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Antimicrobial Activity of Leaf and Root Extracts of *Parkia biglobosa* (African Locust Bean)

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ABSTRACT

The study of antimicrobial properties of aqueous and ethanol extracts of leaves and roots of *Parkia biglobosa* (African locust bean) was carried out using disc paper impregnation and gel diffusion techniques. The ethanol extracts of both the leaves and roots showed inhibitory activity on all the test organisms. The minimum inhibitory concentration (MIC) of the root extract showed inhibitory activity to be most pronounced on *E. coli* followed by *Proteus mirabilis*, *Pseudomonas* sp., *Klebsiella* sp., *Staphylococcus* sp., *Streptococcus aureus* and *Bacillus* sp. The MIC of the leaf extract, on the other hand, showed inhibitory activity to be most pronounced on *E. coli* followed by *Proteus mirabilis* and least against *Staphylococcus* sp., and *Streptococcus aureus*. However, the minimum bacterial concentration (MBC) of the extracts with different dilutions was found to be bacteriostatic for all the test organisms used in the study. The water extracts of all plant parts showed no appreciable antibacterial activity towards any of the bacteria tested. The findings from this study corroborate the use of *P. biglobosa* for medicinal purposes by herbal healers.

Keywords: African locust bean, antimicrobial, bacteriostatic, inhibition, medicinal plant

INTRODUCTION

The use of plant leaves and roots for medicinal purposes has been a common practice from antiquity. It has been established that crude extracts of some plants as well as pure compounds isolated from such plants can exhibit antibiotic activities (Marquez 2005; Smith *et al*. 2007). The medicinal use of those plants is made possible due to the antimicrobial effects of their extracts. Plants used medicinally are of different species in numerous genera or families, and natural products present in different medicinal plants are found in different organisms.

Three species of the Meliaceae family (*Azadirachta indica*, *Cedrela odorata* and *Guanea multiflora*) are used traditionally in Africa as Antipyretics. A herb, *Artimeisia annua* has been used for centuries in China for treatment of malaria (Klayman 1985), the stems of *Veronia amydalina* and *Musularia accuminanta*, the roots of *Carica papaya*, the root bark of *Eudea natalensis*, roots of *Seccuilnega virosa* and leaves of *Phyllatus reiculanta* are used in Africa for treatment of gonorrhea, and other bacterial infections (Stuart 1977).

Parkia biglobosa (the African locust bean tree) belongs to the family Mimosaceae, order Leguminosae, and is native to Africa. *P. biglobosa* occurs in a diversity of agro-ecological zones from tropical rainforests to arid zones (Udobi and Onaolapo 2009).

When fermented, *P. biglobosa* is commonly used as a food condiment known as *dorowa*, *igba* or *irugba* in Yoruba and *ogiri* in Ibo. *P. biglobosa* is used by most traditional healers in the Northern part of Nigeria for treating stomach upset and diarrhea (Lewis and Lewis 1977). The roots, fruits and stem bark of *P. biglobosa* is used in the treatment of infertility and veterinary medicine respectively among the Igede people of Benue State in Nigeria (Igoli *et al*. 2003). *P. biglobosa* leaves are traditionally used as antihypertensive agent in Benin, Nigeria (Tokoudagba *et al*. 2010), A decoction of the skin bark of *P. biglobosa* is used as a mouth wash to steam and relieve toothache as well as a bath for fever (Ajaiyeoba 2002). A bark infusion of the plant is used as a tonic for diarrhea and anemia in both Nigeria and Cote d'Ivoire (Dakar-Eshua *et al*. 2001; Aguru *et al*. 2005).

This paper reports the study on the antimicrobial activity of leaf and root extracts of *P. biglobosa*.

MATERIALS AND METHODS

Plant extracts

Fresh roots and leaves of African locust bean plant were collected from Nsukka, Enugu State, Nigeria, dried and grind into powder. The active ingredients in the samples were extracted by dissolving 90 g of the dry powdered leaves and roots of test plant in 250 ml of hot water and 250 ml of absolute methanol, respectively. The mixtures were allowed to stand for 24 h the supernatants were decanted into clean conical flasks and fresh solvent (hot water and methanol) were poured into the respective residues for further extraction. The respective extracts were concentrated by leaving the alcohol extract open inside a fume cupboard for 48 h while the water extracts were evaporated in an evaporating dish. The residue extracts were weighed and stored at 4°C in a refrigerator until when needed.

Test organisms

Test organisms collected from clinical samples (urine, urethra smear, high vaginal swab and nasal swabs) at the microbiology department of Imo State University Teaching Hospital Orlu. The samples were primarily cultured on blood agar and McConkey agar following the technique of Cheesbrough (1998). The test organisms were identified using their group morphology, cultural characteristics, Gram reaction and other biochemical tests as done by Cruickshark *et al*. (1995). The findings of the biochemical test and morphological characteristics of the isolates were compared with Bergey's Manual of determinative bacteriology (Krieg and Holt 1984).

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Research Note

Table 1 Susceptibility pattern of extract on test organisms.

Sample	Technique	Zone of Inhibition of test organism (mm)						
		Pseudomonas sp.	*Escherichia coli*	*Proteus mirabilis*	*Bacillus* sp.	*Staphylococcus aereus*	*Streptococcus* sp.	*Klebsiella* sp.
Leaf/Alcohol	Paper	12.0 ± 0.05	6.4 ± 0.2	7.0 ± 0.06	8.4 ± 0.03	12.8 ± 0.1	11.4 ± 0.07	8.38 ± 0.05
	Well	12.8 ± 0.03	18.3 ± 0.1	18.9 ± 0.1	13.1 ± 0.08	19.8 ± 0.1	12.6 ± 0.05	11.88 ± 0.05
Root/Alcohol	Paper	11.6 ± 0.02	15.9 ± 0.2	9.1 ± 0.05	9.1 ± 0.07	12.8 ± 0.2	9.0 ± 0.1	7.25 ± 0.08
	Well	12.2 ± 0.15	22.4 ± 0.1	15.3 ± 0.1	14.3 ± 0.04	17.0 ± 0.1	11.0 ± 0.09	9.13 ± 0.05
Root/Hot water	Paper	10.0 ± 0.04	-	-	-	-	-	-
	Well	-	-	-	-	-	-	-
Leaf/Hot water	Paper	-	-	-	-	-	-	-
	Well	-	-	-	-	-	-	-

Key: Paper = Disc paper impregnation technique, Well = Well in Agar (gel diffusion) technique, Zone of inhibition = diameter in millimeter

Testing for antibacterial activity

The antibacterial activity (growth inhibitory effect) of the extract was tested on the selected test organisms using the disc paper impregnation technique as done by Obiajuru (1995) and Frobischer (1968). The antibacterial activity of the extracts was compared with selected commercially available antibiotics obtained from a pharmaceutical store in Owerri, Nigeria.

Statistical analyses

The data obtained from this study were analysed statistically using simple percentage analysis (Philips 1970). Values were expressed as mean ± standard error of means (SEM).

RESULTS AND DISCUSSION

Percentage yield of extracts

In this study, alcohol was found to be a more effective extraction solvent than hot water (**Fig. 1**). The result also shows that the root extract contains more active principles than the leaf extract. The extraction of the active principles in the roots and leaves of the plant using absolute methanol proved better than aqueous extract. This finding corroborate with works carried out on other plants with antimicrobial activities as their active principles were seen to be more soluble in alcohol than water (Farnsworth *et al*. 1985).

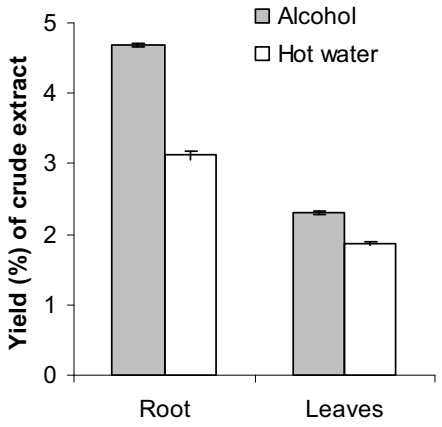

Fig. 1 Percentage yield of crude extract in alcohol and hot water.

Table 2 MIC of root extract on bacterial species.

Test organism	Dilution of extract						
	1/5	1/10	1/20	1/40	1/80	1/160	MIC
Staph. aureus	-	-	-	+	+	+	1/20
Streptococcus sp.	-	-	-	+	+	+	1/20
Bacillus sp.	-	-	-	+	+	+	1/20
E. coli	-	-	-	-	+	+	1/40
Proteus mirabilis	-	-	-	-	+	+	1/40
Pseudomonas sp.	-	-	-	-	+	+	1/40
Klebsiella sp.	-	-	-	+	+	+	1/20

+ = Bacterial growth, - = Bacterial growth inhibited

Susceptibility pattern of extracts on test organisms

The root and leaf extracts of *P. biglobosa* showed different levels of susceptibility on selected test organisms using well in agar (gel diffusion) and disc paper impregnation method (**Table 1**). The observed activity using the two methods was almost the same, but in comparison, the gel diffusion technique showed higher growth inhibitory activity than the disc paper method. This result is similar to that observed by Obiajuru (1995). The results also show that the alcohol extracts exhibited growth inhibitory effects on the test organisms. Using the disc impregnation technique, the root extract of the test plant showed its highest inhibitory effect on *Staphylococcus aereus* (12.8 mm) while the lowest effect was recorded in *E. coli* (6.4 mm). In the gel diffusion technique, the highest inhibition was also recorded in *Staphylococcus aereus* (19.8 mm) while the least was recorded in *Klebsiella* sp. (11.88 mm). The leaf extract (in both techniques) showed the highest inhibitory effect on *E. coli* and the least effect on *Klebsiella* sp. The susceptibility of the extract to the test organisms which comprised both Gram-positive and Gram-negative microorganisms showed that it is broad spectrum in effect. A similar finding was reported by Kandakai-Olukemi *et al*. (1996) in their work on the stem bark of *P. biglobosa.* The high susceptibility of the extract to *Staphylococcus* sp. is of great interest since multiple antibiotic strains of *Staphylococcus aureus* exist in clinical settings worldwide (WHO 1983) as well as in Nigeria (Kandakai-Olukemi *et al*. 1996). With additional research, *P. biglobosa* may be useful in treatment of staphylococcal infections.

Inhibitory effect of extracts on test organisms

The minimum inhibitory concentration (MIC) of the root extract on *E. coli*, *Proteus mirabilis* and *Pseudomonas* sp. was 1/40 while the MIC for *Staphylococcus aureus*, *Streptococcus* sp., *Bacillus* sp. and *Klebsiella* sp. was 1/20 (**Table 2**). The MIC of the leaf extract on the test organisms were 1/10 for *Staphylococcus aureus* and *Streptococcus* sp., 1/20 for *Bacillus* sp., *Pseudomonas* sp., and *Klebsiella* sp., and 1/40 for *E. coli* and *Proteus mirabilis* (**Table 3**).

Minimum bacteriocidal concentration of extracts

The minimum bactericidal concentration (MBC) of the extract is recorded in **Table 4**. All the plates showed positive bacterial growth showing that the extracts were bacteriostatic and not bactericidal on the organisms. The positive bacterial growth observed in all the plates showed that the extracts were not too toxic. It is obvious that when purified, the active principles in the plant will exhibit higher antimicrobial activity than observed in the research.

Table 3 MIC of leaf extract on bacterial species.

Test organism	Dilution of extract						
	1/5	1/10	1/20	1/40	1/80	1/160	MIC
Staph. aureus	-	-	+	+	+	+	1/10
Streptococcus sp.	-	-	+	+	+	+	1/10
Bacillus sp.	-	-	-	+	+	+	1/20
E. coli	-	-	-	-	+	+	1/40
Proteus mirabilis	-	-	-	-	+	+	1/40
Pseudomonas sp.	-	-	-	-	+	+	1/20
Klebsiella sp.	-	-	-	+	+	+	1/20

+ = Bacterial growth, - = Bacterial growth inhibited

Table 4 MBC of extract on bacterial species.

Test organism	Dilution of extract				
	1/5	1/10	1/20	1/40	MBC
Staph. aureus	+	+	+	+	ND
Streptococcus sp.	+	+	+	+	ND
Bacillus sp.	+	+	+	+	ND
E. coli	+	+	+	+	ND
Proteus mirabilis	+	+	+	+	ND
Pseudomonas sp.	+	+	+	+	ND
Klebsiella sp.	+	+	+	+	ND

+ = Bacterial growth, ND = Not determined

Table 5 Comparative study of the mean zones of inhibition of test plant and some selected antibiotics in mm.

Test organism	Ofloxacin	Ciprofluxacin	Erythromycin	Augumentin	Plant root	Plant leaf
Staph. aureus	28.6 ± 0.01	20.0 ± 0.05	10.0 ± 0.01	12.4 ± 0.05	19.8 ± 0.02	17.8 ± 0.1
Streptococcus sp.	29.0 ± 0.3	22.0 ± 0.04	18.4 ± 0.05	14.0 ± 0.02	18.3 ± 0.1	22.4 ± 0.15
Bacillus sp.	24.2 ± 0.05	25.5 ± 0.12	13.8 ± 0.1	13.0 ± 0.08	18.9 ± 0.05	15.3 ± 0.05
E. coli	21.2 ± 0.04	20.4 ± 0.06	3.0 ± 0.2	4.0 ± 0.01	12.6 ± 0.2	11.0 ± 0.07
Proteus mirabilis	20.6 ± 0.15	25.5 ± 0.02	16.5 ± 0.05	10.8 ± 0.1	11.8 ± 0.04	9.1 ± 0.03
Pseudomonas sp.	20.6 ± 0. 2	22.5 ± 0.04	18.0 ± 0.03	12.0 ± 0.05	12.8 ± 0.02	12.2 ± 0.01
Klebsiella sp.	20.0 ± 0.1	24.0 ± 0.03	10.5 ± 0.03	10.5 ± 0.01	13.1 ± 0.01	14.3 ± 0.06

Comparison of zones of inhibition of extracts with selected antibiotics

Comparison of the mean zones of inhibition of selected antibiotics on the test organisms with that of the root and leaf extracts on the test organisms is summarized in **Table 5**. It showed that the antibiotics Ofloxacin and Ciprofloxacin exhibited higher zones of inhibitions than the test plant extracts; while the root and leaf extracts exhibited higher zones of inhibition on the test organisms than Erythromycin and Augmentin.

CONCLUSION

This study has shown that the leaves and roots of *P. biglobosa* (African locust bean) contain some active principles, which have antimicrobial effects on both Gram-positive and Gram-negative bacteria. The positive results of the test justifies the traditional use of the leaves and roots of the plant in treatment of infectious diseases and ailments of the digestive systems especially diarrhea, wounds and ulcers.

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