

Full Length Research Paper

Antioxidant and phytochemical outling of aqueous and ethanolic extract of *Garcinia kola*

*Ibrahim Bello, Yakubu Chris and Herbert Akintola

Department of Biochemistry, Faculty of Pharmacy, Bayero University Kano, Kano, Nigeria.

Accepted 10 March, 2019

Garcinia kola is widely consumed habitually and used as traditional remedy for various diseases in Nigeria. However, information with respect to the effect of using different solvents of extraction on its antioxidant properties is scanty. Therefore, the present study investigates the effect of different solvents on the phytochemical constituents and antioxidant properties of *G. kola*. Phytochemicals measured were the total phenolics, flavonoids and vitamin C content while the antioxidant parameters assessed were the free radical scavenging, Fe (II) - chelating and Fe³⁺ reducing properties. Finally, the ability of both extracts to offer protective benefits to both hepatic and cerebral tissues placed under oxidative assault induced by FeSO₄ and sodium nitroprusside (SNP) was also investigated. The phenolic content of *G. kola* was estimated to be 45.2 ± 0.9 mg/g and 30.3 ± 0.4 mg/g gallic acid equivalent (GAE) for the ethanolic and aqueous extracts, respectively. Whereas the flavonoid content was estimated to be 29.2 ± 0.2 mg/g and 10.8 ± 0.2 mg/g quercetin equivalent (QE) for the ethanolic and aqueous extracts, respectively. In addition, the vitamin C contents were 21.2 ± 0.4 mg/g and 20.8 ± 0.1 mg/g for the ethanolic and aqueous extract of dried *G. kola*, respectively. In all the antioxidant indices measured, the ethanolic extract possessed significantly higher properties than the aqueous extract (p < 0.05). In view of all, the use of *G. kola* for the treatment of cough and liver disorders in folkloric medicines may be related to its phytochemical content. Hence, moderate consumption of the fruit should be encouraged as antioxidant supplement.

Key words: *Garcinia kola*, antioxidant, ethanolic, aqueous and phytochemical.

INTRODUCTION

Plant-derived substances have recently attracted great research interest owing to their versatile applications (Ncube et al., 2008). Specifically, medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube et al., 2008). This is due to the presence of secondary metabolites which accumulate in the various parts of these plants conferring on them their pharmacological relevance (Babalola et al., 2001). Generally, some of these plants especially the edible ones are eaten

habitually without any knowledge of their pharmacological effect. *Garcinia kola* Heckel (Clusiaceae), commonly known as bitter kola (English) and *orogbo* (Yoruba) is a widespread tree of evergreen forest valued in Nigeria for its medicinal nuts which has led to its exploitation in the natural forests in recent times (Farombi et al., 2005). It is chewed extensively in Southern Nigeria as a masticatory to cause nervous alertness and has been proven to exhibit pharmacological uses in treating coughs, and throat infections (Farombi et al., 2005). *G. kola* stem bark has been shown to contain a complex mixture of phenolic compounds such as tannins, guttiferin (Etkin, 1981), biflavonoids, xanthenes, benzophenone, kolaflavanone and garcinia flavanone (Iwu and Igboko, 1982) all of which have antimicrobial activity. Besides, *G. kola* exhibits purgative, antiparasitic, anti-inflammatory, antibacterial and antiviral properties (Akoachere et al., 2002).

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

In addition, the plant possesses hepatoprotective (Braide, 1991; Akintonwa and Essien, 1990), analgesic and hypoglycemic activities (Olaleye et al., 2000; Odeigah et al., 1999). *G. kola* enjoys a folk reputation in the management of sickle cell disease (SCD), as poison antidote (Kabangu et al., 1987, Egunyomi et al., 2009) and in the preservation of lipid food products prone to rancidity (Farombi et al., 2003). Considering the enormous relevance of *G. kola* in folkloric medicine, the present study was focused on the effect of ethanol and water as solvents of extraction on the phytochemical constituents and antioxidant properties of the fruit *in vitro*.

MATERIALS AND METHODS

Chemical reagents

Thiobarbituric acids (TBA), 2, 2 - diphenyl-1-picrylhydrazyl (DPPH) and 1, 10 - phenanthroline were obtained from Sigma (St. Louis, MO). All other chemicals were of analytical grade and obtained from standard chemical suppliers.

Plant material

Fresh fruits of *G. kola* were bought from the Main market in Akure, Nigeria and identified at the crop soil and pest Management Department of the University. The fruits were peeled to remove the shell covering the pulp which was then chopped to small pieces and air - dried. Thereafter, the dried pulp was blended using a Marlex blender and the powdered samples were stored in polythene bags and placed at room temperature until they were used.

Preparation of plant extracts

Five grams (5 g) of powdered samples were weighed in separate extraction bottle and 100 ml of solvent (either distilled water and absolute ethanol) was added to the bottle containing the powdered samples and left to stand for 24 h to allow for extraction at room temperature. Thereafter, each solution was filtered using a Whatman filter paper (No. 1). The filtrates (not evaporated) of both aqueous and ethanolic extracts were stored air tight in a refrigerator until required for use.

Animals

Male adult Wistar rats (200 to 250 g) were used. The animals were used according to the standard guidelines on the Use of Experimental Animal Resources of the Animal Production and Health Department of the Federal University of Technology, Akure, Nigeria.

Determination of total phenol contents

The total phenol contents of the aqueous and ethanolic extracts of *G. kola* were determined according to the method of Singleton et al. (1999) with some modifications. Briefly, 200 μ l of each sample was mixed with an equal volume of water. Folin - Ciocalteu reagent (2.5 ml) and 2 ml of 7.5% sodium carbonate were subsequently added, and the absorbance was measured at 765 nm after

incubating at 45°C for 40 min. The amount of phenols in both extracts was expressed as gallic acid equivalent (GAE).

Determination of total flavonoid content

The flavonoid contents of aqueous and ethanolic extracts of *G. kola* were determined according to the method of Meda et al. (2005) using quercetin (0.01 g / 20 ml ethanol) as a reference compound. Briefly, 500 μ l of stock solution of both extracts were mixed with 50 μ l of aluminum trichloride ($AlCl_3$) and potassium acetate according to Meda et al. (2005). The absorbance (using Spectrumlab digital spectrophotometer) at 415 nm was read after 30 min incubation at room temperature. All determinations were carried out in triplicate. The amount of flavonoids in aqueous and ethanolic extracts of *G. kola* was expressed as quercetin equivalent (QE).

Vitamin C content

The levels of vitamin C in the aqueous and ethanolic extracts of *G. kola* were determined spectrophotometrically as described by Jacques-Silva et al. (2001). Briefly, 1 ml of H_2SO_4 65% (v/v) was added to each solution. The reaction product was determined using a color reagent containing 4.5 mg/ml dinitrophenyl hydrazine (DNPH) and copper (II) tetraoxosulphate (VI) $CuSO_4$ (0.075 mg/ml), and the absorbance of the colored product was measured at 520 nm. The content of ascorbic acid was expressed as ascorbic acid equivalent (AScE).

Free radical scavenging ability

The free radical scavenging abilities of the aqueous and ethanolic extracts of *G. kola* against DPPH free radicals were evaluated according to Gyamfi et al. (1999). Briefly, 600 μ l of either aqueous or ethanolic extracts of *G. kola* was mixed with 600 μ l, 0.3 mM methanolic solution containing DPPH radicals. The mixture was left in the dark for 30 min and the absorbance was measured at 516 nm.

Reducing assay

The reducing property was determined by assessing the abilities of aqueous and ethanolic extracts of *G. kola* to reduce $FeCl_3$ solution as described by Pulido et al. (2000). Extract (0 to 5 mg/ml) were mixed with 250 μ l sodium phosphate buffer (200 mM, pH 6.6) and 250 μ l of potassium ferrocyanide (1%) and the mixture was incubated at 50°C for 20 min. Thereafter, 250 μ l trichloroacetic acid (TCA, 10%) was added, and subsequently centrifuged at 650 rpm for 10 min. 1000 μ l of the supernatant was then mixed with equal volume of water and 100 μ l of ferric chloride (0.1 g / 100 ml). The absorbance was later measured at 700 nm. A higher absorbance indicates a higher reducing power.

Fe^{2+} Chelating property

The Fe^{2+} chelating abilities of aqueous and ethanolic extracts of *G. kola* were determined using a modified method described by Puntel et al. (2005). Freshly prepared $FeSO_4$ (500 μ M) was added to a reaction mixture containing 168 μ l of Tris-HCl (0.1 M, pH 7.4), 218 μ l saline and extract (0 to 5 mg/ml). The reaction mixture was incubated for 5 min before the addition of 13 μ l of 1, 10 - phenanthroline (0.25%, w/v). The absorbance was subsequently measured at 510 nm in a spectrophotometer. The Fe (II) chelating

Table 1. Phytochemical constituents of *G. kola*.

Parameter	Ethanollic extract	Aqueous extract
Total Phenols	45.2 ± 0.9 mg/g (GAE)	30.3 ± 0.4 mg/g (GAE)
Total flavonoids	29.2 ± 0.2 mg/g(QE)	10.8 ± 0.2 mg/g(QE)
Ascorbic acid	21.2 ± 0.4 mg/g (AscE)	20.8 ± 0.1 mg/g (AscE)

Each observation is a mean ± SD of 3 to 4 independent experiments performed in triplicate. Statistical significance is set at $P < 0.05$. GAE, Gallic acid equivalent; QE, quercetin equivalent; AscE, ascorbic acid equivalent.

abilities were subsequently calculated with respect to the reference (which contains all the reagents without the extracts).

Lipid peroxidation

Rats were decapitated via cervical dislocation and the cerebral (whole brain) and hepatic (liver) tissues were rapidly dissected, placed on ice and weighed. Tissues were immediately homogenized in cold 50 mM Tris-HCl, pH 7.4. The homogenates were centrifuged for 10 min at 4000 rpm to yield a pellet that was discarded and a clear supernatant (S1). An aliquot of 100 µl of S1 was incubated for 1 h at 37°C in the presence of both extracts, with and without pro-oxidants, iron (10 µM) and sodium nitroprusside (SNP) (30 µM). This was then used for determining lipid peroxidation. Level of thiobarbituric acid reactive species (TBARS) produced was measured as described by Ohkawa et al. (1979), excepting that the buffer of the colored reaction has a pH of 3.4. The color reaction was developed by adding 300 µl of sodium dodecyl sulphate (SDS, 8.1%) to S1, followed by sequential addition of 500 µl of TBA (0.8%). This mixture was incubated at 95°C for 1 h. The absorbance was measured at 532 nm relative to the controls.

Statistical analysis

The results were expressed as mean ± standard deviation (SD) of four independent experiments performed in triplicate and were analyzed by appropriate analysis of variance (ANOVA), followed by Duncan's multiple range test. Differences between groups were considered significant when $p < 0.05$.

RESULTS

Antioxidant contents of *Garcinia kola*

The total phenols, flavonoids and vitamin C contents of extracts are shown in Table 1. The phenolic content of *G. kola* was estimated to be 45.2 ± 0.9 mg/g (GAE) for ethanolic and 30.3 ± 0.4 mg/g (GAE) for aqueous extract whereas the flavonoid content was 29.2 ± 0.2 mg/g and 10.8 ± 0.2 mg/g (QE) for aqueous extracts. In addition, the vitamin C contents were 21.2 ± 0.4 mg/g and 20.8 ± 0.1 mg/g for ethanolic and aqueous extract of dried *G. kola*, respectively.

Antioxidant mechanisms of *Garcinia kola*

In order to explore the mechanisms involved in the

antioxidant properties of *G. kola*, several *in vitro* antioxidant parameters such as reducing property, metal chelating ability, free radical scavenging properties and inhibitory effect on lipid peroxidation were employed. Generally, *G. kola* exhibited potent antioxidant action in a concentration dependent manner. However, ethanolic extract of *G. kola* demonstrated better antioxidant properties than aqueous extracts in all parameters determined.

DISCUSSION

Interest in medicinal plants has been revived in recent times because of their efficacy in providing cost effective therapy to several diseases due to the presence of secondary metabolites in their body parts. These compounds otherwise known as phytochemicals have been found to be responsible for the antioxidant properties of plants. Of paramount importance are the polyphenols which have been reported to exhibit anticancer, antimicrobial, antiplasmodic, anti-inflammatory, antiulcer, antidiabetic and antihypertensive properties (Balch and Balchi, 2000; Jisika et al., 1992). Interestingly, medicinal plants are rich in these polyphenols which make them a versatile tool for the treatment of ailments in folkloric medicine. Meanwhile, *G. kola* has been reported to exhibit a number of health related benefits that could be harnessed in the management of degenerative disorders (Olaleye et al., 2000). Generally, ethanol and water are commonly used in folkloric medicine for the extraction of plants phytochemicals without any knowledge of their effect on the bioactive constituents of these plants. Hence, the effects of ethanol and water on the phytochemical contents and antioxidant properties of *G. kola* are being investigated using *in vitro* antioxidant parameters.

The phytochemical contents of the extracts of the seeds of *G. kola* shows that it is rich in phenolic acids, flavonoids and vitamin C (Table 1). However, noteworthy is the fact that the phytochemical content of the ethanolic extract was significantly higher than that of the aqueous extract ($P < 0.05$). One rational explanation to this observation is that the solubility of phytochemicals differs depending on the solvent of extraction. As a result, some metabolites which are sparingly soluble in water but

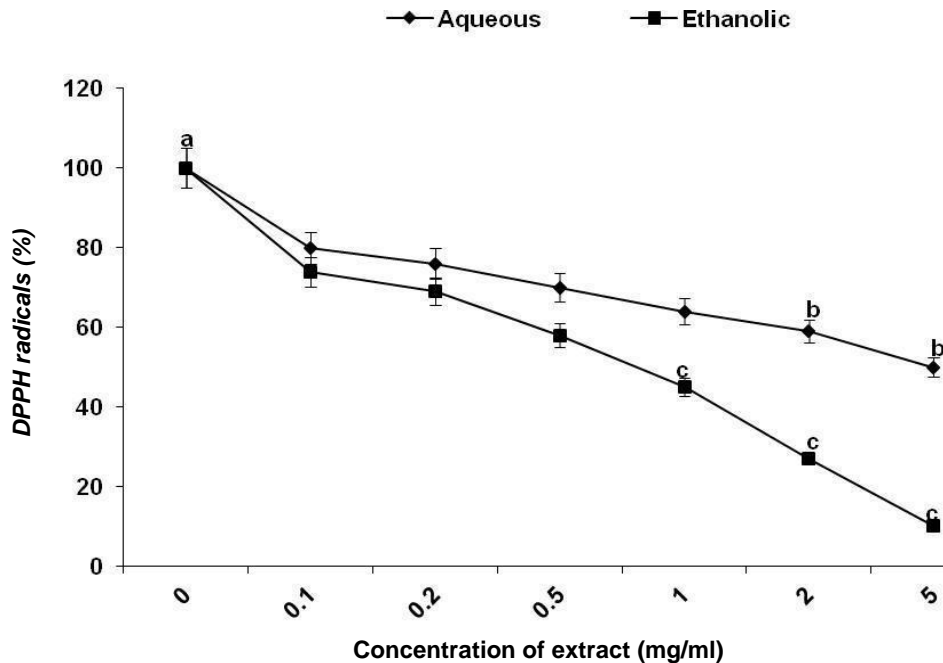


Figure 1. Free radical scavenging abilities of the aqueous and ethanolic extracts of *G. kola*. Data show means \pm SEM values averages from 3 to 4 independent experiments performed in triplicate. 'b' and 'c' indicate a significant difference from the control 'a' at $p < 0.05$.

completely soluble in ethanol would be extracted more in ethanol than in water. For instance, quercetin is insoluble in water but soluble in ethanol (Omololu et al., 2011). Hence, the ethanolic extract of such plants would contain a higher phytochemical content than aqueous extract due to the difference in solubility. This may partly explain why the ethanolic extract contained a significantly ($P < 0.05$) higher phenolic and flavonoid content than aqueous extract. Having unraveled the possible reason for the observed difference in the phytochemical contents in the two extracts of *G. kola*, it is equally important to identify the possible mechanisms involved in its antioxidant activities.

Radical scavenging activity is very crucial to the survival of living organisms, due to the deleterious role of free radicals in foods and biological systems. Routinely, antioxidant potencies of plants are assessed by their ability to scavenge DPPH, an unstable compound which turns to a stable diamagnetic molecule when protonated. This stability is visually noticeable as a discoloration from purple to golden yellow. Interestingly, *G. kola* extracts demonstrated a marked radical scavenging activity (Figure 1). However, the ethanolic extract shows a significantly higher radical scavenging effect than the aqueous extract. Although, the reason behind this observation is still not completely understood, it could be attributed to the difference in the degree of solubility of the bioactive ingredients in the two solvents which guaranteed the extraction of more phytochemicals in the ethanolic than in the aqueous extract. Meanwhile, these phytochemicals

are responsible for the antioxidant properties of plants. For instance, phenolics and flavonoids are commonly known to exhibit anti-allergic, anti-inflammatory, anti-microbial and anticancer activity (Balch and Balchi, 2000; Jisika et al., 1992). From the foregoing, it would be rational to expect that the extract with a higher phytochemical content would exhibit a stronger antioxidant effect.

Moreover, antioxidants can act by chelating transition metals. Interestingly, Figure 2 shows that although both extracts exhibited Fe (II) chelating effect, the ethanolic extract demonstrated a higher ($P < 0.05$) Fe (II) chelating activity than the aqueous extract. This may be related to the quantity of phenolics and flavonoids in the ethanolic extract since earlier reports have indicated that polyphenols exhibit potent iron chelating ability (Omololu et al., 2011). Hence, the increased amount of these polyphenols in the ethanolic extract must have conferred it with a more potent iron chelating property than the aqueous extract.

Besides, antioxidants could reduce and deactivate transition metals (in this case the reduction of Fe^{3+} to Fe^{2+}). Figure 3 shows that the ethanolic extract demonstrated potent ferric reducing power than the aqueous extract. This observation could be attributed to the difference in the levels of the phytochemicals in the extracts which are responsible for metal reduction probably due to their highly nucleophilic nature that enables them to readily donate proton to electron deficient centers to cause reduction.

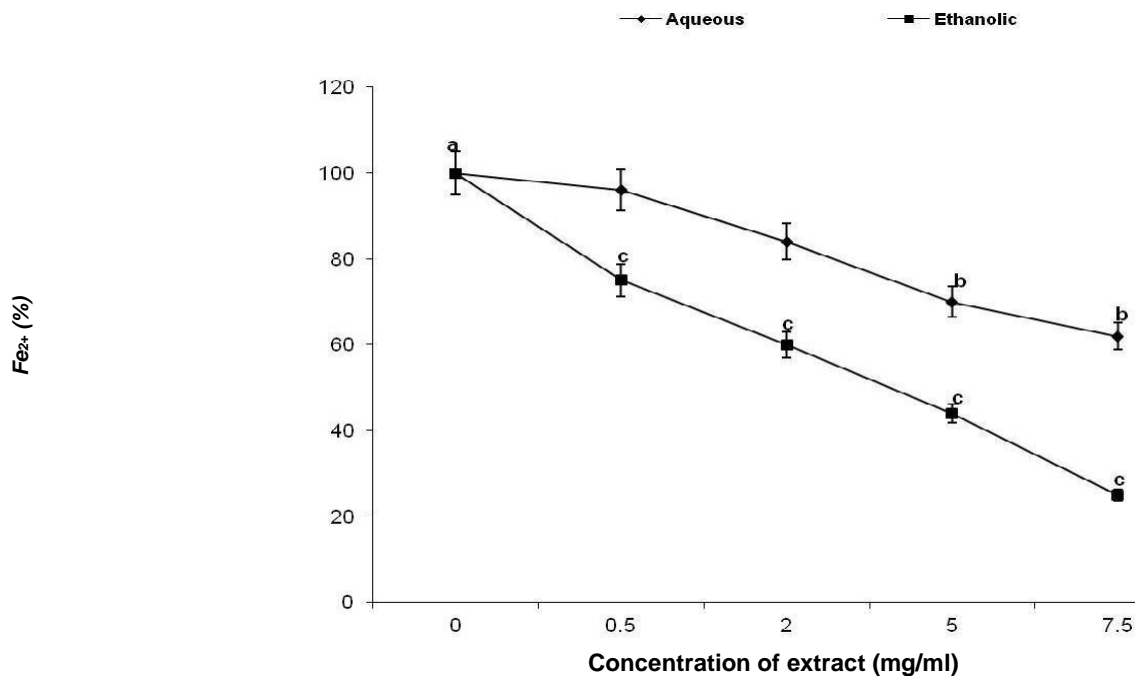


Figure 2. Fe²⁺ - chelating properties of the aqueous and ethanolic extracts of *G. kola*. Data show means \pm SEM values averages from 3 to 4 independent experiments performed in triplicate. 'b' and 'c' indicate a significant difference from the control 'a' at $p < 0.05$.

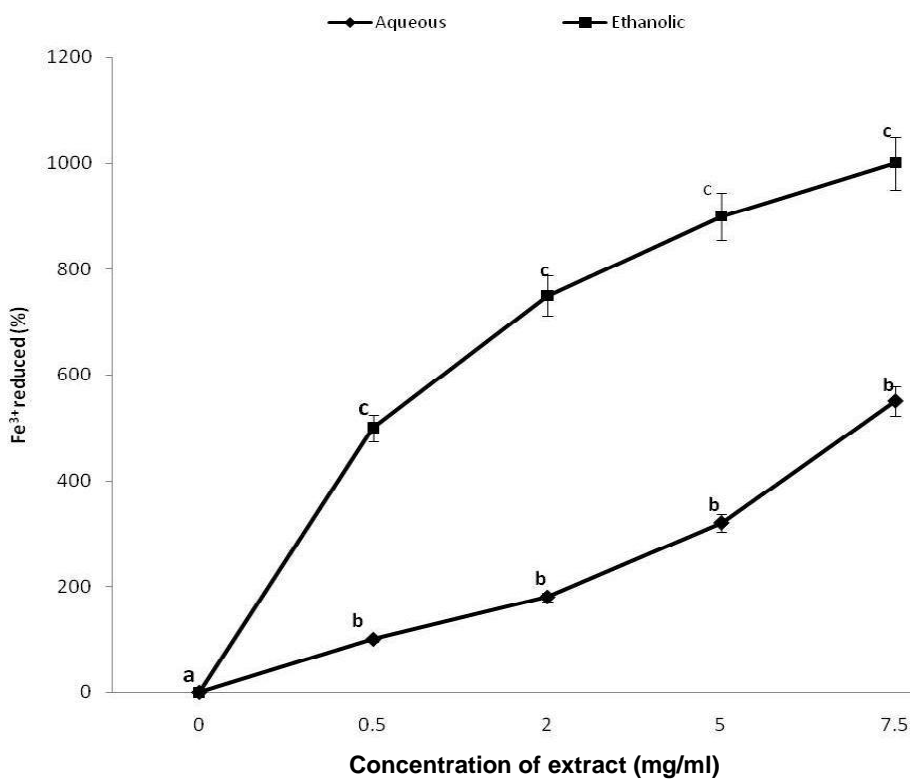


Figure 3. Ferric reducing properties of the aqueous and ethanolic extracts of *G. kola*. Data show means \pm SEM values averages from 3 to 4 independent experiments performed in triplicate. 'b' and 'c' indicate a significant difference from the control 'a' at $p < 0.05$.

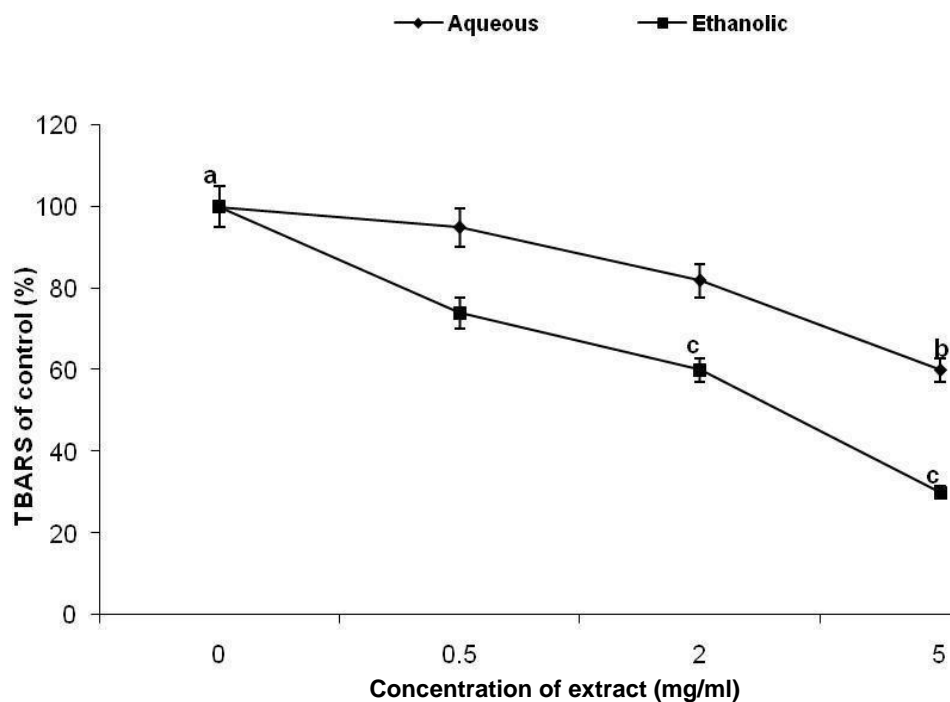


Figure 4a. Inhibitory effects of the aqueous and ethanolic extracts of *G. kola* on Fe^{2+} -induced lipid peroxidation in rat liver. Data show means \pm SEM values averages from 3 to 4 independent experiments performed in triplicate. 'b' and 'c' indicate a significant difference from the control 'a' at $p < 0.05$.

Furthermore, free radicals can attack lipids via an oxidative degeneration process known as lipid peroxidation. The reaction involves the formation of aldehydic compounds which react with TBA to produce a coloured product that could be measured spectrophotometrically. Hence, antioxidants could be assessed based on their ability to prevent the formation of TBARS in a lipid peroxidation process. One major prooxidant commonly employed for oxidative assault on lipids is Fe (II) which has been reported to be detrimental to biological macromolecules by reacting with superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) to produce

the hydroxyl radical (OH^\bullet) via Fenton reaction (Graf et al., 1984) and other reactive oxygen species (ROS) (Klebanoff et al., 1992). Interestingly, Figures 4a and b, respectively shows that *G. kola* extract exhibited marked

inhibitory effect against Fe^{2+} -induced cerebral and hepatic lipid peroxidation. However, the ethanolic extract demonstrated a stronger inhibitory effect probably due to its higher phenolics and flavonoids content which could offer protection to lipids via its potent iron (II) chelating property thereby preventing the generation of ROS and inhibiting oxidative assault in the process.

Besides, SNP have been reported to elicit its cytotoxic effect through the release of cyanide and/or nitric oxide (NO) (Rauhala et al., 1998) both of which have been implicated in the pathophysiology of strokes, traumas, seizures and Alzheimer's, and Parkinson's diseases

(Castill et al., 2000; Prast and Philippou, 2001). In addition, NO can be cleaved photolytically from SNP (Arnold et al., 1984; Singh et al., 1995), leading to generation of reactive intermediates like NO and $[\text{NO}-\text{Fe}(\text{CN})_5]^{2-}$ which can be converted to iron containing $[(\text{CN})_5-\text{Fe}]^{3-}$ and $[(\text{CN})_4-\text{Fe}]^{2-}$ species (Loiacono and Beart, 1992). In fact, after the release of NO, the iron moiety may react with SNP, which could lead to the formation of highly reactive oxygen species, such as hydroxyl radicals via the Fenton chemistry (Graf et al., 1984). The fact that *G. kola* extracts inhibited SNP-induced lipid peroxidation (Figures 5a and b) may possibly suggest that the extracts possibly prevented the breakdown of SNP to its reactive intermediates thereby offering protective shield to both cerebral and hepatic tissues.

Conclusion

Keeping in mind, one could rationally attribute the antioxidant properties of *G. kola* to its phenolic and flavonoid content. Moreover, the present study suggested that ethanol is a better solvent for the full exploitation of the therapeutic potentials of *G. kola* since it exhibited higher antioxidant properties than the aqueous extract. Hence, *G. kola* is a potential antioxidant candidate that could help in the management of degenerative diseases

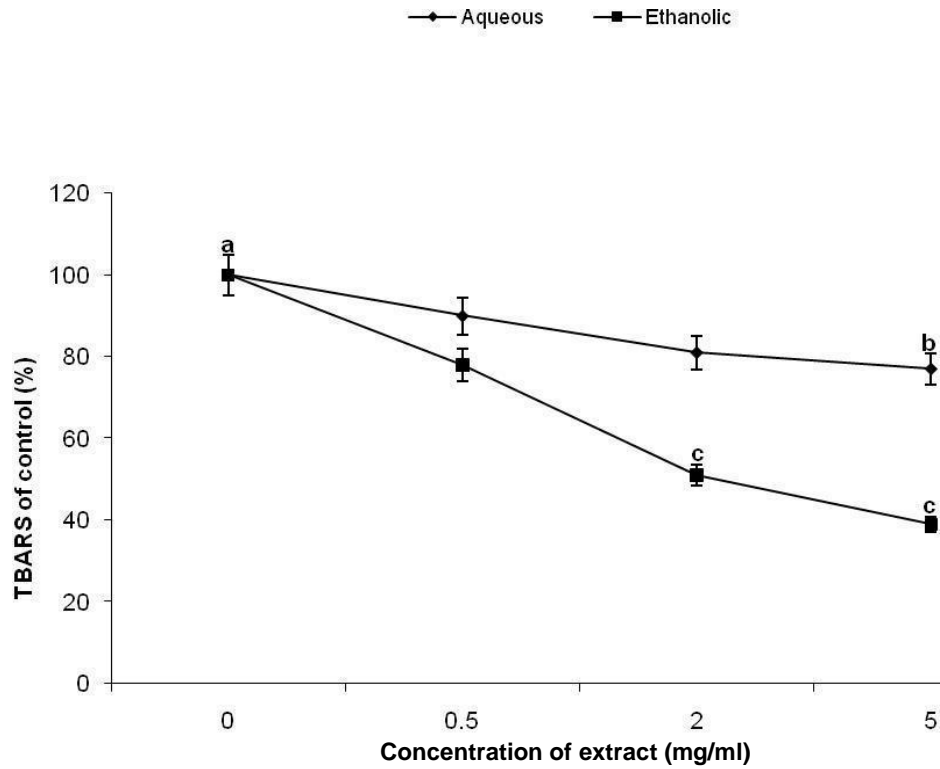


Figure 4b. Inhibitory effects of the aqueous and ethanolic extracts of *G. kola* on Fe²⁺-induced lipid peroxidation in rat brain. Data show means \pm SEM values averages from 3 to 4 independent experiments performed in triplicate. 'b' and 'c' indicate a significant difference from the control 'a' at $p < 0.05$.

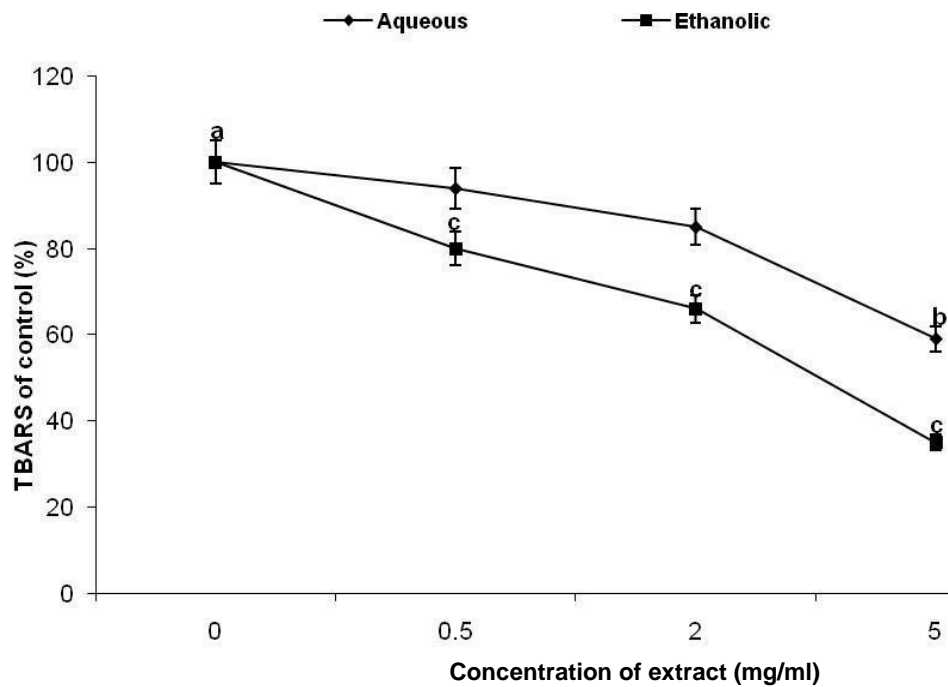


Figure 5a. Inhibitory effects of the aqueous and ethanolic extracts of *G. kola* on SNP - induced lipid peroxidation in rat liver. Data show means \pm SEM values averages from 3 to 4 independent experiments performed in triplicate. 'b' and 'c' indicate a significant difference from the control 'a' at $p < 0.05$.

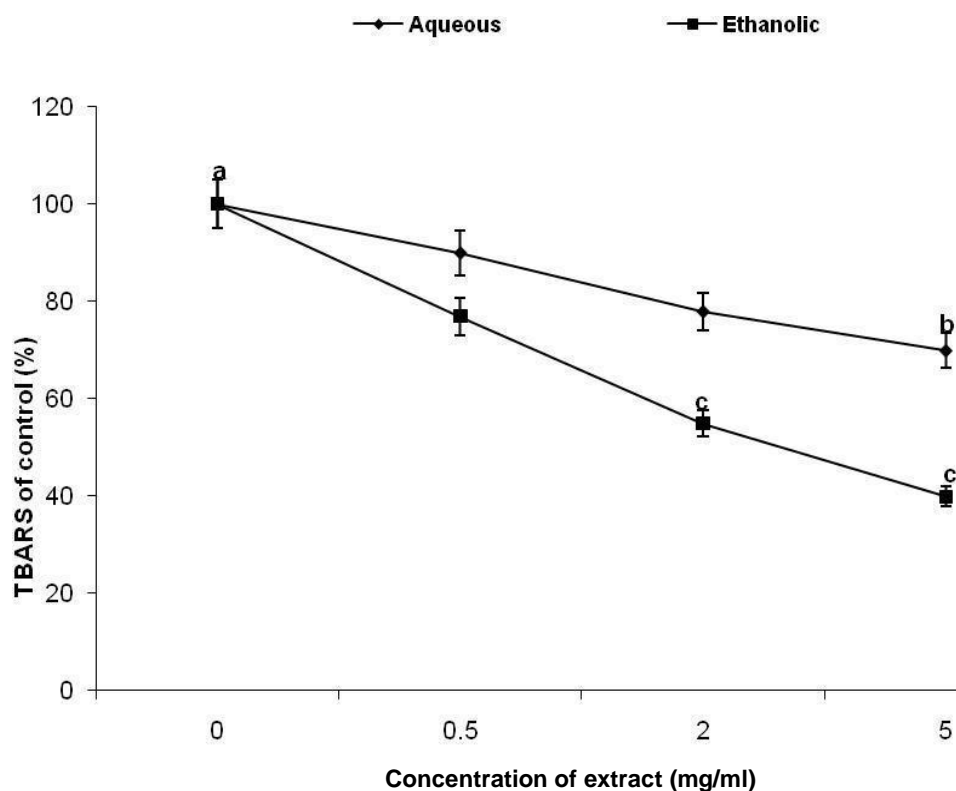


Figure 5b. Inhibitory effects of the aqueous and ethanolic extracts of *G. kola* on SNP - induced lipid peroxidation in rat brain. Data show means \pm SEM values averages from 3 to 4 independent experiments performed in triplicate. 'b' and 'c' indicate a significant difference from the control 'a' at $p < 0.05$.

whose etiology have been linked to oxidative stress.

ACKNOWLEDGMENTS

The authors are grateful for the unflinching support of Prof. JBT Rocha, Brazil. Also the work was partly funded by the DTLC, granted to Biochemistry Department, Federal University of Technology, Akure, Nigeria.

REFERENCES

- Akintonwa A, Essien AR (1990). Protective Effect of *Garcinia kola* seed extract against Paracetamol-induced hepatotoxicity in rats. *J. Ethnopharmacol.* 29: 207-211.
- Arnold WP, Longnecker DE, Epstein RM (1984). Photodegradation of sodium nitroprusside: biologic activity and cyanide release. *Anesthesiology*, 61: 254-260.
- Balch JF, Balch PA (2000). Prescription for Nutritional Healing. New York: A very, Penguin Putnam Inc. pp. 267-270.
- Castill J, Rama R, Davalos A (2000). Nitric oxide-related brain damage in acute ischemic stroke. *Stroke*, 31: 852-857.
- Egunyomi A, Moody JO, Eletu OM (2009). Antisickling activities of two ethnomedicinal plant recipes used for the management of sickle cell anaemia in Ibadan. *Nig. Afr. J. Biotechnol.* 8:20-25.
- Etkin NL (1981). A hausa herbal pharmacopoeia: Biomedical evaluation of commonly used plant medicines. *J. Ethnopharmacol.* 4:75-98.
- Farombi EO, Adepoju BF, Ola-Davies OE, Emerole GO (2005). Chemoprevention of aflatoxin B1- induced genotoxicity and hepatic oxidative damage in rats by kolaviron, a natural bioflavonoid of *Garcinia kola* seeds. *Eur. Cancer J. Prev.* 14:207-214.
- Farombi EO, Nwankwo JO, Emerole GO (2003). Evaluation of the antioxidant and partial characterization of extracts from browned yam flour. *Food Res. Int.* 6:33.
- Graf E, Mahoney JR, Bryant RG, Eaton JW (1984). Iron catalyzed hydroxyl radical formation: stringent requirement for free iron coordination site. *J. Biol. Chem.* 259:3620-3624.
- Gyamfi MA, Yonamine M, Aniya Y (1999). Free-radical scavenging action of medicinal herbs from Ghana: *Thonningia sanguine* on experimentally-induced liver injuries. *Gen. Pharmacol.* 32:661-667.
- Iwu MM, Igboko O (1982). Flavonoids of *Garcinia kola* seeds. *J. Natural. Prod.* 45:650-651.
- Jacques-Silva MC, Nogueira CW, Broch LC, Flores EM, Rocha JBT (2001). Diphenyl diselenide and ascorbic acid changes deposition of selenium and ascorbic acid in liver and brain of mice. *Pharmacol. Toxicol.* 88:119-127.
- Jisika M, Ohigashi H, Nogaka H, Tada T, Hirota M (1992). Bitter steroid glycosides, Vernon sides A1, A2, and A3 and related B1 from the possible medicinal plant *vernonia amygdalina* used by wild Chimp. *Tetrahedron* 48:625-630.
- Kabangu L, Galeffi C, Aonzo E, Nicoletti M, Messana I (1987). New biflavone from the bark of *G. Kola*. *Planta Medica* 11:275 - 277.
- Klebanoff SJ, Gally JI, Goldstein IM, Snyderman R (1992). Oxygen metabolites from phagocytes. Raven Pres, New York pp. 541-588.
- Loiacono RE, Beart PM (1992). Hippocampal-lesions induced by microinjection of the nitric-oxide donor nitroprusside. *Eur. J. Pharmacol.* 216:331-333.
- Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG (2005).

- Determination of the total phenolic, flavonoid and praline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chem.* 91:571-577.
- Ncube NS, Afolayan AJ, Okoh AI (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *Afr. J. Biotechnol.* 7(12):1797-1806.
- Odeigah PG, Taiwo IO, Akomolafe EO, Durojaiye OO (1999). Hypoglycemic action of medicinal plants with tolbutamide in the Albino rats. *Diabetes Int.* 9:71-73.
- Ohkawa H, Ohishi H, Yagi K (1979). Assay for lipid peroxide in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* 95:351-358.
- Olaleye SB, Farombi EO, Adewoye EA, Owoyele VB, Onuasanwo SA (2000). Analgesic and anti inflammatory effect of kolaviron (*A. G. kola* seed extract). *Afr. J. Biomed. Sci.* 3(3):171-174.
- Omololu PA, Rocha JBT, Kade IJ (2011). Attachment of rhamnosyl glucoside on quercetin confers potent iron-chelating ability on its antioxidant properties *Exp. Toxicol. Pathol.* 63(3):249-245.
- Prast H, Philippou A (2001). Nitric oxide as modulator of neuronal function. *Prog. Neurobiol.* 64:51-68.
- Pulido R, Bravo L, Saura-Calixto F (2000). Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *J. Agric. Food. Chem.* 48:3396-3402.
- Puntel RL, Nogueira CW, Rocha JBT (2005). Krebs cycle intermediates modulate thiobarbituric acid reactive species (TBARS) production in rat brain *in vitro*. *Neurochem. Res.* 30:225-235.
- Rauhala P, Khaldil A, Mohanakumar KP, Chiueh CC (1998). Apparent role of hydroxyl radicals in oxidative brain injury induced by sodium nitroprusside. *Free Radic. Biol. Med.* 24:1065-1073.
- Singh RJ, Hogg N, Neese F, Joseph J, Kalyanaraman B (1995). Trapping of nitric oxide formed during photolysis of sodium nitroprusside in aqueous and lipid phases: an electron spin resonance study. *Photochem. Photobiol.* 61:325-330.
- Singleton VL, Orthofer R, Lamuela-Raventos RM (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu's 299:152-170.