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Evaluation of phytochemical, antioxidant and antibacterial activity of *Parkia biglobosa* STEM bark extract

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Abstract
The powdered bark of *Parkia biglobosa* (Jacq.) Br. ex G. Don., is used by the indigenous traditional medicine men of Yobe State to treat bleeding from cuts and wounds, burns, ulcers, and also in the treatment of abnormal menstrual bleeding. Three crude extracts were prepared from the stem bark of *Parkia biglobosa* by successive cold extraction with n-Hexane, ethyl acetate and methanol respectively. The dried extracts were subjected to phytochemical screening and antibacterial/antioxidant studies. Phytochemical screening revealed the presence of Alkaloids, carbohydrates, flavonoids, tannins, terpenoids, glycosides, saponins, sterols, and phenols in all the extracts, but saponins and phenols were only present in the methanolic extracts. Oxalates and quinones were not detected in all the three samples. The antioxidant studies (using DPPH Radical Scavenging Activity) revealed that all the three extracts exhibited tremendously higher antioxidant activities than the standard ascorbic acid. Among them the ethyl acetate extract showed the highest activity (-226.16µg/ml) followed by n-hexane (-21.6µg/ml) and methanol (and -2.11µg/ml). Results from the antibacterial studies of the crude extracts showed significant inhibitory activity against the tested pathogenic organisms with zones of inhibition ranging between 27.5-57.5 mm (*E. coli*), 30-62.5 mm (*B. subtilis*) 22.5-50mm (*S. aureus*) and 25-60mm (*S. typhi*.) as compared to 21-45mm for the control (Ciprofloxacin). These results were taken to indicate that *Parkia biglobosa* is a promising herbal remedy that contains phytochemicals with strong antioxidant and antibacterial properties. This study further validates and strengthens its continuous use in traditional medicine as remedy for cut/wound healing arising from many sources.

Keywords: traditional medicine, phytochemicals, flavonoids, *Parkia biglobosa, Antioxidant, Antibacterial*

Introduction

Medicinal plants have been used to treat human diseases and other ailments for centuries all over the world and Africa in particular. People are becoming increasingly attached to these plants because of their good therapeutic performance, low toxicity and low cost/access. *Parkia biglobosa* (Jacq.) R.Br. ex G. Don is a perennial deciduous tree which belongs to the family of Fabaceae-Mimosoideae formerly Leguminosae. It is popularly known as African Locust Bean tree. In Nigeria, its local names include Dorowa (Hausa), Irugba (Yoruba), ogiri (Igbo), Ruwuno in Kanuri and Nune (Tiv) (Udobi and Onaolapo, 2009) [18]. It is known to be a native of Africa and is an important multipurpose tree of the West African Savannah land and one of the most common species of the parkland agro-forestry system (Sacande and Clethero, 2007) [12]. *P. biglobosa* as a common species of the parkland agro-forestry, plays important roles such as food and wood production (Okafor, 1980; Popoola and Maishanu, 1995) [8, 10]. It provides protein, energy, starch, vitamins and essential minerals to human diet. Above all, it has a therapeutic effect, hence its wide use in traditional medicine (Tee, Ogwuche, and Ikyaagba, 2009) [16]. The plant is considered to be an important medicinal tree in North-central and North-eastern parts of Nigeria (Verinumbe, 1991) [20]. The roots, barks, leaves, stems, flowers, fruits and seeds are all used medicinally to treat a range of ailments including diarrhea, ulcers, pneumonia, burns, coughs, jaundice etc (Sacande and Clethero, 2007) [12]. The Kanuri people of Gujba Local Government Area use the powdered bark of the plant to treat bleeding from cuts, dressing and healing of cuts, wounds and burns, and also in the treatment of abnormal menstrual bleeding (Personal information of Mallam Ibrahim Gana and Baba Mai Saje of Gujba town, Yobe state).

The pulp contains higher cellulose and sucrose but less ascorbic acid than the cotyledons. The pulp also contains other simple sugars except maltose (Alabi *et al*, 2005) [1]. The fruit pods are used in producing insecticide powders for treating crops (Sacande and Clethero, 2007) [12]. The plant is widely distributed throughout Yobe State, and its use as a medicinal plant by traditional healers has been documented in a recently published ethnomedical survey report and recommended for scientific investigation (Umar and Ibrahim, 2015) [19].

For several decades, plant secondary metabolites are studied for their possible therapeutic values (Okafor, 1980) [8]. In continuation of our effort to provide scientific rationale for the use of medicinal plants by traditional medicine men, this paper reports on the phytochemical screening, antioxidants and antibacterial activities of the *P. biglobosa* stem bark.

Materials and methods

Apparatus

Ultrasonicator (Model/AS3120) an ultra-sonic water bath, power sonic 420, with 40KHZ frequency and maximum power of 700w, internal dimension (d): 500 x 300 x 150mm were purchased from Autoscience Ltd USA, analytical weighing balance (Model/PA 214) U.S.A. Analytical weighing balance (Model/PA214) was from Chaus Corporation, Pine Brook, USA; Blender (Model: HR 2815),

China, Drying cabinet (Model: FSM 140), Japan, Genlab (Water bath) from Thermal Engineers (Model: WBH22/FL), United Kingdom, Unico Spectrophotometer (Model No.:UV2150), USA, Beam weighing balance, and ultrasonic cleaner (AS3120) purchased from Autoscience Instrument Co. Limited., Nephelometer and Vortex mixer Bibby Scientific Limited Stone, Staffordshire,(Model: ST15 0SA), UK. Whatman No.1 filter paper was purchased from Whatman international Ltd, Maidstone, England,

Chemicals and Reagents

Ethyl acetate, n-Hexane and Methanol were purchased from BDH Chemicals, Pools, England; Iodine Crystals, Potassium iodide, mercuric chloride and bismuth iodide were from Hopkins and Williams Co Ltd, England 2, 2-diphenyl-1-picrylhyrazyl (DPPH) was purchased from Sigma-Aldrich Co. St Louis, Germany. Ascorbic acid was obtained from Griffin and George, England. All other chemicals used were of highest analytical grade and purchased from BDH Chemical Ltd, Pools, England. Sabouraud Dextrose Agar (SDA) HCM049, Microbial Sci. & Tech. CO., LTD. Blood Agar Base CM0055, Oxoid Ltd., Basingstoke, Hampshire, England, herein referred to as Nutrient Agar (NA).

Sample Collection

The fresh *Parkia biglobosa* stem bark samples were collected behind Federal Government Girls' College (FGGC) Potiskum, Yobe state, Nigeria. The plant was identified by the combined efforts of the traditional healers and botanists attached to the Ministry of Environment, Yobe State, Damaturu, Nigeria.

Preparation of Extraction

The sample was washed and chopped into small bits and dried under shade in the laboratory at ambient temperature. The dried sample was then pulverized in a mortar and pestle into coarse particles and the drying was continued until a constant weight was obtained. A total of 400g of the coarse powder was then successively extracted with solvents of different polarities namely n-hexane, ethyl acetate and methanol as described previously using Ultrasonicator (Samaram, Mirhosseini, Tan, & Ghazali, 2013) [13]. After the ultrasonication the volume of the extracts were reduced and finally dried in a drying cabinet. Each extract was weighed and labeled appropriately and stored until required for analysis.

Phytochemical Screening

Screening tests for the presence of secondary metabolites were performed using standard methods (Ezekwe, *et al.*, 2013; Sabri, *et al.*, 2012; and Tiwari, *et al.*, 2011) [2, 11, 17]

DPPH Radical Scavenging Activity

The free radical scavenging activity of the plant extracts were measured against DPPH at 517nm wavelength using Ascorbic acid as the standard antioxidant as described by other workers (Gupta *et al.*, 2003; Hatano *et al.*,1988) [4, 5]

Microorganisms

The strains of bacteria used in this study (*Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Salmonella typhimurium*) were clinical isolates obtained from the School of Medical Laboratory Technology and Department of Dermatophilosis, National Veterinary Research Institute, Vom, Plateau State, Nigeria. The strains were maintained and tested on Nutrient Agar (bacteria) with ciprofloxacin as control. The antibacterial activity screening was carried out using agar well diffusion and dilution technique as described elsewhere (Perez *et al.*, 1990) [9].

Results and discussion

Secondary metabolites from plants have long history of therapeutic potentials (Sofowora, 1980; 1993) [15]. The preliminary phytochemical screening results is presented in Table 1 showed the presence of alkaloids, carbohydrates, flavonoids, saponins, tannins, terpenoids, sterols, phenols and cardiac glycosides but oxalates and quinones were not detected in Parkia biglobosa. It also indicated the presence of both polar, semi-polar and non-polar moieties of alkaloids, flavonoids, saponins, tannins, terpenoids, phenols and cardiac glycosides; and carbohydrates, saponins and sterols were found only in the polar methanolic extracts. Phytochemicals such as alkaloids, flavonoids, tannins, terpenoids, etc. have been implicated as being responsible for the therapeutic activities of medicinal plants (Nandagoapalan, Doss & Marimuthu, 2016) [7]. Pharmacological activities such as antimicrobial, anti-inflammatory, antioxidant, anticancer, etc. may be important indicators of a plant's medicinal usefulness. The present study carried out antioxidant and antibacterial assays on the three *P. biglobosa* extracts and report findings in the paragraphs below.

Table 1: Phytochemical Screening of n-Hexane, Ethyl Acetate and Methanol Extracts of *Parkia biglobosa* Stem bark.

S/No	Phytochemicals	Type of Test	Solvents		
			n-Hexane	Ethyl Acetate	Methanol
1	Alkaloids	Mayer's	+	+	+
2	Flavonoids	Sodium Hydroxide	+	+	+
3	Saponins	Foaming	−	−	+
4	Glycosides	Keller-Kelliani's	+	+	+
5	Terpenoids	Salkowski's	+	+	+
6	Tannins	Braymer's	+	+	+
7	Sterols	Libermann-Burchard	−	−	+
8	Phenols	Ferric Chloride	+	+	+
9	Carbohydrates	Fehling's Test	−	−	+
10	Oxalates	Acetic acid	−	−	−
11	Quinones	Concentrated HCl	−	−	−

Key: + = Positive test −= Negative test

Antioxidant activity

The antioxidant activities of different concentrations of the extracts and ascorbic acid were measured at 517nm (Table 2). This data was then converted to percentage (%) inhibition with the aid of Microsoft Excel software (Table 3), from which graph was drawn against concentration (Fig 1). From this graph a straight line formula was generated for each extract in order to calculate their IC_{50} values. The results of the IC_{50} (Table 4) showed that all the three extracts (n-hexane, ethyl acetate and methanol) exhibited extremely high antioxidant activities than the standard ascorbic acid. The highest value was recorded for the ethyl acetate fraction (-226.16) followed by n-hexane (-21.56) and methanol (-2.11) respectively as compared to ascorbic acid (153.54). These negative IC_{50} values are rare to come across and very difficult to interpret, nevertheless it is an indication of how strong antioxidants these extracts can be. If properly exploited they provide strong sources of antioxidants for future generation especially the ethyl acetate fraction.

It is a common practice for traditional medicine men usually apply the powdered stem bark of Parkia biglobosa in form of poultices to heal cut/wounds and decoction/maceration when treating stomach ailments and abnormal menstrual bleeding. For this reason, the three extracts were remixed in the same proportion in which they appeared in the extraction exercise and subjected to further antioxidant assay. The results obtained are presented in Table 5 and Fig 3. It is interesting to note that the IC_{50} value of the mixture (0.13) was tenfold higher than that recorded for ascorbic acid. This level of antioxidant activity is enough to protect cells and tissues from the destructive effects of free radicals. This conclusion draws support from Lodhi *et al* (2016) [6] who reported that wound healing occurs via phytochemicals' ability to exert anti-inflammatory/analgesic and antioxidant activities. Furthermore, according to Gadgoli (2016) [3] wound healing is a complex process which takes place in many phases which may involve the participation of plant derived compounds serving as antibacterial and antibiotics, in addition to exhibiting other pharmacological activities. This view is more logical because some degree of antimicrobial activity of the agents/drugs may be necessary in order to prevent infection and facilitate wound healing.

Table 2: The Antioxidant Activities of Different Concentrations of the Parkia Biglobosa Extracts and Ascorbic Acid Measured as Absorbance at 517nm.

S/No	Concentrations (µg/ml)	Standard Ascorbic acid	Hexane	Ethyl Acetate	Methanol
1	1	0.652	0.525	0.42	0.499
2	5	0.643	0.450	0.344	0.43
3	10	0.547	0.288	0.142	0.376
4	50	0.235	0.093	0.09	0.093
5	100	0.088	0.077	0.089	0.092
6	500	0.068	0.065	0.066	0.065

Table 3: The Conversion of Absorbance (517nm) of Various Concentration of Ascorbic Acid and Parkia biglobosa Extracts in Table 2 to Percentage (%) Inhibition.

Concentrations (µg/ml)	Ascorbic Acid	Hexane	Ethyl Acetate	Methanol
0	0.000	0.000	0.000	0.000
1	4.678	23.246	38.596	27.047
5	5.994	34.211	49.708	37.135
10	20.029	57.895	79.240	45.029
50	65.643	86.404	86.842	86.404
100	87.135	88.743	86.988	86.550
500	90.058	90.497	90.351	90.497

Table 4: The IC_{50} Values of *P. biglobosa* Extracts as compared to Standard Ascorbic Acid and their Percentage Inhibitions Extents.

S/No	Test Sample	IC_{50} (µg/ml)	Percentage (%) Inhibition
1	Ascorbic acid	152.48	100
2	n-Hexane	-21.56	114
3	Ethyl acetate	-226.16	248
4	Methanol	-2.11	101.4

Table 5: The Absorbance at 517nm and the IC_{50} Values of *P. biglobosa* Combined Stem Bark Extracts as compared to Standard Ascorbic Acid.

Concentration (µg/ml)	Standard Ascorbic Acid	Combined Extracts
1	0.374	0.192
10	0.119	0.100
20	0.089	0.101
50	0.052	0.107
100	0.050	0.094
200	0.051	0.089
300	0.049	0.089
400	0.049	0.089
500	0.049	0.094
IC50	**1.31**	**0.13**

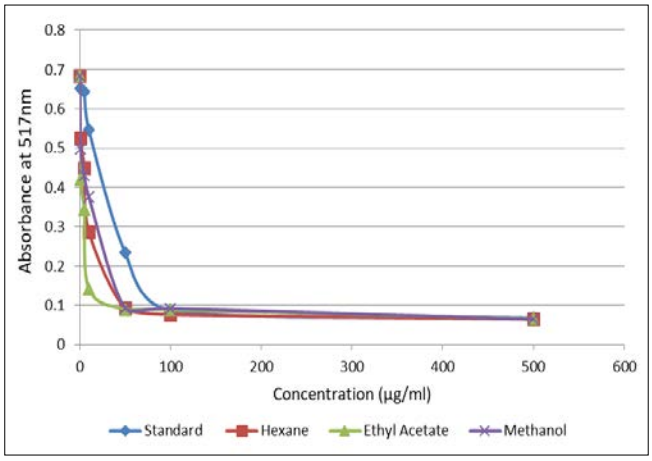

Fig 1: The Graph of Absorbance against Concentration of Ascorbic Acid and *Parkia biglobosa Extracts*

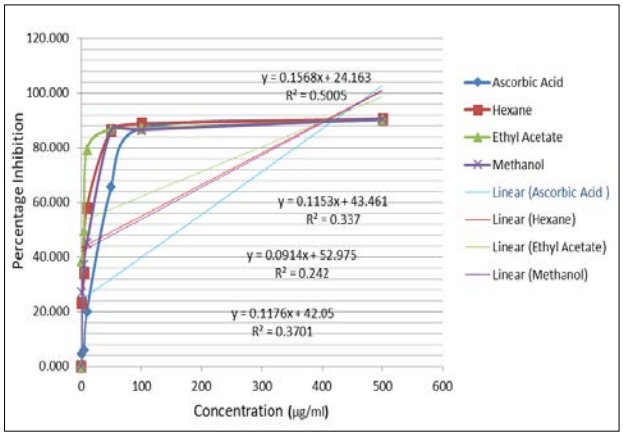

Fig 2: The Graph of Percentage Inhibition Against Concentration Used for the Calculation of IC50 Values.

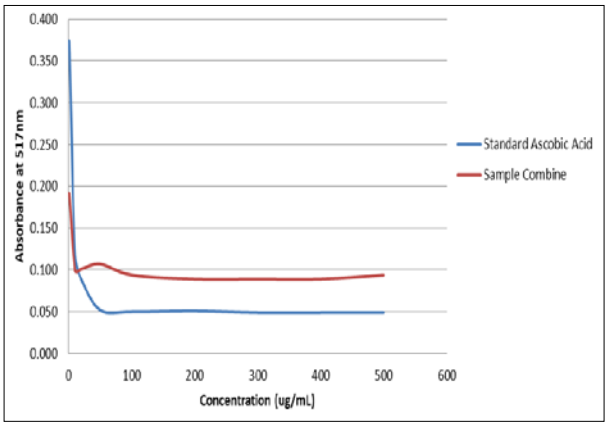

Fig 3: The Graph of Absorbance against Concentration of Ascorbic Acid and *Parkia biglobosa Extracts*

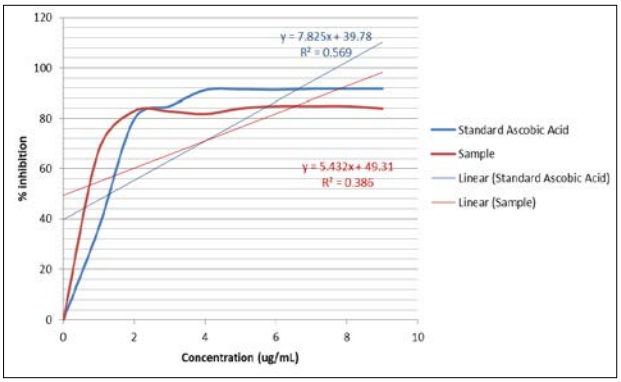

Fig 4: The Graph of Percentage Inhibition against Concentration Used for the Calculation of IC50 Values.

Antibacterial activity

The present study investigated the antibacterial activities of the different concentrations of the n-hexane, ethyl acetate and methanol extracts of Parkia biglobosa stem bark on two Gram positive (G +ve) (Bacillus subtilis & Staphylococcus aureus) and Gram negative (G -ve) (Escherichia coli & Salmonella typhimurium) bacteria. The results of the zone of inhibition (ZOI) are presented in Table 6 and summarized in Fig 5(a-c). The crude extracts showed comparable inhibitory activities with the control ciprofloxacin only at the concentration of 200 mg/ml. At this concentration the G +ve bacteria showed higher zones of inhibition (ZOI = 37-40mm) as compared to the Gram negative (ZOI = 27.5-35mm). These findings suggest that Parkia biglobosa extracts possessed strong antibacterial activities against both Gram positive and Gram negative bacteria, and hence the stem bark of this plant can be used as antibacterial agent in wound healing and other similar ailments.

Table 6: The Results of Antibacterial Activity of Stem bark Extract of *Parkia biglobosa*

Samples	Bacteria	Zones of Inhibition (mm) of Concentration in mg/ml							
		200	100	50	25	12.5	6.25	3.125	Ciprofloxacin
A1	B.S	37.5	12	NCZ	NI	NI	NI	NI	36
	E.C	30	8	NCZ	NI	NI	NI	NI	33
	S.A	37.5	NCZ	NCZ	NI	NI	NI	NI	43
	S.T	27.5	8	NCZ	NCZ	NI	NI	NI	40
B1	B.S	40	11	NI	NI	NI	NI	NI	33
	E.C	30	7	NI	NI	NI	NI	NI	35
	S.A	37.5	11	9	NCZ	NI	NI	NI	40
	S.T	35	8	NCZ	NI	NI	NI	NI	40
C1	B.S	37.5	10	8	NCZ	NCZ	NI	NI	36
	E.C	30	8	NCZ	NI	NI	NI	NI	36
	S.A	37.5	9	NCZ	NI	NI	NI	NI	42
	S.T	27.5	7	NCZ	NI	NI	NI	NI	34

Key:
A1= n-Hexane crude extract B1= Ethyl acetate crude extract C1= Methanol crude extract
NCZ= No clear zone NI = No inhibition
B.S= *Bacillus subtilis* (G +ve) E.C= *Escherichia coli* (G -ve)
S.A= *Staphylococcus aureus* (G +ve) S.T= *Salmonella typhimurium* (G -ve)

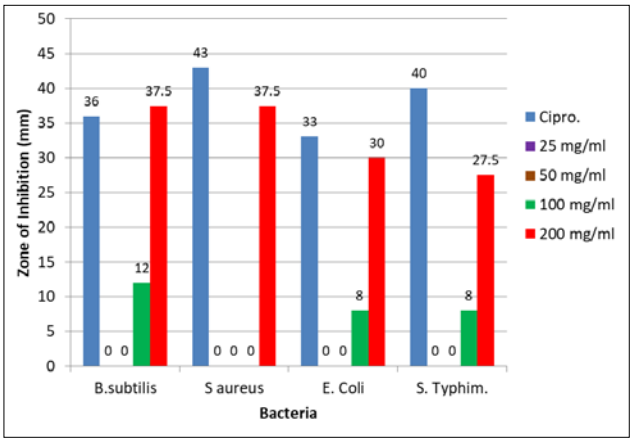

Fig 5a: The Zones of Inhibition Produced by n-Hexane Extracts of *Parkia biglobosa* on Gram negative and Gram positive bacteria.

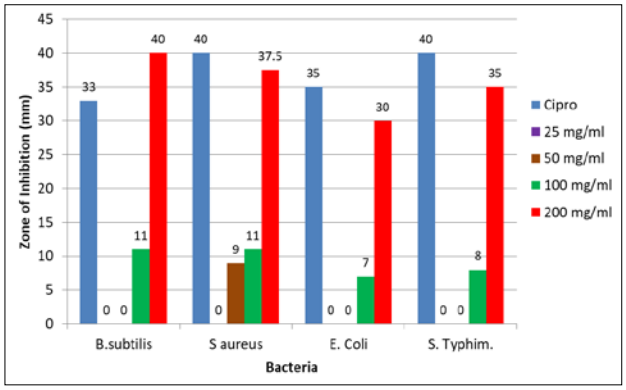

Fig 5b: The Zones of Inhibition Produced by Ethyl Acetate Extracts of Parkia biglobosa on Gram negative and Gram positive bacteria.

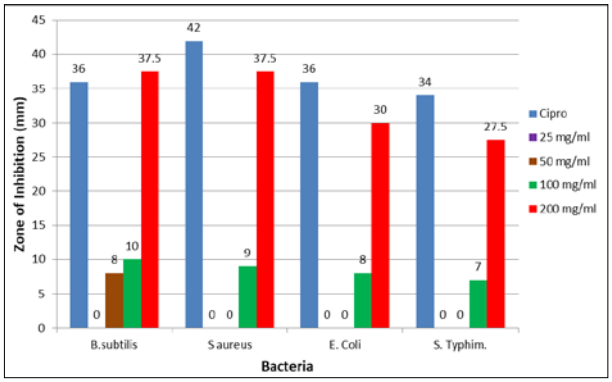

Fig 5b: The Zones of Inhibition Produced by Methanol Extracts of Parkia biglobosa on Gram negative and Gram positive bacteria.

Conclusion

Parkia biglobosa contains essential secondary metabolites, many of which have been implicated previously. The richness of the plant extract in phenolic compounds (anti-oxidants) justify the use of the plant for general health maintenance, control bleeding and in the treatment of wounds and cuts.

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