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HPLC quantification of phenolic content and assessment of methanolic extract of *Antiaris africana* for toxicological study

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The study was aimed at evaluating the toxicological and antioxidant activities of *Antiaris africana* Engl. (family Moraceae), that is used in Nigeria and other West Africa countries as a panacea for the treatment of several ailments. The methanolic extract of *A. africana* (MEA) obtained was analysed for antioxidant activities *in vitro* and screened for various phytochemicals present. Phenolic and flavonoid contents were determined followed with high performance liquid chromatography -diode-array detection (HPLC-DAD) fingerprinting of phenolic content. Furthermore, the sub-acute toxicity of MEA was determined via oral administration of varying doses for 14 consecutive days (0, 50, 100, 200 and 400 mg/kg) in rats. After oral administration for 14 consecutive days in male rats, the toxicity effect was assayed by determining aspartate aminotransferase (AST) and alanine aminotransferase (ALT) for hepatic function; urea and creatinine for renal function; creatinine kinase (CK) for cardiac function; and lipid profile. HPLC results showed that the major phenolics present are quercetin, rutin, caffeic acid, garlic acid and quercetin. MEA was able to scavenge diphenyl picryl hydrazyl, hydroxyl and nitric oxide radicals and prevent lipid peroxidation induced by ferrous sulphate at all concentration tested. The toxicology investigation showed that at low doses, *A. africana* is non-toxic, while at high doses; it is moderately toxic to the animals. In conclusion, *A. Africana* is generally non-toxic; however, care must be taken in administration at higher doses.

Key words: Toxicology, HPLC, phytochemicals, *Antiaris africana.*

INTRODUCTION

Herbal medicine is gaining ground as the treatment of choice in the western world (Dey and De, 2015). Most countries in Africa and other developing countries rely on its usage for their primary healthcare (Eisenberg et al., 1998; World Health Organization, 2008). Nigeria has been planning on integrating herbal medicine as a degree program into the tertiary institution curriculum (Vanguard, 2014). This development is not surprising, looking at the

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fact that traditional medicine has played an important role in disease treatment since the emergence of man's evolution (Ahmada et al., 2013; Pankaj et al., 2009). Most drugs have their origin from natural sources, either they are directly isolated from plants or the isolated compound is modified to improve its efficacy (Fu et al., 2013). Most natural compounds are majorly classified as alkaloids, steroids, tannins, phenolic compounds, flavonoids and saponin (Bishnu et al., 2009). *Antiaris africana* is a plant found in various parts of Nigeria and West Africa. It is commonly called Ooro, Oriro or ako Iroko in the South West part of Nigeria; Farin Loko in the North; and Ojianwu in the South East. The plant is a large tree usually about 15 to 20 m high, but it can grow sometimes up to 40 m, and has white latex and alternate dissymmetric leaves (Berg et al., 1985; Berhaut, 1979), with heavy flat crown and blotchy grey and white bark. The flowers are small with a greenish white color that produces a red velvety fruits (Gill, 1992). It has a wide usage both in industry (timber making) and traditional medicine. Various part of the plant such as leaves, stems and barks are ethnobotanically used in the treatment of various diseases such as rheumatic and respiratory infection (Gill, 1992; Mann et al., 2003), epilepsy, lumbargo, skin irritant, purgative, chest pain (Okogun et al., 1976), syphilis (Berhaut, 1979), throat infection, leprosy, cancer (Kuete et al., 2009), and nervous disorders in the northern part of Nigeria (Moronkola and Faruq, 2013). However, the report on the industrial usage has overshadowed its ethno pharmacological usage as more works are published on it as compared to the latter. This has lead to dearth of report on the scientific rationale behind its ethnopharmarcological usage. Therefore, this experiment was conducted to give scientific insight to its traditional usage.

MATERIALS AND METHODS

Chemicals

Quercetin, rutin, ascorbic acid, tannic acid, Folin-Ciocalteu reagent, sodium carbonate, aluminum chloride, potassium acetate, deoxyribose, 1,1, diphenyl – 2,2- picryl hydrazyl (DPPH), were obtained from Sigma (Chemical Co, St. Louis, MO, USA). ALT, AST, creatinine (CREA), urea, CK, total cholesterol, triglyceride and high density lipoprotein kits were from Randox. All other reagents were of analytical grade. All UV–Vis measurements were recorded on a Shimadzu UV–1800.

Plant collection and preparation of extract

Leaves of *A. africana* were collected at Forest Research Institute of Nigeria (FRIN) garden and authenticated at the herbarium of Botany Department, University of Ibadan, Nigeria by Mr. Esimakhair (voucher number 070613M). The leaves were air dried and pulverized. The powdered sample (1.23 kg) was macerated in 5 L of 80% methanol for 72 h and then filtered. The filtrate was concentrated and then lyophilized to obtain the 81.1 g methanol extract of *A. africana* (MEA) used for the study. This was stored in an amber bottle and refrigerated.

Phytochemical screening

Extracts were phytochemically screened for the presence of alkaloids, saponins, tannins, phlobatannins, anthraquinones, flavonoids, steroids, and terpenoids using standard laboratory procedures (Sofowora, 1993; Trease and Evans, 1985).

Estimation of total phenolic content

The total phenolic content of the extract was estimated according to a modified procedure of Singleton et al. (1999). Briefly, deionised water (0.5 ml) and 125 µl of Folin–Colcalteu reagent were added to 125 µl of MEA dissolved in distilled water. The mixture was allowed to stand for 6 min before adding 1.25 ml of 7% (w/v) Na_2CO_3 solution. The reaction mixture was then allowed to stand for additional 90 min before taking the absorbance at 760 nm against the blank. The tannic acid standard curve was prepared by adding 125 µl of tannic acid dissolved in distilled water (2, 4, 8 and 10 µg/ml final concentrations) in lieu of extract. The amount of total phenolics was expressed as tannic acid equivalents (TAE, mg tannic acid/g sample) through the calibration curve of tannic acid.

Estimation total flavonoid content

Flavonoid content was estimated using the aluminum chloride colorimetric method (Chang et al., 2002). The plant extract in methanol (1 g/ml) was mixed with 0.1 ml of 10% aluminum chloride (w/v), 0.1 ml of 1 M potassium acetate and 2.8 ml distilled water. The mixture was allowed to stand at room temperature for 30 min. The absorbance of the reaction mixture was read at 415 nm. Results were expressed as mg/g quercetin equivalent (QE).

Quantification of compounds in extract by HPLC-DAD

HPLC fingerprinting and reverse phase chromatographic analyses were carried out under gradient conditions using agilent eclipse plus C_{18} column (4.6 x 150 mm) packed with 5 µm diameter particles. Quantification of phenols involved a mobile phase made up of water containing 2% acetic acid (A) and methanol (B). The composition of B was varied starting from the more polar (5% of B) until 2 min and increased gradually till 100% of B as described by the method of Sabir et al. (2012), with slight modification. All chromatography operations were carried out at ambient temperature and in triplicate. High performance liquid chromatography (HPLC-DAD) was performed with a Shimadzu Prominence Auto Sampler (SIL-20A) HPLC system (Shimadzu, Kyoto, Japan), equipped with Shimadzu LC-20AT reciprocating pumps connected to a DGU 20A5 degasser with a CBM 20A integrator, SPD-M20A diode array detector and LC solution 1.22 SP1 software.

Determination of reducing power

Varying amounts of the extract (10 to 800 µg) in 1 ml of distilled water were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide (1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of 10% TCA was added to the mixture which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% $FeCl_3$ and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Nitric oxide radical scavenging assay

10-400 µg of MEA was added in the test tubes to 1 ml of sodium nitroprusside solution (25 mM) and the tubes incubated at 37°C for 2 h. An aliquot (0.5 ml) of the incubation solution was removed and diluted with 0.3 ml of Griess reagent (1% sulphanilamide in 5% H_3PO_4 and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed was immediately read at 570 nm against distilled water as blank with catechin (50 µg) used as standard. Results were expressed as percentage radical scavenging activity (RSA).

Potential to inhibit deoxyribose degradation (deoxyribose assay)

200-1000 µg of sample in 100 µl of distilled water was added to a solution containing 200 µl KH_2PO_4 – KOH (100 mM), 200 ul deoxyribose (15 mM), 200 µl $FeCl_3$ (500 µM) and 100 µl EDTA (1 mM) in a test tube and allowed to mix. The reaction was initiated by addition of 100 µl H_2O_2 (10 mM) and 100 µl ascorbic acid (1 mM). The reaction mixture was incubated at 37°C for 1 h. At the end of the incubation period, 1 ml of 1% w/v TBA was added to each mixture followed by the addition of 1 ml of 2.8% w/v TCA. The solution was heated in a water bath at 80°C for 20 min to develop the pink colored MDA-$(TBA)_2$ adduct. After cooling, the solution was centrifuged and the absorbance of the supernatant measured at 532 nm against distilled water as blank. Results were expressed as the percentage inhibition of deoxyribose degradation.

Inhibition of Fe^{2+}/ascorbate – induced lipid peroxidation

Liver homogenate from rat was prepared by removing liver immediately after sacrifice. The liver was rinsed in ice-cold 1.15% KCl to remove blood stain, blotted and weighed. The weighed tissue was then homogenized in four volumes of ice-cold 0.1 M phosphate buffer; pH 7.4. The reaction mixture containing 0.1 ml of liver homogenate in 30 mM tris buffer, 0.16 mM ferrous ammonium sulphate, 0.06 mM ascorbic acid and different amount of the extract (10-1000 µg), was incubated for 1 h at 37°C. The resulting thiobarbituric reactive species (TBARS) was measured by the method of Varshney and Kale (1990). An aliquot (0.4 ml) of the reaction mixture was mixed with 1.6 ml of 0.15 M tris-KCl buffer to which 0.5 ml of 30% TCA was added. Then 0.5 ml of 0.75% TBA was added and placed in a water bath for 30 min at 85°C, after which it was cooled in ice and centrifuged at room temperature for 3 min at 3000 *g*. The absorbance of the clear supernatant was measured against reference blank of distilled water at 532 nm.

Animal handling and treatment

Animals

Albino rats (Wistar strain) aged four weeks were obtained from the animal house of the Lagos University Teaching Hospital and fed with commercially available standard pelleted feed and water *ad libitum* throughout the period of experiment. All animal experimental protocols conformed to the international guide for the care and use of laboratory animals (National Research Council, 2011).

Rats were randomly divided into five groups with six animals per group. Group I (control) received normal saline (0.9% NaCl) orally. Groups II, III, IV and V received MEA (50, 100, 200 and 400 mg/kg/day, respectively) orally for 14 consecutive days. Twenty four hours after the last administration, blood sample was collected via cervical dislocation for the evaluation of markers of oxidative stress and hepatic, renal and cardiac functions.

There were no observable physical changes in animals administered MEA as compared with the control throughout the duration of the experiment.

ASSAY

Serum collections

At the end of 14-day period, blood was obtained via cardiac puncture under light chlorohydate anaesthesia. Blood was collected in serum bottles. The blood was centrifuged at 4000 rpm at 4°C for 10 min to obtain the serum, which was stored at -20°C until analysis for biochemical parameters.

Biochemical estimations

The activities of albumin, AST, ALT, ALP, CK, CREA, urea, triglyceride (TG), total cholesterol (TC), high density lipoprotein (HDL) and low density lipoprotein (LDL) were estimated using assay kits from Randox Laboratories Ltd., UK according to the instructions of the manufacturer.

Statistical analysis

Results are expressed as mean ± standard deviation. Differences between groups were determined by one-way analysis of variance (ANOVA) using SPSS software package for windows. Post hoc testing was performed for inter-group comparisons using the least significant difference (LSD) test and p-value < 0.05 was considered significant.

RESULTS

HPLC analysis

HPLC fingerprinting of *A. Africana* extract revealed the presence of the garlic acid (t_R = 12.40 min; peak 1), catechin (t_R = 16.35 min; peak 2), chlorogenic acid (t_R = 23.08 min; peak 3), caffeic acid (t_R = 25.39 min; peak 4), ellagic acid (t_R = 30.79 min; peak 5), epigallocatechin (t_R = 33.56 min; peak 6), rutin (t_R = 39.18 min; peak 7), isoquercitrin (t_R = 43.97 min; peak 8), quercitrin (t_R = 47.73 min; peak 9), quercetin (t_R = 52.03 min; peak 10) and kaempferol (t_R = 64.15 min; peak 11) (Figure 1 and Table 3).

Phytochemical screening revealed the presence of saponin, tannin, phlobatannin, flavonoid and terpenoid in the methanol extract of *A. africana* (MEA) leaves. Phytochemical screening also showed that alkaloid is absent in the MEA (Table 1) while quantification of total phenolic content TPC was 243.71±13.18 mg TAE/g extract and total flavonoids was 155.85±9.28 mg QE/g extract (Table 2). HPLC DAD reveals the presence of gallic acid, catechin, chlorogenic acid, caffeic acid, ellagic acid, epigallocatechin, rutin, isoquercitrin, quercetin and kaempferol. Caffeic acid is the most abundant phenolic compound (35.97 ± 0.02 mg/g extract) and rutin is the most abundant flavonoid (30.37 ± 0.04 mg/g extract) (Table 3 and Figure 1).

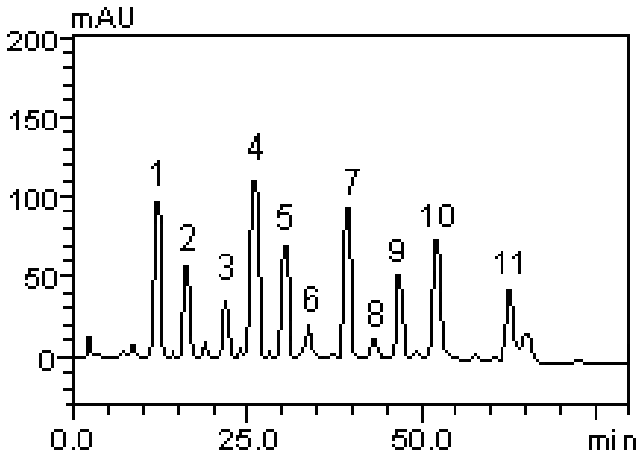

Figure 1. Representative high performance liquid chromatography profile of *Antiaris africana* extract: gallic acid (peak 1), catechin (peak 2), chlorogenic acid (peak 3), caffeic acid (peak 4), ellagic acid (peak 5), epigallocatechin (peak 6), rutin (peak 7), isoquercitrin (peak 8), quercitrin (peak 9), quercetin (peak 10) and kaempferol (peak 11). Chromatographic conditions are described in the methods section.

Table 1. Phytochemical constituents of *Antiaris africana.*

Phytochemical	Observation
Alkaloid	Absent
Saponin	Present
Tannin	Present
Phlobatannins	Present
Anthraquinone	Absent
Flavonoid	Present
Terpenoids	Present

The antioxidant activity of plants is generally attributed to the presence of phytochemicals present. Free radical scavenging and inhibition of TBARS are one of the important assays for the determination of antioxidant activity of plant extracts. Hence, in order to explore and understand these possible mechanisms, several antioxidant assays including NO, DPPH and OH radical scavenging assays were performed and evaluation of the antioxidant activities of the results confirmed that this plant has a broad range of antioxidant properties, including substantial inhibition of lipid peroxidation.

Analysis of the free radical scavenging of NO (Figure 2), DPPH (Figure 3) and OH (Figure 4) of the extracts revealed a concentration-dependent antiradical activity resulting in the conversion of the radicals to non- radical form. MEA significantly scavenge hydroxyl radical generation as observed in the percentage increase in prevention of deoxyribose degradation at all concentration. Also, MEA showed a concentration-dependent anti- DPPH radical scavenging ability. It thus appears that the extracts possess hydrogen donating ability and act as antioxidant. However, the scavenging ability of quercetin, a known antioxidant used as positive control was greater than that of the extracts.

The antioxidant activity was further analyzed by the TBARS method, which is used to quantify lipid peroxidation that corresponds to a cell membrane damage caused by oxidative stress. The Fe (II) induced stimulation in brain TBARS levels. The MEA at all tested concentration was able to prevent lipid peroxidation as observed in the reduction in the amount of TBARS formed (Figure 5), though it was not as potent as the reference drug (quercetin).

Reducing power ability

One of the key activities of antioxidant is their ability to donate electrons and thus reducing radicals to a less active species. This is measured by the reduction of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{2+} formation can be monitored spectrophotometrically. At concentration 400 µg/ml, the methanolic extract (0.7013 ± 0.194) showed greater absorbance than ascorbic acid (0.6016 ± 0.338). Reducing power of the MEA is shown in Figure 6.

Renal function indices

Figure 7 shows the effect of MEA on renal function. All doses of MEA had no significant effect on urea level in the serum as compared to the control ($P<0.05$), with the urea level falling between 18.63 ± 0.4 and 28.0 3± 0.72 mg/dl, except 200 mg/kg urea level of 6.20 ± 1.51 mg/dl, while the creatinine level was significantly decreased in the serum as compared to the control ($P<0.05$) with the concentration falling between 0.46 ± 0.04 to 0.58 ± 0.06 mg/dl.

Hepatic and cardiac function

Figures 8 and 9 reveal the AST, ALT and CK activity respectively in animals administered MEA. There was no significant difference in the activity of AST at all doses as compared to the control, with the activity falling between 58.00 ± 2.8 to 84.20 ± 3.5U/I. The serum ALT activityvaries with each doses of extract administered when compared to the control. MEA at 50 and 100 mg/kg with 20.4 ± 0.19 and 14.8 ± 0.34 U/I, respectively was not significantly different from the control ($P<0.05$), while 200 and 400 mg/kg of MEA showed a significant elevation in the activity of ALT with 98.0 ± 0.41 and 112.8 ± 0.33, respectively as compared to the control ($P<0.05$).

Table 2. Effect of MEA on serum lipid profile.

Group	CHOL (mg/dl)	HDL (mg/dl)	TG (mg/dl)	LDL (mg/dl)
Control	50.28±4.93	24.86±4.58	18.65±2.44	21.67±3.71
50 mg/kg	41.94±6.43*	4.01±1.97*	17.45±3.12	34.44±6.12*
100 mg/kg	37.02±3.04*	5.34±1.93*	21.94±7.48*	27.67±3.24
200 mg/kg	30.22±1.76*	11.95±4.72*	26.12±2.70*	13.05±4.17*
400 mg/kg	23.78±4.80*	9.30±1.40*	29.00±1.92*	8.67±3.03*

Values are expressed as mean ± standard error of the mean. *Significantly different from control.

Table 3. Composition of *Antiaris africana* extract.

A. Africana compounds	Composition		LOD (µg/mL)	LOQ (µg/mL)
	mg/g	%		
Gallic acid	30.74 ± 0.03a	3.07	0.037	0.123
Catechin	16.11 ± 0.02b	1.61	0.028	0.092
Chlorogenic acid	8.36 ± 0.01c	0.83	0.016	0.054
Caffeic acid	35.97 ± 0.02d	3.59	0.012	0.039
Ellagic acid	22.08 ± 0.03e	2.20	0.035	0.115
Epigallocatechin	4.51 ± 0.01f	0.45	0.007	0.023
Rutin	30.37 ± 0.04a	3.03	0.021	0.070
Isoquercitrin*	2.14 ± 0.03g	0.21	-	-
Quercitrin*	15.46 ± 0.01b	1.54	-	-
Quercetin	26.71 ± 0.03e	2.67	0.009	0.029
Kaempferol	13.05 ± 0.02h	1.30	0.014	0.047

Results are expressed as mean ± standard deviations (SD) of three determinations. Averages followed by different letters differ by Turkey test at $p < 0.05$.*Quantified was quercetin. LOD, Limit of detection; LOQ, Limit of quantification.

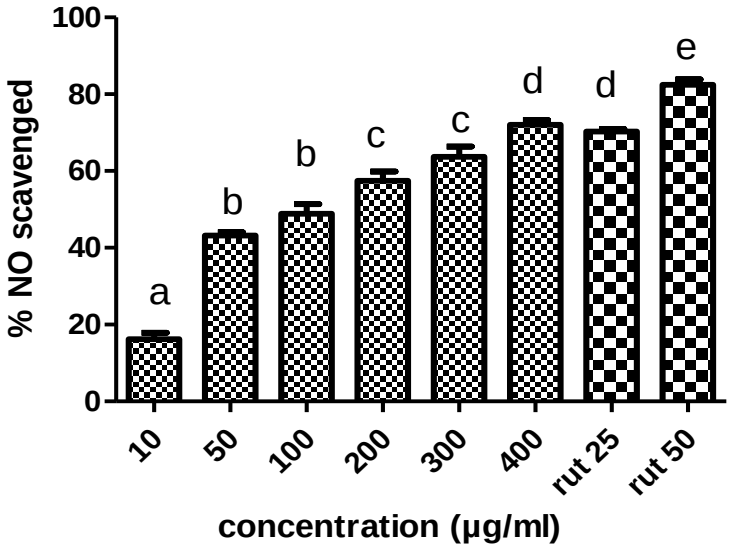

Figure 2. Nitric oxide (NO^{-}) radical scavenging activity (RSA) of methanolic extract of *Antiaris Africana* (MEA). Values represent the mean ± S.E.M. of the values of inhibition *in vitro*; n = 3 experiments in duplicate. $P < 0.05$ versus control. Values with similar alphabets are not significantly different.

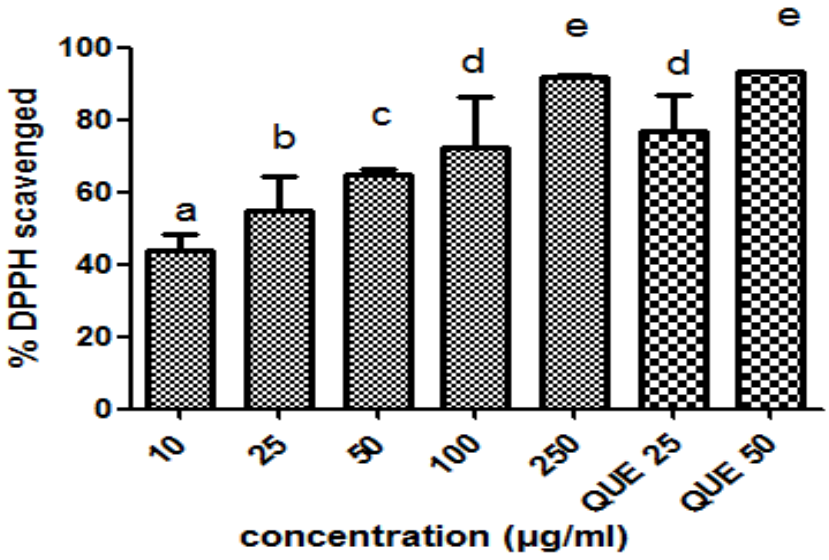

Figure 3. DPPH scavenging activity of MEA. Values represent the mean ± S.E.M. of the values of inhibition *in vitro*, n = 3 experiments in duplicate. P < 0.05 versus control. Values with similar alphabets are not significantly different.

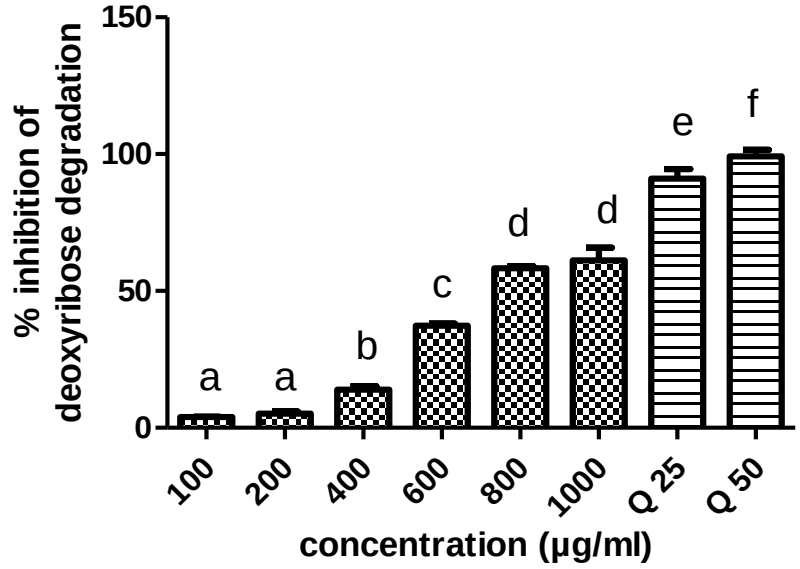

Figure 4. Inhibition of deoxyribose oxidation (hydroxyl radical scavenging activity) by methanolic extract of *Antiaris africana* (MEA). Values represent the mean ± S.E.M. of the values of inhibition *in vitro*; n = 3 experiments in duplicate. P < 0.05 versus control. Values with similar alphabets are not significantly different.

Administration of MEA at all doses generally had no significant effect on CK level, with the activity falling between 52.83 ± 2.88 and 107.83 U/l (P<0.05).

Lipid profile

Table 2 summarizes the effect of varying doses of MEA on lipid profiles in animals. It shows that MEA was able to significantly decrease total cholesterol, triglyceride and LDL-c level in a dose dependent manner when compared to the control; while, a dose dependent elevation of HDL-c level when compared to the control was also observed.

DISCUSSION

The present study is designed to investigate the

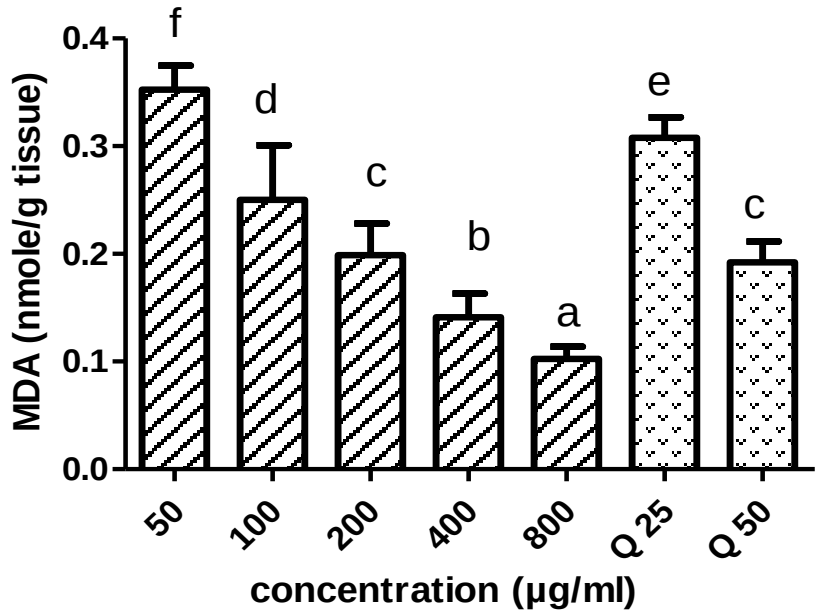

Figure 5. Effect of methanolic extract of *Antiaris Africana* (MEA) on $FeSO_4$ induced lipid peroxidation. Values represent the mean ± S.E.M. of the values of inhibition *in vitro*; n = 3 experiments in duplicate. P < 0.05 versus control. Values with similar alphabets are not significantly different.

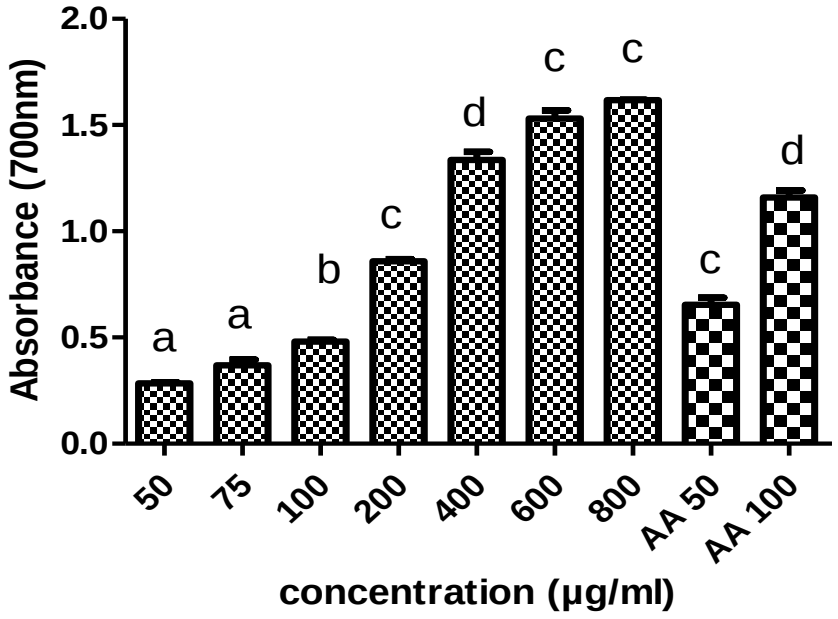

Figure 6. Reducing power of methanolic extract of *Antiaris africana* (MEA). Values represent the mean ± S.E.M. of the values of inhibition *in vitro*, n = 3 experiments in duplicate. P < 0.05 versus control. Values with similar alphabets are not significantly different.

phytochemical constituents present and the biochemical effect of oral administration of MEA on the biomarkers of organ toxicity. *A. africana* (AA) is a plant that is commonly used in Nigeria and other part of West African countries as a panacea in the treatment of several ailments (Kuete et al., 2009). The local usage of AA in

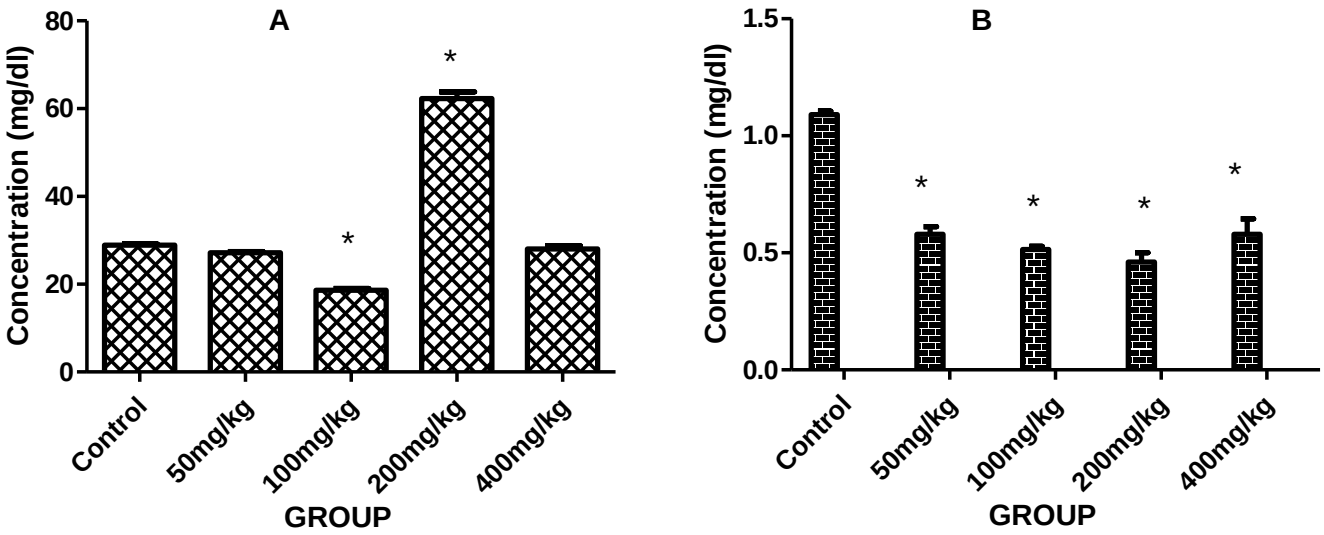

Figure 7. The effect of MEA on marker of renal toxicity A (Urea concentration); B (CREA concentration). Values are expressed as mean ± standard error of the mean. *Significantly different from control (P<0.05).

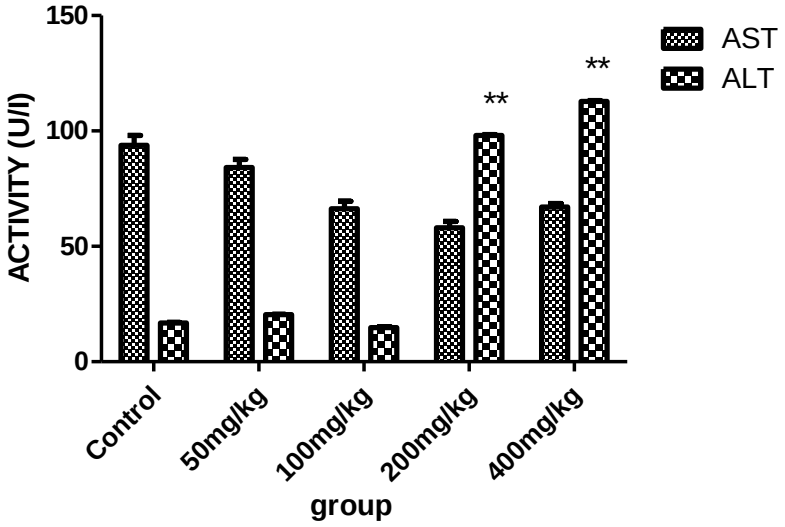

Figure 8. Effects of MEA on markers of hepatotoxic enzyme. Values are expressed as mean ± standard error of the mean. *Significantly different from control (P<0.05).

the treatment of several ailments has been going on for ages past in different part of Nigeria, however there has been no scientific report on the phytochemicals present in it and a proper scientific report on its safety doses upon oral administration of the plant. Phytochemical components such as alkaloids, polyphenols, flavonoid, cardiac glycoside and saponin have been reported to be responsible for both pharmacological and toxic activities in plants (Akinmoladun et al., 2010; Aggarwal et al., 2006). This necessitated a preliminary investigation to identify the various phytochemicals present, assess its antioxidant potentials *in vitro* and possible toxicities of AA. Biomarkers of toxicity include ALT and AST for cytotoxicity and disturbance in hepatic function; urea and creatinine for disturbance in renal function; creatinine kinase is for cardiac function and lipid profile as a link to cardiotoxicity, diabetes and obesity.

Phytochemical screening revealed the presence of saponins, tannins, phlobatannins, flavonoids and terpenoids. Phenolic compounds are not found in animals, majorly synthesized by plants, they are secondary metabolites derived from the shikimate-phenylpropanoids-flavonoids pathways. Flavonoids, one of the largest groups of polyphenols have been reported to be of health

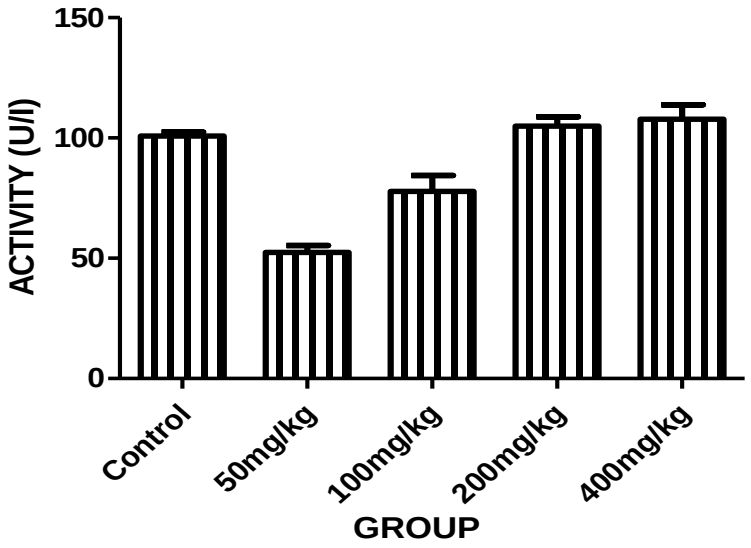

Figure 9. Effect of MEA on creatinine kinase activity. Values are expressed as mean ± standard error of the mean. *Significantly different from control ($P<0.05$).

benefit to human. Several reports has shown that flavonoid possess antioxidative, anti-inflammatory, antimicrobial, cardioprotective and neuroprotective effects (Petti and Scully, 2009; Asensi et al., 2011; Taamalli et al 2013) anti-allergenic, anti-atherogenic, anti-inflammatory, anti-microbial, anti-thrombotic, cardioprotective and vasodilatory effects (Petti and Scully, 2009; Asensi et al., 2011). These metabolites are said to be useful to plant itself but can be toxic to animals, including man. Saponin is a known anti-nutritional phytochemicals that possess the potential to reduce the uptake of certain nutrients including cholesterol and glucose at the gut through intralumenal physicochemical interaction or other yet unidentified activity (Price et al., 1987), suggesting a possible uses in the treatment of diabetes and cardiovascular related diseases; while, tannin, phlobatannin flavonoid and terpenoids are polyphenolic compounds that have been reported to be responsible for most biological activity in plants (Al-Sereiti et al., 1999). Attack of lipid and its derivatives by radicals is one of the mechanisms by which oxidative stress take place, leading to both cell membrane damage and cytotoxicity. Thus the ability of plant extract to inhibit lipid peroxidation is regarded as a key indicator of antioxidant activities. Our data shows that MEA inhibit lipid peroxidation in a concentration dependent manner using brain homogenate. Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity (Babu et al., 2001). The ability of extracts to quench hydroxyl radicals seems to be directly related to the prevention of propagation of the process of lipid peroxidation and they seem to be good scavengers of active oxygen species, thus terminating or reducing the rate of reaction. MEA scavenged the generated hydroxyl radicals as observed in the prevention of 2-deoxy-2-ribose degradation in a concentration dependent manner. The antioxidant potential of plant extracts can also be determined by the ability of plant extract to reduce ferric ion to ferrous ion (Fe^{3+} to Fe^{2+}) by electron donation termed reducing power (RP). The amount of Fe^{2+} formed is now monitored spectrophotometrically (Ebrahimzadeh et al., 2008). Several researchers have reported a direct correlation between antioxidant activities and reducing power of certain plant extract (Akinmoladun et al., 2010; Koncic et al., 2010; Olaleye et al., 2010). MEA showed reducing power at all concentration.

In the safe dose study in rats given the MEA orally at doses ranging from 50-400 mg/kg, all the animals in each group showed no behavioural difference, though there was an initial response to the MEA on first administration which was normalized few hours later. One of the key indicators of adverse reaction to drugs and chemicals is alteration in body weight (Sellers et al., 2007; Raza et al., 2002; Teo et al., 2002; Tofovic and Jackson, 1999). Administration of MEA showed no significant change in body weight (not reported). Biological markers like endogenous enzymes have been shown and established to be organ-specific and can leak from a damaged or an injured organ (Kubavat and Asdaq, 2009). Hepatic function has been monitored by the evaluation of the levels of ALT and AST in conjunction with biochemical analytes such as cholesterol, creatine kinase and creatinine in the serum. The activity of plant extract to lower the serum level of AST and ALT is one of the mechanism of mediating hepatoprotective or

hepatotoxicity effect of plant extract (Adewusi and Afolayan, 2010). Any damage to the hepatic organ shows an increase in the serum level of AST and ALT (Olaleye et al., 2010; Ahsan et al., 2009; Fakurazi et al., 2008; Liu et al., 2006). From the experiment, there was varying observation in the serum level of AST and ALT as compared to the control; MEA showed no toxic effect on AST activity at all administered doses, while the effect on ALT activity showed that 50 and 100 mg/kg dosages were not toxic, however, administration of 200 and 400 mg/kg was toxic as observed in the significant elevation in serum ALT activity. AST is a general marker of toxicity, thus its leakage is not only dependent on hepatic disturbances, it can be implicated in other tisuues as well. However ALT is a major biomarker of hepatic cytotoxicity (James et al., 1993), thus its increased activity at 200 and 400 mg/kg is a sign of toxicity of MEA at high dosages. One of the risk factors implicated in the occurrence of coronary disease is high level of cholesterol (hypercholesterolemia). Recently, it has been reported that the onset of cardiovascular events can be well controlled after reducing the serum LDL-cholesterol level using several therapeutic agents (Cheng et al., 2004). MEA caused significant decrease in the serum levels of triglycerides, total cholesterol, LDL cholesterol, but increased HDL-cholesterol. One of the mechanism of drugs used in the treatment of cardiovascular disease is in their ability to lower lipid level in the blood (Nofer et al., 2001). Thus, it can be deduced that MEA possess hypolipidemic potential and can be a potential drug for treatment of cardiovascular related diseases. This might be linked to a probable role of MEA in lipid metabolism and clearance from the body. The effect of different plant species on lipid lowering has been reported as a key factor in their medicinal use (Kono et al., 1992; Naidu and Thippeswamy, 2002; Devi and Sharma, 2004). Polyphenol is one of the most abundant phytochemical present in plants; it is abundant in various part of the plants, such as the leaves, roots, stems and seeds. One of the key features of the therapeutic uses of polyphenol is based on its antioxidant properties, anti-inflammatory, antitumor and antimicrobial activities (Xu et al., 2012; Wang et al., 2009). Phenolics are compounds possessing one or more aromatic rings with one or more hydroxyl groups. They are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants, with more than 8,000 phenolic structures currently known, ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins (Dai and Mumper, 2010; Harborne and Williams, 2000).

The main classes of polyphenols, based on their structure, are phenolic acids, flavonoids, stilbenes, and lignans. Flavonoids account for approximately two-thirds of the phenolics in our diet (Sathishkumar et al., 2008) and one of the most abundant polyphenols known, it can be classified into six major groups; flavonols, flavones, flavanones, flavan-3-ols, anthocyanins and isoflavones. In support of this,our results shows that flavonoid is the most abundant polyphenols present in *A. africana*. Polyphenols arise from the common intermediate phenylalanine, or a precursor, shikimic acid. Flavonoids are the group of polyphenols most studied and more than 4,000 have been identified and categorized into six subclasses: flavonols, flavones, flavanones, flavan-3-ols, anthocyanins and isoflavones. The interest in flavonoids arises from studies that have shown that consumption of food rich in it protects against many chronic diseases such as liver damage, chronic kidney injury, cancer, cardiovascular diseases and neurodegenerative diseases. This protective property is attributed to their antioxidant, anti-inflammatory and metal chelating abilities (Dai and Mumper, 2010). A variety of phenolic compounds, in addition to flavonoids, are found in fruit, vegetables and many herbs. Phenolic compounds (such as caffeic, ellagic and ferulic acids, sesamol and vanillin) have been reported to have an inhibitory on atherosclerosis disease (Decker, 1995). HPLC fingerprinting reveals the presence of quercetin, rutin, isoquercetirin, caffeic acid and gallic acid. The presence of these chemical constituents in this plant, is an indication that the plant, if properly screened, could yield drugs of pharmaceutical significance.

Conclusion

The oral administration of MEA for 14 consecutive days at doses ranging from 50-400 mg/kg showed no death among the animals. In addition, no significant effect was observed in the animal behavior and activities throughout the duration of administration, also no adverse effect was observed in the organ and body weights of the animals. From the biochemical parameters, it can be deduced that MEA is safe at low doses and significantly reduced cholesterol level as evident from significant reduction in LDLc parameters.

Conflict of Interests

The authors have not declared any conflict of interests.

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