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ANTIOXIDANT POTENTIAL OF *GARCINIA KOLA* (LEAF)

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ABSTRACT

Garcinia kola leaves have long been regarded as pharmacologically potent plant in folk medicine for their medicinal properties in tropical Africa. . In the present study, the ability of ethanolic extract of Garcinia kola leaves at concentrations (3.3-40µg/ml) to prevent 60µM Fe2+ induced lipid peroxidation in rat brain and liver homogenate was assessed using Thiobarbituric acid reactive substance assay (TBARS) invitro . Fe2+ chelating ability of the extract was a;so determined. (1mM FeSO4). The inhibitory effect of Garcinia kola leaves on lipid peroxidation in both liver and brain homogenate and the iron chelating activity were concentration - dependent exhibiting an antioxidant activity against free radicals. The extract showed its highest inhibition at the same concentration (26.7µg/ml) in both liver and brain homogenate with %inhibition of 64.1% and 38.2% respectively. Therefore, the leaves of the plant could be considered to have significant natural antioxidant activity against the initiation of some prevalent diseases.

Keywords: Lipid peroxidation Thiobarbituric acid reactive species Garcinia Kola

INTRODUCTION

Lipid peroxidation is a crucial step in the pathogenesis of several diseases states in adult and infant patients. Reactive oxygen species (ROS) are generated spontaneously in cells during metabolism and are implicated in the aeitology of different degenerative diseases, such as heart diseases, stroke, rheumatoid arthritis, diabetes and cancer (Halliwell, Gutteridge, &Cross, 1992). Consequently, there is a great deal of interest in edible plants that contain antioxidants and health-promoting phytochemicals as potential therapeutic agents. One such plant is *Garcinia kola. Garcinia kola,* generally known as Bitter kola in Nigeria belongs to the family of tropical plants known as *Guttiferae.* Other common English names are Bitter cola, False kola, Garcinia or male kola. The plant is extensively used in herbal medicine and as food, usually found in tropical rain forest region. It prevails as multipurpose tree crops in the home gardens of southern Nigeria (Nzegbule and Mbakwe, 2001).The plant grows as a medium size tree, up to 12-14m high and produces reddish yellowish or orange coloured fruit (Okwu, 2005; Adesanya et al., 2007). Each fruit contains 2-4 yellow seeds and a sour tasting pulp. The seeds when chewed have a bitter astringent taste. *Garcinia kola,* from the family of *Guttiferae,* which is highly valued in Nigeria because of it's edible nut, is a plant that exhibits very potent pharmacological activities such as antioxidant, antibacterial, antifungal, antiviral, anti-inflammatory properties (Adegboye et al., 2008 ; Okwu and Ekeke, 2005 ; Mackeen et al., 2002 ; Iwu et al., 1999 ; Chen and Kang, 1997 ; Iwu , 1993). Phytochemistry of *Garcinia kola* have shown its content to include; prenylated benzophenone, xanthones, biflavonoids (Tereshima et al., 1999) also containing a complex mixture of alkaloid, phenols and tannins (Okwu, 2005). The potency of *Garcinia kola* is revealed because of its phytochemial properties. It is thus referred to as wonder plant because every part of it has been found to be of medicinal importance.

Antioxidants terminate chain reactions in lipid perodixation, by removing free radical intermediates, and inhibit other oxidation reactions. The body's internal production of antioxidants is not enough to neutralize all the free radical, this can be helped by increasing dietary intake of antioxidants in the hope of maintaining health and preventing diseases. The aim of this study is to determine the

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antioxidant potential of *Garcinia kola* leaves using pro-oxidant Iron II sulphate, hence Iron induced lipid peroxidation.

MATERIALS AND METHODS

Plant material

Garcinia kola leaves were collected and authenticated by a taxonomist Mr. F.O Omotayo. Voucher specimens were deposited in the herbarium of the Faculty of Science, University of Ado-Ekiti, Nigeria with Herbarium code number: UHAE 539. The leaves were separated from the other parts of the plants, dried at room temperature and powdered. The powdered Garcinia kola leaves were extracted with cold 70% ethanol at room temperature (270C) for 48hrs. The filtrate (Ethanolic extract) was evaporated to dryness using a rotary evaporator giving a percent yield of 4.5%. Serial dilutions of these were made to obtain the desired concentration of plant for the experiment.

Test animals

All animal procedures were in strict accordance with the NIH Guide for the care and use of laboratory animals. Two to three month old wistar rats (200-250g) were used for the in vitro studies.

Preparation of Thiobarbituric acid Reactive Substance (tbars)

Production of Thiobarbituric acid reactive substance was determined using a method of Ohkawa et al., (1979) as described by Puntel et al., (2005). The rats were killed by cervical dislocation. Liver and brain tissues were quickly removed and placed on ice. One gram of tissues was homogenized in cold 0.1M Tris-buffer pH7.4 (1:10 w/v) up and down in a Teflon homogenizer. The homogenates were centrifuged for 10min at 3000g to yield a pellet that was discarded and the supernatant was used for the assay.

The supernatant (100µl) with or without 50µl of the freshly prepared pro- oxidant (Iron II Sulphate), different concentrations of the plant extracts, and an appropriate volume of distilled water which gives a total volume of 300µl were incubated at 370C for 1hr. The colour reaction was carried out by adding 200, 500 and 500µl each of the 8.1% Sodium dodecyl sulphate(SDS), 1.33M acetic acid (PH 3.4) and 0.6% TBA respectively. The reaction mixtures, including those of serial dilutions of 0.03mM standard MDA, were incubated at 970C for 1hr. The absorbance was read after cooling at a wavelength of 532nm in a UV/VIS spectrometer.

Iron Chelation Assay

The ability of the ethanolic extract to chelate Fe (II) was determined using modified method of Puntel et al; (2005). Briefly 20µl of freshly was prepared and1mM FeSO4 were added to a reaction mixture containing 168µl of 0.1M Tris-Hcl (pH 7.4), 218µl saline (0.9%NaCl) and the ethanolic extract of Garcinia kola (1-100µg/ml). The reaction mixture was incubated for 5min, before the addition of 13µl of 0.25% 1,10 phenanthroline (w/v). The absorbance was subsequently measured at λ 510nm in the spectrophotometer.

STATISTICAL ANALYSIS

Data were analysed statistically by one way ANOVA, followed by Duncan's multiple range test when appropriate.

RESULTS AND DISCUSSION

Several methods have been used to determine antioxidant activity of plants. This present study therefore involves two established methods to evaluate antioxidant activity of Bitter kola leaves namely Thiobarbituric Acid Reactive Substance Assay (TBARS) and Iron Chelation Assay. There is a strong correlation between TBARS (Thiobarbituric acid reactive substance) as a marker in lipid peroxidation and products that reflect oxidative damage to DNA (Chen et al., 2005) Iron overload results in the formation of lipid peroxidation products, which have been demonstrated in a number of tissues including the liver, brain and kidneys (Houglum et al., 1990; Oboh et al.,2007) Storage of iron in the liver leads to liver cirrhosis, rats overloaded with iron showed toxic effects such as heptocellular

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hypertrophy, cardiomyopathy etc (Whittaker et al., 1997). The possible mechanisms of iron toxicity include free radical mediated peroxidative reactions which are readily catalyzed by Iron. The protection offered by the ethanolic extracts of Garcinia Kola suggest that it may be useful in the treatment of liver and brain diseases resulting from iron overload.

Table 1. The inhibitory effect of ethanolic extract of *Garcinia kola* onIron II sulphate induced lipid peroxidation in a rat liver homogenate.

Concentration µg/ml	nMol MDA/mg tissue liver	% Inhibition	Logarithm Equation (r^2)	IC_{50}
Basal	0.067±0.03[a]	…….	Y= 11.09In (x) + 33.43 R^2=0.414	4.9±5.83
Control	0.275±0.25[b]	…….		
Solvent	0.242±0.28	-12.9±6.8		
3.3	0.180±0.18	37.8±5.9		
6.6	0.216±0.24	31.4±18.2		
13.3	0.190±0.22	41.8±18.5		
26.7	0.138±0.19	64.1±24.0		
33.3	0.160±0.17	47.5±10.4		
40	0.070±0.04	51.0±38.6		

The results are expressed as means of three experiments in duplicate ± standard deviation

Table 2. The inhibitory effect of ethanolic extract of *Garcinia kola* onIron II sulphate induced lipid peroxidation in a rat brain homogenate.

Concentration (µg/ml)	nMol MDA/mg tissue liver	% Inhibition	Logarithm Equation (r^2)	IC_{50}
Basal	0.079±0.01[a]	.....	Y=3.477In(x) +23.65 R^2=0.068	19.5±26.3
Control	0.243±0.24[b]	.....		
Solvent	0.203±0.22	-4.4±2.94		
3.3	0.210±0.27	2.69±22.8		
6.6	0.214±0.22	18.9±10.6		
13.3	0.188±0.21	28.1±15.6		
26.7	0.168±0.19	38.2±11.3		
33.3	0.195±0.18	16.5±19.8		
40	0.150±0.15	36.2±39.9		

The results are expressed as means of three experiments in duplicate ± standard deviation

The inhibitory effect of ethanolic extract of Garcinia kola on Fe2+ induced lipid peroxidation in both brain and liver, could be attributed to the presence of antioxidant phytochemicals such as flavonoids. Increase in the formation of TBARS in Iron II Sulphate (60µM) induced lipid peroxidation as compared to the control, suggests possible damage of tissues with an overload of iron.

The results in Table1&2 clearly show that incubation of rat liver and brain in the presence of pro-oxidant Fe2+ caused a significant difference increase in the malonaldehyde (MDA) contents of the rat's liver and brain when compared with control. The increased lipid peroxidation in the presence of Fe2+ could be attributed to the fact that Fe2+ can catalyze one-electron transfer reaction that generate reactive oxygen species, such as OH*, this is formed from H2O2 through Fenton reaction. Iron also decomposes lipid peroxide, thus generating peroxyl and alkoxyl radicals which favour the propagation of lipid oxidation (Zago et al, 2000). Iron overload is a less frequent condition, but high content of tissue iron has been associated with several pathological condition including liver and heart disease (Milman et al., 2001) and neurodegenerative disorders (Berg et al., 2001).

However, extract from Garcinia kola caused a significant decrease in the liver and brain malonaldehyde (MDA) levels, during the Fe2+ induced lipid peroxidation in rat liver and brain tissues.

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(Table 1&2) Ethanolic extract of Garcinia kola had higher inhibitory effect on Fe2+ induced lipid peroxidation in the liver than the brain homogenate. The decrease in the Fe2+ induced lipid peroxidation in rat liver and brain homogenates in the presence of extracts could be as a result of the ability of the antioxidant phytochemicals in extracts to chelate Fe2+ and scavenge free radicals produced by the Fe2+ catalysed product of Reactive Oxygen species (ROS) in the rat brain and liver homogenates.

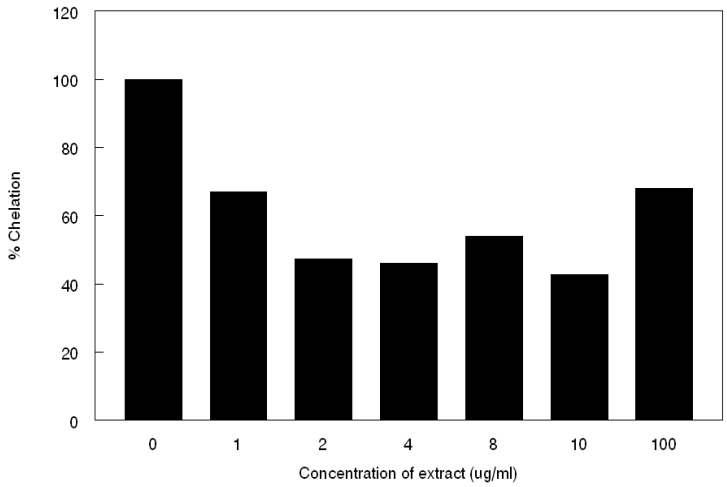

Fig. 1 Iron chelating ability of Ethanolic Extract of *Garcinia kola* leaf

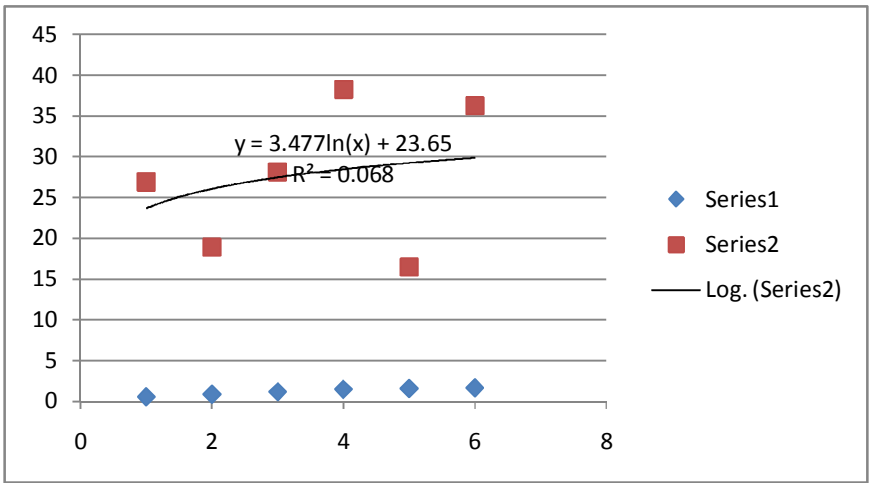

Fig. 2. A graph showing regression equation for the inhibitory effect of the ethanolic extract of *Garcinia kola* leaves on Fe^{2+} induced lipid peroxidation in rat brain homogenate.

The inhibitory effects of the ethanolic extracts from Garcinia kola leaves on 60µM Iron (Fe2+) induced lipid peroxidation at concentrations within the range of 3.3 – 40µg/ml in comparison to the control is showed in Table1&.2. When different concentrations of extracts were added, a significant concentration – dependent inhibition of lipid peroxidation occurred, each extract concentration showed a mild antioxidant activity in inhibiting Fe2+ induced lipid peroxidation. At a concentration of 26.7µg/ml, it is worth noting that there was an agreement between the percentage(%) inhibition of Fe2+ peroxidation with a value of 38.2% and 64.1% in the rat brain and liver tissue respectively, thus reflecting the highest inhibitory effect in both tissues. The minimum inhibitory effect occurred at

6.6µg/ml in the rat liver and brain homogenate and the maximum at 26.7µg/ml, but at the concentration above this, there is a decreased inhibitory effect on lipid peroxidation. The IC50 of Garcinia kola leaf extract on liver homogenate is 4.9 ± 5.8 and in the brain homogenate 19.5 ± 26.3. Antioxidant activity of Garcinia kola leaf was attributed to different compounds like flavonoids (Terashima et al., 2002), phenols, Vitamin C etc.

Ethanolic extracts were assessed for their ability to compete with ferrozine for Fe2+ ions in free solution. In other to provide an explanation for the inhibition of Fe2+ induced lipid peroxidation in rat brain and liver tissue, the Fe2+ chelating ability of the Garcinia kola leaves were determined and the results were shown in Fig 1. All extract concentration demonstrated an ability to chelate 1mM FeSO4 in a concentration-dependent manner. Ethanolic extract of Garcinia kola leaves showed a mild chelating ability of 63.3 ± 9.12% at a concentration of 100µg/ml, but at a concentration of 10µg/ml, the iron chelating ability of Garcinia kola on Fe2+ induced lipid peroxidation is at its minimum of 42.8±1.8%, implying that the chelating ability of Garcinia kola leaves increases as the concentration of the extract increases.

Natural antioxidants present in plants are closely related in their medicinal properties. The antioxidant properties of plant extracts should be evaluated in a variety of model system using several indices to ensure the effectiveness of such plant material. Antioxidants capacity is a widely used parameter for assessing medicinal values of plant, in the treatment of various diseases and this capacity was exhibited by Garcinia kola leaves, hence it can be used in the treatment of various disease associated with the generation of free radicals.

CONCLUSION

In conclusion, the results of this study demonstrates the efficacy of the ethanolic extract of Garcinia kola leaves in the inhibition of Fe2+ induced lipid peroxidation due to its iron chelating ability and this may be associated with its high medicinal use in the treatment of different diseases effectively. Hence, Garcinia kola leaves can be used as an accessible source of natural antioxidants. Further scientific research is recommended to ensure the medicinal properties of the plant in vitro correlate with its antioxidant activity.

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