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Polyphenol Extracts from *Hyptis suaveolens* Leaves Inhibit Fe^{2+}-induced Lipid Peroxidation in Brain

Ganiyu Oboh*

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

Corresponding author: * **goboh2001@yahoo.com**

ABSTRACT

The present study seeks to compare the protective properties of free and bound polyphenols from *Hyptis suaveolens* leaves (a commonly used spice) on Fe^{2+}-induced lipid peroxidation in rat's brain *in vitro.* The free soluble polyphenols were extracted with 80% acetone, while the bound polyphenols were extracted with ethyl acetate from alkaline and acid hydrolyzed residue from free soluble extract. The inhibitory effect of the polyphenol extracts on Fe^{2+} (25μM)-induced lipid peroxidation in rat's brain was determined. The total phenol content and antioxidant properties (1,1-diphenyl -2-picrylhydrazyl (DPPH) free radical scavenging ability, reducing power and Fe (II) chelating ability) of the extracts were determined. The results of the study revealed that the leaf contains 3.88 mg/g total phenol [2.94 (free), 0.94 (bound)]. Incubation of the brain in the presence of Fe (II) caused a significant increase ($P<0.05$) in the malondialdehyde (MDA) content of the brain (256%). However, the polyphenol extracts (0.4–1.6 μg/ml) caused a dose-dependent significant decrease ($P<0.05$) in the MDA contents in the brain. The free soluble polyphenol extracts had a significantly higher ($P<0.05$) inhibitory effect on the Fe (II)-induced lipid peroxidation in brain and higher antioxidant properties. Therefore, the higher antioxidant properties of free soluble polyphenols (which are more) from *H. suaveolens* leaves could have been responsible for its high protection against Fe (II)-induced lipid in brain – *in vitro*.

Keywords: antioxidant, iron, malondialdehyde, oxidative stress

INTRODUCTION

High oxygen demand along with an abundance of readily oxidizable substrates yielding productive oxidative metabolism are required for the normal brain functioning. This demands the existence of a complex and multicomponent antioxidant system in the brain for protection against oxidative damage (Bayir *et al.* 2006). Oxidative stress with depletion of antioxidant reserves and damage to critical biomolecules plays an essential role in brain damage (Maier and Chan 2000; Bayir *et al.* 2003).

Although iron (Fe) is necessary in relatively large amounts for hemoglobin, myoglobin and cytochrome production, and xanthine oxidase, 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 hydroxyl and superoxide radical production (Oboh *et al.* 2007). 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 $O_2^{\bullet}$, which causes massive damage either by itself or by reacting with nitric oxide (NO) to form the powerful peroxynitrite ($ONOO^{\bullet}$) (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 (Parkinson's and Alzheimer's diseases, multiple sclerosis, etc.) (Johnson 2001).

The most likely and practical way to fight against degenerative diseases is to improve body antioxidant status, which could be achieved by higher consumption of vegetables and fruits. Foods from plant origin usually contain natural antioxidants that can scavenge free radicals (Alia *et al.* 2003; Oboh and Rocha 2007a). Epidemiological evidence has clearly shown that diets based on fruits and vegetables, with high content of natural antioxidants, contribute to reduced mortality from cardiovascular and cerebrovascular disease, although their protective effect on cancer risk is less conclusive (Alia *et al.* 2003). Antioxidants are powerful substances that can neutralize free radicals before they damage the body's cells. This is the major reasons why the benefits of antioxidants are talked about often in the media, and why people are advised to eat antioxidant foods i.e., foods high in antioxidants (Oboh and Rocha 2007a). However, in many earlier studies, a correlation had been reported between the antioxidant activities and total phenolic contents of many plant foods such as commonly consumed fruits (Chu *et al.* 2002) and Vegetables (Sun *et al.* 2002).

Phenolic compounds are an important group of secondary metabolites, which are synthesized by plants because of a plant's adaptation to biotic and abiotic stresses (infection, water stress, cold stress, high visible light) (Douglas 1996; Pitchersky and Gang 2000). In recent years, phenolic compounds have attracted the interest of researchers because of their antioxidant capacity; they can protect the human body from free radicals, whose formation is associated with the normal natural metabolism of aerobic cells. The antioxidant activity of phenolics is mainly because of their redox properties, which allow them to act as reducing agents, hydrogen donors, free radical scavenger, singlet oxygen quenchers and metal chelators (Alia *et al.* 2003; Amic *et al.* 2003). 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). However, phenolics in vegetables are present in both free and bound forms. Bound phenolics, mainly in the form of β-glycosides, may survive human stomach and small intestine digestion and reach the colon intact, where they are released and exert their bioactivity (Sosulski *et al.* 1982; Chu *et al.* 2002).

Hyptis suaveolens (hyptis, mintweed) is a perennial

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herb found in dense clumps along roadsides, over-grazed pastures and around stockyards across the tropics, including the Kimberley region of North Western Australia and it is Native to tropical America. The plant is used as an appetizing agent, to combat indigestion, stomach pain, nausea, flatulence, colds, and infection of the gall bladder (Fun *et al.* 1990; Singh *et al.* 1997). The essential oil of *H. suaveolens* has β-caryophyllene, sabinene, 1,8-cineole, *trans*-α-bergamotene, terpinen-4-ol, eugenol and fenchone (Din *et al.* 1988; Asekun and Ekundayo 2000). The oil has been found to possess antibacterial (Asekun *et al*. 1999), antifungal (Menghini *et al.* 1996) and antioxidant (Nantitanon *et al.* 2007) properties. However, there are no information on the distribution of polyphenols in *H. suaveolens* leaves, their antioxidant properties, as well as their potential in the management/prevention of neurodegenerative diseases that are linked with oxidative stresss. This study therefore sought to determine the antioxidant and neuroprotective potentials of free and bound polyphenols from *H. suaveolens* leaves on Fe (II)-induced lipid peroxidation in brain, *in vitro*.

MATERIALS AND METHODS

Materials

Matured leaves of *H. suaveolens* 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 of analytical grade, while the water was glass distilled. In this experiment Wistar strain albino rats weighing 200–230 g were used, and these were collected from the breeding colony of the Biochemistry Department, University of Ilorin, Ilorin, Nigeria. The rats were maintained on a 12 h light/12 h dark cycle, with free access to food and water.

Sample preparation

Extraction of soluble free phenols from samples

For the extraction of the soluble free phenolic compounds, 50 g of the sample was weighed and homogenized with 80% acetone (1:2 w/v) using a chilled Waring blender for 5 min. The sample was homogenized further using a Polytron homogenizer for an additional 3 min to obtain a thoroughly homogenized sample. The homogenate was filtered through Whatman No. 2 filter paper on a Buchner funnel under vacuum. The residue was kept for the extraction of bound phytochemicals. The filtrate was evaporated using a rotary evaporator under vacuum until ~90% of the filtrate was evaporated. The extract was frozen at –4°C (Chu *et al.* 2002).

Extraction of bound phenols from samples

The residue from the above soluble free extraction was hydrolyzed directly with 200 ml of 4 M NaOH solution at room temperature for 1 hr with shaking. The mixture was adjusted to pH 2 with concentrated HCl and the bound phytochemicals were extracted with ethylacetate. The eluate was evaporated under vacuum at 45°C to dryness. Phenolic compounds in this portion were recovered with 10 ml of water and stored at about –4°C until analyis (Chu *et al.* 2002).

Determination of total phenol content

Total phenol content was determined using the method of Singleton *et al.* (1999). Appropriate dilutions of the extract were oxidized with 2.5 ml of 10% Folin-Ciocalteu's reagent (v/v) and neutralized by 2.0 ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45°C and the absorbance was measured at 765 nm in a spectrophotometer (Jenway,UK). Tannic acid (BDH Analar) was used as standard phenol.

Reducing property

The reducing property of the extracts was determined by assessing the ability of the extract to reduce $FeCl_3$ solution as described by Pulido *et al.* (2000), in which 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, after which 5 ml 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: a higher absorbance indicates a greater reducing power.

Fe^{2+} chelation assay

The ability of the extracts to chelate Fe^{2+} was determined using a modified method of Minotti and Aust (1987) with a slight modification 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 (Jenway, UK).

Free radical scavenging ability

The free radical scavenging ability of the extracts against DPPH (1,1-diphenyl-2 picrylhydrazyl) free radical was evaluated (Gyamfi *et al.* 1999). Briefly, appropriate dilution of the extracts (1 ml) was mixed with 1 ml, 0.4 mM methanolic solution containing DPPH radicals, the mixture was left in the dark for 30 min and the absorbance was read at 516 nm.

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 rpm in a Teflon glass homogenizer. The homogenate was centrifuge for 10 min at 3000 × *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 was kept for the lipid peroxidation assay (Belle *et al*. 2004).

Lipid peroxidation and thiobarbibutric acid reactions

The lipid peroxidation assay was carried out using a modified method of Ohkawa *et al.* (1979). Briefly, 100 µl of S1 fraction was mixed with a reaction mixture containing 30 µl of 0.1 M pH 7.4 Tris-HCl buffer, extract (0–100 µl) and 30 µl of the pro-oxidant solution (250 µM freshly prepared $FeSO_4$). The volume was made up to 300 µl by water before incubation at 37°C for 1 h. The colour reaction was developed by adding 300 µl 8.1% SDS (sodium dodecyl sulphate) to the reaction mixture containing S1, this was subsequently followed by the addition of 600 µl of acetic acid/HCl (pH 3.4) mixture and 600 µl 0.8% TBA (thiobarbituric acid) (BDH Analar). This mixture was incubated at 100°C for 1 h. TBARS (thiobarbituric acid reactive species) produced were measured at 532 nm and the absorbance was compared with that of standard curve using MDA (malondialdehyde) (Sigma-Aldrich).

Animal ethics

The handling of animals was carried out in accordance with the recommended international standard (National Research Council, 1988).

Data analysis

The *Hyptis suaveolens* leaves samples were randomly collected from the supermarket and were analysed in triplicate. The results of the three replicates were pooled and expressed as the mean ± standard error (SE). Student's *t*-test, 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

Hyptis suaveolens leaves are popularly used as a flavourant because of its characteristic sweet smell; an infusion of the plant is used to treat catarrhal conditions, affections of the uterus and parasitical cutaneous diseases; the leaf juice is taken internally for colic and stomache-aches. In this study the antioxidant properties of the free and bound polyphenols and their potentials in the management/ prevention of neurodegenerative diseases are highlighted below.

The results of the assessment of free soluble, bound and total phenol contents of *Hyptis suaveolens* leaves are presented in **Fig. 1**. Leaves contained 3.88 mg/g total phenol content, out of which 2.94 mg/g was free soluble phenol and 0.94 mg/g was bound polyphenol. This total phenolic content was higher than the total phenolic content of some commonly consumed vegetables such as broccoli, spinach, onion, carrot, cabbage potato, lettuce, celery and cucumber [19.5 (cucumber) – 101.6 mg/100 g (broccoli)] (Chu *et al.* 2002), some commonly consumed tropical green leafy vegetables (0.1–0.3%) in Nigeria (Oboh and Akindahunsi 2004; Oboh 2005) and, green (73.7–226.3 mg/100 g) and red (103.3–210.0 mg/100 g) hot peppers (Oboh *et al.* 2007). In addition, the total phenol content was higher than that of some commonly consumed fruits such as pineapple, pear, orange, peach, banana, grapefruit, apple, strawberry and lemon (49.6–296.3 mg/100 g) (Sun *et al.* 2002).

However, 75.8% of the total phenols are free soluble polyphenols, while about 24.2% of the total phenols exist in the form of bound polyphenol. This result is in agreement with earlier reports by Chu *et al.* (2002), Sun *et al*. (2002) and Oboh and Rocha (2007b, 2007c) on the distribution of polyphenols in some commonly consumed vegetables, fruits and ripe and unripe pepper. Free phenolics are more readily absorbed, and thus, exert beneficial bioactivities in early digestion. The significance of bound phytochemicals to human health is not clear (Chu *et al*. 2002; Sun *et al.* 2002). However, it is possible those different plant foods with different amounts of bound phytochemicals can be digested and absorbed at different sites of the gastrointestinal tract, and that they also have unique health benefits. Bound phytochemicals, mainly as β-glycosides, can not be digested by human enzymes, and can survive stomach and small intestine digestion to reach the colon where they are digested by bacteria flora releasing phytochemicals with health benefits (Sosulski *et al.* 1982; Chu *et al.* 2002; Sun *et al.* 2002). Epidemiological studies have shown an inverse correlation between vegetable consumption and colon cancer incidence (Voorrips *et al*. 2002).

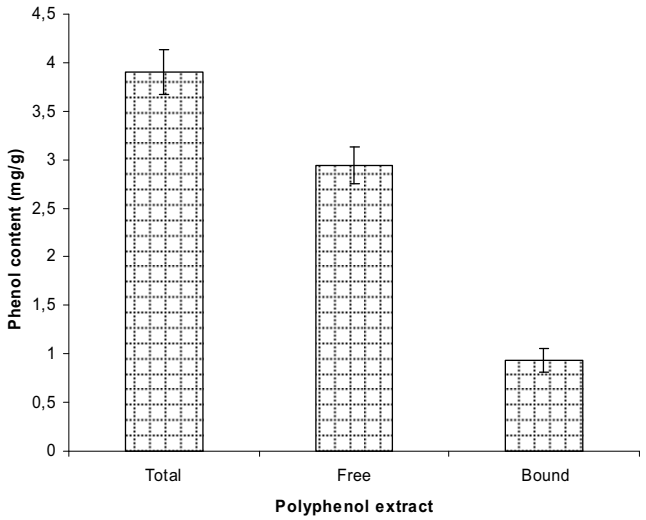

Fig. 1 Distribution of polyphenols in *Hyptis suaveolens* leaves.

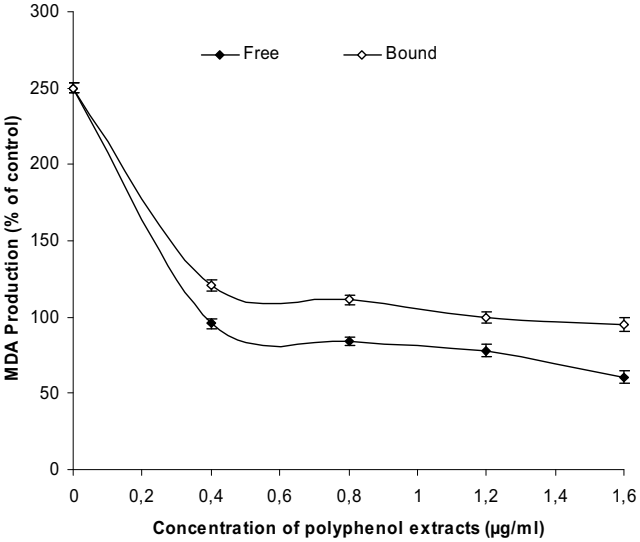

Fig. 2 Inhibition of Fe (II)-induced lipid peroxidation in brain by polyphenolic extract from *Hyptis suaveolens* leaf.

The inhibition of Fe (II)-induced lipid peroxidation in isolated rat's brain homogenates by the extracts is presented in **Fig. 2**. Incubation of the rat's brain in the 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%). These findings agree with our earlier reports on the interaction of Fe (II) with brain (Oboh *et al.* 2007) in which Fe (II) was shown to be a very potent initiator of lipid peroxidation in the brain (pro-oxidant). 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 $OH^{\bullet}$, 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 (Zago *et al.* 2000). 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).

However, the phenolic extracts (0.4–1.6 µg/ml) from the leaves caused a dose-dependent significant decrease ($P<0.05$) in the MDA content of the Fe (II)-stressed brain homogenates [free (60.8–95.6%), bound (95.2–120.9%)], this level of inhibition by both free and bound polyphenols within the concentration of the extract tested were higher than our earlier reports on free and bound polyphenols from ripe and unripe pepper (Oboh and Rocha 2007b, 2007c). Moreover, it is also interesting to know that 1.6 µg/ml of the extract was able to remove the total effect of the Fe (II)-induced lipid peroxidation in the brain. The mode of inhibition of Fe (II)-induced lipid peroxidation cannot be categorically stated, however there is the possibility that the polyphenols could have formed complexes with the Fe (II) thereby preventing them from catalyzing the initiation of lipid peroxidation and/or the possibility that the phytochemical (phenol) could have scavenge the free radical produced by the Fe (II)-catalyzed reaction (Oboh *et al.* 2007).

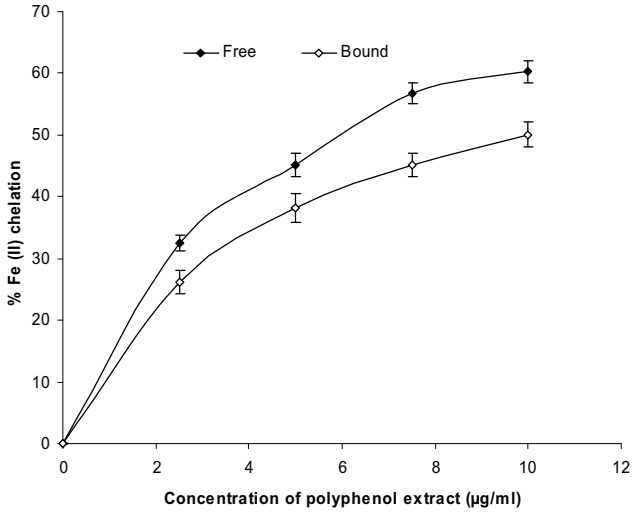

Fig. 3 Fe (II) chelating ability of polyphenolic extract from *Hyptis suaveolens* leaf.

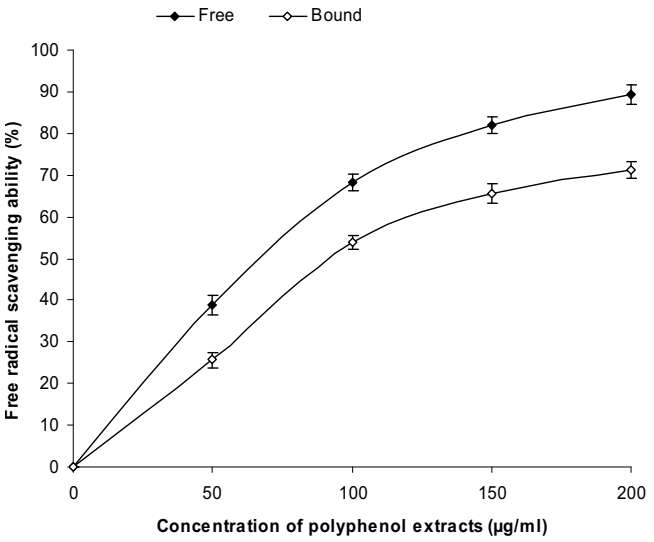

Fig. 4 Free radical scavenging ability of polyphenolic extract from *Hyptis suaveolens* leaf.

Table 1 EC_{50} of polyphenols from *Hyptis suaveolens leaves* on Fe (II)-induced lipid peroxidation in brain.

Polyphenol extract	EC_{50} (µg/ml)
Free	6.9 ± 0.5 b
Bound	8.8 ± 0.3 a

Values represent means of triplicate.
Values with the same alphabet along the same column are not significantly different (P>0.05) according to student's *t*-test.

Nevertheless, the free soluble polyphenols had a significantly higher (P<0.05) inhibitory effect on the Fe (II)-induced lipid peroxidation in the rat's brain than the bound polyphenols. The reason for the higher inhibition of the lipid peroxidation in the brain by the free polyphenol compared to the bound polyphenol cannot be categorically stated, however compositional and structural difference between the free soluble and bound polyphenol extracts may have caused it (Alia *et al.* 2003). Nevertheless, it is significant to know that the free soluble phenol which is more abundant in the plant has higher protective properties.

Antioxidants 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 by reducing/chelating the transition metal composition of foods (Alia *et al.* 2003; Amic *et al.* 2003; Oboh 2006b; Oboh *et al.* 2007). In an attempt to explain the main mechanism through which the phenolic extract prevents Fe (II)-induced lipid peroxidation in the brain, the Fe (II)-chelating ability was assessed. Results are presented in **Fig. 3**. The results revealed that both phenolic extracts significantly (P<0.05) chelate Fe (II) in a dose-dependent manner. The EC_{50} of the extracts are presented in **Table 1**. The EC_{50} of both extracts were low (6.9–8.8 µg/ml), however free soluble phenol (6.9 µg/ml) had a significantly lower (P<0.05) EC_{50} than the bound polyphenol (8.8 µg/ml).

This protective ability of phenolic extracts against Fe (II)-induced oxidative stress by an Fe (II)-chelating mechanism are in agreeent with earlier reports on the antioxidant activity of phenolics, in which one of the mechanisms through which polyphenols exhibit their antioxidant activity is by forming a complex with Fe thereby preventing the initiation of lipid peroxidation (Oboh and Rocha 2007b). This high Fe (II)-chelating ability is of immense importance in the protective ability of polyphenol against oxidative stress, because it is usually too late to attempt to use OH radical scavengers for therapeutic purposes. The reason for this is that extraordinarily high reactivity of hydroxyl radicals towards most biomolecules would require unreasonably high concentrations of intercepting scavengers to outcompete the biomolecules of interest (Bayir *et al.* 2006). These very high concentrations of scavengers are difficult to achieve *in vivo*, and consequently, they are not likely to be used for therapeutic goals (Bayir *et al.* 2006), thereby making Fe (II) chelators a better therapeutic alternative. However, the free soluble phenolic extracts had a significantly higher (P<0.05) Fe (II)-chelating ability than the bound polyphenols, and this may have accounted for its higher protective effect against Fe (II)-induced lipid peroxidation in the rats' brain (**Fig. 2**).

The free radical scavenging ability as typified by their DPPH free radical scavenging ability is presented in **Fig. 4**. The results revealed that both polyphenol extracts [free (38.8–89.3%), bound (25.6–71.2%)] 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). The extract concentration that will scavenge 50% of the DPPH radical (EC_{50}) is also presented in **Table 2**. The EC_{50} for the free soluble and bound polyphenols (87.2–118.6 µg/ml) was lower than that of the essential oil (on DPPH-free radical) which was 3.72 mg/ml (Nantitanon *et al.* 2007), indicating that the free soluble and bound polyphenols from the leaves of *H. suaveolens* are better scavengers of free radicals than the essential oil of the same plant. 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, 1997).

However, free soluble polyphenol extracts (87.2 µg/ml) had a significantly lower (P<0.05) free radical scavenging ability than the bound polyphenol extracts (118.6 µg/ml). This result agrees with the polyphenol distributrion (**Fig. 1**), Fe (II)-chelating ability (**Fig. 3**) and the inhibition of Fe (II)-induced lipid peroxidation in the rat brain homogenates *in vitro* (**Fig. 2**) by the extracts. Furthermore, the fact that the free radical scavenging ability, Fe (II)-chelating ability and the inhibition of Fe (II)-induced lipid peroxidation by 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. However, the

Table 2 EC_{50} of polyphenols from *Hyptis suaveolens* leaves on DPPH free radical scavenging ability.

Polyphenol extract	EC_{50} (µg/ml)
Free	87.2 ± 1.8 b
Bound	118.6 ± 2.1a

Values represent means of triplicate.
Values with the same alphabet along the same column are not significantly different (P>0.05) according student's *t*-test.

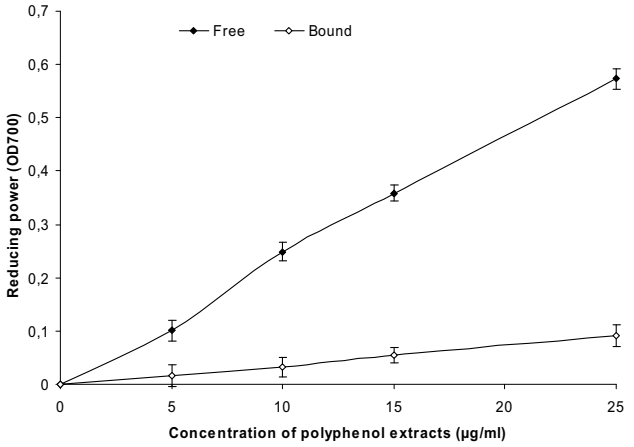

Fig. 5 Reducing power of polyphenolic extract from *Hyptis suaveolens* leaf.

EC_{50} for the Fe (II) chelation (6.9–8.8 µg/ml) as presented in **Table 1** is far below that of the DPPH free radical scavenging ability (87.2–118.6 µg/ml) of the extract (**Table 2**), since a far lower concentration of the extracts is required to chelate Fe (II) when compared to the concentration required to scavenge free radicals. Therefore, these points to the possibility that Fe (II)-chelating ability may be the domineering mechanism through which the polyphenol extracts protect the brain against Fe (II)-induced lipid peroxidation.

The reducing power of the free soluble and polyphenols extract from *H. suaveolens* leaves were determined (**Fig. 5**). The results revealed that both polyphenol extracts (5.0–25 µg/ml) at the concentration tested were able to reduce Fe (III) to Fe (II) in a dose-dependent manner. However free soluble phenolic extracts from *H. suaveolens* leaves had a significantly ($P<0.05$) higher reducing ability than those of bound polyphenols. At the concentration of the extracts tested, the reducing power of the free soluble polyphenols was higher than that of some commonly consumed and underutilized tropical legumes such as cowpea (*Vigna unguiculata*), pigeon pea (*Cajanus cajan*) and African yam bean (*Sphenostylis sternocarpa*)] (Oboh 2006a).

Allhorn *et al* (2005) reported that the reducing property can be a novel antioxidation defense mechanism, 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 (Godvadze *et al.* 2003). Therefore, the higher reducing ability of the free soluble polyphenols may have contributed to the higher protective effect of free soluble polyphenol extracts more than that of the bound fraction. The reason for this higher reducing power of free soluble polyphenols may indicate that free soluble polyphenols may contain polyphenols which have a higher reducing ability than the bound polyphenols. Since the antioxidant activity of phenolics is mainly due to their redox properties, this allows them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Rice-Evans *et al.* 1996, 1997).

CONCLUSION

Free soluble polyphenols are more abundant in *Hyptis suaveolens* leaves than bound polyphenols, and the latter have higher antioxidant properties which may have been responsible for their higher protective effect on Fe (II)-induced lipid in brain *in vitro.* However, Fe (II)-chelating ability appears to be the dominant mechanism through which the extracts protect the brain from Fe (II)-induced lipid peroxidation.

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