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Full Length Research Paper

Phytochemical, Antioxidant and Antimicrobial Properties of *Parkia biglobosa* (African Locust Bean) Pods

Igwo-Ezikpe, M.N[1*], Ogbunugafor, H.A.[2], Gureje A.P.[1] and Ezeonwumelu I.J.[2]
[1]Department of Biochemistry, College of Medicine,University of Lagos, Lagos, Nigeria.
[2]Applied Biochemistry Department, Nnamdi Azikiwe University, Awka, Nigeria.
*Corresponding author email: mimiigwo@yahoo.co.uk; igwo-ezikpe@unilag.edu.ng

ABSTRACT
The use of plant materials for nutrient supply and therapeutic function has necessitated the exploration of the beneficial role of commonly consumed plant materials in multi-disease conditions. Present study investigated the phytochemical, antioxidant and antimicrobial potential of ethanolic extract of *Parkia biglobosa* pod. Preliminary phytochemical screening showed that it contained saponins, flavonoids, terpenoids, cardiac glycosides, glycosides, carotenoids, steroids, tannins and reducing sugar. In addition, the ethanolic extract also exhibited 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging and ferric ion reducing antioxidant activity which occurred at a concentration dependent manner. Furthermore, antimicrobial study showed that the extract is a potent antibacterial agent without antifungal activity at the concentrations investigated. Minimum Inhibitory Concentration (MIC) of the ethanolic pod extract was 12.80 mg/ml for *Staphylococcus aureus, Enterococcus faecalis and Pseudomonas aerugenosa* and 25.60 mg/ml for *Escherichia coli*. This is the same value obtained for the Minimum Bactericidal Concentration (MBC). This study has shown the potential application of ethanolic extract of *Parkia biglobosa* pod in antioxidant requiring and antibacterial treatment.
Key words: *Parkia biglobosa* pod, antioxidant, phytochemical, bacteria, fungi, antimicrobial

1.0 INTRODUCTION
Parkia biglobosa (Jacq) Benth is a perennial tropical plant legume, predominantly distributed within the savannah region of West Africa (Campbell-Platt, 1980). *P. biglobosa* is also known as African locust bean while in Nigeria its local names include; Ogiri (Igbo), Dorowa (Hausa), Irugba (Yoruba) and Nune (Tiv) (Udobi *et al.*, 2008). The African locust bean has gained its popularity from the consumption and economic value of its bean seeds. In West Africa especially Nigeria, the beans are usually fermented to yield a product popularly called 'Dawadawa'. Dawadawa - a black, tasty seasoning, rich in protein is commonly used as condiment in local soups and as a dietary protein source (Wokoma and Aziagba, 2001; Achi, 2005; Chukwu *et al.*, 2010). Furthermore, other parts of the plant such as the fruit and stem have also been exploited. The stem bark was reported to have anti-snake venom activities (Asuzu and Harvey, 2003), the fresh fruit pulp can be used as mineral supplement (Omojola *et al.*, 2011).

Besides the culinary use of the African locust bean, its chemotherapeutic attributes have been explored (Asase *et al.*, 2005; Builder *et al.*, 2012). In traditional medicine practices, a decoction of the stem bark has been used as a hot mouthwash to relieve toothaches (Ajaiyeoba, 2002). A mixture of

the root and leaves has been reported to be an efficacious remedy for the treatment of sore eyes and dental caries (Millogo-Kone *et al.*, 2008).

Free radicals and reactive oxygen species (ROS) have been implicated in the oxidative damage of biomolecules and various organs of the body. Studies have shown the crucial role free radicals play in the pathogenesis of many human diseases namely, cardiovascular and pulmonary diseases, some types of cancer, immune/autoimmune diseases, inflammation, diabetes, cataracts and brain dysfunction such as Parkinson and Alzheimer (Allen and Tresini, 2000; Kusano and Ferrari, 2008; Kumar and Pandey, 2012). However, the deleterious effect of free radicals can be corrected by antioxidants – both enzymatic and non-enzymatic. This has resulted in the search for efficient low cost sources of antioxidants. Researchers and traditional medicine practice have turned to medicinal plants as a means deriving essential bioactive compounds and for cure of diverse alignments.

Many plants have been shown to possess antioxidant properties (Sala *et al.*, 2002; Ivanišová *et al.*, 2013). This has thus raised interest in the investigation of commonly consumed plants for their phytochemicals with nutritional and chemotherapeutic potentials. Therefore, the need to argument synthetic chemotherapeutic compounds with natural products is the drive for the exploitation of natural products from plants; as they may have little or no side effects yet meeting the nutritional, chemotherapeutic and economic needs (Iwu *et al.*, 1999; Hazra *et al.*, 2008). Moreover, despite the efforts of pharmaceutical companies in the production of synthetic antibiotics, there yet exists a marked increase in pathogen population exacerbated by multi drug resistant microorganisms (Nino *et al.*, 2006). Consequently, there is increased research into phytochemicals for the effective therapeutics combat of this menace. The therapeutic effects of plant-based drugs have been documented to be due to the phytochemicals that constitute the plants. These constituents selectively target toxins and pathogens without significant detrimental effect on the human host (Kumar *et al.*, 2012).

Owing to the wide consumption of the seeds of African locust bean coupled with the extensive studies on the beans, effort is geared in this work towards expanding the medicinal potentials of other parts of the plant. There has been dearth of information on the substantive biochemical and antimicrobial investigations on the pods of the African locust bean (Koura *et al.*, 2011); in addition, the continual investigation on the chemotherapeutic potential of medicinal plants cannot be over emphasized. Hence, this work is set to investigate the phytochemical, antioxidant and antimicrobial potentials of the pods of *Parkia biglobosa.*

2.0 MATERIAL AND METHODS

2.1 Sample collection

Freshly harvested pods of *P. biglobosa* were purchased from Kuto market, Abeokuta, Ogun state, Nigeria. They were identified in Department of Botany, Faculty of Science, University of Lagos, Nigeria. The samples without the stalk were oven dried at 35 ± 5 ˚C for 10 hr and then ground to fine powder to a final weight of 403g.

2.2 Extract preparation

The ground pods powder (403 g) was extracted cold by allowing the powder to be covered in 2.5 litre of 80 % ethanol at room temperature for 48 hr. The extraction solution was filtered through Whatman filter paper No. 4 and the filtrate concentrated in water bath (Philips Harris Ltd, England) at a

temperature of 40˚C for 8 hr. The extract was weighed, the percent yield calculated and sample subsequently stored at 4˚C until used.

2.3 Phytochemical Analysis

Aliquots of the ethanolic extract of the plant material were used in the phytochemical studies using standard methods as described by Trease and Evans (1989).

2.4 Antioxidant Assay

The antioxidant properties of the ethanolic extract of *P. biglobosa* pod powder were determined. These include 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity and reducing power potential.

2.4.1 DPPH free radical scavenging Assay

The antioxidant activity of the extracts on the basis of its scavenging of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical was determined by the method described by Leong and Shui (2002). A 0.1 mM solution of DPPH radical in methanol was freshly prepared daily prior to use. Various concentrations of the extract samples were prepared in methanol, an aliquot of the ethanolic extract was mixed with 100 µl of methanolic DPPH radical to give final concentrations of 25, 50, 75, 100 µg/ml respectively. By using microtiter plate reader at 517 nm, the reduction of DPPH radical was measured with continuous monitoring of the decrease of absorption of DPPH every 10 min up to 100 min until the reaction reached the steady state plateau. The percent inhibition of radical scavenging ability of the extract was expressed as Inhibition concentration (IC_{50}) values calculated as:

$$\% \text{ inhibition} = [(Ab - As)/ Ab] *100$$

Where A_b = Absorbance of blank

A_s = Absorbance of sample

2.4.2 Determination of reducing power

Ferric reducing antioxidant power assay was determined by a modification to the method described by Benzie and Strain (1996). A serial dilution of the extract was performed (200, 100, 50, 25 and 12.5 µg/ml) in 0.2 M phosphate buffer pH 6.6 containing 1% ferrocyanate. The mixture was incubated at 50 ºC for 20 min. Trichloroacetic acid (TCA: 10 %, 2.5 ml) was added to a portion of this mixture (5 ml) and centrifuged at 3,000 g for 10 min. The supernatant was separated and mixed with distilled water (2.5 ml) containing 1% ferric chloride (0.5 ml). The absorbance of the mixture was measured at 700 nm.

2.5 Antibacterial activity

The antimicrobial potential of ethanolic extract of *P. biglobosa* pod was evaluated against some pathogenic bacteria and fungi isolates. The antimicrobial assays included sensitivity test, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC).

2.5.1 Sample and reference standard preparation

The sample extract (6 g) was diluted with 5% ethanol (10 ml) to yield a sample concentration of 600 mg/mL which was serially diluted to yield 300 mg/ml and 150 mg/ml. Serial dilutions (320, 160, 80, 40 and 20 µg/ml) of the reference standards (Ciprofloxacin – antibacterial reference standard and Clotrimazole – antifungal reference standard) were used.

2.5.2 Test microorganisms and preparation of starter culture

The test microorganisms used in this study are commonly observed pathogenic clinical isolates. They were obtained from Microbiology Laboratory of the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Lagos, Nigeria. The bacteria strains (*Enterococcus faecalis*,

Escherichia coli, Staphylococcus aureus, and Pseudomonas aerugenosa) were taken from their various slant stock cultures, then subcultured in Mueller-Hinton agar (MHA) and incubated at 37 ^{o}C for 24 hr, while the fungi species (*Candida albicans, Aspergillus niger, Penicillum, Aspergillus flavus*) were subcultured in Sabouraud Dextrose agar (SDA, Oxoid, UK) and incubated at ambient temperature (room temperature; 23- 29^{o}C) for one week. This was to obtain fresh colonies of isolates.

2.5.3 Calibration of organisms in suspension

The spores of *Candida albicans, Aspergillus niger, Penicillum, Aspergillus flavus were* calibrated in 1% tween 80. Serial dilution was done and each dilution was plated out to establish the dilution that gives a confluent growth. The dilution that gave about 10^{8} spore forming unit (SFU) was selected for the assay. *Candida albicans* and the bacteria strains were similarly prepared in sterile normal saline and turbidity adjusted to 0.5 McFarland suspension.

2.5.4 Sensitivity test

Antimicrobial activity of the ethanolic extract of *P. biglobosa* pod was evaluated by agar well diffusion method on Mueller Hinton Agar (MHA) medium (Forbes *et al.*, 1990). The antimicrobial activity was assessed based on the inhibitory effect of the extract on bacterial growth on agar plate. The zone of inhibition (mm) of microbial growth was compared to known antibacterial (ciprofloxacin) and antifungal (clotrimazole) agents. The sterile Mueller Hinton Agar (MHA) medium (20 ml) in petri dishes were uniformly smeared using sterile cotton swabs moistened with pure cultures suspension (0.5 macfarland) of the respective pathogenic bacteria; *Enterococcus faecalis, Escherichia coli, Staphylococcus aureus, and Pseudomonas aerugenosa.* Wells in equidistant were bored round the plate using a sterile cork borer and 0.2 ml of various concentrations of the extracts (150, 300 and 600 mg/ml) were put into the wells while ciprofloxacin and clotrimazole (25, 12.5, 6.25 and 3.125 µg/ml) which were used as positive controls were likewise put into separate well. The plates were left for 1 hr for proper diffusion after which they bacterial plates were incubated at 37 ^{o}C for 24 hr while the fungi plates were incubated at room temperature. The plates were examined for zone of inhibition which was measured with vernier calipers and the value recorded.

2.5.5 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the extracts that has potent antimicrobial activity was determined in Mueller-Hinton broth (MH broth). Freshly prepared pure culture of the isolates were used, the culture was standardized using standard microbiological techniques to have a concentration of very near 1 million cells per milliliter. The antimicrobial agents were diluted to 1:1 using a sterile diluent (MH broth). A mixture (2 ml) of the standardized inoculum and the serially diluted antimicrobial (1:1) was placed in test tubes and incubated at 37 ^{o}C for 24 hr. The series of dilution test tubes were observed for microbial growth, which was indicated by turbidity and/or pellet of microorganisms at the bottom of the test tubes. The test tube with the lowest extract concentration demonstrating no visible growth in the broth was recorded as the Minimum inhibitory concentration (MIC) (Prescot *et al.*, 1996).

2.5.6 Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) assay was performed as an adjunct to the MIC and was used to determine the concentration of the sample extract that is lethal to the target bacteria *in vitro*. From

each MIC broth test tube without visible growth, an aliquot volume of the broth was placed onto MH agar and spread across the entire surface of the plate. The plates were then incubated at 37 ^{o}C for 24 hr. Following the incubation, the MBC plates were examined for colony growth or lack of growth on the various plates. The absence of growth on any of the plates corresponds to the bactericidal effect of that antimicrobial agent. Therefore, the minimum bactericidal concentration (MBC) was recorded as lowest concentration of the extract that showed no growth on the agar.

2.5.7 Minimum Fungicidal Concentration (MFC)

The MFC was determined using the content from the broth dilution of MIC test. The surface of sterile Sabouraud Dextrose Agar (SDA) medium (20 ml) in petri dishes was uniformly smeared with sterile cotton swabs moistened with the contents of the test tubes from MIC test. The plates were then incubated at 37 ^{o}C for 24 hr. The minimum fungicidal concentration (MFC) was recorded as lowest concentration of the extract that showed no growth on the Sabouraud Dextrose Agar (SDA).

2.6 Statistical Analysis

Analyses were carried out in triplicates of each set up. Statistical significant difference ($p < 0.05$) was determined using student's t-test. All data were analyzed using Statistical Package for the Social Science 15.0 for windows (SPSS 15.0).

3.0 RESULTS

The percentage yield of the ethanolic extract of *P. biglobosa* pod was 57.07 %. Phytochemical screening showed the presence of an appreciable number of secondary metabolites which include tannins, saponins, flavonoids, terpenoids, cardiac glycosides, carotenoids and reducing sugars.

The ethanolic pod extract exhibited DPPH free radical scavenging activity and ferric ion reductive capability; these were evident in a concentration and extraction medium-dependent manner (Table 1).

Table 1: DPPH Free radical scavenging activity and Free Reducing antioxidant power (FRAP) potential of ethanolic extract of *Parkia biglobosa* pod

Test sample	Concentration of extract (µg/ml)			
	25	50	75	100
DPPH scavenging (I%)	83.8±0.005*	84.0±0.008*	85.1±0.017*	90.4±0.009*
FRAP (absorbance at 700 nm)	1.00±0.01*	0.41±0.16*	0.12±0.07*	0.00±0.00*

Values are ± SEM, *Significant at $p < 0.05$

The sensitivity test carried out with increasing concentration of the ethanolic extract showed that the extract had antimicrobial activity at 150 mg/ml against the bacteria strains used while at higher concentration of 300 and 600 mg/l all the bacteria strains thrived (Table 2).

Table 2: Dimension of zones of inhibition of ethanolic extract of *Parkia biglobosa* pod against some selected pathogenic bacteria

Bacteria strain	Zone of inhibition*						
	Ethanolic *P. biglobosa* extract (mg/l)			Ciprofloxacin standard (µg/ml)			
	600	300	150	25	12.5	6.25	3.125
S. aureus	0.00	0.00	15.75	33.00	30.00	25.00	18.00
E. coli	0.00	0.00	16.00	25.00	20.00	15.00	0.00
E. faecalis	0.00	0.00	16.00	30.00	23.00	18.50	14.00
P. aerugenosa	0.00	0.00	15.00	35.00	30.00	25.00	20.00

**Diameter of inhibition zones represented as mean of a triplicate*

Conversely, the fungi species flourished at all concentrations evaluated which were 150, 300 and 600 mg/ml (Table 3).

Table 3: Dimension of zones of inhibition of ethanolic extract of *Parkia biglobosa* pod against some selected pathogenic fungi

Fungi strain	Zone of inhibition						
	Ethanolic *P. biglobosa* extract (mg/l)			Clotrimazole standard (µg/ml)			
	600	300	150	25	12.5	6.25	3.125
A.niger	0.00	0.00	0.00	25.00	23.00	20.00	18.00
A. flavus	0.00	0.00	0.00	24.50	22.00	20.00	17.50
C. albicans	0.00	0.00	0.00	27.00	25.00	23.00	20.50
Penicillum sp.	0.00	0.00	0.00	24.00	22.00	20.00	17.50

**Diameter of inhibition zones represented as mean of a triplicate*

Further investigation of the minimum inhibitory concentration (MIC) of the ethanolic pod extract on the susceptible bacteria strain showed that the MIC for *Staphylococcus aureus, Enterococcus faecalis and Pseudomonas aerugenosa* was 12.80 mg/ml and 25.60mg/ml for *Escherichia coli* (Table 4).

Table 4: Minimum inhibitory concentration (MIC) of ethanolic extract of *Parkia biglobosa* pod against some selected pathogenic bacteria

Bacteria strain	Concentration of *Parkia biglobosa* pod extract (mg/ml)									
	0.10	0.20	0.40	0.80	1.60	3.20	6.40	12.80	25.60	51.20
S. aureus	+	+	+	+	+	+	+	-	-	-
E. coli	+	+	+	+	+	+	+	+	-	-
E. faecalis	+	+	+	+	+	+	+	-	-	-
P. aerugenosa	+	+	+	+	+	+	+	-	-	-

+ = Growth; - = no Growth

However, the fungus isolates which were resistant to the ethanolic extract of *P. biglobosa* pod and further subjected to MIC test showed that the extract at the various investigated concentrations had no antifungal activity (Table 5). From the analysis, after obtaining the MIC values, the Mminimum bactericidal concentration (MBC) was observed to be same value with the MIC (Table 6).

Table 5: Minimum inhibitory concentration (MIC) of ethanolic extract of *Parkia biglobosa* pod against some selected pathogenic fungi

Fungi strain	Concentration of *Parkia biglobosa* pod extract (mg/ml)									
	0.1	0.2	0.4	0.8	1.6	3.2	6.4	12.8	25.6	51.2
A.niger	-	-	-	-	-	-	-	-	-	-
A. flavus	-	-	-	-	-	-	-	-	-	-
C. albicans	-	-	-	-	-	-	-	-	-	-
Penicillum sp.	-	-	-	-	-	-	-	-	-	-

Table 6: Minimum bactericidal concentration (MBC) of ethanolic extract of *Parkia biglobosa* pod against some selected pathogenic bacteria

Bacteria strain	MBC (mg/l)
S. aureus	12.80
E. coli	25.60
E. faecalis	12.80
P. aerugenosa	12.80

4.0 DISCUSSION

Investigating the use of plant materials as a potent remedy for various ailments has not only authenticated their efficacies, but has also identified the pharmacological roles of the individual bioconstituents of these plant materials. In this study, the phytochemistry of the pods of *Parkia biglobosa* (Jacq) Benth which showed the presence of an array of medically important secondary metabolites including saponins, tannins, cardiac glycosisides, flavonoids, carotenoids, steroids, terpenoids and reducing sugars is a strong indication that the plant has potential medicinal values. Similar phytochemicals have been reported by studies on other vegetative parts of the plants such as the leaves, stem bark and root bark (Ajaiyeoba, 2002; Abioye *et al.*, 2013).

The presence of these secondary metabolites have been suggested to be responsible for *P. biglobosa* therapeutic application in the treatment of diseases such as cardiac infirmity (Ajaiyeoba, 2002), intestinal disorders and other bacterial infections (Millogo-Kone *et al.* 2008). Adaramola *et al.*, (2012) suggested that the presence of flavonoids in the leaves and stem bark of *P.biglobosa* might account for its relatively high radical scavenging activity. This supports the finding of the present study where the pod exhibited antioxidant activity. Furthermore, investigating the use of plant materials as a potent remedy for various ailments has not only authenticated their efficacies, but has also identified the pharmacological roles of the individual bio-constituents of these plant materials. In addition, in response to the deleterious and economic impact of increased pathogen resistance to orthodox medicine, the quest for a surpassing solution to this health challenge has necessitated increased interest in the field of traditional medicine. In this study, the susceptibility of the bacteria isolates investigated to the ethanolic extract of *P.biglobosa* pod suggested the extract possesses antibacterial potential. The distinct difference between the cell wall compositions of the Gram-positive and Gram-negative bacteria confers a differing degree of antibiotic sensitivity between these classes of bacteria. The presence of the outer layer component of the cell wall of the Gram-negative bacteria confers a unique restrictive permeability of antibiotics on these organisms by limiting the effective dose concentration (EDC) at the target sites. This protective feature inevitably makes them highly tolerant to antibiotics. *Escherichia coli* (Gram negative) which was fairly susceptible at MIC of 25.60 mg L^{-1} confirmed the increased resistance of Gram negative bacteria when compared to the Gram positive bacteria; this is in agreement with studies reported by Ajaiyeoba, 2002; Udobi and Onaolopo, 2010. Interestingly, *Pseudomonas aerugenosa*, a Gram-negative bacterium which was susceptible to the pod extract at the same MIC of 12.80mg/ml with other the Gram-positive strains (*Staphylococcus aureus* and *Enterococcus faecalis*) suggested that the extract may have very high potent efficacy against multi-bacteria infections. Although, the pod extract was a potent bactericidal agent against all the test bacteria strains, the fungi species used in this work were highly resistant to the ethanolic pod extract suggesting that the ethanolic pod extract may lack antifungal potency at the investigated concentrations.

5.0 CONCLUSION

From this present study, *Parkia biglobosa* pod ethanolic extract was observed to be a potent antioxidant and antibacterial agent which could be explored as a therapeutic agent in oxidative stress induced disease condition with concomitant bacterial infection. Further studies on the toxicological status of the extract would help substantiate its nutritional and chemotherapeutic applications.

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