Antimicrobial Evaluation of the Stem Bark Extracts of *Parkia biglobosa* (Jacq.) Benth. Ex G. Don

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Abstract: The stem bark extract of *Parkia biglobosa* (Jacq.) Benth. Ex G. Don was evaluated for antimicrobial activity. Isolates tested were pathogenic strains of *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pnuemoniae*, *Proteus mirabilis*, *Aspergillus flavus* and *Aspergillus fumigatus.* The bark extracts of *P. biglobosa* used were of the solvents hexane, ethanol, petroleum ether and water. The extracts were concentrated at 70^{o}C using a rotary evaporator and then plated at different concentrations with the isolates using the agar well diffusion technique. The ethanolic extract of the stem bark had antimicrobial effect on all the isolates with a minimum inhibitory concentration (MIC) of 40 mg/ml for the isolates except *A. flavus* and *K. pneumonia* which were inhibited the MIC of 66.6 mg/ml. The hexane and petroleum ether extracts had no effect on any of the pathogens however, the aqueous extract was slightly active on *K. pneumonia* at an undiluted concentration. When evaluated with specific broad spectrum antibiotics, ciprofloxacin had efficacy on *K. pneumonia*, *P. mirabilis* and *P. aeruginosa* comparing favourably with the solvent extracts of *P. biglobosa.* [Akintobi OA, Adejuwon AO, Olawale AK. **Antimicrobial Evaluation of the Stem Bark Extracts of *Parkia biglobosa* (Jacq.) Benth. Ex G. Don.** *Rep Opinion* 2013;5(10):41-45]. (ISSN: 1553-9873). http://www.sciencepub.net/report. 7

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1. Introduction

Parkia biglobosa (Jacq.) Benth., commonly called the African locust bean, is a perennial deciduous and leguminous tree which belongs to the Sub family *Mimosdoideae* and Family *Fabaceae* (formerly *Leguminosae*) (Burkhill, 1995). There are about 30 species of *Parkia* distributed pan-tropically with only about 3 occurring in continental Africa with a 4th one in Madagascar (Alabi *et al.*, 2005). In Nigeria, *P. biglobosa* seeds are fermented and processed to make spices and condiments (Ajaiyeoba, 2002). It is of traditional medical importance in West Africa and have used in treating pneumonia, diarrhea, ulcers, malaria and as anti-snake venom (Asuzu and Harvey, 2003; Agunu *et al.*, 2005). The phytochemical screenings of *P. biglobosa* by Millogo-Kone *et al.* (2001a) and Builders *et al.* (2012) have revealed the presence of sterols, triterpenes, saponosides, tannins, phenols, cardiac glycosides, flavonosides and anthocyanoosides in both its bark and leaf at different concentrations. According to Ladokun and Adejuwon (2013), *P. biglobosa* contains protein, fat, crude fibre, ash and moisture whether mashed or unmashed with the possibility of condiment contamination with *Pseudomonas maltophila*, *Streptococcus faecalis*, *Aspergillus niger* and *Aspergillus flavus* during processing.

In the present investigation, the stem bark extract of *P. biglobosa* in different solvents was analysed for antimicrobial activity on selected pathogenic bacteria and fungi in view to determining the most effective.

2. Materials and Methods

2.1 Materials

2.1.1 Collection of plant materials

The stem bark of *Parkia biglobosa* (Jacq.) Benth. used in this study was collected from the Botanical Garden of the University of lbadan, lbadan, Nigeria. Identification was at the Herbarium of the Department of Botany, University of Ibadan.

2.1.2 Test organisms

Pure cultures of bacterial and fungal isolates used for the in vitro antimicrobial assay were obtained from the Laboratory of the Department of Medical Microbiology, University College Hospital, Ibadan, Nigeria. The bacterial cultures were maintained on nutrient agar (NA) slants. The fungi were cultured on Potato dextrose agar (PDA) slants. The bacterial isolates were *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus mirabiliis* while the fungal isolates were *Aspergillus fumigatus* and *Aspergillus flavus* (Olutiola *et al.*, 1991).

2.2 Methods

2.2.1 Plant extracts preparation

The method of Olayemi and Opaleye (2002) was modified and employed for analysis. A piece of the bark of the stem of *Parkia biglobosa* was milled into powder, then 20g of it was weighed and dissolved in 200ml of each of different solvents and allowed to soak for 72 hours at room temperature but stirred regularly every 12 hours. The solvents were sterile distilled water, hexane (70%), petroleum ether (70%) and ethanol (70%). The resultant suspension was filtered into a 500ml beaker using muslin cloth reinforced with Whatman's No 1 filter paper. The extracts were concentrated using a rotary evaporator at 70°C for 4 hours. This was then used for analysis. The excess extract stored in the refrigerator (at 4°C) for further study.

2.2.2 Agar well diffusion technique

The plant extracts were tested for antimicrobial activity using agar well diffusion technique. The diameter of clearance was measured and regarded as being directly proportional to efficacy of extract (Fawole and Oso, 2004).

(a) Inhibitory tests for bacteria

The test organism was first streaked all over the surface of the solidified nutrient agar plates using inoculating loop. A sterile 5mm diameter cork borer was used to make a uniform deep well into the gel. Each well was filled with l ml of different solvent extracts prepared. The dishes were made to stand for 30 minutes at room temperature to allow proper diffusion. Each of the solvents was used as control. Standard antibiotics disc was equally set alongside in the experiments for comparisms. All the plates were incubated for 24 hours at 37^0C after which the diameter of zone of inhibition (clearance) for each extract was measured using transparent metre rule (Harrigan and McCane, 1986).

(b) Inhibitory test for fungi

Agar well diffusion technique was equally used for the test fungi but however, with slight modification. Each extract were poured to cover the surface of PDA gel in Petri dishes and allowed to diffuse before boring with 5mm cork borer where cut rudimental masts cells of the fungal isolates were impregnated. The plates were incubated at 30°C for 72 hours after which the zones of clearance were measured with metre rule (Harrigan and McCane, 1986).

2.2.3 Reactivation of test organism

The bacterial isolates were tested for viability by resuscitating the organisms in buffered peptone broth for 72 hours to allow heavy growth. Each isolate was then subcultured into nutrient agar using the streak plate technique. Incubation was at 37^oC for 24hours (Valya *et al*., 2011; Nwinyi *et al*., 2009; Oboh and Masodje, 2009).

2.2.4 Antibiotic sensitivity test

Antibiotic susceptibility testing was done with the use of antibiotic discs (both Gram negative and Gram positive) by the disk diffusion method (Bauer *et al*., 1966). Before each antibiotic disc was placed on each of the media surface, the pathogenic bacterial isolates from MacConkey agar slants were streaked on the nutrient agar dishes after which the antibiotic disc were aseptically placed on each of the Petri dishes using a sterile forceps. The agar dishes were then incubated at 37°C for 24hours. Afterward, the dishes were examined for zones of inhibition. The zones of inhibition around each antibiotic disc were measured in millimeters using a transparent ruler. Antibiotic susceptibility was evaluated from the reading obtained from the diameter of the zone of inhibition. Antibiogram of isolates was estimated.

2.2.5 Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration for each of the solvent extracts was determined. The mean of the triplicate results was presented.

3. Results and Discussion

Table l shows the diameter zone of inhibition on the selected human pathogenic microorganisms. The Minimum Inhibitory Concentration (MlC) of bark extract from the plant was observed to be 40mg/ml for the isolates except for *Aspergillus flavus and Klebsiella pneumoniae* which had MlC equivalent to 66.6mg/ml (Table 2). The Minimum Inhibitory Concentration was regarded as the lowest concentration of extract required to inhibit test organisms to a significant decrease of over 90% inoculum viability.

Escherichia coli was resistant to all the standard antibiotics engaged in this investigation. *Pseudamonas aeruginosa*, *Klebsiella pneumoniae,* and *Proteus mirabiliis* were sensitive to ciprofloxacin with 8mm, 10mm and 10mm diameter zones of clearance respectively. The isolates resisted the other antibiotics (Table 3).

The antimicrobial activity of the solvent extracts of *Parkia biglobosa* stem bark on selected microorganisms (bacteria and fungi) has revealed the degree of potency of the bark extract as observed in

the values of inhibition zones shown in the tables. Of all the extracts used, ethanol extract was found to be the most active on the tested bacterial strains (*Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Proteus mirabilis*) followed by aqueous extract. *Escherichia coli* was observed to be the most susceptible bacteria to ethanol extract at 40mg/ml MIC. This result is similar to the findings of Obi and Onuocha (2000) who reported that alcohol was the best organic solvent for the extraction of most plant bioactive principles of medical importance. This also tallies with the findings of Millogo-Kone *et al*. (2001b) who expressed that the ethanolic extract of stem bark of *Parkia biglobosa* was active on *Staphylococcus aureus*. Furthermore, Millogo kone *et al*., (2006) later showed again that alcoholic extract of the stem bark of *Parkia biglobosa* was the most active on all his tested microorganisms.

Ethanol extract also showed antimicrobial activity on the fungal species *Aspergillus flavus* and *Aspergillus fumigatus* (Tables 1 & 2).

The phytochemical screening revealed that the stem bark is rich in steroids and triterpenoids, saponins, tannins, reducing compounds and anthocyanins (Sofowora, 2008; Edeoga *et al*., 2005). *Parkia biglobosa* is prescribed in traditional medicine in different parts of West Africa. In some communities, Nigeria inclusive, the bark is soaked in ethanol and used as anti-snake venom that protects against neurotoxic, haematotoxic and cytotoxic effects of poisonous snakes (Agunnu *et al.*, 2005).

4. Conclusion and Recommendation

Diverse claims on the medicinal applications of locust bean (*Parkia biglobosa*) tree in the treatment of multiple aliments have been on the increase. In vitro studies and screening for bioactive ingredients in *Parkia biglobosa* aimed at determining antimicrobial properties using different solvents have shown that the ethanol extract is bactericidal as well as fungicidal. The development and purification of the ethanol extracts of *Parkia biglobosa* in the pharmaceuticals should be encouraged by our governments since *P. biglobosa* is a cheap source of curative.

Table 1: Diameter (mm) of zones of inhibition by *P. biglobosa* bark extracts on selected human pathogenic microorganism

Microbial Isolates used	Solvent of extraction	Undiluted extract 100mg/ml	Control
Escherichia coli	Ethanol	20	0
	Pet. Ether	-	-
	Aqueous	-	-
	Hexane	-	-
Klebsiella pneumoniae	Ethanol	20	11
	Pet. Ether	-	-
	Aqueous	18	-
	Hexane	-	-
Pseudomonas aeruqinosa	Ethanol	26	10
	Pet. Ether	-	-
	Aqueous	-	-
	Hexane	-	-
Proteus mirabilis	Ethanol	20	9
	Pet. Ether	-	-
	Aqueous	-	-
	Hexane	-	-
Aspergillus flavus	Ethanol	22	9
	Pet. Ether	-	-
	Aqueous	-	-
	Hexane	-	-
Aspergillus fumigatus	Ethanol	43	9
	Pet. Ether	-	-
	Aqueous	-	-
	Hexane	-	-

Table 2: Minimum Inhibitory Concentration (MIC) of zones of inhibition with *P. biglobosa* bark extracts on selected human pathogenic microorganism

Microbial Isolates used	**Solvent of extraction**	**Undiluted extract 100mg/ml**	**10ml + 5ml solvent (=66.6mg/ml)**	**10ml + 10ml solvent (50mg/ml)**	**10ml +15ml solvent (=40mg/ml)**
Escherichia coli	Ethanol	20	16	16	12
	Pet. Ether	-	-	-	-
	Aqueous	-	-	-	-
	Hexane	-	-	-	-
Klebsiella pneumoniae	Ethanol	20	15	-	-
	Pet. Ether	-	-	-	-
	Aqueous	18	-	-	-
	Hexane	-	-	-	-
Pseudomonas aeruginosa	Ethanol	15	8	4	3
	Pet. Ether	-	-	-	-
	Aqueous	-	-	-	-
	Hexane	-	-	-	-
Proteus mirabilis	Ethanol	10	8	5	4
	Pet. Ether	-	-	-	-
	Aqueous	-	-	-	-
	Hexane	-	-	-	-
Aspergillus flavus	Ethanol	11	5	-	-
	Pet. Ether	-	-	-	-
	Aqueous	-	-	-	-
	Hexane	-	-	-	-
Aspergillus fumigatus	Ethanol	34	21	7	2
	Pet. Ether	-	-	-	-
	Aqueous	-	-	-	-
	Hexane	-	-	-	-

Table 3: Diameter (mm) zones of inhibition of bacterial isolates using standard antibiotic discs

Antibiotic	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Proteus mirabilis*
Ciprofloxacin	0	10mm	8mm	10mm
Nalixidic acid	0	0	0	0
Amoxicillin	0	0	0	0
Augmentin	0	0	0	0
Nitrofurantoin	0	0	0	0
Streptomycin	0	0	0	0
Gentamycin	0	0	0	0
Tetracycline	0	0	0	0
Chloramphenicol	0	0	0	0
Cloxacillin	0	0	0	0
Cotrimoxazole	0	0	0	0

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