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Qualitative Phytochemical Screening and Antibacterial Activity of Methanolic Stem-Back Extract of *Parkia biglobosa* against Pathogenic Bacterial Isolates

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ABSTRACT

Parkia biglobosa is a medicinal plant traditionally used in West Africa for managing diarrhoeal diseases. This study evaluated the phytochemical composition and antibacterial activity of its methanolic stem-bark extract. The powdered plant material was extracted using Soxhlet apparatus and the extract was screened for the presence of plant secondary metabolites using standard procedures while the antimicrobial activity was determined using both agar well diffusion and broth dilution techniques. Extraction yielded 7.9 g of crude material (15.8%), characterized as dark-brown and gummy. Qualitative screening confirmed the presence of alkaloids, glycosides, flavonoids, saponins, steroids, tannins, terpenoids, and phenols, while reducing sugars were absent. Antibacterial assays revealed dose-dependent inhibition against enteric pathogens, with zones of inhibition ranging from 10.22 mm (Pseudomonas sp.) to 17.09 mm (E. coli) at 250 mg/ml. Minimum Inhibitory Concentration (MIC) values ranged from 3.91 mg/ml (E. coli, K. pneumoniae) to 31.25 mg/ml (P. aeruginosa), while Minimum Bactericidal Concentration (MBC) values extended from 7.81 mg/ml (E. coli) to 125 mg/ml (Proteus mirabilis). These findings highlight the chemical richness and antibacterial potential of P. biglobosa stem bark, supporting its continued investigation as a source of bioactive compounds for therapeutic development. Further studies involving quantitative phytochemical analysis, chromatographic separation and structural characterization of individual constituents are recommended.

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INTRODUCTION

Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world. In rural areas of developing countries, they continue to be used as the primary source of medicine (Chitme *et al.*, 2020). Their biological activities are commonly associated with naturally occurring secondary metabolites, including alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, phenolic compounds and glycosides. These compounds may contribute to the pharmacological properties of plants and provide important starting points for the discovery and development of new therapeutic agents (Chitme *et al.*, 2020).

Parkia biglobosa (Jacq) R.Br.ex. G. Don. is a perennial deciduous legume that reaches 20–30 m high and belongs to the family Fabaceae. The multipurpose tree, which is commonly known as the "African locust bean tree," is indigenous to West Africa and can be found in various subtropical African countries. The tree is widely used in the production of food, beverages, medicine, and non-timber forest resources, with its seeds, fruit pulps, and leaves

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being particularly popular (Komolafe *et al*., 2024). It is indigenous to various West and Central African nations, including Senegal, Guinea, Burkina Faso, Mali, Ghana, Nigeria, Togo, Sudan, and Uganda.

In 1926, the species was named in honor of the esteemed Scottish botanist and explorer, Mungo Park, by Robert Brown (Famojuro *et al*., 2023). The fermented seeds of *Parkia biglobosa* referred to as *Igba* or *Irugba* in Yoruba, *Dorowa* in Hausa, and *Origili* in Igbo—are widely used across Nigeria and much of West Africa as a traditional seasoning in local soups (Salawu *et al*., 2019). The species has considerable nutritional, economic and medicinal importance in the region.

Numerous investigations have indicated that the different parts of the African locust bean plant are abundant in a variety of phytochemicals. According to Zhang *et al*., (2015) the protective role of phytochemicals may be associated with antioxidant activity, since over production of oxidants (reactive oxygen species and reactive oxygen nitrogen species) in the human body is involved in the pathogenesis of many chronic diseases. Several phytochemicals are found in the plant's bark, leaves, and roots (Musara *et al.*, 2020). This extensive ethnomedicinal use has generated interest in investigating the chemical constituents responsible for its biological activities. Previous investigations have reported the presence of several classes of phytochemicals in different parts of *P. biglobosa.* These include flavonoids, tannins, terpenes, saponins, cardiac glycosides, alkaloids and reducing sugars (Salawu *et al.,* 2019). These compounds can act as bacteriostatic or bactericidal agents.

They achieve this by damaging microbial cell walls and membranes, blocking nucleic acid and protein synthesis, disabling key enzymes, and disrupting processes like quorum sensing and biofilm development (Erb and Kliebenstein, 2020). Flavonoids demonstrate antimicrobial activity through multiple mechanisms, such as damaging microbial cell membranes, blocking nucleic acid synthesis, disrupting energy metabolism, and altering microbial signaling pathways (Cushnie and Lamb, 2021).

By interacting with bacterial proteins and enzymes, they hinder essential metabolic functions like DNA gyrase activity and ATP production, ultimately restricting microbial growth and replication (Daglia, 2020). Alkaloids demonstrate strong, wide-ranging antibacterial effects against both Gram-positive and Gram-negative bacteria through diverse mechanisms. Primarily, they compromise the integrity of bacterial cell walls and membranes, leading to disrupted intracellular balance and eventual cell death (Zhang *et al*., 2026).

Antioxidant capacity of Tannins makes it useful in preventing lipid oxidation in foods, extending shelf life, and reducing risks of chronic diseases linked to oxidative stress (Ngoc *et al.*, 2023). Saponins interact with microbial cell membranes due to their amphiphilic structure (hydrophilic sugar chains + lipophilic aglycone), causing pore formation and leakage of cellular contents (Ibrahim *et al*., 2026). However, the phytochemical composition of plant extracts can vary depending on the plant part, geographical origin, extraction method and solvent used.

The Enterobacteriaceae family includes clinically important pathogens such as *E. coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Shigella spp.*, and *Proteus spp.*, which are major causes of diarrhoeal and systemic infections. These Gram-negative bacteria are increasingly associated with multidrug resistance, biofilm formation, and virulence factors that complicate treatment, making them a global public health concern (WHO, 2017). Recent studies confirm that *Parkia biglobosa* stem-bark extracts possess strong antibacterial and antibiofilm activity, attributed to phytochemicals such as alkaloids, flavonoids, tannins, and saponins. Methanolic fractions have shown inhibition zones up to 17 mm against *E. coli* and MIC values as low as 3.91 mg/ml, while also disrupting biofilm formation in resistant strains (Akanni *et al*., 2024; Kabir *et al*., 2024). These findings validate its ethnomedicinal use and highlight its potential as a source of bioactive compounds.

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The choice of extraction solvent is particularly important in phytochemical investigations because compounds differ in their solubility according to their chemical properties. Methanol is widely used for extracting plant constituents because it can solubilize a broad range of polar and moderately non-polar compounds. In the present study, methanol was therefore used to obtain an extract of *P. biglobosa* stem bark for preliminary phytochemical investigation.

The objective of this study was to determine the extraction yield and physical characteristics of the methanolic stem-bark extract of *P. biglobosa*, qualitatively screen the extract for selected phytochemical constituents and determine the Antibacterial Activity of the plant against some members of Enterobacteriaceae family isolated from diarrheic stool sample of under five children (*Escherichia coli, Shigella* spp, *Salmonella typhi*, *Klebsiella* spp, *Proteus* spp and *Pseudomonas* spp), and determine the MIC and MBC of the extract.

MATERIALS AND METHODS

Ethical Approval

Ethical clearance for the collection of isolates was collected from Ethical Committee of the Kano State Ministry of Health with an approval Number NHREC/17/03/2030. And the Isolation of the organisms was conducted at Muhammad Abdullahi Wase Specialist Hospital.

Isolation and Identification of the Test Organisms

The diarrheal sample was collected in a sterile stool container and cultured into liquid and onto solid media for isolation and identification of the bacterial pathogens. Pre-enrichment broth (Selenite F) was used to allow the multiplication of bacteria; and then subsequently sub-cultured onto a MacConkey agar (MCA) and Salmonella - Shigella (SSA) and then incubated aerobically at 37^{o}C for 24 hours (Cheesbrough, 2000). After incubation bacterial growth was observed for colony appearance and morphology. Suspected *E coli* colonies was re-inoculated onto freshly prepared Eosin Methylene Blue (EMB) Agar to obtain a pure colony. The identification process was carried out using Gram staining and Biochemical Test.

Plant Sample Collection and Authentication

The sample of the plant bark was collected from 'Tsakani' Village of Dambatta Local Government, Kano State, Nigeria. The plant was identified at the Herbarium unit, Department of Plant Science and Biotechnology, Bayero University, Kano, Nigeria, with Accession Number BUKHAN 0262.

Drying and Processing of the plant bark

The fresh stem bark of *Parkia biglobosa* collected was prepared as described by (Akwaji *et al.,* 2016). The bark was washed under running tap water, air-dried at room temperature under shade for six days, and then pulverized. The pulverized dried plant bark was homogenized to fine powder. The powdered form was stored in an airtight glass container for further analysis.

Extraction

The powdered material (50g) of the plant was weighed and extracted with 250ml of Methanol using Soxhlet extraction method. The extract was concentrated at 40^{0}C under reduced pressure using rotary evaporator. The concentrated extract was collected in a sterile screw capped bottles and labelled appropriately.

Determination of Percentage Yield

The percentage yield calculated as follows: Percentage yield = Mass of extract /Mass sample taken x 100 (Bunu *et al*., 2021).

Physical Characterization of the Extract

The crude extract was observed for physical characteristics, particularly color and texture, following concentration of the extract.

Phytochemical Screening

Qualitative phytochemical screening was performed to determine the presence or absence of selected secondary metabolites as

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described by (Magashi, A. M. & Abdulmalik, U., 2018) with little modification.

Standardization of the Inoculum

A loopful of the confirmed test isolate was picked using a sterile wire loop from respective nutrient agar slants maintained at 4^0C and standardized by emulsifying in 2ml of sterile physiological saline and match with the 0.5 McFarland standards for sensitivity test as described by Magashi and Abdulmalik, (2018). This turbidity was equivalent to approximately 1.5 × 10^8 colony forming units per ml (cfu/ml).

Preparation of the Test Concentrations

The extract and the standard (Ciprofloxacin) were prepared using doubling dilution method described by Abalaka *et al*., (2010). Stock solution was prepared by weighing 1g of the crude extract and dissolved in 2ml of Dimethyl sulphur oxide (DMSO) which gave 500mg/ml. From the stock solution test concentrations of 250mg/ml, 125mg/ml, 62.5mg/ml and 31.25mg/ml was prepared.

Antibacterial Susceptibility Testing

The plates of Mueller Hinton agar were dried in an oven at 45^0C to remove moisture. Using a sterile swab stick, standardized inoculum of each isolate was swabbed onto the surface of Mueller Hinton agar in separate plate. Four wells of 6mm were made in each plate with a central well for control using sterile Cork borer and 0.1ml of the extracts of the various concentrations was added into the wells. The plates were allowed to stand for 15minutes on the table for free diffusion of the extracts. The plates were incubated at 37^0C and inhibition zones were measured after 24 hours using Vernier caliper (Olukemil and Yvonne, 2005).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The plant extract that showed significant antimicrobial activity was subjected to MIC assay by doubling dilution method in which 1ml of 250mg/ml concentration was added to first test tube containing 1ml of nutrient broth which arrived at 125mg/ml, from there 1ml from the first test tube was serially diluted up to six test tubes that is 125mg/ml, 62.5mg/ml, 31.25mg/ml, 15.63mg/ml, 7.81mg/ml, 3.91mg/ml. 0.1ml of standardized suspension of the test organisms was then added into each test tubes and the tubes were incubated for 24hrs at 37°C. Tubes containing broth and the extracts without inoculum served as positive control, while tubes containing broth and inoculum served as negative control. After incubation, the tube with the lowest dilution with no turbidity was considered as MIC. Subsequently, those tubes that showed no turbidity were sub-cultured on nutrient agar for bacterial organisms, and absence of growth after incubation was considered as MBC (Akinnibosun and Itedjere, 2013).

RESULTS

From Table 1, Methanolic Extract of *P. biglobosa* was dark-brown and Gummy and the crude extract recovered was 7.9g and has percentage yield of 15.8%.

Table 1: Physical Characteristics of *Parkia biglobosa* Methanolic Extract

Parameters	Observation
Weight (g)	7.9
Percentage yield (%)	15.8
Color	Dark brown
Texture	Gummy

From Table 2, the methanolic extract of *P. biglobosa* tested positive for alkaloids, glycosides, flavonoids, saponins, steroids, tannins, terpenoids and phenols. Reducing sugars were not detected in the extract.

Table 2: Phytochemical Profile of *P. biglobosa* Methanolic Stem-bark Extract

Phytochemicals	Inference
Alkaloid	+
Glycoside	+
Flavonoid	+
Reducing sugar	-
Saponins	+
Steroids	+
Tannins	+

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Phytochemicals	Inference
Terpenoid	+
Phenols	+

Key: += positive, -= negative

From Table 3, Antibacterial assays revealed dose-dependent inhibition against enteric pathogens, with zones of inhibition ranging from 10.22 mm (*Pseudomonas sp.*) to 17.09 mm (*E. coli*) at 250 mg/ml.

Table 3: Antibacterial Activity of *Parkia biglobosa* Methanolic Extract against Isolated Organisms

Organism	Concentrations (mg/ml) /zone of inhibition (mm)				
	250	125	62.5	31.25	CONTROL(Ciprofloxacin)
E. coli	17.09±0.04^{b}	15.29±0.17^{c}	14.08±0.11^{d}	11.32±0.13^{e}	23.60±0.14^{a}
Klebsiella sp.	15.26±0.16^{b}	13.49±0.54^{c}	11.58±0.05^{d}	9.45±0.16^{e}	22.04±0.04^{a}
Salmonella sp.	14.31±0.19^{b}	12.83±0.08^{c}	10.53±0.16^{d}	8.11±0.08^{e}	23.49±0.16^{a}
Shigella sp.	12.09±0.00^{b}	10.42±0.43^{c}	9.39±0.15^{d}	7.89±0.00^{e}	23.35±0.11^{a}
Pseudomonas sp.	10.22±0.18^{b}	8.81±0.21^{c}	7.95±0.08^{d}	7.34±0.17^{d}	21.22±0.00^{a}
Proteus sp.	11.16±0.18^{b}	10.02±0.10^{c}	9.39±0.08^{d}	7.03±0.03^{e}	23.27±0.19^{a}

P<0.05

Table 4: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Parkia biglobosa* Methanolic Extracts against the isolated organisms (Concentration in mg/ml)

Isolate	MIC	MBC
E. coli	3.91	7.81
K. pneumoniae	3.91	15.63
Samonella typi	7.81	15.63
Shigella dysentriea	15.63	62.5
P. aeruginosa	31.25	125
Proteus mirabilis	15.63	125

DISCUSSION OF FINDINGS

Extraction of *Parkia biglobosa* with methanol produced 7.9 g of crude extract, corresponding to a percentage yield of 15.8%. The appreciable recovery obtained in the present study suggests that methanol was effective in solubilizing and recovering extractable constituents from the plant material. The efficiency of plant extraction is influenced by several factors, including solvent polarity, plant part, particle size, solvent-to-sample ratio, extraction time and temperature. The recovery of extract, and consequently the percentage yield, is predominantly influenced by the fiber composition of the plant material under investigation. Samples characterized by elevated fiber content exhibited markedly reduced extraction yields, whereas those with lower fiber content demonstrated substantially higher yields (Abdulhamid and Sani, 2019).

Logtong and Quansah (2021) demonstrated that methanol extraction of *P. biglobosa* seeds produced appreciable phenolic constituents and antioxidant activity, further supporting the effectiveness of methanol for recovering bioactive constituents from the plant. The dark-brown color observed in the crude extract may be associated with the concentration of phenolic compounds, tannins, flavonoids and other colored phytoconstituents during solvent evaporation. The gummy texture of the extract indicates that evaporation of methanol resulted in a concentrated semi-solid residue containing non-volatile constituents of the plant and may extract more resinous or mucilaginous compounds.

The detection of multiple secondary metabolite classes indicates the chemically diverse nature of the stem bark and supports the suitability of methanol for extracting a broad spectrum of bioactive constituents. This finding is consistent with a recent study by Sut *et al.* (2024), in which chromatographic profiling of *P. biglobosa* stem-bark extracts identified flavonoids, hydroxycinnamic acid derivatives and gallotannins, with methanolic extracts showing

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particularly appreciable phenolic-related antioxidant activity. Similarly, Kabir *et al*., (2024) reported alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, steroids and phenolic compounds in different parts of *P. biglobosa*, with the bark showing comparatively higher levels of several phytochemical groups. (Umar *et al*., 2021) identified alkaloids, glycosides, tannins, and saponins as dominant constituents in the leaves and bark of *Parkia biglobosa*. Entonu *et al*., (2023) detected Saponins, Tannins, Steroids, Alkaloids, Flavonoids, Cardiac glycosides and Anthraquinone in Aqueous and Ethanolic extract of *P. biglobosa* stem bark.

Cardiac glycosides, Tannins, Alkaloid and Steroids were present in the Hexene, Ethyl acetate, Ethanol and Water Extracts, but there was complete absence of saponins and anthraquinones in both extracts as reported by Edith (2019). In the findings of Saleh *et al*., (2021), the leaves of *P. biglobosa* contain glycosides, tannins, and alkaloids in trace amount. Salawu *et al*., (2019) claimed that Carbohydrates, Free reducing sugars, combined reducing sugars, Tannins, Alkaloids and Saponins were confirmed in the hot water extract of *Parkia biglobosa* stem bark, but Terpenes, Sterols, Anthraquinones, Cardiac glycosides and Flavonoids were absent in the extract. Phenols, Flavonoids, Steroid, Glycosides Terpenoids ,Saponins, Carbohydrate and Reducing Sugar were present in the ethanolic leaf extracts *Parkia biglobosa* but Alkaloids and Tannins were not detected (Soumana *et al.*, 2024).

The results presented in Table 3 demonstrate that the methanolic extract of *Parkia biglobosa* exhibits notable antibacterial activity against a range of pathogenic organisms, though its efficacy varies across species and concentrations. At the highest concentration (250 mg/ml), the extract produced inhibition zones ranging from 10.22 mm against *Pseudomonas* sp. to 17.09 mm against *E. coli*, indicating stronger activity against enteric bacteria compared to opportunistic pathogens. The relatively higher susceptibility of *E. coli* and *Klebsiella* sp. compared to *Pseudomonas* sp. and *Shigella* sp. may reflect differences in cell wall permeability and resistance mechanisms, as Gram-negative bacteria such as *Pseudomonas* are known for their multidrug resistance traits (Poole, 2011).

However, the control (Ciprofloxacin) consistently produced larger inhibition zones (21.22–23.60 mm), suggesting that while *P. biglobosa* extract is effective, it remains less potent than conventional antibiotics under the tested conditions. This is similar with the finding of El-Mahmood and Ameh (2017) who explained that Conventional antibiotics and synthetic antibacterial agents are manufactured through standardized, reproducible processes, ensuring consistency and quality. In contrast, herbal medicinal products, derived from plant and animal sources, are more susceptible to contamination and degradation due to their natural composition and less controlled preparation methods. The significant interaction between concentration and organisms ($p<0.05$) highlights that bacterial response is strongly dependent on dosage, reinforcing the importance of concentration in antimicrobial efficacy.

The results presented in Table 4 demonstrate that the methanolic extracts of *Parkia biglobosa* exhibit varying degrees of antibacterial activity against the tested organisms, with both MIC and MBC values indicating differential sensitivity. The lowest MIC (3.91 mg/ml) was observed against *E. coli* and *K. pneumoniae*, suggesting strong inhibitory potential, while *Pseudomonas aeruginosa* required the highest concentration (31.25 mg/ml), reflecting its relative resistance.

Similarly, bactericidal activity was most effective at lower concentrations for *E. coli* (7.81 mg/ml), but much higher levels were needed for *Shigella dysenteriae* (62.5 mg/ml) and *Proteus mirabilis* (125 mg/ml). This aligns with the result obtained by Akanni *et al* (2024), the antimicrobial agent of aqueous fraction from the stem-bark methanol extract of *parkia biglobosa* demonstrated moderate to strong inhibitory activity across all tested bacteria, with MIC values ranging from 3.125 to 25 mg/ml. However, the corresponding MBC values were consistently higher (up to 50 mg/ml), indicating that while growth inhibition was achievable at lower

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concentrations, complete bacterial eradication required substantially stronger doses.

CONCLUSION

The methanolic stem-bark extract of *Parkia biglobosa* produced a 15.8% extraction yield and was characterized by a dark-brown colour and gummy consistency. Qualitative phytochemical screening revealed the presence of alkaloids, glycosides, flavonoids, saponins, steroids, tannins, terpenoids and phenols, while reducing sugars were not detected. The broad spectrum of phytochemical classes identified provides preliminary evidence of the chemical richness of *P. biglobosa* stem bark and supports further investigation of the plant as a potential source of biologically active compounds.

For the Antibacterial activity of *Parkia biglobosa*, at 250 mg/ml, the extract showed clear antibacterial activity, with inhibition zones ranging from 10.22 mm against *Pseudomonas* sp. to 17.09 mm against *E. coli*. This indicates stronger effectiveness against enteric bacteria compared to opportunistic pathogens. The lowest MIC value (3.91 mg/ml) was recorded for *E. coli* and *K. pneumoniae*, highlighting their high susceptibility to the extract. In contrast, *Pseudomonas aeruginosa* required the highest MIC (31.25 mg/ml), underscoring its greater resistance.

RECOMMENDATIONS

Further studies should:

1. Quantify the individual phytochemical constituents present in the methanolic extract.
2. Separate and purify individual compounds using chromatographic techniques.
3. Characterize isolated compounds using appropriate spectroscopic methods.
4. Investigate the relationship between the identified constituents and specific biological activities.
5. Evaluate the safety and pharmacological properties of purified compounds before any therapeutic application.

Appendix

Figure 1: *Parkia biglobosa* (African Locust bean) Tree

Figure 2: Soxhlet Extraction Set-up

Figure 3: Qualitative Phytochemical Test

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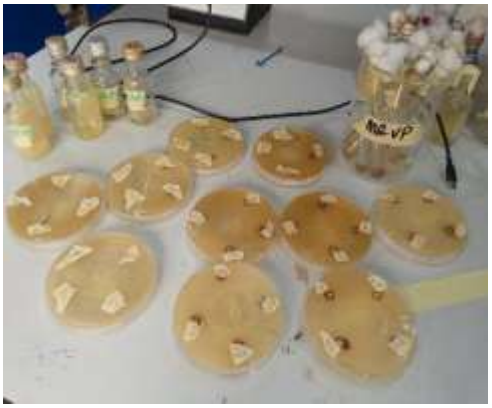

Figure 4

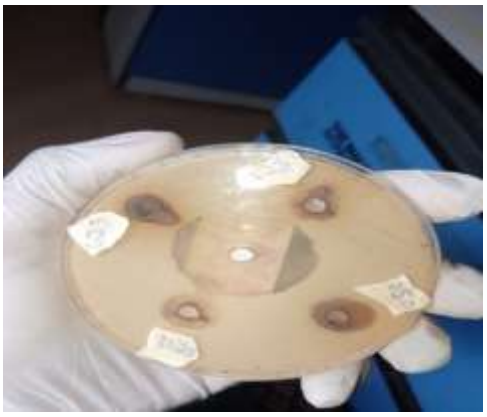

Figure 5
Figure 4 and 5: Zone of Inhibition

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