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IN VITRO ANTI-OXIDATIVE ACTIVITIES OF THE VARIOUS PARTS OF *PARKIA BIGLOBOSA* AND GC-MS ANALYSIS OF EXTRACTS WITH HIGH ACTIVITY

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Abstract:

The anti-oxidative activities of sequentially extracted solvent fractions of different parts of *P. biglobosa* were evaluated in a series of *in vitro* assays. Our findings indicated that all extracts had electron donating and free radical scavenging activities. But the ethanol (EtOH) extracts from all the parts demonstrated more promising anti-oxidative effects in these experimental models. Apart from the aqueous extracts of the stem bark and leaves, all other extracts exhibited hydroxyl radical scavenging (HRS) activity but the ethyl acetate (EtOAc) extract of the stem bark and EtOH extracts of the root and leaves possessed more powerful HRS activity than other corresponding extracts in the parts. Further, nitric oxide (NO) inhibition activities were observed in all the extracts except the EtOAc extract of the stem bark which showed pro-oxidative activity. However, the EtOH extract of the stem bark and root as well as the EtOAc extract of the leaves displayed more potent anti-NO activity than other extracts in the parts. The GC-MS analysis of the EtOH extracts revealed that the most abundant phytochemicals are pyrogallol derivatives. Data from this study suggest that the EtOH extracts from different parts of *P. biglobosa* contained potent anti-oxidative agents and pyrogallol could be the main bioactive constituent.

Keywords: Anti-oxidative, free radicals, GC-MS, *Parkia biglobosa*, pyrogallol

Introduction

Free radicals and reactive oxygen species (ROS) are produced through normal physiological and biochemical processes in living system, and are quenched by a cascade of endogenous antioxidant systems in the body (Young and Woodside, 2001). Over-production of such free radicals and/or inactivation of the antioxidants usually shift the ROS/antioxidants balance in favour of stress, a phenomenon called oxidative stress (OS) (Wiernsperger, 2003). The OS is implicated as a crucial factor in the pathogenesis of a number of diseases which include hypertension, cardiovascular diseases, diabetes mellitus and other metabolic syndromes (Takayanagi et al., 2000; Ceriello and Motz, 2004).

The use of medicinal plants for the treatment of various diseases continues to be an important component of the healthcare delivery system, especially in Africa where more than 5400 medicinal plants were reported to have over 16, 300 medicinal uses (van Wyk, 2008). The influence of these medicinal plants and natural products upon drug discovery is impressive because a number of clinically active drugs are either natural products or have a natural product pharmacophore (Koehn and Carter, 2005).

Parkia biglobosa (Jacq.) Benth. (Mimosaceae), commonly called the African locust bean tree, is native to Nigeria and other West African countries. The fermented seeds are well appreciated as condiments in cooking under various names such as '*dawadawa*' in Nigeria. All of the different parts of this plant are used by traditional healers to cure several metabolic or non-metabolic disorders like hypertension, haemorrhages and dermatosis (Adetola et al., 2006; Udobi and Onaolapo, 2009; Tokoudagba et al., 2010). A recent ethnopharmacological survey in northern Nigeria also revealed that the stem bark part of the plant was among the most commonly used plants for the treatment of diabetes mellitus by traditional healers (Etuk et al., 2010). The analgesic and anti-inflammatory (Kouadio et al., 2000), antivenom (Asuzu and Harvey, 2003), antidiarrhoeal (Agunu et al., 2005), antibacterial (Millogo-Kone et al., 2008), vasorelaxant (Tokoudagba et al., 2010), and wound healing (Adetutu et al., 2011) activities of extracts from different parts of this plant have been demonstrated. Furthermore, the fermented seed extract was also reported to possess hypoglycemic activity in a type 1 diabetes model of rats (Odetola et al., 2006). Although several studies were investigated to examine the above-mentioned effects of this plant, the anti-oxidative effects have however not been examined either *in vitro* or *in vivo* despite its extensive use for the treatment of diabetes mellitus, an OS associated metabolic disorder.

Hence, our present study was firstly designed to conduct a comprehensive investigation on the anti-oxidative effects of the various solvent extracts of the stem bark, root and leaves of this plant with a view to finding compound(s) that could be used to ameliorate the OS mediated metabolic disorders. Based on the results of anti-oxidative activities, the most promising anti-oxidative extracts were subjected to GC-MS analysis in order to identify the phytochemicals contained therein.

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Materials and methods

Chemicals and reagents

Gallic acid, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), 2 deoxy-D-ribose and potassium ferricyanide were Purchased from Sigma-Aldrich through Capital Lab Supplies, New Germany, South Africa. Griess reagent, sodium nitroprusside, thiobarbituric acid and Folin ciocalteau reagent were purhcased from Merck Chemical Company, Durban, South Africa.

Plant material

The stem bark, root and leaf samples of *Parkia biglobosa* were freshly collected in the month of January, 2011 from Zaria, Kaduna State, Nigeria. The plant was identified and authenticated at the herbarium unit of the Biological Science Department, Ahmadu Bello University, Zaria, Nigeria and a voucher specimen number 3017 was deposited accordingly. The stem bark, root and leaves were immediately washed and shade-dried for two weeks to constant weights. The dried samples were ground to a fine powder, and then stored individually in airtight containers for transport to the University of KwaZulu-Natal, Westville campus, South Africa for subsequent analysis.

Preparation of the plant extracts

Forty (40) grams of the fine powdered plant parts were separately defatted with hexane. The defatted materials were sequentially extracted with ethyl acetate, ethanol and water by soaking for 48 hours in 200 ml of the relevant solvent followed by a 2 hour orbital shaking at 200 rpm. After filtration through Whatmann filter paper (No. 1), respective solvents were evaporated under vacuum, using a rotary evaporator (Buchi Rotavapor II, Buchi, Germany) at 40^{o}C under reduced pressure to obtain the different solvent extracts with the exception of the aqueous fractions which were dried on a water bath at 50 ^{o}C. The extracts in each case were weighed, transferred to micro tubes and stored in a refrigerator at 4 ^{o}C until analysis.

Estimation of total phenolic content

The total polyphenol content of each extract was determined (as gallic acid equivalent) according to the method described by "McDonald et al (2001)" with slight modifications. Briefly, 200 µl of the fraction (240 µg/ml) was incubated with 1 ml of 10 times diluted Folin ciocalteau reagent and 800 µl of 0.7 M Na_2CO_3 for 30 minutes at room temperature. Then the absorbance values were determined at 765 nm on a Shimadzu UV mini 1240 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). All measurements were done in triplicate.

Ferric (Fe^{3+}) reducing antioxidant power assay

The ferric reducing antioxidant power method of Oyaizu (1986) was used with slight modifications to measure the reducing capacity of the extracts. To perform this assay, 1 ml of each extract (15, 30, 60, 120 and 240 µg/ml) was incubated with 1 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 1% potassium ferricyanide at 50^{o}C for 30 minutes. After 30 minutes incubation, the reaction mixtures were acidified with 1 ml of 10% trichloroacetic acid. Thereafter, 1 ml of the acidified sample of this solution was mixed with 1 ml of distilled water and 200 µl of $FeCl_3$ (0.1%). The absorbance of the resulting solution was measured at 700 nm in a spectrophotometer. Increased absorbance of the reaction mixture indicated greater reductive capability of the extracts (Gulcin et al., 2004).

Free radical scavenging activity

The total free radical scavenging capacity of the tested extracts was determined and compared to that of ascorbic and Gallic acids as well as trolox by using a slightly modified method described by Tuba and Gulcin (2008). A 0.3 mM solution of DPPH was prepared in methanol and 500 µl of this solution was added to 1 ml of the extracts at different concentrations (15, 30, 60, 120 and 240 µg/ml). These solutions were mixed and incubated in the dark for 30 minutes at room temperature. The absorbance was measured at 517 nm against blank samples lacking the free radical scavengers.

2-Deoxy-D-ribose degradation (hydroxyl radical scavenging) assay

Hydroxyl radical scavenging (HRS) activity was measured by studying the competition between deoxyribose and the extracts for hydroxyl radicals generated by the ascorbate–EDTA– H_2O_2 system (Fenton reaction) as described by Hinnerburg et al. (2006). The assay was performed by adding 200 µl of premixed 100 µM $FeCl_3$ and 100 µM EDTA (1:1 v/v) solution, 100 µl of 10 mM H_2O_2, 360 µl of 10 mM 2-deoxy-D-ribose, 1 ml of different extract concentrations (15–240 µg/ml), 400 µl of 50 mM sodium phosphate buffer (pH 7.4) and 100 µl of 1 mM ascorbic acid in sequence. The mixture was incubated at 50^{o}C for 2 hours. Thereafter, 1 ml of 2.8% TCA and 1 ml of 1.0% thiobarbituric acid (in 0.025 M NaOH) were added to each tube. The samples were further incubated in a water bath at 50^{o}C for 30 minutes to develop the pink chromogen. The extent of oxidation was estimated from the absorbance of the solution at 532 nm and the HRS activity of the extracts is reported as percentage inhibition of deoxyribose degradation.

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Nitric oxide (NO) radical scavenging assay

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide (NO), which interacts with oxygen to produce nitrite ions that can be estimated by use of Griess reagent. Scavengers of NO compete with oxygen leading to reduced production of NO (Kurian et al., 2010). The assay was carried out by incubating 500 µl of 10 mM sodium nitroprusside in phosphate buffer (pH 7.4) and 500 µl of different extract concentrations (15-240 µg/ml) at 37 ^{o}C for 2 hours. The reaction mixture was then mixed with 500 µl of Griess reagent. The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamine were read at 546 nm. Percentage inhibition of nitric oxide generated was measured in comparison with the absorbance value of a control (10 mM sodium nitroprusside in phosphate buffer).

All assays were carried out in triplicate. The scavenging activities of the plant extracts in the case of DPPH, hydroxyl and nitric oxide radical scavenging assays were calculated by using the following formula:

Where As is the absorbance in the presence of the sample and Ac is the absorbance of the control.

Gas chromatography-mass spectrometric (GC-MS) analysis

Based on the results of anti-oxidative assays, the EtOH extracts of the stem bark, root and leaf samples of the plant were subjected to GC-MS analysis. The GC-MS analysis was conducted with an Agilent technologies 6890 GC coupled with (an Agilent) 5973 Mass Selective detector and driven by Agilent chemstation software. A HP-5MS capillary column was used (30 m x 0.25 x mm internal diameter x 0.25 µm film thickness). The carrier gas was ultra-pure helium at a flow rate of 1.0 ml/minute and a linear velocity of 37 cm/second. The injector temperature was set at 250°C. The initial oven temperature of 60°C was programmed to 280°C at the rate of 10 °C/minute with a hold time of 3 minutes. Injections of 1 µl were made in splitless mode with a split ratio of 20:1. The mass spectrometer was operated in the electron ionization mode at 70 eV and electron multiplier voltage at 1859 V. Other MS operating parameters were as follows: ion source temperature 230°C, quadrupole temperature 150°C, solvent delay 4 minutes and scan range 50-700 amu. Compounds were identified by direct comparison of the retention times and mass spectral data with those in the National Institute of Standards and Technology (NIST) library.

Statistical Analysis

All data are presented as mean ± SD of triplicates determination except the data from GC-MS analysis. Data were analysed by using a statistical software package (SPSS for Windows, version 18, IBM Corporation, NY, USA) and Tukey's-HSD multiple range *post-hoc* test. Values were considered significantly different at $P<0.05$.

Results

Higher yields of EtOAc and EtOH extracts were obtained from the leaves in comparison with the stem bark and roots. However, the aqueous extract from the stem bark had the highest yield among the different plant parts. The EtOH extracts contained a significantly ($P<0.05$) higher amount of total polyphenols than other solvent extracts within the parts of the plant (Table 1).

Table 1: Percentage yield and total polyphenol content of various solvent extracts of *P. biglobosa* parts

Samples	% Yield	Total polyphenol (mg/g GAE)
Stem bark		
EtOAc	0.74	57.76 ± 0.88^{f}
EtOH	4.80	114.77 ± 0.99^{h}
Aqueous	3.98	50.96 ± 0.37^{e}
Root		
EtOAc	0.17	0.47 ± 0.04^{a}
EtOH	0.15	168.98 ± 11.32^{i}
Aqueous	1.55	8.95 ± 0.78^{b}
Leaves		
EtOAc	1.54	32.41 ± 0.17^{d}
EtOH	4.95	84.57 ± 1.06^{g}
Aqueous	3.32	31.37 ± 0.65^{c}

Data are presented as mean ± SD values of triplicate determinations. $^{a-i}$Different superscripted letters for a given value within a column are significantly different from each other (Tukey's-HSD multiple range *post hoc* test, $P<0.05$)

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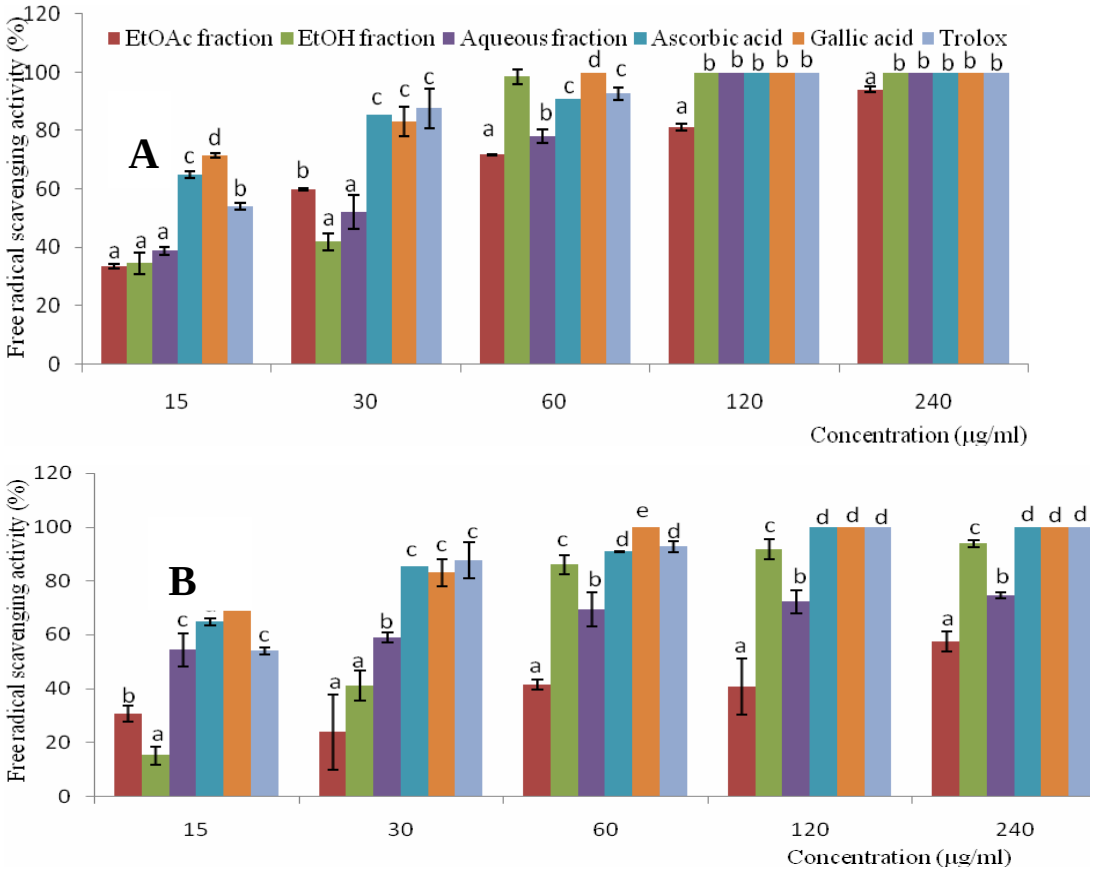
The total reducing power (GAE) of the EtOH extracts of the stem bark and leaves was significantly higher (P<0.05) than those of trolox and other solvent extracts in these parts at all concentrations and significantly higher (P<0.05) than that of ascorbic acid at most concentrations tested. Extracts obtained from the root demonstrated weaker reducing power that were significantly lower (P<0.05) than those of ascorbic acid and trolox (Table 2).

Table 2: Total reducing power (GAE) of solvent extracts from various parts of *P. biglobosa*

	Concentration (µg/ml)				
	15	30	60	120	240
Extracts					
Stem bark					
EtOAc	2.89 ± 0.95^{b}	30.98 ± 1.11^{e}	22.44 ± 1.14^{e}	40.66 ± 1.33^{f}	70.42 ± 4.68^{f}
EtOH	44.11 ± 1.30^{g}	45.60 ± 2.45^{f}	39.65 ± 1.33^{g}	61.71 ± 1.14^{h}	108.76 ± 9.07^{i}
Aqueous	27.69 ± 3.39^{f}	23.93 ± 4.54^{d}	25.88 ± 0.88^{ef}	51.63 ± 5.69^{g}	77.11 ±1.00^{g}
Root					
EtOAc	0.00 ± 0.00^{a}	0.11 ± 0.01^{a}	0.70 ± 0.24^{a}	1.64 ± 0.17^{a}	2.99 ± 0.46^{a}
EtOH	5.55 ± 1.12^{c}	4.35 ± 2.66^{b}	4.92 ± 0.45^{b}	11.87 ± 2.39^{c}	16.27 ± 1.05^{b}
Aqueous	5.15 ± 2.02^{c}	7.38 ± 0.20^{c}	3.94 ± 0.63^{b}	5.98 ± 1.80^{b}	15.79 ± 1.10^{b}
Leaves					
EtOAc	3.30 ± 1.12^{bc}	5.60 ± 1.50^{bc}	7.73 ± 0.55^{c}	15.42 ± 0.65^{d}	25.01 ± 0.38^{c}
EtOH	29.59 ± 6.45^{f}	44.50 ± 6.38^{f}	36.79 ± 2.82^{g}	68.25 ± 3.39^{i}	94.82 ± 0.85^{h}
Aqueous	11.74 ± 3.01^{d}	15.79 ± 1.01^{d}	15.59 ± 1.31^{d}	25.70 ± 3.02^{e}	48.36 ± 3.29^{d}
Ascorbic acid	31.50 ± 4.92^{f}	32.66 ± 3.00^{e}	35.62 ± 5.51^{g}	52.56 ± 5.43^{g}	89.95 ± 6.21^{h}
Trolox	17.84 ± 1.86^{e}	20.88 ± 7.33^{d}	24.42 ± 3.95^{ef}	29.61 ± 1.96^{e}	52.77 ± 0.90^{e}

Data are presented as mean ± SD values of triplicate determinations. $^{a-i}$Different superscripted letters for a given value within a column are significantly different from each other (Tukey's-HSD multiple range *post hoc* test, P<0.05)

The DPPH radical scavenging activities of the various solvent extracts of *P. biglobosa* parts are presented in Figure1.



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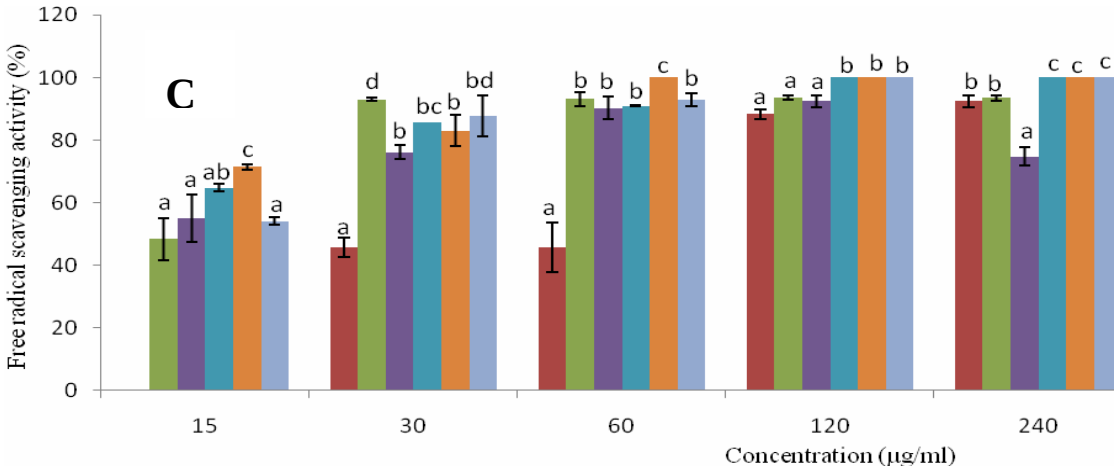

Figure 1: DPPH radical scavenging activity of stem bark (**A**), root (**B**) and leaves (**C**) extracts of *P. biglobosa.* Data are presented as mean ± SD of triplicate determinations. [a-e]Values with different letters over the bars for a given concentration of each extract are significantly different from each other (Tukey's-HSD multiple range *post hoc* test, P<0.05).

While all the extracts showed tendency to quench DPPH free radicals as manifested by the concentration dependent increase in the percentage inhibitions, the EtOH extracts of the stem bark and root displayed significantly higher (P<0.05) radical scavenging activity than other extracts within these parts at higher concentrations (60-240 µg/ml), and the difference was not significant (P>0.05) with the standard antioxidants in some cases. Interestingly, the EtOH extract of the leaves maintained >90% free radical scavenging activity at 30-240 µg/ml. It is important to note that the IC_{50} values of the aqueous extracts of the root and leaves and the EtOH extract of the stem bark were lower than the corresponding values for other extracts in these parts (Table 3).

Table 3: IC_{50} values of various solvent extracts of *P. biglobosa* parts in different anti-oxidative models

	IC_{50} (µg/ml)		
Samples	DPPH	HRS	NO
Stem bark			
EtOAc	24.43	3.63	P
EtOH	1.37	979.22	18.29
Aqueous	22.34	P	8.69*
Root			
EtOAc	177.39	52.79	5.71*
EtOH	34.78	13.21	2.36
Aqueous	7.60	118.16*	>1**
Leaves			
EtOAc	51.69	3.13	4.64
EtOH	4.26	1.18	52.54
Aqueous	1.83	P	252.43
Ascorbic acid	2.56	ND	26.28
Gallic acid	1.27	ND	1.03
Trolox	5.04	2.61	599.21

ND means not determined and P means the extract showed pro-oxidative properties in the experimental model. *The units of these values are mg/ml. ** The unit of this value is g/ml.

The results of the HRS assay indicated that all extracts could scavenge hydroxyl radicals generated by Fenton's reaction except the aqueous extracts of the stem bark and leaves which displayed pro-oxidative tendencies at all concentrations tested (Table 4).

Table 4: Percentage HRS activity of extracts from various parts of *P. biglobosa*

	Concentration (µg/ml)				
	15	30	60	120	240
Extracts					
Stem bark					
EtOAc	20.93 ± 0.85^{f}	39.10 ± 0.68^{e}	19.30 ± 1.48^{d}	8.71 ± 0.40^{c}	-5.57 ± 0.37^{b}
EtOH	0.00 ± 0.00^{c}	10.31 ± 5.49^{c}	17.38 ± 3.00^{d}	33.79 ± 7.69^{e}	28.10 ± 11.80^{e}
Aqueous	-33.31 ± 0.97^{b}	-51.73 ± 3.25^{b}	-69.62 ± 2.00^{a}	-100.00 ± 0.00^{a}	-100.00 ±0.00^{a}
Root					
EtOAc	0.80 ± 0.07^{d}	12.39 ± 3.07^{c}	9.76 ± 1.07^{c}	24.61 ± 1.37^{d,e}	22.39 ± 1.96^{d,e}

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EtOH	46.00 ± 2.86^{h}	48.82 ± 2.73^{f}	51.41 ± 1.01^{f}	44.79 ± 0.78^{f}	38.63 ± 0.39^{e,f}
Aqueous	19.07 ± 0.28^{e}	27.87 ± 0.74^{d}	26.67 ± 0.96^{e}	30.75 ± 1.34^{e}	28.40±2.90^{e}
Leaves					
EtOAc	64.69 ± 1.57^{j}	68.62 ±14.25^{g}	78.81 ± 0.81^{g}	85.02 ± 0.27^{h}	88.63 ± 0.70^{g}
EtOH	29.00 ± 1.32^{g}	15.32 ± 0.31^{c}	7.98 ± 0.57^{b}	1.88 ± 0.23^{b}	1.06 ± 0.23^{c}
Aqueous	-41.26 ± 1.25^{a}	-60.90 ± 3.39^{a}	-81.80 ± 13.10^{a}	-100.00 ± 0.00^{a}	-100.00 ± 0.00^{a}
Trolox	57.32 ± 2.95^{i}	73.11 ± 1.44^{g}	76.04 ± 2.05^{g}	80.09 ± 3.93^{g}	79.82 ± 3.50^{h}

Data are presented as mean ± SD values of triplicate determinations. $^{a-i}$Different superscripted letters for a given value within a column are significantly different from each other (Tukey's-HSD multiple range *post hoc* test, P<0.05)

Although all organic extracts obtained from the stem bark and root exhibited a non-concentration dependent pattern of HRS activity, the EtOAc and EtOH extracts of the stem bark displayed significantly higher (P<0.05) activity at 15-60 µg/ml and 120-240 µg/ml respectively, than other extracts. It is also noteworthy that the EtOH extract of the root consistently demonstrated a significantly higher (P<0.05) HRS activity than other extracts in this part. Contrary to this, the EtOAc extract of the leaves displayed a significantly higher (P<0.05) hydroxyl radical inhibition than other extracts in this part at all concentrations and an activity that inversely correlates with concentration was observed with the EtOH extract of the leaves.

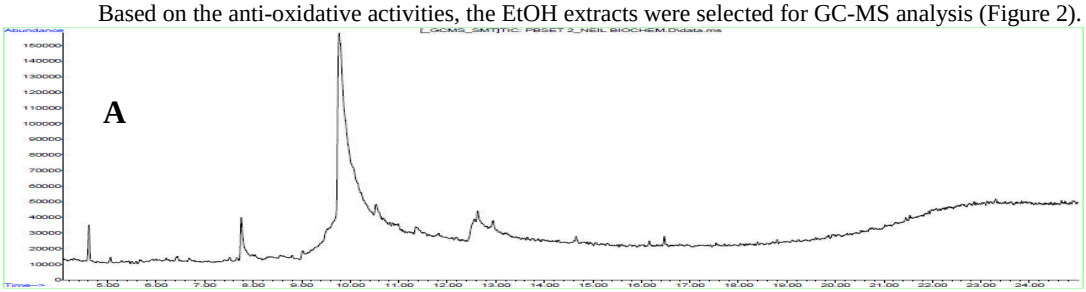
Table 5: Percentage NO scavenging activities of extracts from various parts *P. biglobosa*

Extracts	Concentration (µg/ml)				
	15	30	60	120	240
Stem bark					
EtOAc	-24.74 ± 2..31^{a}	-47.96 ± 1.53^{a}	-47.96 ± 16.07^{a}	-100.00 ± 0.00^{a}	-100.00 ± 0.00^{a}
EtOH	55.47 ± 7.54^{g}	50.78 ± 3.67^{f}	31.60 ± 9.42^{e}	36.91 ± 8.00^{c}	43.45 ± 1.82^{e}
Aqueous	10.73 ± 0.72^{d}	18.53 ±0.69^{d}	33.40 ± 1.33^{e}	32.22 ± 5.05^{c}	22.51 ± 1.14^{b}
Root					
EtOAc	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	14.57 ± 7.66^{b}	24.71 ± 2.93^{b,c}
EtOH	34.39 ± 2.45^{f}	49.67 ± 0.85^{f}	48.73 ± 0.88^{e,f}	39.55 ± 1.62^{c,d}	29.08 ± 0.73^{d}
Aqueous	28.92 ± 1.78^{e}	32.36 ± 0.65^{e}	14.61 ± 2.20^{c}	20.20 ± 6.75^{b}	38.49 ± 6.05^{e}
Leaves					
EtOAc	61.06 ± 4.96^{g,h}	63.54 ± 6.89^{g}	62.74 ± 14.33^{f,g,h}	42.53 ± 9.34^{c,d,f}	60.51 ± 2.86^{g}
EtOH	73.45 ± 1.98^{i}	64.88 ± 7.31^{g}	65.19 ± 3.97^{h}	15.03 ± 6.28^{b}	18.02 ± 3.29^{b}
Aqueous	15.19 ± 7.08^{d}	32.45 ± 1.23^{e}	41.30 ± 7.71^{e}	51.67 ± 3.82^{f}	39.36 ± 4.67^{e}
Ascorbic acid	47.81 ± 0.51^{g}	50.97 ± 1.35^{f}	52.82 ± 1.35^{g}	56.72 ± 3.69^{f,g}	64.08 ± 4.25^{g}
Gallic acid	4.43 ± 2.69^{c}	7.52 ± 1.11^{c}	25.74 ± 0.44^{d,e}	27.66 ± 1.91^{b,c}	31.70 ± 2.51^{d}
Trolox	66.30 ± 1.23^{h}	65.51 ± 1.27^{g}	63.39 ± 1.84^{h}	59.53 ± 1.81^{g}	52.21 ± 2.47^{f}

Data are presented as mean ± SD values of triplicate determinations. $^{a-i}$Different superscripted letters for a given value within a column are significantly different from each other (Tukey's-HSD multiple range *post hoc* test, P<0.05)

Table 5 presents the NO inhibition activities of solvents extracts of *P. biglobosa* parts. Apart from the EtOAc extract of the stem bark, all other extracts were found to exhibit NO inhibition activity. The EtOH and aqueous extracts of the stem bark and root as well as the EtOAc and aqueous extracts of the leaves demonstrated a non-concentration dependent NO inhibition effects, while the EtOH extract of the leaves showed an NO inhibition activity which inversely correlates with concentration. Furthermore, the EtOH extracts of the stem bark and root as well as the EtOAc extract of the leaves possessed lower IC_{50} values than the corresponding extracts in these plant parts (Table 3).

Based on the anti-oxidative activities, the EtOH extracts were selected for GC-MS analysis (Figure 2).



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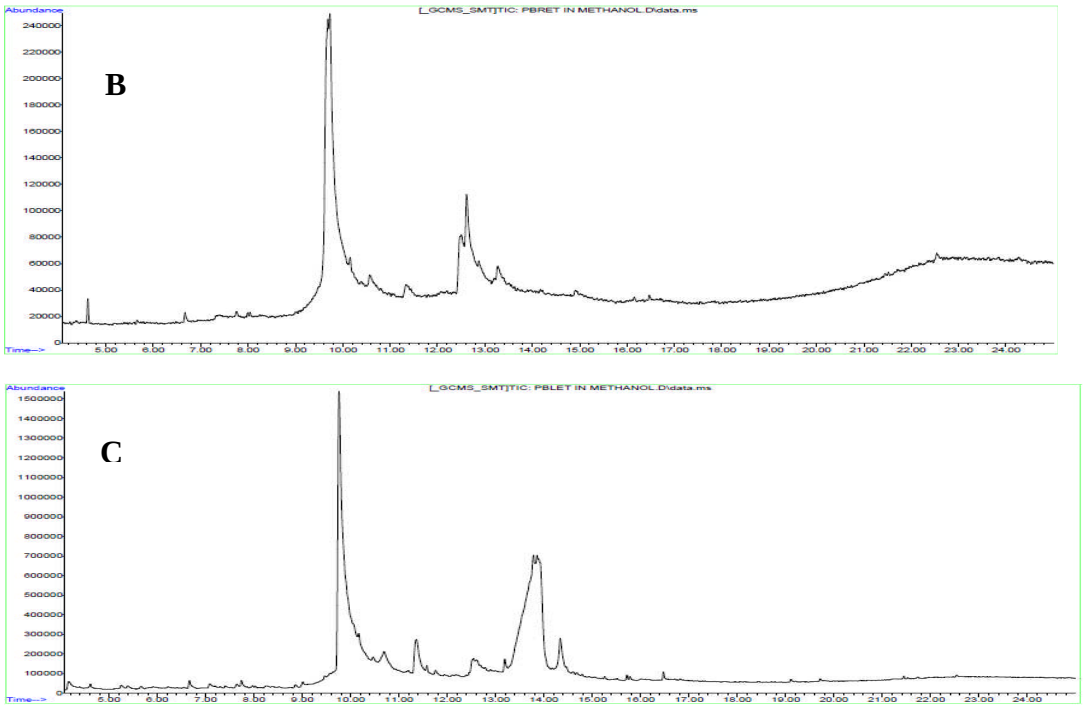

Figure 2: GC-MS chromatogram of EtOH extracts of stem bark (A), root (B) and leaves (C) of *Parkia biglobosa*

The major peak detected in the chromatogram of the EtOH extracts of the stem bark and root were due to those of a 1,2,3-trioxygenated benzene compound (pyragallol) with a hydroxy, methoxy and acetoxy group at each of the oxygenated positions, while an additional phloroglucinol derivative with the same substituents as well as an alloside of the pyrogallol derivative and two sugars, 3-O-methyl-D-fructose and 4-O-methyl-mannose were detected in the EtOH extract of the leaves (Table 6). The exact pyrogallol and phloroglucinol derivatives were not detected by the library of the instrument. Nevertheless, a parent ion at M^+ 182 is evident in the mass spectrum as well as fragments at *m/z* 167 and 125 for the loss of the methyl group and the loss of the methyl and acyl groups respectively. The resultant fragmentation pattern at *m/z* 125 and below is consistent with that of pyrogallol and therefore the oxygenation pattern is probably consistent with that of pyrogallol.

Table 6: Identified compounds from the EtOH extracts of different parts of *P. biglobosa* by GC-MS

Compounds	Retention time (min)	Molecular mass	Relative abundance (%)
Stem bark			
Pyrogallol derivative (acetoxy, hydroxyl and methoxy substituents)	9.76	182	100
Root			
Pyrogallol derivative (acetoxy, hydroxyl and methoxy substituents)	9.73	182	100
Leaves			
Pyrogallol derivative (acetoxy, hydroxyl and methoxy substituents)	9.76	182	52.27
*Pyragallol derivative - alloside	11.36	ND	3.19
Phloroglucinol derivative	12.54	182	0.46
3-O-methyl-D-fructose	13.78	194.18	25.33
4-O-methyl mannose	13.92	194.18	6.92

* The Molecular ion was not detected but the fragmentation pattern is indicative of the compound.

Discussion

The genus *Parkia* comprises over 70 species across the world but only a preliminary report on the antioxidant activity of a single plant (*P. speciosa)* from the genus appears in the literature (Razab and Abdul-aziz, 2010). Ethnobotanical surveys have indicated that different parts of *P. biglobosa* are used in the traditional treatment of diabetes mellitus and other diseases whose pathogeneses are, in part, linked to OS. This study investigated the anti-oxidative activities of various solvent extracts from different parts of *P. biglobosa* as a prelude to finding agent(s) that could be used to ameliorate OS-mediated metabolic disorders.

In the present study, four experimental models for *in vitro* anti-oxidative studies were used because a single model cannot give a full evaluation of the anti-oxidative capabilities of the different extracts tested due to the multiple mechanisms via which antioxidant compounds act. The total reducing ability was measured as Fe^{3+}–Fe^{2+} transformation in the presence of

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the extracts using the method of Oyaizu (1986). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity and therefore the FRAP assay provides a reliable method to study the antioxidant activity of various extracts and/or compounds. The strong reducing power of the EtOH extract of the stem bark and leaf samples indicates that the phytochemical components of this plant with high redox potential, at least in these parts, are EtOH extractable.

DPPH is a stable nitrogen-centred free radical with characteristic colour changes from violet to yellow upon reduction by either the process of hydrogen- or electron-donation. Substances which are able to perform this reaction can be considered as free radical scavengers and therefore antioxidants (Hinnerburg et al., 2006). The high DPPH radical scavenging activities of the various solvent extracts, especially the EtOH extracts that were comparable to standard antioxidants used in most cases, suggest that these extracts have high proton donating ability and could serve as free radical inhibitors. However, the EtOH extracts of the stem bark and leaves as well as the aqueous extracts of the leaves exhibited stronger DPPH radicals quenching ability with lower IC_{50} values than trolox. The computed IC_{50} values for the EtOH extract of the stem bark (1.37 µg/ml) and aqueous extract of the leaves (1.83 µg/ml) are especially remarkable because only a few authors (Wu et al., 2010) reported such low IC_{50} values as found with these extracts in spite of hundreds of reports on the DPPH radical scavenging activity of extracts from plants in different parts of the world. This result could further indicate that these extracts contain powerful free radical scavenging phytochemicals that might have the ability to inhibit free radical upsurge as well as OS and therefore could be useful therapeutic agents for treating radical-related pathological changes.

The hydroxyl radical is an extremely reactive free radical form in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living systems (Kalaivani and Matthew, 2010). Thus, identification of compounds with excellent hydroxyl scavenging activity would be important for diseases where OS is important for the disease initiation and/or progression. From the present study, all organic extracts from the various parts of *P. biglobosa* contain phytochemicals with hydroxyl radical inhibition activity but the activity is more pronounced in the EtOH extracts except in that of the stem bark. The non-concentration dependent pattern of HRS activity displayed by the organic extracts of the stem bark and root as well as the reciprocal activity by the EtOH extract of the leaves could be attributed to a hormesis phenomenon by these extracts in this experimental model. Hormesis is a dose-response relationship for a single endpoint that is characterised by reversal of response between low and high doses of chemicals, biological molecules, physical stressors, or any other initiators of a response (Calabrese and Baldwin et al., 2001) and its occurrence has been documented in numerous biological, toxicological and pharmacological investigations (Kendig et al., 2010). The foregoing therefore indicates that maximum scavenging activity towards hydroxyl radicals by these extracts occurs at optimal points. On the other hand, the pro-oxidative activities of the aqueous extract of the stem bark and leaves in the hydroxyl radical based anti-oxidative model further indicated the need to use a multi method approach before a definite statement can be made on the anti-oxidative effects of a plant extract, extract or pure compound.

Nitric oxide is a free radical generated by endothelial cells, macrophages, neurons among others; and is involved in the regulation of various physiological processes (Lata and Ahuja, 2003). Exoncentration of NO is implicated in the pathology of several diseases including T2D (Ceriello, 2006). Oxygen reacts with the excess NO to generate nitrite and peroxy nitrite anions, which act as free radicals (Baskar et al., 2007). In the present study, incubation of solutions of sodium nitroprusside in phosphate buffer saline resulted in a linear time-dependent nitrite production (Arya et al., 2011), which is reduced by all the tested extracts except the EtoAc extract of the stem bark. This may be due to the anti-NO principles (mostly phenolics) in the extracts which could compete with oxygen to react with NO, thereby inhibiting the generation of nitrite. Further, the hormetic responses shown by some extracts toward scavenging NO indicate that the anti-NO principles have some optimal points of effective inhibition and/or antagonism which occur with other phytochemicals at certain concentrations.

Polyphenols are a well-known class of secondary metabolites with antioxidant activity. This is believed to be mainly due to their high redox properties which play an important role in absorbing and neutralising free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Zheng and Wang, 2001). Although correlation analysis was not performed, our results tend to suggest strong positive correlation between the polyphenolic content and anti-oxidative activity because a broad view of the results indicates that the EtOH extracts with highest polyphenolic content within the plants demonstrated a higher anti-oxidative activity with no pro-oxidative activity in any of the experimental models used. Thus, these extracts were further subjected to direct GC-MS analysis. It is evident from the results that a pyrogallol derivative is the only phytochemical detected in the stem bark and root sample of the plant and could therefore be the main bioactive anti-oxidative agent. However, the presence of the pyrogallol derivative peaks coupled with other phenolics in the leaf sample could account for the observed higher anti-oxidative activities in this part of the plant. On a general note, the observed quantitative difference in the anti-oxidative activities among the various parts of this plant could be attributed to the variations in concentrations and compositions of the anti-oxidative principles in the different parts.

From the results of this study, we can conclude that the EtOH extracts of the various parts of *P. biglobosa* contain more potent anti-oxidative agents than other extracts and that a pyrogallol derivative could be the main bioactive agent. Future work would entail isolating the pyrogallol derivative and other phenolics as well as evaluating the anti-oxidative activity of the pure isolates and mixtures of isolates.

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References

1. Adetutu, A., Morgan, W.A. and Corcoran, O. (2011). Ethnopharmacological survey and *in vitro* evaluation of wound-healing plants used in South-western Nigeria. J Ethnopharmacol. **137**: 50-56.
2. Agunu, A., Yusuf, S., Andrew, G.O., Zezi, A.U. and Abdurahman, E.M. (2005). Evaluation of five medicinal plants used in diarrhoea treatment in Nigeria. J Ethnopharmacol. **101**: 27-30.
3. Arya, V., Yadav, S., Kumar, S. and Yadav, J.P. (2011). Antioxidant activity of organic and aqueous extracts of *Cassia occidentalis* L in relation to their phenolic content. Nat Prod Res. **25**: 1473-1479.
4. Asuzu, I.U. and Harvey, A.L. (2003). The antisnake venom activities of *Parkia biglobosa* (Mimosaceae) stem bark extract. Toxicon. **42:** 763-768.
5. Baskar, P., Rajeswari, V. and Kumar, T.S. (2007). *In vitro* antioxidant studies in leaves of *Annona* species. Ind J Exp Biol. **45**: 480-485.
6. Calabrese, E.J. and Baldwin, L.A. (2001). U-shaped dose-responses in biology toxicology, and public health. Ann Rev Pub Health. **22**: 15-33.
7. Ceriello, A. (2006). Oxidative stress and diabetes-associated complications. Endocr Prac **12**: 60-62.
8. Ceriello, A. and Motz, E. (2004). Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. Arterioscler Thromb Vasc Biol. **24:** 816-823. cereillo
9. Etuk, E.U., Bello, S.O., Isezuo, S.A. and Mohammed, B.J. (2010). Ethnobotanical survey of medicinal plants used for the treatment of diabetes mellitus in the north western region of Nigeria. Asian J Exp Biol Sci. **1**: 55-59.
10. Gulcin, I., Beydemir, H.A., Alici, H.A., Elmastas, M. and Buyukokuroglu, M.E. (2004). *In vitro* antioxidant properties of morphine. Pharmacol Res. **49**: 59-66.
11. Hinnerburg, I., Damien, H.J. and Hiltumen, R. (2006). Antioxidant activities of extracts from selected culinary herbs and spices. Food Chem. **97**: 122-129.
12. Kalaivani, T. and Mathew, L. (2010). Free radical scavenging activity from leaves of *Acacia nilotica* (L) Wild ex Delile, an Indian medicinal tree. Food Chem Toxicol. **48:** 298-305.
13. Kendig, E.L., Le, H.H., Belcher, S.M. (2010). Defining hormesis: Evaluation of a complex concentration response phenomenon. Int J Toxicol. **29**: 235-246.
14. Koehn, F.E. and Carter, G.T. (2005). The evolving role of natural products in drug discovery. Nat Rev Drug Disc. **4**: 206–220.
15. Kouadio, F., Kanko, C., Juge, M., Grimaud, N., Jean, A., N'Guessan, Y.T, Petit, J.Y. (2000). Analgesic and anti-inflammatory activities of an extract from *Parkia biglobosa* used in traditional medicine in the Ivory Coast. Phytother Res. **14**: 635-637.
16. Kurian, A.G., Suryanarayanan, S., Raman, A. and Pidakalla, J. (2010). Antioxidant effects of ethyl acetate extract of *Desmodium gangeticum* root on myocardial ischemia reperfusion injury in rat hearts. Chin Med. **5**: 1-7.
17. Lata, H. and Ahuja, G.K. (2003) Role of free radicals in health and disease. Ind J Physiol Allied Sci. **57**: 124.
18. McDonald, S., Prenzier, P.D., Autokiwich, M. and Robards, K. (2001). Phenolics content and antioxidant activity of olive oil extracts. Food Chem. **73**: 73-84.
19. Millogo-Kone, H., Guissou, I.P., Nacoulma, O. and Traore, A.S. (2008). Comparative study of leaf and stem bark extracts of *Parkia biglobosa* against enterobacteria. Afr J Trad CAM. **5**: 238-243.
20. Odetola, A.A., Akinloye, O., Egunjobi, C., Adekunle, W.A. and Ayoola, A.O. (2006). Possible antidiabetic and antihyperlipidemic effect of fermented *Parkia biglobosa* (Jacq) extract in alloxan-induced diabetic rats. Clin Exp Pharmacol Physiol. **33**: 808-812.
21. Oyaizu, M. (1986). Studies on potent of browning reactions: Antioxidative activities of products of browning reaction prepared from glucosamine. Japan J Nutr. **44**: 307-315.
22. Razab, R. and Abdul-Aziz, A. (2010). Antioxidants from tropical herbs. Nat Prod Comm. **5:** 441-445.
23. Takayanagi, R., Inoguchi, T. and Ohnaka, K. (2000). Clinical and experimental evidence for oxidative stress as an exacerbating factor of diabetes mellitus. J Clin Biochem Nutr. **48:** 72-77.
24. Tokoudagba, J., Auger, C., Breant, L., N'Gom, S., Chabert, P., Idris-Khodja, N., Gbauidi, F., Gbenou, J., Moudachirou, M., Lobstein, A. and Kerth-Schini, V.B. (2010). Procyanidin-rich extracts from *Parkia biglobosa* (Mimosaceae) leaves cause redox-sensitive endothelium-dependent relaxation involving NO and EDHF in procine coronary artery. J Ethnopharmacol. **132**: 246-50.
25. Tuba, A.K. and Gulcin, I. (2008). Antioxidant and radical scavenging properties of curcumin. Chemico-Biol Inter. **174**: 27-37.
26. Udobi, C.E. and Onaolapo, J.A. (2009). Phytochemical analysis and antibacterial evaluation of leaf stem bark and root of the African locust bean (*Parkia biglobosa*). J Med Plants Res. **3:** 338-344.
27. van Wyk, B.E. (2008). A broad review of commercially important southern African plants. J Ethnopharmacol. **119**: 342-355.
28. Wiernsperger, N.F. (2003). Oxidative stress as a therapeutic target in diabetes: revisiting the controversy. Diabetes Metab. **29:** 579-585.
29. Wu, N., Zu, Y., Fu, Y., Kong, Y., Zhao, J., Li, X., Li, J., Wink, M. and Efferth, T. (2010). Antioxidant activities and xanthine oxidase inhibitory effects of extracts and main polyphenolic compounds obtained from *Geranium sibiricum* L. J Agric Food Chem. **58:** 4737-4743.
30. Young, I.S. and Woodside, J.V. (2001). Antioxidants in health and disease. J Clin Pathol. **54**: 176-186.
31. Zheng, W. and Wang, S.Y. (2001) Antioxidant activity and phenolic compounds in selected herbs. J Agric Food Chem. **49:** 5165-5170.