https://dx.doi.org/10.4314/dujopas.v9i2a.25
ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 9 No. 2a June 2023*

Phytochemical and Antibacterial Activities of *Vitellaria paradoxa and Sclerocarya birrea* Stem Bark and Leaves

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Abstract

Increasing resistance developed by many bacterial species has been reported to commonly prescribed antibiotics for bacterial infections. Therefore, the need to search for natural products for remedy of this problem cannot be overemphasized. The aim of the study was to evaluate the phytochemical and antibacterial activities of Vitellaria paradoxa and Sclerocarya birrea 'stem barks and leaves' extracts against some human pathogenic bacteria. The methanol and aqueous extracts of Vitellaria paradoxa and Sclerocarya birrea stem barks and leaves were carried out using cold maceration extraction method. Phytochemical screening and acute toxicity studies were carried out using standard methods. Agar well diffusion and agar dilution methods were employed to determine the zone of inhibition, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC). Phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, tannins, steroid, carbohydrates and triterpenes in both aqueous and methanolic extracts. All the extracts showed broad spectrum of activity at 500 mg/ml but the methanol extract had larger zones of inhibition and lower M.I.C and M.B.C values ranging from 15.125 mg/ml and 31.25 mg/ml against Staphylococcus aureus and Escherichia coli. The LD_{50} of Vitellaria paradoxa and Sclerocarya birrea stem barks and leaves were found to be greater than 5000 mg /kg and could be considered safe for consumption. This study has justified the traditional use of Vitellaria paradoxa and Sclerocarya birrea stem barks and leaves extracts in the treatment of

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infections caused by bacteria. Further investigations should be carried out to isolate pure compounds and determine the mechanisms of action of the plant.

Keywords: Acute toxicity, Agar well diffusion**,** Bacterial, Inhibition, Phytochemical

INTRODUCTION

A discussion of human life on this planet would not be complete without a look at the role of plants, because plants have been an integral part of human society since the start of civilization (Namadina, 2021). Since the beginning of civilization, people have used plants as medicine (Namadina, 2021). Also, plants continue to be a major source of medicines, as they have been throughout human history. It has been said that between 35, 000 and 70, 000 species of plants have been used at one time or another for medicinal purposes (Namadina, 2021). Medicinal plants thus play a vital role in the maintenance of human health throughout the world and notably in the tropics (Shah *et al.,* 2013). Interestingly, many of today's drugs have been derived from plant sources. It is estimated that, plant materials are present in or have provided the models for 50% of western drugs (Shah *et al.,* 2013; Namadina, 2021). The chronic consumption of herbal medicine has for long been traditionally claimed to improve drug resistant. Infectious diseases are the number one cause of death world-wide and in tropical countries it accounts for approximately 50% of death (Namadina, 2021). Apart from resistance, some antibiotics have serious undesirable side effects which limit their applications. Therefore, there is a serious need to develop new antimicrobial agents that are very effective with minimal side effects. Higher plants represent a potential source of novel antibiotic prototypes (Shah *et al.,* 2013).

Sclerocarya birrea {(A. Rich.) Hochst.}Subsp. *caffra* (Sond.) Kokwaro, Family: Anacardiaceae, commonly known as marula tree in English; Danya in Hausa is a common and important tree in Africa with multifaceted uses recognized as a commercially, medicinally and culturally important plant species in the continent (Ojewole *et al.,* 2010; Muhammad, 2017). Ojewole *et al.* (2010) reported that, the stem, leaves and roots of the *Sclerocarya birrea* were used in folk medicine to treat stomach-related morbidities and other illness including malaria, gastritis, peptic and stomach ulcer, dysentery, fever and diarrhea, headaches, body pains, toothache backache, infertility, childhood convulsion, hypertensions, inflammations) diabetic mellitus schistosomiasis and epilepsy in most part of region.

Vitellaria paradoxa commonly Shea butter tree (*Sapotaceae*) is the onlyspecies in the genus indigenous to Africa (Namadina *et al.,* 2020). The Shea tree fruit consist of a thin tart nutritious pulp that surrounds a relatively large oil-rich seed from which Shea butter is extracted (Akihisa *et al.,* 2010). The importance of the Shea nut is second to the numerous benefits of shea butter derived from it (Namadina *et al.,* 2020). 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 (Akihisa *et al.,* 2010).

Infectious diseases represent a serious health problem worldwide today, mostly due to the appearance of antibiotic resistant strains (Shah *et al.,* 2013). There has been an intense search for newer antibacterial agents. The efficacies of plant based drugs used in traditional medicines are being investigated. The plants used in this study have not well documented for antibacterial activities.

MATERIALS AND METHODS

Collection of Clinical Specimen

The patients were asked to clean their external genitalia with disinfectant and their midstream urine was collected in a sterilized cap. Samples were kept in an ice bag and transported to microbiological laboratory (Karzan *et al.,* 2017).

Urine Culturing

Urine samples were cultured on Nutrient agar, blood agar and MacConkey agar media and incubated over night at 37°C. Significant growth was evaluated as ≥ 105 colony-forming units CFU/mL of midstream urine (Karzan *et al.,* 2017).

Culture Characteristics

Each of the colour, size, elevation, margins and texture of colonies were screened. The morphological different colonies on MacConkey agar, nutrient agar and blood agar were sub-cultured into nutrient agar medium, in order to purify the isolated bacteria from each patient urine specimen (Karzan *et al.,* 2017).

Microscopic examination

Pure isolates were examined microscopically, on the basis of their cell wall composition and presence of capsule (Karzan *et al.,* 2017).

Isolation of Bacterial Species

The specimens were cultured on sterile blood agar, chocolate agar and MacConkey agar plates at 37 °C for 24 h in an incubator. Discrete colonies were picked based on their morphology and further sub-cultured on blood agar and chocolate agar to obtain pure culture. The isolated colonies were Gram stained and based on their Gram reactions were inoculated on different selective media — mannitol salt agar, cetrimide agar, eosin methylene blue agar. Different biochemical tests were conducted (catalase, coagulase, and oxidase tests). All the isolates that grew on selected agar media were then placed on nutrient agar and chocolate agar slants and maintained in a refrigerator at 4 °C (Cheesbrough, 2006).

Identification and Characterization of Test Organisms using Rapid Test Kits

Identification and characterization of the bacteria was carried out using Microgen Identification Kit (XYZ). The test was performed according to the manufacturer's specifications (API biomerieux). It was performed by adding saline suspension of the test bacteria to each of the wells, and appropriate wells (1, 2, 3 and 9) were overlaid with sterile paraffin oil. After overnight incubation (18-24 hours) at 37 °C, suitable reagents such as Nitrate A and B, Kovacs, Typtophan deaminase (TDA), Voges-proskauer (VPI and II) were added to wells 8, 10 and 12 for additional tests and colour changes of the different tests done. The results were converted into four to eight digits codes depending on the organisms being tested and interpreted using the Microgen Identification Software Package (MID-60) (Sylvester, 2016).

Collection and Identification of Plant Materials

The *Vitellaria paradoxa* and *Sclerocarya birrea* stem barks and leaves were collected from a local farm in January, 2023 at Minjibir Local Government Area, Kano State, Nigeria. The plants were identified and authenticated in the herbarium of the Plant Biology Department of Bayero University, Kano, Kano State, Nigeria and a voucher specimen number was deposited.

Preparation of Plant extracts

The stem barks and leaves of *Vitellaria paradoxa* and *Sclerocarya birrea* were cleaned, air dried and ground to coarse powder using grinding machine. The powder was stored in air tight containers for further use. Two hundred grams (200 g) each of the powdered stem bark and leaves was soaked into 2000 mL of methanol. The mixtures were allowed to stand for 3 days at room temperature (28 ±2 ^{o}C) with hourly agitations. Each extract was sieved through a muslin cloth, filtered through a Whatman (No.1) filter paper, poured unto a clean evaporating dish and placed on a water bath at 50 ^{o}C until all the solvent evaporated.

Qualitative Phytochemical screening Methanolic extract of *Vitellaria paradoxa* and *Sclerocarya birrea* stem barks and Leaves

The plant extracts were subjected to phytochemical screening in order to identify its phytochemical constituents of the plant using the methods described below.

Tests for carbohydrates

Molish's (General) test for Carbohydrates: To 1 mL of the filtrate, 1 ml of Molish's reagent was added in a test tube, followed by 1 mL of concentrated sulphuric acid down the test tube to form a lower layer. A reddish colour at the interfacial ring indicated the presence of carbohydrate (Evans, 2009).

Tests for Saponins

Frothing test: About 10 mL of distilled water was added to a portion of the extract and was shaken vigorously for 30 seconds. The tube was allowed to stand in a vertical position and was observed for 30 mins. A honeycomb froth that persisted for 10-15 mins indicated presence of saponins (Evans, 2009).

Test for flavonoids

Shinoda Test: A portion of the extract was dissolved in 1-2 mL of 50% methanol in the presence of heated metallic magnesium chips and a few drops of concentrated hydrochloric acid were added. Appearance of red color indicated the presence of flavonoids (Evan, 2009).

Test for alkaloids

Wagner's Test: Few drops of Wagner's reagent was added into a portion of the extract, whitish precipitate indicated the presence of alkaloids (Evans, 2009).

Test for steroids and triterpenes

Liebermann-Burchard's test:

Equal volumes of acetic acid anhydride was added to the portion of the extract and mixed gently. Concentrated sulphuric acid (1 mL) was added down the side of the test tube to form a lower layer. A colour change observed immediately and later indicates the presence of steroid and triterpenes. Red, pink or purple colour indicated the presence of triterpenes while blue or blue green indicates steroids (Evans, 2009).

Test for cardiac glycosides

Kella-killiani's test:

A portion of the extract was dissolved in 1mL of glacial acetic acid containing traces of ferric chloride solution. This was then transferred into a dry test tube and 1mL of concentrated sulphuric acid was added down the side of the test tube to form a lower layer at the bottom. Interphase for purple-brown ring was carefully observed, this indicates the presence of deoxy sugars and pale green colour in the upper acetic acid layer indicated the presence of cardiac glycosides (Evans, 2009).

Test for tannins

Ferric chloride test:

Exactly 3-5 drops of ferric chloride solution was added to the portion of the extract. A greenish black precipitate indicated the presence of condensed tannins while hydrolysable tannins give a blue or brownish blue precipitates (Evans, 2009).

Test for Anthraquinones

Borntrager's test:

Exactly 5 mL of chloroform was added to the portion of the extract in a dry test tube and shaken for at least 5 mins. This was filtered, and the filtrate shaken with equal volume of 10 % ammonium solution, bright pink colour in the aqueous upper layer indicated the presence of free anthraquinones (Evans, 2009).

Antibacterial susceptibility test

Preparation of extract concentration

This was carried out according to the method described by Srinivasan *et al.* (2009). Stock solution of the plant extracts were prepared by adding 0.5 g of each crude plant extract in 1mL dimethyl sulphuroxide (DMSO). From each of the stock solutions, 500 mg/mL, 250 mg/mL, 125 mg/mL and 62.5 mg/mL concentrations were prepared using Two-fold serial dilution method (Srinivasan *et al.,* 2009).

Standardization of bacterial inocula.

Using a sterilized inoculum loop, loopful from an over-night grown agar culture plates containing a test bacteria was transferred into a test tube containing normal saline until the turbidity of the suspension matched the turbidity of the 0.5 McFarland Standard as described by the National committee for clinical laboratory standard (2008).

Susceptibility Test of Bacterial isolates to Different Concentrations of the Extracts

The antibacterial activity of *Vitellaria paradoxa* and *Sclerocarya birrea* stem barks and leaves crude extracts (Methanol) against *Staphylococcus aureus* and *Escherichia coli* was evaluated using agar well diffusion method of susceptibility test (Srinivasan *et al.,* 2009). Mueller-Hinton agar plates were inoculated with 0.1 mL of standardized inoculum of each bacterium (in triplicates) using 0.1ml pipette and spread uniformly with sterile swab sticks. Three wells of 6 mm size were made with sterile cork borer (6 mm) into the inoculated agar plates. Using micropipette, 0.1 mL volume of the various concentrations; 500 mg/mL, 250 mg/mL, 125 mg/ml and 62.5 mg/mL each of the crude extracts were dispensed into wells of inoculated plates. . DMSO was used as negative control. Commercially available standard antibiotic, ampicillin was used as positive control parallel with the extract. The prepared plates were then left at room temperature (37 ^{o}C) for 10 minutes, allowing the diffusion of the extracts into the incubation at 37 ^{o}C for 24 hrs in an incubator. The diameter of inhibition zones (DIZ) were measured and expressed.

Determination of Minimum Inhibitory Concentration

The method used by Adesokan *et al*. (2007) was adopted for tube dilution. Thus, the plant extracts were serially diluted from 500 mg/mL solution to obtain varying concentration. The concentrations were; 250 mg/mL, 125 mg/mL, 62.5mg/mL, and 31.25 mg/mL. Doubling dilutions of the extract were incorporated in Muller Hinton broth (Oxoid, UK), and then inoculated with 0.1 mL each of standardized suspension of the test organisms into the various test tube containing varying concentrations. Another set of test tubes containing only Mueller Hinton broth were used as negative control, and another test tube containing Mueller Hinton

broth and test organisms were used as positive control. All the test tubes and controls were then incubated at 37 ^{o}C for 24 hours. After incubation period, the presence or absence of growth on each tube was observed. A loopful from each tube was further sub cultured onto nutrient agar to confirm whether the bacterial growth was inhibited.

Determination of Minimum Bactericidal Concentration

The minimum bactericidal concentration (MBC) was determined by collecting 1 mL of broth culture from the tubes used for the minimum inhibitory concentration (MIC) determination and sub culturing into fresh solid nutrient agar plates. The plates were incubated at 37 ^{0}C for 24 hours. The least concentration that did not show any growth after incubation was regarded as the MBC (Adesokan *et al*., 2007).

Acute toxicity studies of methanol extract of *Vitellaria paradoxa* and *Sclerocarya birrea* Stem barks and Leaves

Lethal Dose (LD_{50}) Determination

The method of Lorke (1983) was employed. Thus, the phase I involved the oral administration of three different doses of 10, 100 and 1,000 mg/kg of the crude extract, to three different groups of three adult wister albino rats. In a fourth group, three adult male wister albino rats were administered with equivalent/volume of distilled water to serve as control. All the animals were orally administered with the extracts using a curved needle to which acatheter had been fixed. The animals were monitored closely every 30 minutes for the first 3 hours after administration of the crude extracts, and then hourly for the next 6hours for any adverse effects. Then the animals were left for 72 hours for further observation.

When no death occurred, the phase II was employed, only one animal was required in each group. Groups 1–4, animals were orally given 1,500, 2,200, 3250 and 5,000 mg/kg dose levels of the crude extract. All the animals were left for observation as in stage one.

RESULTS

The powdered stem barks and leaves of *Vitellaria paradoxa* and *Sclerocarya birrea* were extracted with methanol and distilled water. Methanol yield more extract than distilled water. (Table 1).

Table 1: Mass and Percentage Yield of *Vitellaria paradoxa* and *Sclerocarya birrea* stem barks and leaves

Extract	Yield	Percentage Yield (%)	Texture	Extract Appearance
VPLA	20.4	10.2	Dried	Brown
VPSA	26.4	13.2	Dried	Light brown
SBLA	21.5	10.8	Dried	brown
SBSA	25.8	12.9	Dried	brown
VPLM	19.1	9.6	Gummy	Dark brown
VPSM	24.7	12.4	Gummy	Brown
SBLM	20.3	10.2	Gummy	Dark green
SBSM	26.4	13.2	Gummy	Brown

Key: VPSA (*Vitellaria paradoxa* stem bark-Aqueous), VPLA (*Vitellaria paradoxa* Leaves- Aqueous), SBS (*Sclerocarya birrea* stem barks- Aqueous), SBLA (*Sclerocarya birrea* Leaves- Aqueous).

Table 2 depicts the phytochemical screening of the aqueous and methanol extracts of *Vitellaria paradoxa* and *Sclerocarya birrea* stem barks and leaves revealed the presence of alkaloids, flavonoids, saponins, carbohydrate, cardiac glycosides, triterpenes, steroids, phenols and tannins. Anthraquinones were absent.

Table 2: Qualitative Phytochemical screening of the Methanolic extracts *Vitellaria paradoxa* and *Sclerocarya birrea* stem barks and leaves

Phytochemicals	***Vitellaria paradoxa***		***Sclerocarya birrea***	
	stem barks	Leaves	stem barks	leaves
Alkaloid	+	+	+	+
Flavonoid	+	+	+	+
Saponins	+	+	+	+
Cardiac glycoside	+	+	+	+
Tannins	+	+	+	+
Steroid	+	+	+	+
Triterpenes	+	+	+	+
Phenol	+	+	+	+
Anthraquinones	-	-	-	-
Carbohydrate	+	+	+	+

Antibacterial activities of aqueous extracts of *Vitellaria paradoxa* and *Sclerocarya birrea* stem barks and leaves showed inhibition on all the tested clinical isolates; *Staphylococcus aureus* and *Escherichia coli* at 500 mg/mL, 250 mg/mL, 125 mg/mL and 62.5 mg/mL (Table 3). The MIC and MBC of the *Sclerocarya birrea* leaf extract recorded respective values of 15.125 mg/mL and 31.25 mg/mL against *Staphylococcus aureus* and *Escherichia coli.* The MIC and MBC of the *Vitellaria paradoxa* stem bark, leaves and *Sclerocarya birrea* stem bark extracts recorded respective values of 31.25 mg/mL and 62.5 mg/mL against *Staphylococcus aureus* and *Escherichia coli.*

Table 3: Antimicrobial activity of aqueous extract of *Vitellaria paradoxa* and *Sclerocarya birrea* stem barks and leaves

Extract	Clinical isolates	Concentration(mg/mL)/Diameter zone of inhibition (mm)						MIC	MBC
		500	**250**	**125**	**62.5**	**Ampicillin**	**DMSO**		
VPL	*S. aureus*	20	20	18	16	36	06	31.25	62.5
	E. coli	22	20	18	16	38	06	31.25	62.5
VPS	*S. aureus*	16	14	13	10	36	06	31.25	62.5
	E. coli	18	16	13	11	40	06	31.25	62.5
SBL	*S. aureus*	15	13	11	09	36	06	15.125	31.25
	E. coli	17	14	12	10	38	06	15.125	31.25
SBS	*S. aureus*	14	12	10	08	36	06	31.25	62.5
	E. coli	13	12	11	09	38	06	31.25	62.5

Key: VPS (*Vitellaria paradoxa* stem bark), VPL (*Vitellaria paradoxa* Leaves), SBS (*Sclerocarya birrea* stem barks), SBL (*Sclerocarya birrea* Leaves).

Antibacterial activities of methanolic extracts of *Vitellaria paradoxa* and *Sclerocarya birrea* stem barks and leaves showed inhibition on all the tested clinical isolates *Staphylococcus aureus* and *Escherichia coli* at 500 mg/mL, 250 mg/mL, 125 mg/mL and 62.5 mg/mL (Table 4). The MIC and MBC of the *Sclerocarya birrea* stem bark extract recorded respective values of 31.25 mg/mL and 62.5 mg/mL against *Staphylococcus aureus* and *Escherichia coli.* The MIC and MBC of the *Vitellaria paradoxa* stem bark, leaves and *Sclerocarya birrea* leaves extracts recorded respective values of 15.125 mg/mL and 31.25 mg/mL against *Staphylococcus aureus* and *Escherichia coli.*

Table 4: Antimicrobial activity of Methanol extract of *Vitellaria paradoxa* and *Sclerocarya birrea* stem barks and leaves

Extract	Clinical isolates	Concentration(mg/mL)/Diameter zone of inhibition (mm)						MIC	MBC
		500	**250**	**125**	**62.5**	**Ampicillin**	**DMSO**		
VPL	*S. aureus*	26	24	20	18	36	06	15.125	31.25
	E. coli	25	23	18	16	38	06	15.125	31.25
VPS	*S. aureus*	21	18	14	12	36	06	15.125	31.25
	E. coli	20	17	13	10	40	06	15.125	31.25
SBL	*S. aureus*	18	15	14	11	36	06	15.125	31.25
	E. coli	19	16	14	09	38	06	15.125	31.25
SBS	*S. aureus*	14	12	10	08	36	06	31.25	62.5
	E. coli	14	13	11	10	38	06	31.25	62.5

Key: VPS (*Vitellaria paradoxa* stem bark), VPL (*Vitellaria paradoxa* Leaves), SBS (*Sclerocarya birrea* stem barks), SBL (*Sclerocarya birrea* Leaves).

From the results of the acute toxicity studies (Table 5), no death was recorded in the first phase. In the second phase, doses of 1500, 2250, 3250 and 5000 mg/kg were used and no death was also recorded. The oral median lethal dose (LD_{50}) for the Methanolic extracts of *Vitellaria paradoxa* and *Sclerocarya birrea* stem barks and leaves was therefore estimated to be greater than 5000 mg/kg and no sign of behaviourial changes were also observed.

Table 5: Acute toxicity studies of Methanolic extracts of *Vitellaria paradoxa* and *Sclerocarya birrea* stem barks and leaves

Treatment	Group	Number of Animals	Dose (mg/kg)	Mortality recorded after 24 hours
Phase I	I	3	10	0/3
	II	3	100	0/3
	III	3	1000	0/3
Phase II	I	1	1500	0/1
	II	1	2250	0/1
	III	1	3250	0/1
	IV	1	5000	0/1

DISCUSSION

The polarity of the solvent (methanol and distilled water) used in the extraction of the plant samples allowed it to draw similar constituents, could be the reason why both solvent are polar. The choice of the method of extraction (cold maceration) was due to the fact that it is similar with the extraction method by the traditional herbalist (Isaiah, 2016).

The results of phytochemical screening carried out on the aqueous and methanol extracts revealed the presence of alkaloids, flavonoids, saponins, carbohydrate, cardiac glycosides, triterpenes, steroids, phenols and tannins. Anthraquinones were absent. These phytochemical constituents are known to possess many biological activities, a saponin possess anti-hepatobiliary disorders and anti-inflammatory property (Isaiah, 2016). Tannins which is a major constituent of canberry juice has for long been used to treat bacterial infection of the bladder (Evans, 2002). While some flavonoids have anti-tumor, antibacterial or antifungal property, they are used in domestic veterinary medicine, particularly in the form of ointment for treating dermal diseases (Trease and Evans, 2002). Many researchers have reported the presence of secondary metabolites in *Vitellaria paradoxa* and *Sclerocarya birrea* stem barks and leaves. These secondary metabolites are generally found as components of plants (Isaiah, 2016). The secondary metabolites reported by several researchers possessed appreciable inhibitory activities against various organisms (Isaiah, 2016). In this study, the phytochemical

screening of both extracts revealed the presence of phenolic compounds and flavonoids. These secondary metabolites might be responsible for the antibacterial activity of the extracts as they exert their antimicrobial activities through different mechanisms. Phenols are generally protoplasmic poisons toxic to all types of cells. Precipitation of proteins occurs with high concentration of phenol, while at low concentrations it denatures proteins without coagulating them (Isaiah, 2016). It freely to penetrates the tissue because of its denaturing activity (Adeshina *et al*., 2012). Flavonoids have been reported to be synthesized by plants in response to microbial infection, hence they exhibit antibacterial activities (Isaiah, 2016). The presence of flavonoids suggest that it can be used as anti–spasmodic and antioxidant, and confirms the reason for the use of the plant in the treatment of spasmodic bronchitis and other microbial infections (Isaiah, 2016).

The diameter zones of inhibition, showed that the methanol extract had more activity than the aqueous extract. The degree of activity varied with the isolates and the extracts. This variation of activity could be due to the differences in the solubility of the secondary metabolite in the different solvents used and also the structural or morphological variability of the tested isolates thus, larger zones of inhibition were produced by the susceptible organisms than the resistant ones (Isaiah, 2016). It could also be due to the polarity of the solvents, water been more polar dissolve more of the secondary metabolites. The observed differences may be as a result of variation of plants location and method of extraction (Isaiah, 2016). This result is in line with the work of Adeshina *et al*., (2012) which showed that the methanol extract of *A. cordifolia* leaf was relatively more active than the aqueous extract against type isolates of *E. coli*, *S. aureus*, *P. aeruginosa* and *Candida albicans*. The result is also not in line with the work of Osumah *et al*. (2012) that showed that the aqueous extract had more activity than the methanol extract against *E. coli*, *P. aeruginosa*, *S. aureus* and *S. typhi* isolates from fecal material and wounds. The diameter zone of inhibition showed a concentration dependent result and the result also showed that the zone of inhibition values of the extracts were far lower than that of the positive control amphicillin. This may be attributed to the fact that conventional antibiotics are usually prepared from synthetic materials by means of reproducible manufacturing techniques and procedures, while herbal medicinal plants products are still crude, prepared from plant and animal origins and are subjected to contamination and deterioration most of the time (EL – Mahmood and Ameh, 2007, Isaiah, 2016).

The M.I.C and M.B.C values were generally lower for the methanolic extract against the test isolates compared to those of the aqueous extract. The MIC and MBC of the *Vitellaria paradoxa* stem bark, leaves and *Sclerocarya birrea* leaves extracts recorded. This is of great importance as it has been reported that this organism has developed resistance to many antibiotics, which sometimes makes its clinical management difficult (Adewunmi *et al*., 2001, Isaiah, 2016). The differences in the susceptibilities of the isolates to the plant extracts can be related to the cell wall composition of the organisms. Gram-positive bacteria have cell wall composed of peptidoglycan with techoic acid in between, therefore they are more susceptible than Gram-negative bacteria that have their cell wall surrounded by bi-lipid layers of Gram-negative lipopolysaccharides and lipoproteins, which prevent ready penetration of antibiotics through their cell wall (Isaiah, 2016).

The observed low MIC values from methanol extract could be due to the fact that methanol extract, it contains the secondary metabolites responsible for the antibacterial activity of the methanol extract.

No death was recorded in the first phase of the study in rats. In the second phase, doses of 1500, 2250, 3250 and 5000 mg/kg were used and no death was also recorded. The oral median

lethal dose (LD_{50}) for the methanol extracts of *Vitellaria paradoxa* and *Sclerocarya birrea* stem barks and leaves was therefore estimated to be greater than 5000 mg/kg and no sign of behaviourial changes were observed. This suggests that the plant extract was non-toxic as no death was recorded. Acute toxicity studies are usually carried out to determine the dose that will cause death or serious toxic manifestations when administered singly or severally at few doses in order to establish doses that should be used in subsequent studies (Wanda *et al.,* 2002). The Organization for Economic Cooperation and Development (OECD), recommended chemical labelling and classification of acute systemic toxicity based on oral median lethal dose values as: very toxic if ≤ 5 mg/kg, toxic if > 5 mg/kg but ≤ 50 mg/kg, harmful if > 50 mg/kg but ≤500 mg/kg, and non-toxic or not harmful if > 500 mg/kg or ≤ 2000 mg/kg (Walum, 1998). Based on this classification, the oral median lethal dose obtained for rats found to be above 5000 mg/kg, was relatively safe orally. The LD_{50} was found to be greater than 5000 mg/kg body weight orally and this suggests that the extract had low acute toxicity when administered orally. This may be attributed to the incomplete absorption brought about by inherent factors limiting absorption in the gastro intestinal tract. The present study agrees with the work done by Kofi *et al*. (2014); Prasanth *et al*. (2015); Adesegun *et al.* (2016) and Ugbogu *et al*. (2016). Bruce (2006) reported that any substance with LD_{50} estimated to be greater than 2000-5000 mg/kg body weight given orally could be considered to be of low toxicity and safe. The very high LD_{50} observed is not a conclusive finding about the safety of the extracts of selected plant species. Higher doses could be tested for better understanding of its effects if used for a long period of time and for proper recommendation on its future utilization (Ogbonnia *et al.,* 2011).

CONCLUSION

Phytochemical screening of the aqueous and methanol extracts of *Vitellaria paradoxa* and *Sclerocarya birrea* stem barks and leaves revealed the presence of alkaloids, flavonoids, saponins, carbohydrate, cardiac glycosides, triterpenes, steroids, phenols and tannins. Anthraquinones were absent. The aqueous and methanol extracts of *Vitellaria paradoxa* and *Sclerocarya birrea* stem barks and leaves were found to possess antibacterial activity against *S. aureus*, *E. coli.* . The acute toxicity is one of the major concerns of indigenous therapeutic preparations and it can be confirmed that the LD_{50} of the methanol extract was above 5000 mg/kg and did not cause mortality in all the tested rats. This study has justified the use of both plant species in the treatment of some bacterial diseases in folkloric herbal medicine.

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