Afr. J. Biomed. Res. Vol. 5; 125 – 129

Original Article

PHYTOCHEMICAL AND ANTIBACTERIAL PROPERTIES OF *PARKIA BIGLOBOSA AND PARKIA BICOLOR* LEAF EXTRACTS

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The comparative studies of different extracts of the leaves of Parkia biglobosa (Jacq) Benth and Parkia bicolor A. Chev (Mimosaceae) with respect to their photochemical and antimicrobial properties was carried out. Preliminary phytochemical screening showed that both plants had similar constituents namely cardiac glycosides, steroids, tannins and alkaloids. The thin layer chromatography of the hexane and ethanol extracts of both plants were also investigated and two of the components of the ethanol extracts of both plants were found to display similar properties. The antimicrobial screening of the hexane, ethyl acetate, ethanol and water extracts of both plant leaves were done using standard strains of Staphylococcus aureus, Bacillus cereus, Esherichia coli, Pseudomonas aeruginosa, Aspergillus niger and Candida utilis. The ethyl acetate, ethanol and water extracts exhibited a concentration dependent antibacterial, inhibiting the growth of the gram-positive bacteria used in the study. Extracts of P. bicolor were slightly more active than those of P. biglobosa.

Key words: Parkia biglobosa, P. bicolor, phytochemical , antibacterial, plants

INTRODUCTION

Parkia biglobosa (Jacq) Benth and *Parkia bicolor* A. Chev belong to the plant family Mimosaceae of the order Leguminisae. In Yoruba, *P. bicolor* is referred to as Igba Odo; Dorowa, in Hausa, and in Ibo as Origili Okpi. *P. biglobosa* popularly known as the African locust bean tree is known in Yoruba as Igba, or Irugba, in Hausa as Dorowa and in Ibo as Origili. The fermented seeds of *P. biglobosa* are used in all parts of Nigeria and indeed the West Coast of Africa for seasoning traditional soups. Similarly, both trees form a crown so are often grown as shade trees (Daziel, 1937; Hutchinson, 1959). However, there are some distinctive characteristic differences. P. *bicolor* is a tree usually found by the river bank and can grow up to about 100m high. On the other hand *P. biglobosa* is found commonly everywhere in the Savannah and it grows up to about 20m high. The pinnae of the former is about 10 - 26 pairs while that of the latter is about 6 - 11 pairs. The leaflets of *P. bicolor* occur in 20 - 55 pairs, those of *P. biglobosa* in 14 - 30 pairs (Andrew, 1956).

Parkia species have found use traditionally as foods, medicinal agents and are of high commercial value. The pulverized bark of *P. bicolor* is employed in wound healing. *P. biglobosa* is known to provide an ingredient that is used in treating leprosy, and for treating hypertension. In Gambia, the leaves and roots are used in preparing a lotion for sore eyes. A decoction of the bark of *P. biglobosa* is also used as a bath for fever, as a hot mouthwash to steam and relieve toothache. The pulped bark is used along with lemon for wound and ulcers (Irvine, 1961).

Parkia plants have been identified as source of tannins, saponins, gums, fuel and wood Seeds of various species of *Parkia* have also been investigated for their protein and amino acid contents (Fetuga *et. al*., 1974). In continuation of our study of chemical constituents of different parts of *P. bicolor and P. biglobos* (Aiyelaagbe, *et. al*., 1996) and plant foods (Ajaiyeoba, 1998) for their

medicinal and food values, the photochemical screening and antimicrobial studies of *P. bicolor and P. biglobosa* is presented.

MATERIALS AND METHODS

Plant Collection and Authentication and Extraction: The leaves of *Parkia bicolor* were collected at the Forestry Research Institute of Nigeria (FRIN), Ibadan. It was authenticated by Mr. F. Akinwunmi of the Herbarium Section were a voucher specimen was deposited while the leaves of *P. biglobosa* were collected in the University of Ibadan, Ibadan and authenticated by Mr. E. Ogunduyilemi of the Department of Botany and Microbiology where a voucher specimen was also deposited. The leaves were sun dried for five days, milled respectively with an electric blender, prior to extraction with the different solvents.

For photochemical analysis, the powdered leaf samples of both plants (100g) were respectively extracted with hexane for 6 hours using a Soxhlet apparatus and the defatted plant materials were air-dried and divided into 5 batches (20g) respectively. Each batch was then individually extracted with a suitable solvent for the various identification tests.

Phytochemical studies: The defatted of samples of both species of *Parkia* were each extracted with ethanol and tested for alkaloids. The ethanol extracts were also tested for free and glycoside, bound anthraquinones (Kapoor et. at., 1969) and for tannins, solutions of $FeCl_3$ was used. The defatted leaf extracts were also tested for sterols and for terpines - using the Libermann - Burchard reagent and for cardiac glycosides using the Killer-Kilani test. The presence of saponins was identified by subjecting the aqueous extract to frothing and red blood cell haemolysis tests (Harborne, 1993; Ajaiyeoba, 1998).

Thin Layer Chromatography of Extracts: Thin Layer Chromatographic (TLC) analyses of the hexane and ethanol extracts of both plants were done using pre-coated silica gel and alumina plates (Merck, GF254, 20 X 20 cm, 0.2 mm thickness). Increasingly polar mobile phases were made with varying mixtures of Hexane, ethyl acetate and ethanol. Spots were visualized with UV lamp fluorescent at 254 nm, Dragendorf spray and Ferric Chloride reagent.

Micro organisms: The following strains of bacteria were used: *Staphylococcus aureus* (NCTC 6571), *Bacillus cereus,* (laboratory stock), *Escherichia Coll* (NCTC 9001) *Pseudomonas aeruginosa* (NCTC 6570)

Media: Nutrient broth No 2, pH 7.4; nutrient agar pH 7.4; tryptone soya broth and tryptone soya agar; all products of Oxoid Laboratories, UK were utilized in this study.

Antimicrobial agents: The following chemotherapeutic agents were included in the test as control; gentamycin sulphate (1μg/ml, Nicholas Laboratories Ltd., UK) Ampicillin 2.5μg/ml, (Lab Oftalmiso, Spain).

Antimicrobial activity determination: The agar cup diffusion and dilution (Kavanagh, 1972), similar to our previous (Ajaiyeoba et. al., 1998) method was used. An overnight broth culture of 1-2 x 107 CFU (Colony Forming Unit) of each bacterium was used to seed sterile molten nutrient agar medium maintained at 450C. They were allowed to set and wells (8mm in diameter) were made on them using a sterile standard cork borer and 60μL of the compound d dissolved in methanol was added to each well. Each plate had wells filled with methanol, gentamicin and ampicillin when seeded with bacteria.

RESULTS

Phytochemical analysis: The results of the phytochemical analysis are presented in Table 1. Both species of

Parkia contained cardiac glycosides, though it gave a much more positive test for that of *P. biglobosa.* Tannins were also indicated in the two leaf extracts. There was a slight presence of alkaloids in both plant materials. The steroidal content of both of them was negligible. However, there was complete absence of saponins and anthraquinones in both extracts.

Table 1
Results of Phytochemical analysis'

Test		*Parkia bicolor*	*Parkia biglobosa*
i.	Cardiac glycosides (Killer-Killani Kedde Tests)	+	+++
ii.	Tannins (Extract + 0. I% $FeCl_3$)	+++	+++
iii.	Saponin glycosides (Extract & 20% NaOH & Benedicts solution	-	-
iv.	Alkaloid Extract- 10% HCl Dragendoff s Wagner's reagent	++ ++	++ +++
V.	Anthraquinones Extract, 12% H2SO4 $CHCl_3$, 10% NH_3 Solution	-	-
vi.	Steroids Libernmann - Burchard	+	+

a. +++, appreciable amount; ++ moderate amount; + trace; complete absence

Table 2:
Thin Layer Chromatography of Extracts of *P. biglobosa and P. bicolor*

Plant Extract	Adsorbent	Mobile Phase*	Spot	R
P. biglobosa (Hexane)	Silica gel	A	1 2	0.40 0.55
P. bicolor (Hexane)	Silica gel	A	1 2	0.53 0.69 0.82
P. biglobosa (Ethanol)	Alumina	B	1 2 3 4	0.74 0.80 0.82 0.86
P. bicolor (Ethanol)	Alumina	B	1 2	0.74 0.76 0.86

H = Hexane; E = Ethanol; A= Hexane/ ethyl acetate (3: 1), B= Ethanol/ chloroform (4: 1)

Thin layer chromatographic (TLC) analysis on silica gel, of the different extracts showed the presence of 2 main components in *P. biglobosa* and 3 components in P. *bicolor,* of the hexane extracts (hexane: ethyl acetate ratio 3:1) as shown in Table 2. In the TLC analysis of the ethanol extracts on alumina (neutral), four compounds were identified in *P. biglobosa* and three (ethanol: chloroform; ratio 9:2) in P. *bicolor.* All the compounds shown on the chromatograms gave a negative Dragendorff reagent (absence of alkaloidal compounds).

Antimicrobial testing: The extracts were tested at two concentrations (50mg/ml and 100mg/ml) in the respective solvents. The drug susceptibility testing of the extracts were done in serial dilutions of the extracts to a concentration of 25mg/ml and 12.5mg/ml of the crude extracts respectively, no antibacterial activities were observed.

Table 3
Antimicrobial screening of extracts of *Parkia bicolor and P. biglobosa*. Zones of Inhibition of Plant Extracts (100mg/ml)[a]

Extracts	Microorganisms			
	S. aureus	*B. cercus*	*E. coli*	*Ps. aeruginosa*
P. biglobosa				
Hexane	-	-	-	-
Ethyl acetate	+	+++	-	-
Ethanol	+	++++	-	-
Water	+	++++	-	-
P. bicolor				
Hexane	-	-	-	-
Ethyl acetate	+	++++	-	-
Ethanol	+	++++	-	-
Water	+	++++	-	-
Methanol	-	-	-	-
Amp (2.5[μg/ml)	++	++++	+	-
Gent [1μg/ml)	+++	++++	++++	++++

[a]*zones of inhibition: 8 - 12 mm- ++, 13 - 15 mm, +++, 16-19*
++++, 20 and above bic, *P. bicolor; big, P. biglobosa* Amp, Ampicillin; Gent, Gentamicin;
[b]Solution of extracts were made in methanol

At 50mg/ml, the growth of *S. a reus and B. cereus* were inhibited by ethyl acetate, ethanol and water extracts of both species of *Parkia* to the same extent. When the concentration of the plant extract was increased to 100mg/ml, the extracts generally exhibited a higher activity. Methanol was included as control while Ampicillin and Gentamicin were included as reference drugs. ` The results of the antimicrobial screening of extracts of both leaf samples are presented in Table 3.

DISCUSSION

The plant family Mimosaceae has been the subject of investigation in nutritional value of the seeds (Krans & Reiboth, 197'); Fetuga et. al., 1974; Ajaiyeoba, et.al. 1996). Locally in Nigeria, the seeds of *Parkia sp* are used for food seasoning, obtained by boiling and fermentation of the seeds, popularly known as *Iru* in Yoruba. Odunfa (1981) identified the microorganisms associated with *Iru* fermentation as *Staphylococcus hominis, S saprophytoccus, xylolus and Bacillus subtilis. P. biglobosa* fruit pulp and seeds are also known to be rich in protein and amino acids, with a high concentration of glutamic acid (Busson et. al. 1958; Lanza et. al., 1962; Krans and Reiboth, 1973). In a previous study, (Aiyelaagbe, 1996), the seed oils of *P. biglobosa and P. bicolor* were analyzed for their possible edible utility The two oils contained similar fatty acids with Arachidic acid being most abundant.

The result obtained in the phytochemical screening as presented in Table 1, seems to justify the use of the leaf of *Parkia biglobosa* for cardiac conditions as appreciable amount was present in *P. biglobosa* alone and not *P. bicolor.*

From the TLC analyses, the two compounds resolved in hexane extract of *P. biglobosa* (R_f 0.40, R_f 0. 5 5 in Hexane: Ethyl acetate: ratio 3:1) respectively were not alkaloids and were not cardiac glycosides. They gave the positive colour reaction for tannins as was detected in the preliminary phytochemical screening. Three spots were observed in the TLC of *P. bicolor* (with R_f of .5-'I, 0.69 and 0.82). They were different from the spots in the former plant extract and were also non-alkaloidal. The ethanol extracts of both plant materials had two spots (R_f 0.74, 0.86) with similar properties as shown on TLC on alumina (Table 2).

All extracts were most active against *B. cereus* and the growth of *S aureus was* moderately inhibited by the extracts. The extracts had a concentration dependent antibacterial activity with sensitivity for only the Gram-positive bacteria used in the study. Ethanol and water extracts of both samples were most active having Minimum inhibitory concentrations of 50 mg/ml. The hexane extracts of both plants were inactive to all the microorganisms used in the study. They were also not susceptible to the growth of *Ps. aeruginosa, Aspergill s niger and Candida utilis* utilized in the study. The results obtained showed that the folklore use of these plants in some of the above -mentioned conditions is justified.

ACKNOWLEDGEMENTS

This work was supported in part by the University of Ibadan Senate grant (No. SRG/COM/91/92/079a). The technical assistance of Mr. M.S. David is hereby acknowledged.

REFERENCES

Aiyelaagbe, O.O., Ajaiyeoba, E.O., Ekundayo, O.O. (1996): Studies on the seed oils of Parkia *biglobosa and Parkia bicolor.* Plant Foods for Human Nutrition 49: 229 - 233.

Ajaiyeoba E.O., Okogun J.1. and Adeniyl B.A. (1996): Antimicrobial activity' of Crenatine, an indole alkaloid synthesized from indole. Phytotherapy Res. 9: 69 - 71.

Ajaiyeoba E.O., (1998): Comparative Phytochemical and Antimicrobial studies of *Solan m macrocarpum and Solan m torv m* leaves. Fitoterapia 70: 184 - 186.

Andrews, F.W. (1956): The flowering plants of Anglo-Egyptian Sudan, Vol. 11, Sudan Government.

Busson F., Perisse, J. and Jasger, P. (I 9 5 9): Chromatographic determination of

amino acids in seeds of *P. biglobosa.* Physiol. Chem. 310:1-3).

Dalziel, J.M. (I 937): The useful plants of West Africa. Crown Agents for Oversea Government and Administration, p. 217.

Dutta, A.C. (I 970): Botany for Degree students 4"' ed., Oxford University Press, London, pp. 724 - 729.

Fetuga, B.L., Babatunde, G.M., Oyenuga, V.A. (1974): Protein quality of some unusual protein foods -African Locust bean seed. Brit. J. Nutri. 3: I- 6.

Harborne, J.B. (1991): Phytochemical Methods, 2'd ed., Chapman & Hull, London, p. 288.

Hutchinson, J. (1959): Families of flowering plants. Dicotyledon. Vol 1. Oxford University Press London, pp. 240 - 247.

Irvine, F.R (I 96 1): Wood Plants of Ghana Oxford University Press, London, p. 104.

Kapoor, L.D., Singh, A., Kapoor, S.L. and Srivastave, S.N. (1969): Survey of Indian Medicinal Plants for Saponins, alkaloids and flavonoids. Lloydia 32: 279 -302.

Krans, G.Y.J. and Reiboth, H. (1973): Structural difference and distribution pattern of amino acids in Mimosaseae. Phytochem.12:125-142.

Lanza, M., Regli, P. and Busson, F. (I 962). Hydroxyproline content in the protein of the fruit pulp of Parkia biglobosa. Med. Trop. 22: 377-384.

Odunfa, S.A. (I 98 1): Identification of the microorganisms associated to Iru' fermentation. J. Plant Foods 3: 245-250.

Received: February 2002

Accepted: September 2002