



Chemical composition, *in vitro* antioxidant and antimicrobial activities of methanol extracts of different parts of *Vitellaria paradoxa*

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Abstract

Due to the unique floral biodiversity, the greener nature of natural products, the resistance of disease-causing microorganisms to synthetic drugs, the appreciable level of toxicity of synthetic drugs, and the trend in infectious diseases globally, there is a need to source new drug templates from locally sourced medicinal plants. This work is aimed at investigating the chemical compositions and *in vitro* antioxidant and antimicrobial activities of methanol extracts of the different parts of the *Vitellaria paradoxa* plant, including the seed cake left after traditional oil extraction.

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The antioxidant activity of the leaves, stem bark, seed cake, and root extracts of *V. paradoxa* was investigated using standard antioxidant assays. The antimicrobial properties of the extracts via the disc diffusion method were also investigated. The seed cake had the highest calorific value (850 kcal). Calcium was found to be highest in the stem bark (1105.83 mg/kg), while potassium was highest in the root sample (4086.50 mg/kg). Flavonoids and phenolics were present in abundance in the root sample (171.60 mg/100g and 396.48 mg/100 g, respectively). The stem bark extract had the lowest IC₅₀ value of 51.52% in diphenyl - 1 - picrylhydrazyl (DPPH) scavenging, while the leaf extract was better in nitric oxide (NO) scavenging with an IC₅₀ value of 54.15%. The total phenolic content of the extracts correlated positively with DPPH scavenging activity. Total flavonoids correlated positively with NO scavenging activity. The *V. paradoxa* extracts inhibited the growth of

Burtholderia cepacia, *Staphylococcus aureus*, and *Salmonella typhi*, among others. *V. paradoxa* contains some chemical elements that can be used to manage some of the chronic diseases in our environment and can serve as a template for drug discovery.

Keywords: Bioassays; *Burtholderia cepacia*; DPPH; *Staphylococcus aureus*; Total flavonoids; Total phenols

1.0 Introduction

From time immemorial and up until now, the traditional medicine in common practice across the African continent and indeed other parts of the world has involved the use of medicinal plants as one of the major sources of therapeutics. In many parts of Africa, medicinal plants are the most easily accessible health care resource available to rural dwellers, partly due to their abundance and partly due to a lack of access to conventional medicine (Ojo *et al.*, 2021). Medicinal plants are those plants that have been scientifically proven to possess the beneficial functions of ameliorating the diseases of humans as well as improving their well-being (Chukwuma *et al.*, 2015). These plants are rich in phyto constituents that can serve as a template for new drug discovery, preparation, and manufacturing of new medicinal products. These plants contain secondary metabolites such as flavonoids, quinine, terpenoids, and so on that have certain biological and therapeutic activities like anti-carcinogenic, anti-mutagenic, anti-inflammatory, and anti-oxidative-stressed disease properties (Isaac *et al.*, 2022a; El-Saber Batiha *et al.*, 2020). Medicinal products of natural origin show minimal negative consequences and have proven to be more efficient when used for therapy than their various synthetic counterparts (Yadav and Agarwala, 2011). Despite the wide scope of clinically active synthetic and semi-

synthetic drugs, the search for new efficacious anti-infective and safer drugs remains a paramount quest due to resistance and the emergence of new parasite strains (Okpuzor *et al.*, 2021).

Vitellaria paradoxa (Sapotaceae) is a plant that is regionally available in Nigeria and some other West, Central, and East African nations, especially near towns and villages. The seed of *V. paradoxa* is rich in triglyceride oil that can replace the oil palm as a supply of suitable eating oil in south-western, central, and northern Nigeria (Iwegbue *et al.*, 2019). The plant species (*Vitellaria*) is easily identified by means of its very lengthy leaf stalks, more widely spaced nerves, and plentiful white exudate when cut in the petiole. Shea fruit is a green, oval-shaped fruit that ranges in size from 2 cm to 5 cm in diameter and has a relatively hard shell. The fleshy pulp is sweet and is eaten as food, while the pulp is used to make jam (Muotono *et al.*, 2017). Shea butter, which is the fat extracted from the kernel of the shea tree, is turning into an increasingly popular constituent of beauty formulas in the cosmetic industry, and its use as a cocoa butter substitute in the chocolate industry has been reported (Ndukwe *et al.*, 2007). *Vitellaria paradoxa*, commonly called shea butter tree, is a conventional medicinal plant of the family Sapotaceae that has been widely used in traditional medicine to treat a wide range of diseases for many years in tropical Africa (Ojo *et al.*, 2021). Shea butter also finds applications in confectionery, cosmetics, soaps, and pharmaceutical products both locally and internationally, and it is sometimes referred to as the "wonder tree due to its versatile application (Isaac *et al.*, 2022b). Plants generally consist of various kinds of chemical constituents that serve the vegetation by contributing to some secondary functions like helping in plant growth, safeguarding the plant's

life by means of the capability of activating protection mechanisms in adverse conditions, and imparting colour, odour, and style to the plants (Yadav and Agarwala, 2011).

In view of the trend in infectious diseases and the like globally, it is therefore of immense scientific interest to investigate the health potentials of the available medicinal plants in our communities with the aim of finding lasting solutions to these global health pandemics and proposing action plans to address future challenges in global health care delivery (Isaac *et al.*, 2022a). Hence, this research is aimed at investigating the phytochemical, proximate, elemental, and amino acid constituents of the methanol extract of the leaves, stem bark, root, and seed cake of *V. paradoxa*. The *in vitro* antioxidant and antimicrobial activities of these extracts are also investigated.

2.0 Materials and Methods

2.1 Sample Collection and Identification

The plant's parts, namely the nuts, root, stem bark, and leaves, were collected from its natural habitat on farmland in Kishi, a town in Irepo Local Government Area of Oyo State, Nigeria. The fresh samples were transported in paper bags to the Department of Chemistry laboratory at Akwa Ibom State University for preparation. The plant materials were authenticated through the use of a publication on the handbook of African medicinal plants (Iwu, 2014). The voucher specimens were labeled as NPH 39 (nuts), NPH 40 (root), NPH 41 (stem bark), and NPH 42 (leaf) and were subsequently preserved in the herbarium of the Natural Products Chemistry (NPH) unit of the Department of Chemistry, Akwa Ibom State University, Akwa Ibom State, Nigeria.

2.2 Sample Preparation

Fresh leaves of *Vitellaria paradoxa*, after sorting and washing, were shade-dried at room temperature, while the seeds, stem bark, and roots were dried in a laboratory oven at 60 °C. The dry samples were ground into powder with a manual grinding machine. The dried powdered specimen of each plant part was extracted with methanol following the methods described in the literature (Ogbole *et al.*, 2018; Isaac *et al.*, 2022a). The crude extracts were preserved in a refrigerator for further analysis. Methanol was the chosen solvent for this research because of its amphiphilic nature and its ability to dissolve a wider range of compounds than water, including polar and, to a large extent, some non-polar compounds (Ogbole *et al.*, 2018). Also, methanol is volatile and evaporates easily in order to separate it from the extract, compared to water. These and other reasons, such as the volatile nature of methanol compared to water, make it a preferred solvent for phytochemical extraction.

2.3 Sample Defatting and Extraction with Methanol

The dry and pulverized seed sample was first treated with petroleum ether (40–60 °C) to remove the lipid or oil via the solvent extraction method described by Isaac and Ekpa (2014) with a slight modification. About 100 g of the sample was thoroughly macerated in 300 ml of petroleum ether in a 500-ml beaker for 30 minutes. The mixture was then filtered using a muslin cloth, and the residue (cake) was washed several times with petroleum ether until the oil was completely removed. The seed cake was dried in an oven at 60 °C to exclude petroleum ether and kept for extraction with methanol. Each of the powdered plant specimens was extracted with methanol via the methods stated in the literature (Isaac *et al.*, 2022a; Ezeokeke *et al.*,

2015). 20 g of each sample was soaked in 300 ml of analytical-grade methanol for 14 days with constant agitation. The samples were filtered using Whatman No. 1 filter paper, and the filtrates were concentrated under reduced pressure at 60 °C to obtain the methanol extract

of the different parts of *V. paradoxa*. The percentage yield of the extract was calculated using Equation 1. The samples were then preserved in a refrigerator prior to biological analysis.

$$\text{Percentage yield (\%)} = \frac{\text{Weight of extract (g)}}{\text{Weight of plant material (g)}} \times \frac{100}{1} \quad (1)$$

2.4 Proximate Analysis

The recommended methods of the Association of Official Analytical Chemists (AOAC, 2012) were used for the determination of total carbohydrate, crude fiber, lipid, ash content, and crude protein while the calorific values were determined using the method described by Isaac and Ekpa (2009). Crude protein, lipid, and carbohydrate were multiplied by factors of 4, 9, and 4 Kcal, respectively, and summed to have the calorific value.

2.5 Determination of Mineral Elements

The elemental composition of the samples was determined by the method described in the literature (Kumaravel and Alagusundaram, 2014) using inductively coupled plasma-optical emission spectroscopy (ICP-OES). One gram (1.00 g) of the samples was weighed into a digestion flask of 250 ml capacity, and 25 ml of nitric acid, perchloric acid, and sulfuric acid were added to each sample. The flask was fixed to a clamp and kept overnight. When the initial reaction was complete, the temperature of the micro-digestion bench was slowly increased from 180 °C to 200 °C. The digestion was continued at that temperature until no visible particles were observed; the temperature was raised to 240 °C, and the digestion acid was evaporated until dense white fumes formed within the digestion flask. After the digestion was complete, the contents of

the flask were filtered, and the digested material was kept in a dust-proof glass chamber. The samples were digested with the disappearance of brown fumes and diluted to 100 ml for elemental analysis by ICP-OES as follows:

The Agilent 720-ES with a megapixel charge-coupled device (CCD) detector was used, which provided simultaneous measurement, while the Agilent SPS3 auto sampler was used for sample introduction. Agilent Expert II software was used to control the instrument and acquire data. Appropriate concentration ranges of working standards from the multi-element stock standard were prepared through the serial dilution method. A new worksheet was created from the ICP-OES expert software, into which were programmed the individual sample codes as well as the methods. After the instrument was programmed, the calibration curves were obtained by running the standards, and then the samples were also analyzed according to the sequence specified in the sequence parameters of the newly created worksheet.

2.6 Phytoconstituent Analysis

2.6.1 Determination of Alkaloids

Alkaloids in the extracts were quantified according to the method described by Ezeonu and Ejikeme (2016). In a 250 cm³ beaker, 2.50 g of each sample were added, followed by the addition of 200 cm³ of 10% acetic acid in

ethanol. The mixture was allowed to stand for 4 hours. The extract was concentrated in a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide (15 drops) was added in drops to the mixture until the precipitation was completed. The mixture was allowed to stand for 3 hours for the precipitates to settle. The solution was decanted and the supernatant was discarded, while the precipitates were washed with 20 cm³ of 0.1M ammonium hydroxide and then filtered using Gem filter paper (12.5 cm). The residue was weighed using an electronic weighing balance, Model B218, dried in an oven, and weighed again. The percentage of alkaloid was determined using this mathematical expression in Equation 2.

$$(\%) \text{ Alkaloid} = \frac{\text{Weigh of alkaloid}(g)}{\text{Weigh of sample}(g)} \times \frac{100}{1} \quad (2)$$

2.6.2 Determination of Steroids

The total steroid was measured by the method described by Salomi *et al.* (2019), with slight modifications. 1 ml of the extract was transferred into 10 ml volumetric flasks, and 2.0 ml of sulphuric acid (4 N) and 2.0 ml of iron (III) chloride (0.5% w/v) were added, followed by the addition of 0.5 ml of potassium hexacyanoferrate (III) solution (0.5% w/v). The mixture was heated in a water bath maintained at 70±2 °C for 30 minutes with occasional shaking. The mixture was then diluted to 10 ml with distilled water. The absorbance was measured at 780 nm against the reagent blank. Total steroids in the extract were expressed in terms of cholesterol equivalents (mg of CHO/g of extract). The total steroid content in the *V. paradoxa* extracts was calculated using the relationship: total steroid content = CHOE × V/m, where CHOE is the cholesterol equivalence (mg/g) determined from the calibration curve (Y = 0.229X + 0.0564; r² = 0.9357); V is the volume of extract (ml); and m is the weight (g) of the pure plant extract.

2.6.3 Determination of Total Flavonoids

The method reported by Ejikeme *et al.* (2014) was used in the determination of total flavonoids. In a 250 cm³ beaker, 2.50 g of sample were added, followed by the addition of 50 cm³ of an 80% (v/v) aqueous methanol solution to the sample. The beaker was covered and allowed to stand for 24 hours at room temperature. After discarding the supernatant, the residue was re-extracted three times with the same volume of ethanol. Whatman filter paper number 42 (125 mm) was used to filter the whole solution of each sample. Each plant sample's filtrate was later transferred into a crucible and evaporated to dryness over a water bath. The contents of the crucible were cooled in a desiccator and weighed until a constant weight was obtained. The percentage of flavonoids was calculated using the formula in Equation 3.

$$(\%) \text{ Flavonoid} = \frac{\text{Weigh of flavonoid}(g)}{\text{Weigh of sample}(g)} \times \frac{100}{1} \quad (3)$$

2.6.4 Determination of Total Phenolics

The total phenolic contents in the methanol extracts were determined using the method of Adaramoye and Akanni (2016) and expressed as gallic acid equivalent per gram of dry weight (mg GAE/g) of extracts. The total phenolic content of the extract was calculated using the relationship: total phenolic content = GAE×V/m, where GAE is the gallic acid equivalence (mg/ml) determined from the linear calibration curve (Y = 0.321X + 0.0562; r² = 0.9175); V is the volume of extract (ml); and m is the weight (g) of the pure plant extract.

2.6.5 Determination of Tannins

The analytical method for the quantitative determination of tannin was according to Amadiet *al.* (2012) and Ejikeme *et al.* (2014). The Folin-Denis reagent was first prepared by dissolving 50 g of sodium tungstate (Na₂WO₄) in

37 cm³ of distilled water, followed by the addition of 10 g of phosphomolybdic acid (H₃PMO₁₂O₄₀) and 25 cm³ of orthophosphoric acid (H₃PO₄). The mixture was refluxed for 2 hours, cooled, and diluted to 500 cm³ with distilled water. One gram (1.0 g) of each sample was added to 100 cm³ of distilled water in a conical flask. This was boiled gently for 1 hour on an electric hot plate and filtered using number 42 (125 mm) Whatman filter paper into a 100 cm³ volumetric flask. Folin-Denis reagent, 5.0 cm³, 10 cm³ of saturated Na₂CO₃ solution, and 50 cm³ of distilled water were added to 10 cm³ of diluted extract (aliquot volume) in a 100 cm³ volumetric flask and made up to the mark with distilled water for colour development. The mixture was thoroughly agitated and allowed to stand for 30 minutes in a water bath at a temperature of 25 °C. With the aid of a Spectrum Lab 23A spectrophotometer, optical density was measured at 700 nm and compared to a standard tannic acid curve. A tannic acid standard curve was obtained by dissolving 0.20 g of tannic acid in distilled water in a 200 cm³ volumetric flask and adding distilled water to make it up to the mark to serve as a stock solution. Varying concentrations (0.2–1.0 mg/cm³) of the standard tannic acid solution were prepared by adding Folin-Denis reagent (5 cm³) and 10 cm³ of saturated Na₂CO₃ (10 cm³) solution and making up to the 100 cm³ mark with distilled water. The solution was thoroughly agitated and left to stand for 30 minutes in a water bath at 25 °C. Optical density was measured at 700 nm with the aid of a Spectrum Lab 23A spectrophotometer. The optical density (absorbance) versus tannic acid concentration was plotted. The following formula (Equation 4) was used in the calculation of the percentage of tannin in the samples:

$$\begin{aligned} & \text{Tannic acid} \left(\frac{\text{mg}}{100\text{g}} \right) \\ &= \frac{C \times \text{extract volume} \times 100}{\text{Aliquot volume} \times \text{Weight of sample (g)}} \quad (4) \end{aligned}$$

where C is the concentration of tannic acid read off the graph.

2.7 Antioxidant Activities

The antioxidant activities of the extracts were investigated through their DPPH and nitric oxide scavenging activities and by their ferric antioxidant reducing power (FRAP), as described below.

2.7.1 DPPH Radical Scavenging Assay

The DPPH radical scavenging activity of the extracts was determined based on the method described by Shimada *et al.* (1992). Briefly, a 0.1 mM solution of DPPH in ethanol was freshly prepared; 1 ml of the solution was added to 1 ml of extract or ascorbic acid as a control in water at different concentrations (25–100 µg/ml). The mixture was shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517 nm by using a UV-visible spectrophotometer. The lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The percent DPPH scavenging effect was calculated using Equation 5.

$$(\%) \text{ DPPH Scavenging} = \frac{A_0 - A_1}{A_0} \times \frac{100}{1} \quad (5)$$

where A₀ is the absorbance of the control and A₁ is the absorbance of the standard or extract. The IC₅₀ value represents the concentration of the compounds that cause 50% inhibition of DPPH radical formation.

2.7.2 Nitric Oxide Scavenging

Sodium nitroprusside (10 mM) in phosphate buffered saline was mixed with different concentrations (25–100 µg/ml) of each extract

and incubated for 30 min. After the incubation period, 0.5 ml of Griess reagent (1% sulfanilamide, 2% H₂PO₄, and 0.1% N-(1-naphthyl) ethylenediaminedihydrochloride) was added (Isaac *et al.*, 2022a). Absorbance was taken at 550 nm, and ascorbic acid was used as a standard. The IC₅₀, which is the inhibitory concentration of each extract required to reduce 50% of nitric oxide formation, was determined. The same reaction mixture was used without the extract, but an equivalent amount of methylated spirit was used as a control. The nitric oxide inhibition percentage was calculated using the Equation 6.

(%) Nitric oxide inhibition

$$= \frac{A_{(control)} - A_{(sample)}}{A_{(control)}} \times \frac{100}{1} \quad (6)$$

2.7.3 Ferric-Reducing Antioxidant Power Assay

The methods reported in the literature (Jimoh *et al.*, 2010; Isaac *et al.*, 2022a) were adopted for the FRAP assay with slight modifications. The stock solutions included 300 mM sodium acetate trihydrate buffer (3.1 g C₂H₃NaO₂·3H₂O and 16 ml glacial acetic acid, C₂H₄O₂), pH 3.6, 10 mM TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) solution in 40 mM HCl, and 20 mM FeCl₃·6H₂O solution. The fresh working solution was prepared by mixing 25 ml of acetate buffer, 2.5 ml of TPTZ, and 2.5 ml of FeCl₃·6H₂O. The temperature of the solution was increased to 37 °C before use. Plant extracts (150 µl) were allowed to react with 2850 µl of the FRAP solution for 30 min in the dark. The absorbance of the coloured product (ferrous tripyridyltriazine complex) was measured spectrophotometrically at 593 nm. The results are expressed in µM Fe (II)/mg dry mass and compared with those of ascorbic acid using equation 7.

Ferric reducing power

$$= \frac{Fe \times \text{volume of sample}(\mu\text{l})}{\text{Weight of sample}(\text{mg})} \quad (7)$$

where Fe is the milligram equivalent of FeSO₄, the volume of the extract in µl, and the mass of the sample in milligrams.

2.8 Antimicrobial Test

The antimicrobial activity test was carried out using the disc diffusion method as described by Arokiyaraj *et al.* (2012) with minor modifications. Petri plates were prepared with 20 ml of sterile Mueller-Hinton agar. The test cultures were swabbed over the solidified media, and the plates were further allowed to dry for 10 minutes. Sterile discs were loaded with methanolic extracts of the various *V. paradoxa* parts. The loaded discs were placed on the surface of the medium and left to diffuse for 30 minutes at room temperature. Negative control was prepared using solvents. Cyproxin was used as the positive control. The plates were then incubated at 37 °C for 24 h. The zone of inhibition was recorded in millimeters, and the experiment was performed three times.

Microbial broth preparation: 1.5 g of peptone salt was weighed into 100 ml of distilled water in a conical flask. This was mixed gently until it was fully dissolved. 5 ml of the preparation was dispensed into each Bijou bottle and sterilized in an autoclave at 121 °C for 15 minutes. This was then allowed to cool, followed by the inoculation of isolates in each bottle.

Nutrient Agar Plate Preparation: About 28.0 g of nutrient agar was weighed into a conical flask. Distilled water was added up to the 1000-ml mark to dissolve the nutrient agar. The solution was placed in an autoclave set at 121 °C for 30 minutes and thereafter brought out to cool. About 15 ml of the preparation was poured into each Petri dish and allowed to set. The dishes were

dried in a hot air oven set at 60 °C and then allowed to cool before use. These nutrient agar plates were properly labeled and impregnated with an identified microbial isolate, followed by loading known concentrations of the extract on punched filter paper. The plates were allowed to incubate at 30 °C for 24 hours, and the zones of growth inhibition were measured and recorded. The plates were prepared in duplicate, and the average reading was taken for each set.

2.9. Data Analysis

All the experiments were conducted in triplicate, and the results were expressed as mean \pm SD (standard deviation). A one-way ANOVA with Tukey's HSD test was used for the analysis of the data at a significant level of $P < 0.05$. Pearson's correlation analysis and multiple linear regression were used for the analysis of the correlation between antioxidant activity and the different phytochemicals.

3.0 Results and Discussion

3.1 Percentage Yield of the Extracts

The percentage yield of the methanol extract of the different parts of *Vitellaria paradoxa* is shown in Table 1. Phytochemical extraction with methanol yielded 13.06% of phytomaterials from the leaves and a maximum of 19.91% from the

roots, as shown in Table 1. There is a significant difference among the percentage yields of the different plant parts at $P < 0.05$. The relatively lower percentage yield of the different samples, which varied between 13.06% and 19.91% (Table 1), recorded in this experiment could be attributed to the cold maceration solvent extraction method, which yields less compared to other methods such as Soxhlet extraction at higher temperatures (Azwanida, 2015). The percentage yields of the extracts in decreasing order were root > seed cake > stem bark > leaves.

3.2 Proximate Composition of the Different *V. paradoxa* Parts

Proximate analysis results showed that the different parts of *V. paradoxa* plants, namely the roots, stem bark, leaves, and seed cake, are rich in carbohydrates and crude fiber with moderate protein content but low in crude fat, as shown in Table 2. The seed cake has a much higher calorific value than the other plant parts. There was a significant difference at $P < 0.05$ in the proximate composition of root stem bark, leaves, and seed cake extracts of the *V. paradoxa* plant. The calorific values were also found to be significant at $P < 0.05$ among the mean values of the stem bark, root, leaves, and seed cake of the *V. paradoxaplant*.

Table 1: Percentage (%) yield for the different extracts

Sample (<i>V. paradoxa</i>)	Weight of plant material (g)	Weight of extract (g)	% Yield
Root	20	3.983	19.910
Stem bark	20	3.018	15.090
Leaves	20	2.613	13.060
Seed cake	20	3.683	18.410

Table 2: Proximate composition of different *Vitellaria paradoxa* plant parts

Plant part	Carbohydrates (%)	Protein (%)	Crude Fat (%)	Crude Fibre (%)	Ash (%)	Calorific value(kcal)
Root	63.95 ± 0.156	9.70 ± 0.092	1.23 ± 0.021	17.12 ± 0.311	3.70 ± 0.014	305.67±0.509
Stem bark	67.36 ± 0.156	8.27 ± 0.057	1.15 ± 0.014	15.33 ± 0.212	3.34 ± 0.028	312.87±0.099
Leaves	66.14 ± 0.255	9.28 ± 0.120	1.89 ± 0.057	14.37 ± 0.141	4.42 ± 0.042	318.69±0.042
Seed cake	60.52 ± 0.325	9.95 ± 0.156	3.84 ± 0.021	16.75 ± 0.290	4.35 ± 0.028	850.68±0.467

The various plant parts have a high carbohydrate value, moderate crude fiber, and low ash content. According to Isaac and Ekpa (2009), these values are important indicators in terms of suitability for compounding animal feed. The seed cake, in addition to this, has a very high calorific value (850.68 kcal), as shown in Table 2. The ash content obtained in this study, which varied between 3.34 and 4.42%, is lower than those reported in the literature for *Solanum nigrum* (13.0%), *Leonotisleonorus* (12.0%), *Ipomeabatatas* (11.10%), *Vernoniacolorate* (15.86%), and *Moringa oleifera* (15.09%) (Jimoh *et al.*, 2010; Antia *et al.*, 2006; Lockett *et al.*, 2000). Dietary carbohydrates are believed to provide well over half of the energy needed for maintenance, growth, and production in farm animals (Nafikov and Beitz, 2007). Traditionally, fish meal, which usually contains between 5 and 50% protein, is the main source of dietary protein in feed compounding (Dersjant-Li, 2021). The protein content of about 9 percent in shea nut cake suggests that this can serve as a good alternative dietary protein source in animal feed, requiring less protein intake, especially with the increase in demand for global animal feed input protein.

3.3 Anti-nutritive Composition of *V. paradoxa* Plant

Chemical compounds that reduce the nutrient utilization and/or food intake of plants or plant products used as human or animal foods are classified as anti-nutritional factors. They play a vital role in determining the use of plants for human or animal consumption. The common anti-nutritional compounds include hydrocyanic acid, phytic acid, oxalic acid, and tannins (Akwaowo *et al.*, 2000). When taken at low concentrations, however, anti-nutrients like phytate, lectin, tannins, amylase inhibitors, and saponins have also been shown to have beneficial actions such as reducing blood glucose and insulin responses to starchy foods and/or plasma cholesterol and triglycerides (Thakur *et al.*, 2019). It is also reported that saponins enhance immunological activity, anti-inflammatory activity, anti-tumor activity, anti-aging activity, and hepatoprotective activity (Guet *et al.*, 2020). Martins *et al.* (2020) referred to condensed tannins as proanthocyanidins, a major class of phenolic compounds that are chemically referred to as flavonoid polymers, and phenolics and flavonoid compounds have been associated with hydroxyl radical scavenging activity and antimicrobial activity (Wang *et al.*, 2021; Chaves *et al.*, 2020; Guet *et al.*, 2020).

Saponin as reported by Gu *et al.* (2020) in *Moringa oleifera* seed after extraction with

ethanol, petroleum ether, and water, respectively, was 20.621.67, 28.723.53, and 77.190.27 mg GEs/g. These values are higher than those obtained in this research, and this could be attributed to the solvent used in the preparation of the extract, which in this research was methanol. The maximum allowable intake of some of these anti-nutrients is 250 mg/kg for hydrocyanic acid, 40 to 50 mg/day for oxalic acid, and 100 to 400 mg/day for phytic acid (Akwaowo *et al.*, 2000). The low tannin concentration, especially in the seed cake (Table 3), further suggests it can be used as a component in animal food compounding. Low values of tannins in this research corroborate the literature report of Gu *et*

al. (2020), who also reported low values of tannins in *Moringa oleifera* seed extracted using ethanol, petroleum ether, and water of 0.450.06, 1.530.49, and 0.480.12 mg CEs/g, respectively.

3.4 Elemental composition of *V. paradoxa* parts

The mineral composition profile of *V. paradoxain* mg/kg is shown in Figure 1. There is no significant difference at $P < 0.05$ in the elemental composition of *V. paradoxa* among the root, stem bark, leaves, and seed cake.

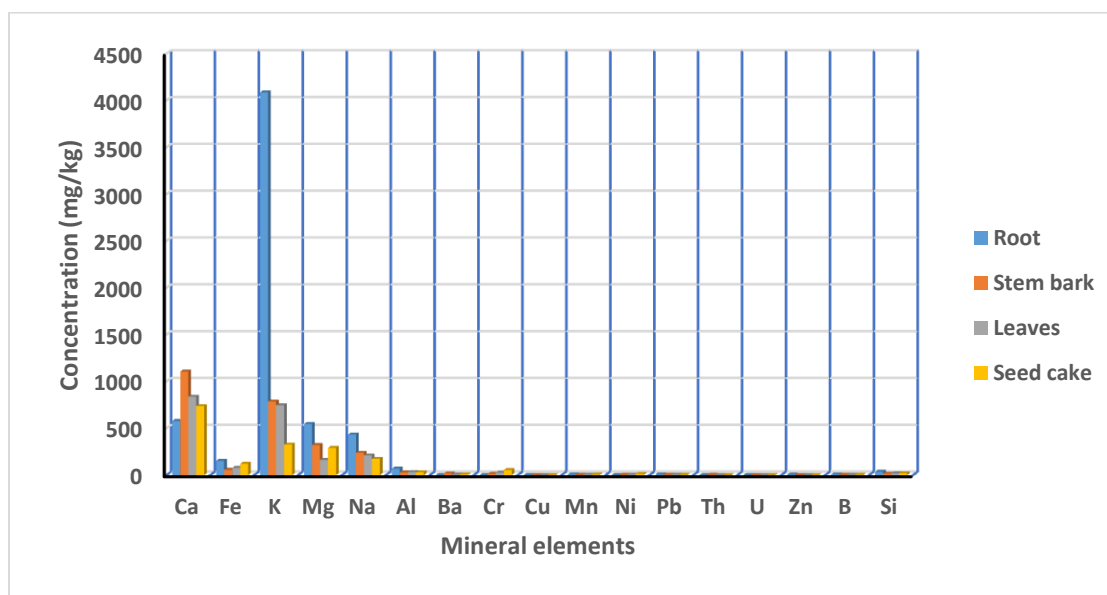


Figure 1: Elemental constituents of *V. paradoxa* parts

The importance of mineral elements in human, animal, and plant nutrition has been well recognized; they are needed for vital biological functions such as the construction and maintenance of bones and the normal function of nerves and muscles in humans and other vertebrates, a function that requires large amounts of calcium; the synthesis of adenosine triphosphate (ATP); and nucleic acid, of which phosphorus is an important constituent; and they

are also essential for acid-base balance, bone formation, and tooth formation (Alagawany *et al.*, 2021). Red blood cells cannot function properly without iron in hemoglobin, the oxygen-carrying pigment of red blood cells. Like vitamins and other essential food nutrients, mineral requirements vary with species. Minerals are inorganic nutrients that are usually required in small amounts, from less than 1 to 2500 mg per day, depending on the mineral (Soetan *et al.*,

2010). Humans and other organisms get their required minerals from food intake. A deficiency of some of these minerals can lead to disease conditions. Major minerals such as potassium (K) and phosphorus (P) are required in amounts of up to 10 g d^{-1} . The daily requirement of secondary and microminerals ranges from 400 to 1 500 mg d^{-1} and $45 \mu\text{g d}^{-1}$ to 11 mg d^{-1} (Gupta and Gupta, 2014). In poultry, minerals are required as an activator of hormones and enzymes, for the skeleton and eggshell formation and replacement, and for the maintenance of acid-base balance and osmotic homeostasis. Sodium (Na), potassium (K), and chlorine (Cl) are indispensable. The presence of important mineral elements such as potassium (K), calcium (Ca), sodium (Na), iron (Fe), and magnesium (Mg) (Figure 1) in appreciable quantities in all plant parts implies that *V. paradoxa* could serve as a potential

alternative source of these elements for consumption. Potassium is the most abundant of all the elements found in the roots of *V. paradoxa*, having the highest amount of 4086.50 mg/kg (Figure 1). Potassium plays a critical role in blood clotting in addition to other metabolic functions, according to Hinsinger (2020).

3.5 Phytochemical Composition

The phytochemicals present in the different parts of the plant were quantified using standard methods, and the results are presented in Table 3. There is no significant difference at $P < 0.05$ in the occurrence of alkaloids, flavonoids, tannins, saponins, and phenolics in the root, stem bark, leaves, and seed cake of *V. paradoxa*. In decreasing order, phytochemical content was: phenolics > flavonoids > tannins > saponins > alkaloids.

Table 3: Quantitative Phytochemicals composition of *V. paradoxa* plant parts

Phytochemicals	Plant parts(mg/100g)			
	Root	Stem bark	Leaves	Seed cake
Alkaloids (280 nm)	0.76±0.071	0.82±0.030	0.05±0.004	0.61±0.004
Flavonoids (280 nm)	171.60±0.849	135.94±0.113	80.60±0.071	37.69±0.226
Tannins (700 nm)	8.93±0.099	7.67±0.028	8.18±0.099	7.24±0.000
Saponins (780)	2.49±0.028	3.26±0.057	2.73±0.028	4.33±0.304
Phenolics (320 nm)	396.48±0.226	379.62±0.042	324.26±0.042	333.49±0.099

This study has revealed the presence of several phytochemical constituents in the root, leaves, stem bark, and seed cake of *V. paradoxa* (Table 3). The constituents are tannin, saponin, flavonoids, alkaloids, and phenolic acids. Egunyomi *et al.* (2009) observed similar phytochemical compositions in the tropical plants growing in Nigeria and other West African countries. Some of which have been shown to exhibit varying biological activities. They are known to show medicinal activity as well as

physiological activity (Ezeonu and Ejikeme, 2016). Ndukwe *et al.* (2007) evaluated methanol extracts of the root, stem bark, and leaf of *V. paradoxa* for preliminary phytochemical screening. Tannin, saponin, steroids, and alkaloids were present in all the parts, while phlobatannin, phenolic acids, and flavonoids were absent in the plant parts, which are however present in small quantities in this study. This agrees with the report by Ojo *et al.* (2021), who found flavonoids, tannins, and alkaloids in the

methanol solution of the leaf extract of *V. paradoxa*. However, phenolic acids were absent in his report but are present in this study. The presence of these phytochemical constituents may be responsible for some of the reported antibacterial activities observed in this study. Total phenolic content of 101.3 mg gallic acid/g and total flavonoids content of 29.3 mg ruting/g were reported for the leaf extract of *Sisymbrium officinale* in ethanol (Khalid *et al.*, 2022), and these values are lower than 324.26 mg/100g for phenolic content and 80.60 mg/100g of total flavonoids content reported in this research. The differences may be due to different plant species and different solvents used for the extraction.

The importance of alkaloids, saponins, and tannins in various antibiotics used in treating common pathogenic strains was reported by El-Mahmood *et al.* (2008). Also, phytochemicals such as alkaloids, tannins, flavonoids, and saponins have been known to produce a definite physiological action on the human body (Edeoga *et al.*, 2005). The presence of alkaloids indicates that they may act as good sedatives and have antispasmodic properties (Makin and Gwarzo, 2020). The presence of phytochemical constituents such as saponins, tannins, alkaloids, and phenolic acids in the plant parts under study is also an indication that the plant is a potential antimicrobial agent; it also possesses some antioxidant, vasodilating, and anticancer potential. Flavonoids are reported to have many pharmacological actions by acting as anti-cancer, antioxidant, anti-hypertensive, and anti-cholinesterase agents. Flavonoids have shown positive results in the treatment of pregnancy-induced disorders. They had also been proven to show greater potential in the treatment of neurodegenerative diseases (Mondal and

Rahaman, 2020). Phenolics are important antimicrobial compounds with excellent inhibitory activity against pathogenic bacteria and fungi, which further extends to drug-resistant and biofilm-forming microorganisms (Ahmad *et al.*, 2021). These two phytochemicals, flavonoids and phenolics, are found in the highest concentrations in all *V. paradoxa* parts examined (Table 3). These may be responsible for the traditional use of the plant in the treatment of infections, as reported by Ojo *et al.* (2021).

3.6 Antioxidant Activities of the Methanol Extracts of *V. paradoxa* Parts

The antioxidant activities of extracts from the different plant parts were evaluated using both DPPH scavenging, NO scavenging, and ferric reducing antioxidant power (FRAP) assays. Analysis for total phenols and flavonoids was also carried out to determine the total antioxidant capacity (TAC) of the extracts. Table 4 shows the results of the total phenol, total flavonoid, and TAC for the different plant parts. There was a significant difference at $P < 0.05$ between the percentage total antioxidant capacity and the total phenolics ($\mu\text{g/ml}$) and total flavonoids ($\mu\text{g/ml}$). The total antioxidant was weakly negatively correlated with the total phenolics ($R = -0.0905$) and was not significant at $P < 0.05$ with a p-value of 0.885563. While the total antioxidant was strongly negatively correlated with total flavonoids ($R = -0.9187$), the result was significant at $P < 0.05$ with a p-value of 0.27485. Many authors link antioxidant activity to phenolic and flavonoid compounds present in medicinal plant extracts or natural products (Hernanz *et al.*, 2023; Osset *et al.*, 2023; Ramon-Sierra *et al.*, 2023; Isaac *et al.*, 2022a; Otmani *et al.*, 2021).

Table 4: Total antioxidant activity

Plant Extract	Total Antioxidant Capacity (%)	Total Phenol ($\mu\text{g/ml}$)	Total Flavonoid ($\mu\text{g/ml}$)
Root	42.41 \pm 0.35	37.39 \pm 0.44	26.43 \pm 0.38
Stem bark	41.59 \pm 0.23	36.30 \pm 0.44	26.80 \pm 1.03
Leaves	36.07 \pm 0.28	35.13 \pm 0.57	29.84 \pm 0.45
Seed cake	42.24 \pm 0.23	32.79 \pm 0.45	24.84 \pm 0.33

The antioxidant activity of polyphenols is mainly due to their ability to act as hydrogen donors, reducing agents, and/or radical scavengers (Lee *et al.*, 2017). The relationship between the studied parameters was assessed through Pearson correlation. This study revealed positive and significantly high correlation coefficient values between total phenolics and DPPH scavenging ($r^2 = 0.8977$) (Figure 2, Table 5) activity and a weak negative correlation to nitric oxide scavenging ($r^2 = -0.0578$) (Figure 3, Table 6) activity. On the other hand, total flavonoids showed a weak negative correlation coefficient of $r^2 = -0.0754$ with DPPH scavenging (Figure 2) and a strong positive correlation ($r^2 = 0.8633$) with nitric oxide scavenging (Figure 3) activity. This indicates that the flavonoid compounds present are significantly contributing to the total antioxidant activities of the *V. paradoxa* plant, corroborating a previous report by Kouakou *et al.* (2021). Free radicals assimilated from the environment or generated from cellular processes have been found to be responsible for oxidative stress, which in turn could lead to many physiological conditions including diabetes, cancer, cardiovascular diseases, and so on (Engwa, 2018). Phytochemicals with high free radical scavenging potential, such as flavonoids, are now being used in the prevention and management of oxidative stress-related diseases.

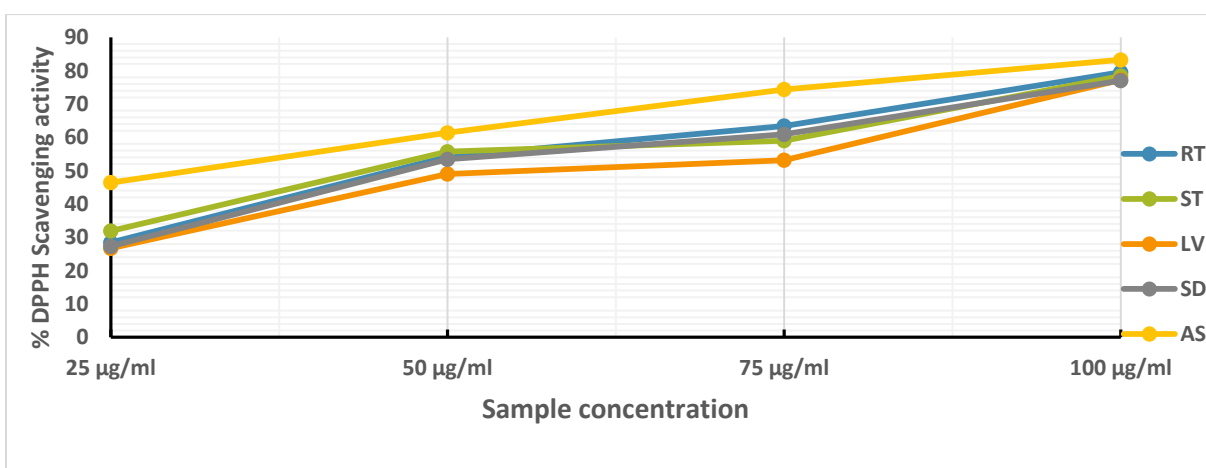
3.6.1 DPPH Scavenging Activity and IC_{50}

The 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging test was carried out based on standard methods, and half maximal inhibitory concentration (IC_{50}) values were calculated from the results. These results were compared with the scavenging activities of ascorbic acid, a common commercial antioxidant, and the results are shown in Table 5. The trend of DPPH scavenging across the different *V. paradoxa* parts is graphically shown in Figure 2.

DPPH is a stable free radical, and it is widely used to assess the radical scavenging activity of antioxidant compounds. The method is based on the reduction of DPPH in methanol solution in the presence of hydrogen-donating antioxidants due to the formation of the non-radical form DPPH-H (Zhang *et al.*, 2014). According to Gomes de Melo *et al.* (2010), antioxidant activity can be classified based on the performance of the crude extract as good activity ($IC_{50} < 69 \mu\text{g/ml}$); moderate activity ($69 \mu\text{g/ml} < IC_{50} < 161 \mu\text{g/ml}$); and low activity ($IC_{50} > 161 \mu\text{g/ml}$). The scavenging of DPPH radicals was found to rise with increasing concentrations of the samples (Table 5). The highest scavenging was observed with the root sample at 100 $\mu\text{g/ml}$ with an IC_{50} value of 52.63 $\mu\text{g/ml}$, while the least scavenging was observed with the leaf sample at 25 $\mu\text{g/ml}$ with an IC_{50} value of 60.02 $\mu\text{g/ml}$.

Table 5: DPPH scavenging activity and IC₅₀ result

Sample	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	IC ₅₀	R ²
Root	28.42 ± 1.27	54.24 ± 0.69	63.44 ± 0.57	79.56 ± 0.34	52.63	0.9634
Stem bark	31.92 ± 0.69	55.70 ± 0.69	59.04 ± 1.50	78.42 ± 0.11	51.52	0.9302
Leaves	26.71 ± 0.47	49.03 ± 0.23	53.18 ± 0.81	77.28 ± 0.34	60.02	0.9382
Seed cake	27.28 ± 2.19	53.42 ± 0.69	60.91 ± 0.69	77.07 ± 0.58	54.54	0.9466
Ascorbic Acid	46.46 ± 0.35	61.38 ± 0.13	74.40 ± 0.17	83.25 ± 0.73	29.33	0.9845

**Figure 2:** DPPH Scavenging activity of *V. paradoxa* root (RT), stem bark (ST), leaves (LV), seed cake (SD) and Ascorbic Acid (AS)

This observation suggests that the stem bark of *V. paradoxa* has the highest radical scavenging activity. This finding corroborates an earlier report by Talla et al. (2016). The scavenging activities of the extracts from all the samples closely compete with those of ascorbic acid as the concentrations tend towards 100 µg/ml, as can be seen from Figure 2.

3.6.2. Nitric Oxide Scavenging Activity and IC₅₀

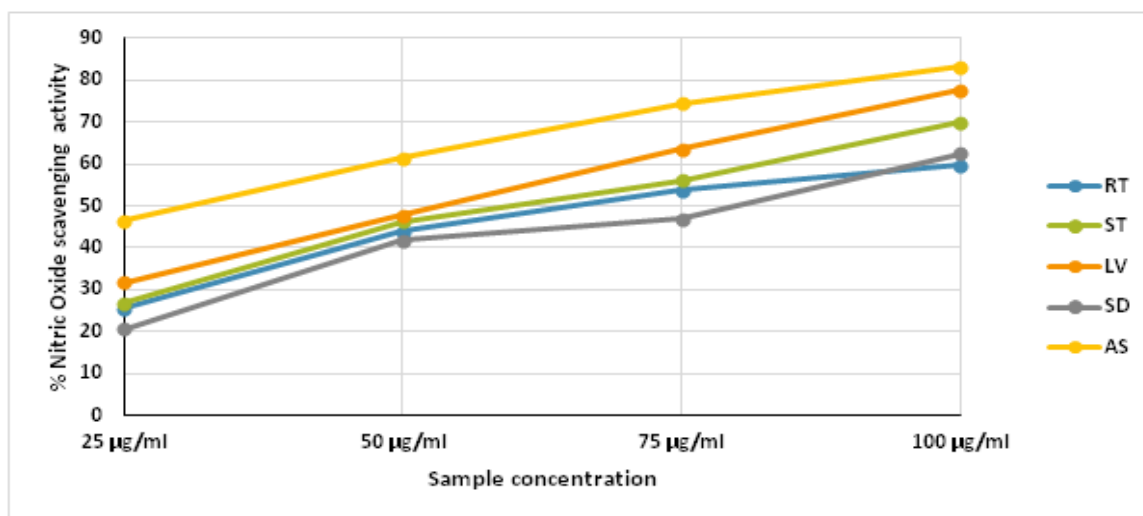
The nitric oxide scavenging activities of the various plant extracts and the IC₅₀ values were compared with those of ascorbic acid. The result

is shown in Table 6. Figure 3 graphically shows the trend of nitric oxide scavenging across the different *V. paradoxa* parts.

Nitric oxide scavenging activities were found to increase with the increase in concentration in all the plant parts extracted (Table 6), similar to the observation in DPPH scavenging. In contrast, however, the highest scavenging was observed in the leaf extract at a 100 µg/ml concentration with an IC₅₀ value of 54.15 µg/ml. The nitric oxide scavenging of ascorbic acid was, however, better than that of the extracts at the concentrations of the experiment, as can be seen in Figure 3.

Table 6: Nitric oxide scavenging activity and IC₅₀ result

Sample	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	IC ₅₀	R ²
Root	25.55 ± 0.39	43.99 ± 0.38	53.76 ± 0.48	59.70 ± 0.58	71.99	0.9403
Stem Bark	26.91 ± 0.78	46.31 ± 0.96	56.08 ± 0.48	70.02 ± 0.09	62.81	0.9763
Leaves	31.70 ± 0.39	47.75 ± 0.29	63.53 ± 0.39	77.67 ± 0.29	54.15	0.9987
Seed Cake	20.76 ± 0.39	41.87 ± 0.48	46.86 ± 0.58	62.43 ± 0.38	76.00	0.9522
Ascorbic Acid	46.46 ± 0.35	61.38 ± 0.13	74.40 ± 0.17	83.25 ± 0.73	29.33	0.9845

**Figure 3:** Nitric Oxide scavenging activities of *V. paradoxa* root (RT), stem bark (ST), leaves (LV), seed cake (SD) and Ascorbic acid (AS).

3.6.3 Ferric Reducing Antioxidant Power (FRAP)

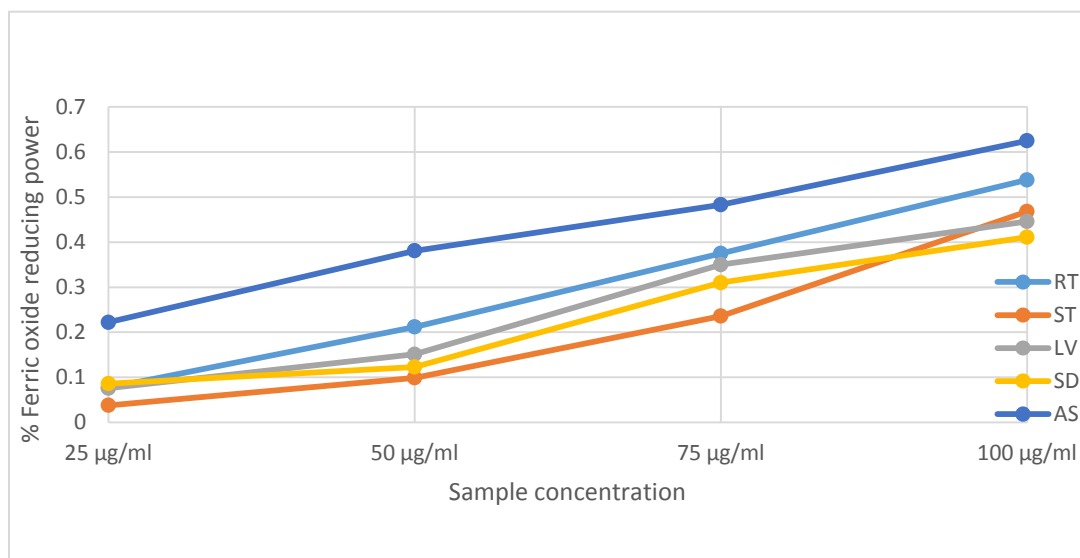
The result of the ferric-reducing antioxidant assay test is presented in Table 7. Ascorbic acid was used as a reference. Figure 4 graphically shows the trend of ferric reducing power of extracts across the different *V. paradoxa* parts.

Fe (III) reduction (Ferric Reducing Power) is often used as an indicator of electron-donating activity, which is an important mechanism of the phenolic antioxidant reaction (Gomes de Melo *et al.*, 2010). Iron is an essential metal for almost all living organisms due to its involvement in many iron-containing proteins and enzymes. However, excess free iron generates oxidative stress,

particularly in the delicate tissues of living organisms, where anti-oxidative defenses are relatively low (Carocci *et al.*, 2018). Antioxidant phytochemicals prevent iron-induced oxidative stress by forming complexes with iron (Fe^{2+} or Fe^{3+}) ions. From the assay results (Table 7), the root extracts showed the highest ferric reduction capacity of $0.538 \pm 0.003 \mu\text{g/ml}$, the closest to the control (ascorbic acid), which is $0.625 \mu\text{g/ml}$, as can be seen in Figure 4. This observation could be responsible for the many traditional therapeutic uses of the root and other parts, as reported by Ndukwe *et al.* (2007) and Falana *et al.* (2016).

Table 7: Ferric reducing antioxidant power assay result

Sample	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml
Root	0.077 ± 0.003	0.212 ± 0.004	0.375 ± 0.006	0.538 ± 0.003
Stem Bark	0.038 ± 0.005	0.099 ± 0.005	0.236 ± 0.011	0.468 ± 0.001
Leaves	0.076 ± 0.005	0.151 ± 0.004	0.350 ± 0.002	0.446 ± 0.003
Seed Cake	0.086 ± 0.002	0.123 ± 0.006	0.310 ± 0.011	0.411 ± 0.003
Ascorbic Acid	0.222 ± 0.005	0.381 ± 0.002	0.483 ± 0.003	0.625 ± 0.001

**Figure 4:** Ferric antioxidant reducing power (FRAP) of *V. paradoxa* root (RT), stem bark (ST), leaves (LV), seed cake (SD) and Ascorbic acid (AS).

3.7 Antimicrobial Activities of Methanol Extracts

In-vitro antimicrobial screening of the methanolic extracts of *Vitellaria paradoxa* plant parts showed appreciable microbial growth inhibition against some gram-positive and gram-negative bacteria, as shown in Table 8. The minimum inhibitory concentration (MIC) was determined by serial dilution of the extract as the

least concentration that showed growth inhibition of the microorganism. Cyproxin (CX), a clinical antibiotic, was used as a positive control. The minimum inhibitory concentration (MIC) for the different extracts against the test organisms and pictures of some selected plates showing growth inhibition after inoculation and incubation are shown in Table 9 and Figure 5, respectively.

Table 8: Antimicrobial screening of methanol extracts of *Vitellaria paradoxa* plant parts

Organism	Diameter of inhibition zone (mm)				
	Root	Stem bark	Leaves	Seed cake	CX
<i>Klebsiella Oxytoca</i>	NI	NI	NI	5	15
<i>Burtholderia Cepacia</i>	10	23	16	25	30
<i>Staphylococcus Aureus</i>	23	25	10	25	28
<i>Enterobacter Faecalis</i>	15	25	10	20	28
<i>Pseudomonas Aeruginosa</i>	20	25	10	20	25
<i>Salmonella Typhi</i>	10	20	15	25	25
<i>Proteus spp</i>	NI	NI	NI	10	20
<i>Monganella Morgani</i>	15	25	10	17	30
<i>Escherichia Coli</i>	NI	15	NI	NI	20

NI = No Inhibition. CX = Cyproxin

Table 9: Minimum inhibitory concentration (MIC) against test organisms

Organisms	Minimum inhibitory concentration (MIC, mg/ml)			
	Root	Stem bark	Leaves	Seed cake
<i>Klebsiella Oxytoca</i>	X	X	X	80
<i>Burkholderia Cepacia</i>	70	40	70	40
<i>Staphylococcus Aureus</i>	40	40	80	40
<i>Enterobacter Faecalis</i>	70	40	80	40
<i>Pseudomonas Aeruginosa</i>	60	40	80	60
<i>Salmonella Typhi</i>	80	60	70	40
<i>Proteus spp</i>	X	X	X	80
<i>Monganella Morgani</i>	70	40	80	70
<i>Escherichia Coli</i>	X	70	X	x

X = MIC could not be determined.

The minimum inhibition concentration of the antimicrobial activity of the ethanol extracts of the root, stem, and leaf of *Petasites hybridus* on *Escherichia coli*, *Salmonella typhimurium*, *Bacillus cerus*, *Staphylococcus aureus*, *Lactobacillus plantarum*, and *Lactobacillus brevis* microorganisms ranges between 0.39 and 87.50 mg/ml for all the extracts and all test microorganisms (Gündüz *et al.*, 2023). The upper limit of the range seems to corroborate this research, whose MIC ranges between 40 and 80

mg/ml. The ethanol extract of the leaf of *Sisymbrium officinale* was also found to show strong antibacterial activity against *E. coli* and *Streptococcus* with a 10 mm and 14 mm zone of inhibition, respectively (Khalid *et al.*, 2022). The strong bacterial activity against gram positive and gram negative bacteria observed in this research could be link to the high phenolic content, flavonoid contents and other phytochemicals present (Khalid *et al.*, 2022).

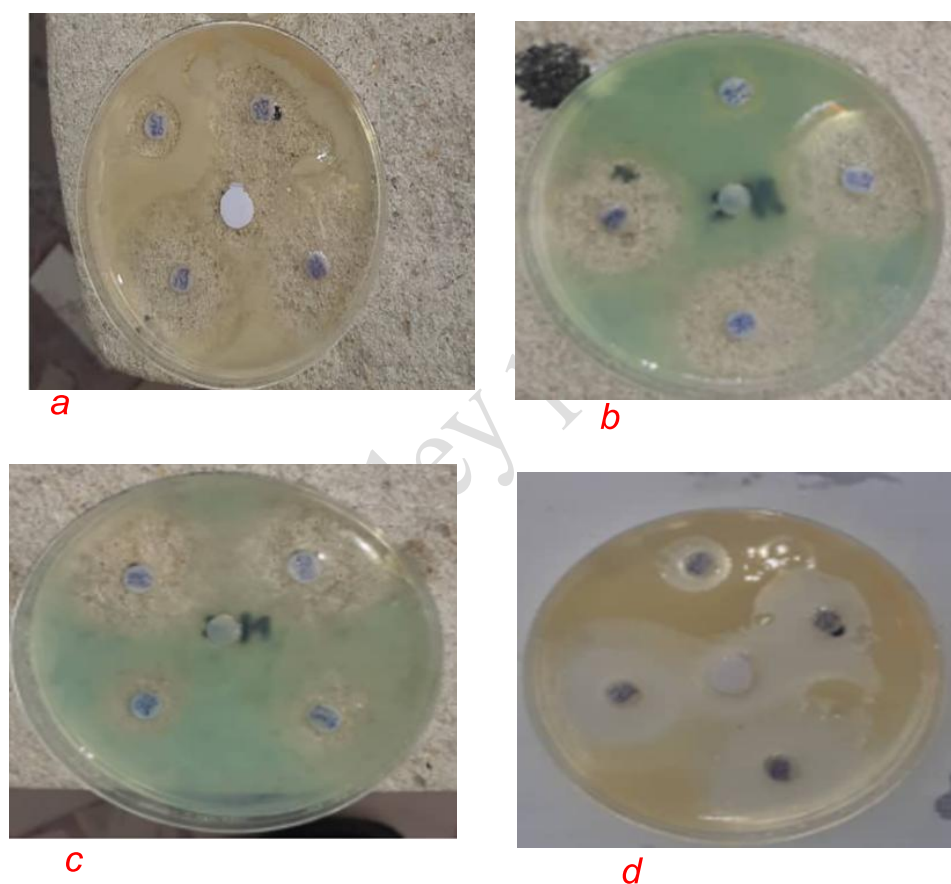


Figure 5: Pictures of the plates showing growth inhibition after inoculation and incubation of *Staphylococcus aureus* (a), *Burkholderiacepacia*(b), *Monganella morgana* (c), and *Salmonella typhi*(d).

Biomedical science has exploited plants as potential sources of drugs to prevent and cure human diseases. The World Health Organization has recognized antimicrobial resistance as a global health security threat that requires action across government sectors and society as a whole

(Kebede *et al.*, 2021). The result of this study showed that all the extracts from the different plant parts demonstrated antimicrobial activity, with the seed cake and stem bark extracts demonstrating the highest activity while the leaf extract had the least activity (Table 8). Variation

in activity among different plant parts and extracting solvents has earlier been reported (Falodun *et al.*, 2006). It was reported that differences in polarity among various solvents could be accountable for the differences in solubility of plant active principles, hence the variation in the degree of activity. Results also showed that the activity of all the extracts was concentration-dependent. Similar results have been reported by several researchers (Ndukwee *et al.*, 2007; Falana *et al.*, 2016; Ojo *et al.*, 2021). The organisms used for the purpose of this investigation are associated with various forms of infections: *Enterobacter faecalis* (stools, dysentery), *K. oxytoca* (pneumonia), *B. cepacia* (wound infections), *E. coli* (gastrointestinal tract infections), and *S. typhi* (typhoid fever) (Prescott *et al.*, 2002). Results of this investigation, therefore, have shown that *V. paradoxa* is a potential source of bioactive phytochemicals with broad-spectrum antimicrobial activity that could be useful in drug development for use against this group of enteric organisms (Table 9, Figure 5). However, so far, preclinical evidence for these activities has not been fully substantiated by clinical studies.

Conclusion

The results obtained from the various screenings carried out on the methanol extracts of the different parts of *Vitellaria paradoxa* and other tests to which these extracts were subjected indicate that the seed cake has a high calorific value (850.68 ± 0.467 Kcal) and crude protein content ($9.95 \pm 0.156\%$). The leaf is rich in carbohydrate ($66.14 \pm 0.255\%$). The ash content varied between 3.34–4.42% among the root, stem bark, leaves, and seed cake. The root of *V. paradoxa* had the highest potassium content (4086.5 mg/kg), while the stem bark had the highest amount of Ca (1105.83 mg/kg). It follows from the results that the different parts of this

plant, particularly the seed cake, can serve as an alternative source of raw materials when compounding animal feed. It was also found that the plant *V. paradoxa* has abundant phytochemicals of biological importance. The appreciable level of antioxidant and antimicrobial activities of the methanol extracts of this plant against some endemic microbes, as seen from the results, also identifies it as a candidate for consideration in the search for phytochemicals of medicinal value in drug discovery.

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