

## Full Length Research Paper

## Comparative studies on the possible antioxidant properties of ethanolic seed extracts of *Cola nitida* (kola nut) and *Garcinia kola* (bitter kola) on hydrogen peroxide induced oxidative stress in rats

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Oxidative stress and impaired antioxidant system have been implicated in the pathophysiology of diverse disease states. This research was done to investigate comparatively the possible enzymatic and non-enzymatic antioxidant properties of ethanol extracts of *Cola nitida* and *Garcinia kola* in H<sub>2</sub>O<sub>2</sub>-challenged rats. Thirty (30) Wistar albino rats were used for this study and were divided into 6 groups of 5 rats per group. Group 1 rats were normal control; group 2 rats were induced with H<sub>2</sub>O<sub>2</sub> only (positive control). Group 3 and 4 were challenged with H<sub>2</sub>O<sub>2</sub> and treated with 100 and 200 mg/kg b.w of ethanol extract of *C. nitida*, respectively. In the same vein, groups 5 and 6 rats represented H<sub>2</sub>O<sub>2</sub>-induced rats treated with 100 and 200 mg/kg b.w of ethanol extract of *G. kola* seed respectively. Group 2 (positive control group) rats showed a significant increase ( $p < 0.05$ ) in malondialdehyde (MDA) concentration compared to rats in group 1 and the treatment groups. Conversely there was a significant decrease ( $p < 0.05$ ) in glutathione concentration of the group when compared to rats in group 2. Group 2 showed a significant decrease ( $p < 0.05$ ) in vitamin C concentration compared to rats in group 1 and the treatment groups. The effects of extracts were accompanied by a significant increase ( $p < 0.05$ ) in the activity of endogenous antioxidant enzymes such as superoxide dismutase (SOD) when compared to the group 2 (positive control group). The two extracts exhibited a significant ferric reducing antioxidant properties (FRAP) in a concentration-dependent manner. This finding indicated that the extracts could contain antioxidant and thus have potential for scavenging free radicals, hence arresting oxidative stress. This may justify their local use in management of some hepatic dysfunction and stress related conditions. However, extracts of *G. kola* was seen to be more potent than that of *C. nitida*.

**Key words:** Oxidative stress, antioxidant, *G. kola*, *C. nitida*.

### INTRODUCTION

Plants are important in our everyday existence. They provide our foods, produce the oxygen we breathe, and

serve as raw materials for many industrial products such as biofuels, dyes, perfumes, pesticides and drugs,

clothes, foot wears, etc. The use of plants in traditional medicinal practice has a long drawn history, and remains the mainstay of primary health care in most of the third world countries especially those living in the rural areas. Historically, plants have provided a source of inspiration for novel drug compounds, as plant-derived medicines have made large contributions to human health and well-being. Traditional medicines are used by about 60% of the world population; in both developing and developed countries where modern medicines are predominantly used (Ogbonnia et al., 2011). Some of the plants include garlic, ginger, pepper, egg plants, *C. nitida*, *G. kola* and many others of which have been investigated. *C. nitida* is known as "kola nut" while *G. kola* is commonly called "bitter kola" in Nigeria. However quite a number of these plant materials mentioned above have antimicrobial and antioxidant properties with tremendous therapeutic potentials (Moon et al., 2010).

*G. kola* has been found to contain Garcinia biflavonones (GB -1, GB-2), kolaflavonone, benzophenone and xanthenes and studies have also shown the ability of these compound in protecting against hepatotoxicity induced by phalloidin, amanita, 2-acetylaminofluorene, carbon tetrachloride, paracetamol, aflatoxin, dimethyl nitrosamine in rodents (Sanchez et al., 2009). *G. kola* also possesses other pharmacological properties like: Hepatoprotective effects (Wegwu and Didia, 2007), hypoglycemic and antioxidant properties (Omaga et al., 2011), antimicrobial effects (Antwi-Boasiako and Abubakari, 2011), antitrichomonal activity (Ibikunle and Ogbadoyi, 2011), and antioxidative and chemopreventive properties (Farombi and Owoye, 2011).

*C. nitida* are effective for refreshing the mouth due to their unique bitter taste, and the twigs are used as a source of alkaloids in pharmaceutical preparations (Oyedade, 1973). Traditionally, the leaves, flowers, twigs, fruit follicles, and bark of *C. nitida* are used to prepare a tonic as remedy for dysentery, coughs, diarrhea, vomiting and chest complaints (Atawodi et al., 2007). Extracts of *C. nitida* nuts has been tested on various pathogenic bacteria including *Staphylococcus aureus*, *Klebsilla pneumonia*, *Psuedomonas aeruginosa*, *Escherichia coli* (Ebana et al., 1991). All extracts showed inhibitory activity against these organisms. Ayebe et al. (2012) reports that *C. nitida* extract inhibited the release of leutinizing hormones (LH) from pituitary cells and may therefore regulate gonadotropin release. This has potential to be used as fertility regulator. Some of the potential medicinal properties of *cola nitida* includes; anticarcinogenic, antimicrobial and antidiabetic activities (Endrini et al., 2011).

Oxidative stress reflects an imbalance between the production of free radicals and the ability of the body to

counteract or detoxify their harmful effect through neutralization by antioxidants. Free radicals are generated in our body during the normal metabolic processes and during exposure to adverse pathophysiological conditions. They are unstable species that are able to induce cellular damage in several ways. Free radicals can adversely alter lipids, proteins and DNA and have been implicated in aging and a number of human diseases. However the most deleterious effect of free radical is damage to DNA, which is associated with the process of carcinogenesis. Antioxidants from plants scavenge free radicals and prevent reactive oxygen species from having damaging effects in common ailments such as inflammation, atherosclerosis, and Alzheimer's disease (Bahorun et al., 2006). This study is aimed at investigating comparatively the possible enzymatic and non-enzymatic antioxidant properties of ethanol extracts of *C. nitida* and *G. kola* in H<sub>2</sub>O<sub>2</sub>-challenged rats.

## MATERIALS AND METHODS

### Plant materials

Fresh seed plant of *C. nitida* and *G. kola* were collected from the University of Nigeria, Nsukka environ on the 7<sup>th</sup> day of May 2015 and were identified by Mr. Alfred Ozioko of the herbarium Botany Dept., University of Nigeria, Nsukka. The seeds were air-dried separately at room temperature and ground to powdery form using electrical grinding machine.

### Animals

Adult male Wistar albino rats of 10 to 16 weeks and average weight of 160±15 g were obtained from the Animal House of the Faculty of Biological Sciences, University of Nigeria, Nsukka. The animals were acclimatised for duration of 7 days under standard environmental conditions with a 12 hour light/dark cycle maintained on a regular feed (vital feed) and water *ad libitum*.

### Extraction of the seeds *C. nitida* and *G. kola*

Large quantities of the seeds of *C. nitida* and *G. kola* were collected from the University of Nigeria, Nsukka environ and were identified by Mr. Ozioko of the herbarium Botany Department, University of Nigeria, Nsukka. The seeds of *C. nitida* and *G. kola* were air-dried separately at room temperature, then into powdery form using electrical grinding machine. The ground samples extracted with 95% ethanol solution, using cold maceration techniques. The samples were filtered using Whatman filter paper. The filtrates concentrated to solid matter using rotary evaporators, which then become the stock sample of the ethanol seed extracts which were used for the analysis. These extracts were stored in the refrigerator compartment.

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### Preparation of prooxidant (Hydrogen peroxide)

Stock concentration (0.2%) of analytical grade of hydrogen peroxidant ( $H_2O_2$ ) was prepared by obtaining 0.2% of  $H_2O_2$  from the analytical concentration. A dose of 0.5 ml/kg of 0.2% of  $H_2O_2$  was used.

### Experimental design

Thirty (30) male albino Wistar rats were acclimatized at the same conditions of temperature and pressure, and the same animal feeds were used for all the rats. The rats were divided evenly into 6 groups with 5 rats per group shown as:

- Group 1: Normal/negative rats (Control).
- Group 2: Positive control ( $H_2O_2$ -induced rats).
- Group 3:  $H_2O_2$ -challenged rats + 100 mg/kg b.w. of ethanol seed extract of *C. nitida*.
- Group 4:  $H_2O_2$ -Challenged rats + 200 mg/kg b.w. of ethanol seed extract of *C. nitida*.
- Group 5:  $H_2O_2$ -Challenged rats + 100 mg/kg b.w. of ethanol seed extract of *G. kola*.
- Group 6:  $H_2O_2$ -Challenged rats + 200 mg/kg b.w. of ethanol seed extract of *G. kola*.

### Biochemical test

Rats were sacrificed after 7 days of the experiment and blood was collected and used for biochemical test.

### Determination of malondialdehyde concentration

Lipid peroxide assay was done by determining the concentration of malondialdehyde (MDA) formed using the method of Varshney and Kale (1990).

### Assay of superoxide dismutase activity

Superoxide dismutase (SOD) activity was assayed using the method as described by Fridorich (1989) as contained in Randox commercial kit.

### Assay of catalase activity

Catalase activity was assayed using the method of Aebi (1983).

### Determination of glutathione concentration

The concentration of glutathione was determined according to the method of Habig et al. (1974).

### Determination of vitamin C

The concentration vitamin C (ascorbic acid) was determined according to the method of Baker et al. (1971).

### Ferric reducing antioxidant properties (FRAP) test

The method of Benzie and Strain (1999) was used for FRAP test.

### Statistical analysis

The results were expressed as mean  $\pm$  SEM and test of statistical significance was carried out using one-way analysis of various (ANOVA). The means were separated using Duncan multiple test. The statistical packaged used was the statistical package for social sciences (SPSS), version 17.

## RESULTS AND DISCUSSION

Hydrogen peroxide is a chemical compound used as an oxidizer, bleaching agent and disinfectant which acts mostly by oxidation. However when used *in vivo* it is a potent oxidant (ROS) which breaks down macro molecules in the body via reduction- oxidation reactions (Nadkarn, 2001). As such, it can initiate "free radical" generation which could cause damage to cell and tissues (Valko et al., 2007) giving rise to some serious side effects which include stomach bleeding, ulcerative colitis intestinal gangrene, gastrointestinal problems, stroke and death.

As shown in Table 1 there was a significant increase ( $p < 0.05$ ) in MDA concentration after  $H_2O_2$  was administered to group 2 rats when compared to other groups which suggests increase in lipid peroxidation. This suggests oxidative deterioration of unsaturated fatty acids of the cell membrane and other macro molecules of the body by free radicals generated by  $H_2O_2$ . This would lead to membrane fluidity and the death of cell. This is consistent with Raghavendran et al. (2004) who reported that free radical intoxication leads to membrane damage. Thus treatment with both extracts caused a dose dependent significant decrease in the MDA concentration of the treatment groups (3, 4, 5, and 6) when compared to the untreated group (2), thus this fit in with the finding of Wegwu and Didia (2007) which suggest that these plant materials may possess the normal antioxidants necessary for protection against free radical damage induced by  $H_2O_2$  in rats bio-system.

Table 2 shows a significant decrease ( $p < 0.05$ ) in the vitamin C concentration on administration of  $H_2O_2$  in group 2 rats (untreated group). This trend depicts increased serum level of reactive oxygen species. This corroborates with Cacciatore et al. (2012), who reported that increased level of free radicals leads to oxidative stress and subsequent damages to vital organs like liver and kidney. The treatment with extracts of both *C. nitida* and *G. kola* shows remarkable increase in serum vitamin C level suggests that extract may contain natural vitamins C which are potent anti-oxidant enhancing metabolic processes including cellular respiration and nutrient metabolism (Rekha et al., 2012). Antioxidant effects of vitamin C was also noticed to be more in *G. kola* than *C. nitida* which may be attributed to the presence of Biflavones in *G. kola* (Farombi and Owoeye, 2011).

Table 2 shows a significant decrease ( $p < 0.05$ ) in the Glutathione concentration of the group 2 untreated rats after  $H_2O_2$  administration when compared to the

**Table 1.** Comparative effects of ethanol extract of *Cola nitida* and *Garcinia kola* seeds on Malondialdehyde (MDA) concentration in hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)-challenged rats.

Groups	MDA (mg/dl)
GROUP 1- Normal/Negative rats (Control)	1.667 ± 0.333
GROUP 2- Positive Control (H <sub>2</sub> O <sub>2</sub> only)	7.100 ± 0.463
GROUP 3- H <sub>2</sub> O <sub>2</sub> + 100 mg/kg b.w. <i>C. nitida</i>	2.590 ± 0.629
GROUP 4- H <sub>2</sub> O <sub>2</sub> + 200 mg/kg b.w. <i>C. nitida</i>	1.267 ± 0.258
GROUP 5- H <sub>2</sub> O <sub>2</sub> + 100 mg/kg b.w. <i>G. kola</i>	2.620 ± 0.450
GROUP 6- H <sub>2</sub> O <sub>2</sub> + 200 mg/kg b.w. <i>G. kola</i>	1.917 ± 0.289

Values are Mean ± SEM; n = 3 animals in each group; P<0.05 is considered significant when compared with positive control group using one-way analysis of variance.

**Table 2.** Comparative effects of ethanol extract of *C. nitida* and *G. kola* (*G. kola*) seeds on glutathione (GSH) concentration and vitamin C concentration in hydrogen peroxide(H<sub>2</sub>O<sub>2</sub>)-challenged rats.

Groups	GSH (mg/dl)	Vitamin C (mg/dl)
Group 1- normal/negative rats (Control)	6.00 ± 0.58	7.81 ± 0.34
Group 2- positive control (H <sub>2</sub> O <sub>2</sub> only)	2.00 ± 0.35	1.96 ± 0.43
Group 3- H <sub>2</sub> O <sub>2</sub> + 100 mg/kg b.w. <i>C. nitida</i>	4.00 ± 0.58	6.33 ± 0.33
Group 4- H <sub>2</sub> O <sub>2</sub> + 200 mg/kg b.w. <i>C. nitida</i>	5.00 ± 0.68	5.33 ± 0.33
Group 5- H <sub>2</sub> O <sub>2</sub> + 100 mg/kg b.w. <i>G. kola</i>	4.67 ± 0.67	6.67 ± 0.33
Group 6- H <sub>2</sub> O <sub>2</sub> + 200 mg/kg b.w. <i>G. kola</i>	5.33 ± 0.33	7.00 ± 0.33

Values are Mean ± SEM; n = 3 animals in each group; P<0.05 is considered significant when compared with positive control group using one-way analysis of variance.

**Table 3.** Comparative effects of ethanol extract of *C. nitida* and *G. kola* seeds on catalase activity in hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) challenged albino rats.

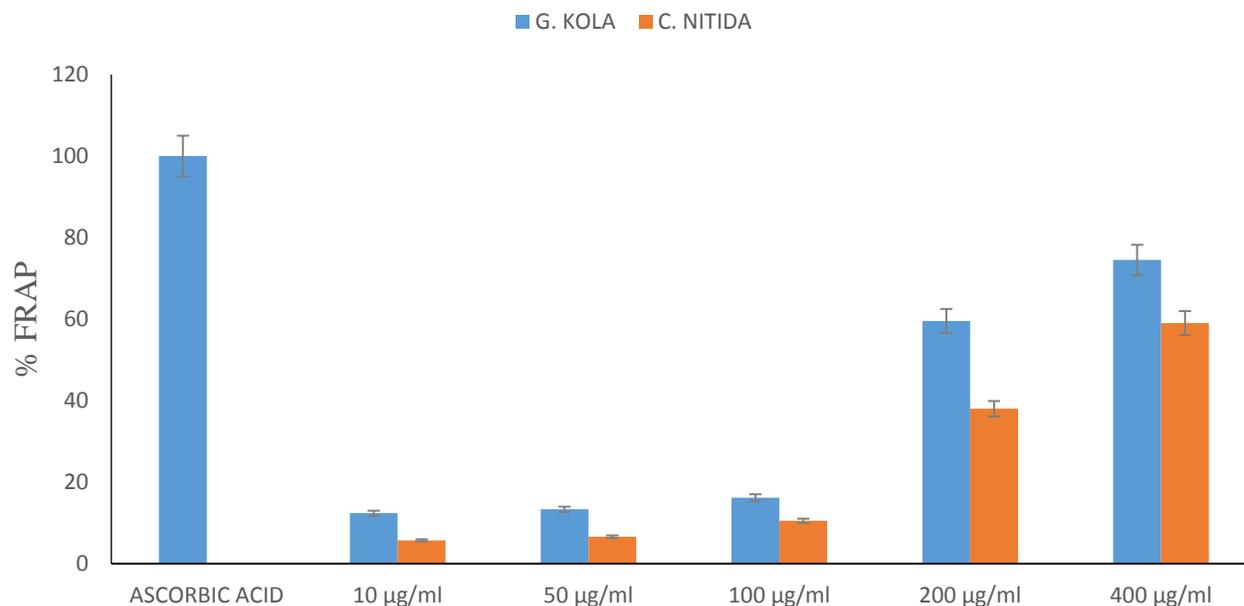
Groups	SOD (IU/L)	Catalase (IU/L)
Group 1- Normal/Negative rats (Control)	18.75 ± 0.56	9.08 ± 1.32
Group 2- Positive Control (H <sub>2</sub> O <sub>2</sub> only)	7.50 ± 0.92	3.00 ± 0.78
Group 3- H <sub>2</sub> O <sub>2</sub> + 100 mg/kg b.w. <i>C. nitida</i>	14.31 ± 0.99	7.11 ± 0.56
Group 4- H <sub>2</sub> O <sub>2</sub> + 200 mg/kg b.w. <i>C. nitida</i>	14.98 ± 0.78	7.92 ± 0.45
Group 5- H <sub>2</sub> O <sub>2</sub> + 100 mg/kg b.w. <i>G. kola</i> .	14.89 ± 0.86	7.79 ± 0.60
Group 6- H <sub>2</sub> O <sub>2</sub> + 200 mg/kg b.w. <i>G. kola</i>	16.43 ± 0.79	8.89 ± 0.36

Values are Mean ± SEM; n = 3 animals in each group; P<0.05 is considered significant when compared with positive control group using one-way analysis of variance.

treatment groups. This depicts damages in the liver because glutathione is a potent antioxidant which is capable of preventing damage to important cellular components caused by free radicals generated after H<sub>2</sub>O<sub>2</sub> administration. This result is consistent with the findings of Wegwu and Dibia (2007) who reported the above as a result of liver damage. When glutathione cannot reduce pro-oxidants thus cellular damage occurs. But treatment with extract as depicted in groups 3, 4, 5 and 6 shows return to near normalcy suggesting the antioxidant potential of the extracts. Thus the result of this work is in tan den with the works of Ibiyinka et al.

(2014) where the antioxidant activity and total phenolic content of some nuts commonly consumed in South-Western Nigeria was evaluated.

From Table 3 administration of hydrogen peroxide alone to group 2 rats caused a significant (P<0.05) decrease in catalase and SOD activity when compared with the normal control. This result is consistent with Jamaludin et al. (2012) who reported that administration of hydrogen peroxide caused a significant decrease in the catalase and SOD activity as a result of the actions of free radicals and other reactive oxygen species. Thus increase in production of free radicals and other reactive



**Figure 1.** Ferric reducing antioxidant power of *Garcinia kola* and *Cola nitida* in varying concentrations.

oxygen species causes depletion in the enzymatic antioxidants of the bio-system. However on administration of different doses (100 and 200 mg/kg b. w.) of the two extracts (*C. nitida* and *G. kola*) reduced the tissue damage induced by  $H_2O_2$  in a dose-dependent manner and hence, significantly ( $p < 0.05$ ) increased the activities of CAT and SOD. Hence, suggesting that the extract may contain antioxidants which are proved by the works of Ganiyu et al. (2014) and Ogunmoyole et al. (2012). The results of this project work is consistent with the findings of Blades (2000) who stated that *C. nitida* showed a powerful scavenging activity of hydroxyl radicals and acted as a chelator of iron, showed a significant inhibitory effect on the peroxidation of rat brain and prevented DNA damage. It also coincides with the findings of Cowan (2010) who showed that *Garcinia kola* is a stimulant and herbal remedy for some disease conditions. *G. kola* can influence indices such as cholesterol, total protein and some vital organs of the body (heart, liver, brains, etc).

The FRAP assay is done to evaluate the antioxidant potential of compounds. From Figure 1 it shows the results of the ferric reducing antioxidant power (FRAP) of *G. kola* and *C. nitida*. The results shows that both extracts have the antioxidant ability to donate an electron to ferric ( $Fe_{3+}$ ) to convert it to ferrous ( $Fe_{2+}$ ) however the trend in FRAP assay results also favors *G. kola* over *C. nitida*. This agrees with research outcome of Ayebe et al. (2012) who asserts that *C. nitida* shows delayed expression of antioxidant manifestation compared to other cola types and Ibiyinka et al. (2014) where the antioxidant activity and total phenolic content of some nuts commonly consumed in South-Western Nigeria were assessed.

## Conclusion

From these research work, reduction in elevated MDA level gives inference in the improvement of GSH level, SOD activity, catalase activity and increase in vitamin C level; hence, suggesting that both *C. nitida* and *G. kola* poses possible non enzymatic anti-oxidant properties, however that of *G. kola* seems to be more efficient in reducing oxidative stress.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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