Antibacterial Activity of *Vitellaria paradoxa* onsome Enteric Bacteria

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Abstract: This research was carried out to evaluate the antibacterial activity of *vitellaria paradoxa* crude extract on two Enteric Organisms namely; *Salmonella typhi* and *Shigella flexaeri*. The clinical isolate of *Salmonella typhi* and *Shigella flexaeri* were subjected to antimicrobial susceptibility test using the agar diffusion technique. Methanolic and aqueous crude extract produced clear zones of inhibition at concentration ranging from 50 to 200mg/ml. The Minimum inhibitory concentration was 60 and 120mg/ml and the minimum bacteriocidal concentration were 80 and 140mg/ml. Two thousand milligram per kilogram body weight of the crude extracts were administered to the mice orally, only one died. In vivo antimicrobial assay revealed that mice treated with crude methanolic and aqueous extracts after being infected with the test organism survived and showed no pathological effect. Similarly, untreated mice (control) died after 48 hours of inoculation with *Salmonella typhi* and *Shigella flexaeri* respectively. Phytochemistry of the *Vitellaria Paradoxa* crude extracts revealed the presence of flavonoids, alkaloids carbohydrates, saponin, and cardiac glycosides, *Vitellaria Paradoxa* crude extract could be a potential source for the treatment of disease associated with *Salmonella typhi* and *Shigella flexaeri*. Further studies should be directed towards isolation and characterization of the active compounds in the crude extracts.

***Keywords**: Antibacterial activity, Enteric bacteria, Vitellaria paradoxa, Susceptibility test, Methanolic extract and Aqueous extracts.*

Introduction

Enteric bacteria are referred to as Gram-negative bacteria that are associated with gastrointestinal flora or diseases. These bacteria are rod-shaped and possess the following characteristics; facultative anaerobes, catalase positives, ferment glucose by one of two major pathways to a variety of end products, oxidase-negative and possess the enterobacterial common antigen in the cell wall (McMahon, 2014).

Enterics can be found in a variety of habitats, not just in the intestinal tract. They are said to be chemoorganotrophs and they exhibit both respiratory and fermentative metabolism (AL-Ouqaili, 2014). The optimum growth temperature is 22 and 35°C on media containing peptone or beef extract. Selective isolation of these organisms is assisted by the use of media containing agents which inhibit the growth of Gram-positive organisms (McMahon, 2014).

Furthermore the enteric bacteria are said to possess four-surface antigens namely: O antigen (Heat-stable cell wall antigen located on the most external surface of Lipolysaccharides); H antigen (Antigen located on flagella and only those bacteria with "flagella" will have an H antigen such as *Salmonella*); K antigen (Antigen associated with the capsule of fimbriae); and pili (Antigenic fimbriae responsible for attachment and colonization of the host (Flashcard of enteric bacteria, 2011).

Most enterics are motile by peritrichous flagella; two major exceptions are *Klebsiella* and *Shigella*. Most can reduce nitrate to nitrite but never to nitrogen gas.

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Depending on the specific organism, various sugars may be fermented and/or respired (yielding energy), and one or more amino acids (such as lysine, ornithine, arginine, glutamic acid and histidine) may be decarboxylated (McMahon, 2014). Fermentation and decarboxylation are anaerobic processes and will result in acid and alkaline reactions, respectively. Another anaerobic process - production of hydrogen sulfide from thiosulfate – is also possessed by some of the enteric. Many are anaerobic, a trait which allows them to thrive in the environment of the gut, and most produce energy by feeding on the sugars and converting them into lactic acid (McMahon, 2014).

Vitelleria paradoxa is generally regarded as a multipurpose plant, found and used in Africa. The plant is said to have its main product known as Shea butter (Blaze *et al*., 2013). The size of the mature tree varies from 7 to 25m.The bole is short, 3-4m, sometimes up to 8m with diameter less than 1m. It has a thick bark that protects it from bush fires; the plant is said to have a reddish slash and it is also said to possess white latex. Most of the leaves are borne in terminal whorls, 20-30 together. Flowers are usually hermaphroditic and they are mainly in clusters, each individual cluster is made up of 10-40 flowers, with cream-yellowish coloration and a good fragrance (Joker, 2000). The Shea tree grows naturally in the wild 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. Decoction of young leaves is used as a vapor bath for headaches in Ghana. The roots are used as chewing sticks in Nigeria, most commonly in savannah areas (Iswawumi, 1978). While the roots and root bark are ground to a paste and taken orally to cure jaundice, stomach ache and diarrhea (Fobil *et al*., 2002). Due to adverse effects of some common antibiotics,

proliferation of fake and counterfeit drugs in Nigeria, emerging drug resistant strains of microorganisms coupled with economic predicament in most developing countries like Nigeria the treatment of bacterial infection has become difficult to sustain. The continuous search for cheap, less toxic and available herbs that could be used to treat infectious diseases is very essential. The study therefore, is an attempt to determine the phytochemical compounds that could be used to treat disease associated with enteric bacteria.

Materials and Methods

Collection and identification of the plant materials

Fresh samples of the leaves, stems and roots were collected from Garatu in Bosso local government area of Niger State. The geographic location of Garatu lies on the Longitude 6.44ºN, and Latitude 9.4ºE. The plant materials were taken to the Department of Biological Sciences, Federal University of Technology, Minna, for identification. Voucher number GBL 2562 MX was deposited in the Department of Biological sciences for reference purpose.

Drying procedure

The leaves, stems and roots were thoroughly washed, air dried at room temperature (28°C) and ground into coarse powder using a sterile mortar and pestle. The dried plant parts were further ground into a fine powder using an electric blender. This was done to enhance the penetration of the extracting solvent, thus facilitating the release of active principles (Iyamabo, 1991).

Extraction

One hundred grams (100g) of each ground part was macerated successively for three days (with occasional shaking) using a cold maceration technique. One thousand millilitres (1000ml) of distilled water, methanol, and acetone were used as extraction solvents respectively. The macerated samples were sieved with Whatman filter paper No1 and evaporated to dryness using a steam bath. The dried extracts were weighed and stored in sterile sample bottles and kept in the refrigerator for further studies (Iyamabo, 1991).

Phytochemical screening

The phytochemical screening of the crude extracts was carried out to detect the presence or absence of some secondary metabolites. The methods by Harbone (1984) and Trease *et al.* (1987) were employed as described below:

Test for Alkaloids: Half of a gram (0.5g) of the crude extract was stirred with five millilitres (5ml) of 1% Hydrochloric acid on a water bath for thirty minutes and filtered. One millilitre (1ml) of the filtrate was put in a test tube. A few drops of Dragendorff's reagent were added to the test tube. Observation for an orange-red precipitate was done and the result recorded (Harbone, 1984; Trease *et al*., 1987).

Test for Carbohydrates: Test for reducing sugars (Fehling's test): 0.2g of the crude extract were dissolved in water and filtered into a test tube. The filtrate was then heated with 5ml of Fehling's A and B solutions each. Observation for the formation of red precipitate of cuprous oxide was done and the result was recorded (Harbone, 1984; Trease *et al*., 1987).

Test for Flavonoids: Extract of about 0.2g were diluted with a few drops of dilute sodium hydroxide. Observation for the appearance of a yellow solution was done. Few drops of dilute acid were again added and observation of colour change was done and the result was recorded (Harbone, 1984; Trease *et al*., 1987).

Test for Phlobatannins: Extract of about zero point two grams (0.2g) of the extract was boiled with water and filtered into a test tube. Five milliliters (5ml) of 1% aqueous Hydrochloric acid were added to the filtrate. Observation for red precipitate was done and the result was recorded (Harbone, 1984; Trease *et al*., 1987).

Test for Saponins: Five millilitres (5ml) of the crude extract was shaken vigorously for 2 minutes with ten milliliters (10ml) with distilled water. Observation for frothing was done and the result recorded (Harbone, 1984; Trease *et al*., 1987).

Test for the steroidal nucleus (Salkowskii's test): Zero point five grams (0.5g) of the crude extract were dissolved in two millilitres (2ml) of chloroform followed by the addition of three millilitres (3ml) of concentrated sulphuric acid to form a lower layer. Observation for a reddish brown ring colour at the interface was done and the result was recorded (Harbone, 1984; Trease *et al*., 1987).

Test for Tannins: Zero point two, five grams (0.25g) of the crude extract was dissolved in ten millilitres (10ml) of distilled water and heated on a water bath. Ferric chloride solution was added. Observation of blue- black, green or blue-green coloration was done and the result was recorded (Harbone, 1984; Trease *et al*., 1987).

Test for cardiac glycosides (Keller-Killani test): Zero point five grams (0.5g) were treated with two millilitres (2ml) of glacial acetic acid containing one drop of ferric chloride solution. This was under layered with one millilitre (1ml) of concentrated sulphuric acid. Observation for a brown ring coloration of the interface was done and recorded (Harbone, 1984; Trease *et al*., 1987).

Culture media

Six point three grams (6.3g) of *Salmonella Shigella* agar was dissolved in one hundred millilitres (100ml) of water through heating and was used as a selective medium for the confirmation of the test organisms. Two point eight grams (2.8g) of nutrient agar dissolved in one hundred milliliters (100ml) of water was sterilized and used for susceptibility testing (Idu *et al*., 2012).

Identification of the test organisms

The test organisms (*Salmonella typhi* and *Shigella flexneri*) were obtained from the stock culture in the Microbiology Laboratory, General Hospital, Minna, Niger State. The isolates were identified using the schemes of Cheesbrough (2006).

Standardization of the test organisms

The McFarland standard was employed in the standardization of the test organisms. Morphologically similar colonies of each test organisms were transferred aseptically from an agar plate culture into a tube containing 4 to 5 ml of a suitable broth medium (nutrient broth). The broth was shaken and incubated at 37°C until it achieved or exceeded the turbidity of the 0.5 McFarland standards (usually in 2 to 6 hours). The turbidity of the actively growing culture in the broth was adjusted with sterile saline or broth to obtain turbidity that was optically comparable to that of the 0.5 McFarland standards (Lalitha, 2004).

Bacterial assay of the extracts

The antibacterial assay of the crude extracts and bioactive fractions was done using a punch well method described by (Idu *et al*., 2012). The plates were prepared by dispensing 20ml of nutrient agar into sterile Petri plates and allowed to set. A 4mm cork borer was used to punch holes in the medium. Four wells were made on each Petri plate, adequately spaced out after inoculation. About zero point two millilitres (0.2ml) of the different concentrations was introduced into each well. The petri plates were incubated at a temperature of 37ºC for 24 hours, after which observation for the zones of inhibition was conducted, measurement of the zones of inhibition were carried out and the results recorded in comparison with the effect of the standard antibiotic (Chloramphenicol) as the control (Idu *et al*., 2012).

Minimum inhibitory concentration (MIC)

The MIC of the antimicrobial compounds was determined by broth dilution methods. Five millilitres (5 ml) of nutrient broth were dispensed into each test tube. Zero point five millilitres (0.5mL) of bacteria suspension (1.0×10^6) was inoculated in each test tube containing the broth. This was followed by the introduction of different concentrations (80mg/mL, 100mg/mL 120mg/mL, 140mg/mL and 160mg/mL) of the crude extract into the test tubes. In the control tubes, the antimicrobial compounds were not added. The uninoculated test tube was used to check the sterility of the medium and as the negative control while the positive control tube (which was the inoculated test tube) was used to check the suitability of the medium for growth of the microorganisms and the viability of the inoculums. The final volume in all the test tubes was adjusted to ten milliliters (10ml) using distilled water. All the test tubes were properly shaken and then incubated at 37ºC for 24 hours. The MIC was determined by the lowest concentration of the extract that inhibited visible growth (Abalaka *et al*., 2011).

Minimum bactericidal concentration (MBC)

The MBC of the extracts was determined by sub culturing the contents of the tube(s) that showed inhibition or no turbidity on nutrient agar plate and absence of growth on incubation for 24 hours was confirmatory for MBC (Vogel *et al*., 1996).

Thin–layer chromatography

Thin layer chromatography was performed on a sheet of glass which was coated with a thin layer of adsorbent material such as silica gel or aluminum oxide. The sample was then applied at one end of the plate and placed in a TLC tank containing a shallow amount of the solvent or mixture of solvents (mobile phase) to be used. After the sample had been applied on the plate and placed in the beaker, the solvent was drawn up the plate via capillary action. The different analytes ascended the TLC plate at different rates, and so separation was achieved (Mukinda *et al*., 2007).

Acute oral toxicity studies

Acute toxicity study was performed on 30 animals using a single dose of 2000mg/ kg body weight. The animals were divided into 6 groups, each containing 5 animals. The animals were starved overnight before they were administered with a crude extract orally. After drug administration the animals were provided with food and water immediately and were under observation for any mortality/ adverse signs (Canadian Council on Animal Care, 1997).

***In vivo* antibacterial activity of the crude extracts**

Experimental animals

Mice within the age of 8-12 weeks with body weight of 18-22g were acquired from Ibrahim Badamosi Babangida University Lapai. The mice were kept in standard cages with adequate food, water and under hygienic conditions for 2 weeks, before the inoculation (Canadian Council on Animal Care, 1997).

Challenge culture preparation (preparation of inoculum)

Salmonella-Shigella agar was inoculated with a loop full of the test organisms, in order to activate it. The test organisms were further transferred into test tubes containing ten millilitres (10 ml) of sterilized nutrient broth and incubated at 37ºC for 18-24 hours.

The activated culture was serially diluted in test tubes with normal saline to a cell concentration of 1.0×10^5 cfu/ml was obtained (Eman *et al*., 2008).

Inoculation of test organisms and administration of plant extracts and antibiotic to albino mice

The mice were divided into 2 major groups (A and B). Each major group consists of 9 sub- groups, each of 5 replicates (n=5). The volume of the inoculums introduced into each mouse was given, as prescribed by Itelima *et al*. (2014). Inoculation of the mice and administration of both the extracts and antibiotics was done orally in accordance with the procedure of (Itelima *et al*., 2014).

Observation of mortality rate, survival rate and other pathological manifestations

The mortality rate and survival rate of the mice in the subgroups were calculated as numbers of the mice that died and survived during the course of the experiment in relation to all the mice that were used (Eman *et al*., 2008). The animals were observed to note the consistency, frequency and colour of their faecal waste. The mice were also observed for any abnormalities and pathological manifestations (such as loss of appetite, loss of weight and body weakness) during the period of the experiment (Itelima *et al*., 2014). At the end of the study, the infected mice were killed using chloroform to prevent the spread of the infection associated with enteric pathogens in the environment (Itelima *et al*., 2014).

Statistical analysis

Two way ANOVA was used to determine the significant differences between the two extracts and in comparison with the standard antibiotic (Chloramphenicol).

Results

The phytochemical components of *Vitellaria paradoxa* include: Saponins, Steroids, Alkaloids Flavonoids, Cardiac glycosides, Starch and Carbohydrates in both methanolic and aqueous extracts. Other compounds such as Carbohydrates and Tannins were present in only the methanolic stem extract while phenolics were present only in the aqueous stem extracts; phlobatannins were not detected in both methanol and aqueous stem extracts of the plant.

Table 1. Phytochemical constituents of *Vitellaria paradoxa*

phytochemicals compounds	Leaf		Stem		Root	
	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous
Carbohydrates	+	+	+	-	+	+
Cardiac glycosides	-	-	+	+	+	+
Saponins	+	+	+	+	+	+
Steroids	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Tannins	-	-	+	-	+	+
Phlobatannins	-	-	-	-	-	-

Key + = Presence of the phytochemical compound; - = Absence of the phytochemical compound

Table 2. Reveals that VPML, VPMS, VPAR and VPMR (in ascending order) had significant antibacterial activity on *Salmonella typhi* and *Shigella flexneri* at 50mg for 24hours and there was decreased in the antibacterial activity after 48hours.

Table 2. Zones of Inhibitionproduced by *V. paradoxa*crude extractat 50mg/ml (mm)

	24 hours		48 hours		72hours	
PlantExtracts	*Salmonella typhi*	*Shigella flexneri*	*Salmonella typhi*	*Shigella flexneri*	*Salmonella typhi*	*Shigella flexneri*
VPML	5.67 ± 0.33^b	4.67 ± 0.33^c	5.33 ± 0.33^b	3.33 ± 0.33^b	4.00 ± 0.58^b	3.00 ± 0.58^b
VPAL	0.67 ± 0.33^a	0.00 ± 0.00^a	0.00 ± 0.33^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a
VPMS	6.00 ± 0.58^b	5.00 ± 0.58^c	5.00 ± 0.58^b	3.67 ± 0.88^b	4.33 ± 0.33^b	2.33 ± 0.33^b
VPAS	1.33 ± 0.02^a	0.33 ± 0.33^a	0.67 ± 0.33^a	0.33 ± 0.03^a	0.00 ± 0.00^a	0.00 ± 0.00^a
VPMR	9.33 ± 0.33^c	7.00 ± 0.58^d	7.00 ± 0.58^c	5.33 ± 0.33^c	6.00 ± 0.58^c	3.00 ± 0.58^b

VPAR	9.00±0.58[c]	5. 33±0.58[b]	7.00±0.58[c]	1.33±0.33[a]	5.67±0.33[c]	0.00±0.00[a]
CONTROL	18.00±0.58[d]	17.33±0.67[c]	16.00±0.58[d]	15.33±0.67[d]	15.67±0.33[d]	14.33±0.33[c]

Values are Mean±SEM of triplicate determinations. Values followed by a different superscript along a column are significantly (p<0.05) different.

Key:VPML---Methanolic leaf extract of *Vitellaria paradoxa*; VPAL---Aqueous leaf extract of *Vitellaria paradoxa* ; VPMS---Methanolic stem extract of *Vitellaria paradoxa* ; VPAS---- Aqueous stem extract of *Vitellaria paradoxa* ; VPMR---- Methanolic root extract of *Vitellaria paradoxa* ; VPAR---- Aqueous root extract of *Vitellaria paradoxa.*

Table3. Revealsthat at 100mg concentration of both methanolic and aqueous extracts of *Vitellaria paradoxa*, there were significant antibacterial activities on *S.typhi* and *S. flexneri* after 24 and 48 hours

Table 3.Zones of Inhibitionproduced by*V. paradoxa* crude extract at 100mg/ml

	24hours		48hours		72hours	
PlantExtracts	*Salmonella typhi*	*Shigella flexneri*	*Salmonella typhi*	*Shigella flexneri*	*Salmonella typhi*	*Shigella flexneri*
VPML	7.00±0.58[a]	5.33±0.88[a]	5.67±0.33[a]	4.00±0.58[a]	4.33±0.33[a]	2.67±0.33[a]
VPAL	6.00±0.58[b]	4.00±0.58[a]	4.67±0.33[a]	2.67±0.33[a]	2.67±0.33[a]	1.33±0.33[a]
VPMS	12.00±0.58[c]	10.00±0.58[b]	11.00±0.58[c]	9.00±0.58[b]	10.33±0.33[c]	8.67±0.33[b]
VPAS	10.33±0.33[c]	9.67±0.33[b]	9.00±0.58[b]	8.33±0.33[b]	8.33±0.33[b]	7.67±0.33[b]
VPMR	12.67±0.33[c]	11.33±0.33[b]	11.67±0.33[c]	10.33±0.33[b]	10.67±0.33[c]	9.00±0.58[b]
VPAR	11.00±0.58[c]	10. 00±0.58[b]	10.00±0.58[c]	9.33±0.67[b]	8.00±0.58[b]	10.00±0.58[b]
CONTROL	24.67±0.33[d]	24.00±0.00[c]	24.00±0.58[d]	23.00±0.58[c]	23.00±0.58[d]	20.00±0.58[c]

Values are Mean±SEM of triplicate determinations. Values followed by a different superscript along a column are significantly (p<0.05) different.

Key:VPML---Methanolic leaf extract of *Vitellaria paradoxa*; VPAL---Aqueous leaf extract of *Vitellaria paradoxa* ; VPMS---Methanolic stem extract of *Vitellaria paradoxa* ; VPAS---- Aqueous stem extract of *Vitellaria paradoxa* ; VPMR---- Methanolic root extract of *Vitellaria paradoxa* ; VPAR---- Aqueous root extract of *Vitellaria paradoxa*.

Table 4. Indicates that at 150mg concentration of both methanolic and aqueous extracts, there were significant activities on the test organisms after 24, 48 and 72 hours respectively.

Table 4. Zones of Inhibition (mm)produced by *V.paradoxa* crude extract 150mg/ml

		24 hour		48hour	72hour	
PlantExtracts	*Salmonella typhi*	*Shigella flexneri*	*Salmonella typhi*	*Shigella flexneri*	*Salmonella typhi*	*Shigella flexneri*
VPML	11.00±0.58[a]	10.00±0.58[a]	9.33±0.88[a]	9.00±0.58[a]	8.67±0.67[a]	8.00±0.58[a]
VPAL	10.33±0.33[a]	9.33±0.33[a]	9.00±0.58[a]	8.00±0.58[a]	8.33±0.33[a]	7.33±0.33[a]
VPMS	13.00±0.58[ab]	11.67±0.88[ab]	12.33±0.67[ab]	11.00±0.58[b]	11.67±0.33[bc]	10.33±0.67[b]
VPAS	12.00±0.58[b]	11.00±0.58[ab]	11.00±0.58[ab]	10.00±0.58[ab]	10.00±0.58[ab]	9.00±0.58[a]
VPMR	14.67±0.33[b]	14.33±0.33[c]	13.67±0.33[c]	13.33±0.33[c]	12.33±0.33[d]	12.67±0.33[c]
VPAR	13.33±0.58[ab]	12.00±0.58[ab]	12.33±0.67[ab]	11.33±0.67[ab]	11.67±0.33[bc]	10.33±0.33[ab]
CONTROL	27.00±0.58[c]	26.33±0.33[d]	26.00±0.58[d]	25.67±0.33[d]	25.33±0.67[e]	25.00±0.58[d]

Values are Mean±SEM of triplicate determinations. Values followed by a different superscript along a column are significantly (p <0.05) different

Key:VPML---Methanolic leaf extract of *Vitellaria paradoxa*; VPAL---Aqueous leaf extract of *Vitellaria paradoxa* ; VPMS---Methanolic stem extract of *Vitellaria paradoxa* ; VPAS---- Aqueous stem extract of *Vitellaria paradoxa* ; VPMR---- Methanolic root extract of *Vitellaria paradoxa* ; VPAR---- Aqueous root extract of *Vitellaria paradoxa*.

Table 5. Indicates that at 200mg concentrations of both methanolic and aqueous extracts, there were significant activities on the test organisms after 24, 48 and 72 hours respectively.

Table 5.Zones of Inhibition (mm) produced by*V.paradoxa* crude extract 200mg/ml

	24hours		48hours		72hours	
Plant Extracts	*Salmonella typhi*	*Shigella flexneri*	*Salmonella typhi*	*Shigella flexneri*	*Salmonella typhi*	*Shigella flexneri*
VPML	12.00±0.58^{a}	11.33±0.33^{a}	11.00±0.58^{a}	7.67±0.33^{a}	10.00±0.58^{a}	10.00±0.00^{a}
VPAL	11.67±0.33^{a}	11.00±0.58^{a}	11.67±0.33^{a}	10.33±0.33^{ab}	10.33±0.33^{a}	9.67±0.33^{a}
VPMS	15.33±0.33^{b}	12.00±0.58^{a}	12.33±0.33^{a}	11.33±0.33^{ab}	11.33±0.33^{a}	10.33±0.33^{a}
VPAS	13.00±0.58^{a}	14.67±0.33^{b}	15.00±0.58^{b}	13.67±0.33^{b}	14.00±0.58^{b}	12.33±0.33^{b}
VPMR	17.00±0.58^{b}	15.00±0.58^{b}	16.00±0.58^{b}	14.00±0.58^{b}	14.00±0.58^{b}	12.67±0.40^{b}
VPAR	15.68±0.88^{b}	14.67±0.67^{b}	14.00±0.58^{ab}	13.33±0.33^{b}	13.00±0.58^{b}	12.00±0.58^{b}
CONTROL	30.00±0.58^{c}	29.67±0.33^{c}	29.33±0.67^{c}	29.00±0.58^{c}	28.33±0.67^{c}	28.00±0.58^{c}

Values are Mean±SEM of triplicate determinations. Values followed by a different superscript along a column are significantly ($p < 0.05$) different

Key:VPML---Methanolic leaf extract of *Vitellaria paradoxa*; VPAL---Aqueous leaf extract of *Vitellaria paradoxa* ; VPMS--- Methanolic stem extract of *Vitellaria paradoxa* ; VPAS---- Aqueous stem extract of *Vitellaria paradoxa* ; VPMR---- Methanolic root extract of *Vitellaria paradoxa* ; VPAR---- Aqueous root extract of *Vitellaria paradoxa*.

Table 6. Reveals theminimum inhibitory and bactericidal concentrations from 60-120mg and 80-140mg for both organisms

Table 6. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) in (mg/ml) of Methanolic and Aqueous extracts of *Vitellaria paradoxa*

	MIC		MBC	
Plant Extract	*Salmonella typhi*	*Shigella flexneri*	*Salmonella typhi*	*Shigella flexneri*
VPML	80	80	120	120
VPAL	100	120	140	140
VPMS	60	60	80	80
VPAS	80	80	120	120
VPMR	60	60	80	80
VPAR	80	80	100	100
CONTROL(+)	_	_	-	-
CONTROL(-)	+	+	+	+

Key:VPML---Methanolic leaf extract of *Vitellaria paradoxa*; VPAL---Aqueous leaf extract of *Vitellaria paradoxa* ; VPMS--- Methanolic stem extract of *Vitellaria paradoxa* ; VPAS---- Aqueous stem extract of *Vitellaria paradoxa* ; VPMR---- Methanolic root extract of *Vitellaria paradoxa* ; VPAR---- Aqueous root extract of *Vitellaria paradoxa* ; control (+)----- control with antibiotics, control (-)----- control without antibiotics.

Table 7. Reveals that only methanolic extracts of the plant had a toxic effect on the mice at 2000mg/kgbw. Only methanolic leaf, stem and root extracts exhibited the toxic effect, which translated to the death of 3 mice.

Table 7. Toxicity of the crude extracts on mice before infection

Extract	**No of mice per extract**	**Dose**	**No of mice that died**
Methanolic leaf extract of *Vitellaria paradoxa*	5	2000mg/kgbw	1/5
Aqueous leaf extract of *Vitellaria paradoxa*	5	2000mg/kgbw	0/5
Methanolic stem extract of *Vitellaria paradoxa*	5	2000mg/kgbw	1/5

Aqueous stem extract of *Vitellaria paradoxa*	5	2000mg/kgbw	0/5
Methanolic root extract of *Vitellaria paradoxa*	5	2000mg/kgbw	1/5
Aqueous stem extract of *Vitellaria paradoxa*	5	2000mg/kgbw	0/5

Key: Mg/kgbw---Milligram per kilogram body weight

Table 8. Reveals the mortality rate and pathological effects of the crude extracts on mice at 2000mg/kgbw after infection with*S.typhi*. At the end of the 7days treatment with the various extracts; only 5 mice had diarrhoea, 8 experienced weight and 8 experienced loss of appetite.

Table 8. Effects of the crude extracts on the mice during treatment of *S.typhi*

Sub-group	No of Mice	Dilution Factor	Mortality rate (%)	W.D (%) 1-3Days	W.D (%) 4-6 Days	W.D (0%) >7days	Loss of appetite (%) >7days	Loss of Weight (%) >7days	Body weakness/slow Movement (%) >7days
I	5	10^{-5}	0/5(0%)	1/5(20%)	0/5(0%)	0/5(0%)	0/5(0%)	0/5(0%)	0/5(0%)
I	5	10^{-5}	0/5(0%)	3/5(60%)	2/5(40%)	0/5(0%)	1/5(20%)	1/5(20%)	1/5(20%)
I	5	10^{-5}	0/5 (0%)	2/5(40%)	0/5(0%)	0/5(0%)	0/5(0%)	0/5(0%)	0/5(0%)
II	5	10^{-5}	0/5(0%)	3/5(60%)	1/5(20%)	0/5(0%)	1/5(20%)	1/5(20%)	1/5(20%)
V	5	10^{-5}	0/5(0%)	1/5(20%)	0/5(0%)	0/5(0%)	0/5(0%)	0/5(0%)	0/5(0%)
V	5	10^{-5}	0/5(0%)	4/5(80%)	1/5(20%)	0/5(0%)	1/5(20%)	1/5(20%)	1/5(20%)
I	5	10^{-5}	0/5 (0%)	0/5(0%)	0/5(0%)	0/5(0%)	0/5(0%)	0/5(0%)	0/5(0%)
II	5	10^{-5}	5/5(100%)	5/5(100%)	5/5(100%)	5/5(100%)	5/5(100%)	5/5(100%)	5/5(100%)
III	5	10^{-5}	0/5(0%)	0/5(0%)	0/5(0%)	0/5(0%)	0/5(0%)	0/5(0%)	0/5(0%)
X									

Key: Sub group I= Treated with VPML; II= Treated with VPAL: III = Treated with VPMS; IV = Treated with VPAS; V=Treated with VPMR; VI= Treated with VPAR; VII=Treated with Ciprofloxacin;VIII= Infected not treated; IX= Not infected not treated; W.D= Watery diarrhea

Table 9. Reveals the mortality rate and pathological effects of the crude extracts on mice at 2000mg/kgbw after infection with *S.flexneri*. At the end of the 7days treatment with the various extracts; only 5 mice had diarrhoea, 11 experienced weight loss and 11 experienced loss of appetite.

Table 9. Effects of the crude extracts on the mice during treatment of *Shigella flexneri.*

Subgroup	No of Mice	Dilution Factor	Mortality rate (%)	W.D (%) 1-3 Days	W.D (%) 4-6days	W.D (%) >7days	Loss ofappetite (%) >7days	Loss of weight (%) >7days	Body weakness/slow Movement (%) >7days
I	5	10^{-5}	0/5(0%)	5/5 (100%)	1/5(20%)	0/5(0%)	1/5(20%)	1/5(20%)	1/5(20%)
II	5	10^{-5}	0/5(0%)	5/5 (100%)	2/5 (40%)	0/5(0%)	2/5(40%)	2/5(40%)	2/5(40%)
III	5	10^{-5}	0/5 (0%)	4/5 (80%)	0/5(0%)	0/5(0%)	0/5(0%)	0/5(0%)	0/5(0%)
IV	5	10^{-5}	0/5(0%)	5/5 (100%)	2/5 (40%)	0/5(0%)	2/5(40%)	2/5(40%)	2/5(40%)
V	5	10^{-5}	0/5(0%)	4/5 (80%)	0/5(0%)	0/5(0%)	0/5(0%)	0/5(0%)	0/5(0%)
VI	5	10^{-5}	0/5(0%)	5/5 (100%)	1/5 (20%)	0/5(0%)	1/5(20%)	1/5(20%)	1/5(20%)
VII	5	10^{-5}	0/5 (0%)	0/5(0%)	0/5(0%)	0/5(0%)	0/5(0%)	0/5(0%)	0/5(0%)
VIII	5	10^{-5}	5/5(100%)	5/5 (100%)	5/5(100%)	5/5(100%)	5/5(100%)	5/5(100%)	5/5(100%)
IX	5	10^{-5}	0/5(0%)	0/5 (0%)	0/5(0%)	0/5(0%)	0/5(0%)	0/5(0%)	0/5(0%)

Key: Sub-group I= Treated with VPML; II= Treated with VPAL: III = Treated with VPMS; IV = Treated with VPAS; V=Treated with VPMR; VI= Treated with VPAR; VII=Treated with Ciprofloxacin; VIII = Infected not treated; IX= Not infected not treated; W.D= Watery diarrheoa.

Table 10. Reveals the different number of bioactive components found in each extract. Samples A-D gave rise to two bioactive components each while samples E and F gave rise to three bioactive components each.

Table 10. Fractionated crude extracts of the leaf, stem and roots

Sample	Solvent system	No of spots	Bioactive components
A	Chloroform: methanohydroxide 4: 1	2	A1
			A2
B	,,	2	B1
			B2
C	,,	2	C1
			C2
D	,,	2	D1
			D2
E	,,	3	E1
			E2
			E3
F	,,	3	F1
			F2
			F3

Key:VPML---Methanolic leaf extract of *Vitellaria paradoxa*; VPAL---Aqueous leaf extract of *Vitellaria paradoxa* ; VPMS--- Methanolic stem extract of *Vitellaria paradoxa* ; VPAS---- Aqueous stem extract of *Vitellaria paradoxa* ; VPMR---- Methanolic root extract of *Vitellaria paradoxa* ; VPAR---- Aqueous root extract of *Vitellaria paradoxa* ; control (+)----- control with antibiotics, control (-)----- control without antibiotics.

Table 11. Reveals the first bioactive components (A1, B1 and D1) of compounds A, B and D had no antibacterial activity on the test organisms while the second bioactive components (A2, B2 and D2) of these compounds along with all the bioactive components of E and F had antibacterial activity on the test organisms.

Table 11. Antibacterial Activity of each bioactive component

Compounds	Bioactive components	*Salmonella typhi*	*Shigella flexneri*
A	A1	_	-
	A2	+	+
B	B1	_	_
	B2	+	+
C	C1	+	+
	C2	_	_
D	D1	+	+
	D2	+	+
E	E1	+	+
	E2	+	+
	E3	+	+
F	F1	+	+
	F2	+	+
	F3	+	+

Key: + = exhibition of antibacterial activity; - =no antibacterial activity

Discussion

The findings of this study revealed that the methanolic and aqueous leaf, stem and root extracts of *Vitelleria paradoxa* had various bioactive compounds which are well known for their therapeutic abilities. The study shows the three parts of the plant, all contain carbohydrates, saponins, steroids, alkaloids and flavonoids. Other phytochemical components such as cardiac glycoside and tannins were also detected in the crude plant extracts. The findings agree with the previous report by Ndukwe *et al*. (2007).

The methanol crude extracts showed significant activity at 50mg/ml concentrations, after 24 and 48 hours on both organisms, when compared to the aqueous extracts. Reason to account for the difference in antibacterial activity between the methanolic and

aqueous extracts at same concentration (50mg/ml) could be attributed to variations in polarity of the solvents and solubility of the bioactive compounds in the plant parts as reported by Elmahood *et al*. (2005). Similarly, the inability of the aqueous extracts to show significant antibacterial activity could be attributed to the release of some enzymes such as phenolases and hydrolases that might have effects on the activity of the active compounds, or perhaps due to incomplete extraction of the active component when water was used as a solvent, as earlier reported by Elmahood *et al*. (2005).

may be due to the fact that the active component in 100mg/ml of *Vitellaria paradoxa* is insufficient to exhibit significant antibacterial activities as reported by Mbata *et al*. (2008).

However the antibacterial activities of methanolic and aqueous crude extracts of *Vitellaria paradoxa* at 150mg/ml and 200mg/ml were significance on *S.typhi* and *S.flexneri* after 24 hours, 48 hours and 72 hours as compared to the antibacterial activities of methanolic crude extracts of *Vitellaria paradoxa*. The high antibacterial activities of methanolic and aqueous crude extract observed at 150mg/ml and 200mg/ml could be due to the enhanced effect of the plant extracts based on the increased concentration of the individual extract, which are said to contain more phytochemical constituents. The outcome of this, conform to the result obtained in a study by (Ahmed *et al*. 2012).

The minimum inhibitory concentration (MIC) of the stem and rootof *Vitellaria paradoxa* was observed to be low, when compared to the MIC of the leaf of *Vitellaria paradoxa* (Table 6) on both test organisms. The low MIC observed in both stem and root is as a result of the fact that these parts of the plant have more bioactive compounds than the leaves. Such observation has also been reported by Emeuwa (1982) and El-Faraley *et al*. (1983). In addition, the low MIC observed in the stem and root crude extracts of *Vitellaria paradoxa* could also be attributed to the presence of latex, which is believed to enhance the antibacterial activities of the plant as reported by Okeniyi *et al*. (2012).

The result of this study revealed that the minimum bacteriocidal concentration (MBC) of the crude plant extracts indicated higher concentrations than that of the MIC. This observation is based on the fact that the concentration of the crude extract required to completely eliminate an organism must be higher than the concentration required to inhibit the growth, as reported by Acheampong *et al*. (1984). The lowest MBC of these plant parts was observed in methanolic stem and root extracts while the highest MBC was observed in the aqueous leaf extract. This may be based on the fact that most of the active component in the methanolic stem and root are highly bactericidal on these test organisms than their aqueous leaf counterpart. This result agrees with the findings of Ndukwe *et al*. (2007)

The acute oral toxicity study on the mice revealed that the oral administration of *Vitellaria paradoxa*, at a single dose of 2000mg/kg body weight, caused single deaths in each group of mice administered with methanolic crude extract, when compared with the group of mice administered with aqueous crude extracts. The death of mice administered with 2000mg/kg bw of methanolic extracts could be attributed to the fact that this high single dose of *Vitellaria paradoxa* was toxic in relation to their body weights. However, this result disagrees with the findings of Arsad *et al.* (2013), who reported that treatment at any dose of *Rhaphidophoria decursiva* irrespective of the weights of the mice was non-toxic.

The *Invivo* determination of the efficacy of both the methanolic and aqueous extracts of *Vitellaria paradoxa* in mice infected with *Salmonella typhi* and *Shigella flexneri* revealed that the extract showed significant antibacterial effect upon administration for the treatment of the infected mice. The extent of the extract's activities in the treated mice was dose and time- dependent and daily observation of the mice also revealed that after seven days most clinical symptoms observed in the mice few days after inoculation disappeared.

The presence of different bioactive components in the crude extracts of *Vitellaria paradoxa* indicates that *Vitellaria paradoxa* contains diverse potent active ingredients .This result agrees with the findings of Jayashree (2013). All bioactive components obtained in the crude extracts of the roots showed antibacterial activity against the two test organisms in this study. This suggests that these individual components or bioactive compounds that were not able to exhibit antibacterial activity on the test organisms, may be due to their inactive nature or requires a synergistic relationship with other bioactive components, as reported by Harbone (1984) and Oyeleke *et al*. (2008).

Conclusion

The methanolic and aqueous extracts of various parts of *Vitellaria paradoxa*, especially the root, contained more phytochemicals that were active against *Salmonella typhi* and *Shigella flexneri* at a concentration as low as 50 milligram, indicating that the plant is potent and contains therapeutic properties. The crude extracts of the plant was found to be safe at higher dose of 2000mg/kg body weight. The activity of the crude extracts at concentrations ranging from 50-200mg/ml showed antibacterial activity, particularly after 24 to 48 hours but the activity declined after 72hours.

Recommendations

Based on the results obtained from this study, the following recommendations are made:

Administration of extracts should be time and dose dependent to ensure complete eradication of the pathogenic organisms and prevent development of resistant genes.

Since administering methanolic crude extracts caused a single death. It is therefore recommended that before the administration of extracts, side effects of the extracts and appropriate dose in relation to the weight of the mouse should be ascertained.

Prior to administration of crude extracts, solvents should be completely dry to avoid its side effects on mice.

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