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Full Length Research Paper

Phytochemical and antimicrobial screening of the crude extracts from the root, stem bark and leaves of *Vitellaria paradoxa* (GAERTN. F)

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The root, stem and leaves of *Vitellaria paradoxa,* belonging to the Sapotaceae family and which have some ethnomedicinal applications were studied. Phytochemical screening of the plant parts reveals the presence of carbohydrates (free reducing sugars, ketoses, pentoses and starch), saponins, steroids, tannins and alkaloids. The antimicrobial screening of the crude methanol extract carried out *in vitro* on the following clinical isolates; *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pnuemoniae and Salmonella typhi* showed that the crude methanol extracts had wider range of activity on these organisms than the petroleum ether extracts. The crude stem extracts inhibited the growth of *P. aeruginosa, K. pneumoniae, B. cereus and S. typhi* at concentration of 50 mg/ml while the leaf had a minimal inhibition concentration (MIC) of 70 mg/ml on *S. aureus, E. coli and S. typhi*. The root had an MIC of 60 mg/ml on *S. aureus, E. coli, P. aeruginosa, K. pneumoniae* and *S. typhi*. The MBC in all the cases were slightly higher than the MIC and was lowest in the stem extracts which indicates that the stem bark may contain the most active components.

Key words: Sapotaceae, *Vitellaria paradoxa*, antimicrobial activity, clinical isolates, *in vitro*.

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). 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).

Shea butter contains high level of uv-absorbing triterpenes esters which include cinnamic acid, tocopherols (vitamin A), and phytosterols (Wiesman et al., 2003). Badifu (1989) confirmed that shea butter contains a high level of unsaponifiables (5 -1 5%) which include phytosterols that is,. campesterol, stigmasterol, β-sitosterol and α-spinosterol and triterpenes such as cinnamic acid ester, α- and β-amyrin, parkeol, butyrospermol. lupeol and a hydrocarbon called karitene. Analysis of the kernel reveals the presence of phenolic compounds such as gallic acid, catechin, epicatechin, epicateachin gallate, gallocatechin, epigallocatechin, epigallocatehin gallate, as well as quercetin and trans-cinnamic acid (Steven et al., 2003). 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. Nothing was reported on the anti-microbial studies on leaves and stem bark of the plant and this opened up our research on the study.

MATERIALS AND METHOD

The plant materials (root, stem bark and leaves) were collected from Zaria, Kaduna State, Nigeria in March 2005 and identified by Mallam Abdullahi Musa of the Herbarium of the Faculty of Sciences, Ahmadu Bello University, Zaria, Nigeria. The voucher specimen, number 105347, was kept in the Herbarium. The sam-

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les were air-dried, pulverized using wooden pestle and mortar and stored in polythene bags and kept away from moisture until needed for use.

Extraction procedure

The air-dried pulverized plant materials (60 g each of root, stem bark and leaves) was packed in three soxhlet extractors and labeled accordingly. These were defatted using redistilled petroleum spirit (60 – 80°C) to yield 1.1 g (1.8%), 1.3 g (2.2%) and 0.7 g (1.2%) of fatty acids and their derivatives, respectively. These were then exhaustively and respectively extracted with redistilled methanol and concentrated *in vacuo* at 40°C using rotary evaporator to obtain 5.94 g (9.9%), 6.3 g (10.5%) and 5.7 g (9.7%) of crude methanol extracts respectively. The various crude extracts were later subjected to bioassay analyses.

Phytochemical analysis of the plants material

The various plant parts (root, stem bark and leaves) were screened for plant metabolites using the pulverized materials respectively. Standard techniques of Brain and Turner (1975) were employed in the phytochemical screening. These metabolites include carbohydrates, free reducing sugars, ketoses, pentoses, soluble starch, glycosides, steroidal aglycone, tannins, saponins, flavonoids and anthracene derivatives.

Antimicrobial screening test

Pure clinical isolates of *Salmonella pneumoniae, Vibrio cholerae, Staphylococcus aureus, Klebsiella Pneumoniae, Pseudomonas aeruginosa, Bacillus cereus, Escherichia coli* and *Salmonella typhi* obtained from the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria were grown on a nutrient agar slant in bijou bottles in an incubator at 37°C for 24 h. Stock solutions of the respective plant extracts were prepared by initially dissolving 5 g of each methanol extract (root, stem bark and leaves) in 5 ml of DMSO to obtain stock solutions of concentration 1000 mg/ml each. From the stock solution, concentration of 200, 160, 120, 100, 80, 60, 20 and 10 mg/ml were prepared by serial dilution. The cork and bore diffusion method of Bauer et al. (1966) and Barry and Thornsberry (1985) were used in the anti-microbial screening. Inoculation of the prepared plates with the organism was done using a wire loop to transfer a strand of the organism into the plate followed by cross-streaking with the same wire loop to achieve uniform spread on the plate. A control was set up alongside using pure DMS0 for each strain of organism. The plates were incubated at 37°C for 24 h after which they were examined for zones of inhibition of growth.

Determination of the minimum inhibitory concentration (MIC)

The determination of the minimum inhibitory concentration (MIC) was carried out on the methanol extract because it showed more sensitivity against the growths of the test organisms. The medium was nutrient agar solution which was prepared according to the manufacturers standard of 28 g/1000 ml. In this case double strength was prepared by dissolving 28 g in 500 ml of distilled water which was swirled and mixed thoroughly by heating to allow uniform dissolution after which 5 ml of it was dispensed into 30 sets of universal bottles and sterilized in an autoclave at 121°C for 15 min. The agar was allowed to cool to 45°C and each graded solution was then mixed gently with molten double strength nutrient agar in a Petri-dish and allow to solidify for one hour. Extracts' concentrations of 100, 80, 60, 50, 40, 30, 10 and 0.5 mg/ml were prepared by serial dilution. Each plate was divided into six equal sections and labeled accordingly to correspond to six test organisms. Two 5 mm diameter paper discs (Whatman No.1) were placed aseptically into each labeled section of the plate using sterilized forceps. With an automatic micropipette, 0.1 ml of each bacterial suspension was taken and transferred aseptically and carefully into each appropriate pre-labeled paper disc on the agar plates. The plates were incubated for 24 h at 37°C after which they were observed for growths or death of the test organisms. The lowest concentration inhibiting growth was taken as the minimum inhibitory concentration (MIC).

Table 1. Phytochemical screening of *Vitellarin paradoxa* root, stem bark and leaves.

Phytochemical	Root	Stem bark	Leaves
Carbohydrate	+	+	+
Free reducing sugar	+	+	+
Ketoses	+	+	+
Pentoses	+	+	+
Starch	+	+	+
Cardiac glycosides	-	-	-
Saponins	+	+	+
Steroids	+	+	+
Alkaloids	+	+	+
Flavonoids	-	-	-
Anthracene	-	-	-

+ = positive; - = negative.

Determination of the minimum bactericidal concentration (MBC)

This was carried out to know if the organisms could be killed completely or their growths could only be inhibited. Another 30 sets of plates of nutrient agar were prepared according to the manufacturers' standard and sterilized in an autoclave as earlier described. The paper discs in all the plates from the MIC tests were re-activated. Emphasis was mostly paid to the MIC plates and the preceding plates. The re-activation was done in a mixture of 0.5% egg lecithin and 3% Tween 80 solution in a test tube. The reactivated organisms were sub-cultured into appropriately labeled quadrants of the sterilized nutrient agar plates using wire loop into each test tube and streaking uniformly on the labeled quadrants. This was then incubated for 24 h at 37°C after which they were observed for growths. The MBC was the quadrant with the lowest concentration of the extract without growth.

RESULTS AND DISCUSSION

The phytochemical screening of the root, stem bark and leaves of *Vitellaria paradoxa* revealed the presence of carbohydrates, simple reducing sugars, soluble starch, saponins, alkaloids and tannins in all the plant parts studied as shown in Table 1. The presence of saponins, tannins, alkaloids in all the plant parts studied (root, stem bark and leaves) calls for an in-depth study on the plant. The metabolites are of various pharmacological impor-

Table 2. Antimicrobial screening of methanol and petroleum spirit extracts of *Vitellarin paradoxa* root, stem bark and leaves.

Test organism	Diameters of inhibition zones (mm)					
	Methanol extract			Petroleum spirit extract		
	Leaf	Stem	Root	Leaf	Stem	Root
Strep. Pneumoniae	19	19	20	20	11	X
Vibrio cholerae	17	25	20	X	X	X
Staph. aureus	16	17	19	16	X	14
Kleb. pneumoniae	15	17	21	6	12	11
Ps. aeruginosa	15	20	17	12	10	X
Bacillus cereus	17	30	18	11	X	8
E. coli	15	15	17	14	11	7
Sal. typhi	17	17	17	11	X	X

X = No inhibition.

Table 3. MIC of the methanol extracts of the leaves of *Vitellarin paradoxa*.

Test organisms	Concentration (mg/ml) of extract								
	100	80	70	60	50	40	20	10	0.5
Bacillus cereus	- -	*- -	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Staph aureus	- -	- -	* - -	+ +	+ +	+ +	+ +	+ +	+ +
E. coli	- -	- -	*- -	+ +	+ +	+ +	+ +	+ +	+ +
Pseudo aeruginosa	- -	*- -	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Kleb. Pneumoniae	- -	*- -	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Salm. typhi	- -	- -	*- -	+ +	+ +	+ +	+ +	+ +	+ +

Key * = MIC concentration; - - = No growth; + + = growth.

Table 4. MIC of the methanol extract of the stem of *Vitellarin paradoxa*.

Test organism	Concentration (mg/ml) of extract								
	100	80	70	60	50	40	20	10	0.5
B. cereus	- -	- -	- -	- -	*- -	+ +	+ +	+ +	+ +
S. aureus	- -	- -	- -	*- -	+ +	+ +	+ +	+ +	+ +
E. coli	- -	- -	- -	*- -	+ +	+ +	+ +	+ +	+ +
P. aeruginosa	- -	- -	- -	- -	*- -	+ +	+ +	+ +	+ +
K. Pneumoniae	- -	- -	- -	- -	- -	+ +	+ +	+ +	+ +
S. typhi	- -	- -	- -	- -	- -	+ +	+ +	+ +	+ +

* = MIC concentration; - - = No growth; + + = growth.

tances. Many triterpene saponins and their aglycones have been reported by Hostettmann and Martson (1995) as in Ndukwe et al. (2005) to have varied uses as antiulcerogenic, anti-inflamatory, fibrinolytic, antipyretic, analgesic and anti-edematous in action. 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 antimicrobial screening of the extract of the root, stem bark and leaves of this plant showed that the methanol extracts are more active than the petroleum ether extracts as is seen in Table 2. This could be due to the presence of some very active bioactive substances in this fraction. The MIC results are presented in Tables 2 to 6. The crude extract of the stem inhibited and fully prevented the growths of *B. cereus, P. aeruginosa, K. pnemoniae* and *S. typhi* at a concentration of 50 mg/ml as recorded in Tables 2, 4 and 8 while the extract from the leaf is minimal at 70 mg/ml against *S. aureus, E. coli* and *S. typhi* (Table 3) and the MIC for the root is 60 mg/ml against *S. aureus, E. coli, P. aeruginosa, K. pneu-*

Table 5. MIC of the methanol extract of the root of *Vitellarin paradoxa*.

Test organism	Concentration (mg/ml) of extract								
	100	80	70	60	50	40	20	10	0.5
Bacillus cereus	- -	*- -	++	++	++	+ +	+ +	+ +	+ +
Staph aureus	- -	- -	- -	*- -	+ +	+ +	+ +	+ +	+ +
E. coli	- -	- -	- -	*- -	+ +	+ +	+ +	+ +	+ +
Pseudo aeruginosa	- -	- -	- -	*- -	+ +	+ +	+ +	+ +	+ +
Kleb. Pneumoniae	- -	- -	- -	*- -	+ +	+ +	+ +	+ +	+ +
Salm. typhi	- -	- -	- -	*- -	+ +	+ +	+ +	+ +	+ +

* = MIC concentration; - - = No growth; + + = growth.

Table 6. Summary of MIC of the methanol extract of *Vitellarin paradoxa* root, stem bark and leaves.

Test organism	Minimum inhibitory concentration (MIC, mg/ml)		
	Leaf	Stem	Root
Bacillus cereus	80.0	50.0	80.0
Staph aureus	70.0	60.0	60.0
E. coli	70.0	60.0	60.0
Pseudo aeruginosa	80.0	50.0	60.0
Kleb. Pneumoniae	80.0	50.0	60.0
Salm. typhi	70.0	50.0	60.0

Table 7. MBC of the methanol extract of the leaf of *Vitellarin paradoxa*.

Test organism	Concentration (mg/ml) of extract								
	100	80	70	60	50	40	20	10	0.5
Bacillus cereus	*- -	+ +	++	++	++	+ +	+ +	+ +	+ +
Staph aureus	- -	*- -	++	++	+ +	+ +	+ +	+ +	+ +
E. coli	- -	*- -	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Pseudo aeruginosa	*- -	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Kleb. Pneumoniae	*- -	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Salm. typhi	*- -	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +

* = MBC concentration, - - = No growth , + + = growth

Table 8. MBC of the methanol extract of the stem of *Vitellarin paradoxa*.

Test organism	Concentration (mg/ml) of extract								
	100	80	70	60	50	40	20	10	0.5
Bacillus cereus	- -	- -	- -	*--	++	+ +	+ +	+ +	+ +
Staph aureus	- -	- -	*--	++	+ +	+ +	+ +	+ +	+ +
E. coli	- -	- -	*--	+ +	+ +	+ +	+ +	+ +	+ +
Pseudo aeruginosa	- -	- -	- -	*--	+ +	+ +	+ +	+ +	+ +
Kleb. Pneumoniae	- -	- -	- -	*--	+ +	+ +	+ +	+ +	+ +
Salm. typhi	- -	- -	- -	*--	+ +	+ +	+ +	+ +	+ +

* = MBC concentration; - - = No growth; + + = growth.

monia and *S. typhi* as recorded in Table 5. The MBC (Tables 7 to 10) generally showed slightly higher concentrations than that of the MIC and the methanol extracts could completely exterminate the test organisms from growing. The MBC was lowest in the stem extract which indicates that the stem bark contains the highest

Table 9. MBC of the methanol extract of the root of *Vitellarin paradoxa*.

Test organism	Concentration (mg/ml) of extract								
	100	80	70	60	50	40	20	10	0.5
Bacillus cereus	*- -	+ +	++	++	++	+ +	+ +	+ +	+ +
Staph aureus	- -	- -	*--	+ +	+ +	+ +	+ +	+ +	+ +
E. coli	- -	- -	*--	+ +	+ +	+ +	+ +	+ +	+ +
Pseudo aeruginosa	- -	- -	*--	+ +	+ +	+ +	+ +	+ +	+ +
Kleb. Pneumoniae	- -	- -	*--	+ +	+ +	+ +	+ +	+ +	+ +
Salm. typhi	- -	- -	*--	+ +	+ +	+ +	+ +	+ +	+ +

* = MBC; - - = No growth; + + = Growth.

Table 10. Summary of the MBC of the methanol extract of *Vitellarin paradoxa* root, stem bark and leaves.

Test organism	Minimum bactericidal concentration (MIC) mg/ml		
	Leaf	Stem	Root
Bacillus cereus	100	60	100
Staph aureus	80.0	70	70
E. coli	80.0	70	70
Pseudo aeruginosa	100.0	60	70
Kleb. Pneumoniae	100.0	60	70

concentrations of the bioactive constituents as shown in Table 10. The findings in this work have justified the use of this plant in ethnomedicinal treatment of wounds, sprain, cold, etc which are caused by some of these organism used in this study. Work is still on in the isolation and characterization of the bioactive compounds in this plant.

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