American Journal of Food Technology

ISSN 1557-4571

Academic Journals Inc.

www.academicjournals.com

American Journal of Food Technology 3 (5): 325-334, 2008
ISSN 1557-4571

Antioxidative Potential of *Ocimum gratissimum* and *Ocimum canum* Leaf Polyphenols and Protective Effects on Some Pro-Oxidants Induced Lipid Peroxidation in Rat Brain: An *in vitro* Study

Ganiyu Oboh
Department of Biochemistry,
Federal University of Technology, P.M.B. 704, Akure, Nigeria

Abstract: This study seeks to determine the antioxidant properties and the ability of polyphenol extracts from *Ocimum gratissimum* (OGP) and *Ocimum canum* (OCP) leaves (commonly used Spices in Tropical Africa, Asia and South America) to inhibit some pro-oxidants (Fe^{2+} and sodium nitroprusside) induced lipid peroxidation in rat's brain homogenates-*in vitro*. The free soluble polyphenols were extracted with 80% acetone; thereafter the ability of the extracts to inhibit 25 μM $FeSO_4$ and 7.0 μM sodium nitroprusside induced lipid peroxidation in isolated rat's brain was determined. The antioxidant properties of the extracts as typified by their total phenol content, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging ability, reducing power and Fe (II) chelating ability were also determined. The results of the study revealed that both pro-oxidants [Fe^{2+} (256%) and sodium nitroprusside (160%)] caused a significant increase ($p<0.05$) in the malondialdehyde (MDA) content of the brain. However, polyphenol extracts (0.4-1.6 μg mL^{-1}) from both species of *Ocimum* caused a dose-dependent significant decrease ($p<0.05$) in the malondialdehyde (MDA) contents of the brain. However, polyphenol from *Ocimum canum* had a significantly higher ($p<0.05$) inhibitory effect on both Fe (II) and sodium nitroprusside induced lipid peroxidation in the rat's brain homogenates than that of *Ocimum gratissimum*. This higher inhibitory effect of *Ocimum canum* could be attributed to its significantly higher ($p<0.05$) total phenol content, Fe (II) chelating ability, reducing power and free radical scavenging ability. Therefore, Fe (II) and sodium nitroprusside induced oxidative stress in the brain could be potentially prevented/ managed by dietary intake of *Ocimum gratissimum* (OGP) and *Ocimum canum* (OCP) leaves, however *Ocimum canum* (OCP) leaf extract is more active. These antioxidant properties of the *Ocimum* spp. polyphenol may have contributed to the use of the leaves in the treatment of mental illness in folk medicine.

Key words: *Ocimum*, antioxidant, polyphenols, Fe (II), sodium nitroprusside, brain

INTRODUCTION

Oxidative stress results from either a decrease of natural cell antioxidant capacity or an increased amount of Reactive Oxygen Species (ROS) in organisms. Free radicals are chemically active atoms or molecular fragments that have a charge due to an excess or deficient number of electrons. Examples of free radicals are the super oxide anion, hydroxyl radical, transition metals such as iron and copper, nitric acid and ozone. Free radicals are highly unstable because they have one or more unpaired electron. They scavenge in the body to grab or donate electrons, thereby damaging cells, proteins and DNA (genetic materials) (Martin *et al*., 2003). For years researchers have known that free radicals are associated with process that lead to cell degeneration, especially in the brain (Shulman *et al*., 2004).

They have been implicated as important factors that contributed to development of neurodegenerative disorders such as Lou Gehrig's disease and Huntington's disease (Martin *et al*., 2003). The brain and nervous system are particularly vulnerable to oxidative stress due to limited antioxidant capacity (Vega-Naredo *et al*., 2005).

Although Fe is necessary in relatively large amounts for hemoglobin, myoglobin and cytochrome production, xanthine oxidase and the other Fe proteins require rather small amounts of Fe. On the other hand, free Fe in the cytosol and in the mitochondria can cause considerable oxidative damage by increasing superoxide production. Through Fenton reactions and by activating xanthine oxidase, which produces both uric acid (an antioxidant that recycles ascorbic acid in the cell and is therefore vital to the animals that do not produce ascorbic acid, such as primates) and O_2*, which causes massive damage either by itself or by reacting with nitric oxide (NO) to form the powerful peroxynitrite (ONOO*) (Johnson, 2001). High levels of both Cu and Fe, with relatively low levels of Zn and Mn play a crucial role in brain cancer and in degenerative diseases of the brain (Johnson, 2001). Sodium nitroprusside is an anti-hypertensive drug, it acts by relaxation of vascular smooth muscle; consequently, it dilates peripheral arteries and veins. However, sodium nitroprusside (SNP) has been reported to cause cytotoxicity through the release of cyanide and/or nitric oxide (NO). NO can act independently, it may cause neuronal damage in cooperation with other Reactive Oxygen Species (ROS) (Bellé *et al*., 2004; Posser *et al*., 2006; Oboh and Rocha, 2007a).

The human body is equipped with an antioxidant defense system that deactivates these highly reactive free radicals, through the activities of antioxidants enzymes (made in the body) and antioxidant phytochemicals that soak up the excess reactivity that these free radicals have, turning them to harmless particles or waste products that can be get rid of (Doblado *et al*., 2002; Oboh, 2005, 2006). Phenolic compounds are an important group of secondary metabolites, which are synthesized by plants because of plant adaptation to biotic and abiotic stress condition (infection, water stress, cold stress, high visible light) (Oboh and Rocha, 2007a). In recent years, phenolic compounds have attracted the interest of researchers because of their antioxidants capacity; they can protect the human body from free radicals, whose formation is associated with the normal natural metabolism of aerobic cells. The antiradical activity of flavonoids and phenols is principally based on the structural relationship between different parts of their chemical structure (Rice-Evans *et al*., 1996).

Ocimum canum and *Ocimum gratissimum* are the two varieties of *Ocimum* spp. commonly found in tropical Africa. *Ocimum canum* Sims-(Family-Lamiacaeae) occurs wild in tropical Africa, where it is used in the treatment of malaria, headache and eye infections as well as for aromatic purpose (Bassole *et al*., 2005). Aqueous extract of *Ocimum canum* lowers blood glucose levels and facilitates insulin release by isolated pancreatic beta-islet cells. *Ocimum canum* is also used in the treatment of diseases of the kidneys, bladder and urethra (Nyarko *et al*., 2002). *Ocimum gratissimum* (family Labiatae) is the most abundant specie of *Ocimum*. The volatile aromatic oil from the leaves of *Ocimum gratissimum* consists mainly of thymol (32-65%), eugenol, xanthones, terpenes and lactones. Nutritional importance of this plant centres on it's usefulness as a seasoning because of its aromatic flavour. In folk medicine, *Ocimum gratissimum* is extensively used as anti-malaria, anti-convulsant and against cough. The crushed leaf juice is used in the treatment of convulsion, stomach pain and catarrh. Its oil has antiseptic, antibacterial and antifungal activity (Fadohan *et al*., 2004). The decoction of the leaves is used in the treatment of mental illness (Onajobi, 1986). Although a lot had been reported on the phytochemistry of *Ocimum canum* and *Ocimum gratissimum*, however there is limited information on the antioxidant activity of their polyphenols and their potential used in the management of neurodegenerative diseases associated with oxidative stress. This study therefore sought to characterize the antioxidant properties of polyphenol extracts of *Ocimum canum* and *Ocimum gratissimum* and their inhibitory effects on some neurotoxins (Fe^{2+} and sodium nitroprusside) induced lipid peroxidation in Rat's brain homogenates *in vitro*.

MATERIALS AND METHODS

Sample Collection

Ocimum gratissimum (Efinrin Nila) and *Ocimum canum* (Efinrin Wewe) leaves were purchased from a local market in Akure South Local Government Area of Ondo State, Nigeria and authenticated in Biology Department, Federal University of Technology, Akure, Nigeria. All the chemicals used were analytical grade, while the water was glass distilled and the study was carried out between January and October, 2007. In this experiment Wistar strain albino rats weighing 200-230 g were used and these were collected from the breeding colony of Biochemistry Department, University of Ilorin, Nigeria. The rats were maintained on a 12 h light/12 h dark cycle, with free access to food and water.

Sample Preparation

The free soluble polyphenols of the *Ocimum* spp. were extracted using 80% acetone as described Chu *et al*. (2002).

Determination of Total Phenol Content

The total phenol content of the extracts was determined using Folin-Ciocalteu's reagent as reported by Singleton *et al*. (1999).

Determination of Reducing Property

The reducing property of the extracts was determined by assessing the ability of the extracts to reduce $FeCl_3$ solution as described by Pulido *et al*. (2000). 2.5 mL aliquot was mixed with 2.5 mL 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min and then 2.5 mL 10% trichloroacetic acid was added. This was then centrifuged at 650 rpm for 10 min. Five milliliter of the supernatant was mixed with an equal volume of water and 1 mL 0.1% ferric chloride. The absorbance was measured at 700 nm and a higher absorbance indicates a greater reducing power.

Fe^{2+} Chelation Assay

The ability of the extracts to chelate Fe^{2+} was determined using the method reported by Puntel *et al*. (2005). Freshly prepared 500 μM $FeSO_4$ (150 μL) was added to a reaction mixture containing 168 μL 0.1 M Tris-HCl (pH 7.4), 218 μL saline and the extracts (0-25 μL). The reaction mixture was incubated for 5 min, before the addition of 13 μL 0.25% 1, 10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm in a spectrophotometer.

Free Radical Scavenging Ability

The free radical scavenging ability of the extracts against DPPH (1,1-diphenyl-2 picrylhydrazyl) free radical was evaluated using the method reported by Burits and Bucar (2000).

Lipid Peroxidation Assay

Preparation of Tissue Homogenates

The rats were decapitated under mild diethyl ether anaesthesia and the cerebral tissue (whole brain) was rapidly dissected and 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 1200 rev min^{-1} in a Teflon glass homogenizer. The homogenate was centrifuge for 10 min at 3000 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), DNA and RNA that was kept for lipid peroxidation assay (Bellé *et al*., 2004).

Lipid Peroxidation and Thiobarbibutric Acid Reactions

The lipid peroxidation assay was carried out using the modified method of Ohkawa *et al*. (1979).

Data Analysis

The results of the three replicates were pooled and expressed as mean±standard error (SE). A one-way analysis of variance (ANOVA) and the Least Significance Difference (LSD) were carried out (Zar, 1984). Significance was accepted at $p \leq 0.05$.

RESULTS AND DISCUSSION

The results revealed that the total phenol contents of both plants were high (1.6-2.0 mg g^{-1}), however that of *Ocimum canum* (2.0 mg g^{-1}) was significantly higher ($p<0.05$) than that of *Ocimum gratissimum* (1.6 mg g^{-1}) (Fig. 1). However, it is worth noting that the total phenol content of the two *Ocimum* species were higher than that of the total phenolic content of broccoli, spinach, onion, carrot, cabbage potato, lettuce, celery and cucumber (Chu *et al*., 2002) and as well as some commonly consumed green leafy vegetables in Nigeria (Oboh, 2005) and, green and red hot peppers (Oboh *et al*., 2007). In addition, the total phenolic content of the two *Ocimum* spp. were higher than that of some commonly consumed fruits (cranberry, apple, red grape, strawberry, peach, lemon, pear, banana, orange, grapefruit and pineapple) reported by Sun *et al*. (2002).

The result revealed that incubation of the rat's brain in presence of Fe (II) caused a significant increase ($p<0.05$) in the MDA content of the brain (256%) when compared with the basal brain without Fe (II) (100%) (Fig. 2). These findings agree with our earlier reports on the interaction of Fe (II) with brain (Oboh *et al*., 2007; Oboh and Rocha, 2007a); in that Fe (II) is a very potent initiator of lipid peroxidation in brain (pro-oxidant). The increase in the MDA content of the brain 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 us 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 favours the propagation of lipid oxidation (Puntel *et al*., 2005; Oboh *et al*., 2007; Oboh and Rocha, 2007b). Elevated Fe (II) content in the brain had been linked to Parkinson's disease. Although the aetiology of Parkinson's disease remains obscure, various studies point to a central role of Fe-induced oxidative stress mechanism. Elevated Fe levels have been localized to degenerate regions of brains from Parkinson's disease patients, a finding also demonstrated in animal models of the disease (Martin *et al*., 2003).

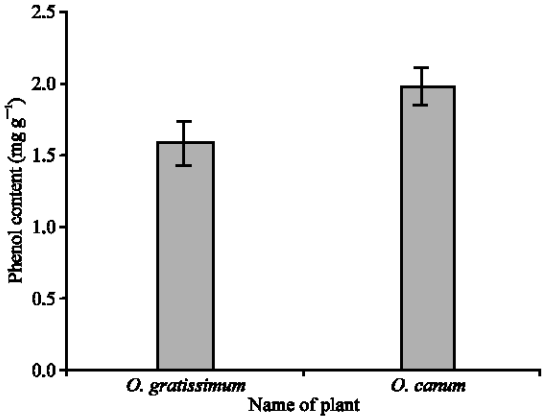

Fig. 1: Total phenol content of *O. gratissimum* and *O. canum* leaves

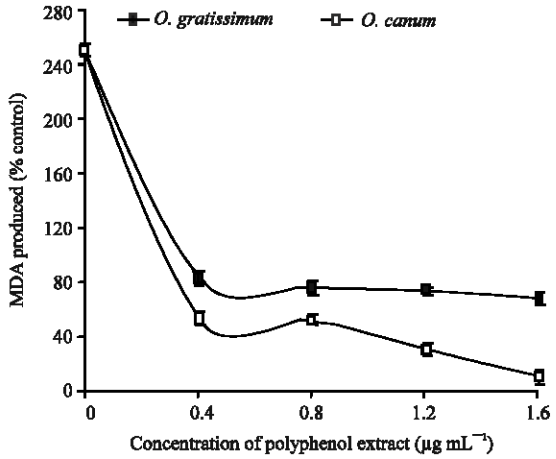

Fig. 2: Inhibition of Fe (II) induced lipid peroxidation in rat's brain by polyphenol extract from *Ocimum* spp.

However, the phenolic extracts (0.4-1.6 µg mL^{-1}) from both *Ocimum* spp. caused a dose dependent decrease in the MDA content of the Fe (II) stressed brain homogenates [*Ocimum canum* (11.5-55%), *Ocimum gratissimum* (69.3-89.8%)], this level of inhibition is highly remarkable, as the level of inhibition is higher than what was earlier reported by our group on ripe and unripe hot peppers (Oboh *et al*., 2007; Oboh and Rocha, 2007a, b). The reason for the high inhibition of the lipid peroxidation in the brain by the polyphenol extracts from *Ocimum* spp. cannot be categorically stated, however, it will not be far fetch from the possibility that the polyphenols could form complexes with the Fe (II) thereby preventing them from catalyzing the initiation of lipid peroxidation, or/and the possibility that the phytochemical (phenol) could have scavenge the free radicals produced by the Fe (II) catalyzed lipid peroxidation reaction (Oboh *et al*., 2007).

Antioxidant carry out their protective properties on cells either by preventing the production of free radicals or by neutralizing/scavenging free radicals produced in the body or reducing/ chelating the transition metal composition of foods (Oboh, 2006; Oboh *et al*., 2007). In an attempt to explain the main mechanism through which the phenolic extracts prevent Fe (II) induced lipid peroxidation in the brain; the Fe (II) chelating ability was assessed. The results revealed that both phenolic extracts significantly ($p<0.05$) chelate Fe (II) in a dose dependent manner (Fig. 3). This protective ability of phenolic extracts against Fe (II) induced oxidative stress by Fe (II) chelating mechanism agrees with earlier reports on phenolic, in that one of the mechanism through which they exhibit their antioxidant activity is by forming complex with Fe thereby preventing the initiation of lipid peroxidation (Oboh and Rocha, 2007a, b).

However, the phenolic extracts from both *Ocimum* spp. had higher Fe (II) chelating ability than phenolic extracts from ripe and unripe hot peppers (Oboh and Rocha, 2007a, b). This high Fe (II) chelating effect of the phenolic extracts may have contributed immensely to the inhibition of Fe (II) induced lipid peroxidation in the isolated Rat's brain-*in vitro*. However, polyphenol extracts from *Ocimum canum* had a significantly higher ($p<0.05$) Fe (II) chelating ability than that of *Ocimum gratissimum*, the reason for the significantly higher Fe (II) chelating ability of the *Ocimum canum* extract may not be far from its significantly higher total phenol content (Fig. 1). This higher Fe (II) chelating ability of the *Ocimum canum* phenolics extract may have consequently increased its protective ability against Fe (II) induced lipid peroxidation in brain (Fig. 2).

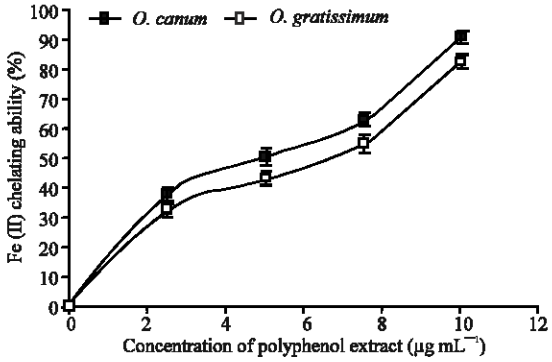

Fig. 3: Fe (II) chelating ability of polyphenol extract from *Ocimum* spp.

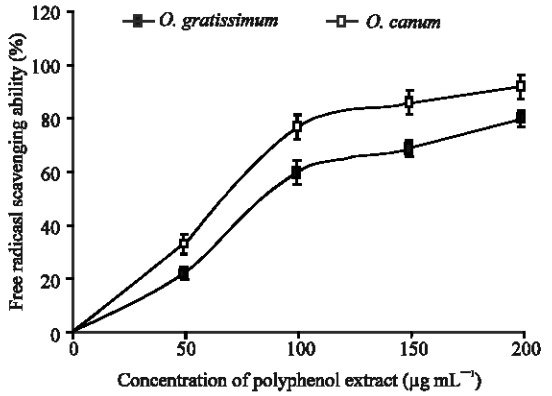

Fig. 4: Free radical scavenging ability of polyphenol extract from *Ocimum* spp.

Furthermore, the polyphenols extract from both plants [*Ocimum canum* (33.0-92.4%), *Ocimum gratissimum* (22.4-80.1%)] were able to scavenge the DPPH free radical in a dose-dependent manner, within the concentration of the phenolic extracts tested (50-200 µg mL^{-1}). Phenolic compounds have attracted the interest of researchers because they show promise of being powerful antioxidants that can protect the human body from free radicals (Fig. 4). The antiradical activity of polyphenols is principally based on the redox properties of their hydroxyl groups and the structural relationships between different parts of their chemical structure (Rice-Evans *et al*., 1996).

However, polyphenol extracts from *Ocimum canum* (33.0-92.4%) had a significantly higher ($p<0.05$) free radical scavenging ability than that of *Ocimum gratissimum* (22.4-80.1%) within the concentration tested. This free radical scavenging ability result agrees with the total phenol content (Fig. 1), Fe (II) chelating ability (Fig. 3) and the inhibition of Fe (II) induced lipid peroxidation in the rat's brain homogenates-*in vitro* (Fig. 2) by the extracts. This finding agrees with many earlier reports, in that there is a correlation between the antioxidant activities and total phenolic contents of many plants. Furthermore, the fact that the free radical scavenging ability, Fe (II) chelating ability and the inhibition of Fe (II) induced lipid peroxidation by the phenolics follow the same trend suggest that free radical scavenging ability and Fe (II) chelation mechanisms may be involved in the protective ability of the polyphenol against Fe (II) induced lipid peroxidation in the brain.

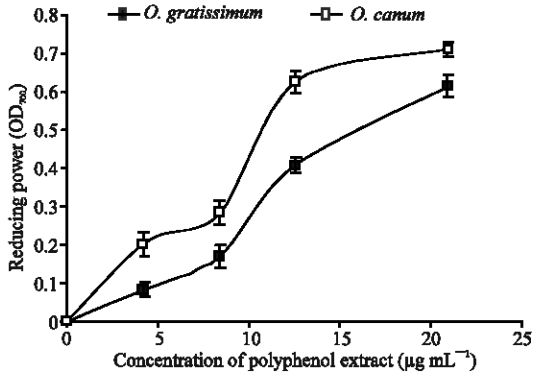

Fig. 5: Reducing power of polyphenol extract from *Ocimum* spp.

The polyphenol extracts (5.0-25 µg mL^{-1}) at the concentration tested were able to reduce Fe (III) to Fe (II) in a dose-dependent manner. However phenolic extracts from *Ocimum canum* had a significantly ($p<0.05$) higher reducing ability than those of *Ocimum gratissimum* (Fig. 5). At the concentration of the extracts tested, reducing power of the two *Ocimum* species were higher than that of some commonly consumed and underutilized tropical legumes (Oboh, 2006) and some tropical green leafy vegetable (Oboh, 2005).

Allhorn *et al*. (2005) reported that the reducing property can be a novel antioxidation defense mechanism; this is possibly through the ability of the antioxidant compound to reduce transition metals. Reduced metals such as Fe (II) or Cu (I) rapidly react with lipid hydroperoxides, leading to the formation of reactive lipid radicals and conversion of the reduced metal to its oxidized form. Furthermore, it is worth noting that there was an agreement between the phenolic content of the two *Ocimum* spp., DPPH free radical scavenging ability, reducing power and their reducing ability. Therefore, reducing ability may have also be major contributory mechanism to the higher protective effect of *Ocimum canum* polyphenol extracts as against that of *Ocimum gratissimum*. The reason for this higher reducing power of *Ocimum canum* polyphenols may not be far fetch from the fact that antioxidant activity of phenolics is mainly due to their redox properties which allowed them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Rice-Evans *et al*., 1996). Therefore, the high protective effect of *Ocimum* spp. against Fe (II) induced lipid peroxidation may be due to their Fe (II) chelating ability, reducing power and free radical scavenging ability. However the higher protective effect of *Ocimum canum* may not be far fetch from its higher phenolic content which resulted in higher Fe (II) chelating ability, free radical scavenging ability and reducing power.

Incubation of the isolated rats brain homogenates in the presence of 7 µM sodium nitroprusside caused a significant increase ($p<0.05$) in the (MDA) (160%) content of the isolated brain compared to the unstressed brain (100%) (Fig. 6), this result agrees with earlier reports on the interaction of sodium nitroprusside with isolated rat's brain (Oboh *et al*., 2007; Oboh and Rocha, 2007a). 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 (Bellé *et al*., 2004; Puntel *et al*., 2005). 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 (Oboh *et al*., 2007).

However, poly phenols extract from both species of *Ocimum* significantly ($p<0.05$) inhibited 7 µM sodium nitroprusside induced lipid peroxidation in rat's brain in a dose-dependent manner. This

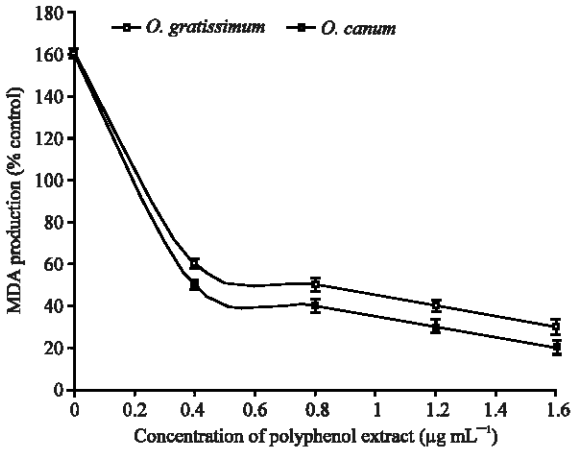

Fig. 6: Inhibition of sodium nitroprusside induced lipid peroxidation in rat's brain by polyphenol extract from *Ocimum* spp.

is an indication that both plants polyphenol extracts were able to scavenge the NO* produced by sodium nitroprususside and chelate the Fe produced as a result of the decomposition of the sodium nitroprusside. However, polyphenols from *Ocimum canum* had a significantly higher ($p<0.05$) inhibitory effects on sodium nitroprusside induced lipid peroxidation in the isolated rat's brain than that of *Ocimum gratissimum*. The basis for this may not be far fetch from its higher free radical scavenging ability and Fe (II) chelating effects, which makes it possible to scavenge more NO radical and to chelate the Fe produced from the decomposed sodium nitroprusside.

CONCLUSION

Polyphenol extracts from both *Ocimum canum* and *Ocimum gratissimum* were able to protect the brain against Fe (II) and sodium nitroprusside induced lipid peroxidation. However polyphenol extracts from *Ocimum canum* had a higher protective effect against both Fe (II) and sodium nitroprusside induced lipid peroxidation brain (*in vitro*). The higher protective effect may due to their high Fe (II) chelating ability, free radical scavenging and reducing power (antioxidant properties). These antioxidant properties of the *Ocimum* polyphenol may have contributed to the use of the leaves in the treatment of mental illness in folk medicine.

ACKNOWLEDGMENT

Post-doctoral fellowship from Alexander von Humboldt (AvH) is graciously acknowledged during which period the manuscript was written.

REFERENCES

Allhorn, M., A. Klapyta and B. Akerstrom, 2005. Redox properties of the lipocalin α1-microglobulin: Reduction of cytochrome c, hemoglobin and free iron. Free Radic. Biol. Med., 38 (5): 557-567.

Bassole, I.H.N., R. Nebie, A. Savadogo, C.T. Ouattara, N. Barro and S.A. Traore, 2005. Composition and antimicrobial activities of the leaf and flower essential oils of *Lippia chevalieri* and *Ocimum canum* from Burkina Faso. Afr. J. Biotech., 4 (10): 1156-1160.

Bellé, N.A.V., G.D. Dalmolin, G. Fonini, M.A. Rubim and J.B.T. Rocha, 2004. Polyamines reduces lipid peroxidation induced by different pro-oxidant agents. Brain Res., 1008 (2): 245-251.

Burits M. and F. Bucar, 2000. Antioxidant activity of *Nigella sativa* essential oil. Phytother Res., 14 (5): 323-328.

Chu, Y., J. Sun, X. Wu and R.H. Liu, 2002. Antioxidant and antiproliferative activity of common vegetables. J. Agric. Food Chem., 50 (25): 6910-6916.

Doblado, R., H. Zielinski, M. Piskula, H. Kozlowska, R. Munoz, J. Frias and C. Vidal-Valverde, 2002. Effect of processing on the antioxidant vitamins and antioxidant capacity of *Vigna sinensis* Var. Carilla, J. Agric. Food Chem., 53 (4):1215-1222.

Fadohan, P., J.D. Gbenou, B. Gnonlonfin, K. Hell, W.F. Marasas and M.J. Wingfoeld, 2004. Effect of essential oils on the growth of *Fusarium verticillioides* and fumonisin contamination in corn. J. Agric. Food Chem., 52 (22): 6824-6829.

Johnson, S., 2001. The possible crucial role of iron accumulation combined with low tryptophan, zinc and manganese in carcinogenesis. Med. Hypotheses, 57 (5): 539-543.

Martin, F.L., S.J.M. Williamson, K.E. Paleologou, R. Hewitt, O.M.A. El-Agnaf and D. Allsop, 2003. Fe (II)-induced DNA damage in α-synuclein-transfected human dopaminergic BE (2)-M17 neuroblastoma cells: Detection by the Comet assay. J. Neurochem., 87 (3): 620-630.

Nyarko, A.K., H. Asare-Anane, M. Ofosuhene and M.E. Addy, 2002. Extract of *Ocimum canum* lowers blood glucose and facilitates insulin release by isolated pancreatic beta-islet cells. Phytomedicine, 9 (4): 346-351.

Oboh, G., 2005. Effect of Blanching on the Antioxidant property of some tropical green leafy vegetables. Lebensm.-Wiss.u.-Technol., 38 (5): 513-517.

Oboh, G., 2006. Antioxidant properties of some commonly consumed and underutilized tropical legumes. Eur. Food Res. Technol., 224 (1): 61-65.

Oboh, G., R.L. Puntel and J.B.T. Rocha, 2007. Hot pepper (*Capsicum annuum*, *Tepin* and *Capsicum chinese*, *Habanero*) Prevents Fe^{2+}-induced lipid peroxidation in Brain *in vitro*. Food Chem., 102 (1): 178-185.

Oboh, G. and J.B.T. Rocha, 2007a. Polyphenols in Red Pepper [*Capsicum annuum* var. *aviculare* (Tepin)] and their protective effect on some Pro-oxidants induced lipid peroxidation in Brain and Liver. Eur. Food Res. Technol., 225 (2): 239-247.

Oboh, G. and J.B.T. Rocha, 2007b. Distribution and antioxidant activity of polyphenols in ripe and unripe tree pepper (*Capsicum pubescens*). J. Food Biochem., 31 (4): 456-473.

Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95 (2): 351-358.

Onajobi, D., 1986. Smooth muscle contracting lipid-soluble principles in chromatographic fractions of *Ocimum gratissimum*. J. Ethnopharmacol., 18 (1): 3-11.

Posser, T., M.B. Moretto, A. Luiz Dafre, M. Farina, J.B.T. Rocha, C.W. Nogueira, G. Zeni, J. Dos Santos Ferreira, R. Bainy Leal and J.I. Franco, 2006.Antioxidant effect of diphenyl diselenide against sodium nitroprusside (SNP) induced lipid peroxidation in human platelets and erythrocyte membranes: An *in vitro* evaluation. Chem. Biol. Interact., 164 (1-2):126-135.

Pulido, R., L. Bravo and F. Saura-Calixto, 2000. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. J. Agric. Food Chem., 48 (8): 3396-3402.

Puntel, R.L., C.W. Nogueira and J.B.T. Rocha, 2005. Krebs cycle intermediates modulate thiobarbituric acid reactive species (TBARS) production in Rat Brain *in vitro*. Neurochem. Res., 30 (2): 225-235.

Rice-Evans, C., N.J. Miller and G. Paganga, 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic. Biol. Med., 20 (7): 933-956.

Shulman, R.G., D.L. Rothman, K.L. Behar and F. Hyder, 2004. Energetic basis of brain activity: Implications for neuroimaging. Trends Neurosci., 27 (8): 489-495.

Singleton, V.L., R. Orthofer and R.M. Lamuela-Raventos, 1999. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. Methods in Enzymology 299 (Oxidants and Antioxidants, Part A); Academic Press: San Diego, CA, pp: 152-178.

Sun, J., Y. Chu, X. Wu and R. Liu, 2002. Antioxidant and antiproliferative activities of common fruits. J. Agric. Food Chem., 50 (25): 7449-7454.

Vega-Naredo, I., B. Poeggeler, V. Sierra-Sanchez, B. Caballero, C. Tomas-Zapico, O. Alvarez-Garcia, D. Tolivia, M.J. Rodriguez-Colunga and A. Coto-Montes, 2005. Melatonin neutralizes neurotoxicity induced by quinolinic acid in brain tissue culture. J. Pineal Res., 39 (3): 266-275.

Zar, J.H., 1984. Biostatistical Analysis. Prentice-Hall, Inc. USA, pp: 620.