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ANTIMICROBIAL AND ANTIHELMINTHIC PROPERTIES OF SHEA BUTTER (*Vitellaria paradoxa*).

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ABSTRACT.

The phytochemical, antimicrobial and antihelminthic screening of the crude extract of three types of Vitellaria paradoxa (shea butter) was investigated in this study. The crude extracts were dissolved in di-methylsulfoxide. The phytochemical constituents of the crude extracts were accessed and compared. Clinical isolates under aseptic conditions were collected from the Medical Microbiology Laboratory, University of Port Harcourt Teaching Hospital and further morphological and biochemical tests were carried out to identify this clinical isolates as; Staphylococcus sp., Escherichia sp., Pseudomonas sp., Klebsiella sp., Streptococcus sp and Candida sp. Inoculums were prepared and adjusted to 0.5ml Mc Farland standard of each test bacterium. It was spread onto sterile Muller Hinton Agar plates so as to achieve even growth. The plates were allowed to dry and a sterile cork borer (6.0mm diameter) was used to bore wells in the agar plates. Ofloxacine and Fluconazole was used as bacteria and fungi control respectively. The crude yellow extracts exhibited inhibitory activities that were found to be higher than crude white and ivory colored extract on all the test organisms. Despite the crude yellow extract exhibited higher inhibitory activities than the other extracts; the antibacterial activity was low in 10^{-1} to 10^{-4} dilutions for some bacteria. The crude extracts revealed the presence of Alkaloids, flavonoids, Cardiac glycosides, saponnin, and carbohydrates. Anthraquinnone and phlobatannin were absent in the extracts. This study also revealed that shea butter has no anti-helminth effect after 24hours exposure of the eggs of Ascaris lumbricoides and Trichuris trichuria in the shea oil sample rather; the DMSO used as control killed the eggs. This calls for further investigation.

KEYWORDS: Phytochemical, Antimicrobial, Antihelminthic, Vitellaria and Paradoxa

INTRODUCTION

Shea butter is an off white or ivory colored fat extracted from the nut of the African shea tree (*Vitellaria paradoxa*) (Alfred Thomas *et al.,* 2002). It is a triglyceride fat derived mainly from steric acid and oleic acid.

Shea butter tree is indigenous to sub-Saharan Africa and belongs to the family *Sapotaceae,* two species of the plant has been identified namely *Vitellaria paradoxa* and *Vitellaria nilotica.* Based on distribution, *Vitellaria paradoxa* is produced mainly in the West African sub-region while *Vitellaria nilotica* grows mainly in northern Uganda and southern Sudan. Shea butter tree grows in the wild and has a huge economic and ecological potential (Okullo *et al.,* 2010). It is a dicotyledonous woody plant. The plant thrives naturally in the dry savannah belt of West Africa from Senegal in the west to Sudan in the east, and onto the foothills of the Ethiopian highlands. It occurs in 19 countries across the African continent, namely Benin, Ghana, Chad, Burkina Faso, Cameroon, Central African Republic, Ethiopia, Guinea Bissau, Cote D'Ivoire, Mali, Niger, Nigeria, Senegal, Sierra Leone, Sudan, Togo, Uganda, Zaire and Guinea (FAO, 1998). It covers a swath of the continent, some 5,000km long and 400 – 750 km wide. Nigeria has a comparative advantage in the production and export of shea nut in Africa over her counterparts due to the large available arable land and suitable climatic conditions for its production. It currently grows in the wild in many states including Niger, Nasarawa, Kebbi, Kwara, Kogi, Lagos, Adamawa, Benue, Edo, Katsina, Plateau, Sokoto, Zamfara, Taraba, Borno and Oyo. Although it appears to be a rather obscure wild specie growing side by side with arable crops, it is widely known, valued and exploited by the natives in all the areas where it grows. The English call it shea, while the french call it Karite. In Nigeria, the Igbos call it Okwuma; Yorubas call it Orioyo

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while Hausas call it markade (Odebiyi, 2004).

Composition of shea butter.

Shea butter contains high level of UV-absorbing triterpenes esters which include cinnamic acid, tocopherols (vitamin A), and phytosterols (Wiesman *et al.,* 2003). Shea butter fat extract is a complex fat that in addition to many nonsaponifiable components (substances that cannot be fully converted into soap by treatment with alkali) contains the following fatty acid.

Oleic acid (40-60%), Steric acid (20-50%), Linoleic acid (3-11%) Palmitic acid (2-9%), Linolenic acid (<1%), Arachidic acid (<1%) (Bouvet *et al.,* 2010)

Shea butter melts at body temperature. Proponent of its use for skin care maintains that it absorbs rapidly into the skin, acts as a 'refatting' agent and has a good water binding property (Hemat *et al.,* 2003)

Uses of shea butter

Shea butter is used as a base for medicinal ointments in traditional medicine.

It is also used in pharmaceuticals and cosmetics because it is naturally rich in Vitamins A, E, and F.

Some of the isolated chemical constituents are reported to have antimicrobial, anti-inflammatory, emollient and humectant properties (Manosroi *et al.,* 2010)

The butter is also locally used in cosmetics, chocolates, candle and pastries as cocoa butter substitute.

Furthermore shea butter is widely utilized for domestic purposes such as cooking, skin moisturizer, edible products (Alander, 2004).

Traditionally, Shea butter are used as cream for dressing hair, protecting skin from extreme weather and sun, relieving rheumatic and joint pains, healing wounds/swelling/bruising, and massaging pregnant women and children.

It is also used in treatments of eczema, rashes, burns, ulcers, psoriasis and dermatitis.

Shea butter protects the skin from both environmental and free radical damage.

It is used as a natural hair conditioner, makeup remover or as a treatment for buns, cuts, scrapes, sun burns and diaper rash.

Classification of Shea butter.

The United States Agency for International Development and other companies (US Agency for International Development, October 2006) suggested a classification system for shea butter separating it into five grades

A (raw or unrefined, extracted using water)

B (refined)

C (highly refined and extracted with solvent such as hexane.)

D (lowest uncontaminated grade)

E (with contaminants)

Raw unrefined shea butter in its natural state are extracted manually or mechanically. It has a mild nutty-smoky scent and a golden to light ivory colour.

Refined shea butter are extracted with chemical process (with hexane mostly) which involves bleaching, deodorizing and overheating. All of these deeply altering chemical processes yield a white odorless shea butter that has lost its deep moisturizing, anti-ageing, protection and healing property.

Butter extraction and refining

The traditional method of preparing unrefined shea butter consist of the following steps.

Separating and cracking

Crushing

Roasting

Grinding

Separating the oils

Collecting and shaping

Separating/cracking: The outer pulp of the fruit is removed. When dry, the nut, which is the source of shea butter, must be separated from the outer shell. This is a social activity, traditionally done by Women Elders and young girls who sit on the ground and break the shells with small rocks.

Crushing: To make the shea nuts into butter, they must be crushed. Traditionally, this is done with mortars and pestles. It requires lifting the pestles and grinding the nuts into the mortars to crush the nuts so they can be roasted.

Roasting: The crushed nuts are then roasted in huge pots over open, wood fires. The pots must be stirred constantly with wooden paddles so the butter does not burn. The butter is heavy and stirring it is hot, smoky work, done under the sun. This is where the slight smoky smell of traditional shea butter originates.

Grinding: The roasted shea nuts are ground into a smoother paste, water is gradually added and the paste is mixed well by hand.

Separating the oils: The paste is kneaded by hand in large basins and water is gradually added to help separate out the butter oils. As they float to the top, the butter oils, which are in a curd state, are removed and excess water squeezed out. The butter oil curds are then melted in large open pots over slow fires. A period of slow boiling will remove any remaining water, by evaporation.

Collecting and shaping: The shea butter, which is creamy or golden yellow at this point, is ladled from the top of the pots and put in cool places to harden. Then it is formed into balls.

Industrially, a mechanical sheller such as the Universal Nut Sheller may be used. The refined butter may be extracted with chemicals such as hexane, or by clay filtering.

The specific objective is to

Determine the presence and actions of some phytochemicals in crude yellow and ivory coloured shea butter

Determine the antimicrobial activities of crude white, crude yellow and ivory shea butter against clinical isolates.

Determine the antihelminthic properties of crude white, crude yellow and ivory shea butter

MATERIALS AND METHODS.

Shea Butter Source.

Locally extracted shea butter from Ghana was bought into a neat nylon from fruit garden market, Port Harcourt and packed in a sterile container. Shea butter from Osun state, Nigeria was also bought from mile three market in Port Harcourt, River State.

Materials

The materials/apparatus used for this research project include; petri dishes, conical flasks, autoclave, gloves, cotton wool, Bunsen burner, cork borer, wire loop, incubator, ethanol, weighing balance, meter rule, bijou bottles, peptone water, test tubes, pipette, Mueller

hinton agar, Mc Farland standard (0.5), dimethylsulfoxide (DMSO), fluconazole (fungi control), ofluxacine (bacteria control), normal saline, wooden applicator stick, glass slides, cover slip, iodine, graduated bulb pipette and a microscope.

Method of sterilization.

Glasswares such as conical flasks, test tubes, bijou bottles, petri dishes, were sterilized by autoclaving at 121^0C for 15minutes at 15psi. Using Bunsen burner, other materials such as inoculating wire loop was used aseptically by heating to bright red, holding the loop straight down into the flame so that the whole loop and lower part of the handle is heated. This will destroy all living organism on the loop while the cork borer was sterilized by dipping in ethanol and passing over the flame of Bunsen burner.

Methods of media preparation.

Nutrient agar

This is a general purpose medium used for the isolation of bacteria. It was prepared according to manufacturer's instruction. 28g of powder was suspended in 1000ml of distilled water. It was autoclaved at 121^0C for 15minutes at 15psi. allow to cool to 50^0C and pour into petri dishes.

Muller hinton agar (MHA)

This medium is used in testing the sensitivity of various isolates to the plant extract (shea oil) and the antibiotics as control. 38g of MHA powder was dissolved in 1 liter of distilled water. The powder was allowed to soak for 5 minutes, boiled and was sterilized by autoclaving at 121^0C for 15mins at 15psi. It was allowed to cool then dispensed into petri dishes and dried using hot air oven.

Collection of clinical isolates

Pure clinical isolates of *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella sp, Streptococcus sp and Candida albecans* was obtained from the patients at the University of Port Harcourt Teaching Hospital, Medical Laboratory Department and was cultured on nutrient agar (NA), incubated at 37^0C for 24hours. Further tests were carried out to characterize and identity the isolates in the hospital laboratory.

Characterization of the isolates

The isolation and characterization of bacterial isolates were based on cultural, morphological and biochemical tests characteristics of the isolates using standard methods.

The cultural characteristics which include the colony shape, size, surface texture, elevation, pigmentation and appearance were determined by direct observation of the colonies on the sub-cultured plates also the grams staining technique for differentiating grams positive bacteria from grams negative bacteria.

The biochemical analysis carried out include the citrate test, catalase test, indole test, motility test, starch hydrolysis test, oxidize test, lactose fermentation reaction

The fungal identification was carried out using the microscope. Using lactophenol cotton blue method, fungal organisms were identified.

Morphological tests

Gram staining

Procedures

A thin smear of the cultures were made on a clean grease-free slides, air dried and heat fixed. Crystal violet (primary dye) were added till the slide is covered, stained for one minute and rinsed in slow running water and drained. The smear was then covered with Lugol's gram's iodine, allowed for one minute rinsed in slow running water. 95% ethanol was added to decolorize the slides content till stain no longer washes off, this step was done rapidly, the slides were gently rinsed with water. The smears were counter-stained with safranin (a secondary dye) stained for 30 seconds, rinsed with water and allowed to air-dry. The stained slides were then observed under the light microscope using 100x objective.

2.8. Biochemical tests.

Oxidase test: Oxidase regeant which contain 1.0% (w/v) of N, N, N-tetramethyl-p-phenylenediamine dihydrochloride was prepared by dissolving 0.1g of the compound in 100ml deionized water. A filter paper was soaked with the solution, allowed to absorbed, and smears of the isolates from the pure cultures were made on the oxidase paper strips and observed for colour change. A purple colouraton within 5 seconds indicates positive, the colour change is due to the possession of cytochrome.

Catalase test: Hydrogen peroxide was used for this test. A loopful of the bacteria from 24 hours old cultures was transferred to a clean-grease free slides and a drop of hydrogen peroxide (H^2O^2) was added to the bacteria on the slide. Effervescence caused by the libration of free oxygen as a gas bubble reveals a positive result, indicating the presence of catalase in the culture. Catalase is an enzyme that breaks down hydrogen peroxide to water and oxygen.

Coagulase test: this test is used to identify *Staphylococcus aureus* which produces the enzyme coagulase. A drop of distilled water was placed on each end of a slide. A colony of the test organism was emulsified in each of the drops to make two thick suspensions. A loopful of plasma was added to one of the suspensions and mixed g ently. Clumping of the organism was checked within 10 seconds. Clumping within 10 second indicates that the organism is *Staphylococcus aureus*.

Motility Test: The motility test was done to determine if the organism is motile or not motile by moving away from the line of inoculation.

A sterile wire loop was used to pick an isolate, stabbed directly into the centre of the test tubes containing the motility agar and incubated for 18-24 hours at 37°C. a diffuse growth away from the line of inoculation indicates a positive result, no diffused growth indicates a negative result.

Citrate Test: This test was used to determine if an organism can utilize citrate as its sole source of carbon and energy. The citrate test uses a medium in which sodium citrate is the source of carbon and energy. In Simon's citrate agar, the pH indicator is bromothymol blue, which is green neutral pH and becomes blue when the medium become alkaline.

Slopes slant of Simon's citrate agar was prepared in bijou bottles and the test organisms will be inoculated by streaking the surface and stabbing the butt with a sterilized Inoculating needle and will be incubated at 35°C for 48 hours and was observed for a bright blue colour in the medium which indicates a positive result.

Indole test: indole is nitrogen-containing compound formed when the amino acid tryptophan is hydrolyzed by

bacteria that have the enzyme tryptophanases, this were detected using Kovac's reagent. Isolates were inoculated in peptone water prepared by dissolving 5.0g of peptone in 500ml of distilled water, dispensed into bijou bottles and sterilized. After 24 hours of incubation, few drops of the kovac's reagent were added. A red layer in the medium indicated indole formation.

Determination of Antimicrobial Activity of Extracts

Fresh plates of the test bacteria were made from the isolate cultures obtained on agar slants. Colonies of fresh cultures of the different bacterial isolates was picked and suspended in 5ml nutrient broth in well labeled sterile bijou bottles and incubated for 24 hours at 30^0C. The stock solution of the samples was prepared by dissolving 1ml of each sample in 9ml of di-methyl sulfoxide to give a concentration of 10fold serial dilution.

The Agar well diffusion method as described by Lino and Deogracious (2006) was used. Standardized inoculums (0.5 McFarland turbidity standard equivalent to 5 x 10^8cfu/ml) (NCCLS, 1999) of each test bacterium was spread onto sterile Muller Hinton Agar plates so as to achieve even growth. The plates were allowed to dry and a sterile cork borer (6.0mm diameter) was use d to bore wells in the agar plates. The samples were prepared by double dilution method. This is done by adding 1ml of the stock solution to the test-tube containing 9ml of Di-methyl sulfoxide to achieve a concentration of 200mg/ml. This concentration (500ml/ml) was further diluted to concentrations of 100mg/ml and 50mg/ml. Subsequently, 0.1ml of each sample was introduced in wells earlier bored in the agar plate cultures. Ofloxacine and fluconazole were used as control for bacteria and fungi respectively. The plates were then incubated at 37^0C for 24h. The fungi plate was left for 48hours. Antimicrobial activity of the samples was determined after incubation period by measurement of zones of inhibition.

Collection and maintenance of worms

Eggs of *Ascaris lumbricoides* and *Trichuris trichuria* were identified from a freshly collected stool sample from a patient in the University of Port Harcourt Teaching Hospital by viewing under the microscope and then dropped in a test-tube containing normal saline.

15 mls of normal saline is added to a test-tube and marked on the 15 ml meniscus using a grease pencil. 1ml is then removed from it to have 14 mls normal saline in the test-tube. The stool sample containing *Ascaris lumbricoides* and *Trichuris trichuria* was added to the test-tube and mixed until normal saline-stool mixture reaches the 15 ml mark on the test-tube. It was mixed properly using a wooden applicator stick to get a slightly homogenous mixture.

Determinaton of anti-helmintic activities shea butter.

1ml of saline-stool mixture was pipetted into different dilutions of DMSO and shea butter in the ratio of 1:1, 1:2, 1:3 in various test-tubes and DMSO used as control of the anti-helminth test substance and then mixed. It was incubated for 24 hours at room temperature in the dark. After 24 hours, 0.15ml of the mixture of anti-helminth test helminthic stool solution was measured onto a clean grease free slide and a drop of iodine was added. It was then examined microscopically for helminth eggs. The number of eggs counted was multiplied by 100eggs/ml.

Preparation of normal or physiological saline

Normal saline was prepared from sodium chloride and distilled water. 8.5grams of Sodium chloride was dissolved into 1 liter of distilled water in a leak-proof bottle pre-marked to hold 1 liter. It was mixed properly till salt was fully dissolved.

Phytochemical Screening

The shea butter oil samples was screened for their qualitative Phytochemical screening using qualitative analysis showed the presence of many constituents, including alkaloids, flavonoids, saponins, anthraquinnones, tannins,carbohydrates, phlobatannins, and cardiac glycosides.

50 grams of the sample (natural butter) was macerated in 100 mls of n-hexane for 24 hours and filtered. Partition with 50 mls of dichloromethane in a separation funnel, remove the dichloromethane layer and dry the n-hexane layer containing the sample. Dry to remove n-hexane using a steam water bath at 45^oC. In this case, the n-hexane gradually evaporate leaving the butter in liquid and or pasty form, allow to cool.

Alkaloids: 0.5 g of each extract (shea butter) was heated with 5% hydrochloric acid in a steam bath for 10min until the acid is mixed with sample. Remove filter and allow to cool for the following test; Drangendroff, Hagers and Mayer. The presence of a yellow precipitate in Hagers indicates a positive result. A brown precipitate in drangendroff indicates a positive result, a cream precipitate in Mayer also indicates a positive result.

Flavonoids: equal volume of distilled water was boiled with the sample for 5mins. The oily part was collected from the measuring cylinder and transferred into a test tube. A piece of magnesium metal and 2mls of concent rated sulphuric acid were added to it reaching with the metal to give a pink colour. This observation indicates a pink result.

Selivanoff: the sample was treated with selivanoff to form white precipitate.

Tannins: About 5 drops of ferric chloride is heated with sample to observe the presence of blue-black colour indicating the presence of tannin. Bromine water was also treated with the samples; a decolouration of bromine water at sample layer shows a positive result.

Anthraquinnones:

Free hydroxyl anthraquinnone; benzene or chloroform is dissolved with samples with 5 mls of 10% ammonia solution to form a violet or pink colour at the ammonia lower layer with a ring at interface.

Combined hydroxyl bontragers; extract sample with benzene filters and partition ammonia, remove the ammonia through separate funnel and treat benzene layer with concentrated H_2SO_4. Observation of violet, pink or red colour indicates a positive result.

Carbohydrate: the sample is shaken thoroughly with distilled water until well mixed. Heat and use cotton wool to filter the mixture.10% alpha naphtol and concentrated H_2SO_4 is used to concentrate the mixture. A bulky precipitate with brown ring at the interface where the liquid meets indicates the presence of simple sugars.

Fehling test for sugar reduction: boil a well mixed fehling solution A*B with the sample for 10 minutes. A change in colour with precipitate from blue-green to yellow or red indicates presence of cuppous oxide or sugar reduction.

Saponosides: frothing test, just a preliminary test. The samples were well shaken in a test tube to obtain froth at the upper layer of the sample tube. After two vigorous shakes, the tubes were left to stand for 10-15 minutes. If the froth persists, it indicates the presence of saponin.

Emulsion test; after the observation of froth test, add 5 mls of olive oil and shake vigorously. An observation of cloudy precipitation by the side of the test tube shows emulsion.

Phlobatannins: 50mls of absolute methanol is used to extract the content of the sample and filtered with filter paper to remove the oily part of the sample and prepare a 10% NaOH with 3-5 mls of dinitrobenzoic acid. Heat to evaporate out the acid. An immediate change from brown to green indicates the presence of phlobatannin.

Cardiac glycosides:

Salwoski; mix extract with chloroform, partition with 20 mls of dichloromethane and shake vigorously. Remove chloroform layer and treat with concentrated H_2SO_4 to form a ring at the interphase with a yellow colour at the upper layer.

RESULTS

Phytochemical screening using qualitative analysis of the crude yellow, crude white and n-hexane extract of Shea butter (*Vitellaria paradoxa*) revealed the presence of medicinal active constituents. It showed the presence of Alkaloids, cardiac glycosides, flavornoids, saponnin, and carbohydrates. Anthraquinnone and phlobatannin

were abscent in the shea oil. The phytochemical characteristics of the various shea butter oil investigated are summarized in Table 3.1. The percentage yield of the chemical constituents of the various shea butter extracts studied is summarized in Table 3.2.

Antimicrobial screening result.

Based on the colonial morphology of the bacteria isolates from the University of Port Harcourt Teaching Hospital, Medical Laboratory Department, the different arrangement of the identified test organisms include; edge, size, colour, elevation, shape, opacity, texture, staining morphology. Also the morphological and biochemical characteristics of the isolates were identified as, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas* sp., *Klebsiella* sp., *Streptococcus* sp. and *Candida albicans.* The antimicrobial activites of the various extracts (crude white, crude yellow and ivory colored) of shea butter revealed that the crude white extract was sensitive to four test organism and resistance to two as shown in table 3.3. The crude yellow extract was resistant to the six test isolates as seen in table 3.4. The ivory coloured extract was resistant to three test isolates and sensitive to three test isolates as shown in table 3.

Anti-helmintic activity of crude yellow, n-hexane and crude white extracts of shea butter.

The 24 hour exposure of the eggs of *Ascaris lumbricoides* and *Trichuris trichuria* to different concentrations of crude yellow, n-hexane and crude white extracts of shea butter revealed that shea butter (*Vitelleria paradoxa*) has no anti-helminthic effect compared to the di-methylsulfoxide used as control. The anti-helminthic effects by the different concentrations of shea butter extracts and DMSO are given in Table 3.8.

PHYTOCHEMICALS TESTED	CRUDE YELLOW	N-HEXANE EXTRACT	CRUDE WHITE
Alkaloids			
Mayer	+	++	++
Drangendroffs	+	++	++
Hagars	+	++	++
Tannins			
Bromine water	-	-	-
Ferric chloride	+	++	++
Anthraquinnones			
Free hydroxy	-	-	-
Combined hydroxyl	-	-	-
Flavonoides			
Shinoda	++	++	+
Selivaniffs	++	++	+
Saponnins			
Frothing test	+	+	+
Emulsion test	+	+	+
Phlobatannins			
Sodium bi-carbonate	-	-	-
Hydrochloric test	-	-	-
Cardiac glycosides			
Legal test	+	++	++
Salwoski	+	++	++
Carbohydrate			
Molisch sugar test	+	++	+
Fehling for sugar reduction	+	++	+

Key (+) present,
(++) highly present,
(-) absent or undetected

Table 3. 1: Qualitative analysis of the phytochemicals of shea butter extracts.

PHYTOCHEICALS TESTED	CRUDE YELLOW	N-HEXANE EXTRACT	CRUDE WHITE
Alkaloids	10.92	10.92	10.92
Tannins	11.13	11.13	11.10
Anthraquinnones	0.12	0.12	0.12
Flavonoides	15.02	15.06	14.06
Saponnins	9.10	9.19	9.10
Phlobatannins	1.12	1.14	1.12
Cardiac glycosides	13.02	13.04	13.02
Carbohydrate	10.08	10.08	10.08

Table 3.2. Percentage yield for quantitative analysis of chemical constituent of the various crude extracts.

Crude white extract Zones of inhibition by extract in mm

Microorganisms	**Neat (mm)**	**10^{-1} (mm)**	**10^{-2} (mm)**	**10^{-3} (mm)**	**10^{-4} (mm)**	**Control (mm)**
Staphylococcus sp.	10	0	0	0	0	27
Pseudomonas sp	10	10	6	6	4	40
Escherichia coli	0	0	0	0	0	40
Klebsiella sp	0	0	0	0	0	18
Streptococcussp	8	0	0	0	0	25
Candida albicans	13	0	0	0	0	18

Controls
Ofloxacine was used for bacteria
Fluconazole was used for fungi

Antimicrobial activities of crude white extracts.

Crude yellow extract Zones of inhibition by extract in mm

Microorganism	Neat (mm)	10^{-1} (mm)	10^{-2} (mm)	10^{-3} (mm)	10^{-4} (mm)	Control (mm)
Staphylococcus sp	22	12	0	0	0	30
Pseudomonas sp	11	0	0	0	0	20
Escherichia coli	20	20	16	16	15	33
Klebsiella sp	11	0	0	0	0	15
Streptococcus sp	16	6	6	0	0	25
Candida albicans	11	0	0	0	0	15

Controls
Ofloxacine was used for bacteria
Fluconazole was used for fungi

Table 3.4: Antimicrobial activities of crude yellow extracts Ivory coloured extract Zones of inhibition by extract in mm

Microorganisms	Neat (mm)	10-1 (mm)	10-2 (mm)	10-3 (mm)	10-4 (mm)	Control (mm)
Staphylococcus sp	0	0	0	0	0	30
Pseudomonas sp	15	12	10	10	6	22
Escherichia coli	0	0	0	0	0	26
Klebsiella sp	22	0	0	0	0	15
Streptococcus sp	0	0	0	0	0	25
Candida albicans	22	0	0	0	0	16

Controls
Ofloxacine was used for bacteria
Fluconazole was used for fungi

Table 3.5: Antimicrobial activities of ivory colored extracts.

	0 hours	24 hours		
Concentrations	All extracts (eggs/ml)	Crude yellow (eggs/ml)	Crude white (eggs/ml)	Ivory colour (eggs/ml)
Neat	200	200	200	200
50% (1:1)	200	200	200	200
100% (1:2)	200	200	200	100
150% (1:3)	200	100	200	100
Control (DMSO)	200	0	100	0

Required sample size is 200eggs/ml

Anti-helminthic activities of the various shea extracts

DISCUSSION

The phytochemical screening of *V. paradoxa* (shea butter) showed the presence of alkaloid, tannins, flavonoids, saponnins, cardiac glycosides and carbohydrates. Extract with n-hexane gave the highest yield, followed by crude white extract, while crude yellow gave the lowest yield of the phytochemical constituents. The percentage concentration of the bioactive constituents present in the extracts are as follows;

For crude yellow extract, Alkaloids (10.92%), Tannin (11.13%), phlobatannin (0.12%), Anthraquinnone (0.12%), Flavonoids (15.02), Carbohydrates (10.08%), Saponin (9.10%) and Cardiac glycosides (13.02%).

For n-hexane extract, Alkaloids (10.92%), Tannin (11.13%), phlobatannin (0.14%), Anthraquinnone (0.12%), Flavonoids (15.06), Carbohydrates (10.08%), Saponin (9.19%) and Cardiac glycosides (13.04%).

For crude white extract, Alkaloids (10.92%), Tannin (11.10%), phlobatannin (1.12%), Anthraquinnone (0.12%), Flavonoids (14.06), Carbohydrates (10.08%), Saponin (9.10%) and Cardiac glycosides (13.02%).

The extracted phytochemicals have also been reported in similar studies (Okeke and Elekwa, 2006; Adamu *et al.,* 2007; Okullo *et al*., 2010). The presence of the phytochemicals observed in the present study may have attributed to the antimicrobial, anti-inflammatory, emollient and humectants properties of *V. paradoxa* reported by other researchers (Alander 2004; Oku llo *et al*., 2010; Manosroi *et al*., 2010). Flavonoids have been reported to have anti-oxidant free radical scavenging properties. They prevent oxidative cell damage and are anti-casinogenic (Pietta, 2000.) Okwu *et al.,* (2004) also opined that its presence in the intestinal tract reduces the risk of heart diseases while preventing inflammation. This bioactive ingredient had been observed to be highly present in *V. paradoxa* examined. The presence of flavonoids in the present study revealed that it can be edible and also could be attributed to its use as an anti-oxidant agent in cosmetics industries (Essiett *et al.*, 2010).

Tannins have reported to have astringent and detergent properties (Bruneton, 1999 and Bouquet *et al.,* 2008), suggesting it could be effective as an anti-fungal agent.

The hemolytic, expectorant, anti-inflammatory and immune-stimulating efficacy of saponin has been documented (Ray *et al.,* 2014). The presence of saponin in this plant could be responsible for the traditional use of shea butter in the relaxation of muscles and in the treatment of sprains, wounds and colds as practiced generally in Nigeria.

The role of cardiac glycosides in the correction of heart disorders as well as the slowing and strengthening effect it possess on failing hearts has been well documented (Terease *et el.,* 2002). This phytochemical was highly present in n-hexane and crude white extract but was found in trace quantity in crude yellow extract. The presence of this compound in shea butter could be useful in the treatment of diseases associated with the heart Anyasor *et al.,* 2011. Anthraquinones and Phlobatanin were absent in the three extracts screened. Generally, the presence of these bioactive compounds in these extracts of *Vitelleria paradoxa* thus suggest a

scientific verification to its usage in traditional, industrial and medicinal use.

In this investigation, the isolates that was used to carry out this antimicrobial sensitivity were identified as; *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas* sp, *Klebsiella* sp, *Steptococcus* sp and *Candida albecans.* The organisms tested are based on their implication in human diseases such as skin diseases, typhoid, pneumonia, dysentery, urinary tract, respiratory problems and others.

From table 3, the result showed that the crude white extracts of Shea oil show no activity on *Escherichia coli* and *Klebsiella* sp., but showed activities on *Pseudomonas* sp., *Staphylococcus aureus*, *Streptococcus* sp., and *candida* sp., with 10mm, 10mm, 8mm and 13mm zones of inhibition respectively.

From table 4, the crude yellow extract showed activity on the six test organisms with higher zones of inhibition of 22mm, 20mm and 16mm for *Staphylococcus aureus Escherichia coli* and *Streptococcus* sp., respectively.

The ivory colour extract showed no activity to *Staphylococcus aureus, Streptococcus* sp., and *Escherichia coli.* It showed activities on *Klebsiella sp., Candida* and *Pseudomonas* with 22mm, 12mm, and 15mm, zones of inhibition respectively. Table 5 shows the activities of the shea butter on the organisms.

The crude yellow extracts exhibited inhibitory activities that were found to be higher than crude white extract on all the test organisms. Despite the crude yellow extract exhibited higher inhibitory activities than the other extracts, the antibacterial activity was low in 10^{-1} to 10^{-4} dilutions for some bacteria. Consequently, the minimum inhibitory concentration (MIC) determined did not show any zone of inhibition except for *Pseudomonas* sp. The MIC result is traceable to the fact that the tenfold serial dilution reduced its initial concentration which had no visible effect on the organisms resulting in no zone of inhibition. A low level of activity at a low extract concentration may suggest that the concentrations of the active constituent in the extracts are too low for any appreciable antibacterial activity (Uchechi *et al*., 2010).

In table 6, the anti-helminthic result revealed that after 24 hours the crude white extract could not destroy the helminth eggs while one egg was found at 100% and 150% concentration in ivory coloured shea oil and 150% concentration for crude yellow extract respectively. It was observed that the di-methylsulfoxide (DMSO) used as control destroyed the helminth eggs indicating that DMSO has anti-helmintic effect on the eggs of *lumbricoides* and *Trichuris trichuria*.

CONCLUSION

In conclusion, this research work has proved beyond doubt that the crude extracts of *Vitellaria paradoxa* (Shea butter) has antimicrobial effects on some organisms. The presence of naturally occurring bioactive chemical compounds found in shea butter are of various pharmacological importance. The anti-helminthic study, showed that the crude extracts of *Vitellaria paradoxa* do not have anti-helminthic effect rather, the di-methylsulfoxide (DMSO) used as control inhibited the eggs of *Ascaris lumbricoides* and *Trichuris trichuria*. This calls for further investigation.

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