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Antagonistic Activities of Microorganisms Associated with Indigenous Black Soap on Some Selected Skin Pathogens

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Introduction: The indigenous black soap has been reported to possess antimicrobial activities. The aim of this research is to screen three different indigenous black soaps for extremophiles and then investigate their antagonistic effects on selected skin associated pathogens.
Methods: Microbiological analyses of three different indigenous black soap samples were investigated using serial dilution technique. Antagonistic activities of *Bacillus vedderi*, B*acillus faraginis, Chrysosporium* spp, *Aspergilus granulosus*, *Aspergilus flavus* and R*amulispora javanicus* microbial isolates from indigenous black soaps on clinical test pathogens: *Staphylococcus aureus*, *Staphylococcus epidermidis Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Trichophyton rubrum*, *Candida albicans,* were investigated by co-culture method. Physicochemical analysis of the black soap was accomplished according to standard analytical methods. Analysis of variance (anova) was performed followed by Duncan multiple range post hoc tests, considered the value p =.05.
Results: Antagonism assay showed that *B. vedderi* and *B. faraginis* inhibited the growth of *E. coli,* and *S. epidermidis* with inhibition zones ranging from 15-34 mm. Fungal isolates do not show antagonism towards the test isolates. Physicochemical properties of the black soaps implied they

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provide an extreme environment as evident in their pH of 10.30, 10.82 and10.43 respectively.
Conclusion: Antimicrobial activities of indigenous black soap could be as a result of its bacterial flora. These extremophiles could be source of unique metabolites of clinical appraisal.

Keywords: Extremophiles; antagonism; indigenous black soap; Staphylococcus epidermidis; Escherichia coli; Bacillus vedderi; Bacillus faraginis.

1. INTRODUCTION

The consistently evolving scourge of antimicrobial resistance now requires a smart diversion from the current seemingly stereotypic trends and practices [1]. It is therefore a hightime medical science explored other novel, weird, scientific means of tackling this challenge. Extremophiles are organisms capable of surviving in an environment which ordinarily would not permit such [2]. These organisms are believed to have evolved unique adaptations chiefly for this purpose. Extremophiles are considered to be very useful in the sense that the ability to survive in such extreme condition relatively confer on them relevant traits of research interest. These organisms produce useful metabolites of medical and industrial importance. The indigenous black soap is native to West Africa and it is widely used for bathing as well as a medium for traditional therapy [3]. Antimicrobial activities of the black soap as previously established; could be as a result of its microbial flora. The soap provides an extreme environment that would not allow many organisms to survive; the few present are regarded as extremophiles. Investigating the black soap for microorganisms with useful metabolites will not only be a panacea to the evolving antimicrobial resistance threat, but also a vista, an avenue for characterizing new set of extremophiles.

2. METHODOLOGY

2.1 Collection of Test Organisms and Black Soap Materials

Purified clinical isolates: *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis Pseudomonas aeruginosa*, *Candida albicans* and *Trichophyton rubrum* were collected from The University College Hospital UCH Ibadan. Their identities were confirmed by means by sub culturing on appropriate agar and were maintained at 4°C till further analysis. Plain Indigenous black soaps were procured from the local market (A). Indigenous black soaps were also prepared by saponification and fortified separately with brown (B) and white (C) eggshell powder, effectively at 20 g/250 mL of the molten soap.

2.2 Microbiological Analysis of the Black Soap Samples

Dilution plate method was employed. Exactly 1 g of the soap samples were suspended into 9 mL of sterile distilled water in properly labeled test tubes to make a stock solution. Dilutions were made to 10^{-5} by removing 1 mL from the first test tube (10^{-1}) aseptically and adding to successive tubes. With a sterile 1 mL pipette, 1 mL aliquot from the higher dilutions (10^{-4} and 10^{-5}) obtained for each sample were aseptically transferred to already labeled nutrient and potato dextrose agar plates and spread with the aid of sterile glass spreader. The nutrient agar plates were incubated upside down at 37°C for 24 h while Potato dextrose agar plates at 25°C 72 h.

2.3 Characterization and Identification of Isolates

The pure culture of the bacterial isolates were studied and subjected to: Microscopic examination, staining techniques (Gram and Spore) and the following biochemical tests: Motility, Catalase, Oxidase, Starch hydrolysis, Carbon sources utilization, *In-vitro* growth at pH10 according to the methods described by [4]. Bacterial isolates were identified with reference to the Bergey's Manual of Systematic Bacteriology and the Advanced Bacterial Identification Software (ABIS) online Encyclopedia (The Great Bacteria Book). Pure Fungal isolates were identified based on macroscopic and microscopic characteristics with robust reference to Barnet and Hunter's illustrated genera of imperfect fungi [5].

2.4 Preparation of Culture Media

Nutrient agar (Oxoid) 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 using Floor *Model* AA13 at 121°C for 15 min. Mueller Hinton agar (Oxoid) was prepared by suspending 38 g of the commercially prepared medium into 1000 mL of distilled water. Potato dextrose agar (Oxoid) was prepared by suspending 39 g of the commercially prepared medium into 1000 mL of distilled water. After sterilization, the medium was allowed to cool and 1% of streptomycin was added to inhibit bacteria.

2.5 Preparation of Standard Inoculum

The test organisms from growth in 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 with the aid of a spectrophotometer (Unico1100RS) to match a turbidity of 0.5 McFarland standards at wavelength of 540 nm according to the method described by [6]. 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.

2.6 Physicochemical Analysis of Indigenous Black Soap Samples

All reagents were analytical grade. Analyses were carried out according to the methods described by [7]. The following parameters were analyzed: Moisture content, Total Fatty Matter, Alcohol Insoluble Matter, Water Insoluble Matter, Free Caustic Alkali, Unsaponified Neutral Fat, Rate of Wear, Bulk Density, pH, Lathering ability, Colour and Foam stability.

2.7 Screening of Indigenous Black Soap Isolates for Antagonistic Activities on Test Pathogens

A single streak was made from 24 h old culture of bacteria, 23 mm away from the centre of petri-dish. The plates were then incubated for 24 h 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 [8]. 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.

2.8 Statistical Analysis

All experiments were conducted in triplicates and data collected from the study were subjected to Analysis of Variance (ANOVA). Treatment means were compared using Duncan New Multiple Range Test (DNMRT) at 5% level of significance. These analyses were carried out using SPSS version 17.

3. RESULTS

3.1 Bacterial Isolates from Indigenous 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 bacterial isolates were motile, catalase positive and were consistent in carbon source utilization. The names of the identified isolates were: *Bacillus vedderi* and *Bacillus faraginis*. The isolates also demonstrated *in vitro* growth at pH 10 (Table 1).

Table 1. Morphological and biochemical characteristics of bacterial isolates

Characteristics	Isolate A1	Isolate C1
Macroscopic		
Colony shape	Circular	Circular
Colony colour	White	Cream
Colony edge	Rough	Irregular
Elevation	Raised	Slightly raised
Microscopic		
Gram reaction	+	-
Shape/structure	Rod	Rod
Spore	+ (Terminal)	+ (Subterminal)
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: A1: Bacillus vedderi, C1: Bacillus farraginis

3.2 Fungal Isolates from Indigenous Black Soap Samples

Four different fungi were isolated from the black soap samples. Their microscopic and macroscopic niceties varied uniquely as shown in (Table 2). For instance, colonies of *R. javanicus* had bright-grey colour, regular colony margin with moderate growth. Sporodochia (Microscopic details) are small out-buds from the substomatal stromata and pushing through stomata. *Chrysosporium* spp has colonies that are granular, cottony, raised and folded in appearance. When observed from the front, the colour is white cream. Colonies of *A. flavus* on potato dextrose agar at 25°C were olive to lime green with cream reverse. More fungal growths were recovered from the market sampled black soap.

3.3 Physicochemical Properties of Indigenous Black Soap Samples

The physicochemical traits of the indigenous soap samples suggest the soaps with eggshell powder generally has lower moisture content at 10.195%, and 9.560% for indigenous soaps fortified with brown and white eggshells respectively and the market sample at 12.026%. The black soap market sample recorded the highest level of total fatty matter at 78.107%. While black soaps with brown and white eggshells had 44.304 and 50.314% respectively (Table 3).

3.4 Antagonistic Activities of Indigenous Black Soap Microbial Isolates

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

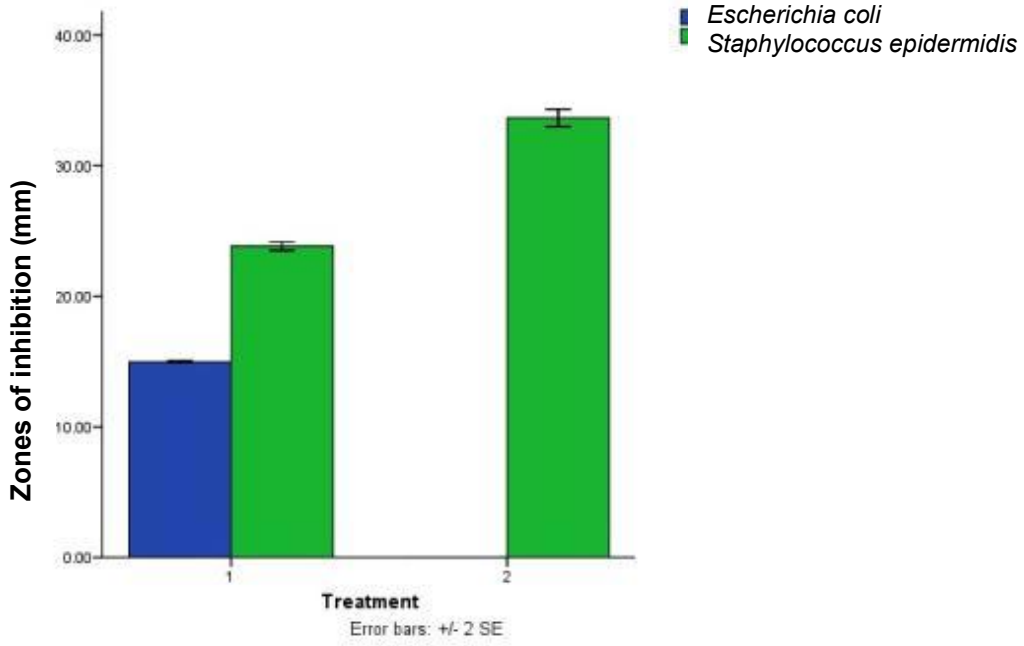

Fig. 1. Comparative antagonistic activities of bacterial isolates from indigenous black soap on *Escherichia coli and Staphylococcus epidermidis*
Keys: *1= Bacillus vedderi, 2= Bacillus farraginis*

Table 2. Macroscopic and microscopic characteristics of fungal isolates

Macroscopic	Microscopic	Isolates
Colonies on potato dextrose agar at 25°C are olive to lime green with cream reverse. Growth is rapid. Texture is woolly to cottony to somewhat granular.	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), metulae (8 – 10 x 5 – 7 µm). Conidia are smooth globose, 3 - 6 µm in diameter.	C1
Colonies grow moderately rapidly at 25°C. They are granular, cottony and flat, raised and folded in appearance. From the front, the colour is white cream, yellow or tan to pale brown. The reverse is white to brown.	Conidiophores are poorly differentiated, much like vegetative hyphae, mostly erect and branching irregularly, hyaline; conidia (aleuriospores or arthrospores) hyaline, 1-celled, globose to pyriform, terminal or intercalary, single or in short chains, usually with a broad basal scar.	A1
Colony is grey- bright grey, colony margin is regular; grow moderately.	Sporodochia are small, arising from substomatal stromata and pushing through stomata; conidiophores hyaline, simple or branched, short; conidia hyaline, filiform, septate, with short lateral branches. sclerotia present.	B2
Colonies on potato dextrose agar at 25°C are dull brown, irregularly furrowed, mostly floccose, uneven in texture, with 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.	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 biseriate, with almost the entire surface being covered. Conidia are globose, pale green, delicately roughened, and measure 3.5-5.5 µm.	A2

Tentative identity: C1: Aspergilus flavus, A1: Chrysosporium spp, B2: Ramulispora javanicus, A2: Aspergilus granulosus

Table 3. Physicochemical properties of indigenous black soap

Parameter	A	B	C
Moisture content (%)	12.02 ± 0.00^{c}	10.22 ± 0.05^{b}	9.44 ± 0.02^{a}
Total fatty matter (%)	78.11 ± 0.05^{c}	44.30 ± 0.00^{a}	50.31 ± 0.05^{b}
Ethanol insoluble matter (%)	18.04 ± 0.17^{c}	17.23 ± 0.01^{b}	17.01 ± 0.01^{a}
Water insoluble matter (%)	10.96 ± 0.00^{a}	23.93 ± 0.02^{b}	24.44 ± 0.00^{c}
Free caustic alkali (%)	3.53 ± 0.05^{c}	1.54 ± 0.01^{a}	2.02 ± 0.01^{b}
Unsaponified neutral fat (%)	0.92 ± 0.05^{a}	3.16 ± 0.01^{b}	1.14 ± 0.01^{a}
Rate of wear (%)	94.74 ± 0.00^{a}	96.18 ± 0.01^{c}	95.13 ± 0.00^{b}
Bulk density (MG/CM) ^{3}	1.36 ± 0.03^{b}	0.95 ± 0.03^{a}	0.95 ± 0.03^{a}
pH	10.30 ± 0.00^{a}	10.82 ± 0.04^{b}	10.43 ± 0.11^{a}
Lathering ability	Good	Good	Good
Colour	Black	Black	Black
Foam stability	Stable	Stable	Stable

Values represent means ± standard deviation of triplicate readings. Superscripts of the same letter in a row are not significantly different at P=.05.

Key: A=Black soap market sample, B=Black soap with brown eggshell powder, C=Black soap with white eggshell powder, %=Percentage, MG/CM^{3}=Milligram/Centimetre cubic

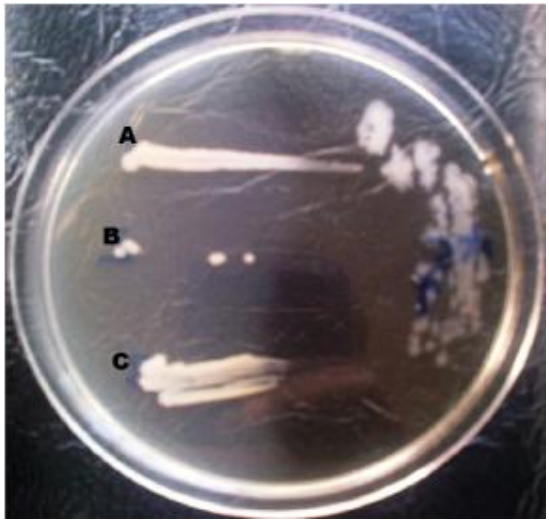

Plate 1. Antagonistic properties of *Bacillus vedderi* on *S. epidermidis* and *E. coli*
Keys: A: S. aureus, B: S. epidermidis, C: E. coli

Plate 2. Control showing the pathogens growing unchallenged
Keys: A: S. aureus, B: S. epidermidis, C: E. coli

4. DISCUSSION

Findings from the present study have posited that the indigenous black soap provides an extreme environment and may not support the growth of many microorganisms. The few present can be regarded as alkaliphiles due to its high pH as such organisms have capability to thrive in this condition. Alkaliphilic microorganisms have become a cynosure of medical interest because of their important natural metabolites, their habitat diversity and ecological significance [9]. These are subdivision of microbes that grow under extremely high or low conditions with intrinsic adaptive response to environmental stress. [10] concluded that a true alkaliphile is an organism that exhibits optimal growth at pH values above 9-9.5.

Bacterial isolates from the indigenous black soap: *Bacillus vedderi* and *Bacillus farraginis* further corroborate the submission of [11] that *Bacillus* spp have developed several molecular based adaptive mechanism of survival in an extreme environment. Their ability to produce extracellular compounds toxic to other pathogens does not only ensure their survival in such hostile environment; these extracellular products could as well be useful in therapeutic medicine. *Bacillus vederi* for instance has potential for production of broad spectrum antimicrobial agent of medical importance. The fungal isolates are supported by previous submission of [12] that bacteria and fungi are capable of colonizing almost every niche and that the urgent need for novel antimicrobial compounds requires the characterization of microbial ecosystem to yield new information with respect to metabolic functions of the different microorganisms inhabiting such a complex environment. Non-antagonistic activities of the fungal isolates here could be explained in terms of different inherent mechanisms of survival that may be predicated on genetics without conspicuous notable inhibitory activities. Physicochemical properties of any soap are ordinarily very relevant to its utmost quality. The current study is more interested in the fact that these traits confirm the soap actually provides extreme condition as the indigenous black soap is made from alkaline rich compounds. The pH as the sole interest; has definitely shown that the condition is alkaline and that microorganisms associated are indeed alkaliphiles following their demonstration of *in-vitro* growth at pH 10.

5. CONCLUSION

This study provides information on the plausible means of antimicrobial activities and physicochemical characteristics of different indigenous black soap samples with interest in the antagonistic potentials of their alkaliphiles. These extremophiles could be source of novel metabolites of medical importance.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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