International Scholars Journals

International Journal of Medicinal Plants Research ISSN 2169-303X Vol. 14 (10), pp. 001-007, October, 2025. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Antibacterial Activity and Phytochemical Profile of Parkia biglobosa: A Study of Leaf, Stem Bark, and Root Extracts

C. E. Udobi[1*] and J. A. Onaolapo[2]

[1]Department of Food Technology, College of Science and Technology, Kaduna Polytechnic, Kaduna, Nigeria.
[2]Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University Zaria, Nigeria.

Accepted 6 August, 2025

Antibacterial evaluation of the methanolic extract and aqueous fractions of the Leaf, Stem Bark and root of the African locust bean tree, *Parkia biglobosa* was carried out using the agar- well diffusion method. The extracts and their fractions were tested against two gram positive organisms – *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* NCTC 8326B76 and two gram negative organisms–*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC-27853. Results obtained confirmed a broad spectrum of activity as all the organisms used were inhibited by the extracts and their aqueous fractions at concentrations between 2.5 – 20 mg. Zones of clearing observed against *S. aureus* were more pronounced, distinct and wider than those against other organisms tested. It was closely followed by *P. aeruginosa*. Results of the Minimum Inhibitory Concentrations (MIC) test using the aqueous fractions showed that the MIC ranged from 1.562 - 25mg/ml. This confirms the high activity of the aqueous fraction of the methanolic extract of the stem bark (WS) against *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853. The WS showed higher activity than the aqueous fraction of the methanolic extract of the leaf (WL) and the aqueous fraction of the methanolic extract of the root (WR). When the extracts were screened for secondary metabolites, tannins, flavonoids and saponins were detected among others. This was further confirmed by the aqueous fractions. These secondary metabolites are most likely responsible for the observed activity of the plant parts. Results obtained in this work justify the medicinal uses to which *P. biglobosa* parts have been employed traditionally in recipes for infection.

Key words: Antibacterial evaluation, *parkia biglobosa*, aqueous fraction, minimum inhibitory concentration, secondary metabolite.

INTRODUCTION

The quest for solutions to the global problems of anti- biotic resistance in pathogenic bacteria has often focused on the isolation and characterization of new antimicrobial compounds from a variety of sources including medicinal plants (Sibanda and Okoh, 2007). This is probably because the efficacies of these plant products have been confirmed in different disease situations in different parts of the world and that their little or no known side effects have made them succeed where most synthetic or conventional agents have failed. It may also be because scientists have established that crude extracts of some plants and some pure compounds from such plants can potentiate the activity of antibiotics in-vitro (Marquez, 2005; Smith et al., 2007).

In Africa, medicinal preparations from plants have been used over a long period for the treatment of ailments. This is because orthodox medicine is not available in some places due to a wide range of reasons, among which includes that the first line drugs which are cheap and affordable have become ineffective because of resistance. Now however, these plant preparations are becoming more widely used by people all over the world as they understand the gentle strength in them and the fact that most of them can be used safely without the known side effect of drugs (Steve, 2004). Now, workers in the field of plant medicines research, regard higher

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

plants as living chemical factories that provide a vast number of unusual chemical substances that display a variety of biological actions (Oyi, 2001).

Plants are able to produce compounds which though have no apparent function in the primary metabolism of the plant (Robinson, 1991), have good activity against bacterial pathogens when they are able to find their way into and accumulate in them. These compounds have had an extensive history of use as therapeutic agents (Tyler et al 1988).

Parkia biglobosa is a multipurpose fodder tree that belongs to the family MIMOSACEAE (Sabiti and Cobbina, 1992). Popularly called the "African locust bean tree", they are known to occur in a diversity of agro- ecological zones from tropical rainforest where the rain is high to the arid zone where it is low. The height ranges from 7 - 30 m. It is crown large and spreads wide with low branches, the leaves are alternate, dark green, bipinnate and about 8 – 30 mm x 1.5 – 8 mm in size with about 13 - 60 pairs of leaflets of distinct venation on a long rachis. The pods are pink brown to dark brown when matured, they are up to 45 cm long and 2 cm wide. Each pod contains up to 30 seeds embedded in a yellow peri- carp. The seeds are relatively large with an average weight of 0.26 g and have a hard testa. (Agroforestree Database, 2008).

P. biglobosa is found in many countries of the world especially along the West African coast where the seeds are fermented into iru or dawadawa (daddawa), a popular food seasoning known to be rich in protein and vitamin B2. Among a wide range of uses which includes apiculture, fodder, tanning, food, etc, this plant has been used exten- sively for medicinal purposes by the Hausa people of Nor- thern Nigeria and other parts of West Africa. A decoction of the stem bark is used as a mouthwash to steam and relieve toothache as well as a bath for fever (Ajaiyeoba, 2002). The bark is also used with lemon for wounds and ulcers. In cote d' Ivoire and Nigeria, a bark infusion is used as a tonic for diarrhea and as an enema (Duker-Eshun et al., 2001; Agunu et al., 2005.) Among the Hausa people of Nor- thern Nigeria, *P. biglobosa* is used against bronchitis, pneumonia, diarrhea, violent colic, vomiting, sores and ulcers (Mallam Abbas, Samaru-Zaria, personal communi- cation). The leaves are also used for burns and toothache as well as for sore eyes in Gambia (Banwo et al 2004). The root of P*. biglobosa* has been reported to be used in lotions for sore eyes when combined with leaves, they are active against bronchitis, pile, cough, amoebiasis, dental carries and conjunctivitis (Millogo-Kone et al., 2006). This paper reports the results of the phytochemical analysis and the antibacterial evaluation of the leaf, stem bark and root of *P. biglobosa.*

MATERIALS AND METHODS

Methods

Plant collection and authentication: The plant materials namely leaf, stem bark and roots were collected from Samaru–Zaria in Kaduna state, Nigeria. They were authenticated in the herbarium section of the Biological Science Department of the Ahmadu Bello University Zaria, Nigeria where a voucher with specimen number 2846 was kept.

Preparation and extraction of Plant Samples: Each plant material was air dried for five days on the laboratory bench (Oboh et al, 2007) and then ground into powder in a mortar. Exactly 250 g each were then extracted to exhaustion with methanol using a Soxhlet apparatus.

Afterwards, the solvents were removed and the extracts obtained were stored in the desiccator until needed.

Fractionation of Extract: Each of the extracts (Leaf, Stem bark and Root) was fractionated using Petroleum ether, Chloroform and water. About 20 g of each dried extract was ground in a mortar and dissolved in 200 mls of water before shaking vigorously in a separating flask. The mixture obtained was filtered using a filter paper to remove debris. Thereafter, 200 mls of petroleum ether was added to the mixture, shaken vigorously and allowed to settle. The petroleum ether layer (on top) was removed and concentrated while a further 200 mls of chloroform was added to the aqueous layer and also vigorously shaken and allowed to settle.

The aqueous and the chloroform layers were further separated while the chloroform portion was concentrated to dryness by allowing to stand on the laboratory bench until all the solvent evaporated. The aqueous layer was concentrated to dryness using mild heat. The resulting fractions were appropriately stored in a desiccator until needed.

Phytochemical screening

All the fractions were subjected to Phytochemical screening to test for the presence of saponins, tannins, flavonoids, carbohydrates, alkaloids and steroids among other secondary metabolites using standard methods as described by Trease and Evans (1989); Harbone, (1991) (Table 1)

TEST ORGANISMS

Purification of test organisms

The test organisms were obtained from Professor, J. A. Onaolapo of the Pharmaceutical Microbiology section of the Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria, Nigeria.

Their purity was confirmed by sub-culturing into nutrient broth incubated at 37°C for 18 h, T streaked unto sterile nutrient agar plates and incubated at 37°C for further 18 h. The developed colonies were observed under the microscope after simple staining. Pure cultures were kept on agar slopes at 4°C until needed. They were sub-cultured once every month.

Preparation of inoculums

When needed, the test organisms were grown overnight in nutrient broth at 37°C. They were further diluted 1:1000 for gram positive organisms and 1:5000 for gram negative organisms in normal saline to obtain about one million cells/ml (Anderson, 1970).

SUSCEPTIBILITY TESTING

The agar cup diffusion method was used for this test. Sterile nu- trient agar plates were flooded with appropriately diluted organism

Table 1. Results of Primary Phytochemical screening.

Metabolites	WR	WS	WL	PR	PS	PL	CR	CS	CL
Saponin	+	+	+	–	–	–	–	–	–
Carbohydrate	+	+	+	–	–	–	–	–	–
Alkaloid	–	–	–	–	–	–	+	+	–
Tannins	+	+	+	–	–	–	–	–	–
Flavonoids	+	+	+	–	–	–	–	–	–
Anthraquinones	–	–	–	–	–	–	–	–	–
Cardiac glycosides	+	+	+	–	–	–	–	–	–
Resins	–	–	–	+	+	+	–	–	

Key: + = Present, - = Absent. WR=Aqueous fraction of root, WS= Aqueous fraction of stem bark, WL=Aqueous fraction of leaf, PR=Petroleum ether fraction of root, PS=Petroleum ether fraction of stem bark, PL= Petroleum ether fraction of leaf, CR=chloroform fraction of root, CS=chloroform fraction of stem bark, CL=chloroform fraction of leaf.

organism, the excess was aseptically drained and the surface, allowed to dry at room temperature. Wells were bored into the agar plates using a 4 mm sterile cork borer and 0.1 ml each of different concentrations of the extract and fraction ranging from 200 – 25 mg/ml made using water was introduced into each well. The plates were allowed a pre-diffusion time of 1 h at room temperature and then incubated at 37°C for 24 h after which zones of inhibition were read to the nearest millimeter.

Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of the fraction was determined using the tube dilution method. (Sahm and Washington, 1990). 1 ml of the extract solution at concentrations of 25 mg/ml was added to 1 ml of nutrient broth and subsequently transferred thus, 1 ml from the 1st tube to the next up to the nineth tube. Then, 1 ml of 24 h culture of test organism diluted 1:1000 for gram nega- tive or 1:5000 for gram positive was inoculated into each test tube and mixed thoroughly on a vortex mixer. The tube with the lowest dilution with no detectable growth was considered as the MIC.

RESULTS

Phytochemical analysis

Results of Phytochemical Screening showed the presence of a number of secondary metabolites including tannins, saponins, flavonoids and alkaloids.

Activity of the aqueous fractions of the methanolic extracts of the Leaf, stem bark and root of *P. biglobosa* against the test organisms

Results obtained showed that the aqueous fractions of the methanolic extracts of the leaf, stem bark and root displayed reasonable activity against the organisms tested. The observed zones of inhibition due to the aqueous fraction of the stem bark against *S. aureus* were clearer and more distinct than it was for other organisms. The results are presented in Figures 1 - 3.

They show that the stem bark fraction (WS) showed the best activity followed by the leaf fraction (WL) and root fraction (WR) (Table 2)

DISCUSSION

Phytochemical screening

The presence of different secondary metabolites especially those known to be responsible for antimicrobial activity was confirmed. Some of these include saponins, tannins and flavonoids among others. The presences of these metabolites in plants have been linked to the antimicrobial activities of the plants (Lewis and Ausubel, 2006; Cowan, 1999).

Antimicrobial activity tests

The results confirmed that the extracts obtained with polar solvents (Ethanol and Methanol) showed more antimicrobial activity to the test organisms than the non-polar solvents, for example, chloroform. This is explained by the fact that the secondary metabolites identified in the extracts which are known to have antibacterial properties are also polar compounds and may have been easily extracted by the polar solvents since it is known that “like dissolves like”. Ajaiyeoba, (2002) had earlier on reported that hexane (a non polar solvent) extract of leaves of *P. biglobosa* were inactive when tested on similar organisms.

The same work also confirmed that ethanolic and aqueous (polar solvents) extracts of the leaves of the same plant were more active than extracts of other non- polar solvents.

All the extracts tested showed antimicrobial activity against both gram positive (*S. aureus*) and gram negative organisms (*P. aeruginosa* and *E. coli*). This suggests that they may have a broad spectrum of activity.

The antimicrobial activity test results of the fractions of methanolic extract confirmed that its activity level is very close to that of the crude extracts. This may be because the same metabolites in the crude extracts have all been

Table 2. Antibacterial activity of extracts of *P. biglobosa* against the test organisms.

Solvent	CONC (mg/ml)	Zones of inhibition (mm)								
		S. aureus ATCC 25923			*E. coli* ATCC 25922			*P. aeruginosa* ATCC 27853		
		Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
Hot Water	50	15	14	15	20	20	18	17	15	15
	100	17	20	23	22	25	25	20	20	20
	200	19	23	25	26	27	26	23	21	21
Methanol	50	13	16	12	17	11	12	14	15	14
	100	15	20	17	22	21	19	19	21	21
	200	18	21	20	24	23	25	21	22	22
Ethanol	50	12	15	12	13	13	12	13	12	12
	100	16	18	15	16	22	15	16	15	16
	200	17	20	19	18	25	20	18	18	18
Chloroform	50	0	10	10	0	14	11	NT	NT	NT
	100	0	15	12	0	16	13	NT	NT	NT
	200	10	17	13	11	18	15	NT	NT	NT

NT = Not tested.

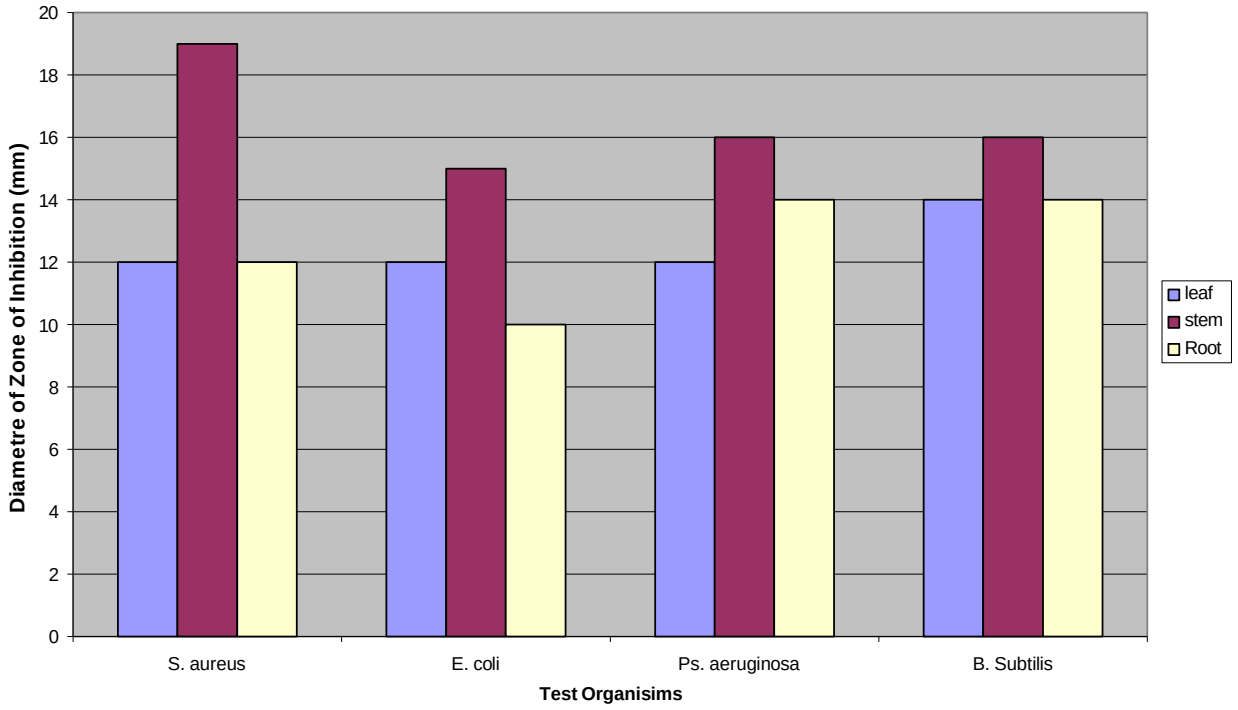

Figure 1. Bar chart showing results of antibacterial susceptibility test of aqueous fractions of leaves, root and stem bark of *P. biglobosa* at a concentration of 5mg.

all been extracted into the aqueous fraction. The zones of inhibition obtained were clearer and more distinct when the fractions were used compared to that of the crude extract. This may be confirming the fact that all the active metabolites have been concentrated in the fractions and that the metabolites are in a purer form. The observed zones of inhibition due to the stem bark fraction against *S. aureus* and *P. aeruginosa* were wider, clearer and more distinct than those for the other organisms.

P. aeruginosa is a known difficult organism resistant to a wide range of antibacterial agents (Jawetz and Melnick, 1982). It has been implicated in wounds, burns and urinary tract infections. It is also known to multiply in in- use dilutions of disinfectants (Cruickshack et al., 1973). The good results obtained against it with the extracts, also justifies its use by trado-medical practitioners in the treatment of wounds, burns, sores and ulcers. (Banwo et al., 2004; Personal communication).

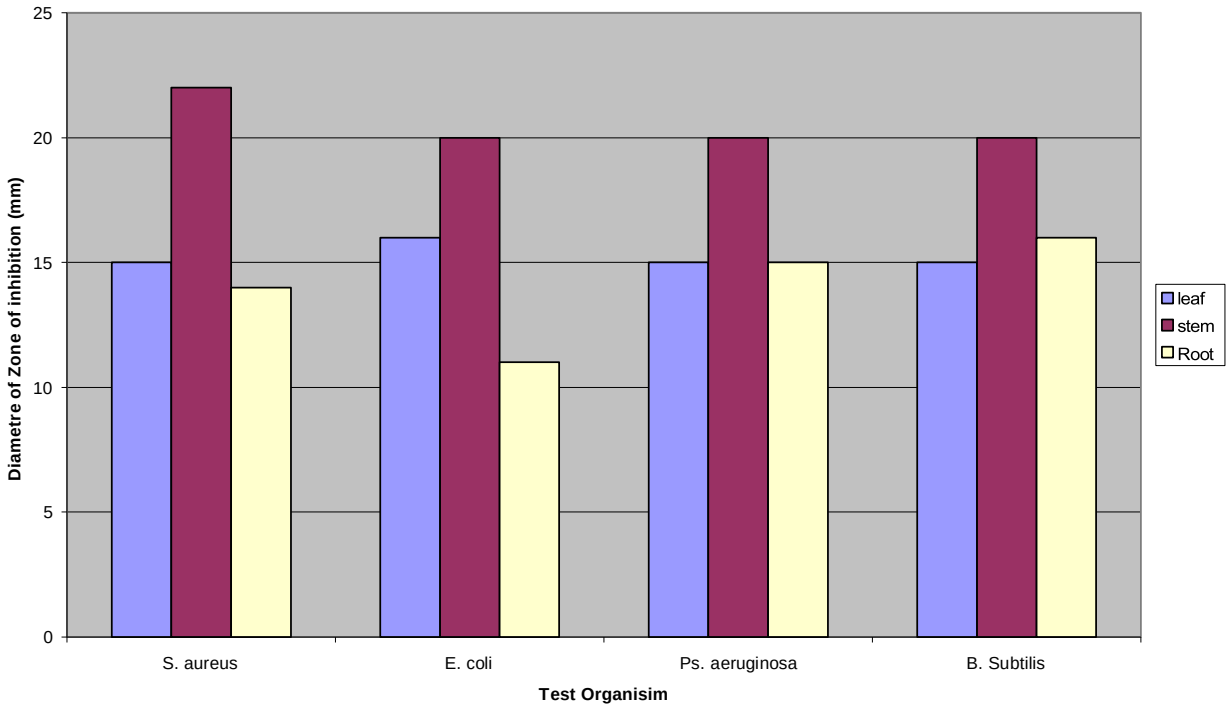

Figure 2. Bar chart showing results of antibacterial susceptibility test of aqueous fractions of leaves, root and stem bark of *P. biglobosa* at a concentration of 10mg.

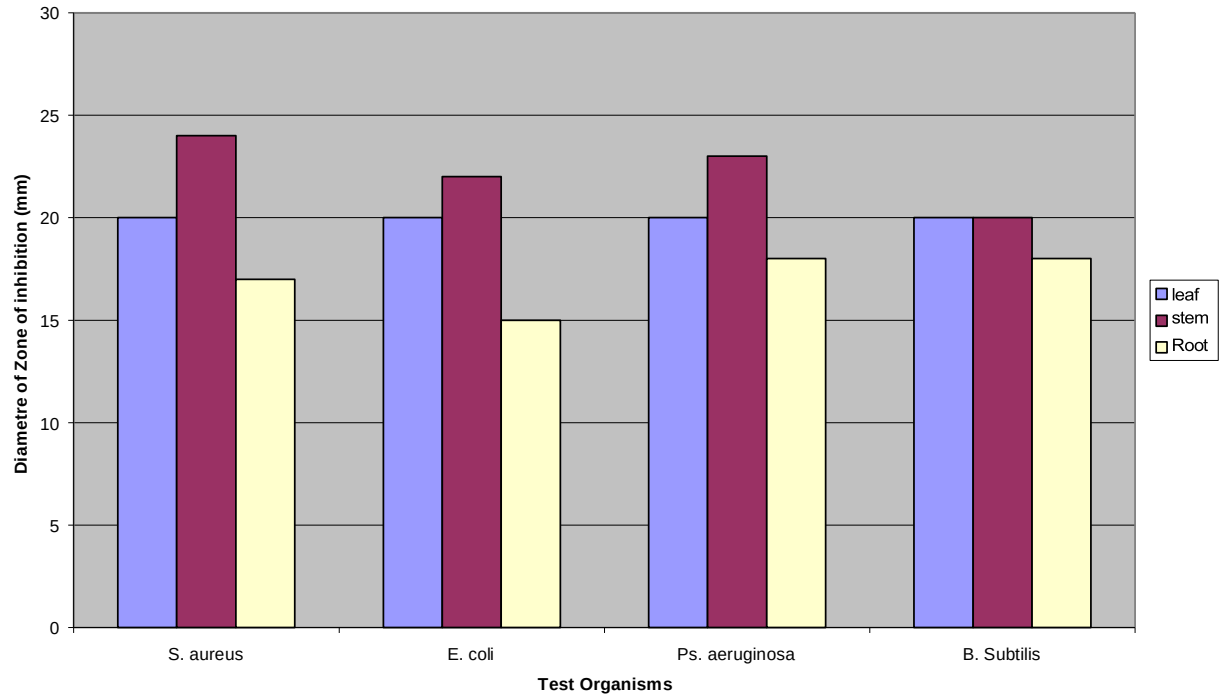

Figure 3. Bar chart showing results of antibacterial susceptibility test of aqueous fractions of leaves, root and stem bark of *P. biglobosa* at a concentration of 20 mg.

MINIMUM INHIBITORY CONCENTRATION (MIC)

The results of MIC showed that they ranged from 1.562 – 25 mg/ml. The values were lowest for *S. aureus* and *Ps. aeruginosa* (1.562 mg/ml) then followed by *B. Subtilis* and *E. coli* (6.25 and 12.5 mg/ml) respectively. The low MIC values of 1.562 mg/ml for *S .aureus* and *P. aeruginosa* further confirmed the good activity of the fractions against the test

Table 3. Minimum Inhibitory Concentration (MIC) of the aqueous fractions of the leaf, stem bark and root of *P. biglobosa* against the test organisms. The MIC obtained ranged from 1.562 mg/ml for *S .aureus* and *P. aeruginosa* to 25mg/ml for *E. coli*.

Fractions	Organism	25	12.5	6.25	3.125	1.562	0.781	0.390	0.195	0.897	0.048	MIC (mg/ml)	MBC (mg/ml)
WS	BS	-	-	-	+	+	+	+	+	+	+	6.25	>25.0
	PS	-	-	-	-	-	+	+	+	+	+	1.562	3.125
	SA	-	-	-	-	-	+	+	+	+	+	1.562	3.125
	EC	-	+	+	+	+	+	+	+	+	+	25.0	>25.0
WR	BS	-	-	+	+	+	+	+	+	+	+	12.5	NT
	PS	-	-	-	-	-	+	+	+	+	+	1.562	NT
	SA	-	-	-	+	+	+	+	+	+	+	6.25	NT
	EC	-	-	+	+	+	+	+	+	+	+	12.5	NT
WL	BS	-	-	-	+	+	+	+	+	+	+	6.25	NT
	PS	-	-	-	-	-	+	+	+	+	+	1.562	NT
	SA	-	-	-	-	-	+	+	+	+	+	6.25	NT
	EC	-	+	+	+	+	+	+	+	+	+	25.0	NT

Key: WL, WS and WR=Aqueous fractions of the leaf, stem bark and root of *Parkia biglobosa.*
BS=*Bacillus subtilis*, PS=*Pseudomonas aeruginosa*, SA=*Staphylococcus aureus*, EC= *Escherichia coli*.

organisms obtained during the antimicrobial sensitivity test. (Table 3)

Conclusions

The use of the leaf, stem bark and root of *P. biglobosa* by traditional healers in the treatment of many infectious diseases whose causative agents include common bacteria used in this study has been justified. The activity of the plant parts is confirmed to be due to secondary metabolites tannins, saponins and flavonoids. All the extracts inhibited both gram negative and gram positive organisms used confirming them to have broad spectrum of activity.

The aqueous fractions of the methanolic extracts of the plant parts showed activity which is close to that shown by the crude extracts confirming that the polar secondary metabolites namely tannins, saponin and flavonoids which are contained therein are responsible for the activity. The slight difference is explained by the fact that crude extracts may contain inactive substances which may also antagonize the antimicrobial actions of one another (Ebi and Ofoefule, 1997). All the organisms tested grew at their determined inhibitory concentrations when plated out on solid media before finally being killed at higher concentrations. The extracts are therefore confirmed to be bacteriostatic at lower concentrations but bactericidal at higher concentrations.

The aqueous fractions are known to be non- toxic (Udobi et al., 2008). Since the organisms used for the work could be common contaminants of pharmaceutical products, it may be reasonable to propose the use of the plant extracts as preservatives.

The production of ameliorated traditional drug which will be affordable, effective and acceptable using the stem bark of *P. biglobosa* as suggested by Millogo-Kone (2007) is recommended.

REFERENCES

Agroforestree Database. International Centre for Research in Agroforestry (ICRAF).

Agunu A, Yusuf S, Andrew GO, Zezi AU, Abdulrahman EM (2005). Evaluation of five medicinal plants used in diarrhea treatment in Nigeria. J. Ethnopharmacol. 101(1-3): 27-30

Ajaiyeoba EO (2002). Phytochemical and antibacterial properties of *Parkia biglobosa* and *Parkia bicolor* leaf extracts. Afr. J. Biomed. Res. 5(3):125 -129.

Anderson GT (1970). "Testing of Susceptibility of antimicrobial agents and assay of antimicrobial agents in body fluids". Manual of clinical microbiology by American Society for Microbiology 1st ed pp 299 - 310.

Audu R, Umilabug SA, Renner J, Awodiji JA (2000). Diarrhea Management. J. Nig. Inf. cont. Assoc. 3:15.

Banwo GO, Abdullahi I, Duguryil M (2004). The antimicrobial activity of the stem bark of *Parkia clappertoniana* keay family Leguminosae against selected microorganisms. Nig. J. Pharm. Res. 3(1):16-22.

Cowan MM (1999). Plant products as antimicrobial agents .Clin. Microbial. Rev. 12(4): 564 – 582.

Cruickshack R, Duguid J P, Marmoin BP, Swain RH A (1973). Medical Microbiology 12th Ed. ELBS, Edingburgh Duker-Eshun GD, Beni CT, Asonaming WA, Akwamoah RA (2001). Chemical investigation of the stem bark of *Parkia*

clappertoniana Keay. Journal of Ghana Science Assoc. 3:2.
Ebi GC, Ofoefule SI (1997). Investigations into the forkloric antimicrobial Activities of Landolphia Owerrience. Phytother. Res. 11: 149-151.
El-Mahmood AM, Ameh JM (2007). In-vitro Antibacterial activity of Parkia biglobosa(Jacq) root bark extract against some micro-orga- nisms associated with urinary tract infections. Afr. J. Biotech. 6(11): 1272-1275.
Harborne JB (1991). Phytochemical Methods, 2nd ed.; Chapman & Hull London.
Jawetz E, Melnick JL (1982). Review of Medical Microbiology. 15th ed. Large Med. Pub. California.
Lewis K, Ausubel FM (2006). Prospects for plant derived antibacterials. Nat. Biotechnol. 24(12):1504-1507.
Millogo-Kone H, Guisson IP, Nacoulna O, Traore AS (2006). Study of the antibacterial activity of the stem bark and leaf extracts of *Parkia biglobosa* (Jacq) Benth on *Staphylococcus aureus*. Afr. J. Trad. Comp. Alter Med. 3(2): 74- 78.
Oyi AR (2001). A study of antimiciobial and phytochemical properties of the latex of *Jatropha Curcas* Linn (Euphorbiacea). Phd. Dissertation. A. B. U. Zaria, Nigeria.
Robinson T (1991). The Organic Constituents of higher plants. 6th ed. Cardus Press, North Amherst, Massachusetts.
Sabiti EN, Cobbina J (1992). Parkia biglobosa: A potential multipurpose fodder tree legume in West Africa. Int. Tree Crops. J. 7:113 – 139.
Sahm DF, Washington JA (1990). Antibacterial ssusceptibility test dilution methods: In: Manual of clinical Microbiology. Lennette E.H. 5th ed Am. Soc. Microbiol. Washington DC, pp. 1105-1116.
Sibanda T, Okoh AI (2007). The challenges of overcoming antibiotic resistance; plant extracts as potential sources of antimicrobial and resistance modifying agents Afri. J. Biotech. 6(25): 2886-2896.
Smith ECJ, Williamson EM, Wareham N, Kaatz GN and Gibbons S (2007). Antibacterials and modulators of bacterial resistance from the immature cones of *chamacyparis lawsoniana*. Phytochem. 68(2): 210-217.
Steve B (2004). Herbal Property Dictionary. Lifelong Press.
Trease GE, Evans WC (1989). Pharmacognosy. 15th edn. Brailliar Tridel can, Macmillan Publishers.
Tyler VE, Brady LR, Robbers JE (1988). Pharmacognosy, 9th ed. Lea and Fabiger, Philadephia.
Udobi CE, Onaolapo JA, Agunu A (2008). Antibacterial activities and bioactive components of the aqueous fraction of the stem bark of *Parkia biglobosa* (Jacq) (Mimosacea). Nig. J. Pharm. Sci., 7(1): 76-82.