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Bioactivity of Crude Extracts of Stem Bark of *Vetillaria Paradoxa*

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Abstract

This research aimed to study the bioactivity of crude extracts of stem bark of *Vetillaria paradoxa*"Shea-nut tree" as used in traditional medicine for treatment of stomach ache and control of diarrhea, using Hexane, Acetone and Ethanol as the extraction solvents. Phytochemical screening of stem bark of extracts of *Vitellaria paradoxa* revealed the presence of alkaloids, steroids, phenolic compounds, flavonoids, Saponins, tannins, and cardiac glycosides. Ethanol, acetone, and hexane extracts inhibited the growth of pathogenic *Escherichia coli, salmonella typhi,* and *stapilycoccus aureus* with varying degrees of activity with the ehanol extract demonstrating the highest activity against all the tested bacterial organisms.

Keywords: Phytochemiscals, antimicrobial, secondary metabolites, bioactivity, inhibition.

1. Introduction

Vitellaria paradoxa (Sapotaceae) is a plant that is locally abundant in Nigeria in the derived Savannah zones, particularly near towns and villages. It is rich in oil and replaces the oil palm as a source of edible oil in Northern Nigeria (Keay, 1989; Borokini, 2014). The plant species *(Vitellaria)* is easily distinguished by its very long leaf stalks, more widely spaced nerves and abundant white latex when slashed and in the petiole. Shea-butter is the fat extracted from the kernel of this plant. It is becoming increasingly popular as a component of cosmetic formulation in addition to its long standing use as a cocoa butter substitute in the chocolate industry (Hall et al, 1996;Omwirhiren, James, & Abass, 2016; Keay, 1989; Borokini, 2014)

Shea butter contains high level of uv-absorbing triterpenes esters which include cinnamic acid, tocopherols (vitamin A), and phytosterols (Leke, 2015). Badifu (1989) confirmed that shea butter contains a high level of unsaponifiables (5 – 15 %) which include: phytosterols ,campesterol, stigmasterol, sitosterol, spinosterol and triterpenes such as cinnamic acid ester and amyrin, parkeol, butyrospermol. lupeoland and a hydrocarbon called karitene. Analysis of the kernel reveals the presence of phenolic compounds such asgallic acid, catechin, epicatechin, epicateachingallate, gallocatechin, epigallocatechin, epigallocatehingallate, as well as quercetin and trans-cinnamic acid (Steven et al., 2001; Leke, 2015). Collinson and Zewdie-Bosuener (1999) and Bauer and Moll (1942) variously reported works on this plant which focused essentially on the fruit, kernel, seed and the fat from the seed.

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V. paradoxa (formally *Butryspermumparadoxum*), *(Sa-potaceae)* is an immensely popular tree with many applications in folkloric medicine. It is commonly called Shea butter (English), Kareje (Fulfulde, Nigeria), Kadanya (Hausa, Nigeria) Koita (Gbagi, Nigeria), mameng (Cham, Nigeria). The tree grows naturally in the wild of the dry savanna belt of West Africa, from Senegal in the West to Sudan in the East and onto the foothills of the Ethiopian mountains. V. paradoxa is considered a sacred tree by many communities and ethnic groups and plays important roles in religious and cultural ceremonies. It is also believed to have some spiritual protective powers (Agbahungba and Depommier, 1989; Rajeshwar et al., 2016). Different parts of the plant including leaves, roots, seeds, fruit and stem bark have been used in the treatment of enteric infections such as diarrhea, dysentery, helminthes and other gastrointestinal tract infections, skin diseases and wound infections (Soladoye et al., 1989). The bark is used to suppress cough and also to treat leprosy (Ferry et al., 1974).

The research aims to assess the phytochemicals and antibacterial activities of the plant using three solvents and to compare same.

2. Materials and Methods

Preparation of reagents

Wagner's reagent

2.0 g iodine crystals and 3.0 g potassium iodide were dissolved in minimum amount of water and then made up to 100 ml with distilled water.

Mayer's reagent

13 g of mercuric chloride was dissolved in 50ml of distilled water. Also 50 g of potassium iodide was dissolved in 20ml of distilled water. The two solutions were mixed and the volume was made up to 100ml with distilled water.

Dragendroff's reagent

Bismuth (0.85 g) was dissolved in 100ml of glacial acetic acid and 40ml of water to give solution A. Another solution designated solution B was prepared by dissolving 8.0 g of potassium iodide in 20ml of distilled water. Both solutions were mixed to give a stock solution.

Ferric chloride solution

5.0g ferric chloride was dissolved in 100ml of distilled water.

Ethanol solution

45ml of absolute ethanol was mixed with 55ml of distilled water.

Dilute sulphuric acid

A 10.9ml aliquot of concentrated sulphuric acid was mixed with 5ml of distilled water and made up to 100ml.

Lead acetate solution

45ml of 15 % leads acetate (that is 15.0 g of lead acetate in 100ml of distilled water)
Was dissolve in 20ml of absolute ethanol and made up to 100ml with distilled water.

Dil. Hydrochloric acid

Measured volume, 2.0 ml concentrated hydrochloric acid was diluted with some distilled water and made up to 100ml.

Preparation of 0.3 % Sodium hydroxide

Sodium hydroxide (0.3 g) was dissolved in a little amount of water and made up to 100ml mark with distilled water.

Preparation of 2 % glacial acetic acid

2ml of glacial acetic acid was dissolved in distilled water and made up to 100ml.

Sample collection and preparation

Fresh stem bark of *Vitellaria paradoxa* (2.5 kg) were collected from Janligo village of Yana, Shira L.G.A in Bauchi State, Nigeria. The plant was identified by a local medical practitioner Mr. Kabiru Adamu (Dan-Chakwati) from Shira L.G.A Bauchi State and authenticated by a Botanist. The Sample was dried under (shade) room temperature for over 21 days and grinded using mortar and pestle, sieved in order to obtain a pure powdery form of the sample using 300mics sieve. The powdered material was stored in polythene bags at room temperature until needed.

Extraction

50 g of the dried and pulverized fiber-free stem bark of *Vitellaria paradoxa* was extracted exhaustively via maceration with 3*300cm^3 of Hexane (HE), acetone(ACE) and ethanol(EE), each

at room temperature for 24 hrs. Excess solvent was removed in to give crude extracts of HE, ACE, and EE, from Hexane, Acetone and Ethanol respectively.

Phytochemicals Screening

Crude Hexane extract (HE) was screened for phytochemicals using standard procedures (Harborne, 1984; Sofowora, 1984).

Phytochemicals screening methods

Detection for Alkaloids

Standard methods were used for the detection of alkaloids using Dragendroff's reagent (Harborne, 1984; Sofowora, 1984).

Detection for flavonoids

Standard methods were used for the detection of the presence of Ammonia (Harborne, 1984; Sofowora, 1984).

Detection for Tannins

Standard methods were used for the detection of Ferric chloride (Harborne, 1984; Sofowora, 1984).

Detection for Phenolic compounds

Standard methods were used for the detection of Phenols (Harborne, 1984; Sofowora, 1984).

Detection for Saponins Test

Standard methods were used for the detection of saponins (Harborne, 1984; Sofowora, 1984).

Detectionfor Terpenoids

Standard methods were used for the detection of Terpeniods (Harborne, 1984; Sofowora, 1984).

Detection of Glycosides

Standard methods were used for the detection Glycosides.

Detection for Sterols

Standard methods were used for the detection of sterols (Harborne, 1984; Sofowora, 1984).

Thin layer chromatography of *V. paradoxa Crude* Extract

Three chromatography papers of the same length (30cm) were used. Three different mixtures of solvent system varying polarities were also used to enhance ideal separation in different chromatography tanks: hexane/chloroform (1:1), chloroform, chloroform/ethyl acetate (1:1) and Ethyl acetate/Ethanol (1:1).

The solvent front and the separation were later calculated and the relation factor was obtained using the formula.

$R_f = m^1/m$

Were R_f=relation factor. M^1=separations (cm). M= solvent front (cm).

The disc diffusion method

The paper disc diffusion assay technique (Akpuaka et al., 2003 and Nuhu et al., 2000) was done.

Preparation of the medium

The nutrient agar medium was prepared by dissolving 7.0 g of agar in 250 ml of distilled water in a conical flask and heated to dissolve. The solution was sterilized in an autoclave at 121°C for 15 mins, cooled and poured into Petri dishes to set.

Preparation of culture media and inoculation

Culture of *Staphylococcus aureus*, *salmonella typhi*, and *Escherichia coli* were obtained from the microbiology laboratory of Abubakar Tafawa Balewa University, Bauchi, Nigeria. Pure isolates were obtained by sub-culturing unto fresh nutrient agar plates. The bacteria were separately used to inoculate the Petri dishes. The plates were incubated at 37 ± 2°C for 24 hours

Assay of extracts

Two different concentrations of each extract were obtained by suspending 250 mg of each extract in 6.0 ml of absolute ethanol and the volume was made up to 10.0 ml using sterile distilled water to give a concentration of 25 mg/ml (25,000 µg/ml). These also served as stock solutions. The second concentrations were obtained by diluting 2.0 ml each of the stock solutions with 2.0 ml of sterile distilled water giving a concentration of 12.5 mg/ml (12,500 µg/ml). Sterile 6 mm Whatman's filter paper discs were soaked in the extracts and placed on the plates and incubated for 24 h at 37 ± 2°C. The plates were examined for clear zones of activity. The zones of inhibition were measured using a transparent plastic meter ruler

Broth diffusion method

Equal volumes (1.0 ml) each of the extract solutions (25 and 12.5mg/ml) were mixed with same volume (1.0 ml) of an overnight broth culture of *S. aureus* and *E. coli* to give solutions of final concentrations of 12.5 and 6.25 mg/ml, respectively, in a test tube. These were then incubated at 37 ± 2°C for 24 h and observed for the presence of bacterial growth using a compound microscope

Inoculation and application of extracts

Bacterial strains were inoculated in 15ml of sterile nutrient broth an inoculated at 37°c for 24 hours. The concentration of inoculants was set to 0.5mc for loud standards after 24 hours the plate was removed from the oven and 3 different extracts 0.5ml was introduced to the plates and leaved for 24 hours.

Minimum Inhibitory Concentration (MIC)

This is the lowest drug concentration that prevents visible microorganisms' growth after overnight incubation, a plate of solid growth media. After a pure culture is isolated was examined and minimum inhibitory concentration was determined.

3. Results

Table 1. Weight of various macerated fractions of Vetillari aparadoxa

Fraction	Observation	Weight (g)
V. paradoxa + Ethanol	**Reddish brown**	**2.60**
V. paradoxa + Acetone	**Dark brown**	**3.70**
V. paradoxa + N-hexane	**Yellow brown**	**1.70**

Table 2. Result of phytochemical screening

Key:

Phytocompounds	Regents used	Extracts		
		ACE	HE	EE
Alkaloid	**Dragendroff's** **Mayer's**	+ +	+ +	- -
Flavonoids	**1 % ammonia,** **2 m NaOH, + HCl**	++ ++	+ +	- -
Tannins	**5 % FeCl**	+	-	+
Saponins	**Olive Oil**	+	+	-
Terpenoids	**Salkowski**	++	+	-
Glycosides	**Legal's** **Kelarkillani**	+ +	- -	+ +
Steroids	**Salkowski**	+	-	+
Phenols	**1 % gelatin solution** **10 % NaCl**	+	+	+

ACE = Acetone Extract
HE = Hexane Extract
EE = Ethanol Extract
+ = Slightly Present
++ = Moderately Present
+++ = Highly Present
- = Absent

Table 3. Antibacterial activity and zone of inhibition (mm) of V. paradoxa disc diffusion

Extracts	Concentration	E. coli	S. Typhi	S. aureous
EE	**25mg/ml** **12.5mg/ml**	**6** **-**	**8** **-**	**10** **-**
ACE	**25mg/ml** **12.5mg/ml**	7 -	7 -	**2** **-**
Hexane	**25mg/ml** **12.5mg/ml**	**1** **-**	**2** **-**	**3** **-**
Sreptomycin		**25**	**NT**	**NT**

NT = not tested/done: = no inhibition

Table 4. Various fractions from thin layer chromatography of stem bark of V.paradoxa

Extracts	Solvent System	Retention Faction (R.F)
Hexane	**hexane/Chloroform** **Chloroform/Ethyl acetate** **Ethyl acetone/Ethanol**	**0.6** **0.3** **0.5**
ACE	**hexane/Chloroform** **Chloroform/Ethyl acetate** **Ethyl acetone/Ethanol**	**0.7** **0.8** **0.8**
EE	**hexane/Chloroform** **Chloroform/Ethyl acetate** **Ethyl acetone/Ethanol**	**0.5** **0.4** **0.3**

HE =-Hexane Extract
ACE = Acetone Extract
E = Ethanol Extract

4. Conclusion

Phytochemical screening of Hexane, Acetone, and Ethanol, extracts revealed the presence of flavonoids, tannins, terpenoids, saponins, sterols, alkaloids and cardiac glycosides by positive reaction with the respective test reagents. Phytochemical screening showed that maximum presence of secondary metabolites was in Acetone extract than in the Hexane, and Ethanol, where almost all the phytocompounds appeared in the acetone extract (Table 1), whereas tannins, glycosides, and steroids where absent in hexane extract; alkaloids, flavonoids and Saponins, were absent in the Ethanol extract. Antimicrobial susceptibility of the extracts (50 mg/ml) against the test organisms showed that in all the three solvents used, the ethanol extracts demonstrated the highest activity, followed by Acetone, whereas hexane demonstrated the least activity on the tested bacteria as seen in (Table 3).

Preliminary phytochemical investigations of stem bark of *vitellaria paradoxa* revealed the presence of some/many secondary metabolites. These secondary metabolites are linked to microbial activity of the plant material and the extracts of this plant has antimicrobial effects on the tested enteric bacteria, hence serve as potential therapeutic agents against diarrhea and other microbial afflictions.

References

Agbahungba, Depommier, 1989 – *Agbahungba, G., Depommier, D.* (1989). Shea and African locust beans parklands aspects in southern Borgou. Bois et Forêts des Tropiques, 222, 41-54.

Badifu, 1989 – *Badifu, G.I.O.* (1989). Lipid composition of Nigerian Butyrospermum paradoxum. *Kernel Journal of Food Composition and Analysis*, 2(3), 238-244.

Bauer, Moll, 1942 – *Bauer K.H., Moll H.* (1942). The contents of the shea butter nut, the seed of Butryospermumparkii. Arch. Pharm. 280: 37-45.

Borokini, 2014 – *Borokini, T.* (2014). A Systematic Compilation of IUCN Red-listed Threatened Plant Species in Nigeria. *International Journal of Environmental Sciences*, 3(3), 104–133.

Collinson, Zewdie-Bosuener, 1999 – *Collinson C., Zewdie-Bosuener A.* (1999). Shea butter Markets: Their Implications for Ghanaian Shea butter Processors and Exporters; Project A0772, Report 2403; Natural Resources Institute, University of Greenwich, U.K. P. 20.

Ferry et al., 1974 – *Ferry M.P., Gessain M., Geeain R.* (1974). Vegetative Propagation of Shea, Kola and Pentadesma. Cocoa research institute, *Ghana Annual Report* (1987/88): 98-100.

Hall, 1996 – *Hall J.B., Aebischer D.P., Tomlison H.F., Osei-Amaning E., Hindle J.R.* (1996). Vitellaria paradox. A monograph: school of agricultural and forest sciences. University of wales. Bangor U.K.

Harborne, 1984 – *Harborne J.B.* (1984). Methods of Plant Analysis. In: Phytochemical Methods. Springer, Dordrecht.

Keay, 1989 – *Keay R.W.J.* (1989).Trees of Nigeria. A revised version of Nigerian trees (1960, 1964) by Keay RWJ, Onochie CFA, Stanfield DP. Clarendon press Oxford, pp. 389-390.

Leke, 2015 – *Leke, O.* (2015). Medical plants and natural products chemistry in Achebe' s" things fall apart": The metaphor of kola. *International Journal of Arts and Humanities*, 3(3), 70–76.

Omwirhiren et al., 2016 – Omwirhiren, E. M., James, S. A., Abass, A. O. (2016). Saudi Journal of Medical and Pharmaceutical Sciences The Phytochemical Properties and Antimicrobial Potentials of Aqueous and Methanolic seed Extract of Cola nitida (Vent.) and Cola acuminata (Beauvoir) grown in South West, Nigeria. *Saudi Journal of Nedical and Pharmaceutical Sciences*, 354–363. https://doi.org/10.21276/sjmps.2016.2.12.6

Rajeshwar et al., 2016 – *Rajeshwar, Y., Sreekanth, T., Kahsay, G., Hussen, D. A., Eticha, T.* (2016). Hepatoprotective activity of Anogeissus latifolia bark in ethanol intoxicated rats. The American Journal of Science and Medical Research, 1(2), 198–201. URL: https://doi.org/10.17812/ ajsmr1210.Published

Steven et al., 2003 – *Steven M., Zeev W., Nissim G.* (2003). Phenolic constituents of shea (Vitellriaparadoxa) kernel. *J. Agric. Food Chem*. 51: 6268-6273.

Sofowora, 1984 – *Sofowora A.* (1984). African Medicinal Plants, University of Ife Press, IIe-Ife, Nigeria, P. 104.

Soladoye et al., 1989 – *Soladoye M.O., Orhiere S.S., Ibimode B.M.* (1989). Ethanobotanical Study of two Indigenous Multipurpose Plants in the Guinea Savanna of Kwara State – Vitellariaparadoxa and Parkiabiglobosa Biennial Conference of Ecological Society of Nigeria, 14 August, Forestry Research Institute, Ibadan. P. 13.