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Antioxidant and Inhibitory Effect of Scent Leaf (*Ocimum gratissimum*) on Fe2+ and Sodium Nitroprusside Induced Lipid Peroxidation in Rat Brain *In vitro*

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Abstract: Neurodegenerative diseases have been linked to oxidative stress arising from peroxidation of membrane biomolecules and high levels of Fe and Sodium nitroprusside have been reported to play an important role in neurodegenerative diseases and other brain disorder. Therefore, this study sought to investigate the inhibitory effect of aqueous, ethanolic and ethyl acetate extract of *Ocimum gratissimum* leaves on $FeSO_4$ and Sodium Nitroprusside (SNP) induced lipid peroxidation in rat brain *in vitro*. Incubation of the brain tissue homogenate in the presence of $FeSO_4$ and SNP showed both pro-oxidants [Fe^{2+} and sodium nitroprusside (SNP)] caused a significantly reduction in (p<0.05) the accumulation of lipid peroxides in a concentration dependent manner. However, the ethyl acetate fraction significantly ($P < 0.05$) inhibited Fe^{2+} induced oxidative stress in the rat brain tissue homogenates than the aqueous and ethanolic extract respectively. This higher inhibitory effect of *Ocimum gratissimum* could be attributed to its significantly higher phytochemical content, Fe^{2+} chelating ability, hydroxyl scavenging ability, total phenolic content and reducing power. However, part of the mechanisms through which the extractable phytochemicals in *Ocimum gratissimum* protect the brain may be through their antioxidant activity, Fe^{2+} chelating, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and OH● scavenging ability. Therefore, oxidative stress in the brain could be potentially managed/prevented by dietary intake of *Ocimum gratissimum*.

Key words: Fe^{2+} Chelation · Lipid Peroxidation · Phenolics · Antioxidants · Rat · Brain

INTRODUCTION

Oxidative stress occurs where there is an imbalance between the production of reactive oxygen and the biological system's ability to readily detoxify the reactive intermediate or easily repair the resulting damage [1]. All forms of life maintain a reducing environment within their cells by enzymes through a constant input of metabolic energy. Disturbances in this normal redox state could lead to the production of peroxides and free radicals that damage all compounds of the cell, including proteins, Lipids and DNA [2]. Numerous *in vitro* and *in vivo* studies reported that polyphenolic compounds protect against oxidative stress [3, 4]. Some of these medicinal plants have been investigated for their antioxidative properties and treatment of such diseases [5, 6]. Many of the metabolites from these medicinal plants especially flavonoids exhibited potent antioxidant activity *in vitro* and *in vivo* [7-9]. Most of the free radical scavenging potential in herbs and spices is due to the redox properties of phenolic compounds which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers [10, 11]. The importance of free radicals and reactive oxygen species (ROS) has attracted increasing attention. ROS and free radical mediated reactions are involved in degenerative or pathological processes such as aging, cancer, rheumatoid arthritis, coronary heart disease and Alzheimer's disease. Many antioxidant compounds, naturally occurring in plant sources have been identified on free radical or active

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oxygen scavenger. Many synthetic antioxidant components have shown toxic or mutagenic effects, which have shifted the attention onto the naturally occurring antioxidants [12]. Water, ethanol and ethyl acetate has been used widely to extract bioactive components from plants. It is noted that an extraction solvent system is selected according to the purpose of extraction such as the nature of interested components, the availability of reagents and equipments, cost and safety concerns and so on [13]. *Ocimum gratissimum* Linn (Labiatae) is grown for the essential oils in its leaves and stems. Eugenol, thymol, citral, geraniol and linalool have been extracted from the oil [14]. This study was to characterize the antioxidative properties of aqueous, ethanolic and ethyl acetate extracts of *Ocimum gratissimum* and their inhibitory effects on some neurototoxin (Fe^{2+} and sodium nitroprusside) induced lipid peroxidation in Rat's brain homogenates *in vitro*.

MATERIALS AND METHODS

Collection of Plant: Fresh leaves of *O. gratissimum* were bought in the market at Ado, Nigeria. The plant was identified and authenticated by a plant scientist in the Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria and a voucher specimen (U.H.A.E 15) was deposited accordingly at the herbarium of the Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria. The yield was calculated and the dry extract was stored in a refrigerator at-4°C until use for the experiments.

Chemicals and Reagents: Chemicals and reagents used such as 1,10-phenanthroline, gallic acid, Folin-Ciocalteau's reagent were procured from Sigma-Aldrich, Inc., (St. Louis, MO), trichloroacetic acid (TCA) was sourced from Sigma-Aldrich, Chemie GmbH (Steinheim, Germany), dinitrophenyl hydrazine (DNPH) from ACROS Organics (New Jersey, USA), hydrogen peroxide, methanol, acetic acid and Fecl3 were sourced from BDH Chemicals Ltd., (Poole, England), $CuSO_4 \cdot 5H_2O$, H_2SO4, sodium carbonate, AlCl34, potassium acetate, Tris-HCl buffer, sodium dodecyl sulphate, FeSO4, potassium ferricyanide and ferric chloride were of analytical grade while the water was glass distilled.

Preparation of Extract

Aqueous Extract Preparation: 50g of the powdered sample were extracted with distilled water of 500mls (via maceration) for 48hrs using the method of Aguawa And Mittal [15]. The mixtures were decanted and filtered using sterile whatman paper No1. The filterate measured up to 425ml and evaporated to dryness using a freeze dryer to obtain 8 % yield. The crude extracts were later subjected to bioassay analyses. From the stock solution, concentrations of 10, 20, 40, 80 and 100mg/ml were obtained by serial dilution. These were stored until further use.

The percentage yield of extraction calculated as follows was 8 %.

$$\text{Percentage yield} = \frac{\text{Weight of the dry extract}}{\text{Weight of powdered leaves}} \times 100\%$$

Ethanolic Extract Preparation: 120g of the powdered sample were extracted with solvent combination (via maceration) of 70% ethanol for 48hrs using the maceration method described by Malairjan *et al*. [16]. One litre of 70% ethanol were used. The mixtures were decanted and filtered using sterile whatman paper No1. The filterate measured up to 600mls and evaporated to dryness using a freeze dryer to obtain 9.92 % yield.

Ethyl Acetate Preparation: 120g of the powdered sample were extracted with solvent combination (via maceration) of 70% ethanol for 48hrs using the method of Hong *et al.* [17]. One liter of 70% ethanol were used. The percentage yield of extraction calculated as follows was 2.08 %.

Determination of Total Phenolic Content: The extractable phenol content were determined on the extracts using the method reported by Singleton *et al*. [18]. Appropriate dilutions of the extracts were mixed with 2.5 mL of 10% Folin-Ciocalteau's reagent (v/v) and neutralized by 2.0 mL of 7.5% sodium carbonate. The reaction mixtures were incubated for 40 min at 45 °C and the absorbance were measured at 765nm in the spectrophotometer. The total phenol content were subsequently calculated using gallic acid as standard. The absorbance of the blue colour that developed were read at 765 nm. The concentrations of total phenols were expressed as mg/gm of dry extract.

The concentrations of total phenolic compounds in the extract were determined by using the formula:

$T = CV/M$

Where,

T = Total phenolic content mg/gm of plant extract in GAE,
C = Concentration of Gallic acid from the calibration curve,
V = volume of the extract in ml,
M = wt of the pure plant extract.

Determination of Reductive Ability: The reducing property of the ginger extracts were determined by assessing the ability of the extract to reduce a $FeCl_3$ solution as described by Pulido *et al*. [19]. A 2.5 mL aliquot were mixed with 2.5 mL, 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL, 1% potassium ferricyanide. The mixtures were incubated at 50 °C for 20 min and then 2.5 mL, 10% TCA were added. These were then centrifuged at 650 g for 10 min. A 5 mL of the supernatant were mixed with an equal volume of water and 1 mL, 0.1% ferric chloride. The same treatments were performed to a standard ascorbic acid solution and the absorbance taken at 700 nm. The reducing power were then calculated and expressed as ascorbic acid equivalent.

Determination of DPPH Radical Scavenging Activity: The free radical scavenging activity of the hydroalcoholic extract of *Ocimum gratissimum* was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH) using the method of Blois; a method based on the reduction of a methanolic solution of the coloured DPPH radical. [20,21] Used as a reagent, DPPH evidently offers a convenient and accurate method for titrating 0.1 mM solution of DPPH in methanol was prepared and 1 mL of this solution was added to 3 mL of the extract suspension in water at different concentrations (1, 2, 3, 4 and 5 mg). After 30 minutes of incubation, absorbance was measured at 517 nm. Ascorbic acid was used as reference material. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. All the tests were performed in triplicate and the results averaged. The percentage reduction in absorbance was calculated from the initial and final absorbance of each solution [22, 23]. The percentage inhibition was calculated by comparing the absorbance values of control and samples. Percentage scavenging of DPPH radical was calculated using the formula,

$$\% \text{ Scavenging of DPPH} = (Abs_{control} - Abs_{test\ sample})/Abscontrol \times 100$$

Determination of Iron Chelation Ability: The Fe^{2+} chelating ability of both extracts were determined using a modified method of [24] with a slight modification by Puntel *et al*.[25]. Freshly prepared 500 µmol L^{-1} $FeSO_4$ (150 µL) were added to a reaction mixture containing 168 µL of 0.1 mol L^{-1} Tris-HCl (pH 7.4), 218 µL saline and the extracts (0-25 µL). The reaction mixtures were incubated for 5 min, before the addition of 13 µL of 0.25% 1, 10-phenanthroline (w/v). The absorbances were subsequently measured at 510 nm in a spectrophotometer. The Fe^{2+} chelating ability were calculated with respect to the control.

$$\text{Percentage } Fe^{2+} \text{ chelating ability (\%)} = (Abs_{control} - Abs_{test\ sample})/Abscontrol \times 100$$

where $Abs_{control}$ = absorbance of the control (reacting mixture without the test sample) and, $Abs_{test\ sample}$ = absorbance of reacting mixture with the test sample.

Determination of Hydroxyl Radical Scavenging Effect: Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and test compounds for hydroxyl radical generated by Fe^{3+}-Ascorbate-EDTA H_2O_2 system (Fenton reaction) according to the method of Kunchandy and Rao [26] The hydroxyl radicals attack deoxyribose that eventually results in TBARS formation. The reaction mixture contained in a final volume of 1.0 mL, 100 ìl of 2-deoxy-2-ribose (28 mM in KH_2PO_4-K_2HPO_4 buffer, pH 7.4), 500ìl solutions of various concentrations of hydroalcoholic extract (1, 2, 3, 4 and 5 mg) in KH_2PO_4 -KOH buffer (20 mM, pH 7.4), 200 ìl of 1.04 mM EDTA and 200 ìM $FeCl_3$ (1:1 v/v), 100 ìl of 1.0 mM H_2O_2 and 100 ìl of 1.0 mM ascorbic acid was incubated at 37 °C for 1 h. The free radical damage imposed on the substrate, deoxyribose was measured as TBARS by the method of Ohkawa *et al*. [27] 1.0 mL of thiobarbituric acid (1%) and 1.0 mL of trichloroacetic acid (2.8%) were added to the test tubes and the incubation was continued at 100 °C for further 20 min. After cooling, absorbance was measured at 532 nm against control containing deoxyribose and buffer.

Lipid Peroxidation Assay

Preparation of Tissue Homogenates: Normal healthy rats were decapitated under mild diethyl ether anaesthesia and the brain was rapidly dissected, placed on ice and weighed. This tissue was subsequently homogenized in cold saline (1/10 w/v) with about 10 up-and-down strokes at approximately 1,200 rpm in a Teflon glass homogenizer. The homogenate was centrifuged for 10 min at 3,000 x g to yield a pellet that was discarded and a low-speed supernatant (S1) containing mainly water, proteins, lipids (cholesterol, galactolipid, individual phospholipids, gangliosides), DNAand RNA that was kept for lipid peroxidation assay [28]. The ethical committee of the Afe Babalola University approved this study.

Lipid Peroxidation and TBA Reactions: The lipid peroxidation assay was carried out using the modified method of Ohkawa *et al*.[29].

Statistical Analysis: Statistical analysis of difference between groups was evaluated by one-way ANOVA followed by student t test. The values P < 0.05 were regarded as significant.

RESULT

The total phenolic content of aqueous, ethyl acetate and ethanolic extract of *Ocimum gratissimum* is 4.00±0.00 mg GAE/g, 10.34±0.47 mg GAE/g and 1.56±0.06 mg GAE/g respectively.

The ethyl acetate extracts show significantly ($P < 0.05$) higher reducing power than ethanol and water extracts of *Ocimum gratissimum* Figure 1. IC_{50} value for reducing power by the ethanolic, ethyl acetate and aqueous extracts of *O. gratissimum* were 0.94, 1.55 and 0.49 mg/ml, respectively.

The scavenging activities of DPPH exerted by each extract were summarized in figure 2. The scavenging effect of extracts in the range 1-5 mg/ml on the DPPH radical increased with an increasing concentration of OG extracts figure 2. IC_{50} value for DPPH scavenging by the ethanolic, ethyl acetate and aqueous extracts of *O. gratissimum* were 2.47, 1.58 and 5.29 mg/ml, respectively.

The metal chelating effect of ethanol, ethyl acetate, water extracts of *Ocimum gratissimum* decreased in the order of ethyl acetate >_ethanol >_aqueous extract (P <_0.05) and were increased with the increased concentration of *Ocimum gratissimum* extracts figure 3.

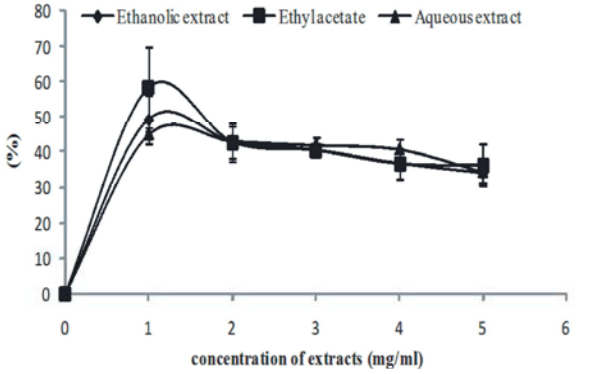

Fig. 1: Ferric reducing antioxidant properties of ethanolic, ethyl acetate and aqueous extracts of *Ocimum gratissimum*.

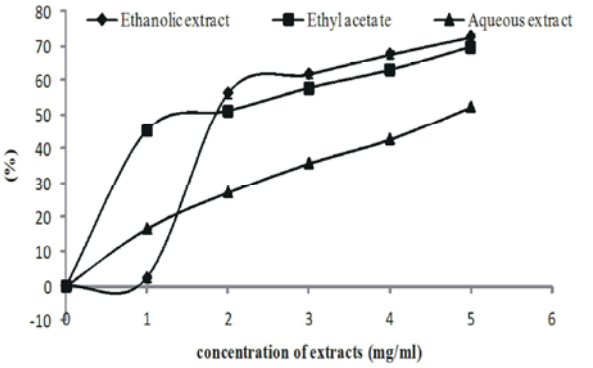

Fig. 2: DPPH free radical scavenging ability of ethanolic, ethyl acetate and aqueous extracts of *Ocimum gratissimum*.

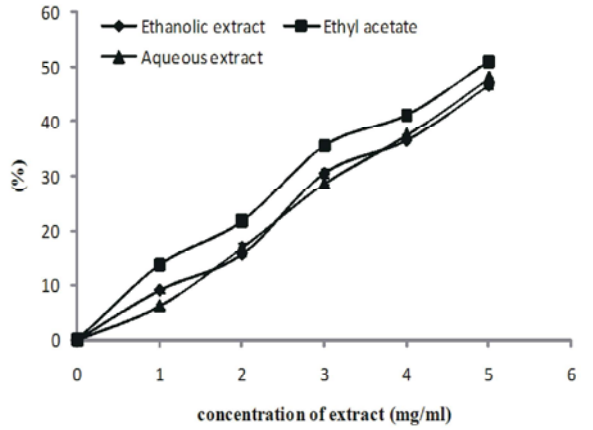

Fig. 3: Iron chelating ability of ethanolic, ethyl acetate and aqueous extract of *Ocimum gratissimum*.

Ocimum gratissimum ethyl acetate extract had significantly (P <_0.05) higher chelating effect than ethanolic and aqueous extracts. IC_{50} values for chelating effect of ethanolic, ethyl acetate and aqueous extracts of

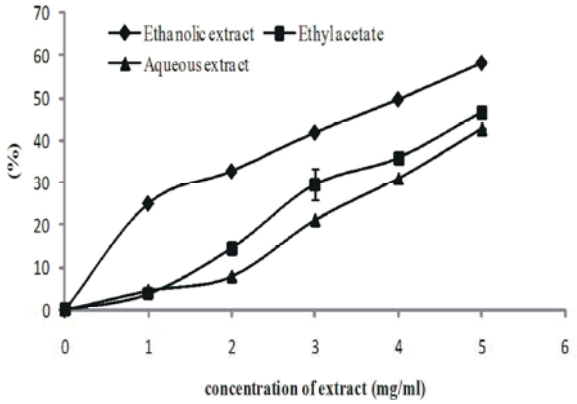

Fig. 4: Hydroxyl free radical scavenging ability of ethanolic, ethyl acetate and aqueous extracts of *Ocimum gratissimum*.

O. gratissimum were 6.81, 5.51 and 6.34 mg/ml, respectively. This shows that ethyl acetate extract of *O. gratissimum* has a higher chelating ability than aqueous and ethanolic form.

Ferric-EDTA was incubated with H2O2 and ascorbic acid at pH 7.4. Any hydroxyl radical scavenger added to the reaction would compete with deoxyribose for the availability of hydroxyl radicals. IC_{50} values for hydroxyl radical effect of ethanolic, ethyl acetate and aqueous extracts of *O. gratissimum* were 3.98, 6.48 and 8.83 mg/ml, respectively as shown in figure 4.

The inhibition of aqueous extracts with Fe^{2+} and SNP induced lipid peroxidation in isolated rat brain homogenates by the extracts is presented in (Table 1). The highest inhibition was observed to be 0.33mg/ml concentrations in the brain for Fe^{2+} while for SNP 1.33mg/ml was exceptionally high. The result shows that the plant is has a high potency to inhibit TBARS production in the liver induced with Fe^{2+}, as a high correlation was observed ($r^2 = 0.742$) than that of SNP with ($r^2 = 0.510$).

Table 2 shows the interaction (inhibition) of the plant extracts with Fe (II) and SNP induced lipid peroxidation in rat brain. The highest inhibition was observed with the 2.67mg/ml concentration in the brain, with that of the Fe^{2+} being exceptionally high. *Ocimum gratissimum* caused a significant inhibition ($p < 0.05$) in SNP induced lipid peroxidation in the brain in a concentration dependent manner (0.33-3.33mg/ml). The plant shows a high potency to inhibit TBARS production in the liver induced with SNP, as a high correlation was observed ($r^2 = 0.794$) than that of Fe^{2+} with ($r^2 = 0.234$).

Table 3 shows the inhibition of the ethyl acetate extracts with Fe (II) and SNP induced lipid peroxidation in brain of rat. *Ocimum gratissimum* caused a significant inhibition ($p < 0.05$) in Fe^{2+} induced lipid peroxidation in the brain in a concentration dependent manner (0.33-3.33mg/ml). The highest inhibition was observed to be 0.66mg/ml concentration in the liver for SNP.

Table 1: Antioxidant activity of aqueous extract of *Ocimum gratissimum* (OG) on iron sulphate induced and Sodium nitroprusside (SNP) induced lipid peroxidation in brain homogenate *in vitro*.

Treatments	*OG concentration (mg/ml)*	*% Inhibition*	*Logarithm equation*	*IC_{50} (mg/ml)*
Basal	-	78.98±0.39	Y = -7.27In(x) + 82.88	2.41±0.02
Control	-	-	$R^2 = 0.742$	
Iron + OG	0.33	79.70±3.04		
Iron + OG	0.66	72.61±2.74		
Iron + OG	1.33	71.12±3.81		
Iron + OG	2.67	72.44±0.91		
Iron + OG	3.33	70.70±0.31		
Basal	-	66.72±1.24	Y = -3.92In(x) + 71.02	2.87±0.02
Control	-	-	$R^2 = 0.510$	
SNP + OG	0.33	67.47±1.04		
SNP + OG	0.66	66.42±1.02		
SNP + OG	1.33	68.40±2.42		
SNP + OG	2.67	64.55±1.51		
SNP + OG	3.33	62.45±0.75		

*Results are expressed as means of three experiments in duplicate ± standard deviation

Table 2: Antioxidant activity of ethanolic extract of *Ocimum gratissimum* (OG) on iron sulphate induced and Sodium nitroprusside (SNP) induced lipid peroxidation in brain homogenate *in vitro*.

Treatments	*OG concentration (mg/ml)*	*% Inhibition*	*Logarithm equation*	*IC_{50} (mg/ml)*
Basal	-	73.96±5.72	Y = -7.98In(x) + 79.76	2.28±0.02
Control	-	-	R^2 = 0.234	
Iron + OG	0.33	71.32±1.47		
Iron + OG	0.66	71.16±2.14		
Iron + OG	1.33	73.01±2.50		
Iron + OG	2.67	74.13±8.77		
Iron + OG	3.33	56.63±5.14		
Basal	-	70.50±1.92	Y = -6.16In(x) + 72.98	2.45±0.02
Control	-	-	R^2 = 0.794	
SNP + OG	0.33	67.48±1.45		
SNP + OG	0.66	67.38±1.50		
SNP + OG	1.33	65.48±0.56		
SNP + OG	2.67	63.81±1.34		
SNP + OG	3.33	60.25±1.20		

*Results are expressed as means of three experiments in duplicate ± standard deviation

Table 3: Antioxidant activity of ethyl acetate fraction of *Ocimum gratissimum* (OG) on iron sulphate induced and Sodium nitroprusside (SNP) induced lipid peroxidation in brain homogenate *in vitro*.

Treatments	*OG concentration (mg/ml)*	*% Inhibition*	*Logarithm equation*	*IC_{50} (mg/ml)*
Basal	-	68.82±2.98	Y = -3.26In(x) + 71.60	3.07±0.02
Control	-	-	R^2 = 0.942	
Iron + OG	0.33	69.13±3.65		
Iron + OG	0.66	68.11±3.75		
Iron + OG	1.33	67.31±3.31		
Iron + OG	2.67	66.72±3.40		
Iron + OG	3.33	65.26±3.80		
Basal	-	68.92±2.98	Y = -4.96In(x) + 73.47	2.65±0.05
Control	-	-	R^2 = 0.651	
SNP + OG	0.33	68.37±1.77		
SNP + OG	0.66	70.11±1.25		
SNP + OG	1.33	67.48±2.22		
SNP + OG	2.67	65.58±2.28		
SNP + OG	3.33	63.16±2.92		

*Results are expressed as means of three experiments in duplicate ± standard deviation

The result shows that the plant is has a high potency to inhibit TBARS production in the liver induced with Fe^{2+}, as a high correlation was observed (r^2 = 0.942) than that of SNP with (r^2 = 0.651).

DISCUSSION

Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups [30]. The phenolic compounds may contribute directly to antioxidative action [31].

The antioxidant activity has been reported to be concomitant with the development of reducing power [32]. The reducing power of extract might be due to its hydrogen donating ability, as described by Shimada *et al.* [33]. This result shows that the aqueous extract of *O. gratissimum* has a higher reducing power than ethanolic and ethyl acetate extract.

DPPH is a stable free radical in aqueous or methanol and ethanol solution and accept an electron or hydrogen radical to become a stable diamagnetic molecule. It is usually used as a substrate to evaluate the antioxidative activity of antioxidants [31]. DPPH scavenging activity has been used by various researchers as a quick and reliable parameter to assess the *in vitro* antioxidant activity of crude plant extracts [34,35]. In DPPH test the

ability of a compound to act as donor for hydrogen atoms or electrons was measured spectrophotometrically. In the present investigation *Ocimum gratissimum* at different doses demonstrated significant DPPH scavenging activity indicating their abilities to act as radical scavengers.

Iron can stimulate lipid peroxidation by the Fenton reaction and also accelerates peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation [36]. Metal ions play an important role in the acceleration of oxidation of important biological molecules, for instance they may catalyze the formation of first few radicals that can lead to propagation of the radical chain reaction in lipid peroxidation [37]. This indicates that the chelation property of the extracts on the Fe^{2+} ions may be able to afford protection against oxidative damage. It was reported that chelating agents, which form d-bond with a metal, are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ion [37], Chelating agents inhibit the radical mediated oxidative chain reactions in biological or food systems and consequently improve human health and food quality, stability and safety. Citric acid and its salts phosphates and salts of EDTA are among the most commonly used chelators. In addition, plant phenolic compounds have also been found to be good metal ion chelators [38].

Hydroxyl radicals are the major active species causing lipid oxidation and enormous biological damage [39,40]. The deoxyribose method is a simple assay to determine the rate constants for reactions of hydroxyl radicals [41]. Hydroxyl radicals were formed in free solution and were detected by their ability to degrade 2-deoxy-2-ribose in to fragments that on heating with TBA at low PH form a pink chromogen [42, 43].

The finding that Fe^{2+} caused a significant increase in the MDA content of the brain agreed with earlier report where Fe^{2+} was shown to be a potent initiator of lipid peroxidation in the brain (pro-oxidant) [44]. The increased lipid peroxidation in the presence of Fe^{2+} could be attributed to the fact that Fe^{2+} can catalyze one-electron transfer reactions that generate reactive oxygen species, such as the reactive OH●, which is formed from H_2O_2 through the Fenton reaction. Iron also decomposes lipid peroxides, thus generating peroxyl and alkoxyl radicals, which favors the propagation of lipid oxidation [45]. Elevated Fe^{2+} content in the brain had been linked to a host of neurodegenerative diseases. Elevated Fe^{2+} levels have been localized to degenerate regions of brains from Parkinson's disease patients, a finding also demonstrated in animal models of the disease. However, the ethanolic, aqueous and ethyl acetate extractable phytochemicals from the spices caused a concentration dependent (0.33-3.33mg/ml) significant decrease in the inhibition of the Fe^{2+} stressed brain homogenates and ethyl acetate fraction significantly ($P < 0.05$) inhibited Fe^{2+} induced oxidative stress in the rat brain tissue homogenates than the aqueous and ethanolic extract respectively. The decrease in the Fe^{2+} induced lipid peroxidation in the rat brain homogenates in the presence of the extracts could be as result of the ability of the extracts to chelate Fe^{2+} and/or scavenge free radicals produced by the Fe^{2+} catalyzed production of reactive oxygen species (ROS) in the rat brain. However, the higher ability of the ethyl acetate extract to protect the rats' brain could be because of its higher extractable phytochemical content. Antioxidants carry out their protective role on cells either by preventing the production of free radicals or by neutralizing/scavenging free radicals produced in the body or by reducing/chelating the transition metal composition of foods [44, 46, 47]. All the extracts chelate Fe^{2+} at the concentration of the extracts tested with ethyl acetate better chelator than aqueous and ethanolic extract. This result however, is in agreement with the Fe^{2+} induced lipid peroxidation (Table 1, Table 2, Table 3), phenolic content and antioxidant activity of the extracts, suggesting that Fe chelation may be one of the possible mechanisms through which antioxidant phytochemicals from *Ocimum gratissimum* prevent lipid peroxidation in brain by forming a complex with Fe, thus preventing the initiation of lipid peroxidation. Sodium nitroprusside (SNP) can cause brain damage through the release of cyanide and/or nitric oxide (NO) which can acts either alone or in conjunction with other reactive oxygen species (ROS) such as superoxide radical to cause neuronal damage [28,25]. The Fe produced from the decomposition of the sodium nitroprusside could also sustain the lipid peroxidation, by initiating the production of OH radical through Fenton's reaction [44].

However, the ethanolic, aqueous and ethyl acetate extract of *Ocimum gratissimum* inhibited 7 µM sodium nitroprusside induced lipid peroxidation in rat's brain in a concentration dependent manner. This indicates that all the plant extract were able to scavenge the NO^{*} produced by sodium nitroprusside and chelate the Fe produced as a result of the decomposition of the sodium nitroprusside.

CONCLUSION

The aqueous, ethanolic and ethyl acetate extract of *Ocimum gratissimum* were able to protect brain from Fe^{2+} induced lipid peroxidation. However, the ethyl acetate extract of *Ocimum gratissimum* had a higher protective effect against both Fe^{2+} induced lipid peroxidation in brain (*in vitro*) than that of aqueous and ethanolic extracts. The higher protective effect of *Ocimum gratissimum* may be due to presence of higher antioxidant phytochemicals. And part of the mechanisms through which the extractable phytochemicals in *Ocimum gratissimum* protect the brain may be through their antioxidant properties.

Author Disclosure Statement: No competing financial interests exist

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