

ANTIMICROBIAL ACTIVITIES OF THE INDIGENOUS BLACK SOAP FORTIFIED WITH HONEY ON SOME SELECTED SKIN PATHOGENS

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ABSTRACT

Antimicrobial activities of the black soap and the antagonistic potentials on the following skin pathogens: *Escherichia coli*, *Klebsiellapneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Trichophytonrubrum*, *Candida albicans*, *Escheichia coli* ATCC-25922, *Klebsiellapneumoniae* ATCC-11930, *Staphylococcus aureus* ATCC 25923, *Stahylococcus epidermidis* ATCC-12228, *Pseudomonas aeruginosa* ATCC-27853, and *Candidia albicans* ATCC-10261 were investigated using agar diffusion method. Black soap was prepared using ashes from dried plantain skin by saponification and then fortified with honey at 20g/250 ml of molten soap before allowing it to cure in a mould for two weeks. Black soap was also got from the market. Commercial antiseptic soaps and creams: Meriko, Mekako, Septol, Delta and Dettol, Tribact, Tricutan, Funbact A, Sivoderm and Acneaway and broad spectrum standard antibiotic served for comparative and control purposes. Bacteria isolates from the black soap sources are *Bacillus brevis* and *Bacillus megaterium*. Fungal isolates: *Chrysosporium spp* and *Aspergillus granulosis* were isolated from the black soap market sample, *Aspergillus flavus* from black soap with honey. Plasmid profiling of the bacteria revealed the presence of plasmids. *B. brevis* contained plasmid with an estimated molecular weight of 17578bp. Proximate analysis of the honey revealed they contain high organic matter. Physicochemical properties of the black soaps showed they are suitable for bathing as evident in their total fatty matter (TFM %) of 72.107 and 48.314 for market sampled black soap, black soap with honey respectively. The antagonism assay indicated that *B. brevis* and *B. magaterium* inhibited the growth of *E. coli* and *S. epidermidis* with inhibition zones ranging from 15-34 mm. black soap had more inhibitory effects on all pathogens. Black soap with honey had the highest inhibition zone of 17.83 mm on *Candida albicans* ATCC-10261 followed by the black soap market sample at 15.83 mm on the same organism, Dettol had the highest inhibitory action at 12.7 mm of inhibition zone against *Klebsiella pneumomiae* ATCC-11930 of the selected antiseptic soaps. The comparative minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of the black and commercial antiseptic soaps ranged from 62.5 mg/mL and 500 mg/mL. the mechanism of action showed that sodium and potassium ions were leaked by the black soaps from the cell of the organisms. 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. This study has shown that the antimicrobial activities of the black soap may also be attributed to its bacterial flora.

Keywords: Black soap, Antimicrobial activities, Plasmid profiling, Honey

{**Citation:** Bashua O.C., Oladunmoye M.K. Antimicrobial activities of the indigenous black soap fortified with honey on some selected skin pathogens. American Journal of Research Communication, 2017, 5(10): 28-64} www.usa-journals.com, ISSN: 2325-4076.

INTRODUCTION

Vegetable or animal derived oils, made into salts are known as soaps (Al-Doori *et al.*, 2003). Modified detergents are added in their formulation to enhance their antibacterial activity. Such soaps have potential ability to remove 65 to 85% bacterial population, prevalent on human skin (Larson *et al.*, 2004). Homemade soap using local raw materials is an amazing method that is used in producing soaps for the family use in the ancient days. Technically, soap making involves the use of sodium salts (Isaac, 2005). Soaps are salts of fatty acids and it may be hard or soft soap depending on the type of ingredients used (Okeke, 2009). Soaps are made by the hydrolysis of fats with caustic soda (Sodium hydroxide), thus converting the glycosides of stearic, oleic and palmitic acids into sodium salts and glycerol. Soaps have a cleansing action because they contain negative ions composed of a long hydrocarbon chains attached to a carboxyl group (Okeke, 2009). The hydrocarbon chain has an affinity for grease and oil and the carboxyl group has an affinity for water thus soaps are mostly used with water for bathing, washing and cleaning. Indigenous black soap has been used by West African natives from time immemorial. This dark brown, mild and cleansing substance is known as 'sabulum salo' in Hausa, 'anago', 'alata samina' in Ghana and 'eko-zhiko' in Nupe (Getradeghana, 2000; David, 2005; Aliyu *et al.*, 2012). It is also referred to as 'ôsedúdu' or 'abuwe' among the Yoruba-speaking people in the south western Nigeria (Bella, 2011). In Southwest Nigeria, indigenous black soap may be made from either roasted plantain skins or dried waste cocoa pods and vegetable oil, palm oil or palm nut oil (Ikpor *et al.*, 2012). In the Northern part of Nigeria, it is produced from a mixture of vegetable oil, palm kernel oil and sheabutter (Aliyu *et al.*, 2012). Black soap is preferred for bathing due to its natural source of vitamins A and E (Getradeghana, 2000). Because of its phenolic contents, it is generally used to cure skin rashes (Mike, 2008; Ekwenye and Ijeomah 2005). Black soap enjoys a reputation for improving or eliminating uneven skin tone, razor bumps caused by ingrown hairs and skin rashes. It is not scented and can be used by anyone who wishes to improve the quality of his/her skin. Excellent for clearing up oily skin and priced for its antiseptic properties. African people also use black soap to prevent the skin from rashes, ring worm, measles, eczema and body odour. It is used as a natural shampoo to avoid dry itchy scalp. Black soap is used in the treatment of many infectious diseases caused by micro-organisms. In traditional medicine, soaps are very common vehicles for application of medicinal plants especially for external use and also for the treatment of skin diseases (Ajaiyeoba *et al.*, 2003; Ahmed *et al.*, 2005; Ajose, 2007). Locally manufactured soap is made from lye obtained

from ash of burnt cocoa husks, plantain peels, palm wastes, wood and other plant debris and is known to have some antimicrobial properties (Moody *et al.*, 2004).

Studies have shown that soaps containing active antimicrobial ingredients remove more bacteria as compared to plain soap (Lucet *et al.*, 2002). In recent time, the soap has been improved industrially into more presentable forms (although many people still prefer the traditionally prepared one) with different trade names such as 'Dudu Osun' 'Zee Black Soap' etc. The attribute of the soap includes mildness on the skin, rich lather, protection against skin disorders (including rashes, eczema, and scabies) treatment of skin infection (such as ringworm), protection of even skin toning and smoothness of the skin (Ekwenye and Ijeomah 2005).

MATERIALS AND METHODS

Collection of black soap and its materials

Plantain peels were collected from local dump sites and sun-dried for three weeks. Pure honey was also collected. The Local palm kernel oil from processed palm nut (Adin Eyan), earthen pot and wooden stirrer were also procured from the local market. The market sample black soap was also gotten.

Preparation of black soap by saponification (hot method)

Dried plantain peels were incinerated to complete ashes. The ashes were gathered and added to water in a basin in order to leach the alkali contents of the ash. The resulting solution was filtered with the aid of Muslin cloth to obtain lye without particulate matters. This was followed by a brief heat treatment. The base oil (Adin) was heated in an earthen pot until it has thawed and thoroughly heated through. The lye was then stirred into the heated oil until the desired shade of dark brown or black colour was obtained. The liquid soap was scooped from the earthen pot as it is being formed into another basin in order to allow the addition of pure honey to the molten soap, followed by thorough stirring in order to ensure even dispersion of the honey. It was then transferred into mould and cured via a process of hardening for two weeks.

Collection of test organisms

The following clinical test microorganisms *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis* *Pseudomonas aeruginosa*, *Candida albicans* and *Trichophyton rubrum* and reference strains: *Escherichia coli* ATCC-25922, *Klebsiella pneumoniae* ATCC-11930, *Staphylococcus aureus* ATCC-25923, *Staphylococcus epidermidis* ATCC-12228 *Pseudomonas aeruginosa* ATCC-27853 and *Candida albicans* ATCC-10261 were collected from The University College Hospital UCH Ibadan. Their identities were confirmed, tagged with proper labels to avoid ambiguity and were maintained at 4°C till further analysis.

Sterilization methods

All glasswares were washed with detergent and rinsed with tap water followed by distilled water. It was allowed to drain for about 10-20 minutes. Petri dishes and pipettes were placed in canisters and autoclaved at 121 °C for 15 mins; while the test tubes and conical flasks were carefully wrapped in Aluminum foil and sterilized in the oven at 170°C for 2 hrs.

Preparation of culture media

Media were prepared according to the appropriate manufacturer's specifications.

Preparation of Nutrient Agar medium

Nutrient Agar was prepared by suspending 28 g of the commercially prepared medium into 1000 mL of distilled water. A suspension was formed which on heating with occasional swirling of the flask dissolved. The conical flask was plugged with cotton wool and wrapped carefully with aluminum foil. This was autoclaved at 121 °C for 15 mins.

Preparation of Mueller Hinton Agar medium

Mueller Hinton agar was prepared by suspending 38 g of the commercially prepared medium into 1000 mL of distilled water. A suspension was formed which on heating with occasional swirling of the flask dissolved. The conical flask was plugged with cotton wool and wrapped carefully with aluminum foil. This was autoclaved at 121 °C for 15 mins.

Preparation of Potato dextrose Agar medium

Potato dextrose agar was prepared by suspending 39 g of the commercially prepared medium into 1000 mL of distilled water. A suspension was formed which on heating with occasional swirling of the flask dissolved. The conical flask was plugged with cotton wool and wrapped carefully with aluminum foil. This was autoclaved at 121 °C for 15 mins. After sterilization in the autoclave, the medium was allowed to cool and 1% of streptomycin was added to inhibit bacteria.

Preparation of Sabouraud dextrose Agar medium

Sabouraud dextrose agar was prepared by suspending 65 g of the commercially prepared medium into 1000mL of distilled water (supplemented with 5% Sodium chloride to enhance dermatophytes growth). A suspension was formed which on heating with occasional swirling of the flask dissolved. The conical flask was plugged with cotton wool and wrapped carefully with aluminum foil. This was autoclaved at 121 °C for 15 mins.

Microbiological analysis of the black soap samples**Isolation of bacteria from black soap**

1g of each soap sample (market, laboratory prepared and honey fortified black soap) were weighed and introduced into a petri dish of already prepared nutrient agar. It was incubated at 37C for 24 hours. After incubation, the colonies on the surface were counted. Total, microbial count expressed as colony forming units (cfu), were recorded for each plate.

Isolation of fungi from black soap

Potato dextrose agar was used for the isolation of fungi. 1g of the black soap samples (market, laboratory prepared, and fortified with honey) were weighed and flooded with potato dextrose agar and afterwards incubated at room 25C for 72hours. (Warcup method). The number of colonies that developed at the end of incubation was counted and expressed in cfu/ml of the soap samples. (Dubey and meheshwari, 2005)

Preparation and maintenance of pure cultures

The plates were examined and colonies were counted. The average colony obtained was used to calculate the number of bacteria per gram of the soap samples. These observed colonies on the plates were further examined microscopically. Isolates of different bacteria colonies and fungi were sub-cultured repeatedly by streaking on Nutrient and Potato dextrose medium until pure cultures were obtained. Different bacteria pure cultures were then inoculated into Nutrient agar slants, incubated for 24hrs in order to ensure proper growth and then kept as stock cultures in the refrigerator for identification. Pure fungi Isolates were also maintained on Potato dextrose agar slants in the refrigerator until further investigative procedure.

Characterization of Bacteria isolates

The pure culture of each isolate was examined. Microscopic examination, staining techniques and biochemical tests were carried out on the isolates according to the methods described by Olutiola *et al.* (2000);

Gram staining

A heat-fixed smear of each isolate was made from 24hr old culture on different slides. The slides were stained with 2 drops of crystal violet and washed gently with water. This was then rinsed off with Gram's iodine solution and was allowed to react for about one minute after which the iodine was poured off and the slide washed with 95% alcohol until no more violet colour runs from the slide. The slides were then rinsed under a gentle-running tap water and were counterstained with 2 drops of safranin for about 2 minutes, blot dried and examined under an oil immersion lens.

Spore staining

Heat-fixed smear of the isolates were prepared and carefully labeled as appropriate. Slides were initially flooded and steamed for about 10 minutes with continuous flooding with Malachite Green and later washed with water followed by the addition of 0.25% Safranin. Slides were then washed with water and observed under the microscope using oil immersion lens (Olutiola *et al.*, 2000).

Motility test

A little Vaseline was placed around the edge of the hollow in a clean cavity slide. A loopful of 24hrs old culture of the bacteria was aseptically transferred to the centre of a clean cover-slip laid on the bench without spreading it. The cavity slide was then carefully inverted over the cover-slip in such a way that the drop is in the centre of the cavity and the slide was gently pressed firmly enough to enable the Vaseline seal the cover-slip in position. The slide was then smoothly inverted in order to allow the drop of culture in hanging position. The preparation was immediately observed under a reduced illumination (Olutiola *et al.*, 2000).

Catalase test

This test was also carried out on a clean, sterile new slide. A fairly thick emulsion of the isolate was made on a clean slide and a loop full of 3% hydrogen peroxide (H_2O_2) was added to it. The presence of catalase will be indicated by effervescence (Olutiola *et al.*, 2000).

Oxidase test

The filter paper spot was first wet with three drops of sterile distilled water after which a large mass of pure 24hrs old bacteria culture was aseptically transferred to the wet spot. This was then followed by the addition of one drop of the oxidase reagent. The spots were observed for blue colour within 10 seconds and the results compared with the control.

Starch hydrolysis test

Starch agar was prepared by fortifying the appropriate medium with 1% of soluble starch and autoclaved at 121°C for 15mins after which the plates were poured and allowed to gel. The organisms were then streaked once across the surface of the plates and incubated at 37°C for 24h. The plates were then flooded with some quantity of Gram's iodine. Unhydrolyzed starch will form blue black colour with the iodine while hydrolyzed starch appears as a clear zone which results from α -amylase activity. Reddish brown zones around the colony indicate partial hydrolysis of starch (Olutiola *et al.*, 2000).

Carbon sources utilization

The sugars used for this test were eight in number and they include: Glucose, lactose, Fructose, Mannitol, Galactose, Maltose, Arabinose and Sucrose. 1.0% of each sugar was prepared by dispersing 1gram in 100ml of distilled water. 1.0% peptone was also prepared the same way and 0.01% methyl red was also prepared by diluting 0.01ml in 100ml of distilled water. 0.5ml of the indicator was added to one tube of each carbon type and peptone, 10ml in quantity with Durham tube carefully inverted in the tubes were then sterilized at 121°C for 15 minutes in the autoclave before each carbon source was inoculated with the same organism and incubated at 37°C for three to four days. Change in colour and accumulation of gas is an indication of positive result (Olutiola *et al.*, 2000).

Identification of isolates

The identification of bacterial isolates was carried out after the physical, morphological and biochemical characterization of the isolates. The isolates were identified with reference to the Bergey's manual of determinative bacteriology and ABIS online Encyclopedia (The great bacteria book). Pure Fungi isolates were identified based in macroscopic and microscopic characteristics with robust reference to Barnet and Hunter's illustrated genera of imperfect fungi (Barnet and Hunter, 1998).

Plasmid analysis of bacteria isolates

Plasmid extraction

Plasmid extraction was carried out based on the methods of Molina - Aja *et al.* (2009) with little modification. A single bacterial colony was picked up and grown in 5.0mL of Muller Hilton broth overnight in an Eppendorf tube and centrifuged at 10,000rpm for 2 min. The cell pellets obtained were re-suspended in 150µL EDTA-Tris buffer and vortexes to mix. This was followed by the addition of 175 µL of 2% Sodium Dodecyl Sulphate (SDS) and 175µL of 0.4N NaOH. The tube was mixed vigorously, 250 µL of cold 5M potassium acetate was added vigorously, the tube was centrifuged at 12, 000 rpm for 5 min and the supernatant was transferred to a sterile 1.5mLEppendorf tube and equal volume of cold isopropanol was added. After inverting gently, the mixture was immediately centrifuged at 12,000 rpm for 10 min and the DNA pellet was washed with 650µL of cold (40°C) 70% ethanol by centrifuging at 12,000 rpm for 15 min. The supernatant was discarded and the pellet was dried for 30 min and re-suspended in 40µL of sterile deionised water. Agarose Gel Electrophoresis was carried out by weighing 0.8g of agarose powder and 100mL of 1X Tris Borate Buffer (TBE buffer) was added, the buffer was dissolved by boiling in a microwave oven and allowed to cool to about 60°C and then 10 µL of ethidium bromide was added and mixed by swirling. The agarose was then poured into electrophoresis tank with the comb in place to obtain a gel thickness of about 4-5mm and was allowed to solidify for about 20 minutes and the comb was removed, the tray was then placed in the electrophoresis tank. This was followed by the addition of 1X TBE buffer; this was then poured into the tank ensuring that the buffer covered the surface of the gel. The sample 15 µL was mixed with 2 µL of the loading dye and was carefully loaded into the wells created by the combs (marker was loaded in line 1). Electrodes were connected to the power pack in such a way that the negative terminal is at the end where the sample was loaded; electrophoresis was run at 60-100V until loading dye has migrated about three-quarter of gel. Electrodes were disconnected and gel was removed from the tank and visualized in UV- trans-illuminator.

Physicochemical analysis of indigenous black soap samples

All reagents were analytical grade. Analyses were carried out according to the methods described by A.O.A.C (2007)

Determination of moisture content

5 g of samples was accurately weighed using analytical balance (sensitivity 0.1mg) into dried, tarred moisture dish and dried in an oven (Memmert, Germany) for 2hr at $101 \pm 1^{\circ}\text{C}$ and repeated until a constant weight (difference between two measurement not exceed 0.5mg/g of sample) was reached. The % moisture was calculated using the following formula (A. O. A. C. 2006).

$$\% \text{ Moisture} = \frac{C_s - C_h}{C_s - C_w} \times 100$$

C_w = weight of crucible

C_s = weight of crucible + sample

C_h = weight of crucible + sample after heating.

Determination of total fatty matter (TFM)

The total fatty matter test is carried out by reacting soap with acid in the presence of hot water and measuring the fatty acids obtained. 10 g of soap was weighed and 150 ml distilled water was added and heated. The soap was dissolved in 20 ml of 15 % H₂SO₄ while heating until a clear solution was obtained. Fatty acids on surface of the resulting solution was solidified by adding 7g of bee wax and reheated. The set up was allowed to cool to form cake. Cake was removed and blotted to dry and weighed to obtain the total fatty matter using a formula (A. O. A. C. 2006).

$$\% \text{ TFM} = \frac{A - X}{W} \times 100$$

A= weight of wax + oil

X= weight of wax

W= weight of soap.

Determination of matter insoluble in Ethanol

5g weight (W) of soap sample was weighed into 250ml beaker. 150ml of ethanol was added and heated to dissolve. Few drops of distilled water were added to it to increase the solubility of the ethanol. Using 41 Whatman filter paper of 12cm diameter it was dried in the oven, cooled, in the desiccator and weighed (W₁). The sample solution was filtered under a boiling ethanol. The alcohol was dried leaving the filter paper and the residue. It was later cooled in the desiccator and quickly reweighed. The later was repeated to get a constant weight (W₂)

Calculation

$$\% \text{ MIIE} = \frac{W_2 - W_1}{W} \times 100$$

Determination of matter insoluble in water

5g weight (W) of grated soap sample was weighed into 250mL of water, leveled with 150mL of water and heated to dissolve on hot plate using 41 Whatman filter paper of 12.5cm diameter. This was dried in the oven, cooled in the desiccator. Weighed (W₁) and the sample solution was filtered under boiling alcohol. The water on the filter paper and the reweighed and residue cooled in the desiccator. Reweighed and reweighing was repeated to get a constant weight (W₂).

Calculation:

$$(\%) \text{ MIIW} = \frac{W_2 - W_1}{W} \times 100$$

Determination of free caustic alkali

5g of finished soap was weighed and dissolved in 30mL of ethanol. Few drops of phenolphthalein indicator and 10mL of 20% BaCl₂ were added. The resulting solution was titrated against 0.05M H₂SO₄. Free caustic alkali- the volume of the acid obtained was calculated using the formula;

$$\text{NaOH} = \frac{0.31 \times V_a}{W}$$

V_a = Vol. of acid

W = weight of soap

Determination of rate of wear

The weight (W) of soap was taken before the soap was immersed in distilled water and rubbed against the palm with water. This was done for about two hundred times and the soap dipped in water after rubbing against the palm for every twenty five times. The soap was left for 24 hours and the procedure was repeated. This was done for five days but the new weight (which is usually less due to the loss in weight) was recorded before the daily washings. The daily new weights, losses in weight, percentage losses in weight, and cumulative losses in weight were noted.

Calculation:

$$\%ROW = \frac{\Sigma Cum}{W} \times 100$$

Determination of Bulk density

An irregular shape soap sample was totally immersed in water displacing an equal volume of water which was recorded. The volume of the water was calculated mathematically. The soap was weighed and its weight recorded before immersing it in the water.

Calculation:

$$\text{Bulk Density} = \frac{\text{Mass (g)}}{\text{Volume (cm}^{-3}\text{)}}$$

Determination of pH

2.0 g of the soaps were dissolved in 50 ml of deionised water and the pH determined using a pH meter (Kent EIL 7055). This was done twice for each soap sample and the mean computed.

Determination of lathering ability

2.00 g of the soap was dissolved in 50 mL of distilled water in a 100 mL measuring cylinder and shaken vigorously for 2 min. It was allowed to stand for 10 min after which the height of the foam was determined. This was repeated thrice for each soap sample and the mean computed.

Determination of colour

This was done based on visual examination.

Determination of foam stability

Five 100ml conical flasks were numbered serially, 1 to 14. Sixteen (16) ml of distilled water was added to each of the flasks followed by 8g of soap. The mixture was warmed to get a solution. 1ml of soap solution was added to 3ml of distilled water. The process was repeated for each soap solution in different test tubes. With the mouth of the test tubes closed, each was shaken vigorously with equal force for 1 min. The timer was set immediately, the rate of disappearance of 5mm of froth was observed.

Preparation of standard inoculum

The test organisms from growth on nutrient broth incubated at 37°C for 18 h for the bacteria and potato dextrose broth incubated at 35°C for 48 h for the yeast were suspended in saline solution (0.85% NaCl) and adjusted to match a turbidity of 0.5 McFarland standards at wavelength 540nm according to the method described by CLSI (2010). For the dermatophyte, the surface of the Petri-plates with the filamentous fungus in confluence growth was flooded with ten (10) mL of sterile distilled water. 0.5 ml containing approximately 2.4×10^6 cells/mL was used.

Determination of Minimum inhibitory concentrations MIC, Minimum bactericidal concentration MBC and Minimum fungicidal concentration MFC of Soap samples on test pathogens

The tube dilution susceptibility test was used to determine the MIC, MBC and MFC values for the soap samples: A series of Mueller-Hinton broth tubes containing varying two fold concentrations of the various soap samples in the range of 500 mg/mL to 62.5 mg/mL was prepared and incubated with a previously standardized density of the test organisms (0.5ml). The lowest concentration of the soap sample resulting in no growth following visual inspection after 18-24 hours of incubation for bacteria and 24 – 27 h for yeast and mould was recorded as the MIC. The MBC and MFC were determined appropriately by sub-culturing tubes showing no growth including the MIC on a fresh medium. The lowest soap samples concentration from which the microorganisms do not grow when transferred to a fresh medium were recorded applicably as MBC and MFC.

Screening of black soap isolates for antagonistic activities on test pathogens by Fokkema method

A single streak was made from 24hrs old culture of bacteria, 23 mm away from the centre of petri-dish. The plates were then incubated for 24hrs in order to allow antagonistic substances to be produced; after which a single streak of each test organism was put perpendicularly to the antagonists without touching it (Fokkema, 1973). These were incubated at 37°C for twenty four hours before proper comparison with the control experiment was made and horizontal zones of inhibition measured. Standard antibiotic discs were also used against the test pathogenic organisms as positive control and comparative purposes.

Comparative antimicrobial activities of the Black soaps and selected commercial antiseptic soap by Agar well diffusion.

The susceptibilities of the test organisms to black soaps and selected antiseptic soaps were assayed using agar-well diffusion method (Aliyu, *et al.*, 2009; Ikpoh *et al.*, 2012). 5g of soap was scraped with sterile blade and dissolved in 10mL of sterile distilled water to give a stock solution this was also done for selected topical creams. 0.5ml of the standardized suspension was seeded into Mueller Hinton agar plates and PDA plates (90mm in diameter) using pour plate method. Three wells were made on each plate using sterile core borer (8 mm) and 0.2 mL of the black soap solutions was transferred into each of the three wells (appropriately labelled) and sterile distilled water into the fourth well as the negative control. The responses of the test organisms to the selected commercial antiseptic soaps were evaluated by making six wells on each plate using sterile cork borer (8mm) followed by the addition of 0.2 mL of the soap solution to each of the five wells (appropriately labelled) and distilled water into the sixth well as the negative control. The antimicrobial activities of the selected commercial topical creams were investigated as follow: six wells were made on each plate using sterile cork borer (8 mm) and 0.2 mL of the cream solution was transferred into each of the five wells (appropriately labelled) and distilled water into the sixth well as the negative control.

For the selected broad spectrum antibiotics, standardized suspension of test organisms was used to swab the surface of already set Mueller Hinton agar with the aid of sterile cotton swab sticks. The plates were allowed to drain properly before the antibiotic discs were aseptically placed on its surface with the aid of a sterile forceps.

The plates were incubated at 37⁰C for 24 h for the bacteria, 48 h for *C. albicans* and at room temperature for five days for the dermatophyte. The plates were observed for zone of inhibition around the wells and the zones of inhibition were then measured and recorded using transparent metre rule. The test was conducted in duplicates.

Determination of mechanism of action of the Black Soap

A 0.5 mL each of the standardized organism was added to 5 mL of the prepared Mueller Hinton and Sabouraud dextrose broth to which 0.5mL 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 h and 72 h for bacteria, yeast and the mould respectively. The solution was centrifuged at 7000 revolution per minute (r. p. m.) and 3 mL of the supernatant was diluted with 50ml sterile distilled water and analyzed using a flame photometer at 589 nm and 766 nm for sodium and potassium ion leakage respectively.

Qualitative screening of black soaps for phytochemicals

All reagents were analytical grade. Analyses were carried out according to the methods described by A.O.A.C (2007)

Alkaloid determination

About 0.5g of the sample was stirred 5mL of 1% aqueous HCL on a stream water bath, 1mL 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 determination

About 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 determination

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 the presence of tannin.

Terpenoid determination

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.

Phlobatannin determination

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

Flavonoid determination

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 1ml conc. H_2SO_4 indicate the presence of flavonoid.

Quantitative screening of the black soaps for phytochemicals.

All reagents were analytical grade. Analyses were carried out according to the methods described by A.O.A.C (2007)

Determination of saponin content

A 2 g of sample was weighed into a 250 ml beaker and 100ml of isobutyl alcohol was added. Shaker was used to shake the mixture for 5 hours to ensure uniform mixing. The mixture was filtered with No 1 whatman filter paper into 100 ml beaker containing 20ml of 40% saturated solution of magnesium carbonate ($MgCO_3$). The mixture obtained was filtered again using No 1 Whatman filter paper to obtain a clean colourless solution. 1 ml of the colourless solution was taken 150ml volumetric flask using pipette and 2ml 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 30minutes for the colour to develop. The absorbance was read against the blank at 380mm.

Determination of flavonoid content

A 0.5ml of appropriately diluted sample was mixed with 0.5ml methanol, 50 μ L, 10% $AlCl_3$, 50 μ L, 1M potassium acetate and 1.4 ml water and allowed to incubate at room temperature for 30 minutes. The absorbance of the reaction mixture was subsequently measured at 415nm; 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 50ml sample bottle. 10 ml of 70% aqueous acetone was added and properly covered. The bottle were put in an ice bath shaker and shaken for 2h at 30C. Each solution was then centrifuged and the supernatant stored in ice 0.2ml of each solution was pipetted into the test tube and 0.8ml of distilled water was added. Standard tannin acid solutions were prepared from a 0.5mg/ml of the stock and the solution made up to 1ml with distilled water. 0.5ml of Folin-cicocateau reagent was added to both sample and standard followed by 2.5ml of 20% Na₂CO₃ the solution were then vortexed and allowed to incubate for 40minutes at room temperature, its absorbance was read at 725nm against a reagent blank concentration of the same solution from a standard tannic acid curve that was prepared.

Determination of terpenoid content

A 2.0g of soap sample was soaked in 50ml of 95% ethanol for 24hrs. the extract was filtered and the filtrate extracted with petroleum ether (60 to 80C) and concentrated to dryness. The dried ether extract was treated as total terpenoids.

Preparation of black soap for GCMS analysis

This was done according to the methods described by A.O.A.C (2007). About 1.0g of soap was dissolved in 0.2ml of toluene. To the solution, 1.5ml of methanol and 0.3ml of the 8.0% hydrogen chloride (HCl) solution were added in this order. The final HCl concentration was 1.2% (w/v) or 0.39M, which correspond to 0.06 ml of conc. HCl in a total volume of 2ml. The tube was vortexed and then incubated at 45C overnight (14h or longer) for mild methanolysis/methylation. After cooling to room temperature, 3μL of sample was injected into the column of GCMS (QP2010 Plus Shimadzu, Japan).

Statistical analysis of data obtained

Analysis of Variance (ANOVA), was used to investigate significant differences between treatments at 5% confidence level (P = 0.05).

RESULTS

Microbiological analysis of the Black soap samples

The Bacteria colony forming units per gram (cfu/g) obtained for the black soap market sample, prepared black soap, and black soap with honey is shown on tables 1-3. Black soap market sample and laboratory prepared sample have the highest bacterial cfu/g of 9.1×10^6 at the same dilutions of 10^{-5} . Black soap fortified with honey demonstrated lowest bacterial cfu/g of 1.2×10^6 at dilution 10^{-5} . There were significant differences ($P \leq 0.05$) between the bacterial mean colony counts at the considered dilutions for black soap market sample and black soap with honey (Tables 1-3).

The mean fungal counts obtained for the black soap market sample, laboratory prepared black soap and black soap fortified with honey is shown on table 1 - 3. Laboratory prepared black soap has higher fungal counts at both dilutions while black soap with honey demonstrated lowest fungal cfu/g of 1.0×10^6 . There were no significant differences ($P \leq 0.05$) between the fungal mean colony counts at the considered dilutions for black soap market sample (Tables 1-3).

Bacterial isolates from Black Soap samples

Two different *Bacillus* spp were isolated from the black soap samples in this research. Gram staining showed the presence of Gram positive rod-like bacteria. It was observed that the bacteria isolated were motile, catalase positive (Table 4). The names of the identified isolates were: *Bacillus brevis* and *Bacillus megaterium* (Table 4).

Table 1: Mean Microbial Count in Black Soap Obtained from Market, laboratory prepared and honey fortified black soap

Dilution factor	Mean colony count	
Bacteria		CFU/g
10 ⁻⁴	16.33±0.57 ^a	1.6×10 ⁵
10 ⁻⁵	9.17±0.17 ^b	9.1×10 ⁶
Fungi		SFU/g
10 ⁻⁴	28.33±2.89 ^a	2.8×10 ³
10 ⁻⁵	28.33±2.89 ^a	2.8×10 ⁴
Black soap		CFU/G/g
10 ⁻⁴	13.33±0.57 ^b	1.3×10 ⁵
10 ⁻⁵	9.17±0.28 ^a	9.1×10 ⁶
Fungi		SFU/g
10 ⁻⁴	46.67±5.77 ^b	4.6×10 ³
10 ⁻⁵	36.67±5.77 ^a	3.6×10 ⁴
Bacteria		CFU/g
10 ⁻⁴	13.32±0.25 ^b	1.3×10 ⁵
10 ⁻⁵	12±0.12 ^b	1.2×10 ⁶
Fungi		SFU/g
10 ⁻⁴	12.67±5.77 ^b	1.2×10 ⁵
10 ⁻⁵	10.00±10.00 ^a	1.0×10 ⁶

Values followed by the same letter in a column are not significantly different at P≤0.05.

Keys: CFU/g=Colony Forming Units per Gram, SPU/g=Spore Forming Units per Gram.

Fungal isolates from black soap samples

Three different fungi were isolated from the black soap samples. Their microscopic and macroscopic niceties varied uniquely (Table 5). The isolates are then matched with their respective sources in furtherance to proper elucidation.

Plasmid profiles of Bacteria isolates from black soap samples

The bacteria isolates from black soap samples (*Bacillus brevis* and *Bacillus megaterium*) were investigated for the presence of DNA plasmids using Agarose gel electrophoresis. The results obtained revealed that the organisms actually contained plasmids. The molecular weights of the plasmids were determined using DNA- Hind III molecular weight marker (Plate 1). It was observed that *B. brevis* contained plasmid with an estimated molecular weight of 20145bp. The band pattern obtained for *Bacillus megaterium* does not clearly represent a plasmid when compared with that of *Bacillus brevis*.

Table 2: Morphological and biochemical characteristics of bacteria isolates

Characteristics	Isolate AB1	Isolate C1
Macroscopic Characteristics		
Colony shape	Circular	Circular
Colony colour	White	Cream
Colony edge	Rough	Smooth
Elevation raised	Raised	Slightly
Microscopic Characteristics		
Gram reaction	+	
+		
Shape/structure		Rod
Rod		
Spore (Subterminal)	+ (Terminal)	+
Motility	+	+
Catalase	+	+
Oxidase	+	+
Starch hydrolysis	-	-
Carbon sources utilization		
Glucose	-	-
Lactose	+	+
Fructose	+	-
Mannitol	-	+
Sucrose	+	-
Galactose	+	-
Maltose	+	+
Arabinose	-	-
Growth at pH 10	+	
+		

Key; + Positive, - Negative

Probable identity; AB1: *Bacillus brevis*, C1: *Bacillus megaterium*

Table 3: Microscopic and Macroscopic characterization of fungal isolates

Isolates	Macroscopic	Microscopic
C1	Colonies on potato dextrose agar at 25°C are olive to lime green with cream reverse. Rapid growth. Texture is woolly to cottony to somewhat granular. Sclerotia, present, are dark	Hyphae are septate and hyaline. Conidial heads are radiate to loosely columnar. Conidiophores are coarsely roughened, uncoloured, up to 800 µm long x 15 – 20 µm wide, vesicles globose to subglobose (20 – 45 µm),
A1	Colonies grow moderately rapidly at 25°C. They are granular, cottony and flat, raised and folded in appearance. From the	Conidiophores are poorly differentiated, much like vegetative hyphae, mostly erect and branching irregularly, hyaline; conidia (aleuriospores or arthrospores)
A2	Colonies on potato dextrose agar at 25°C are dull brown, irregularly furrowed, mostly floccose, uneven in texture, with	Hyphae are septate and hyaline. Conidiophores are thin walled, smooth, pale brown measuring 350-500 µm long, Vesicles are elliptical, 12-18 µm in diameter, and biseriolate, with almost the entire surface being covered. Conidia
Ten	granular appearance due to the production of small aggregates of Hülle cells. The reverse is dark yellow to reddish brown. Growth rate is moderate to rapid. Conidial heads are pale blue-green.	are globose, pale green, delicately roughened, and measure 3.5-5.5 µm.

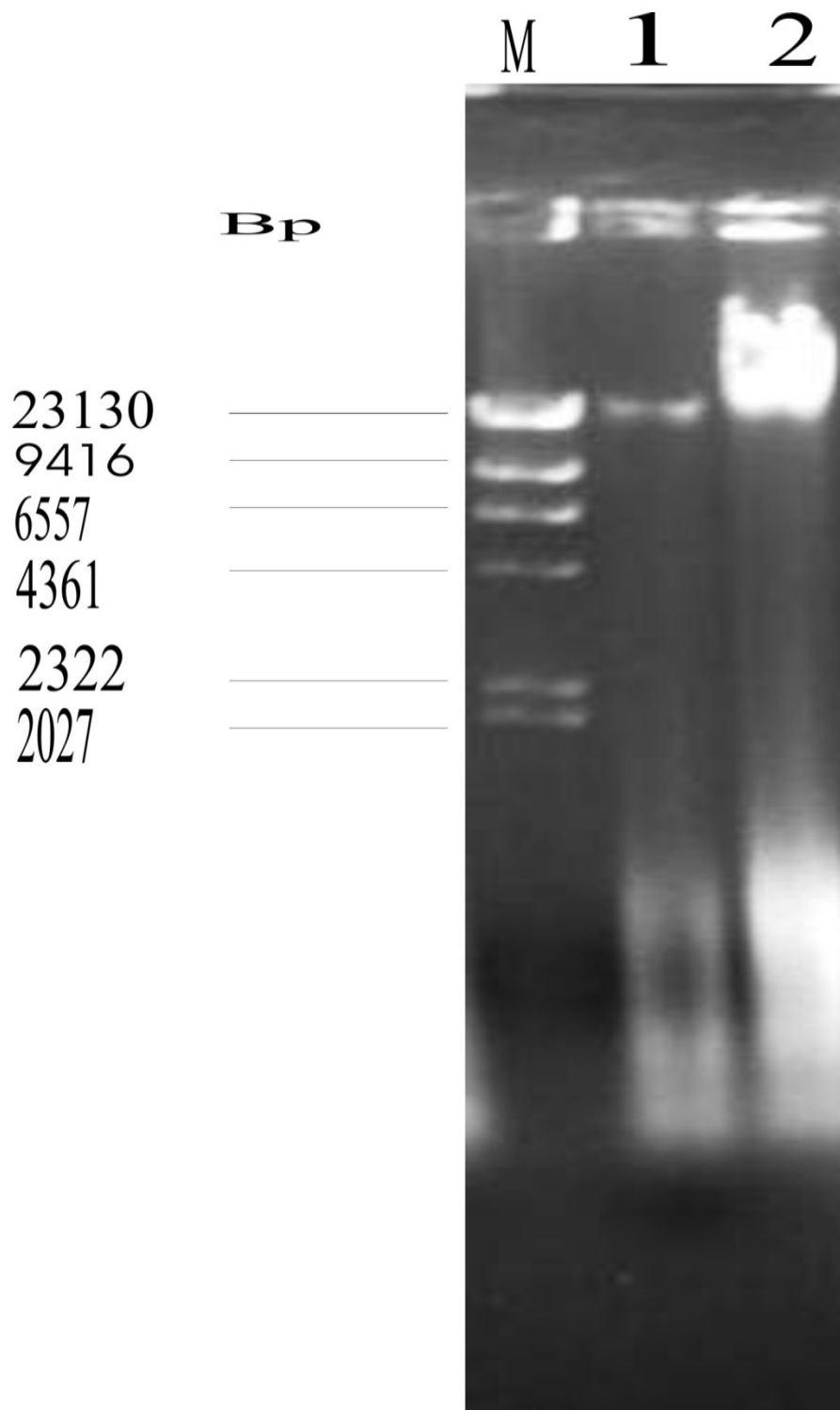


Plate 1: Electrophoretic pattern for plasmid profile of bacteria isolates from black Soap samples.

Keys: M=Molecular weight marker, 1=*Bacillus brevis*, 2= *Bacillus megaterium*, bp= basepair, 1bp=3.4 Angstrom (Å) while 1000bp= 1 kilo basepairs.

Physicochemical properties of Black soap Samples

The physicochemical traits of the black soap samples suggest that black soaps with honey generally has lower moisture content at 9.89 %. While the market sample at 11.50 %. The black

soap market sample recorded the highest level of total fatty matter at 28.41 %. While black soap with honey recorded 12.16%. There were significant differences ($P \leq 0.05$) between the Unsaponified Neutral Fat of the black soap market sample and black soap with honey (Table 7).

Minimum inhibitory concentrations MIC of Soap samples on test pathogens

The comparative minimum inhibitory concentration MIC tests of the Black soaps and some selected commercial antiseptic soap on the test organisms as shown in Table 8 indicates that black soaps were more active as evident in their lower MIC values (MIC, 62.5 mg/mL, 125 mg/mL for *T. rubrum*) when compared with the commercial soaps mostly at 500 mg/mL on majority of the test pathogens.

Minimum Bactericidal concentrations MBC of Soap samples on test pathogens

It was discovered in this research that the MIC for the Black soaps is also its MBC, (MBC, 62.5 mg/mL, 125mg/mL for *T. rubrum*) while that of selected commercial soaps ranged appropriately (MBC, 250-500 mg/mL) (Table 9)

Minimum Fungicidal concentrations MFC of Soap samples on test pathogens

Black soaps have MFC value at 62.5 mg/mL and 125mg/mL for *T. rubrum* while commercial antiseptic soaps at 250-500 mg/mL (Table 10). MFC value could not be detected for septol on *T. rubrum*. *T. rubrum* on a general note required higher MFC when compared with *Candida albicans* (table 10).

Table 4: Physicochemical properties of Black Soap

Parameter	BB	CC
Moisture Content (%)	11.50± 0.10 ^f	9.89±0.08 ^e
Total Fatty Matter (%)	28.41 ± 0.50 ^h	12.16±0.17 ^h
Matter Insoluble in Ethanol (%)	18.056 ± 0.100 ^g	17.010±0.090 ^f
Matter Insoluble in Water (%)	10.963 ± 0.020 ^e	24.445±0.050 ^g
Free Caustic Alkali (%)	15.74 ± 0.40 ^c	12.95±0.30 ^c
Unsaponified Neutral Fat (%)	0.886 ± 0.000 ^a	1.212±0.001 ^b
Rate of Wear (%)	94.738 ± 0.000 ⁱ	95.134±0.120 ⁱ
Bulk Density (MG/CM) ³	1.340 ± 0.000 ^b	1.73±0.03 ^a
pH	9.40± 0.04 ^d	9.80±0.07 ^e
Lathering ability	Good	Good
Colour	Black	Black
Foam stability	Stable	Stable

Values represent means ±standard deviation of triplicate readings. Superscripts of the same letter in a row are not significantly different at $P \leq 0.05$.

Key: BB=Black soap market sample, CC=Black soap with honey, %=Percentage, MG/CM³=Milligram/Centimetre cubic.

Table 5: Comparative Minimum Inhibitory Concentration MIC (mg/mL) of Black soaps and Commercial soaps

Organisms	Source	BB	CC	1	2	3	4	5
* <i>S. epidermidis</i>		60.5	60.5	250	250	250	125	125
<i>S. epidermidis</i>	ATCC-12228	60.5	60.5	250	250	250	125	125
* <i>S. aureus</i>		60.5	60.5	500	250	500	125	250
<i>S. aureus</i>	ATCC-25923	60.5	60.5	125	250	500	125	250
* <i>E. coli</i>		60.5	60.5	500	500	500	250	500
<i>E. coli</i>	ATCC-25922	60.5	60.5	500	500	500	250	500
* <i>P. aeruginosa</i>		60.5	60.5	500	500	500	500	500
<i>P. aeruginosa</i>	ATCC-27853	60.5	60.5	500	500	500	500	ND
* <i>K. pneumoniae</i>		60.5	60.5	250	500	250	500	500
<i>K. pneumoniae</i>	ATCC-11930	60.5	60.5	250	500	250	500	500
* <i>C. albicans</i>		60.5	60.5	250	250	500	125	500
<i>C. albicans</i>	ATCC-10261	60.5	60.5	250	500	500	125	500
* <i>T. rubrum</i>		125	125	500	500	ND	500	500

Key: BB=Black soap market sample, CC=Black soap with honey, 1=Meriko, 2=Mekako, 3=Septol, 4=Delta, 5=Dettol, ND=Not detectable.
 *= Clinical isolate

Table 6: Comparative Minimum Bactericidal Concentration MBC (mg/mL) of Black soaps and Commercial soaps.

Organisms	Source	A	B	1	2	3	4	5
* <i>S. epidermidis</i>		60.5	60.5	500	500	500	250	250
<i>S. epidermidis</i>	ATCC-12228	60.5	60.5	500	500	500	250	250
* <i>S. aureus</i>		60.5	60.5	500	500	500	250	250
<i>S. aureus</i>	ATCC-25923	60.5	60.5	500	500	500	250	250
* <i>E. coli</i>		60.5	60.5	500	500	500	500	500
<i>E. coli</i>	ATCC-25922	60.5	60.5	500	500	500	500	500
* <i>P. aeruginosa</i>		60.5	60.5	500	500	500	500	500
<i>P. aeruginosa</i>	ATCC-27853	60.5	60.5	500	500	500	500	500
* <i>K. pneumoniae</i>		60.5	60.5	500	500	500	500	500
<i>K. pneumoniae</i>	ATCC-11930	60.5	60.5	500	500	500	500	500

Key: BB=Black soap market sample, CC=Black soap with honey, 1=Meriko, 2=Mekako, 3=Septol, 4=Delta, 5=Dettol.
 *= Clinical isolate

Table 7: Comparative Minimum Fungicidal Concentration MFC (mg/mL) of Black soaps and Commercial soaps

Organisms	Source	BB	CC	1	2	3	4	5
* <i>C. albicans</i>		60.5	60.5	500	250	500	250	500
<i>C. albicans</i>	ATCC-10261	60.5	60.5	250	500	500	125	500
* <i>T. rubrum</i>		125	125	500	500	ND	500	500

Key: BB=Black soap market sample, CC=Black soap with honey, 1=Meriko, 2=Mekako, 3=Septol, 4=Delta, 5=Dettol, ND=Not detectable
 * = Clinicals

Screening of Black soap isolates for antagonistic activities on test pathogens.

It was determined that the two Bacteria isolates: *B. brevis* and *B. megaterium* which represent 100% of the bacterial isolates from the black soap samples produced inhibitory substances against *Escherichia coli*, and *Staphylococcus epidermidis*; with *B. brevis* inhibiting the growth of both organisms. *B. megaterium* was able to inhibit the growth of *E. coli* only. The active isolates suppressed or inhibited bacteria growth (both clinical and reference strains) with inhibition zones ranging from 15 to 34 mm. (Figure 1). None of the fungal isolates showed antagonism towards the test pathogens.

Comparative antimicrobial activities of the Black soaps and selected commercial antiseptic soap.

It was found out that Black soap had more inhibitory effects on all the pathogens used in this investigation. Black soap with honey had the highest inhibition zone of 17.83mm with *Candida albicans* ATCC-10261 followed by the black soap market sample at 15.83mm on the same organism, (Table 11). There were significant differences ($P \leq .05$) between the inhibitory activities of the black soaps and other soaps. Selected commercial antiseptic soaps have inhibition zones ranging from 0-12.17mm, with Dettol having the highest inhibitory action of 12.17 mm against *Klebsiella pneumoniae* ATCC-11930 (Table 11).

The commercial topical skin creams with both antibacterial and antimycotic activities used in this study for positive control and as well as broadened comparative purposes inhibited the growth of most test organisms with zones of inhibition between 0-14mm. It was discovered that Tricutan, Sivoderm and Acneaway were not active against *Klebsiella pneumoniae*. Acneaway had an inhibitory effect of 14 mm in zone of inhibition against *Staphylococcus aureus* ATCC-25923. There were significant differences ($P \leq .05$) between the inhibitory activities of the black soaps and the selected skin creams (Table 12).

The commercial broad spectrum antibiotics used for also both positive control also exhibited inhibitory action against the test pathogens. Nitrofurantoin had the highest inhibitory action of 10mm of inhibition zone against *Klebsiella pneumoniae* clinical and reference strains. Type Cultures were relatively more susceptible to the antibiotics when placed side by side with clinical isolates. Other antibiotics with inhibitory activities against one or two test organisms are; Gentamycin, Ofloxacin, Cotrimoxazole, Tetracycline and Nalidixic acid. Zones of inhibition ranged from 1-10 mm. There were significant differences ($P \leq .05$) between the inhibitory activities of Nitrofurantoin and other antibiotics. (Table 13).

Mechanisms of action of Black Soap

Sodium and potassium ions were leaked by the black soaps from the cell of the organisms as illustrated in Figure 2. Sodium ion was leaked to a value of 833 ppm for *S. aureus* while potassium ion to the lowest value of 20 ppm for *T. rubrum*

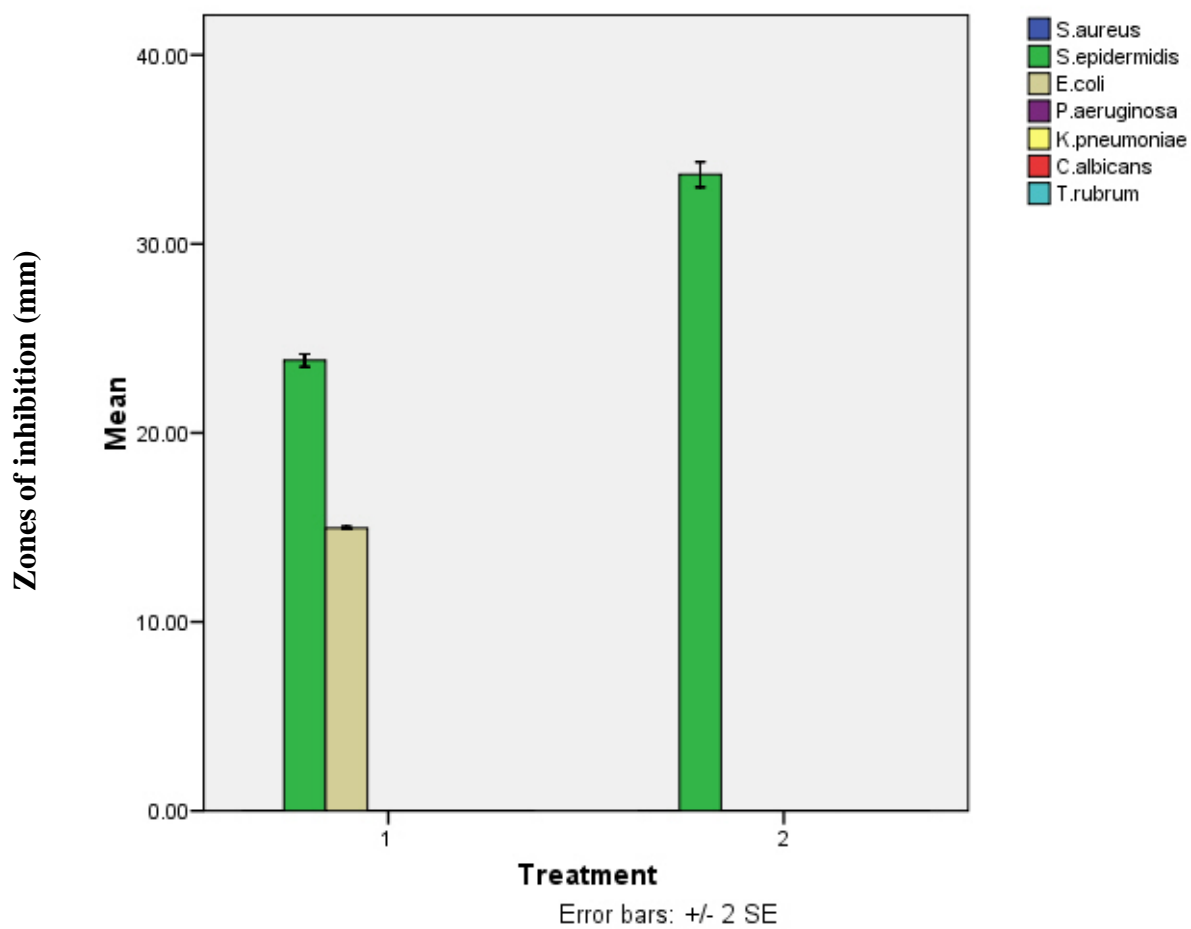


Figure 1: Comparative antagonistic activities of bacterial isolates from the black soap on test pathogens.

Keys: 1= *Bacillus brevis*, 2= *Bacillus megaterium*

Table 8: Comparative antimicrobial activities of black soap and selected commercial soap at 60mg/mL

Organisms	Source	A	B	C	1	2	3	4	5	Control
* <i>S. epidermidis</i>		10.01±0.01 ^e	13.63±0.55 ^g	11.83±0.29 ^f	6.01±0.01 ^d	5.00±0.00 ^c	4.00±0.01 ^b	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00
<i>S. epidermidis</i>	ATCC-12228	11.93±0.12 ^e	14.50±0.01 ^f	11.83±0.29 ^e	7.00±0.00 ^d	6.00±0.00 ^c	5.67±0.58 ^c	2.00±0.00 ^b	2.02±0.01 ^b	0.00±0.00
* <i>S. aureus</i>		10.83±0.28 ^c	13.63±0.55 ^d	10.90±0.17 ^c	0.00±0.00 ^a	5.00±0.06 ^b	5.01±0.02 ^b	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00
<i>S. aureus</i>	ATCC-25923	11.83±0.29 ^e	15.00±0.00 ^g	10.90±0.17 ^d	3.00±0.00 ^b	7.00±0.00 ^d	6.00±0.00 ^c	3.00±0.00 ^b	2.67±0.56 ^b	0.00±0.00
* <i>E. coli</i>		11.00±0.03 ^c	12.00±0.00 ^c	11.90±0.17 ^c	0.00±0.00 ^a	3.00±0.01 ^b	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00
<i>E. coli</i>	ATCC-25922	13.83±0.29 ^f	12.83±0.28 ^e	11.90±0.17 ^d	0.00±0.00 ^a	3.00±0.01 ^c	1.00±0.00 ^b	1.00±0.00 ^b	0.00±0.00 ^a	0.00±0.00
* <i>P. aeruginosa</i>		11.98±0.29 ^c	13.67±0.28 ^d	9.83±0.29 ^b	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00
<i>P. aeruginosa</i>	ATCC-27853	12.83±0.29 ^f	15.17±0.28 ^g	9.83±0.28 ^e	1.00±0.00 ^b	0.00±0.00 ^a	0.00±0.00 ^a	2.00±0.00 ^c	1.00±0.00 ^b	0.00±0.00
* <i>K. pneumoniae</i>		8.83±0.29 ^e	9.83±0.29 ^f	8.67±0.29 ^e	7.00±0.01 ^d	6.17±0.29 ^c	10.00±0.00 ^f	2.98±0.32 ^b	10.00±0.00 ^f	0.00±0.00
<i>K. pneumoniae</i>	ATCC-11930	13.83±0.29 ^g	15.83±0.29 ^h	8.67±0.28 ^d	10.00±0.00 ^e	10.00±0.00 ^e	7.17±0.29 ^c	3.16±0.28 ^b	12.17±0.28 ^f	0.00±0.00
* <i>C. albicans</i>		13.67±0.76 ^g	13.10±0.12 ^f	13.17±0.29 ^f	3.98±0.03 ^b	4.00±0.00 ^b	6.00±0.00 ^d	9.00±0.01 ^e	5.00±0.00 ^e	0.00±0.00
<i>C. albicans</i>	ATCC-10261	15.83±0.29 ^f	17.83±0.29 ^g	13.17±0.28 ^e	4.83±0.28 ^c	4.00±0.00 ^b	5.00±0.00 ^c	10.00±0.00 ^e	6.00±0.00 ^d	0.00±0.00
* <i>T. rubrum</i>		6.00±0.00 ^e	4.00±0.01 ^d	3.83±0.29 ^d	4.00±0.01 ^d	0.00±0.00 ^a	2.00±0.00 ^c	0.00±0.00 ^a	1.00±0.01 ^b	0.00±0.00

Values followed by the same letter in a row are not significantly different at P≤0.05.

Key: A=Black soap market sample, B=Prepared Black soap, C= Black soap with honey, 1=Meriko, 2=Mekako, 3=Septol, 4=Delta, 5=Dettol, Control=Sterile distilled water. * = Clinicals

Table 9: Comparative antimicrobial activities of black soap and selected commercial skin creams at 60mg/mL

Organisms	Source	A	B	C	1	2	3	4	5	Control
* <i>S. epidermidis</i>		10.01±0.01 ^e	13.63±0.55 ^g	11.83±0.29 ^f	8.00±0.00 ^d	2.97±0.58 ^b	6.99±0.03 ^c	2.97±0.03 ^b	2.98±0.03 ^b	0.00±0.00
<i>S. epidermidis</i>	ATCC-12228	11.93±0.12 ^e	14.50±0.01 ^f	11.83±0.29 ^e	10.00±0.00 ^d	4.17±0.29 ^b	7.33±0.50 ^c	4.17±0.29 ^b	4.70±0.57 ^b	0.00±0.00
* <i>S. aureus</i>		10.83±0.28 ^c	13.63±0.55 ^d	10.90±0.17 ^c	7.98±0.03 ^b	9.00±0.01 ^c	9.00±0.00 ^c	9.98±0.03 ^d	13.00±0.00 ^f	0.00±0.00
<i>S. aureus</i>	ATCC-25923	11.83±0.29 ^e	15.00±0.00 ^g	10.90±0.17 ^d	9.17±0.29 ^b	10.00±0.00 ^c	10.10±0.01 ^c	10.01±0.02 ^c	14.00±0.00 ^f	0.00±0.00
* <i>E. coli</i>		11.00±0.03 ^c	12.00±0.00 ^c	11.90±0.17 ^c	7.80±0.03 ^b	9.00±0.00 ^c	8.00±0.01 ^b	9.00±0.01 ^e	9.00±0.01 ^e	0.00±0.00
<i>E. coli</i>	ATCC-25922	13.83±0.29 ^f	12.83±0.28 ^e	11.90±0.17 ^d	9.67±0.58 ^c	9.83±0.27 ^b	9.17±0.70 ^b	10.00±0.00 ^c	10.17±0.29 ^c	0.00±0.00
* <i>P. aeruginosa</i>		11.98±0.29 ^e	13.67±0.28 ^f	9.83±0.29 ^d	1.05±0.01 ^a	1.99±0.04 ^b	2.05±0.01 ^b	3.00±0.00 ^c	2.00±0.00 ^b	0.00±0.00
<i>P. aeruginosa</i>	ATCC-27853	12.83±0.29 ^f	15.17±0.28 ^g	9.83±0.28 ^e	1.05±0.01 ^a	0.00±0.00 ^a	2.00±0.01 ^b	4.00±0.00 ^d	3.00±0.00 ^c	0.00±0.00

* <i>K. pneumoniae</i>		8.83±0.29 ^e	9.83±0.29 ^f	8.67±0.29 ^c	3.99±0.04 ^b	0.00±0.00 ^a	4.03±0.05 ^b	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00
<i>K. pneumoniae</i>	ATCC-11930	13.83±0.29 ^g	15.83±0.29 ^h	8.67±0.28 ^f	4.67±0.57 ^e	1.00±0.00 ^b	5.00±0.00 ^e	2.00±0.00 ^c	3.33±0.57 ^d	0.00±0.00
* <i>C. albicans</i>		13.67±0.76 ^g	13.10±0.12 ^f	13.17±0.29 ^f	4.03±0.06 ^b	9.01±0.01 ^c	9.33±0.28 ^c	4.07±0.06 ^b	10.20±0.27 ^d	0.00±0.00
<i>C. albicans</i>	ATCC-10261	15.83±0.29 ^f	17.83±0.29 ^g	13.17±0.28 ^e	4.83±0.28 ^b	9.50±0.50 ^c	9.83±0.29 ^d	4.67±0.29 ^b	10.17±0.29 ^d	0.00±0.00
* <i>T. rubrum</i>		6.00±0.00 ^d	4.00±0.01 ^b	3.83±0.29 ^b	3.99±0.04 ^b	4.00±0.00 ^b	5.10±0.17 ^c	8.17±0.29 ^e	5.00±0.00 ^c	0.00±0.00

Values followed by the same letter in a row are not significantly different at $P \leq 0.05$.

Key: A=Black soap market sample, B=Prepared Black soap, C=Black soap with honey, 1=Tribact, 2=Tricutan, 3=funbact A, 4=Sivoderm, 5=Acneaway, Control=Sterile distilled water. * = Clinicals

Table 10: Comparative antibacterial activities of some broad spectrum antibiotics on selected bacteria pathogens

Organisms	Source	AMX	COT	TET	AUG	OFL	GEN	NAL	NIT
* <i>S. epidermidis</i>		0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	8.00±0.01 ^c	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
<i>S. epidermidis</i>	ATCC-12228	2.00±0.00 ^e	1.00±0.01 ^b	1.10±0.01 ^b	2.00±0.00 ^c	8.00±0.00 ^d	1.00±0.00 ^b	0.00±0.00	7.68±0.54 ^d
* <i>S. aureus</i>		0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	6.17±0.28 ^b	0.00±0.00 ^a	8.00±0.00 ^c
<i>S. aureus</i>	ATCC-25923	1.00±0.00 ^b	2.00±0.00 ^c	0.00±0.00 ^a	2.00±0.00 ^c	0.00±0.00 ^a	7.00±0.00 ^d	0.67±0.50 ^b	9.00±0.00 ^e
* <i>E. coli</i>		0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	3.00±0.01 ^b	0.00±0.00 ^a	7.00±0.01 ^c
<i>E. coli</i>	ATCC-25922	1.00±0.00 ^b	1.00±0.00 ^b	2.00±0.00 ^c	0.00±0.00 ^a	0.00±0.00 ^a	4.00±0.00 ^d	1.00±0.00 ^b	7.93±0.12 ^e
* <i>P. aeruginosa</i>		0.00±0.00 ^a	3.01±0.01 ^c	0.00±0.00 ^a	0.00±0.00 ^a	8.00±0.00 ^e	2.98±0.03 ^b	4.00±0.00 ^d	0.00±0.00 ^a
<i>P. aeruginosa</i>	ATCC-27853	0.00±0.00 ^a	3.01±0.01 ^c	0.00±0.00 ^a	0.00±0.00 ^a	8.02±0.01 ^e	2.98±0.03 ^b	4.00±0.00 ^d	0.00±0.00 ^a
* <i>K. pneumoniae</i>		0.00±0.00 ^a	5.00±0.00 ^c	1.00±0.01 ^b	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	10.00±0.00 ^c
<i>K. pneumoniae</i>	ATCC-11930	3.17±0.29 ^c	6.00±0.00 ^d	2.00±0.00 ^b	0.00±0.00 ^a	2.17±0.29 ^b	3.17±0.29 ^c	2.00±0.00 ^b	10.00±0.00 ^c

Values followed by the same letter in a row are not significantly different at $P \leq 0.05$.

Key: AMX=Amoxicillin, COT=Cotrimoxazole, TET=Tetracycline, AUG=Augumentin, OFL=Ofloxacin, GEN=Gentamicin, NAL=Nalidixic Acid
NIT=Nitrofurantoin.

* = Clinical isolates.

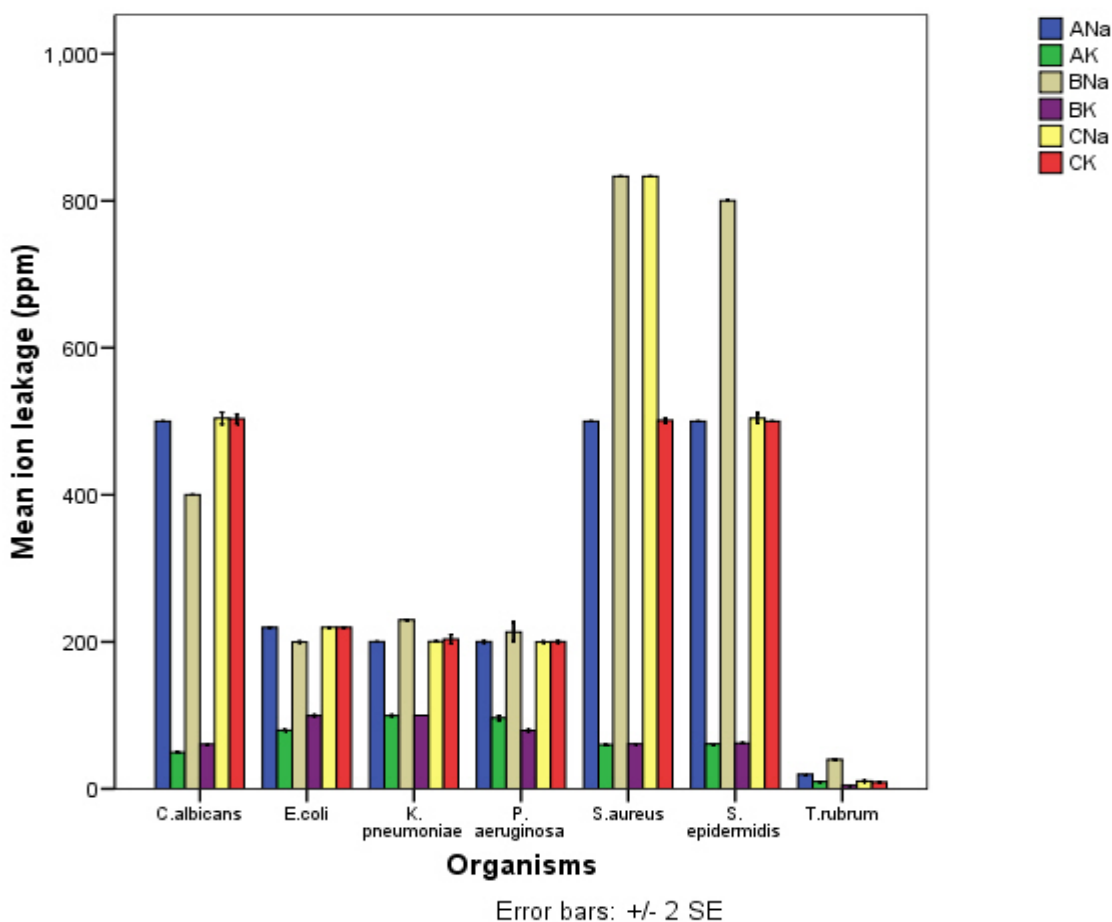


Figure 2: Comparative Sodium and Potassium ions leakage from test organisms by black soaps.

Keys: A=black soap market sample, B= prepared black soap, C=black soap with honey

Qualitative phytochemical profile of black soaps

Qualitative phytochemical screening has shown that black soaps contain Saponin, Tannin, Flavonoid, and Terpenoid, while Alkaloid and phlobatannin was absent (Table 14)

Quantitative phytochemical profile of indigenous black soaps

The quantitative screening of the black soaps for phytochemicals revealed variation in the compositions. The occurrence levels are reported in Table 15. Saponin has the highest occurrence and ranged between 20.0 to 33.31 mg/g in the studied samples. Tannin has the lowest occurrence with the values ranging between 1.0 to 2.05 mg/g in the samples. The values were not significantly different except for black soap fortified with honey.

Structurally elucidated black soaps

The result of the GC/MS analysis of methylated fractions of the black soaps showed different functional groups. A total of 19 peaks were obtained from three black soap samples. 9 peaks were obtained from prepared black soap sample and market sample, while 10 peaks were gotten from black soap sample fortified with honey.

Table 11: Qualitative phytochemical profile of indigenous black soaps

Parameters	A	B	C
Saponin	+	+	+
Tannin	+	+	+
Alkaloid	-	-	-
Phlobatannin	-	-	-
Flavonoid	+	+	+
Terpenoid	+	+	+

Key: A = Black soap market sample, B= Prepared black soap, C= Black soap fortified with honey, + = present, - = absent

Table 12; Quantitative phytochemical composition (mg/g) of indigenous black soaps

Parameters	A	B	C
Saponin	20.50±0.92 ^c	28.36±0.23 ^c	33.31±0.82 ^b
Tannin	1.05±0.03 ^a	2.05±0.02 ^a	2.05±0.03 ^a
Flavonoid	3.50±0.89 ^c	4.33±0.00 ^c	3.83±0.89 ^b
Terpenoid	9.96±0.84 ^d	9.09±0.72 ^c	6.66±0.04 ^a

Values represent means ± standard deviation of triplicate readings. Superscripts with the same alphabets in a row are significantly different ($P \leq 0.05$).

Key: A = Black soap market sample, B= Prepared black soap, C= Black soap fortified with honey, Mg/g= Milligram/gram .

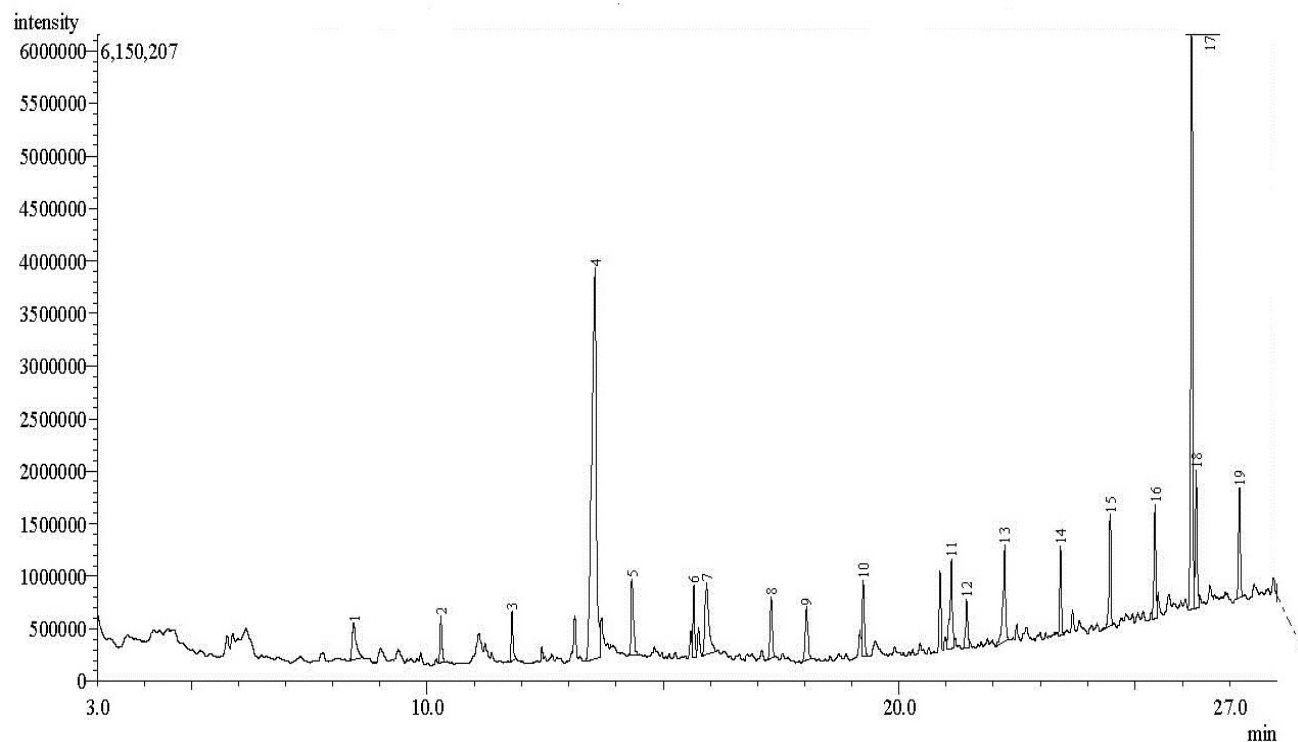


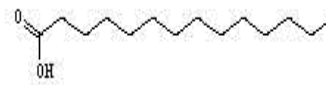

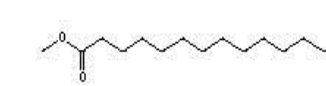






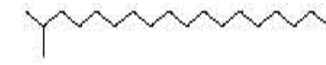
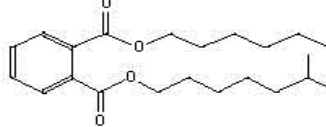

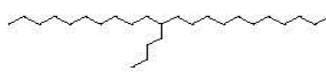


Figure 3. Chromatogram analysis (GC/MS) of black soap sample.

Table 13: Retention time of separated compounds from indigenous black soap

Peak/No	Retention Time (Minutes)	Compounds	Functional group
1	8.450	n-Nonanoic acid	<chem>CCCCCCCCC(=O)O</chem>
2	10.292	n-Tridecane acid	<chem>CCCCCCCCCCCC(=O)O</chem>
3	11.800	n-Cetane acid	<chem>CCCCCCCCCCCCC(=O)O</chem>
4	13.558	Dodecyclic acid	<chem>CCCCCCCCCCCC(=O)O</chem>

5	14.342	n-Hexadecane acid	
6	15.650	Tetradecane acid	
7	15.925	Tetradecanoic acid	
8	17.292	2-methlnonadecane	
9	18.042	Pentadecanoic acid	
10	19.242	Nonadecane acid	
11	21.108	11-Octadecenoic acid	
12	21.433	n-Octadecenoic acid	
13	22.242	Eicosane	
14	23.425	Adakane 12 acid	
15	24.475	n-Tetratetracontane acid	
16	25.425	7 - Hexylicosane acid	
17	26.208	Phthalic acid	
18	26.300	2- methylicosane	
19	27.208	Heptadecane	

DISCUSSION

Findings from this research reveal that the black soap provides an extreme environment and do not support the growth of many microorganisms. Few can be regarded as alkaliphiles due to its

high pH and such organisms have capability to thrive in such conditions. Alkaliphilic microorganisms have attracted interest because of their valuable natural products, their physiological interest and the intrinsic interest of both their ecological niches and their diversity. These microorganisms are a subset of organism that grow under extreme conditions with respect to an environmental stress, organisms that are now called “extremophiles” A true alkaliphile is generally considered to be an organism that exhibits optimal growth at pH values above 9-9.5.(Brian et al., 2011). A true alkaliphile must be able to maintain a cytoplasmic pH that is more acidic than the external pH; this is in distinction to a much larger group of alkaline tolerant microorganisms that have more modest capacities for pH homeostasis and that can tolerate but not grow optimally in a pH range of 9-10 (Terry, 2002).

The bacteria isolated from the black soap *Bacillus brevis* and *Bacillus megaterium* further corroborate the submission of Gao *et al.* (2011) that *Bacillus* spp have evolved several genetic mediated mechanisms to survive in extreme environment. Terry, (2002) also submitted that the most intensively studied aerobic alkaliphiles are *Bacillus* species. The isolated fungi are *Aspergillus flavus*, *Chrysosporium* spp, and *Aspergillus granulosis*. These findings are supported by Bastian and Alabouvette, (2009) that Bacteria and fungi are capable of colonizing almost every niche and that there is need to characterize the microbial ecosystem and yield new information regarding the metabolic functions of the different microorganisms inhabiting in such a complex environment. The presence of plasmid DNA in the Bacteria isolates especially *B. brevis* has further corroborated the previous findings of Gao *et al.*, (2011) as mention earlier. Before now, no attention has been placed both on the microbiological analysis of the black soap and plasmid profiling of its isolates. This will indeed be a vista for potential extremophiles with useful novel metabolites. Plasmids frequently carry genes for antibiotic resistance, toxigenicity and can as well confer extremophily status on microorganisms. Plasmids are useful markers in Recombinant DNA technology and as such this makes plasmids indispensable tool in Molecular Biology (Agbagwa *et al.*, 2012). The results from this investigative study can be used as an epidemiological tool for the typing of isolates from black soap samples. The more plasmids exist in an organism, the more specific is the plasmid profile as a marker for a single isolate. Most *Bacillus* species, regardless of their source, harbour at least one indigenous plasmid (Molina-Aja *et al.*, 2009). The functions of these plasmids have classically been correlated with phenotypical properties, including drug resistance, carbohydrate metabolism, amino-acid metabolism, carotenoids, cholic acid derivatives, organic acids and bacteriocins production (Molina-Aja *et al.*, 2009). The presence of these plasmids may be responsible for their survival in black soap.

The past submission of Subramanian *et al.*,(2007), that honey contain biological active compounds which corroborate with the beneficial antibacterial property of honey used in this research. The results have also shown that, honey is a good source of hydrogen peroxide which is the major contributor to its antimicrobial activity.

Physicochemical properties of any soap are vital to its utmost quality. The moisture content of the black soap with honey generally was found to be lower than that of the market sample which retained more moisture. This could be as a result of the absorbent nature of the honey. Moisture content is a parameter that is used in accessing that shelf life of a product. The moisture content of 10.195% recorded in the black soap with honey was still in line with the recommended percentage (10-15%) Encyclopaedia of Industrial Chemical Analysis, (2007). Implication of high moisture content in soap is that the excess water could possibly react with any unsaponified neutral fat to give free fatty acid and glycerol in a process called hydrolysis of soap on storage (Tewari, 2004). The amount of matter insoluble indicated the level of purity of the soap. The

hike in the level of impurities as shown in the black soaps generally may be attributed to the level of impurity of the alkali used for its production and other added ingredients.

Free caustic alkali is one of the parameters that determine the abrasiveness of any given soap, (Onyango, 2014). This mostly results from improper or incomplete saponification. The maximum and minimum recommended value is 0.25% for laundry soap and 0.2% for toilet soap (Encyclopaedia of Industrial Chemical Analysis, 2005). The Free alkali content of the soaps revealed that the black soaps with honey has the lowest percentage; this is plausibly due to the local palm kernel oil (Adin Eyan) used which was highly saponified. The measure of the suitability of any soap for bathing and washing of material is its Total Fatty Matter. The recommended values are 20.0% and 50.0% for laundry and toilet soaps respectively (Encyclopaedia of Industrial Chemical Analysis, 2005). The values obtained for all the soaps indicated that the black soaps generally would be most suitable for bathing rather than for laundry due to its high total fatty matter.

The unsaponified neutral fat (UNF) is expected to be low if all present in any soap. However soap which displays any presence of caustic alkali are expected to have no or lower UNF than free caustic alkali. The market sample black soap with 3.571% free caustic alkali, 0.886% UNF was recorded while black soap fortified with honey 1.212% UNF. This value for the black soaps with honey is relatively higher than that of the corresponding black soaps market sample, but still follows trend. This findings still agree with the submission of Onyegbado *et al.* (2002)

The Minimum Inhibitory Concentrations MIC, Minimum Bactericidal Concentration MBC and Minimum Fungicidal Concentration MFC showed that the black soaps in this research are generally more effective than the commercial antibacterial soaps. Both black soap samples had MIC, MBC and MFC of 62.5 mg/mL-125 mg/mL on all the test pathogens; while the commercial soaps had higher and varied activities mostly at 500 mg/mL specifically for the MBC. The significance of low MIC, MBC and MFC of any antimicrobial agent is that it is always a safer and better drug. The relatively high MIC, MBC and MFC values of the commercial soaps could be as a result of several factors ranging from evolving antimicrobial resistance mechanisms among pathogens to unwholesome, quality degrading production processes on the part of manufacturers of these soaps.

It has been shown that *Bacillus brevis* and *Bacillus megaterium* are effective antagonists of *Staphylococcus epidermidis*, *Escherichia coli*. *Bacillus brevis* is effective against both *S. epidermidis* and *Escherichia coli*. *Bacillus megaterium* had the highest zone of inhibition of 34mm against *S.epidermidis*. This observation is in conformity with the report of Fujinami and Fujisawa, (2010) that Alkaliphilic *Bacillus* strains are of industrial importance because they produce various alkaline extracellular enzymes exhibiting great values in industrial application. Agarry *et al.* (2004) also submitted that the antagonists are more adapted to environmental extremes than pathogens. Alkaliphilic *Bacillus* strains have also been described by Gao *at al.* (2011) as promising metabolite producers of carotenoids, cholic acid derivatives, and organic acids. These inherent abilities of the *Bacillus* spp may be responsible for the antagonistic action of the isolates in this study against some of the test bacteria pathogens.

Comparative antimicrobial activities of the black soaps and selected commercial antiseptic soap by agar well diffusion revealed the black soaps had more significant inhibitory effects in the overall than others. The pattern of growth inhibitions of the test pathogens shows the varying abilities of the organism to resist the antimicrobial effect of the soaps. However, these variations could be due to the differences in the nature and structures of the bacteria cell wall in view of the fact that it is the ultimate target of any antimicrobial agent or disinfectant. The result shows that

the black soaps exhibited high levels of antimicrobial activity which is the ability of the soaps to inhibit the growth or destroy the normal micro-biome. The active ingredient in the soap is what draws a thin line between one type of soap and another. The commercial antiseptic soaps in this study mainly contain trichlocarban and triclosan as the active agents. These chemical compounds exhibit antimicrobial effects by denaturing or disrupting cell activity and interfering with microbial metabolism. This, to all intents and purposes, is a function of factors such as the intrinsic properties of the organisms, contact time, soap content (e.g triclosan), concentration of individual formulation, skin sensitivity and even personal beliefs and orientations.

Traditional black soap does not have a key active ingredient such as triclosan, instead it releases oil that kills bacteria, rinses microorganisms away on the skin, and ultimately preventing the emergence of mutant bacteria strains. This is in conformity with previous findings of Ikpoh *et al.*, (2012). Findings from this study is also at parity with the previous discovery of Aliyu *et al.*, (2012) which attributed the antimicrobial action of the traditional medicated soap specifically against gram positive organisms like *S. aureus* to the ability of the soap due to its long chain fatty acid content to distort the peptidoglycan present in its cell wall. The activity of the soap against *S. aureus* therefore, could be attributable to the palm kernel oil present in the soap (Ugbogu, 2006). Ugbogu, (2006) opined that palm kernel oil has inhibitory effect on *S. aureus* and *Streptococcus* sp. The major fatty acids in palm kernel oil used for the production of Black soap are lauric acid, myristic acid and oleic acid. The inhibitory actions of the black soap with honey on gram negative bacteria could be due to the addition of honey. Honey has been used as home remedy for skin irritation and other associated problems like wounds (Subramanian *et al.*, 2007).

The commercial antibiotics were observed to be less effective in inhibiting the test organisms especially the clinical isolates. This is as a result of excessive exposure of the bacteria pathogens to these antibiotics in a clinical setting and possibility of evolution of antibiotic resistant strains. Doughari *et al.* (2007) reported that the state of administration of an antimicrobial agent affects the effectiveness of such agent. However, the sensitivity of *Pseudomonas aeruginosa* to Nitrofurantoin further corroborates its contributory prowess to increasing menace of antimicrobial resistance. Microbes may develop resistance to antibiotics under selective pressure, or they may acquire antibiotic resistance determinants without direct exposure to antibiotics. Inappropriate usage of antimicrobial agents has resulted in the development of antibiotic resistance which recently has become a major problem. (Goldstein, 2000). Agbagwa *et al.* (2012); Onifade and Oladoja (2015) observed recently that bacterial antibiotics resistance patterns are sometimes associated with the presence of large plasmids and the ability of plasmids for conjugation process. Plasmid which can be trans-conjugated usually possess a high molecular weight thereby making these plasmids to harbour the antibiotic resistance genes. These invariably increase their ability to threaten human consumers since strains carrying resistance genes qualified them as potential human pathogens.

Nasreen *et al.* (2009) reported that studies carried out on plasmid-less isolates showed that multiple antibiotics resistances pattern with high antibiotic indicates that resistance to most of the antibiotics is of chromosomal origin or on mobile genetic elements that may help in the disseminations of resistant genes to other bacteria of human clinical significance. However, the antibacterial activities of the black soaps fortified with honey compared favourably with standard antibiotics, which if purified and improved upon may exhibit higher zones of inhibition on the test pathogens.

The inhibitory effect of the topical skin creams with antimycotic and antibacterial activities on the test organisms showed that it competes finely with the soaps with varying significant differences. Only notable outright resistance here is that of *Klebsiella pneumoniae* to Tricutan, Sivoderm and Acneaway which could be as a result of negative interactions between the ingredients of these creams which mainly contain Clotrimoxazole and Neomycin sulphate in appropriate proportions. Findings from the present investigative study are in conformity with that of Azubuikwe *et al.* (2013) in which he posited that Funbact A cream had higher antimicrobial activities against *S. aureus*, *E. coli* and *Kleb sp.* and *C. albicans*.

The mechanism of action of the 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. 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. Antifungal action of the black soap 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.

Phytochemicals are chemicals which have been evolved by plants for their self defence, among other biological functions and reduce the maximum utilization of nutrients especially proteins, vitamins, and minerals, thus preventing optimal exploitation of the nutrients present in a food and decreasing the nutritive value (Ugwu and Oranye, 2006).

Anti – nutritional factors are also known as secondary metabolites in plants and they have been shown to be highly biologically active. These are secondary compound produced as side products of processes leading to the synthesis of primary metabolites (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 honey had highest saponin value of 33.31 mg/g which could be predicted on the interactions of compounds at fortification level.

The structural complexity of saponins results in a number of physical, chemical, and biological properties, which include sweetness and bitterness, foaming and emulsifying properties, pharmacological and medicinal properties as well as antimicrobial activities. Saponins are surface active compounds with detergent, wetting, emulsifying and foaming properties due to the presence of a lipid-soluble aglycone and water soluble sugar chain(s) in their structure (Shanthakumari *et al.*, 2008) Tannins are heat stable and they decreased protein digestibility in animals and humans, probably by either making protein partially unavailable or inhibiting digestive enzymes and increasing faecal nitrogen (Felix and Mello, 2000). Tannins are known to prevent skin eruptions (Encyclopaedia Britannica Online, 2014). Antimicrobial activities of the indigenous black soap could be attributed to the presence of these compounds.

Results from the GC-MS analysis of the methylated fraction of the black soap have revealed the presence of 19 compounds and Phthalic acid showed high percentage of peak and retention time across the soap samples. Apart from these compounds, fatty acids were the mostly identified. Previous submission by Lograda *et al.*(2012) that fatty acids have been demonstrated to be bactericidal to important pathogenic microorganisms including antibiotic resistant *S. aureus*. Facts from the GC-MS analyses of the black soap have once again further corroborated the

position of Aliyu *et al.* (2012) which attributed the antimicrobial action of the traditional medicated soap specifically against gram positive organisms like *S.aureus* to the ability of the soap due to its long chain fatty acid content to distort the peptidoglycan present in its cell wall. Antimicrobial activities of the indigenous black soap could be as a result of the presence of these oils in their relatively partially unsaponified state.

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