Volume-1 | Issue-2 | November-2018 | ISSN - 2617-4421 (Print) | ISSN - 2617-7188 (Online) |

**East African Scholars Journal of Medical Sciences**
(An Open Access, International, Indexed, Peer-Reviewed Journal)
A Publication of East African Scholars Publisher, Kenya
www.easpublisher.com

**Original Research Article**

# Assessment of Anti-Oxidative Potential of Ethanol Seed Extract of *Cola lepidota* in High Fat Fed Female Wistar Albino Rats

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Article History
Received: 14.11.2018 | Accepted: 22.11.2018 | Published: 30.11.2018

**Abstract:** This study assessed the anti-oxidative potentials of ethanol seed extract of *Cola lepidota* in High Fat Fed (HFD) female Wistar Albino rats. Thirty (30) apparently healthy female albino wistar rats weighing between 80 – 90g were divided into five (5) equal groups for the study which lasted for 90 days. Group I which served as negative control was fed with 200g of normal rat chow and water daily. Group II which served as positive control received 200g of high fat diet (HFD) daily. Group III received 200g of HFD for the first 30 days and 200g of HFD + 300mg/kg of extract for the remaining 60 days. Group IV received 200g of HFD for the first 30 days and 200g of HFD + 300mg/kg of ascorbic acid for the remaining 60 days. Group V received 200g of HFD for the first 30 days and 200g of HFD + 300mg/kg of extract + 300mg/kg of ascorbic acid for the remaining 60 days. All administrations were done orally; standard analytical methods were used for the determination of serum superoxide dismutase (SOD) activity, serum malondialdehyde (MDA) level, serum catalase activity and whole blood glutathione (GSH) level. The result indicated a significant reduction in the concentration of all the antioxidants in the HFD untreated group (group II) compared to other groups with the concentrations of SOD, CAT and GSH in group II being 7.2U/ml, 7.0mmol/mg and 0.04µg/ml respectively. It showed a significant increase in the concentration of MDA (6.2mmol/mg protein) in group II compared to the other groups. Also, the result showed that concentrations of all the antioxidants significantly increased in groups IV and V compared to group II with the concentrations of SOD, CAT and GSH being 14U/ml, 14mmol/mg and 0.1µg/ml respectively in group IV and 14U/ml, 13mmol/mg and 1.4µg/ml respectively in group V. In conclusion, seed extracts of *Cola lepidota* has antioxidant properties which become most potent when combined with the non-enzymatic antioxidant ascorbic acid and therefore may be used for prevention and management of oxidative stress related health disorders like cancer, Alzheimer's disease, diabetes, autoimmune disorders and cardiovascular diseases**.**
**Keywords:** Cola Lepidota, Antioxidant parameters, High Fat Diet, and Wistar Rats.

## INTRODUCTION

Plants are one of the richest sources of bioactive compounds and have been the basis of many traditional medicines throughout the world for thousands of years (Ingale and Hivrale, 2010). The use of plants as sources of remedies for the treatment of diseases date back to prehistoric times and people of all continents are used to this old tradition (Bijana, 2012). Natural products, such as plant extracts, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity (Cos *et al,* 2006). According to World Health Organization (WHO, 2001), 60% of the world's population depends on traditional medicine and 80% of the population in developing countries depends almost entirely on traditional medicine practices and herbal medicines for their primary health care needs (Abdel-Azim *et al.,* 2011; Zhang and Moller, 2000). The reasons for this, especially in developing nations, includes ease and cost of assessing orthodox medicine as well as cost of procuring prescribed medications (Chan, 2003). Tropical African sub-regions are home to many useful medicinal plants, however, there are many of them whose potentials are yet to be harnessed or domesticated in tropical African Sub-regions (Ogbu *et al.,* 2014).

*Cola lepidota* is one of the varieties of the cola species commonly referred to as Monkey Kola. Monkey kola includes *Cola lepidota* (yellow variety with yellow pod), Cola parachycarpa (white variety with white pod) and Cola lateritia (red variety with red pod) (Meregini, 2005). They belong to the family Malvaceae, sub-

family Sterculioideae and a group called drupes (Pamplona-Roger, 2008), thus sharing botanical family and sub- family with the popular West African plantation kola nuts (*Cola nitida*). *Cola lepidota* is cultivated throughout the tropical regions of the world and commonly found in Southern Nigeria between the months of June and November (Ogbu *et al.,* 2007). It grows wild and up to the height of 18m with twisted trunk, edible fruit which is crunchy and tasty with yellow pulp (Ogbu *et al.,* 2007, Singh *et al.,* 2010), roundish pod and mostly consumed fresh. It is commonly called monkey cola in Western Cameroon (Iwu, 1993). In Nigeria, it is known as "Achicha" in Igbo and "Ndiyah" in Ibibio and Efik (Iwu, 1993). It was reported that local people of southern Nigeria and Cameroon relish the fruits including wild primate animals such as monkeys, baboons and other species which probably led to the name 'Monkey Cola' (Ogbu and Umeokechukwu, 2014).

Phytochemical screenings of C. lepidota K. schum reveals that it contains alkaloids, saponins, terpenoids, flavonoids, phenols, steroids, glycosides, tannins and beta-carotene (Essien *et al.,* 2015; Okudu *et al.,* 2015; Ene-Obong *et al.,* 2016). It also contains significant amounts of calcium, potassium, iron, selenium, zinc, copper, sodium, vitamins A, B and C (Okudu *et al.,* 2015; Ene-obong, 2016). Not much has been documented about the medicinal and health-care importance of *C. lepidota*. It was reported that *Cola lepidota* is employed in Nigerian folk medicine as febrifuge and for the treatment of pulmomary disorders (Engel *et al.,* 2011; Ogbenerebo *et al.,* 2013), also reported was its anticancer and antioxidant activities of its leaf and bark extracts (Engel *et al.,* 2011; Ogbenerebo *et al.,* 2013).

Free radicals are short lived and highly reactive molecules. They are produced either from external sources (pollution, cigarette smoke, radiation, medication etc) or generated in situ as a result of normal cellular metabolism (Parke and Sapota, 1996). They are utilized by immune system for lyses of micro-organisms, but become toxic when produced in excess quantity (Senthil and Manoharan, 2004). One significant feature of free radicals is lipid peroxidation which leads to tissue damage and cell death (Bandyopadhyay 1999). Free radicals are implicated in the pathogenesis of various chronic and degenerative disorders such as cancer, aging, Alzheimer's disease, diabetes, autoimmune disorders and cardiovascular diseases. (Ames *et al.,* 1993; Halliwell *et al.,* 1994). Overweight and obese people are likely to have high aerobic cellular metabolism thus producing enormous free radicals with its associated oxidative stress. According to Furukawa *et al* (2004), increased oxidative stress in obesity was due to increased ROS production from accumulated fat.

Free radicals are scavenged by antioxidant defense mechanisms. The enzymatic antioxidants and non-enzymatic antioxidants (Vitamin C, E and GSH) protect the tissues from free radical toxicity (Yu 1994). World Health Organization (WHO) estimated that at least 2.8 million people die each year worldwide as a result of overweight and obesity (WHO, 2016). Nutrition was implicated as the major cause of obesity in both men and women (Vandevijvere and Swinburn, 2014). Eating more plant-based foods which are rich in phytochemicals according to Heather and Talcott (2009) prevent oxidative stress in the body, a process associated with obesity. However, there is paucity of information on the antioxidative potential of *Cola lepidota* seeds on increase in fat intake. This study therefore aimed at assessing the anti-oxidative potentials of ethanol seed extract of *Cola lepidota* in high fat fed (HFD) female wistar albino rats.

## MATERIALS AND METHODS

*Cola lepidota* fruits were purchased at the Eke-Ukwu Owerri Market in Imo State, Nigeria. The fruits were properly washed and the seeds obtained by peeling the bark and cutting open the fruit pulps. The seeds were chopped into pieces, air-dried under room temperature for 21days and pulverized using an electric blender. 100g of the seed powder was macerated with 100mls of 100% absolute ethanol. Extraction and filtration was done after 48 hours and the extract was concentrated by evaporation using rotary evaporator RE52. High fat feed formulation was done according to the method of Nna *et al.,* (2014).

Thirty (30) apparently healthy female albino wistar rats weighing between 80 – 90g at 6 weeks (42 days) of age were used for the study. The rats were bought from laboratory animal facility of the Department of Biochemistry, University of Nigeria Nsukka and were taken to the Department of Veterinary Medicine, Micheal Okpara University of Agriculture, Umudike where the study was conducted. They were confined in cages under standard environmental conditions (temperature of 22 - $25^0$C, 12hrs light and 12hrs dark cycle) for purpose of acclimatization. During acclimatization which lasted for 7 days, the rats were fed with standard rat chow and clean water *ad libitum*. The experiment lasted for 3 months (90 days) and proceeded according to the University's ethics on animal handling.

The animals were randomly divided into five groups labeled groups I, II, III, IV and V with each group consisting of 6 rats. Group I (negative control group) received 200g of normal rat chow and water daily throughout the 90 days of experiment. Group II (positive control group) received 200g of high fat diet (HFD) daily for 90 days. Group III received 200g of HFD for the first 30 days and 200g of HFD + 300mg/kg

of extract for the remaining 60 days. Group IV received 200g of HFD for the first 30 days and 200g of HFD + 300mg/kg of ascorbic acid for the remaining 60 days. Group V received 200g of HFD for the first 30 days and 200g of HFD + 300mg/kg of extract + 300mg/kg of ascorbic acid for the remaining 60 days. At the end of the experimental period (90 days), the rats were fasted overnight (12 – 14 hours) and then sacrificed under ethyl ether anaesthesia. Blood samples were collected via cardiac puncture and allowed to clot for 1 hour. Serum was obtained by centrifuging at 400rpm for 10 minutes in a wisperfuge centrifuge (Model 1384). The serum obtained was used for the determination of serum superoxide dismutase (SOD) activity, determination of serum malondialdehyde (MDA) level according to the modified method of Draper and Hadley (1990), determination of serum catalase activity according to the method of Atawobi (2011) and the determination of whole blood glutathione (GSH) according to the method of Ellman (1959).

Statistical analysis of data generated in this study was done using the SPSS/IBM version 21 software. The means and standard error of means were calculated for all parameters under investigation. Statistical differences between the experimental and control groups were determined using ANOVA and students t-test. Values were considered significant at $p<0.05$.

**RESULTS**

The results are presented in figures (bar charts) with the y-axis showing concentration and x-axis showing group.

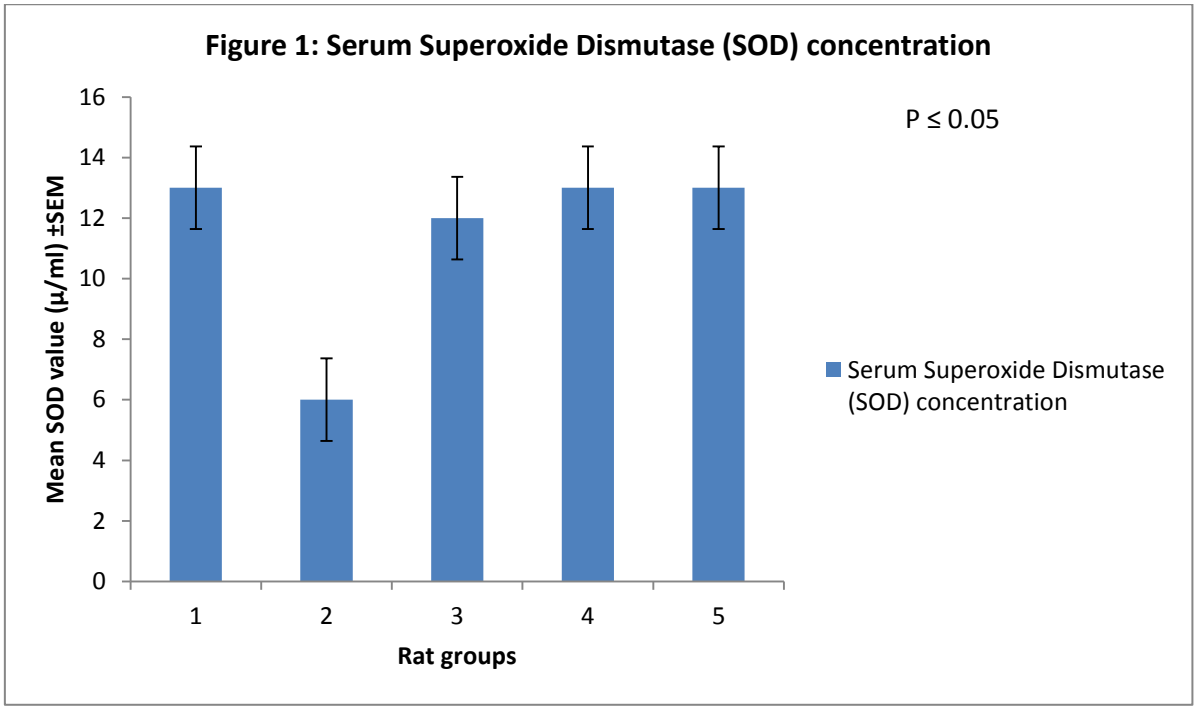


**Figure 1 graphically represents the mean serum concentration of SOD in µ/ml ± SEM of the groups. Group 2 has the least SOD concentration than the rest of the groups.**

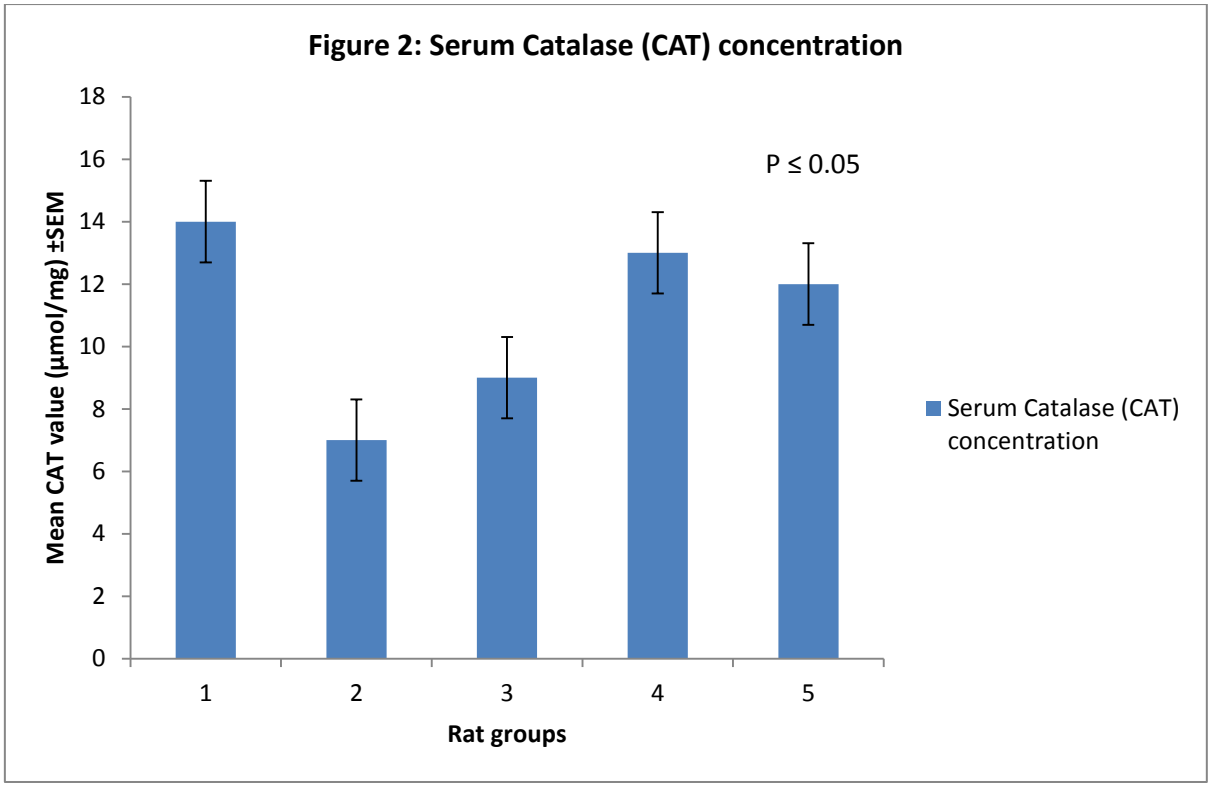


**Figure 2 graphically represents the mean serum concentration of CAT in µmol/mg ± SEM of the groups. Group 2 has the least significant serum CAT concentration followed by group 3.**

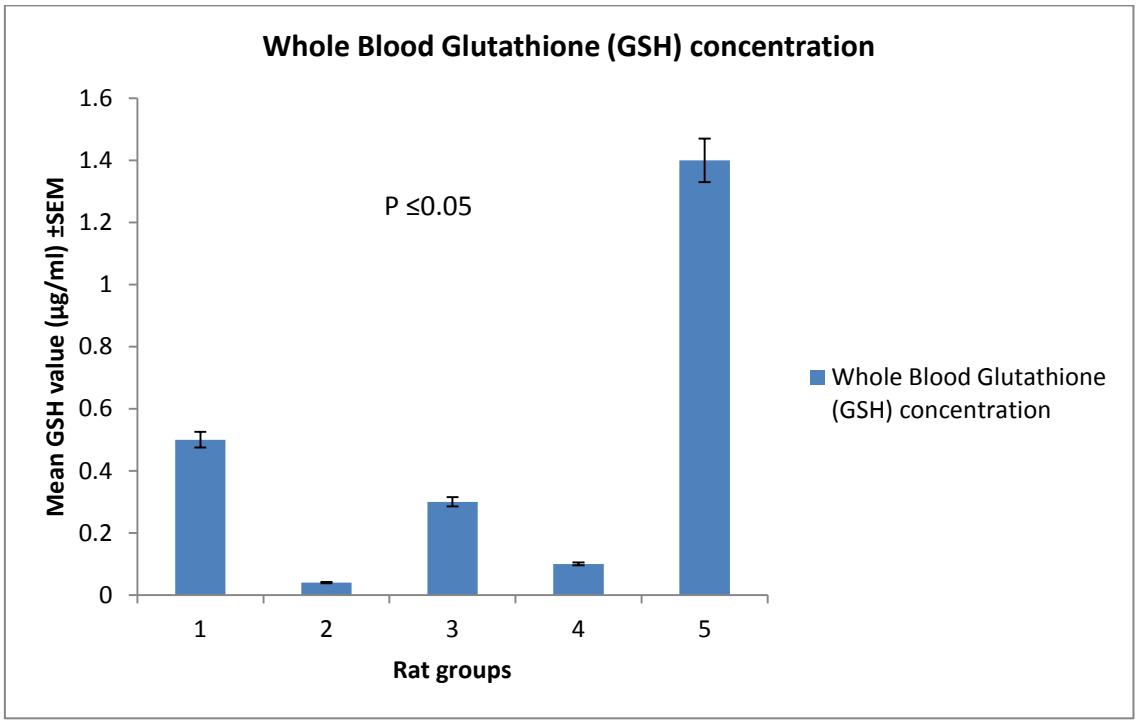


**Figure 3 graphically represents the mean serum concentration of GSH in µg/ml ± SEM of the groups.**

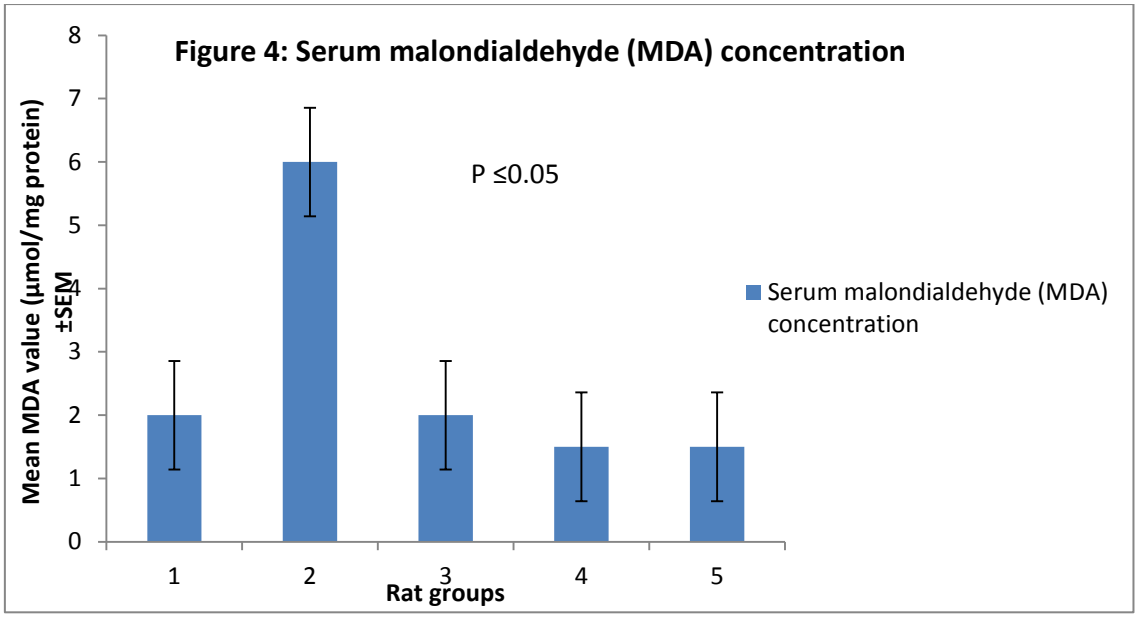


**Figure 4 graphically represents the mean serum concentration of MDA in µmol/mg ± SEM of the groups. Group 2 has the highest significant serum MDA concentration while groups 4 and 5 have the least.**

**DISCUSSION**

Antioxidants are powerful substances that prevent oxidation and delay or inhibit the damaging effects of reactive oxygen species (ROS) to a target molecule. They have the ability to trap or scavenge free radicals thus protecting the body from free radical induced oxidative stress. Oxidative stress affects all cell macromolecules including DNA, proteins and lipids causing mutation, protein inactivation and lipid peroxidation respectively (Esterbauer *et al.*, 1990). There are enzymatic antioxidants (SOD, CAT, GPx) and non-enzymatic antioxidants (Vitamin C, E and GSH), both protect the tissues from free radical toxicity (Yu 1994). Many medicinal plants have been reported to exhibit antioxidant property with minimal side effects. This study was undertaken to assess the antioxidative potential of ethanol seed extract of Cola lepidota in high fat fed female wistar rats.

From the present study, group 2 rats which were exclusively fed with HFD showed a significant decrease in the concentrations of the antioxidants (SOD, CAT and GSH). This decrease in the concentration of the antioxidants is suspected to have resulted from the depletion of antioxidants while trying to scavenge and mop up free radicals associated with intake of high fat diet. High fat intake cause increase in peroxidation of polyunsaturated lipids in cell membranes, phospholipids degradation (Nwankpa *et al.*, 2012) and changes in lipoprotein synthesis thereby depleting the antioxidants. Antioxidant enzymes such as SOD, CAT and GPx play important roles in scavenging the free radicals and preventing cell injury (Bergendi *et al.*, 1999). There is also a significant increase in the concentration of MDA among the exclusive HFD group. This is suggestive of the role of HFD in promoting lipid peroxidation. Malondialdehyde which is the most abundant lipid peroxide is widely used as an indicator of lipid peroxidation (Kawase *et al.*, 1989). Also observed in this study is the decrease in blood GSH concentration in group 4 rats fed with HFD and ascorbic acid. Finally, there is increase in antioxidants with the increase in GSH being most significant and a significant decrease in MDA in group 6 rats which were given combination of HFD, ascorbic acid and extract. Similar results have been reported on leaves of *Sida accuta* (Nwankpa *et al.*, 2014) and *Phyllanthus nuriri* (Nwajo *et al.*, 2007) on reducing lipid peroxidation hence reduction of reactive oxygen species (ROS) which causes oxidative damage.

The findings of this study suggest that high fat diet increases the level of free radicals, increases lipid peroxidation and increases depletion of antioxidants. It is also a pointer to the weak effect of non-enzymatic antioxidants vitamin C in scavenging free radicals. It however showed that synergistic effect of extract of *Cola lepidota* and vitamin C has a very powerful antioxidant property which is far more potent than administration of either the extract or vitamin C alone. The result of this study agrees with the findings of Oghenerobo and Falodun (2013), and Nwankpa *et al.*, (2012). This may be explained by the presence of secondary metabolites which have the tendency to mop free radicals.

In conclusion, seed extracts of *Cola lepidota* has antioxidant properties which become most potent when combined with ascorbic acid. This therefore lends support to the ethnomedicinal use of *C. lepidota* in

Nigerian folk medicine in the treatment of oxidative stress related health disorders.

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