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Evaluation of Antibacterial Activity of *Parkia biglobosa* (African Locust Beans) Extracts against Multidrug Resistant *Salmonella* Species

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Abstract

There is global resurgence in the use of herbal preparations and in some developing countries like Nigeria and it is being gradually integrated into the primary and secondary health care systems. The study aimed to evaluate the antibacterial activity of P. biglobosa leaf extracts against clinical isolates of Salmonella species. Phytochemical screening of the extracts was conducted using conventional method. Agar well diffusion method was used to evaluate the antibacterial activity of the extract while broth dilution method was employed for determination of Minimum inhibitory concentration (MIC). The results showed presence of flavonoids, terpenoids, tannins, alkaloids, cardiac glycosides and anthraquinones. Antibacterial activity of the extract show activity against the test isolates, with the inhibition zones increasing with concentration. The n-hexane extracts generally exhibits a higher inhibitory effect (with average activity of 12.5mm) compared to the aqueous extract (10.6mm). The MIC of the extracts indicated that the n-hexane extract inhibited the growth of the isolate at 12.5 – 25 mg/ml while aqueous extract at 25 – 50 mg/ml. the MBC of n-hexane extract ranges from 12.5 – 50 mg/ml while aqueous extract ranges from 25 – 75 mg/ml. It is concluded that the extract of P. biglobosa leaf was active against Salmonella species isolated from gastrointestinal infection patients

Keywords: *Antibacterial activity, multidrug resistance, Parkia biglobosa, Salmonella*

Introduction

The development of resistant genes in bacteria is one of the mechanisms that support their natural adaptation to the presence of antimicrobial agents. Resistance to drugs by microorganisms is increasing despite the fact that pharmaceutical industries are producing a number of new antibiotics (Clark *et al.*, 2023). Infections as a result of recurrent multiple antimicrobial resistant have claimed at least 50,000 lives in many parts of the world. It is estimated that if there is a continuous rise in resistance levels by 2050 it would lead to 10 million deaths annually (Fida *et al.*, 2021). There is global resurgence in the use of herbal preparations and in some developing countries like Nigeria; it is being gradually integrated into the primary and secondary health care systems. Nearly all societies have used herbal materials as sources of medicines and the development of these herbal medicines depended on local botanical flora (Ain *et al.*, 2022). Several plants are indicated in folk and other traditional systems of medicines as anti-infective agents. As a result, different remedies evolved in different regions of the world as communications got improved. The scientific literature is full of reports of antimicrobial activity of plants and their secondary metabolites and scientific evaluation of these plants remains an area of intense investigations (Patel and Gohil, 2018).

Plants play a pivotal role in the prevention or treatment of diseases and thus, reduce the adverse effects of conventional treatments. An essential part of the investigation of plants is the identification of biologically active compounds in the plant, leading to further biological and pharmacological studies (Hayat *et al.*, 2020). The plant kingdom represents a resource pool of species with potent medicinal potentials. They constitute the richest source of pharmaceuticals, nutraceutical and folk medicine products across the globe. The increasing side-effects of synthetic drugs on humans and their influence on the evolution of resistant microbial strains triggered research into plant resources and their derivatives as suitable alternative therapeutics. The natural products in plants will be continued to be exploited towards meeting the urgent need to develop new and effective drugs, since plant plays a leading role in the treatment of human diseases (Jacob *et al.*, 2021).

Parkia (tree) is a flowering plant genus that belongs to the Fabaceae family (subfamily, Mimosoideae) and has a pan-tropical range. In 1995, thirty-one species of this genus will be reported (Fitria *et al.*, 2019). In 2009, four more species will be identified. Ten species are found in Asia, four in Africa, and twenty in the Neotropics. Meanwhile, according to a plant list (2018), the genus Parkia has 80 scientific names, with 41 approved names and 39 synonym species (Behuria and Sahu, 2020). The presence of nutrients and phytochemicals in African locust beans has been linked to their medicinal importance. Enormous reports demonstrate that plants from genus *Parkia* possess medicinal values, attributable to the presence of pharmacological active compounds. Taken together, two most studied species, *P. biglobosa* and *P. speciosa*, show potential as antidiabetic, antihypertensive, and antimicrobial, to name a few (Ramu *et al.*, 2016). Phytochemical investigations indicated terpenoids (monoterpenoids, diterpenoids, and triterpenoids), phenolics acids and flavonoids (flavonols, isoflavone, flavanone, and flavan-3-ols) are the major chemical constituents present in the species of this genus, which are responsible for their diverse pharmacological activities. It seems that certain phytoconstituents in Parkia have their unique pharmacological effects (Ward *et al.*, 2017). β-Sitosterol and stigmasterol, for instance, could be investigated further and be developed as hypoglycemic agents; cyclic polysulfides, such as antimicrobials; lectins and monosaccharide saponins for anticancer treatment; and polyphenols, most possibly catechin and its derivatives, and active peptides for blood pressure-lowering effect. In view of that the, study aim to evaluate the antibacterial activity of *P. biglobosa* leaf extracts against clinical isolates of *Salmonella*

Materials and Methods

Study Sites

The plant samples were collected at Darki town, Wudil local Government and stool samples were collected at Murtala Muhammad Specialist Hospital while the study was conducted at Aliko Dangote University of Science and Technology, Wudil Kano State. Wudil local government area is located in the east-central area of Kano state and in the central Kano region between longitude 11'80"5 and latitude 11'08"17 North with its headquarters in Wudil town. The area had an average amount of rainfall of about 800 – 900 mm and also had an annual temperature range between 26 – 33°C and a population figure of 176,623 people while covering an area of about 640 square kilometers.

Ethical Clearance

Ethical approval with reference number SHREC/2023/4034 was obtained from Kano State Ministry of Health with consent of the management of Murtala Muhammad Specialist Hospital (Ref No.: MMSHZ/GEN/0832/I). Confidentiality was ensured in accordance with standards of medical practice as previously described by Zakir *et al.* (2021).

Collection and Identification of Plant Material

The fresh plant (leaves) was obtained in Darki town, Wudil Local Government Area. The plant was identified at the herbarium section, Department of Plant Biology, Bayero University, Kano, and an accession number was obtained (BUKHAN 0262). The samples were taken to the Microbiology laboratory, Aliko Dangote University of Science and Technology, Wudil for further studies as previously described by Aliyu *et al*., (2020).

Preparation of Extract

The leaves of the *P. biglobosa* tree obtained were dried under the shade to reduce the moisture content. The dried leaves were ground into fine powder using mortar and pestle. One hundred and seventy grams (175g) were transferred into a Soxhlet extractor with 250 ml of n-hexane. The method of Jamilah *et al.* (2020) was employed to obtain an aqueous extract from the plant with some modifications. This was performed using a Soxhlet extractor by soaking 30g of the pulverized sample in 300 ml of distilled water for one hour and filtering it with Whatman No. 1 filter paper. The extracts were stored at 4 °C before use**.**

Phytochemical Screening of the Plant Extracts

The leaf extract of the plant (*Parkia biglobosa*) was subjected to the following phytochemical screenings: alkaloids, flavonoids, phenols, triterpenes, steroids, saponins, and tannins, according to the method described by Wang *et al.* (2022).

Test isolates

Clinical isolates of *Salmonella* were isolated from gastrointestinal infected patient attending Microbiology laboratory of Murtala Muhammad Hospital Kano. Isolates were further subjected to the following biochemical identification protocols after which the isolates were inoculated onto nutrient agar slants for molecular characterization (Yu *et al*., 2021).

Determination of Multidrug Resistance Isolates

The antibiotic susceptibility test (AST) was done by the disk diffusion method as adopted by Aliyu *et al*. (2021). Seven commonly used antibiotics under seven classes were employed: penicillins (amoxicillin- 30 μg), fluoroquinolones (ciprofloxacin- 5 μg), amphenicols (chloramphenicol 30 μg), polypeptides (colistin- 10 μg), aminoglycosides (gentamicin- 10 μg), tetracyclines (tetracycline- 30 μg), and cephalosporins (ceftazidime- 30 μg).

Determination of Antibacterial Activity

The method of Patel and Gohil (2018) was adopted. Mueller-Hinton agar was prepared according to the manufacturer's instructions. The inoculum of the test organism was prepared by picking discrete colonies of the organisms from the nutrient agar and transferring them into sterile distilled water in a bijou bottle to obtain a turbidity equivalent to 0.5 McFarland turbidity standards. The suspension was seeded evenly onto the surface of Mueller-Hinton agar plates in duplicates with a sterile swab stick. Using a 6mm diameter sterile Cork borer, 4 wells were made in the agar and labeled as 100 (mg/ml), 50 (mg/ml), 25 (mg/ml), and 12.5 (mg/ml) representing the pure extract. The labeled wells were aseptically filled with their corresponding concentrations of the extract. A control was made using ciprofloxacin. The plates were incubated at 37°C for 24 hours and examined for zones of inhibition (Fida *et al*., 2021).

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentrations of the extract and fractions were prepared by serial doubling dilution using distilled water to obtain concentrations of 2000 μg/ml, 1000 μg/ml, and 500 μg/ml. Equal volumes (2 ml) of extract and Mueller-Hinton broth were mixed. Specifically, 0.1 ml of standardized inocula (1.0×10^8 CFU/ml) was added to each of the test tubes (Wang *et al*., 2022). The tubes were incubated aerobically at 35°C for 24 hours. Tubes containing broth and leaf extracts without inocula served as positive controls, while tubes containing broth and inocula were observed as negative controls. The tubes were observed after 24 hours of incubation to determine the minimum inhibitory concentration, which is the lowest concentration that showed no evidence of growth (Rasheed *et al*., 2020).

Determination of Minimum Bactericidal Concentration (MBC)

The tubes that showed MIC (turbidity) for the test organism and the tubes preceding it in the serial dilution were sub-cultured onto appropriately labeled nutrient agar plates. The plates were incubated at 37°C for 24 hours. A cidal effect was indicated by a lack of visible growth, while a static effect was indicated by visible growth, as described by Aliyu *et al*. (2021).

Results

Phytochemical Constituents of the Extracts

The phytochemical screening of *Parkia biglobosa* in Table 1 shows that both n-hexane and aqueous extracts contain flavonoids, terpenoids, and tannins. However, alkaloids and cardiac glycosides are present only in the aqueous extract, while anthraquinones are present only in the n-hexane extract. Steroids and saponins are absent in both extracts.

Table 1: Qualitative of Phytochemical Constituents of *Parkia biglobosa* leaf

S/N	Phytochemical	n-hexane	Aqueous
1	Alkaloids	-	+
1	Flavonoids	+	+
3	Terponoid	+	+
4	Cardiac Glycoside	+	-
5	Steroids	-	-
6	Tannins	+	+
7	Saponins	-	-
8	Anthraquinones	+	-

Key; + = Positive; - = Negative

Table 2: Distribution of Multi-drug resistant isolate among study subject

Isolates	No. identified	Percentage (%)	*P*-value
MDR	5	50	1.0000
NMDR	5	50	
Total	10	100	

Key: MDR=Multi-drug resistant, NMDR=Non-Multi-drug resistant

Antibacterial Activity of the Extracts

The n-hexane and aqueous extracts of *Parkia biglobosa* show antimicrobial activity against the test isolates, with the inhibition zones increasing with concentration. The n-hexane extract generally exhibits a higher inhibitory effect compared to the aqueous extract, and the control shows the largest inhibition zones, suggesting standard antibiotic effectiveness. The significant difference (P≤0.05) among the means implies variable responses to the extracts (Table 3).

Table 3 Inhibitory activity of n – Hexane and Aqueous Extracts of *P. biglobosa* against Test Isolates

	Isolates/Zone of Inhibition (mm)				
Conc. (mg/m)	**C**	**E**	**G**	**I**	**J**
n-Hexane			.		
12.5	8.62±0.9120[a]	7.22±1.0213[b]	6.9±1.0943[b]	7.54±1.0283[b]	6.25±1.0756[b]
25	10.12±1.91211[a]	9.19±1.8967[b]	9.19±1.1256[b]	9.19±1.9856[b]	9.19±1.7595[b]
50	14.85±2.36891[a]	14.03±2.89543[a]	14.03±3.43257[a]	14.03±3.25678[a]	14.03±2.58199[a]
100	18.25±1.9382[a]	16.67±2.5356[b]	16.76±3.4567[b]	16.87±2.3467[b]	16.83±2.5356[b]

Aqueous					
12.5	6.75±1.03280[a]	6.49±0.567[a]	6.50±0.564[a]	6.50±0.947[a]	6.50±0.925[a]
25	8.74±1.99896[a]	8.80±1.9988[a]	8.75±1.9988[a]	8.60±1.9988[a]	8.70±1.9988[a]
50	12.19±2.40052[a]	11.79±1.994[b]	11.99±1.994[b]	11.80±1.994[b]	11.89±1.994[b]
100	15.85±2.59487[a]	14.35±1.4762[a]	13.37±1.6758[a]	13.25±1.7569[a]	13.25±1.2456[a]
Control	22.19[e]	21.67[e]	21.67[e]	21.67[e]	21.67[e]

Keywords; Salmonella Test isolates - C, E, G, I, J

NOTE; Mean values on the column with different super scripts are significantly different (P≤0.05). Mean ± Standard deviation.

MIC and MBC of the Extracts

Table 4 shows the MIC values indicate the lowest concentration at which the extracts inhibit bacterial growth. The n-hexane extract shows a lower MIC for isolates C, G, and I compared to E and J, suggesting better efficacy. The aqueous extract requires higher concentrations for effective inhibition, reflecting its lower potency relative to the n-hexane extract. The MBC values indicate the lowest concentration needed to kill the bacteria. The n-hexane extract again shows better effectiveness, with lower MBC values compared to the aqueous extract. The higher MBC values for the aqueous extract suggest that it is less potent in killing the bacteria outright as shown in table 4.

Table 4: The Minimum Inhibitory and Bactericidal Concentrations (MIC and MBC) of n – hexane and Aqueous Extracts of *P. biglobosa* against the Test Isolates

	n-Hexane Extract		Aqueous Extract	
Isolates	**MIC (mg/mL)**	**MBC (mg/mL)**	**MIC (mg/mL)**	**MBC (mg/mL)**
C	12.5	25	12.5	25
E	25	50	50	75
G	12.5	25	25	50
I	12.5	25	50	75
J	25	50	50	75

Key: Sample Code (C, E, G, I, J) – Salmonella Test isolates

Discussion

The phytochemical screening of *Parkia biglobosa* reveals the presence of various bioactive compounds. Both extracts contain flavonoids, terpenoids, and tannins, which are known for their antimicrobial properties. However, alkaloids and cardiac glycosides are present only in the aqueous extract, while anthraquinones are exclusive to the n-hexane extract. The absence of steroids and saponins in both extracts is also noted. Phytochemicals are secondary constituents of plants which are responsible for biological actions and some have also been reported to possess anti-oxidative potentials (Lezoul *et al.*, 2020). Phytochemicals are particularly implicated in antimicrobial potentials of various medicinal plants as supported by different researchers.

The results demonstrate that both extracts exhibit antimicrobial properties, with inhibition zones increasing in size with higher concentrations of the extracts. The n-hexane extract generally shows a higher inhibitory effect compared to the aqueous extract, suggesting that the bioactive compounds in the n-hexane extract are more potent or more effectively extracted. The antimicrobial activity of plant extracts has been linked by many researchers to be due to the presence of phytochemicals in them (Radha *et al.*, 2021). The antimicrobial activity of the extracts tested *in vitro* could be higher than they are reported if active ingredients from the extracts are isolated and tested. Hayat *et al.* (2020) reported that crude extracts may contain inactive substances which may also antagonize the antimicrobial actions of one other.

The Minimum Inhibitory Concentration (MIC) values indicate that the n-hexane extract is more effective at lower concentrations for most isolates compared to the aqueous extract. This is further supported by the Minimum Bactericidal Concentration (MBC) values, where the n-hexane extract again shows better effectiveness, requiring lower concentrations to achieve bactericidal effects. This confirms the study of Saleh *et al.* (2021) who reported that the antimicrobial activity resided solely in the alcohol soluble fraction of volatile oil. This observation is in accordance with the findings of Yu *et al*. (2021) and Ain *et al*. (2022), who reported a strong antibacterial effect of alcoholic extract of tested plants in comparison to aqueous extract. The differences in the activities of alcoholic and water extracts may be due to varying degrees of solubility of the active constituents in these two solvents. From this observation we can infer that alcohol is relatively a more efficient solvent than water for the extraction of bioactive compounds of *Parkia biglobosa* than water. Difference in potency and diversity of compounds extracted by using organic solvents and water has also been reported previously (Clark *et al.*, 2023). However, in contrast to our results, one study done in Iraq has shown that the aqueous extract was more potent than the alcoholic form in inhibiting microorganisms (Rasheed *et al.*, 2020). Therefore, MIC and MBC values, the lowest concentration of antibacterial substance required to produce a sterile culture has also been computed here. Demonstration of antibacterial activity against the test bacteria is an indication that alternative antibiotic substances in these plants could be used for the development of newer antibacterial agents in future. The observed low MIC and MBC values against these bacteria suggest that the plant extracts has the potential to treat ailments arising from the bacterial pathogens effectively.

Conclusion

Finding of the present study indicate the presence of flavonoids, terpenoids, and tannins in both the extract. However, alkaloids and cardiac glycosides are present only in the aqueous extract, while anthraquinones are exclusive to the n-hexane extract. Antibacterial activity of the extract show activity against the test isolates, with the inhibition zones increasing with concentration. The n-hexane extract generally exhibits a higher inhibitory effect compared to the aqueous extract, and the control shows the largest inhibition zones, suggesting standard antibiotic effectiveness. The MIC of the extracts indicated that the n-hexane extract inhibited the growth of the isolate at 12.5 – 25 mg/ml while aqueous extract at 25 – 50 mg/ml. the MBC of n-hexane extract ranges from 12.5 – 50 mg/ml while aqueous extract ranges from 25 – 75 mg/ml.

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