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Investigations into Antibacterial, Phytochemical and Antioxidant Properties of *Vitellaria paradoxa* (Gaertn.) Stem Bark Extracts

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Authors' contributions

This work was carried out in collaboration between all authors. Author OOO was involved in microbiological aspect of the experiments which include mode of action of the plant extract. Author DAA designed the project and proof read the manuscript. Author POA carried out the chemistry aspect of the experiment. Author OFA collected the plant sample and prepared it for extraction of the biological components. Author JOA carried out the preparation of media and other chemicals used for the experiments. Author FOO assisted in the microbiological analysis of the plant extract. All authors read and approved the final manuscript.

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ABSTRACT

This study investigated the antibacterial potentials and mechanisms of action of crude extract and fractions from stem bark of *Vitellaria paradoxa* on susceptible bacterial isolates. It also assessed the phytochemical constituents and antioxidant properties of the plant. This was with a view to tackling problem of multidrug resistance development by microorganisms.
The stem bark of *V. paradoxa* was harvested from Ijagbo, Kwara State, Nigeria, in the month of April, 2016. The plant sample was oven dried at 40°C using hot-air oven and ground into fine

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powder. The powdered sample was cold extracted using methanol and sterile distilled water in ratio 3:2 (v/v). The mixture obtained was concentrated *in vacuo* using a rotary evaporator and then lyophilized. The crude extract collected was screened for antimicrobial, phytochemical and antioxidant properties. The crude extract was later partitioned into fractions using different organic solvents in the order of their polarity. The antimicrobial potentials of the various solvent fractions were determined using agar-well diffusion method. The active fractions were further partially purified by combination of thin layer and column chromatography. The rate of killing, protein, nucleotide and potassium ions leakages of the active fractions were determined using *Staphylococcus aureus* and *Escherichia coli* as representatives of Gram positive and Gram negative bacteria respectively. The most active partially purified fraction was analysed using GC-MS.

Phytochemical screening of the extract revealed the presence of alkaloids, flavonoids, saponins, tannins, reducing sugar and cardiac glycosides. The minimum inhibitory concentrations of the crude extract ranged between 0.545 mg/mL and 2.187 mg/mL while those of aqueous, butanol and ethylacetate fractions ranged between 0.31 mg/mL and 5.00 mg/mL, 0.31 mg/mL and 2.50 mg/mL and 0.31 mg/mL and 2.50 mg/mL respectively. The time kill assay showed that the percentage of the cells killed increased with increasing concentrations of the fractions, as well as, contact time intervals. Leakages of protein, potassium ions and nucleotides followed the same trend observed for killing rate. *Vitellaria paradoxa* extract exhibited 50% inhibition of DPPH free radicals at 0.008777 mg/mL, whereas ascorbic acid used as standard had IC_{50} of 0.078777 mg/mL. The major active constituent of the purified sample was identified as 14-methylhexadecanoic acid.

The study concluded that *V. paradoxa* stem bark extract possessed antioxidant properties and exhibited appreciable antimicrobial activities against the test pathogens.

Keywords: Vitellaria paradoxa; antioxidant; antimicrobial; antibacterial; phytochemicals; killing rate.

1. INTRODUCTION

Medicinal plants are of great importance in health care delivery due to the presence of chemical substances found in them which produce a definite physiological action on human body. Many drugs of herbal origin used for the treatment of microbial infections have shown interesting results. Majority of these drugs have shown increasing promises *in vivo* against multiple drug resistant bacteria and fungi. Thus, many of these medicinal plants present good sources of therapeutic agents which are used traditionally for different purposes, chiefly for the treatment of ailments caused by pathogens. About 80% of the population in developing countries rely on infusion or poultices of plants in folklore remedies for their health [1]. Therefore, plant kingdom is quite unavoidably useful in the life of man both in medicine, food and shelter.

Infectious diseases caused by microorganisms have reached an outrageous stage and thus act as threat to public health. This has warranted efforts by ethno scientists to search for new biologically active compounds especially from medicinal plants for the development of more potent antimicrobials which could be used to overcome the multiple resistances developed by these organisms towards the synthetic antimicrobial agents. There are many microbial infections that are being experienced by humans which are highly difficult to manage due to multidrug resistant nature of these pathogens. Hence, there is an urgent need to detect newer source for more potent antimicrobial agents, from nature to combat the activities of these pathogens. This work is one of such attempt.

Vitellaria paradoxa belongs to Sapotaceae family [2] and is popularly known as shea butter tree. It grows naturally in the wild of the dry savannah belt of West Africa. This tree stretches in abundance onto the foothills of the Ethiopian mountains [3].*Vitellaria paradoxa* is considered a sacred tree by many communities and ethnic groups in Africa and plays important roles in religious and cultural ceremonies. Shea butter obtained from the kernel of this plant is used in the treatment of several ailments which include inflammation, rashes, dermatitis, ulcers as well as rheumatism [4]. Decoctions prepared from *V. paradoxa* leaves are used to treat stomach ache, headache, oral infection and can as well be used as an eye lotion [5]. Different parts of this plant, eg., leaves, roots, seeds and stem bark are useful in treating enteric infections such as diarrhoea, helminthes, skin diseases and wound infections [6].

Vitellaria paradoxa is used traditionally in Cameroon for the treatment of dysentery,

cutaneous infection, diarrhoea, microbial infections and fever [7,8]. Studies have shown that triterpene alcohols extracted from shea butter possess anti-inflammatory activity [9]. Stem bark extracts of *V. Paradoxa* had been found to exhibit significant antifungal activity [10]. The kernels of this plant contain fat which is used extensively in cosmetics and chocolate industries [5,11].

2. MATERIALS AND METHODS

2.1 Panel of Microorganisms Used for the Study

Panel of standard strains of bacteria from National Collection of Industrial Bacteria (NCIB) and locally isolated organisms (LIO) used were obtained from culture collections of Prof. Akinpelu, at Department of Microbiology, Obafemi Awolowo University, Ile Ife, Osun State, Nigeria. Various clinical strains of bacteria used for this study were collected from stock culture collection of Microbiology Laboratory of Obafemi Awolowo University Teaching Hospitals Complex, Ile Ife, Osun State, Nigeria.

The inoculum of the bacterial strains was prepared using colony suspension method as described by European Committee for Antimicrobial Susceptibility Testing (EUCAST) [12].

2.2 Culture Media Employed

Nutrient agar and nutrient broth (LAB M Lancashire BL97JJ, UK) were used for subculturing the organisms while Mueller-Hinton agar (LAB M Lancashire BL97JJ, UK) was used for sensitivity testing. The media were initially sterilized by autoclaving at 121°C for 15 minutes.

2.3 Preparation of Plant Sample

Fresh stem bark of *V. paradoxa* used for the investigation was collected from Ijagbo, Kwara State, Nigeria, in the month of April, 2016. The plant was authenticated in Herbarium of Department of Botany, Obafemi Awolowo University, Ile Ife, Osun State, Nigeria. Voucher specimen of the plant sample was prepared and deposited for reference purposes with Voucher number IFE- 17576. The dried plant sample was milled into fine powder and stored in an air-tight container for further use.

2.4 Extraction of Bioactive Components of the Plant Sample

Exactly 1.85 kg of powdered sample was soaked in solution of methanol and sterile distilled water in ratio 3:2 (v/v) for 4 days with regular agitation. The mixture was filtered into a clean, sterile flask. The supernatant collected was later concentrated *in vacuo* in rotary evaporator to eliminate the organic solvent leaving aqueous solvent. The aqueous part was lyophilized to collect crude extract of the plant sample which was dark in colour.

2.5 Determination of Phytochemical Compounds in the Plant Extract

The phytochemical components in the plant extract were determined using methods described by Trease and Evans [13] and Harborne [14]. The test included determination of the presence of saponins, tannins, flavonoids, alkaloids, cardiac glycosides and reducing sugars in the extract.

2.6 Fractionation of the Crude Extract from *V. paradoxa* Stem Bark

The fractionation of the crude extract was done using different organic solvents in order of their polarity starting with n-hexane and graduated to n-butanol with the highest polarity. The fractions collected were kept in an air-tight container for further use.

2.7 Sensitivity Testing of *V. paradoxa* Crude Extract and the Fractions on Bacterial Strains

The antimicrobial activity of the crude extract along with those of the fractions was determined using agar-well diffusion method [15]. The bacterial strains were first re-activated in nutrient broth for 18 h before use. Exactly 0.1 mL of standardized bacterial strains (10^8 cfu/mL of 0.5 McFarland standard) was transferred into molten Mueller-Hinton agar medium at 40°C, thoroughly mixed and then poured into a sterile Petri dish. The plates were allowed to set and sterile 6 mm cork borer was used to bore wells into the medium. The wells were later filled up with the solution of the crude extract and the fractions. The concentration of the crude extract used was 35 mg/mL while that of the fraction was 10 mg/mL. Streptomycin and ampicillin were used as positive controls at a concentration of 1

mg/mL. Care was taken not to allow solution to spill on the surface of the medium. The plates were left on the laboratory bench for an hour to allow for proper inflow of the solution into the medium and incubated at 37°C for 24 h. The plates were not stock-pile to allow even distribution of temperature round the plates. The plates were later observed for zones of inhibition which is an indication of susceptibility of the organisms to the extract.

2.8 The Minimum Inhibitory Concentrations (MIC) of the Fractions against Susceptible Bacterial Strains

The MIC of the fractions was determined by preparing two-fold dilution of the fraction [15] and 2 mL of different concentrations of the solution was added to 18 mL of pre-sterilized molten nutrient agar. This gave final concentrations range of 0.31 mg/mL to 5.00 mg/mL. The medium was then poured into sterile Petri dishes and allowed to set and left on the laboratory bench overnight to ascertain that there were no contaminants in the prepared plates. Dry surfaces of the plates were later streaked with a 18 h old standardized inoculum of the susceptible bacterial strains. The plates were later incubated aerobically at 37°C for 48 h after which they were examined for the presence or absence of bacterial growth. The MIC was taken as the lowest concentration of the fraction that inhibited the growth of the bacterial strains.

2.9 The Minimum Bactericidal Concentrations (MBC) of the Fractions against Susceptible Bacterial Strains

The MBC of the fractions were determined against the susceptible bacterial strains [16] by taking sample from line of streaked onto extract-free freshly prepared nutrient agar medium plates and incubated at 37°C for 72 h. The MBC was taken as the concentration of the fraction that did not show any bacterial growth on the plates.

2.10 Determination of Rate of Kill of Susceptible Bacterial Strains by the Fractions

The rate of kill of the susceptible bacterial strains was determined using the active fractions on the viability of *E. coli* representing Gram negative and *Staph. aureus* representing Gram positive organisms [17]. Cultures of these bacterial strains were first standardized to approximately 10^6 cfu/mL before use. Exactly 0.5 mL of the standardized suspension of the culture was added to 4.5 mL of different concentrations of the fraction relative to MIC. The experiment was held at room temperature over a period of 2 h to determine the killing rate of the organisms. A volume of 0.5 mL of each of the suspension was withdrawn at time interval and transferred to 4.5 mL of recovery medium containing 3% “Tween 80” to shake off the effect of the extract carry-over from the bacterial cells. The suspension was then serially diluted and plated out for viable counts. These plates were then incubated at 37°C for 48 h before determining the survival cells. Control plates containing the test cells without the inclusion of antimicrobial agents were set up along with the experimental. Viable counts were made in triplicates for each sample and compared with the counts of the control. Depression in viable counts indicated killing by the fractions.

2.11 Determination of Protein Leakage from the Susceptible Bacterial Strains by the Fractions

Eighteen hour old cells of *E. coli* and *Staph aureus* were separately washed in 0.9% (w/v) normal saline (NaCl). Washed suspension of *E. coli* and *Staph aureus* cells (inoculum size approximately 10^6 cells 0.5 McFarland standards) were treated with various concentrations of the fraction relative to the MIC at various time intervals for 2 h. Each suspension was then centrifuged at 7000 rpm and supernatant collected was assayed for protein [18]. In assaying for the protein, 0.4 mL Bradford reagent was added to 1.6 mL sample (0.2 mL supernatant added to 1.4 mL sterile distilled water) to make up 2 mL total volume. Optical density (OD) of the resulting solution was thereafter taking at 595 nm after 5 minutes but not later than 1 h. The optical density of each of the samples was calculated from the equation of the best-fit linear regression line obtained from the graph of the Bovine Serum Albumin (BSA) standard curve.

2.12 Preparation of Bovine Serum Albumin Standard Curve

Bovine serum albumin stock solution of concentration 100 µg/mL was first prepared. Varying concentrations of the bovine serum albumin was thus prepared from the stock solution. Bradford reagent (0.4 mL) was added

to the various bovine serum albumin concentrations. This was allowed to stand for 5 minutes after which the optical density was measured at 600 nm. The various optical density values obtained were thereafter plotted against bovine serum albumin concentrations to form standard albumin curve. The concentrations of proteins in the samples were then calculated from the equation of the best-fit linear regression line obtained from the graph of the bovine serum albumin standard curve.

2.13 Determination of Potassium Ions Leakage from the Susceptible Bacterial Strains by the Fractions

Potassium ion leakage from the cells of the susceptible bacterial strains representatives was determined using the active fractions [19]. Exactly 50 mL of harvested and washed cells (OD_{470nm} = 1.5) were placed in a clean 100 mL beaker which was magnetically stirred. A 5.0 mL of ionic strength adjustment buffer (ISAB; 18.37 g of tetraethylammonium chloride in deionized water and made up to 100 mL) was added to the beaker. This ensured that the background ionic strength of all solution was kept constant. The potassium ion sensing electrode (Qualiprobe QSE 314, EDT Instruments Waldershare Park, Dover, UK) and its reference electrode (Qualiprobe double junction reference electrode E8092, EDT Instruments) were placed into the cell suspension. The potential difference (mV) derived by the electrodes was measured using a Whattman PHA 220 pH/mV meter (Whattmann Maidstone, UK). Bacterial cells were treated with various concentrations of the fractions relative to the MIC. The potassium ion efflux from the cells in the suspension was measured at time interval over a period of 2 h as a potential difference in mV. These values were converted to concentrations of potassium ions by reference to a conversion graph, which had been constructed earlier using KCL standard solutions. The concentration of potassium ions released was plotted against time.

2.14 Determination of Nucleotides Leakage from the Susceptible Bacterial Cells by the Fractions

Cells of *E. coli* and *Staph aureus* from 18 h old nutrient broth culture were washed in 0.9% (w/v) normal saline and standardized (inoculum size 10^6 cells cfu/mL) and treated with different concentrations of the fractions relative to the MIC at various contact time interval over a period of 2 h [20]. Each suspension was centrifuged at 10000 rpm and decanted. Wavelength of the supernatant collected was determined at 260_{nm} to quantify the amount of nucleotide leaked out of the cells by comparing with the standard curve. A range of concentrations from 0.5 to 5 µM of dNTP mix (dA, dC, dG, dT) (INQABA Biotech., Pretoria, South Africa) was prepared in TE buffer and used to generate the standard curve. The blank used constitute sterile distilled water inoculated with the standardized inoculum.

2.15 Determination of Antioxidant Property of *V. paradoxa* Stem Bark Extract

The antioxidant property of the extract was assessed by DPPH free radical assay [21]. The stem bark extract of V*. paradoxa* was reacted with DPPH radical in ethanol solution. A 0.5 mL solution of the extract was mixed with 3 mL of absolute ethanol and 0.3 mL of DPPH radical solution (0.5 mM) in ethanol. When DPPH reacts with an antioxidant compound which can donate hydrogen, it is reduced. The colour change when DPPH reacted with the extract was read at 517_{nm} after 100 minutes of reaction using UV spectrophotometer. The mixture of ethanol (3.3 mL) and sample (0.5 mL) served as blank. The control solution was prepared by mixing ethanol (3.5 mL) and DPPH radical solution (0.3 mL) [22] which was used to determine the scavenging activity percentage (AA%).

2.16 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of *V. paradoxa* Stem Bark Extract

The GC-MS analysis of the most active partially purified butanol fraction of *V. paradoxa* was performed using Agilent Technologies GC system. The system comprises of an AOC-20i auto-sampler and a Gas Chromatograph interfaced to a triple axis Mass Spectrometer detector equipped with an Elite-5MS (5% diphenyl/95% dimethyl poly siloxane) fused to a capillary column (30 × 0.25 µm ID × 0.25 µm df). For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 mL/min, and an injection volume of 2 µL was employed (a split ratio of 10:1). The injector temperature was maintained at 250°C, the ion-source temperature was

200°C, the oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 second. The solvent delay was 0 to 6 minutes, and the total GC/MS running time was 34.667 seconds. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass-detector used in this analysis was Agilent Technologies-5975C while the gas chromatography model was Agilent Technologies-7890A. The injector model used was Agilent Technologies-7683B, and the software adopted to handle mass spectra and chromatograms was a NIST version 14.0L.

2.17 Statistics and Data Processing

All the experiments were done in triplicates. Data was analysed by a 4 x 4 Latin square design with statistical program using the GLM model (Statistical Analysis Systems, SAS Institute, Cary, NC, USA, 2001). Results were contrasted with negative and a positive control. The mean of the values was compared using independent t-test of significance ($p < 0.05$).

3. RESULTS

The crude extract collected was dark brown in colour and the yield was 117.5 g which was 10.6% of the powdered sample. The aqueous, n-butanol and ethyl acetate fractions were obtained from the crude extract with the exception of n-hexane and chloroform which did not show affinity for the bioactive components of the crude extract in the partitioning process. This is an indication that n-hexane and chloroform are not the best organic solvent to be used for the extraction of bioactive components of *V. paradoxa* stem bark. The crude extract along with the aqueous, ethyl acetate and n-butanol fractions exhibited range of zones of inhibition against the bacterial strains tested (Tables 1 and 2). Table 1 shows zones of inhibition exhibited by the crude extract, streptomycin and ampicillin against the organisms. The zones of inhibition exhibited against the test organisms by the crude extract ranged between 9.67 mm and 13.67 mm, while streptomycin exhibited zones of inhibition ranging between 16.67 mm and 28.33 mm. On the other hand, zones of inhibition exhibited by ampicillin against the test organisms ranged between 12.33 mm and 22.00 mm. The aqueous and ethyl acetate fractions inhibited the growth of all bacterial strains tested (Table 2). The zones of inhibition exhibited by aqueous fraction against the bacterial strains ranged between 7.00 mm and 17.33 mm while zones of inhibition observed for n-butanol ranged between 8.00 mm and 20.33 mm (Table 2). On the other hand, zones of inhibition observed for ethyl acetate fraction were between 8.67 mm and 16.00 mm. Overall, the three fractions compared favourably with the two standard antibiotics – streptomycin and ampicillin used as positive controls. The three fractions along with streptomycin inhibited the growth of all the 32 bacterial strains tested while ampicillin inhibited the growth of 27 organisms (Tables 1 and 2). Table 3 shows varying degrees of activities exhibited by partially purified samples of n-butanol fraction at a concentration of 1 mg/mL against the bacterial strains. The zones of inhibition exhibited by the partially purified samples ranged between 7.00 mm and 12.00 mm. Sample BUT_{64} exhibited the highest activities by inhibiting the growth of 23 out of 32 bacterial strains tested, followed by BUT_{73} and BUT_{74} that inhibited the growth of 4 and 3 test organisms respectively.

Varying degrees of MIC and MBC were exhibited by aqueous, ethyl acetate and n-butanol fractions against susceptible bacterial strains used for this study (Tables 4 and 5). The MIC observed for aqueous, n-butanol and ethyl acetate fractions against the test organisms ranged between 0.31 mg/mL and 5.00 mg/mL; 0.31 mg/mL and 2.50 mg/mL; 0.31 mg/mL and 2.50 mg/mL respectively.

On the other hand, the MBC exhibited by aqueous and n-butanol fractions against the organisms ranged between 0.63 mg/mL and 5.00 mg/mL while those observed for ethyl acetate ranged between 0.63 mg/mL and 5.00 mg/mL as well. The phytochemical analysis of the extract revealed the presence of tannins, saponins, flavonoids, alkaloids, cardiac glycosides and reducing sugars (Table 6).

The mode of action of the three fractions was determined on the susceptible bacterial strains using representatives of Gram positive and Gram negative organisms. These were done by assaying for the killing rate, leakages of proteins, potassium ions and nucleotides from the test cells. The extent and killing rate of the test organisms by aqueous fraction at various concentrations are shown in Fig. 1. The percentage of *E. coli* cells killed by the fraction at 1 x MIC in 15 minutes of contact time with the

organism was 5.0% and this rose to 19.5% after 30 minutes of contact time. At 60 minutes, the population of the cells killed was 49.0% while the percentage killed after 90 minutes of contact time rose to 60.5%. The population of the test cells killed after 120 minutes was 81.0% at the same concentration (Figs. 2 and 3).

This monophasic effects were observed when the concentration of aqueous fraction was increased to 2 x MIC. Finally, 94.9% of the test cells were killed at 3 x MIC after 120 minutes of contact time. The same trend of killing rates was observed for n-butanol and ethyl acetate fractions when the cells were tested. The results show the same patterns as observed in the previous tests against *Staph. aureus* (Figs. 4 and 5).

The bactericidal effects of these fractions were also determined on the test cells through protein leakage from these cells. Fig. 6 shows the effect of aqueous fraction on protein leakage from *E. coli* cells at concentrations of 1 x MIC, 2 x MIC and 3 x MIC. The amount of protein leaked from the test cells at 1 x MIC concentration in 15 minutes was 1.51 µg/mL and this increased to 3.24 µg/mL after 30 minutes of contact time of cells with the solution of the fraction. Leakage of protein from the test cells continued to increase

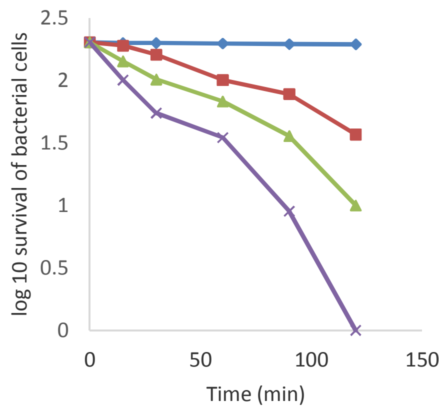

Fig. 1. The extent and rate of killing of *E. coli* cells by aqueous fraction at 1 x MIC (), 2 x MIC (), 3 x MIC () and control ()

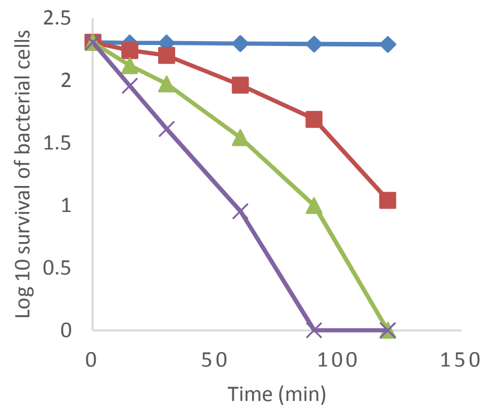

Fig 2. The extent and rate of killing of *E. coli* cells by butanol fraction at 1 x MIC (), 2 x MIC (), 3 x MIC () and control ()

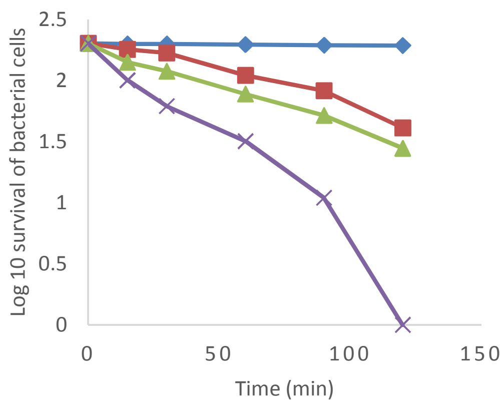

Fig. 3. The extent and rate of killing of *E. coli* cells by ethylacetate fraction at 1 x MIC (), 2 x MIC (), 3 x MIC () and control ()

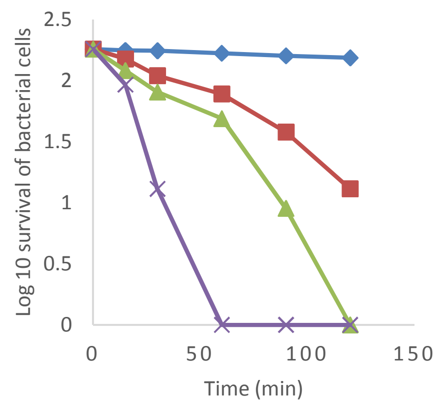

Fig. 4. The extent and rate of killing of *Staph. aureus* cells by butanol fraction at 1 x MIC (), 2 x MIC (), 3 x MIC () and control ()

with time. At 120 minutes of contact time and at the same concentration the amount of protein leaked from the cells was 15.21 µg/mL The same trend of activities was observed when the concentrations of the fraction were increased to 2 x MIC and 3 x MIC. The results of the experiments with n-butanol and ethyl acetate fractions against both *E. coli* and *Staph aureus* followed the same patterns as observed in tests with aqueous fraction (Figs. 7 and 8). The results of other mode of actions of the fractions through leakages of potassium ions and nucleotides from the test cells are shown in Figs. 9 and 10. The same trend of reactions observed in killing rates and protein leakage from the cells occurred in this test.

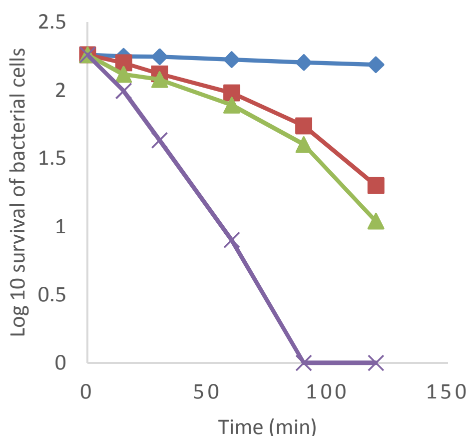

Fig. 5: The extent and rate of killing of *Staph. aureus* cells by ethylacetate fraction at 1 x MIC (—■—), 2 x MIC (—▲—), 3 x MIC (—×—) and control (—◆—)

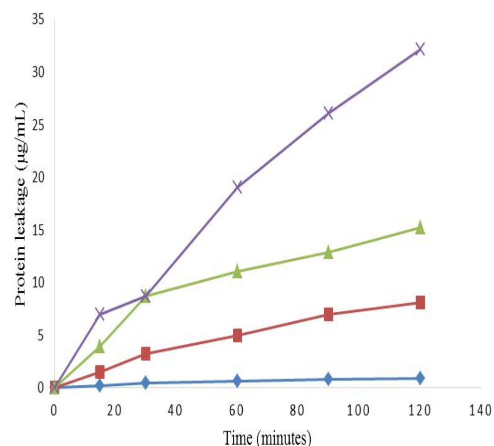

Fig. 6. The effect of aqueous fraction on protein leakage from *E. coli* cells at 1 x MIC (—■—), 2 x MIC (—▲—), 3 x MIC (—×—) and control (—◆—)

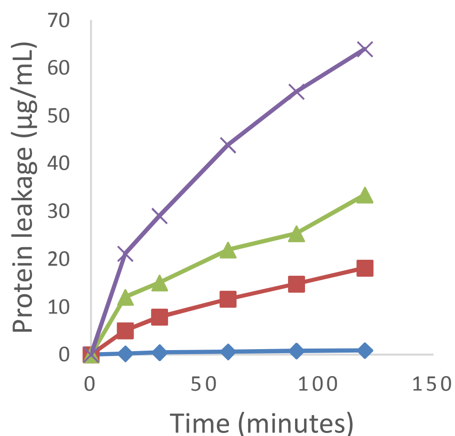

Fig. 7. The effect of butanol fraction on protein leakage from *E. coli* cells at 1 x MIC (—■—), 2 x MIC (—▲—), 3 x MIC (—×—) and control (—◆—).

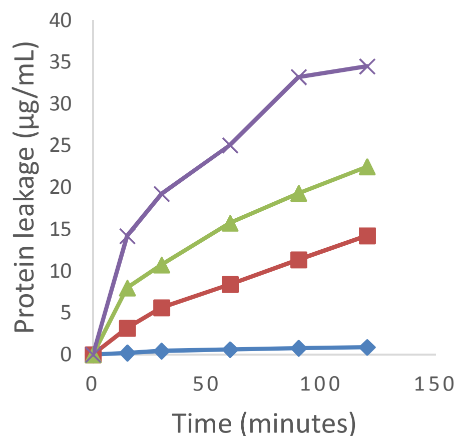

Fig. 8. The effect of ethylacetate fraction on protein leakage from *E. coli* cells at 1 x MIC (—■—), 2 x MIC (—▲—), 3 x MIC (—×—) and control (—◆—)

Table 1. The sensitivity patterns exhibited by *V. paradoxa* crude extract against bacterial strains

Bacterial strains	Zone of Inhibition (mm*)			
	Crude extract (35 mg/mL)	Streptomycin (1 mg/mL)	Ampicillin (1 mg/mL)	M/W(1:1) (V/V)
Bacillus anthracis (LIO)	11.33 ± 1.53	24.33 ± 2.08	0.00	0
Bacillus cereus (NCIB 6349)	13.67 ± 1.53	22.00 ± 2.65	0.00	0
Bacillus polymyxa (LIO)	9.67 ± 1.53	19.33 ± 1.53	16.33 ± 1.53	0
Bacillus stearothermophilus (NCIB 8222)	10.33 ± 1.53	28.00 ± 1.73	15.33 ± 4.51	0
Bacillus subtilis (NCIB 3610)	13.33 ± 1.53	27.67 ± 2.08	16.00 ± 3.61	0
Citrobacter braakii (CIS)	10.33 ± 1.15	17.33 ± 1.53	19.67 ± 2.52	0
Citrobacter freundii (CIS)	11.33 ± 1.53	22.33 ± 2.52	21.00 ± 2.65	0
Citrobacter youngae (CIS)	9.67 ± 0.58	21.00 ± 2.00	15.00	0
Clostridium sporogenes (NCIB 532)	12.00 ± 2.00	24.67 ± 3.51	16.33 ± 1.53	0
Corynebacterium pyogenes (LIO)	10.67 ± 1.53	21.67 ± 2.08	21.00 ± 2.65	0
Enterococcus faecalis (NCIB 775)	11.00 ± 1.73	25.33 ± 1.53	0.00	0
Escherichia coli (NCIB 86)	12.00 ± 1.73	22.33 ± 2.52	16.00 ± 1.73	0
Klebsiella pneumoniae (CISP)	10.00 ± 1.00	20.00	17.33 ± 2.52	0
Klebsiella pneumoniae (CISP)	10.33 ± 1.53	20.33 ± 2.31	19.67 ± 2.52	0
Klebsiella pneumoniae (NCIB 418)	12.67 ± 1.15	20.67 ± 2.52	0.00	0
Micrococcus luteus (NCIB 196)	11.33 ± 1.53	23.33 ± 2.52	0.00	0
Proteus vulgaris (LIO)	11.67 ± 2.52	25.33 ± 2.52	15.33 ± 3.51	0
Pseudomonas aeruginosa (CIW)	11.33 ± 1.53	20.67 ± 2.08	17.00 ± 2.00	0
Pseudomonas aeruginosa (CIW)	13.67 ± 1.15	25.67 ± 1.53	14.00 ± 2.65	0
Pseudomonas aeruginosa (CIU)	12.67 ± 2.52	28.33 ±1.53	16.33 ± 2.89	0
Pseudomonas aeruginosa (CIU)	10.33 ± 1.53	25.33 ± 1.53	14.00	0
Pseudomonas aeruginosa(CIU)	10.00 ± 2.00	22.33 ± 2.52	16.00 ± 2.65	0
Pseudomonas aeruginosa (CIU)	12.67 ± 0.58	19.00± 2.65	15.67 ± 3.21	0
Pseudomonas aeruginosa (NCIB 950)	14.00 ± 2.00	15.00	17.67 ± 2.52	0
Pseudomonas flourescens (NCIB 3756)	11.33 ± 1.15	27.00 ± 2.00	22.00 ± 2.65	0
Shigella sp. (LIO)	13.00	21.00 ± 2.65	19.00 ± 2.65	0
Staphylococcus aureus (CIW)	10.33 ± 2.08	18.67 ± 2.52	20.67 ± 1.15	0
Staphylococcus aureus (CIW)	11.67 ± 1.15	23.67 ± 2.08	12.33 ± 0.58	0
Staphylococcus aureus (CIW)	10.67 ± 1.15	24.67 ± 2.52	14.67 ± 2.08	0
Staphylococcus aureus (NCIB 8588)	13.00	26.67 ± 2.52	17.00 ± 2.65	0
Streptococcus pneumoniae (CIB)	12.33 ± 2.08	18.00 ± 2.00	15.00	0
Streptococcus pneumoniae (CIB)	9.67 ± 2.08	16.67 ± 2.08	16.33 ± 3.21	0

Key: LIO = Locally Isolated Organism, CIS = Clinical Isolate from Stool, CIW = Clinical Isolate from Wound, CISP = Clinical Isolate from Sputum, CIU = Clinical Isolate from Urine, CIB = Clinical Isolate from Blood, NCIB = National Collection of Industrial Bacteria, 0 = Not Sensitive, mm = Mean of three readings, M/W = Methanol/water*

Table 2. The sensitivity patterns of zones of inhibition exhibited by *V. paradoxa* fractions against bacterial strains

Bacterial strains	Zone of Inhibition (mm*)			
	Aqu (10 mg/mL)	**But (10 mg/mL)**	**Etl (10 mg/mL)**	**M/W(1:1) (v/v)**
Bacillus anthracis (LIO)	7.67 ± 1.53	19.00 ± 2.00	13.00 ± 2.00	0
Bacillus cereus (NCIB 6349)	8.67 ± 1.15	8.33 ± 1.53	9.67 ± 1.53	0
Bacillus polymyxa (LIO)	9.00 ± 2.00	13.00 ± 2.00	10.33 ± 2.08	0
Bacillus stearothermophilus (NCIB 8222)	10.00	15.33 ± 2.52	13.33 ± 1.53	0
Bacillus subtilis (NCIB 3610)	8.33 ± 1.53	16.00 ± 3.00	10.00	0
Citrobacter braakii (CIS)	8.33 ± 1.15	19.67 ± 1.53	11.67 ± 1.53	0
Citrobacter freundii (CIS)	8.67 ± 1.15	9.67 ± 1.53	10.33 ± 1.53	0
Citrobacter youngae (CIS)	11.67 ± 1.15	14.67 ± 1.53	13.33 ± 1.53	0
Clostridium sporogenes (NCIB 532)	10.33 ± 1.53	20.33 ± 2.08	10.33 ± 0.58	0
Corynebacterium pyogenes (LIO)	9.67 ± 0.58	12.67 ± 1.53	9.67 ± 1.15	0
Enterococcus faecalis (NCIB 775)	10.00	13.33 ± 1.53	10.67 ± 0.58	0
Escherichia coli (NCIB 86)	9.33 ± 2.31	10.00 ± 1.00	11.00 ± 2.00	0
Klebsiella pneumoniae (CISP)	7.00 ± 1.00	8.00	8.67 ± 0.58	0
Klebsiella pneumoniae (CISP)	11.67 ± 1.53	8.67 ± 1.53	10.33 ± 1.53	0
Klebsiella pneumoniae (NCIB 418)	10.33 ± 0.58	15.33 ± 0.58	15.67 ± 1.53	0
Micrococcus luteus (NCIB 196)	10.33 ± 2.08	12.00 ± 1.00	10.33 ± 0.58	0
Proteus vulgaris (LIO)	16.00 ± 2.00	8.00 ± 1.00	9.67 ± 1.53	0
Pseudomonas aeruginosa (CIU)	13.33 ± 1.53	20.33 ± 0.58	13.00 ± 2.00	0
Pseudomonas aeruginosa (CIU)	11.00 ± 2.00	11.33 ± 1.53	10.00 ± 1.00	0
Pseudomonas aeruginosa(CIU)	17.33 ± 2.08	13.67 ± 1.15	15.00 ± 1.00	0
Pseudomonas aeruginosa (CIU)	11.67 ± 1.53	15.67 ± 1.53	12.67 ± 0.58	0
Pseudomonas aeruginosa (CIW)	12.67 ± 1.53	15.33 ± 0.58	13.00 ± 1.00	0
Pseudomonas aeruginosa (CIW)	10.00	16.00 ± 1.73	12.00 ± 1.73	0
Pseudomonas aeruginosa (NCIB 950)	11.00 ± 2.00	19.33 ± 1.53	16.00 ± 1.73	0
Pseudomonas flourescens (NCIB 3756)	12.67 ± 1.53	17.67 ± 1.53	13.33 ± 2.08	0
Shigella sp. (LIO)	9.67 ± 2.08	14.33 ± 1.15	11.33 ± 1.15	0
Staphylococcus aureus (CIW)	10.67 ± 1.53	13.00 ± 2.00	12.00	0
Staphylococcus aureus (CIW)	9.67 ± 1.15	13.33 ± 1.53	12.00	0
Staphylococcus aureus (CIW)	15.00	14.67 ± 0.58	13.67 ± 1.15	0
Staphylococcus aureus (NCIB 8588)	10.67 ± 2.08	15.00 ± 1.00	10.33 ± 1.53	0
Streptococcus pneumoniae (CIB)	10.67 ± 2.52	18.67 ± 1.53	11.00 ± 1.00	0
Streptococcus pneumoniae (CIB)	13.00 ± 1.73	16.00 ± 1.00	13.00 ± 2.00	0

Key: LIO = Locally Isolated Organism, CIS = Clinical Isolate from Stool, CIW = Clinical Isolate from Wound, CISP = Clinical Isolate from Sputum, CIU = Clinical Isolate from Urine, CIB = Clinical Isolate from Blood, NCIB = National Collection of Industrial Bacteria, 0 = Not Sensitive, mm = Mean of three readings, Aqu = Aqueous fraction, But = Butanol fraction, Etl = Ethylacetate fraction, M/W = Methanol/water*

Table 3. The sensitivity patterns of zones of inhibition exhibited by partially purified samples of butanol fraction against susceptible bacterial strains

Bacterial strains	Zone of Inhibition (mm*)				
	BUT_{73} (1 mg/mL)	BUT_{D73} (1 mg/mL)	BUT_{64} (1 mg/mL)	BUT_{82} (1 mg/mL)	M/W (1:1)
Bacillus anthracis (LIO)	0.0	0.0	7.0 ± 0.6	0.0	0
Bacillus cereus (NCIB 6349)	0.0	0.0	9.0 ± 1.0	0.0	0
Bacillus polymyxa (LIO)	0.0	0.0	0.00	0.0	0
Bacillus stearothermophilus (NCIB 8222)	8.5 ± 1.0	0.0	11.0 ± 1.0	0.0	0
Bacillus subtilis (NCIB 3610)	0.0	0.0	9.7 ± 0.6	0.0	0
Citrobacter braakii (CIS)	0.0	0.0	9.0 ± 0.0	0.0	0
Citrobacter freundii (CIS)	0.0	9.7 ± 1.5	0.0	0.0	0
Citrobacter youngae (CIS)	0.0	0.0	0.0	0.0	0
Clostridium sporogenes (NCIB 532)	8.0 ± 0.0	0.0	11.5 ± 0.6	0.0	0
Corynebacterium pyogenes (LIO)	0.0	0.0	8.0 ± 0.0	0.0	0
Enterococcus faecalis (NCIB 775)	0.0	0.0	0.0	0.0	0
Escherichia coli (NCIB 86)	0.0	0.0	8.0 ± 1.0	0.0	0
Klebsiella pneumoniae (CISP)	0.0	0.0	9.7 ± 0.6	0.0	0
Klebsiella pneumoniae (CISP)	11.0 ± 0.0	0.0	8.5 ± 1.2	0.0	0
Klebsiella pneumoniae (NCIB 418)	0.0	8.0 ± 0.0	0.0	0.0	0
Micrococcus luteus (NCIB 196)	0.0	0.0	10.5 ± 0.6	0.0	0
Proteus vulgaris (LIO)	7.0 ± 0.6	0.0	10.0 ± 0.0	0.0	0
Pseudomonas aeruginosa (CIU)	0.0	0.0	8.7 ± 0.6	0.0	0
Pseudomonas aeruginosa (CIU)	0.0	0.0	11.0 ± 1.0	0.0	0
Pseudomonas aeruginosa(CIU)	0.0	0.0	9.5 ± 0.6	0.0	0
Pseudomonas aeruginosa (CIU)	0.0	0.0	0.0	0.0	0
Pseudomonas aeruginosa (CIW)	0.0	0.0	8.7 ± 1.2	0.0	0
Pseudomonas aeruginosa (CIW)	0.0	0.0	9.5 ± 0.6	0.0	0
Pseudomonas aeruginosa (NCIB 950)	0.0	0.0	11.0 ± 1.0	0.0	0
Pseudomonas flourescens (NCIB 3756)	0.0	0.0	12.0 ± 1.2	0.0	0
Shigella sp. (LIO)	0.0	0.0	9.7 ± 1.5	0.0	0
Staphylococcus aureus (CIW)	0.0	10.0 ± 0.0	10.0 ± 0.0	0.0	0
Staphylococcus aureus (CIW)	0.0	0.0	8.5 ± 1.2	0.0	0
Staphylococcus aureus (CIW)	0.0	0.0	9.0 ± 1.0	0.0	0
Staphylococcus aureus (NCIB 8588)	0.0	0.0	0.0	0.0	0
Streptococcus pneumoniae (CIB)	0.0	0.0	0.0	0.0	0
Streptococcus pneumoniae (CIB)	0.0	0.0	0.0	0.0	0

Key: LIO = Locally Isolated Organism, CIS = Clinical Isolate from Stool, CIW = Clinical Isolate from Wound, CISP = Clinical Isolate from Sputum, CIU = Clinical Isolate from Urine, CIB = Clinical Isolate from Blood, NCIB = National Collection of Industrial Bacteria, 0 = Not Sensitive, mm = Mean of three readings, BUT_{73}, BUT_{D73}, BUT_{64} and BUT_{82} = Partially purified samples from butanol fraction, M/W = Methanol/water*

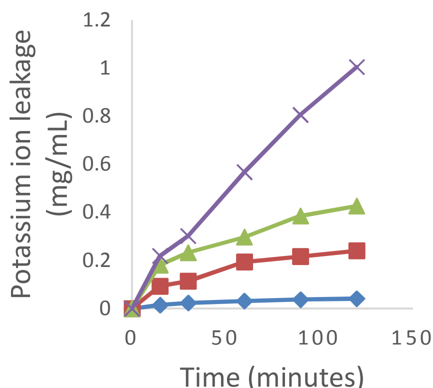

Fig. 9. The effect of aqueous fraction on potassium ion leakage from *E. coli* cells at 1 x MIC (red square), 2 x MIC (green triangle), 3 x MIC (purple cross) and control (blue diamond)

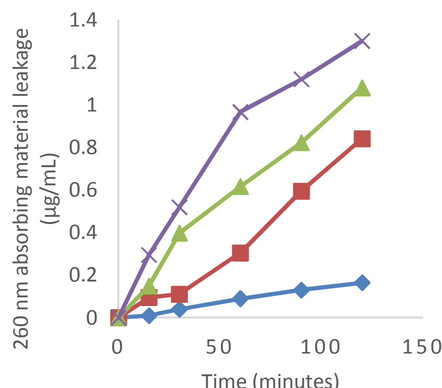

Fig. 10. The effect of aqueous fraction on nucleotides leakage from *E. coli* cells at 1 x MIC (red square), 2 x MIC (green triangle), 3 x MIC (purple cross) and control (blue diamond)

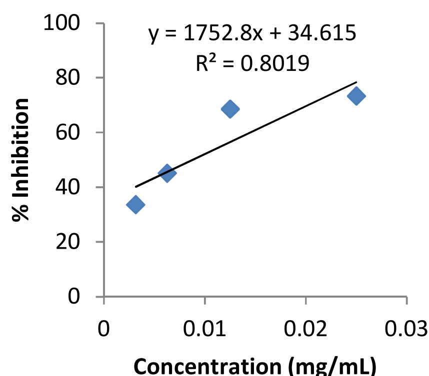

Fig. 11. The antioxidant activity of *Vitellaria paradoxa* stem bark extract (standard curve)

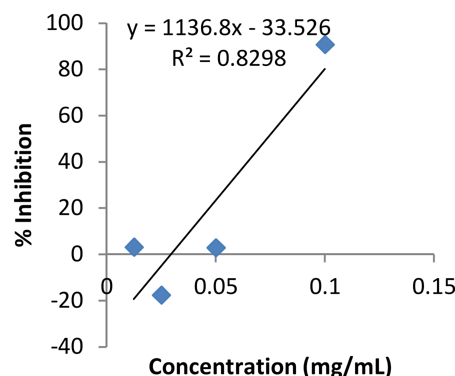

Fig. 12. The antioxidant activity of ascorbic acid (standard curve)

Table 4. Minimum inhibitory concentrations and minimum bactericidal concentrations exhibited by *V. paradoxa* extract against susceptible bacterial strains

Bacterial strains	Crude extract	
	MIC (mg/mL)	MBC (mg/mL)
Bacillus anthracis (LIO)	0.545	1.093
Bacillus cereus (NCIB 6349)	0.545	1.093
Bacillus polymyxa (LIO)	0.545	1.093
Bacillus stearothermophilus (NCIB8222)	0.545	1.093
Bacillus subtilis (NCIB3610)	0.545	1.093
Citrobacter braakii (CIS)	1.093	2.187
Citrobacter freundii (CIS)	1.093	2.187
Citrobacter youngae (CIS)	0.545	1.093
Clostridium sporogenes (NCIB 532)	0.545	1.093
Corynebacterium pyogenes (LIO)	0.545	1.093
Enterococcus faecalis (NCIB 775)	0.545	1.093
Escherichia coli (NCIB 86)	1.093	2.187
Klebsiella pneumoniae (CISP)	1.093	2.187
Klebsiella pneumoniae (CISP)	2.187	4.375
Klebsiella pneumoniae (NCIB 418)	1.093	2.187
Micrococcus luteus (NCIB 196)	1.093	2.187
Proteus vulgaris (LIO)	1.093	2.187
Pseudomonas aeruginosa (CIU)	1.093	2.187
Pseudomonas aeruginosa (CIU)	2.187	4.375
Pseudomonas aeruginosa (CIU)	0.545	1.093
Pseudomonas aeruginosa (CIU)	1.093	2.187
Pseudomonas aeruginosa (CIW)	1.093	2.187
Pseudomonas aeruginosa (CIW)	0.545	1.093
Pseudomonas aeruginosa (NCIB 950)	0.545	1.093
Pseudomonas flourescens (NCIB 3756)	1.093	2.187
Shigella sp. (LIO)	1.093	2.187
Staphylococcus aureus (CIW)	2.187	4.375
Staphylococcus aureus (CIW)	1.093	2.187
Staphylococcus aureus (CIW)	0.545	1.093
Staphylococcus aureus (NCIB 8588)	0.545	1.093
Streptococcus pneumoniae (CIB)	1.093	2.187
Streptococcus pneumoniae (CIB)	2.187	4.375

Key: LIO = Locally Isolated Organism, CIS = Clinical Isolate from Stool, CIW = Clinical Isolate from Wound, CISP = Clinical Isolate from Sputum, CIU = Clinical Isolate from Urine, CIB = Clinical Isolate from Blood, NCIB = National Collection of Industrial Bacteria

Table 5. Minimum inhibitory concentration and minimum bactericidal concentration exhibited by aqueous, butanol and ethylacetate fractions against susceptible bacterial strains

Bacterial strains	Aqueous fraction		Butanol fraction		Ethylacetate fraction	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
Bacillus anthracis (LIO)	0.31	0.63	0.31	0.63	0.31	0.63
Bacillus cereus (NCIB 6349)	1.25	2.50	1.25	2.50	0.63	1.25
Bacillus polymyxa (LIO)	2.50	5.00	0.63	1.25	1.25	2.50
Bacillus stearothermophilus (NCIB 8222)	0.63	1.25	0.31	0.63	0.31	0.63
Bacillus subtilis (NCIB 3610)	0.63	1.25	0.63	1.25	0.63	1.25
Citrobacter braakii (CIS)	1.25	2.50	0.63	1.25	1.25	2.50
Citrobacter freundii (CIS)	1.25	2.50	0.63	1.25	0.63	1.25
Citrobacter youngae (CIS)	0.63	1.25	0.63	1.25	0.63	1.25
Clostridium sporogenes (NCIB 532)	0.63	1.25	0.63	1.25	0.63	1.25
Corynebacterium pyogenes (LIO)	0.31	0.63	0.31	0.63	0.31	0.63
Enterococcus faecalis (NCIB 775)	0.63	1.25	0.31	0.63	0.31	0.63
Escherichia coli (NCIB 86)	2.50	5.00	0.63	1.25	1.25	2.50
Klebsiella pneumoniae (CISP)	0.31	0.63	0.31	0.63	0.31	0.63
Klebsiella pneumoniae (CISP)	0.31	0.63	0.31	0.63	0.31	0.63
Klebsiella pneumoniae (NCIB 418)	1.25	2.50	0.31	0.63	0.63	1.25
Micrococcus luteus (NCIB 196)	2.50	5.00	0.31	0.63	1.25	2.50
Proteus vulgaris (LIO)	1.25	2.50	0.63	1.25	1.25	2.50
Pseudomonas aeruginosa (NCIB 950)	0.63	1.25	0.31	0.63	0.31	0.63
Pseudomonas aeruginosa (CIU)	1.25	2.50	1.25	2.50	2.50	5.00
Pseudomonas aeruginosa (CIU)	2.50	5.00	0.63	1.25	1.25	2.50
Pseudomonas aeruginosa (CIU)	0.31	0.63	0.31	0.63	0.31	0.63
Pseudomonas aeruginosa (CIU)	1.25	2.50	0.63	1.25	0.63	1.25
Pseudomonas aeruginosa (CIW)	2.50	5.00	1.25	2.50	2.50	5.00
Pseudomonas aeruginosa (CIW)	5.00	10.00	2.50	5.00	2.50	5.00
Pseudomonas flourescens (NCIB 3756)	0.63	1.25	0.63	1.25	1.25	2.50
Shigella sp. (LIO)	1.25	2.50	0.63	1.25	1.25	2.50
Staphylococcus aureus (CIW)	0.63	1.25	0.31	0.63	0.31	0.63
Staphylococcus aureus (CIW)	1.25	2.50	0.63	1.25	0.63	1.25
Staphylococcus aureus (CIW)	0.63	1.25	0.31	0.63	0.31	0.63
Staphylococcus aureus (NCIB 8588)	0.63	1.25	0.63	1.25	0.63	1.25
Streptococcus pneumoniae (CIB)	2.50	5.00	0.31	0.63	0.63	1.25
Streptococcus pneumoniae (CIB)	5.00	10.00	1.25	2.50	2.50	5.00

Key: LIO = Locally Isolated Organism, CIS = Clinical Isolate from Stool, CIW = Clinical Isolate from Wound, CISP = Clinical Isolate from Sputum, CIU = Clinical Isolate from Urine, CIB = Clinical Isolate from Blood, NCIB = National Collection of Industrial Bacteria

Table 6. Phytochemical compounds of *V. paradoxa* crude extract

Phytoconstituents	Result
Saponins	Positive
Alkaloids	Positive
Reducing sugar	Positive
Flavonoid	Positive
Tannins	Positive
Cardiac glycoside	Positive
Steroids	Negative

The *in vitro* antioxidant assay of *V. paradoxa* stem bark extract shows appreciable antioxidant potential when compared to ascorbic acid used as standard (Figs. 11 and 12). The extract exhibited highest percentage of 73.34% at a concentration of 0.025 mg/mL when compared with the inhibition exhibited by ascorbic acid. The extract was found to reduce free radicals released by 2,2-diphenyl-1-picrylhydrazyl to half and thus showed appreciable free scavenging activities at the highest concentration of 0.008777 mg/mL.

The GC-MS chromatogram analysis of the partially purified butanol fraction revealed twenty-eight peaks which indicated the presence of twenty-eight constituents (Fig. 13). On comparison of the constituents mass spectra with the National Institute of Standards and Technology (NIST) 14.0 Library, 1,2-diethylcyclooctane, prop-1-en-2-yltetradecylcarbonate, cyclohexylmethylethylsulfite, hexylcyclohexane, cyclotetradecane, 1,2,4,5-tetraethylcyclohexane, octylcyclohexane, tetradec-1-ene,

diethylphthalate, 1-octadecene, 14-methylhexadecanoic acid, tetradecylpentafluoropropionic acid, (Z)-methyl-9-octadecenoic acid, (E)-methyloctadec-11-enoate, 1-tridecene, (1S,15S)-Bicyclo[13.1.0]hexadecan-2one, (Z)-ethylheptadec-9-enoate, 17-pentatriacontene, methyl-2-hydroxyeicosanoate, 11,13-dimethyl-12-tetradecen-1-olacetate,3-methy-4-(phenylthio)-2-prop-2-enyl-2,5-dihydrothiophene-1,1-dioxide, 1-isothiocyanato-3-methyladamantane and 5-butyl-6-hexyloctahydro-1H-indene were identified to be present in the partially purified butanol fraction. Of the twenty-eight compounds identified, the most prevalent compounds were 14-methylhexadecanoic acid, an ester compound with retention time of 20.741 seconds (17.673%), (Z)-methyl-9-octadecenoic acid, an ester (11.751%) and methyl-14-methylheptadecanoate (8.356%).

4. DISCUSSION

The bioactive components of stem bark extract of *V. paradoxa* were investigated for their antibacterial activities against panel of pathogens associated with human infections. The antioxidant property of this plant was also studied. The crude extract from this plant along with the fractions obtained from it exhibited high degree of antimicrobial properties against the test bacterial strains comprising of both typed cultures and clinical isolates. These organisms are made up of Gram positive and Gram negative and they were all susceptible to the plant extracts. This is an indication of broad spectrum activity exhibited by the extract. The results obtained from this study showed that the extracts compared favourably with the standard antibiotics used as positive controls. Thus, stem bark extract from *V. paradoxa* may serve as a source of potent antimicrobial agent of natural origin to combat the infections caused by pathogens and reduced or prevent death of patients. Our findings in this study thus support the usefulness of *V. paradoxa* in folklore remedies for infections caused by microorganisms. *Vitellaria paradoxa* stem bark extract revealed the presence of some phytochemicals which include tannins, alkaloids, flavonoids and others which are known to contribute to the biological activities of medicinal plants [13]. These phytochemicals thus contributed to the antibacterial activities of *V. paradoxa*. Such phytochemicals can be exploited for the development of antimicrobials to combat the infections caused by pathogens especially those that have developed multi-resistance to many of the available antibiotics.

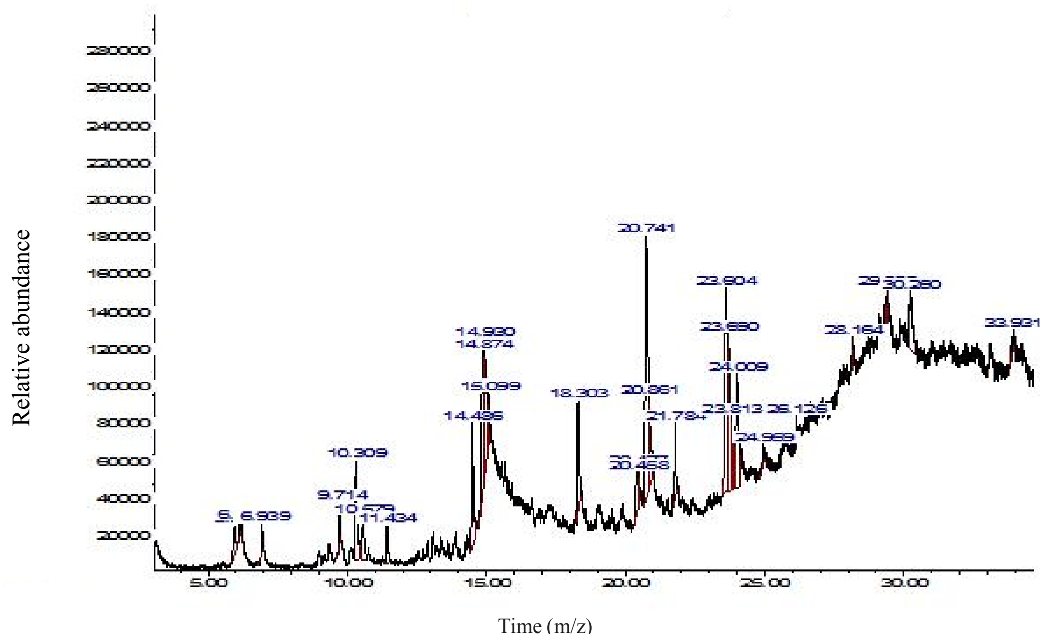

Fig. 13. GC-MS chromatogram of partially purified butanol fraction of *V. paradoxa*

The MIC and MBC of *V. paradoxa* extracts were studied and various concentrations of the extracts exhibited inhibitory or cidal effects on cells of the organisms tested. All the three fractions (aqueous, n-butanol and ethyl acetate fractions) exhibited lowest MIC of 0.31 mg/mL against the test isolates while the lowest MBC observed was 0.63 mg/mL (Table 5). The MIC index of plant extract which is equal or less than 2 mg/mL is considered as bactericidal while those above 2 mg/mL but less than 16 mg/mL are bacteriostatic [23]. The MIC exhibited by these fractions against the bacterial strains tested is lower than 2 mg/mL. This shows that *V. paradoxa* stem bark extract exhibited appreciable antimicrobial property. A low MIC value of medicinal plant extract indicates a better antimicrobial agent [24], thus, supported our findings. Hence potent antimicrobial agents could be developed from this plant for the treatment of infections caused by multi-resistant microorganisms. Such antimicrobial agents could go a long way in health care delivery to safe people's lives.

Mode of action of *V. paradoxa* were investigated through killing rate of the test cells, leakages of proteins, potassium ions and nucleotides from cells of bacterial strains tested. From our observations, the fractions exerted cidal effects on test cells within a shortest period of time which is an indication of a better bactericidal effect. The results from this study showed a relationship between the quantity of cellular constituents leaked out of the test cells and the number of the cells killed with an increase in contact time with the solution of the fraction. For example, the population of *E. coli* cells killed by aqueous fraction at a concentration of 1 x MIC was 19.5% within 30 minutes of contact time while the amount of proteins leaked within the same period of time was 3.24 µg/mL. When the contact time reached 120 minutes, 81.0% of the cells were killed and the amount of proteins leaked at the same time was 8.14 µg/mL (Figs. 1 and 6). Thus, there was correlation between the population of the cells killed with the amount of cellular contents leaked by the fraction. The same trend of effects was observed for n-butanol and ethyl acetate fractions on the cells tested. The results showed that the cells were killed through the leakage of cellular constituents from the cells.

Phenolic compounds are known to react with the cellular membrane of the bacterial cells which resulted in impairing of both its functions and integrity [25,26,27]. These phytochemical compounds that are present in *V. paradoxa* stem bark enhanced the mode of action of the extracts by acting in disruption of the test cells leading to their death. Thus, the bactericidal effects exhibited by *V. paradoxa* stem bark extracts showed a significant therapeutic potential of this plant. This observation supported the usefulness of *V. paradoxa* in folklore remedies for the treatment of microbial infections among many tribes in West Africa.

Vitellaria paradoxa exhibited antioxidant activity and this compared favourably with ascorbic acid used as standard (Figs. 11 and 12). Plants that exhibit antioxidant properties are known to possess free radical scavenging ability [28] and this antioxidant potential in plants is majorly due to the phenolic components present in them [29]. Presence of free radicals in human body may results to clinical disorders like liver diseases, cancer, renal diseases and degenerative diseases [30]. High antioxidant property observed for *V. paradoxa* stem bark extract may serve as a pointer towards development of antioxidant drug of natural origin from this plant and such compounds could be used for the treatment of the ailment mentioned above. *Vitellaria paradoxa* is known to be used among many tribes in West Africa as anti-stress and anti-ageing. Thus, results obtained from our findings support the usefulness of this plant in folklore remedies for stress and ageing.

The major chemical constituent identified in *V. paradoxa* was revealed to be 14-methylhexadecanoic acid. Such compound can be isolated from this plant and used for the development of antimicrobial drug of natural origin for the treatment of microbial infections especially those caused my multi-drug resistant pathogens.

5. CONCLUSIONS

In conclusion, *V. paradoxa* stem bark extracts exhibited both antimicrobial and antioxidant properties. The ability of this plant extract to kill or inhibit the growth of bacteria at minimum contact time has established that broad spectrum antimicrobial drugs of natural origin could be developed from *V. paradoxa* stem bark. Such antimicrobial agent could be used to combat infections caused by multi-drug resistant bacteria. In addition, *V. paradoxa* stem bark could also serve as a good source for the

development of antioxidant drug to treat stress and prevent early ageing in humans.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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