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Full Length Research Paper

In vitro antibacterial activity of *Parkia biglobosa* (Jacq.) root bark extract against some microorganisms associated with urinary tract infections

El-Mahmood, A. M. * and Ameh , J. M.

Department of Microbiology, Federal University of Technology, Yola, Nigeria.

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The medicinal plant *Parkia biglobosa* (Jacq.) was screened for the phytochemical components and antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* which are associated with urinary tract infections. Aqueous solutions are more potent than methanolic solutions and activity is concentration dependent. *P. aeruginosa* is least susceptible than the other organisms.

Key words: Antibacterial activity, Parkia biglobosa, phytochemical, urinary tract infections.

INTRODUCTION

There is global resurgence in the use of herbal prepa-rations 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 deve-lopment of these herbal medicines depended on local botanical flora. 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 (Lino and Deogracious, 2006) . The scientific literature is full of reports of antimicrobial activity of plants and their secondary metabolites (Erdemeier et al., 1996, Hassan and Ahmed, 1996; Darokan et al., 1999; Cutter, 2000; Babu et al., 2002) and scientific evaluation of these plants remains an area of intense investigations.

Growing misuse of antibiotics and chemotherapeutic agents leading to drug resistance is now pushing a con-siderable proportion of people in both developed and developing countries to the use of herbal medicines. As a consequence of this in 1997, the 30th World Assembly adopted a resolution urging national governments of member nations to utilize their traditional systems of medicines with regulations suited to their national health care system. In Nigeria, traditional medicine boards were established in all the states to run parallel with the hospitals management boards and primary health care development agencies. Here, we report the results of a study designed to assess the phytochemical and antibac-terial activity of extracts from a medicinal plant, *Parkia biglobosa* (Jacq.) (The African locust bean tree) widely used in North-Eastern Nigeria as sources of timber, food and medicines.

*Corresponding author. E-mail: elmahmu@yahoo.com.

MATERIALS AND METHODS

Collection and preparation of the plant material

The plant was selected based on reports of its widespread use among the local communities in North-Eastern region of Nigeria. Local vernacular names of the plant (Nareje in Fulfulde, Dorawa in Hausa, Tokoro in Yoruba, Ruh in Yendang and Wupga in Igala) were initially used to assist in the identification of the plant. Proper identification of the plant was done at the botany department of Federal University of Technology, Yola. After collection, the root bark was sun dried for 7 days and pounded using pestle and mortar and stored at 35 - 37°C until required.

Extraction procedure

One hundred grams of the powdered drug was soaked into 400 ml distilled water in a 1 litre capacity conical flask. This was allowed to stand for 24 h at 35 - 37°C and then filtered using Whatman No.1 filter paper. The filtrate evaporated to dryness using rotary evapo-rator and the resultant extract stored in a reagent bottle at 4 –8°C.

Table 1. Phytochemical components of crude extract of *Pakia biglobosa* root bark.

Saponins	++
Glycosides	+++
Tannins	+++
Phenolics	++
Alkaloids	+

+++ = present in appreciable amount. ++ = present in moderate amount. + = present in trace.

Similar procedure was followed to obtain methanolic extract, using 99.5% methanol as a solvent.

Preparation of bacteria

The bacteria used were *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* isolated from clinical specimens obtained from patients diagnosed with urinary tract infections at the Federal Medical Centre, Yola. Isolation and identification of the organisms were done following standard procedures in handling clinical specimens (Potashmercher et al., 1979; Cheesbrough, 2002). The organisms were maintained on nutrient agar slants at 2 - 8°C. Purity of the organisms was checked at regular intervals by plating and staining (Acheampong et al., 1988).

The bacterial cultures were standardized using the method of Baker et al. (1983). The test organisms were suspended into sterile universal bottles containing nutrient broth and normal saline added gradually to it so as to compare the culture turbidity to that of McFarland standard, which corresponded to approxi-mately 1.0 x 10^7cell.ml^{-1}.

Screening for phytochemical components

The method described by Odebiyi and Sofowora (1978) was used to test for the presence of the bioactive constituents of the plant material.

Determination of antibacterial activity

The antibacterial screening was done as described by Lino and Deogracious (2006) and Akoma et al. (2002) . Briefly, 1 ml of the test culture (1.0 x 10^7cell.ml^{-1}) was placed into a sterile plate and 19 ml molten agar at 45ºC poured and the plate shaken for even spread and proper mixing of the organisms and agar. The agar was allowed to solidify. Wells of approximately 6 mm in diameter and 2.5 mm deep were made on the surface of the agar medium using a sterile cork borer. The plates were turned upside down and the wells labeled with a marker. The extract was reconstituted by dissolving 1 g of each in 1 ml of distilled water and each well was filled with 0.5 ml test sample. Pure solvents were used as control. The aqueous solution of 12.5 µg equivalent gentamicin was used as a positive control. The plates were incubated at 37°C for 24 h and zones of inhibition measured with a pair of calipers and a millimeter ruler and results tabulated. The same test procedure was repeated for the methanolic extract.

The MIC of the extracts against the test organisms was determined using the broth dilution method (Sahm and Washington, 1990). Briefly 1 ml of the extract solution at concentration of 200 mgml^{-1} was added to 1 ml of nutrient broth and subsequently transferred. One 1 ml from the first test tube to the next, for up to the seventh test tube. Then 1 ml of 24 h culture of test organism (1.0 x 10^7cell.ml^{-1}) was inoculated into each test tube and mixed thoroughly on a vortex mixer. The test tubes were then incubated at 37°C for 24 h. The tube with the lowest dilution with no detectable growth was considered as the MIC.

RESULTS

The results of the phytochemical analysis are shown in Table 1. Each of the 100 g of the dried powdered root bark yielded a semi solid brown powdery substance of 15 g for the aqueous and 8.6 g for the methanolic extracts. The plant *P. biglobosa* (Jacq.) root bark consists mainly of glycosides and tannins, appreciable amounts of saponins glycosides and phenolics, with trace amount of alkaloids.

The results for antimicrobial screening as measured by diameters of zones of inhibition are shown in Table 2. The activities of the water and methanolic extracts are qualitatively similar and concentration dependent. *P. aeruginosa* is less sensitive and only slightly inhibited even at higher drug concentration of 200 mg ml^{-1}. The other three organisms displayed variable responses with *K. pneumoniea* more vulnerable to the activity of the crude drug extract. The drug susceptibility testing of the extracts were done in serial dilutions of the extracts from 200 to 6.25 mg ml^{-1} and no activity was recorded at the lower concentrations of the extracts.

Another result of the antimicrobial activities of the extracts as determined by measuring the minimum inhibitory concentrations (MIC) are given in Table 3. The MIC data for the organisms are also variable, and concentration dependent similar to the data in Table 2. There were slight growths in aqueous solution of the extract (200 mg ml^{-1}) against *P. aeruginosa*. The MIC which was determined by geometrically diluting the extract was 200 mg ml^{-1} for *P. aeruginosa* and *E. coli*, 100 mg ml^{-1} for *S. aureus* and 50 mg ml^{-1} for *K. pneumoniea*.

DISCUSSION

The African locust bean tree, *P. biglobosa* (Jacq.) is a perennial tree legume, belonging to the sub-family *Mimosoideae* and family *Leguminosae*. The plant has been used as a source of food, medicinal agents, timber and is of high commercial value (Fetuga et al., 1974). In West Africa, the seeds of the plant are used for food seasoning, obtained by boiling and fermentation of the seeds, known as *daddawa* by the Fulani and Hausa tribesmen (Ajaiyeoba, 2002) . A decoction of the leaves, bark and roots are used in treating leprosy, eye sores, toothache, fever and hypertension as well as wound and ulcer and in treating snake bite (Ajaiyeoba, 2002) . Phytochemical results indicated that the root bark of the plant contains a lot of glycosides and tannins, appreciable amounts of saponins and traces of alkaloids. The presence of such

Table 2. Antibacterial screening of crude extracts of *Pakia biglobosa* (Jacq.). Zone (mm) diameter of inhibition.

Extract (mg ml^{-1})	*S. aureus*		*P. aerugenosa*		*E. coli*		*K. pneumoniae*	
	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
200	++	+++	+	+	++	++	+++	++++
100	++	++	-	-	+	+	++	+++
50	+	+	-	-	-	-	+	+
25	-	-	-	-	-	-	-	-
12.5	-	-	-	-	-	-	-	-
6.25	-	-	-	-	-	-	-	-
Control	-		-		-		-	
Gentamicin (12.51µg ml^{-1})	+++		++		+++		++++	

-: 6 mm or absence.
+: 8 - 12 mm.
++: 13 – 15 mm.
++++: 20 mm.

Table 3. Minimum inhibitory concentration (MIC) for aqueous and methanolic extracts of *Pakia biglobosa*.

Extract (mg ml^{-1})	MIC							
	S. aureus		*P. aerugenosa*		*E. coli*		*K. pneumoniae*	
	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
200	-	-	+	-	-	-	-	-
100	-	-	+	+	+	+	-	-
50	+	-	+	+	+	+	-	-
25	+	+	+	+	+	+	+	+
12.5	+	+	+	+	+	+	+	+
6.25	+	+	+	+	+	+	+	+

-: No growth.
+: Growth.

bioactive compounds has been linked to the antibacterial activity such as inhibition of growth (De and Ifeoma, 2002) and offering some protection to the plant against microbial infections (Farnsworth, 1982). The presence of some of the phytochemical components like saponins, tannins and phenolic compounds have been attributed to the activity of the crude drugs observed (De and James, 2002). In his own study, Ajaiyeoba (2002) found out that the leaves contained a lot of cardiac glycosides and or saponin glycoside. The seeds of the plant were also reported to be rich in proteins and amino acids and some simple sugars, lactose and maltose (Alabi et al., 2005). The presence of those compounds in the plant may seem to justify the used of the crude drugs for the treatment of snake bites (Isuzu and Harvey, 2003), diabetes (Odetola et al., 2006), fever and infections caused by some susceptible pathogens (De and Ifeoma, 2002). The presence in the root of substantial quantities of glycosides may justify the use of the decoctions for cardiac conditions and hypertension (Mohamed et al., 2005).

Both the water and methanolic extracts displayed appreciable antibacterial activities against such recalcitrant pathogenic bacteria like *K. pneumoniea*, *S. aureus* and *E. coli* that are known to show above average resistance to most chemical antimicrobial agents. These pathogens are known to cause majority of community and hospital acquired infections and are capable of elaborating several virulence factors including the formation of biofilms on colonized surfaces (Indrayan et al., 2002; De and James, 2002). *P. aeruginosa* is however less susceptible, even to a higher concentration of 200 mg ml^{-1} of the extracts. The lack of susceptibility of *P. aeruginosa* to the extracts could be attributed to the fact that this bacteria is inherently resistant to many antibiotics and non-antibiotic antimicrobial agents as a result of the permeability barrier afforded by its outer membrane (Lino and Deogracious, 2006). The data obtained in this study is similar to those obtained from the study of the leaves and seeds of *P. biglobosa* (Jacq.) against S. *aureus*, *B. cereus*, *P. aeruginosa*, *A. niger* and *C. utilis* by Ajaiyeoba (2002) who reported that *B. cereus* was more susceptible, *S. aureus* and *E. coli* less sus-ceptible while *P. aeruginosa* and the *A. nige*r and *C. utlis* not susceptible. The methanolic extract produced larger zones of inhibitions and lower MICs than aqueous extracts probably because not all the bioactive components have been

extracted in water (De and Ifeoma, 2002). Several workers including Wolinsky and Sote (1982) have made similar observations and attributed the differences in activity to the high solubility of some of the phytochemical components such as tannins and phenols in methanol than water. The conventional antibiotic gentamicin, consistently showed superior activity than either extracts similar to the data presented by other scholars (De and Ifeoma, 2002; Kubmarawa et al., 2002). This may be attributed to the fact that conventional antibiotics and non-antibiotic antibacterial agents are usually prepared from synthetic materials by means of reproducible manufacturing techniques and procedures, herbal medicinal products are prepared from plant and animal origins, most of the time subjected to contamination and deterioration (De and Ifeoma, 2002). The storage of extracts like any other pharmaceuticals requires special conditions of humidity, temperature and light. There is also always the possibility that a given extract which is inactive *in vitro* may exhibit properties of pro- drugs which are administered in an inactive form, but their metabolites may be active (Lino and Deogracious, 2006). The data obtained in this and similar studies seemed to justify the folklore use of *P. biglobosa* (Jacq.) in medical practice by majority of the populations of the sub-Saharan Africa.

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