaps at 50% and 100% bioaccessibility

Sample	Heavy metals											
	Pb		Cd		As		Hg		Cr		50% HI	100% HI
	50% HQ	100% HQ	50% HQ	100% HQ	50% HQ	100% HQ	50% HQ	100% HQ	50% HQ	100% HQ	∑50%HQ	∑100%HQ
RWA	8.39E - 04	1.68E - 03	0.00E+00	0.00E+00	6.12E - 03	1.22E - 02	6.26E - 04	1.25E - 03	0.00E+00	0.00E+00	7.58E -03	1.52E - 02
RWM	6.83E - 04	1.37E - 03	8.34E - 05	1.67E - 04	3.96E - 03	7.92E - 03	7.65E - 04	1.53E - 03	0.00E+00	0.00E+00	5.49E -03	1.10E - 02
REI	6.31E - 04	1.26E - 03	8.13E - 03	1.63E - 02	2.36E - 03	4.73E - 03	2.22E - 03	4.45E - 03	1.22E - 06	2.45E - 06	1.34E - 02	2.67E - 02
RES	4.64E - 04	9.28E - 04	6.26E - 04	1.25E - 03	1.32E - 03	2.64E - 03	8.34E - 04	1.67E - 03	1.25E - 06	2.50E - 06	3.25E - 03	6.49E - 03
RCS	4.95E - 04	9.91E - 04	1.21E - 03	2.42E - 03	2.29E - 03	4.59E - 03	1.04E - 03	2.09E - 03	0.00E+00	0.00E+00	5.04E - 03	1.01E - 02
RCI	7.92E - 04	1.58E - 03	3.67E - 03	7.34E - 03	1.42E - 02	2.84E - 02	6.95E - 04	1.39E - 03	0.00E+00	0.00E+00	1.93E - 02	3.87E - 02
UPS1	2.66E - 04	5.32E - 04	1.25E - 04	2.50E - 04	2.29E - 04	4.59E - 04	1.39E - 04	2.78E - 04	9.73E - 08	1.95E - 07	7.60E - 04	1.52E - 03
UPS2	2.92E - 04	5.84E - 04	0.00E+00	0.00E+00	2.36E - 03	4.73E - 03	2.78E - 04	5.56E - 04	9.73E - 08	1.95E - 07	2.93E - 03	5.87E - 03
UPS3	2.61E - 04	5.21E - 04	0.00E+00	0.00E+00	3.20E - 03	6.40E - 03	4.17E - 04	8.34E - 04	5.56E - 08	1.11E - 07	3.88E - 03	7.75E - 03
NBS1	4.01E - 04	8.03E - 04	3.50E - 03	7.01E - 03	8.06E - 03	1.61E - 02	7.65E - 04	1.53E - 03	6.67E - 07	1.33E - 06	1.27E - 02	2.55E - 02
NBS2	4.28E - 04	8.55E - 04	0.00E+00	0.00E+00	7.92E - 03	1.58E - 02	5.56E - 04	1.11E - 03	4.03E - 07	8.06E - 07	8.91E - 03	1.78E - 02
NBS3	2.71E - 04	5.42E - 04	0.00E+00	0.00E+00	7.37E - 03	1.47E - 02	6.95E - 05	1.39E - 04	1.67E - 07	3.34E - 07	7.71E - 03	1.54E - 02

HQ- hazard quotient, HI- hazard index (sum of all metal HQ for one soap sample); Pb- Lead, Cd- Cadmium, As- Arsenic, Hg- Mercury, Cr- Chromium; RWA, RWM, REI, RES, RCS and RCI are raw African black soaps, UPS1, UPS2 and UPS3 are unregistered African black soaps, NBS1, NBS2 and NBS3 are NAFDAC registered African black soaps

Table 7: Lifetime cancer risk for carcinogenic heavy metals in black soaps at 50% and 100% bioaccessibility

Sample	Heavy metals							
	Pb		Cd		As		50% TLCR	100%TLCR
	50% LCR	100% LCR	50% LCR	100% LCR	50% LCR	100% LCR	∑50%LCR	∑100%LCR
RWA	2.85E - 08	5.71E - 08	0.00E+00	0.00E+00	2.75E - 06	5.51E - 06	2.78E - 06	5.56E - 06
RWM	2.32E - 08	4.64E - 08	2.79E - 07	5.59E - 07	1.78E - 06	3.57E - 06	2.09E - 06	4.17E - 06
REI	2.14E - 08	4.29E - 08	2.72E - 05	5.45E - 05	1.06E - 06	2.13E - 06	2.83E - 05	5.67E - 05
RES	1.58E - 08	3.16E - 08	2.10E - 06	4.19E - 06	5.94E - 07	1.19E - 06	2.71E - 06	5.41E - 06
RCS	1.68E - 08	3.37E - 08	4.05E - 06	8.10E - 06	1.03E - 06	2.06E - 06	5.10E - 06	1.02E - 05
RCI	2.69E - 08	5.39E - 08	1.23E - 05	2.46E - 05	6.38E - 06	1.28E - 05	1.87E - 05	3.74E - 05
UPS1	9.04E - 09	1.81E - 08	4.19E - 07	8.38E - 07	1.03E - 07	2.06E - 07	5.31E - 07	1.06E - 06
UPS2	9.93E - 09	1.99E - 08	0.00E+00	0.00E+00	1.06E - 06	2.13E - 06	1.07E - 06	2.15E - 06
UPS3	8.86E - 09	1.77E - 08	0.00E+00	0.00E+00	1.44E - 06	2.88E - 06	1.45E - 06	2.90E - 06
NBS1	1.36E - 08	2.73E - 08	1.17E - 05	2.35E - 05	3.63E - 06	7.26E - 06	1.54E - 05	3.08E - 05
NBS2	1.45E - 08	2.91E - 08	0.00E+00	0.00E+00	3.57E - 06	7.13E - 06	3.58E - 06	7.16E - 06
NBS3	9.22E - 09	1.84E - 08	0.00E+00	0.00E+00	3.32E - 06	6.63E - 06	3.33E - 06	6.65E - 06

LCR- Lifetime cancer risk, TLCR- Total Lifetime cancer risk (sum of all metal LCR for one soap sample); Pb- Lead, Cd- Cadmium, As- Arsenic; RWA, RWM, REI, RES, RCS and RCI are raw African black soaps, UPS1, UPS2 and UPS3 are unregistered African black soaps, NBS1, NBS2 and NBS3 are NAFDAC registered African black soaps

***Allium cepa* test**

The growth inhibition of the black soap solutions on the root growth of Allium cepa L. is presented in Table 8. Maximum root growth was observed in the negative control (distilled water) at an average root length of 46.78 mm (100%) while the least root growth was observed in UPS1 at -1.78 mm (-3.80% of the negative control) at 1.00% concentration.

Table 8: Relative growth root length in percentage of control of *Allium cepa* L. 72 hours post-exposure to different concentrations of black soaps

	Growth expressed in percentage of control (%)		
Concentration (%)	**RWA**	**UPS1**	**1% SLS**
0.10	7.13±8.07***	0.48 ± 2.57***	-3.09 ± 2.85***
0.50	-2.38± 3.11***	-0.95 ± 1.89 ***	-1.66 ± 10.13***
1.00	-1.66 ± 3.97 ***	-3.80 ± 2.78***	-1.19 ± 3.23 ***
Control		100	

RWA- raw African black soap, UPS1, unregistered African black soap, NBS1- registered African black soap; Negative control- distilled water, 1% SLS- sodium lauryl sulphate -positive control; Data were expressed as mean ± standard deviation with n = 3, p-value p<0.001 was considered significant.

Skin irritation potential

The effects of selected black soaps on human skin such as change in skin surface pH, trans-epidermal water loss and sebum content were determined as shown in Table 8.

Table 9: Wash-off test results post-exposure to the African black soap samples

Parameters	**Samples**			
	RWA n=4	**P-value**	**NBS1 n=4**	**P-value**
Skin Surface pH				
Pre-wash	5.31±0.05 *		5.16±0.17	
Post-wash	5.42±0.28	0.454	5.28±0.10	0.366
Percentage increase (%)	2.01±4.71		2.43±4.45	
Average trans-epidermal water loss (g/hm^2)				
Pre-wash	12.63±0.94		13.70±4.50	
Post-wash	19.05±6.70	0.179	15.98±3.43	0.045
Percentage increase (%)	53.39±61.85		19.35±12.70	
Sebum content ($\mu g/cm^2$)				
Pre-wash	9.50±12.77		1.00±0.82	
Post-wash	0.00±0.00	0.233	0.00±0.00	0.092
Percentage decrease (%)	100.00		100.00	

RWA- raw African black soap, NBS1- registered African black soap. Data were expressed as *mean ± standard deviation with n = 4, A p-value p<0.05 was considered significant.

DISCUSSION

The phytochemical evaluation showed the presence of saponins, steroids and phenols in all the black soaps. Saponin is a very important phytochemical in all soaps. It is a natural surfactant which is notable for its surface activity, emulsifying, wetting, foaming and detergency properties.[31] This finding is similar to a previous study.[32] The presence of steroids suggests that ABS may have some anti-inflammatory characteristics.[33] Phenols have been shown to have antimicrobial properties.[34] The absence of other phytochemicals in this study, though present in some other findings, may be as a result of the fact that the plant parts containing those constituents were not utilized or were lost during the black soap production.

The moisture contents were found to be generally lower in all the raw African black soaps when compared with the refined, unregistered and NAFDAC registered soaps. The results obtained showed that the moisture content ranged from 4.72 to 37.14 % with REI being the lowest and UPS1 being the highest. The moisture content of raw African black soaps ranged from 4.72 % (REI) to 9.90 % (RWM); unregistered African black soaps ranged from 6.00 % (UPS2) to 37.14 % (UPS1); and registered African black soaps ranged from 6.93 % (NBS1) to 19.05 % (NBS2). Amongst all the soaps tested, only samples UPS1, UPS3 and NBS2, exceeded standard maximum acceptable limit of 15 % according to the Tanzania Bureau of Standards (TBS).[35] The variations in the moisture content values of the black soaps could be as a result of varying formulation ingredients as well as the formulation processes adopted by the different producers.[36] Additionally, it could also be attributed to the packaging and storage conditions prior to the sample collection. Some ABS contained humectants such as Aloe vera, honey which help to keep the body moist. Humectants are hygroscopic substances hence they can attract and retain moisture thereby increasing the moisture content. Results of previous studies on moisture contents of ABS have also been variable.[1,10] High moisture content could lead to hydrolysis of soap on storage because of excess water reacting with any unsaponified neutral fat. Moisture content of soaps is regarded as a parameter for assessing the shelf life of products.[37]

Abrasiveness in soaps is usually caused by the amount of FCA in soap. ABS with high content of FCA can cause skin irritation, dryness and scaling. Soluble soap is usually formed by the presence of excess alkali, which saponifies the fats and oils on the skin. The skin can get dried when the soluble soap is washed away. According to Bureau of Indian Standards (BIS), good quality soap should have FCA of < 5%.[38] In this study, the FCA values of all the soaps tested were within the permissible limit of quality.

The percentage water insoluble matter of all the soap samples were higher than the TBS maximum acceptable limit of 0.50 %.[12,35] The percentage alcohol insoluble matter of all the soap samples were also higher than the acceptable limit of 2.5 - 10 % according to BIS,[38] except for samples RCS, UPS1 UPS3 and NBS2. Soaps with higher amount of matters insoluble in water and alcohol suggest that they contain a lot of impurities. These impurities may come from the source of the alkali or additives used during their production. Exceptional high values of water insoluble matter and alcohol insoluble matter obtained for some of the samples might be attributed to high impurities from their raw materials.[8] Most of the soaps studied in a previous study also showed higher values for percentage water insoluble matter and alcohol insoluble matter than the acceptable limits.[12]

High chloride content causes soaps to crack, it also reduces the solubility of soap in water.[12] All the soap except samples RWM and NBS1 were within the acceptable limit of percentage chloride content, which is < 1.50 %.[38] Generally, the percentage chloride content of the soaps compared well with previous studies where the chloride content was 0.91 %[39] and 0.06 - 0.07 %.[40] High chloride content may be due to the use of highly chlorinated water or other forms of contamination during production process.

Total fatty matter (TFM) determines the quality of any soap, its hardness and moisturizing capacity. Soaps with lower TFM depict lower quality, hard soap and low moisturizing capacity while soaps with higher TFM do not cause dryness and are less harmful to the skin.[41,42] According to BIS, the minimum acceptable limit for percentage TFM is 60 - 76 %.[38] Among all the soaps tested, samples RWA, UPS1, UPS3 and NBS1 had low percentage of TFM, particularly, the TFM of samples RWA and NBS1 were very low and far from the minimum acceptable limit. Variations in the percentage of the TFM of the black soaps might be due to their saponification methods.[43] Generally, the percentage TFM of the soaps compared well with previous studies where the TFM was 72.23 %[39] and 56.13 %.[40]

The results obtained for foam height compared well with foam heights (4.00 - 10.20 cm) studied previously.[44] The amount of froth found in the soaps may be as a result of the type of oil used in making the soap. Palm kernel oil is usually used and its major fatty acid is lauric acid, it is known to cause foaming when saponified.[45] All ABS samples' foam heights demonstrated their strong lathering and foaming capabilities.

The pH of raw black soaps ranged from 9.50 (RWA) to 10.37 (REI); unregistered packaged black soaps ranged from 8.97 (UPS1) to 10.10 (UPS3) and the registered packaged black soaps from 9.57 (NBS1) to 10.03 (NBS3). A study has shown that the pH values of most bathing soaps are between 9-10.[46] The pH of some of the black soaps compared well with previous findings.[10,12,47] When soaps are in water, they are alkalinic and can neutralize the body's protective acid mantle, which can act as barriers against bacteria and viruses in the process.[12] The pH of a healthy human skin ranges between 4.1 - 5.8.[48] However, bathing soaps do have a short contact time with the skin before they are washed off, unlike body creams. Soaps with overly higher pH values may cause irritation to the skin. This corrosive action can be ameliorated by adding excess fat or oil to the soap formulations.[12]

Microbial load analysis revealed that no microbes were found present in all the black soap samples. This suggests that black soap provides a harsh environment which does not support the growth of many microorganisms. This "extreme" environment is marked by high alkalinity[49] due to the high pH levels (>8.0) of black soaps. The microbiological quality of the black soaps may also be due to their solid/ semi- solid consistency and low water activity.

This study showed that ABS exhibits antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans*. All the black soaps had activity at 250 and 500 mg/mL concentration except UPS1 and UPS3 at 250 mg/mL concentration; UPS2 at 500 mg/mL concentration that had no activity against Pseudomonas aeruginosa. Sample UPS2 at 250 mg/mL concentration also had no activity against *Pseudomonas aeruginosa* and *Candida albicans*. *S. aureus* and *C. albicans* have been shown to cause skin infections like boils, impetigo, and thrush,[50] while *E. coli*, *P. aeruginosa* and *S. aureus* are known to be wound infection pathogens.[51] Since these black soaps are susceptible to these organisms, this shows the potentials and efficacy of black soaps in treating diseases caused by these organisms. Registered black soap NBS1 seems to show the highest inhibition against all the test organisms except against *E.coli*.

Overall, all the black soaps with antimicrobial activity exhibited considerable level of inhibitory actions when compared to Triclosan (control). The results obtained compare well with the findings of a previous study.[52] It is important to note that the presence of phenol may have also contributed to the anti-microbial activity of these black soaps.[34]

Trace concentrations of the studied heavy metals were found in most of the black soap samples. They were calculated at 50 % (mid-point scenario) and 100 % (worst-case scenario) bioaccessibility. The heavy metal concentration values were generally higher at 100 % bioaccessibility than at 50 % bioaccessibility. Cadmium was below detectable levels in RWA, UPS2, UPS3, NBS2, and NBS3 and its value was designated as 0. Trivalent Chromium was also below detection level in RWA, RWM, RCS and RCI, its value was designated as 0. None of the soaps exceeded the maximum permissible limit of 10 µg/g, 3 µg/g, 3 µg/g, 1 µg/g 53 and 50 µg/g 54 for Pb, Cd, As, Hg and Cr^{3+} respectively. The concentrations of Pb, Cd and Hg of all the African black soaps at 100% bioaccessibility in this current study were lower than the concentrations of Pb, Cd and Hg found in African black soaps in a previous study.[12] The concentrations of As in all the black soaps except sample UPS1 were higher than the mean concentration (0.004 µg/g) of As in shower gel in a previous study; however, the concentrations of Cr^{3+} of all the black soaps except samples REI and RES were lower than the mean concentration (0.03 µg/g) of Cr^{3+} in shower gel in the same study.[17]

The occurrence of these heavy metals in black soaps might be as a result of the contaminated sources of the raw materials and lack of standardization of production processes. Using products with heavy metals for a long time may pose a threat to human health and could have a negative impact on the environment.[55] Hence, good collection practices should be followed when sourcing for the raw materials and good manufacture practice must be adhered to during production.

The systemic exposure dose (SED) of the heavy metals was calculated at 50 % (mid-point scenario) and 100 % (worst-case scenario) bioaccessibility. The SED values at 50 % bioaccessibility were half of the SED values at 100 % bioaccessibility. The SED values of all the metals were below their reference daily doses of 0.004,[20] 0.001,[21] 0.0003,[22] 0.0003[23] and 0.003[24] mg/kg body weight/day for Pb, Cd, As, Hg and Cr^{3+} respectively.

According to the Scientific Committee on Consumer Safety data, the minimum acceptable margin of safety value of any human skin product is 100.[18] Margin of safety below 100 signifies a non-carcinogenic risk to humans. The MOS values obtained in this study showed that there was no apparent non-carcinogenic risk associated with the use of these black soaps as these values were far above 100, except for Cadmium in RWA, UPS2, UPS3, NBS2, and NBS3 samples as well as Cr^{3+} in RWA, RWM, RCS and RCI samples in which their heavy metal concentrations were below detectable levels. The MOS values obtained in this study were comparable to that of a previous study in which the values were also far above 100.[17]

The hazard quotient (HQ) is a parameter for estimating the potential health risks of pollutants which in this case are heavy metals. The HQ is a non-carcinogenic risk assessment.[17] The parameter for estimating the risk to human health using a product containing more than one heavy metal is called the hazard index (HI) and it is the sum of hazard quotients for each heavy metal analyzed.[28] The hazard quotients and hazard indices of all the black soaps were within acceptable limit as they did not exceed the maximum limit of 1, thus further indicating their safety and low potential for non-carcinogenic risk to human health. The HQ and HI values obtained in this study were comparable to that of a previous study in which the values were also below [1.17]

A skin preparation is regarded to be safe if the lifetime cancer risk is within 10-4 and 10-6, meaning there is little chance of it causing cancer. The risk of carcinogenicity is deemed to be significant if the lifetime cancer risk of the substance is greater than 10-6. All of the black soaps generally fell within an acceptable safety level for their lifetime cancer risk and total lifetime cancer risk values, thus demonstrating carcinogenic non-toxicity. The LCR and TLCR values obtained in this current study were also comparable to a previous study.[17]

Allium cepa test is useful in evaluating toxicity in the environment and it also serves as bio-indicator to detect genotoxicity and cytotoxicity.[56] However, in this present study, only the impact of the test samples on the growth of *Allium cepa* root was considered. One soap each from the three classes of soaps was chosen for the *Allium cepa* test; the soap with the lowest pH in each category was selected.

The negative control (distilled water), showed maximum root development of 46.78 ± 8.27 cm which was taken as 100 % after 72 hours. The roots of the test samples and the positive control were wilted after 72 hours. This caused the majority of the roots to break off and their growth length expressed as percentage of control after 72 hours was reduced and was between the range of -3.81 to 7.13 %. The negative values signified that the roots had become shorter than they were after initial growth in water for 24 hours at the start of the experiment. This meant that the black soaps had a negative impact (retardation) on the growth of the Allium cepa roots. By extension, careless disposal of black soaps must be avoided because it may be toxic to both aquatic and terrestrial life. The values obtained for the test samples were comparable to that of SLS which served as the positive control. SLS has been shown to be environmentally toxic.[57] Even though it has been used in many cosmetics, this current study has shown that it also affected the growth of Allium cepa roots. Hence, care should be taken when disposing formulations that contain SLS.

A raw black soap and a NAFDAC registered black soap were used for the skin irritation test. These black soaps had the least pH values in the raw unpackaged soap group and NAFDAC registered and packaged groups respectively. The average skin surface pH for the subjects that used the raw black soap was found to be 5.31 and 5.42 pre-wash and post-wash respectively, while the average skin surface pH for the subjects that used the NAFDAC registered black soap was found to be 5.16 and 5.28 pre-wash and post-wash respectively. All the skin surface pH values for both pre-wash and post-wash for both soaps were between the range of 4.95 - 5.76. This shows that the values were within pH value of 4.1 - 5.8 which was the range obtained from a recent investigation on the pH of healthy individuals.[48] From this study, it is evident that the black soaps did not impact an undesirable pH on the skin. Abnormal skin pH have been linked to skin irritancy and other skin reactions which can lead to skin barrier disruption and different skin diseases.[48]

The average trans-epidermal water loss was found to increase post wash in both groups that used the raw and NAFDAC registered black soaps. There was an increase in the post-wash trans-epidermal water loss in 87.5% of the subjects tested. Although, the difference between the pre and post wash average trans-epidermal water loss values was not statistically significant ($p<0.05$). Low TEWL values indicate that the skin is intact with a good functional barrier.[58] A high TEWL value usually results into an alteration in the skin barrier functionality and propensity for some skin diseases like psoriasis and atopic dermatitis. This can be as a result of low hydration of the stratum corneum. An intact skin do not allow penetration of large particulate substances and dermal absorption of chemicals but when the skin is compromised, these could occur.[59] A previous study has shown that an increased risk of hand dermatitis at TEWL >15 $g/m^2/h$ was observed in some hairdressers however, the increased risk was not statistically significant.[59] Therefore, the average TEWL values obtained for the two groups in this current study call for concern, although different factors like rate of exposure to cleansing agents, race, age and sex employed in the two studies may play a definitive role.

In 62.5 % of all the study participants, sebum was completely stripped off the skin post wash, thus leaving the skin devoid of the natural oily skin moisturizers produced by the sebaceous glands. This means that the black soaps had drying effect on the skin. Although, the difference between the pre and post wash sebum level was not statistically significant. Sebum skin-stripping can be reduced by increasing the moisturizer content of the soap formulation by adding oils and other emollients like shea butter or cocoa butter to mention but a few.

CONCLUSION

The physicochemical evaluation of the black soaps showed that their qualities were fairly acceptable. All the black soaps showed an absence of bacterial and fungal colonies. The antimicrobial evaluation proved that all the black soaps were efficacious against the tested microbes except sample UPS2 which had no anti-bacterial activity against Pseudomonas aeruginosa. The margin of safety, hazard quotients and hazard indices of heavy metals tested showed that the soaps did not have a potential for non-carcinogenic risk; also the lifetime cancer risk evaluation showed that there was no potential carcinogenic risk to human health. Retardation of onion root growth was observed in the Allium cepa test; this could imply that careless disposal of black soaps may cause toxicity to both aquatic and terrestrial life. The skin irritation test showed a possibility of skin barrier dysfunctionality and propensity for some skin diseases due to increased TEWL and reduced sebum content after washing with the soaps. This study has shown that there is a great prospect in the utilization of African black soaps; however, there is a need for regulation of the sources of the raw materials; standardization of manufacturing processes and proper disposal of black soaps in order to safeguard public health.

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