

## Free Radicals Scavenging and Neuroprotective Effects of Ethanolic Leaf Extract of *Combretum zenkeri* Leaf

C. O. Ujowundu<sup>1\*</sup>, C. U. Ogbonna<sup>1</sup>, G. N. Okwu<sup>1</sup> and C. S. Alisi<sup>1</sup>

<sup>1</sup>Department of Biochemistry, Federal University of Technology, P.M.B. 1526 Owerri, Nigeria.

### Authors' contributions

This work was carried out in collaboration between all authors. Authors COU, GNO and CUO designed the study, wrote the protocol and interpreted the data. Authors COU, CUO and CSA anchored the field study, gathered the initial data and performed preliminary data analysis. Author CUO managed the literature searches and produced the initial draft. All authors read and approved the final manuscript

### Article Information

DOI: 10.9734/ARRB/2015/14039

#### Editor(s):

(1) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.

#### Reviewers:

- (1) Anonymous, Jazan University, Egypt.  
(2) Novatus F. Mushi, Institute of Traditional Medicine, Muhimbili University of Health and Allied Sciences, Tanzania.  
(3) Anonymous, Federal University of Piaui, Brazil.  
(4) Anonymous, National Research Center, Egypt

Complete Peer review History: <http://www.sciencedomain.org/review-history.php?iid=797&id=32&aid=7298>

Original Research Article

Received 16<sup>th</sup> September 2014  
Accepted 10<sup>th</sup> November 2014  
Published 15<sup>th</sup> December 2014

### ABSTRACT

*Combretum zenkeri* is a small tree found in the tropical West Africa that has been applied by locals in the treatment of arthritic and other inflammatory diseases. This study investigated the free radical scavenging activities of ethanolic leaf extract of *C. zenkeri* against nitric oxide NO<sup>•</sup> and hydroxyl OH<sup>•</sup> radicals and reducing power (Fe<sup>3+</sup>–Fe<sup>2+</sup> transformation ability). Inhibition of lipid peroxidation through determination of thiobarbituric acid reactive substances (TBARS) in brain homogenates (neuro-protective potentials) was also studied. The plant extract demonstrated the ability to scavenge nitric oxide and hydroxyl radicals in a dose-response manner. In the reducing power assay, the ethanolic extracts gave the optical density in a logistic dose response curve with the concentration of 581.46 µg/ml at optical density of 0.5. The neuroprotective ability of the plant extract indicated good antioxidant capacity. These results provide basis to regard *C. zenkeri* as an asset in the quest to ameliorate oxidative diseases with naturally occurring secondary metabolites in plants.

\*Corresponding author: E-mail: [ujowundu@yahoo.com](mailto:ujowundu@yahoo.com);

**Keywords:** *Combretum zenkeri*; nitric oxide; hydroxyl radical; oxidative stress.

## 1. INTRODUCTION

Humans live in a highly oxidative environment and processes involved in metabolism may result in the production of more oxidants [1]. Oxidants, otherwise referred to as reactive oxygen species (ROS), are various forms of activated oxygen. They are free radicals like superoxide ( $O_2^-$ ), hydroxyl ( $OH^\cdot$ ), nitric oxide (NO) and lipid peroxy ( $LOO^\cdot$ ) radicals [2]. The non-free radical species are lipid peroxide ( $LOOH$ ), hydrogen peroxide ( $H_2O_2$ ) and singlet oxygen ( $O_2^1$ ) [3]. They are produced by aerobic organisms and are known to easily react with most biological molecules viz; proteins, lipoproteins, lipids and DNA [2].

Exposure to free radicals from a variety of sources has led organisms to develop a series of defence mechanisms [4]. Defence mechanisms against free radical-induced oxidative stress involve: (i) preventative mechanisms, (ii) repair mechanisms, (iii) physical defences, and (iv) antioxidant defences [3]. Antioxidants can terminate or retard the oxidation process by scavenging free radicals [2], as possible protection agents for reducing oxidative damage of the human body from ROS [5]. Oxidative stress may set in if the infiltration of ROS overwhelms the antioxidant capacity of the human body. Meanwhile, previous research showed that some of the antioxidant compounds were derived from plant extracts. Some of these antioxidants are polyphenolic compounds which have shown strong antioxidant capacity [6].

The use of leafy vegetable is part of Africa's cultural heritage and they play important roles in the customs, traditions and food culture of the African household [7,8]. In Nigeria, many fruits, shrubs, spices and herbs and leafy vegetables are used as food, food drinks and for medicinal purposes [9,10,11]. Traditional medicine is widely spread throughout the world. It is the total combination of knowledge and practice, whether explicable or not, used in diagnosing, preventing or eliminating a physical, mental or social disease [12].

Most of the protective effects of plants on living cells have been attributed to their non-nutrient constituents e.g. carotenoid, flavonoids, isoflavonoid and phenolic acids [13]. Plant-derived natural products have received considerable attentions in recent years due to the diverse pharmacological properties, including

antioxidant and antitumor activity [14], and plant derived antioxidants are presumed safe [2]. One of such plants is *Combretum zenkeri* (family-Combretaceae), widely distributed and used in tropical West Africa from Guinea to southern Nigeria and Cameroon. It is called *ubi* in Mbaise or *ubegwu* in Owerri both in Eastern Nigeria. *Combretum zenkeri* Engl. and Diels is found in *Utesi* East, Edo State, Nigeria as a rare climbing weed [15]. The leaves are used in Ogun State for the treatment of inflammatory diseases like rheumatoid-arthritis [16]. It has also been recorded that habitants of Umuahia South-eastern Nigeria use the leaf as a source of vegetable [17], while some Mbaise natives use it as spice to prepare delicacies for nursing mothers. All these show dependence on the plant as an anti-inflammatory medicine.

*C. zenkeri* has been shown to contain flavonoids, alkaloids, saponins, vitamins A and C and minerals elements with antioxidant potentials [17]. However, there is no report of its ability to scavenge free radicals. This study, aims to do that and also generate standardized data that will drive safety and efficacy in herbal medicine practice. It is believed that the outcome will give us a scientific insight on its use in certain ethnopharmacological practices against inflammatory diseases.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

The plant leaves of *C. zenkeri* were collected during the morning hours at onset of harmattan, from a farm at Ihiagwa, Owerri-West LGA, Imo-State Nigeria. The plant was identified by Mr. F. O. Iwueze, a plant taxonomist. The plant sample was authenticated by a professional taxonomist Dr. F. N. Mbagwu from Imo State University with specimen voucher number IMSU/0124 and thereafter deposited in the Imo State University Herbarium. The fresh leaves were plucked out from the plant stalk, rinsed with clean water and air-dried for 15 days. The dried leaves were pulverized with a mechanical grinder, packaged in air-tight glass jar and stored at room temperature until analysis was carried-out.

### 2.2 Preparation of Extracts

The pulverized sample was macerated [18], and a 450 g weight was soaked in 1200 ml of absolute ethanol (BDH). The setup was

constantly agitated for four for (4) days and then filtered. The extract was concentrated *in vacuo*, using a rotary evaporator (NYC), to achieve the ethanolic leaf extract of *C. zenkeri* (CZEE) concentrate. The concentrated ethanol extract so obtained was stored in a refrigerator at 4°C until used.

### 2.3 Animal Material

Adult male rabbit was purchased from Relief Market, Owerri Nigeria. The animal was anaesthetized, sacrificed and the brain removed and weighed immediately. This study was approved by the ethics committee of the Department of Biochemistry, Federal University of Technology, Owerri, Nigeria. All experiments were performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

### 2.4 Nitric Oxide Radical (NO<sup>•</sup>) Scavenging Assay

The method of [19] was employed to measure the scavenging effect of *C. zenkeri* extract on nitric oxide, with little modification [20,21]. In this method sodium nitroprusside (SNP) (25 mM) decomposes in the reaction mixture of phosphate buffered saline (pH 7.2) giving NO<sup>•</sup>, which further reacts with oxygen to give nitrate and nitrite whose qualities can be determined with Griess reagent [1% sulfanilamide in 5% phosphoric acid and 0.1% naphthyl ethylenediamine dihydrochloride (NED)]. 4 ml of *C. zenkeri* extract solution at various concentrations was introduced (in the test tubes) to 1 ml of sodium nitroprusside (SNP) solution (10 mM). The tubes were incubated for 2 hours at 29°C. A 2 ml aliquot of the incubation solution was diluted with 1.2 ml Griess reagent [21]. The absorbance of the chromophore formed [during the nitrite's diazotization with sulfanilamide and futher coupling with naphthyl ethylenediamine dihydrochloride (NED)] was immediately read at 550 nm. The concentration was determined from a standard curve based on sodium nitrite solutions of known concentrations treated in like manner with Griess reagent. The experiments were carried-out in triplicates and the data were presented as a mean of three independent determinations. Nitrite formation inhibition by the ethanolic leaf extract of *C. zenkeri* or the standard antioxidant (Quercetin) was calculated relative to the control with the aid of a standard curve.

$$\% \text{Inhibition} = 100 - \frac{\text{Test}}{\text{Control}} \times 100$$

### 2.5 Determination of Hydroxyl Radicals Scavenging Activity

The method of [22], as modified by [23] was used. The competition between deoxyribose and the test compounds for hydroxyl radicals generated from the Fe<sup>3+</sup>/ascorbate/EDTA/H<sub>2</sub>O<sub>2</sub> system was studied to test the hydroxyl radical scavenging ability of ethanolic extract of *C. zenkeri* leaf. This was done using the Fenton oxidant reaction mixture (Fe<sup>3+</sup>/ascorbic acid and H<sub>2</sub>O<sub>2</sub>) [24]. This reaction mixture contained: 14.0 mM Deoxyribose, 0.1 mM FeCl<sub>3</sub>, 0.1 mM EDTA, 10 mM H<sub>2</sub>O<sub>2</sub>, 0.1 mM ascorbic acid, 20 mM KH<sub>2</sub>PO<sub>4</sub>-KOH buffer at pH 7.4 and the ethanolic leaf extract of *C. zenkeri* (0-2000µg/ml) in 1.0 ml final volume. This was incubated for 1 hr at 37°C. The deoxyribose degradation was tested as thiobarbituric acid reactive substances (TBARS). A mixture of 20% acetic acid (pH 3.5) (1.5 ml), 0.8% Thiobarbituric acid (TBA) (1.5 ml) and 8.1% sodium dodecyl sulphate (SDS) (0.2 ml) was prepared in distilled water and incubated at 100°C for 1 hr, cooled and trichloroacetic acid (2 ml) was added. The mixture was vigorously vortexed and centrifuged for 10 minutes at 3000 rpm. The absorbance of the supernatant was measured at a wavelength 532 nm. Concentration of TBARS was calculated using its molar extinction. Hydroxyl radical scavenging of the extract was calculated relative to control [21].

$$\% \text{Inhibition} = 100 - \frac{\text{Test}}{\text{Control}} \times 100$$

### 2.6 Evaluation of Possible Neuro-protective Potential

The ability of ethanolic leaf extract of *C. zenkeri* to inhibit hydrogen peroxide-induced lipid peroxidation in rabbit brain homogenate was used to determine the neuroprotective potential of the extract. Whole rabbit brain was homogenized in 10% w/v phosphate buffered saline. Then, 200 µl of the rabbit brain homogenate was treated with 10 µM of hydrogen peroxide and varying concentrations of ethanol leaf extract of *C. zenkeri* and incubated for one hour [25]. The resulting thiobarbituric acid reacting substances (TBARS) was measured by the method of [22], as modified by [23].

## 2.7 Estimation of Reducing Power

The reducing power of ethanolic extract of *C. zenkeri* was determined by studying the transformation of  $Fe^{3+}/Fe^{2+}$  in the presence of the plant extract [26]. The plant extract (0 - 5.0 mg) or standard (butylated hydroxyl toluene) were dissolved in a buffered medium which give final concentrations of 0 – 800  $\mu\text{g/ml}$  in a reaction mixture consisting of 2.5 ml of phosphate buffer (0.2 mM) at pH 6.6 and 1%  $K_3Fe(CN)_6$  (2.5 ml) as described [21].

## 2.8 Data Analysis

Data obtained from the tests as mean values from triplicate studies were fitted into kinetic model equations. The equations are Log Norm Cum, logistic dose response, decay 1+1 kinetic sigmoid abcd, and sigmoid abc models respectively. Iterative minimization of least squares was employed to estimate the parameters on the software Levenberg-marquardt algorithm (Table curve 2D SYSTAT USA) [27].

## 3. RESULTS

### 3.1 Nitric Oxide Scavenging Activity

The obtained results (Fig. 1) showed a logistic dose response curve, describing the nitric oxide scavenging effect of ethanolic leaf extracts of *Combretum zenkeri*. The hyperbolic curve depicts a steady increase in percentage nitric oxide scavenging ability at concentrations less than 200  $\mu\text{g/ml}$ . At higher concentrations nitric oxide radical scavenging was maintained at 72%, showing that an increase in concentration does not produce further increase in the extract's nitric oxide scavenging ability.

### 3.2 Hydroxyl Radical Scavenging Activity

The extract inhibited hydroxyl radical induced deoxyribose degradation in a concentration dependent manner (Fig. 2). In the result, the hydroxyl radical scavenging capacity of ethanolic leaf extracts of *C. zenkeri* produced a logistic dose response model,  $R^2 = 0.980164965$ . An increase in concentration produced a hyperbolic rise in percentage OH radical scavenging ability. At higher concentration, the effect was maintained at a lag showing that further increase in CZEE concentration does not give rise to any

improved percentage hydroxyl radical scavenging ability.

### 3.3 Possible Neuroprotective Potential

On lipid peroxidation in rabbit brain tissues, there is a marked inhibition of the production of thiobarbituric acid reactive substances (TBARS), by the ethanolic leaf extract of *C. zenkeri* (Fig. 3). Following the logistic dose response model, when compared to a standard antioxidant, quercetin, even though ethanolic leaf extract of *C. zenkeri* showed a lower % inhibition at 500  $\mu\text{g/ml}$  and 1000  $\mu\text{g/ml}$ , it shows  $IC_{50}$  at  $22.4 \pm 1.89$   $\mu\text{g/ml}$ .

### 3.4 Reducing Power ( $Fe^{3+}$ - $Fe^{2+}$ Transformation Ability)

The reducing power measured the concentration of ethanolic leaf extract of *C. zenkeri*, at which the Optical Density at 700 nm equals 0.5. When the result was subjected to logistic dose-response model curve-fitting, a sigmoidal curve with little reducing capacity at lower concentration was observed (Fig. 4). The reducing power of ethanolic leaf extract of *C. zenkeri* from the plot is 581.46  $\mu\text{g/ml}$ .

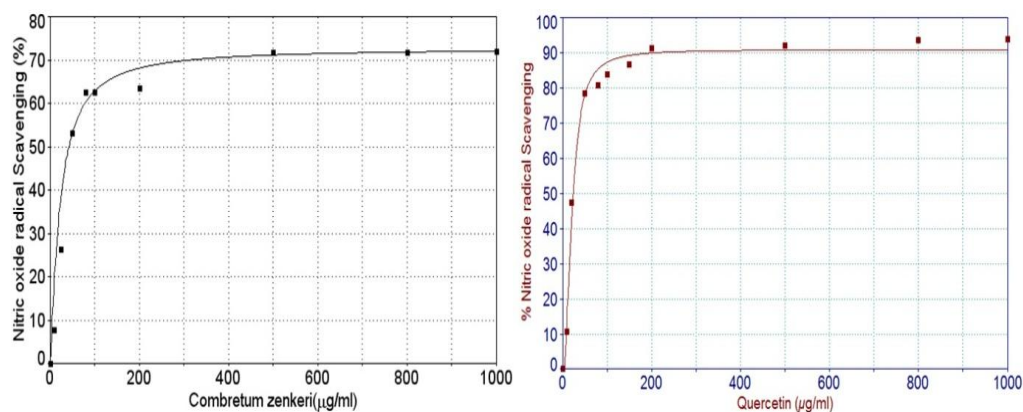
## 4. DISCUSSION

Plants have become extremely important in health and disease conditions, due to their antioxidants properties. In recent past several dietary and herbal formulation, which have free radical scavenging potential have shown relevance in the treatment of diseases [28]. The ability of the ethanolic leaf extract of *C. zenkeri* to scavenge nitric oxide ( $NO^{\cdot}$ ) corroborates the earlier study [17] on its rich phytochemical and vitamin content. Nitric oxide is an oxidative molecule involved in diverse physiological processes such as neurotransmission, regulation of blood pressure, defence mechanisms etc. [29]. Despite these beneficial effects of  $NO^{\cdot}$ , its contribution to oxidative damage is increasingly evident [13]. The antioxidants contents of *C. zenkeri* are mostly polyphenolic compounds [17,30] with high redox potentials that act as reducing agents, hydrogen donors and singlet oxygen quenchers [31]. This ability correlated with other works in which plant extracts were found to contain polyphenolics and possess NO scavenging ability [32,13,12]. The NO scavenging ability was better than that of *Drymaria diandra* [2].

Iron catalysed generation of ferryl-perferryl complex or hydroxyl radicals initiates lipid peroxidation that accelerates peroxidation by decomposing lipid hydro-peroxides into peroxy and alkoxy radicals. The hydroxyl radical is highly reactive and can damage biological molecules. It reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids that eventually yield carbonyl products like malondialdehyde (MDA) [33]. This explains the ability of rabbit brain tissue homogenate to produce MDA and other aldehydes [thiobarbituric acid reactive substances (TBARS)] in the presence of  $H_2O_2$  as a result of lipid peroxidation. Percentage inhibition of lipid peroxidation in rabbit brain tissues as shown in the result indicates the neuroprotective potential of ethanolic leaf extract of *C. zenkeri* [25]. When this is compared with standard antioxidant (quercetin) effect, ethanolic leaf extract of *C. zenkeri* (CZEE) can be said to have a more effective threshold ( $IC_{50}$ ) inhibition against lipid peroxidation. High oxygen utilization, high content of oxidizable polyunsaturated fatty acids and the presence of redox-active metals (Cu, Fe) give the brain tissues away to direct oxidative attack. CZEE is more effective than *Drymaria diandra* in goat liver homogenates [2] and *Chromolaena odorata* in rabbit brain homogenates [21] but less effective when compared to *Axonopus compressus* in egg yolk homogenate [34]. If exposure to organic solvents could show signs of neurotoxicity [35], the ethanolic leaf extract of *C. zenkeri* could serve as a solution towards protecting neurological tissues.

The ability of the CZEE to inhibit the production of the TBARS shows its reducing power as

shown in the reducing power analysis (Fig. 4), which has direct correlation. The reducing power of a compound is related to its electron transfer ability and may therefore serve as a significant indicator of its antioxidant activity [36]. Reducing power is a measure of the reductive ability of antioxidants, which is evaluated by the transformation of Fe (III) to Fe (II) in the presence of the sample extracts [37]. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [38]. In other words, it shows effectiveness in improving the stability of free radicals in a system. Given, that the reducing power measures the concentration of a substance at which the optical density (OD) at 700 nm equals 0.5, *Chromolaena odorata*, *Acalypha wilkesiana*, *Cnidioscolus aconitifolius*, *Vernonia amygdalina* and *Solanun scabrum* leaves have higher reducing power than ethanolic extracts of *C. zenkeri* leaves [21,39]. However, the ethanolic leaf extracts of *C. zenkeri* have higher reducing power than *Minosopu selengi* [40], *Withania somnifera* root [33] and *Morinda lucida* [41]. Plant extracts that possess reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants [42] Ethanolic leaf extract of *C. zenkeri* can possibly reduce oxidized intermediates in systems having shown to possess reducing power.



**Fig. 1. Nitric oxide scavenging effect of ethanolic leaf extracts of *Combretum zenkeri* graded concentrations**

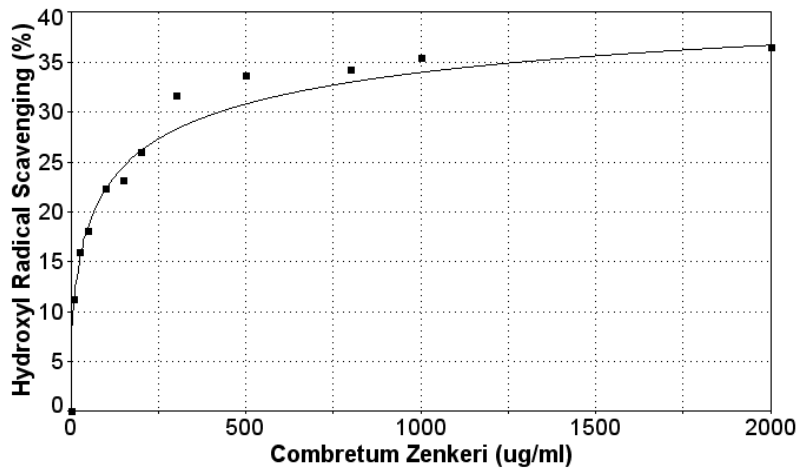


Fig. 2. Percentage Hydroxyl radical scavenging effect of ethanolic leaf extracts of *C. zenkeri* graded concentrations

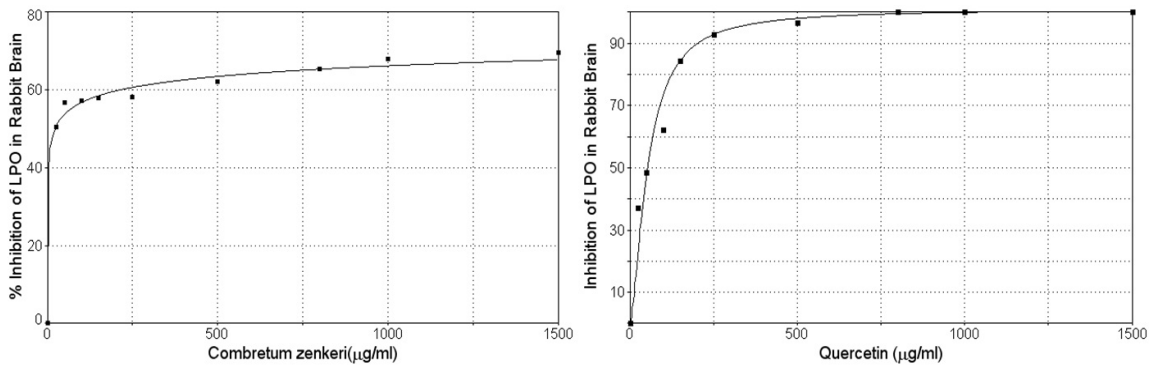


Fig. 3. Graded concentrations inhibition effect of ethanolic leaf extract of *C. zenkeri* on lipid peroxidation in rabbit brain tissues

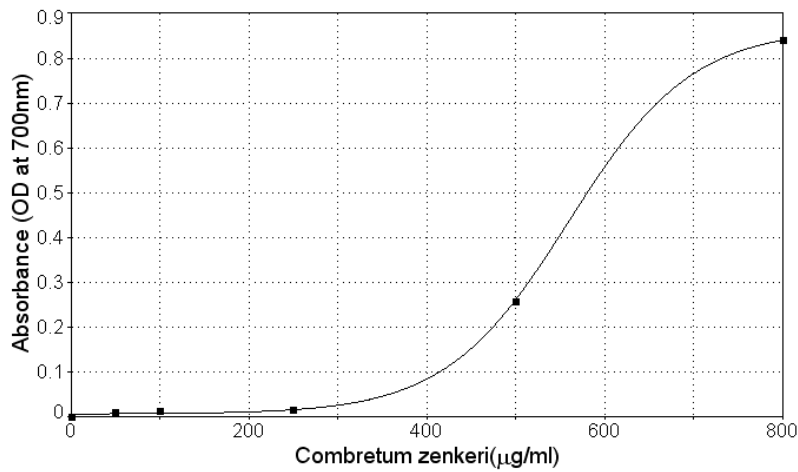


Fig. 4. Reducing power of ethanolic leaf extract of *Combretum zenkeri* = 581.46ug/ml

The low percentage hydroxyl radical scavenging of ethanolic leaf extracts of *C. zenkeri* may be due the affinity of the binding components in the system. The antioxidant(s) in the extract could also be acting as chelators of the Fe<sup>3+</sup> ions in the system making it less possible for them to complex with the deoxyribose, or simply donate hydrogen atoms and accelerate H<sub>2</sub>O<sub>2</sub> conversion to H<sub>2</sub>O [43]. Besides their antioxidant characteristics, flavonoids can chelate trace metals [44]. According to [45], the high binding energy values (of iron-quercetin complex) indicate that antioxidant quercetin is a powerful chelating agent that can sequester iron (II) in such a way to prevent its involvement in the Fenton reaction. The chemical content of leaf extracts of *C. zenkeri* [17] could have contributed in such reaction. The percentage hydroxyl radical scavenging activity of ethanol leaf extracts of *C. zenkeri* is low when compared to *Chromolaena odorata* [21], *Ocimum gratissimum* leaf extract [32] and wild mushroom species [46].

## 5. CONCLUSION

The results provide basis upon which the plant can be regarded as an asset in the quest to ameliorate oxidative diseases using naturally available secondary metabolites in plants. The phyto-metabolites found in the plant in the previous study [17] give reasons for the bio-efficacy of the plant extract.

## ETHICAL APPROVAL

We, hereby declare that this study was approved by the ethics committee of the Department of Biochemistry, Federal University of Technology, Owerri, Nigeria and all experiments were in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

- Rui H, Boyer J. Apple phytochemicals and their health benefits. *Rev Nutr J*. 2004;3:5.
- Mandal P, Misra TK, Ghosal M. Free-radical scavenging activity and phytochemical analysis in the leaf and stem of *Drymaria diandra* Blume. *Intl J Integrative Biol*. 2009;7(2):80-84.
- Valko M, Leibfritz D, Moncola J, Cronin M TD, Mazura, M, Telser J. Free radicals and antioxidants in normal physiological functions and humandisease. *IJBCB*. 2007;39:44-84.
- Cadenas E. Basic mechanisms of antioxidant activity. *Biofactors*. 1997;6:91-397.
- Lai LS, Chou ST, Chao WW. Studies on the antioxidant activities of Hsian-tsao (*Mesona procumbens*, Hemsl). *J Agric Food Chem*. 2001;49:963-968.
- Vinson JA, Liang XQ, Proch J, Hontz BA, Dancel J, Sandone N. Polyphenol antioxidants in citrus juices: *In vitro* and *in vivo* studies relevant to heart disease. *Adv. Exp Med Biol*. 2002;505:113-122.
- Mensah JK, Okoli RI, Ohaju-Obodo JO, Eifediyi K. Phytochemical, nutritional and medical properties of some leafy vegetables consumed by Edo people of Nigeria. *Afr J Biotechnol*. 2008;7(14):2304-2309.
- Ujowundu CO, Igwe CU, Enemor VHA, Nwaogu LA, Okafor OE. Nutritional and Anti-Nutritive Properties of *Boerhavia diffusa* and *Commelina nudiflora* Leaves. *Pak J Nutr*. 2008;7(1):90-92.
- Nwaogu LA, Alisi CS, Ibegbulem CO, Igwe CU. Phytochemical and antimicrobial activity of ethanolic extract of *Landophia Oweriensis* Leaf. *Afr J Biotechnol*. 2007;6(7):890-893.
- Ujowundu CO, Kalu FN, Okafor EO, Agha CN, Alisi CS, Nwaoguikpe RN. Evaluation of the chemical composition of *Dacryodes edulis* (G. Don) seeds. *Int J Biol Chem Sci*. 2010;4(4):1225-1233.
- Ujowundu CO, Kalu FN, Emejulu AA, Okafor OE, Nkwonta CG, Nwosunjoku EC. Evaluation of the chemical composition of *Mucuna Utilis* leaves used in herbal medicine in Southeastern Nigeria. *Afr J Pharmacy Pharmacol*. 2010;4(11):811-816,
- Etim OE, Ekanem SE, Sam SM. *In Vitro* Antioxidant Activity and Nitric Oxide Scavenging Activity of *Citrullus Lanatus* Seeds. *J Natural Sci Res*. 2013;3(12):126-133.
- Etim OE, Egbuna CF, Odo CE, Udo NM, Awah, FM. *In vitro* antioxidant and nitric oxide scavenging activities of *Piper guineense* seeds. *Global J Res Med Plants Indigen Med*. 2013;2(7):475-484.



14. Karthikumar S, Vigneswari K, Jegatheesan K. Screening of antibacterial and antioxidant activities of leaves *Eclipta prostrata* (L). Sci Res Essay. 2007;2(4):101-104.
15. Akinnibosun HA, Omatsola ME. Baseline studies of the floral biodiversity of a proposed crude oil exploration field in Edo State, Nigeria. Sci World J. 2011;1(1):453-457
16. Gbolade AA, Ogbale OO, Ajaiyeoba EO. Ethnobotanical survey of plants used in treatment of inflammatory diseases in Ogun State of Nigeria. Euro J Sci Res. 2010;43(2):183-191.
17. Ujowundu CO, Okafor OE, Agha NC, Nwaogu LA, Igwe KO, Igwe CU. Phytochemical and chemical composition of *Combretum zenkeri* leaves. J Medicinal Plants Res. 2010;4(10):965-968.
18. Handa SS, Khanuja SPS, Longo G, Rakesh DD. Extraction technologies for medicinal and aromatic plants. International centre for science and high technology; 2008. Accessed 20 February 2014. Available:<http://agritech.tnau.ac.in/horticulture>
19. Marcocci I, Marguire JJ, Droy – lefaiz MT, Packer L. The nitric oxide scavenging properties *Ginkgo biloba* extract. *Bioch Biophys. Res Com.* 1994;201:748-755.
20. Alisi CS, Onyeze GOC. Nitric oxide scavenging ability of ethyl acetate fraction of methanolic leaf extracts of *Chromolaena odorata* (Linn.). Afr J Bioch Res. 2008;2(7):145-150.
21. Alisi CS, Ojiako OA, Osuagwu CG, Onyeze GOC. Free radical scavenging and *In-vitro* antioxidant effects of ethanol extract of the medicinal herb *Chromolaena odorata* Linn. Br J Pharm Res. 2011;1(4):141-155.
22. Ohkawa H, Ohishi N, Yagi K. Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95:351–358.
23. Liu J, Edamatsu R, Kabuto H, Mori A. Antioxidant action of Guilingji in the brain of rats with FeCl<sub>3</sub> induced epilepsy. Free Rad Biol Med. 1990;9:451–454.
24. Halliwell B. Oxidants and human disease: Some new concepts. FASEB J. 1987;1:358.
25. Muralikrishnan D, Binu T, Bala VM. Antiparkinson drug *Mucuna pruriens* shows antioxidant and metal chelating activity. Phyto Res. 2008;22:6-11.
26. Oyaizu M. Studies on products of browning reaction prepared from glucoseamine. Japanese J Nutr. 1986;44:307–315.
27. Marquardt DW. An algorithm for least squares estimation of non-linear parameters. J Soc Ind App. Math. 1964;2:431-441.
28. Tiwari OP, Tripathi YB. Antioxidant properties of different fractions of *Vitex negundo* Linn. Food Chem. 2007;100:1170-1176.
29. Bergendi L, Benes L, Durackova Z, Ferencik M. Chemistry, physiology and pathology of free radicals. Life Sci. 1999;65:1865–1874.
30. Acharya JD, Ghaskadbi SS. Protective effect of pterostilbene against free radical mediated oxidative damage. Complementary Alter Med. 2013;13:238
31. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kulaja TS, Heinonen M. Antioxidant activity of plants extracts containing phenolics compounds. J Agric Food Chem. 1999;47:3954-3962.
32. Awah FM, Verla AW. Antioxidant activity, nitric oxide scavenging activity and phenolic contents of *Ocimum gratissimum* leaf extract. J Med Plants Res. 2010;4(24):2479-2487.
33. Chaudhuri D, Ghate NB, Sarkar R, Mandal R. Phytochemical analysis and evaluation of antioxidant and free radical scavenging activity of *Withania somnifera* root. Asian J Pharm Clin Res. 2012;5(4):193-199.
34. Ibeh BO, Maxwell E, Bitrus HJ. Phytochemical compositions and *In vitro* antioxidant capacity of methanolic leaf extract of *Axonopus compressus* (P. Beauv.). Euro J Med Plants. 2013;3(2):254-265.
35. Hegazy NM, Abdel GNB, Metwally FM, Ahmed HH, Abdel RER, Abraham KS, Sharaf NE. Neurotoxic effects of organic solvents in exposed workers: Altered expression of some biochemical markers. New York Sci J. 2010;3(11):171-176.
36. Raja G, Shaker IA, Sailaja I, Swaminathan R, Basha SS, Babu KS. Phytochemical screening and reducing power assay of nut extracts of *Juglans regia* L. Int J Chemical Life Sci. 2012;1(1):1026-1032.
37. Gülçin I, Oktay M, Kireççi E, Küfrevloglu I. Screening of antioxidant and antimicrobial activities of anise (*Pimpinella*



- anisum* L.) seed extracts. Food Chem. 2003;83:371-382.
38. Osawa T. Novel natural antioxidants for utilization in food and biological systems. In: Uritani I, Garcia VV, Mendoza EM. (Eds). Post harvest biochemistry of plant food-materials in the tropics. Japan Scientific Societies Press, Japan. 1994;241-251.
39. Anokwuru CP, Adaramola FB, Ogunnowo AA, Idigo OC, Aina DA. Comparative phenolic, flavonoid contents and antioxidant activity of aqueous ethanol and water extracts of four medicinal plants In Nigeria. *Academ Arena*. 2012;4(7):1-5.
40. Saha MR, Hasan SMR, Akter R, Hossain MM, Alam MS, Alam MA, Mazumder MEH. *In Vitro* free radical scavenging activity of methanol extract of the leaves of *Mimusops Elengi* Linn. *Bangl J Vet Med*. 2008;6(2):197-202.
41. Ogunlana OE, Ogunlana O, Farombi OE, Morinda Lucida. Antioxidant and reducing activities of crude methanolic stem bark extract. *Adv In Nat Appl Sci*. 2008;2(2):49-54.
42. Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J Agric Food Chem*. 1995;43:27-32.
43. Wang H, Liu T, Chen Y, Shiuan D. Protective effect of freeze-dried extract of vegetables and fruits on the hydroxyl radical mediated oxidative damage of DNA and decrease of erythrocytes deformability. *Appl Biochem Biotechnol*. 2007;141(2-3):241-249.
44. Wang BJ, Lien YH, Yu ZR. Supercritical fluid extractive fractionation-study of the antioxidant activities of propolis. *Food Chem*. 2004;86:237-243.
45. Leopoldini M, Russo N, Chiodo S, Toscano M. Iron chelation by the powerful antioxidant flavonoid quercetin. *J. Agric. Food Chem*. 2006;54:6343-6351.
46. Oyetayo VO. Free radical scavenging and antimicrobial properties of extracts of wild mushrooms. *Brazilian J. of Microbiol*. 2009;40:380-386.

© 2015 Ujowundu et al; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

*Peer-review history:*

*The peer review history for this paper can be accessed here:*  
<http://www.sciencedomain.org/review-history.php?iid=797&id=32&aid=7298>