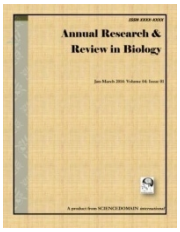


Annual Research & Review in Biology
4(22): 3345-3358, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Neuroprotective and Free Radicals Scavenging Potentials of Some Common Leafy Vegetables Consumed in South - Eastern Nigeria

C. S. Alisi[1*], E. S. Asiwe[1], A. A. Emejulu[1], A. C. Ene[1] and R. N. Nwoguikpe[1]

[1]Department of Biochemistry, Federal University of Technology, PMB 1526 Owerri, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors CSA and RNN designed the study. Authors ESA and ACE performed the statistical analysis. Authors AAE and CSA wrote the protocol. Authors ESA wrote the first draft of the manuscript. Authors CSA, ESA and AAE managed the analyses of the study. Authors ACE and RNN managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

Received 21st February 2014
Accepted 18th May 2014
Published 13th June 2014

ABSTRACT

Antioxidant potential, lipid peroxidation inhibition ability in brain homogenate and total phenolic and flavonoid contents of ethanol extracts of some leafy vegetables (*Vernonia amygdalina, Gongronema latifolium and Ocimum gratissimum*) consumed in south-eastern Nigeria were determined. The antioxidant and neuroprotective potentials of the ethanol extracts were assessed by 2, 2-diphenyl-1-picrylhydrazil (DPPH) assay and through determination of thiobarbituric acid (TBA) reactive substances in brain homogenates. Total phenolic and flavonoid contents were quantitatively determined in the extracts spectrophotometrically. Results obtained from the present study showed that *G. latifolium* and *V. amygdalina* extracts were effective against hydrogen peroxide-induced lipid peroxidation with threshold inhibitory concentrations (IC_{50}) values of 357.36±15.6*µ*g/ml and 629.5±22.72*µ*g/ml respectively while *O. gratissimum* achieved only 20% inhibition (IC_{20}) at 785.73±40.23*µ*g/ml. In the DPPH free radical scavenging assay, 50% of DPPH radicals were scavenged at181.5(µg/ml), 153.33(µg/ml), and 189.67(µg/ml) of *V. amygdalina, G. latifolium* and *O. gratissimum* extracts concentrations respectively. Free radical scavenging ability of the extracts was in the order *G. Latifolium* >*V. Amygdalina* >

**Corresponding author: Email: chinwe.alisi@futo.edu.ng, chinwealisi@yahoo.com;*

O. gratissimum. Total phenolic content was 432.79±42.2mg/100g,274.1±37.3mg/100g, and 490.49±58.1mg/100g catechin equivalent, while flavonoid content was 843.09±21.6 mg/100g, 746.34±13.7mg/100g and 1160.98±24.5mg/100g catechin equivalent in *V. amygdalina, G. latifolium*and *O. gratissimum* respectively. The inhibition of lipid peroxidation and DPPH radical scavenging followed a logistic dose response pattern.These results indicatethat these vegetables possess neuro-protective potentials and free radical scavenging ability. The present data scientifically corroborate with the consumption of these plants as an exogenous sources of antioxidants that could improve nutrition and boost antioxidant defense.

Keywords: Lipid-peroxidation; brain; antioxidant activity; V. amygdalina; G. latifolium; O. gratissimum; phenolic compounds; flavonoids.

1. INTRODUCTION

Plants, and indeed vegetables have long been consumed as integral part of daily diets and in many cases as a potent source of essential nutrients. The nutrient content of different vegetables varies considerably from vitamins, essential amino acids, to minerals and antioxidants that exist together and in varied proportion [1]. Apart from its role in human nutrition, plants have also been reserved for the sick and convalescence because of their peculiar medicinal values [2].

Lipid peroxidation, defined as the oxidative breakdown of polyunsaturated fatty acids, is a free radical-mediated phenomenon which is both transition metals and enzymes catalysed [3]. Lipid peroxidation initiators are reactive oxygen species (ROS) such as hydroxyl (OH•), peroxyl radicals (ROO•) and the superoxide anion radicals ($O_2^{\bullet -}$), which are formed byexogenous chemical factors and endogenous metabolic processes in the human body or in food systems [4]. Free radical production is a well-established mechanism of cellular injury in both plants and animals, and is used as an indicator of oxidative stress in cells and tissues.

Brain is the target for different stressors because of its high sensitivity to stress-induced degenerative conditions [5,6]. Brain has limited access to the bulk of antioxidants produced by the body; neurons are the first cells to be affected by a shortage of antioxidants and are most susceptible to oxidative stress [7].

An update of the researches carried out in the last decades with this focus showed that the antioxidants of plant origin with properties against free-radicals could have great importance as therapeutic agents in several diseases related to oxidative stress, since these compounds are effective as radical scavengers and inhibitors of lipid peroxidation [5,6]. In this context, the use of these natural products has proved to be clinically efficient and relatively less toxic than the existing drugs [5] and produce beneficial effects for human health through metabolic activities such as metabolic regulation, metabolic energy control, and activation/inactivation of biomolecules, signal transduction, cell exchange, endothelium-related vascular functions, and gene expression [8]. Lipid peroxide scavenging activities and antioxidant potentials of plants have been reported [9-12].

O. gratissimum (OG), a perennial plant belonging to the family Lamiaceae is grown in the tropics of Africa and warm temperature regions. In southern Nigeria the leaves are used as

local spice and vegetables in soups. It is also grown for the essential oils in its leaves and stems, which are used as insect repellent and as an antifungal agent [13]. *O. gratissimum* has found wide use in toothpastes and mouth washes. Its leaves are used for colds and fevers, the herbs are popularly used for treatment of diarrhoea and also applied topically as ointments. It is used in the treatment of sore throats and tonsillitis. It is also used as an expectorant and a cough suppressant [11]. Free radical scavenging properties of its leave extract has been demonstrated [10,11].

V. amygdalina (VA) (Asteraceae) is a small tree or shrub (2-5 m) that commonly grows in tropical Africa, and has been domesticated in some parts of West Africa and Nigeria (14). The leaves are green with a characteristic odour and a bitter taste [15]. It is known as 'Ewuro' in Yoruba, 'Etidot' in Ibibio, 'Onugbu' in Igbo and 'Chusa-diki' in Hausa tribes in Nigeria. Leaves of VA are used as vegetables in a wide variety of culinaries in Nigeria. It has also been widely applied in traditional non-orthodox medicine in the treatment of a variety of diseases. Different parts of this plant have been demonstrated to possess pharmacological benefits such as antidiabetic properties [16,17], antihelminthic and antimicrobial properties [18,19], Antimalaria/antiplasmodial activity [20], antioxidant activity [21,22], antihyperlipidemic properties [23] and hepato-protective effect [24,25].

G. latifolium (GL) is a tropical rainforest climbing plant belonging to the Apocynaceae family. In south-eastern Nigeria the plant is commonly known as "Utazi".It is a shrub, with milky or less often, clear latex. The leaves are characterised for its bitter taste, similar to that of *V. amygdalina*. The leaf of *G. latifolium* is consumed in south-eastern Nigeria as a vegetable in a number of local diets. The plant is well known in traditional medicine circus in the West African sub region as a viable cure for a number of diseases and nutritional purposes. Research has revealed anti-diabetic properties of the plant [16,26,17], hepatoprotectve effect [27], antioxidant activity[26]. Phytochemical analysis of *V. amygdalina, G. latifolium* and *O. gratissimum* reveals the presence of saponins, flavonoids and tannins [11,16].

The present work aims at evaluating free radical scavenging ability and the inhibition of lipid peroxidation in brain homogenates by these plants, as an indication of possible neuroprotective potentials.

2. MATERIALS AND METHODS

2.1 Chemicals and Solutions

Thiobarbituric acid, Folin-Denis reagent, Catechin, Trichloroacetic acid and Sodium deodosyl sulphate (Sigma, St. Louis, MO, USA), Hydrogen peroxide and sodium carbonate (Loba-Chemie Mumbai, India), Acetic acid, Sodium dihydrogen phosphate, disodium hydrogen phosphate and Ethanol (BDH Poles England), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Fluka-Chemie, Switzerland). All other reagents used were of analytical grade.

2.2 Collection and Preparation of Plant Extracts

Fresh leaves of *G. latifolium, V. amygdalina and O. gratissimum* were collected from Nekede, Owerri West Local Government Area, Imo State, Nigeria. Collection of fresh leaves was organized in the rainy season of June 2010.The identity of the plant was authenticated by Dr F. N. Mbagwu, of Department of Plant Science and Biotechnology, Imo state University, Owerri, Nigeria. Plant specimens (Voucher Nos 0089-91) were deposited in the

Institution Herbarium. The leaves were sorted to clear debris and extraneous materials, washed and air dried at room temperature (28º±2ºC) and then further dried to constant weight in an oven at 50^{o}C and pulverized in a mill (BL 335 Kenwood). The ethanol extracts were obtained by soaking 400g of the pulverized powder in 2.0L ethanol and left to stand for 5days at room temperature (28º±2ºC). The sediment was removed by coarse filtration using a sieve and afterwards with what man no. 1 filter paper. The filtrates were separately evaporated at 49°C under reduced pressure, using a rotary evaporator (rotavapour), and then dried to solid form in vacuum desiccator to obtain ethanol extracts of *V. amygdalina*, *G. latifolium* and *O. gratissimum* respectively. The extracts were stored at 4ºC till when used.

2.3 Preparation of Brain Homogenates

A healthy male rabbit (450g) was obtained from a commercial breeding home in Owerri, Imo State, Nigeria. The animal was anesthetized, sacrificed by cervical dislocation and whole brain was removed and weighed. 10g of the brain tissue was homogenized in phosphate buffered saline 10% (w/v) using a Homogeniser (BL 335 Kenwood) to obtain a 10% brain homogenate. All experiments were carried out as approved by appropriate ethics committee (SC-BCH-EC-023) and were performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

2.4 Determination of Total Phenolic Content

Total phenolic contentwas determined using the method described by Wetassinghe and Shahidi [28]. Briefly, 50mg of *V. amygdalina*, *G. latifolium* and *O. gratissimum* were dissolved in 100ml of ethanol to obtain a concentration of 0.5mg/ml. Folin-Denis reagent (0.5ml) was added to centrifuge tubes containing 0.5ml of extracts. Tubes were shaken and 1ml of a saturated $NaCO_3$ solution was added into each tube. Volume was then adjusted to10ml by the addition of 8ml of deionised water and the content was vigorously mixed. Tubes were allowed to stand at room temperature for 25 min and then centrifuged for 5min at 4000xg. Absorbance of the supernatants was measured at 725nm. A blank sample for each extract was used for background subtraction. Content of total phenolics in each extract was determined using a standard curve prepared for (+) catechin (R^2=0.998).Total extracted phenolic compound was expressed as mg (+) catechin equivalent/100g extract.

2.5 Determination of Total Flavonoid Content

The total flavonoid content of Plant extract was determined colorimetrically according Zou, Lu, and Wei [29]. In brief, 0.5ml of sample prepared above was mixed with 2ml of distilled water and subsequently with 0.15ml of 5% $NaNO_2$ solution. After 6 min of incubation, 0.15 ml of 10% $AlCl_3$ solution was added and then allowed to stand for 6 min, followed by addition of 2ml of 4% NaOH solution to the mixture. Immediately, water was added to the sample to bring the final volume to 5ml, the mixture was thoroughly mixed and allowed to stand for another 15min. The absorbance was read at 510nm. Content of total flavonoid in each extract was determined using a standard curve (R^2=0.999) prepared for (+) catechin. Total extracted flavonoid was expressed as mg (+) catechin equivalent/100g extract.

2.6 Determination of Free Radical (2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) Scavenging Activity

The DPPH radical is a stable organic free radical with an absorption maximum band around 515–528 nm. It is a useful reagent for evaluation of antioxidant activity of compounds. In the DPPH test, the antioxidants reduce the DPPH radical to a yellow-coloured compound, diphenylpicrylhydrazin, and the extent of the reaction will depend on the hydrogen donating ability of the antioxidant [30].

The scavenging activity of extract for the radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was measured by the method described by Velázquez et al. [31]. Extracts were dissolved in methanol at concentrations of 2.65–170mg/ml, and 0.75ml of each sample was mixed with 1.5 ml of DPPH (Fluka-chemie, Switzerland) (0.02mg/ml) in methanol, with methanol serving as the blank sample. The mixtures were left for 15 min at room temperature and its absorbance then measured at 517nm. Catechin (0–50mg/l) was used as positive control. The radical-scavenging activity was calculated as follows:

$$\%\text{DPPH radical Scavenging} = \frac{\text{Blank absorbance - Sample absorbance}}{\text{Blank absorbance}} \times 100$$

The IC_{50} (concentration causing 50% inhibition) values of the extracts and vitamin E(standard antioxidant substance) were determined graphically.

2.7 Inhibition of hydrogen Peroxide-Induced Lipid Peroxidation in Rabbit Brain Homogenate

The ability of *V. amygdalina*, *G. latifolium* and *O. gratissimum* extract to inhibit lipid peroxidation in rabbit brain was studied by incubating rabbit brain homogenate treated with hydrogen peroxide (10µM) and different concentrations of the extracts (0–1000µg/ml) [32]. A mixture of rabbit brain homogenate (200µl), *V. amygdalina*, *G. latifolium* or *O. gratissimum* extract from 0 to 800µg/ml, and hydrogen peroxide (10µM) were incubated for one hour. Thiobarbituric acid reactive substances were measured according to the criteria established by Liu et al. [33]. Briefly, to the incubation mixture was added 0.75ml of 20% acetic acid (pH 3.5), 0.75ml of thiobarbituric acid (1.0%) and 0.2ml of sodium dodecyl sulphate (8.1%), and heated at 100ºC for one hour. 2ml of TCA (10%) was added and centrifuged at 6000rpm for 5minutes and optical density of the resulting supernatant read at 532nm wavelength. Quercetin was used as standard

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

2.8 Data Analysis

Results of triplicate determinations of percentage Inhibition were calculated as means ± standard deviation. These results were then ploted against concentrations of plant extracts and standards by curve-fitting both symmetric (sigmoid) and asymmetric (logistic dose response) transition eqautions using Table curve 2D (systat USA). This was done by robust minimization of ln (sqrt{1+Residuals^2) and high pearson (VII lim). Maximum iterations were set at 100 with both intercept and zero intercepts. Inhibitory concentrations (IC_5 – IC_{100}) were determined by evaluation of x= root(y). The results of the IC_{50}were then subjected to one-

way analysis ofvariance(ANOVA) and significant differences between means were determined at alpha =0.05 using Excel+Analysi-it (Leeds UK) as formerly described [34,35].

3. RESULTS

3.1 Effect of Ethanol Extracts of *V. amygdalina, G. latifolium, O. gratissimum* and Vitamin E on Scavenging of 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical

Fig. 1 presents the DPPH free radical scavenging abilities of *V. amygdalina, G. latifolium, O. gratissimum*extractsand vitamin E. According to this Figure, the datashowed that the plants results favourably comparedwith vitamin E profile. All the extract achievedover 65% radical scavenging at the tested concentrations.The extracts were alsoeffective at low concentrations (10µg/ml). *G. latifolium* was most effective achieving 75.54±0.80% free radical scavenging at 400µg/ml of extracts. Free radical scavenging ability was in the order *G. latifolium*> *V. amygdalina* > *O. gratissimum*.IC_{50} for *V. amygdalina, G. latifolium and O. gratissimum* as evaluated from the inhibition graph (Fig. 1) were181.5µg/ml,153.33µg/ml, and 189.67µg/ml respectively.

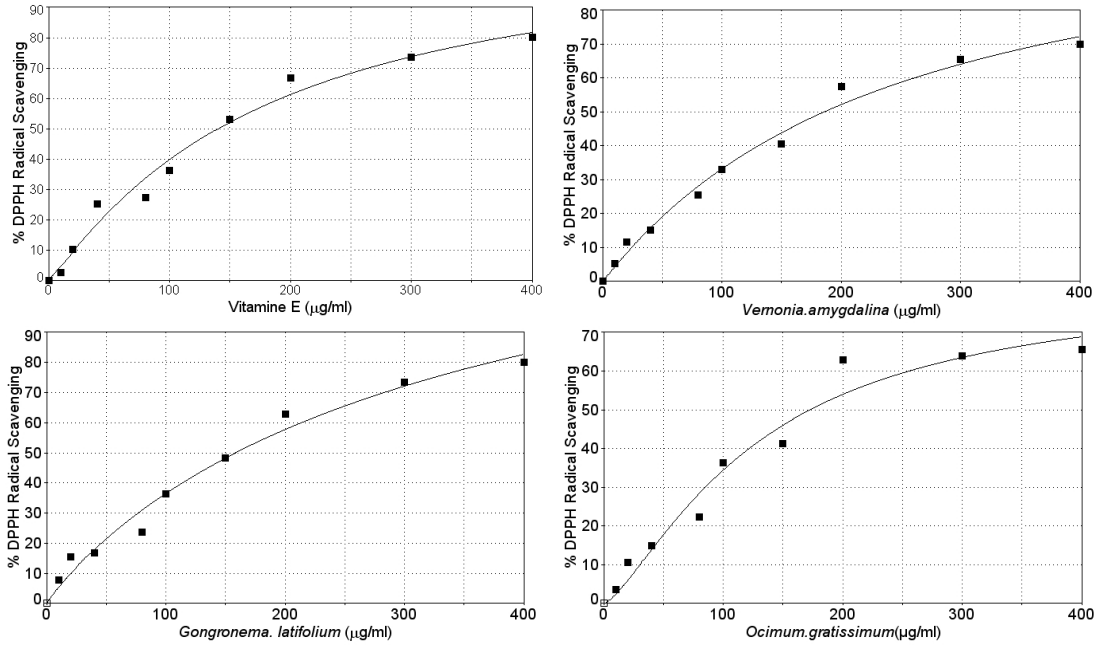


Fig. 1. Effect of ethanol extracts of *V.amygdalina, G.latifolium, O.gratissimum* and vitamin E onscavenging of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) Radical. Note that scavenging of DPPH radical followed a logistic dose response pattern

3.2 Effect of Ethanol Extracts of *V. amygdalina, G. latifolium, O. gratissimum* and quercetin on H_2O_2 Induced lipid Peroxidation in Rabbit Brain Homogenates

The result obtained from this study (Fig. 2) Showedthat ethanolextracts of *V. amygdalina, G. latifolium* and *O. gratissimum* inhibited hydrogen peroxide(H_2O_2)induced lipid peroxidation in rabbit brain homogenates. *G. latifolium* exerted the highest inhibition of lipid peroxidation process among the extracts with an inhibitory concentration (IC_{50}) value of 357.37±15.6µg/ml, followed by *V. amygdalina* with an inhibitory concentration (IC_{50})value of 589.88±22.72*µ*g/ml. *O. gratissimum* was not appreciably effective and IC_{50} was non-determinable. However, its inhibitory concentration (IC_{20}) was 754.28±30.23*µ*g/ml.

Result of the study (Fig. 2) showed that the extracts and quercetin inhibited lipid peroxidation process in a dose dependent fashion. Extracts of *V. amygdalina* and *O. gratissimum* inhibited H_2O_2 induced lipid peroxidation in rabbit brain homogenatesin a logistic dose response (LDR) pattern (R^2=0.998), while *G. latifolium* and quercetin followed a sigmoid abc pattern (R^2= 0.973 and 0.971 respectively) (Table 1).

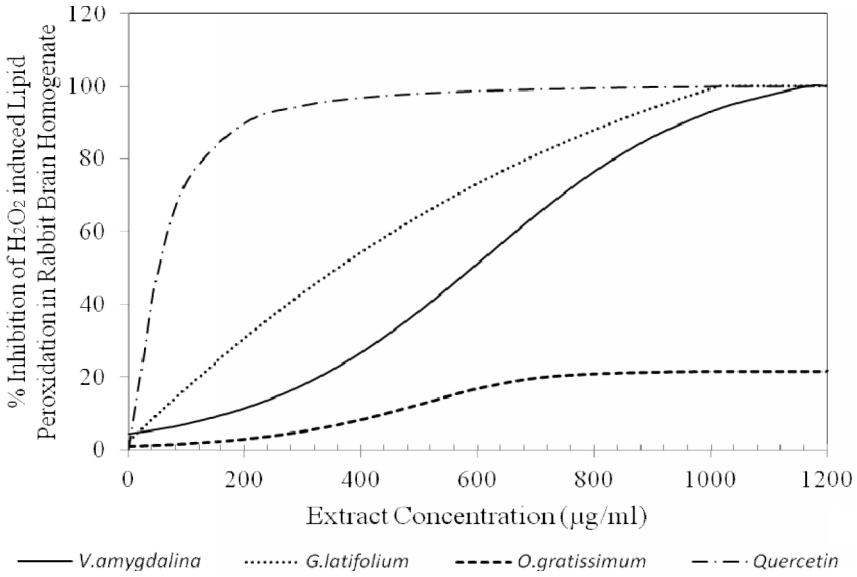
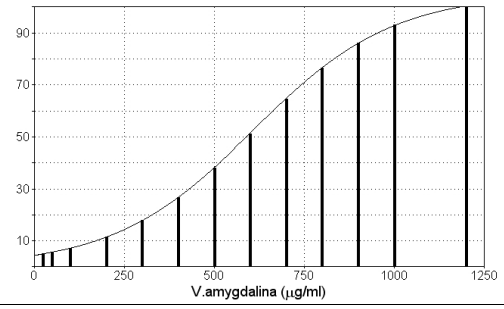
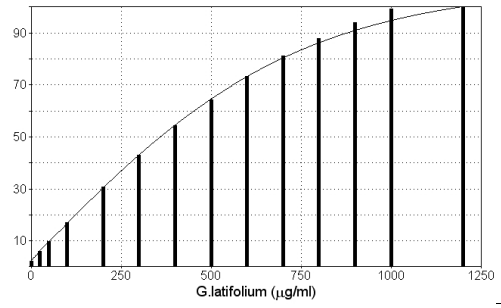
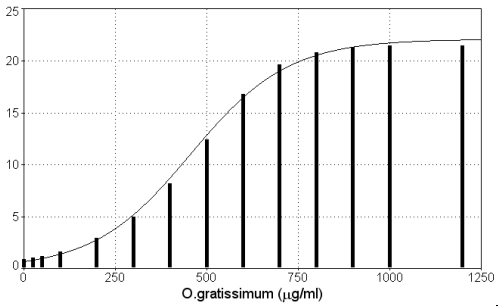
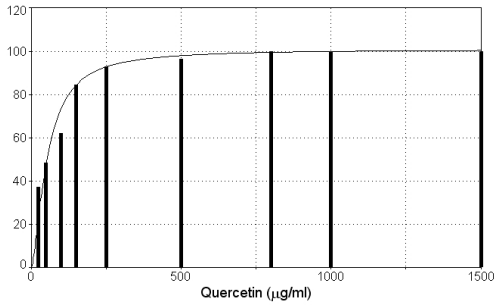


Fig. 2. Effect of ethanol extracts of *V. amygdalina, G. latifolium, O. gratissimum*and quercetin on H_2O_2 induced lipid peroxidation in rabbit brain homogenates

3.3 Total Phenolic and Flavonoid Content of Ethanol Extracts of *V. amygdalina, G. latifolium* and *O. gratissimum*

Fig. 3 shows the total phenolic and flavonoid contents of *V. amygdalina, G. latifolium* and *O. gratissimum*. Flavonoid content was 843.09±21.6mg/100g, 746.34±13.7mg/100g and 1160.98±24.5mg/100g Catechin equivalent; while total phenolic content was 432.79±42.2mg/100g, 274.1±37.3mg/100g, and 490.49±58.1mg/100g Catechin equivalent in *V. amygdalina, G. latifolium* and *O. gratissimum* respectively. Phenolic and flavonoid content of *O. gratissimum* was significantly ($p<0.05$) higher than *V. amygdalina* and *G. latifolium*.

Table 1. Models of inhibition of H_2O_2- induced lipid peroxidation process by extracts of A) *V. amygdalina,* B) *G. latifolium, C) O. gratissimum* and D) Quercetin

	MODEL	IC_{50}	Equation	R^2
% Inhibition of H_2O_2-induced Lipid Peroxidation in Rabbit Brain Homogenate	A 	589.58±22.72	Sigmoid abc	0.998
			$y = \frac{a}{1+\exp\left(-\frac{x-b}{c}\right)}$	
	B 	357.36±15.60	Logistic dose response (LDR) abcd	0.973
			$y = a + \frac{b}{1 + \left(\frac{x}{c}\right)^d}$	
	C 	ND	Sigmoid abc	0.998
			$y = \frac{a}{1+\exp\left(-\frac{x-b}{c}\right)}$	
	D 	51.91 ± 2.01	Logistic dose response (LDR)abcd	0.971
			$y = a + \frac{b}{1 + \left(\frac{x}{c}\right)^d}$	

Where y = % inhibition of peroxidation, x = extract/antioxidant concentration

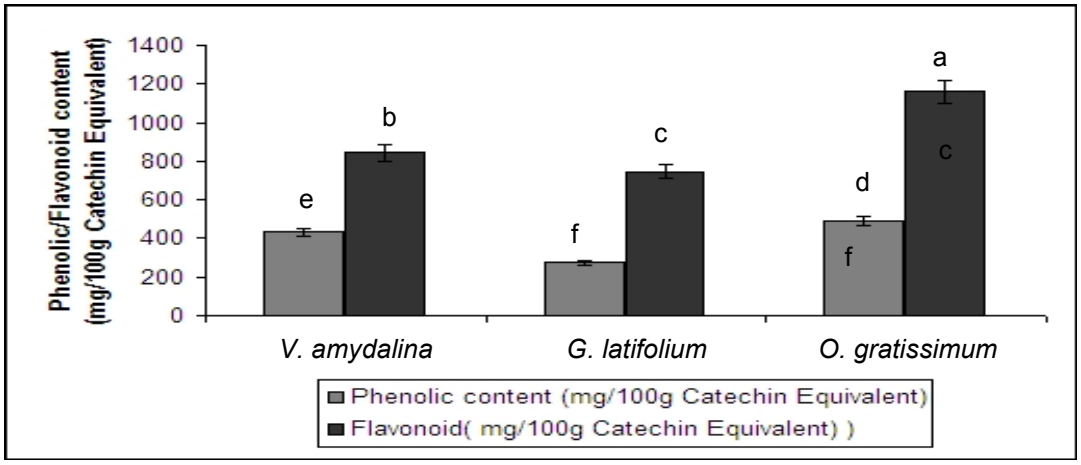


Fig. 3. Phenolic and flavonoid content of *V. amygdalina, G. latifolium and O. gratissimum* expressed as mg/100g Catechin equivalent. (Different superscript showed significant difference at p<0.05)

4. DISCUSSION

The ethanol extracts of *V. amygdalina*, *G. latifolium*, and *O. gratissimum* possessed a high total phenolic and flavonoid content [36,37]. Their phytochemical content is comparable to those of established medicinal plants and vegetables. Flavonoids and phenolic compounds are important plant derived antioxidants, and their *In-vitro* and *In-vivo* properties have been extensively reported [38,39]

DPPH radical has been widely used in *In-vivo* assessment of free radical scavenging ability via the capacity of antioxidants to donate electron or H^+ to DPPH [30]. *V. amygdalina*, *G. latifolium*, and *O. gratissimum* possessed high antiradical property. *G. latifolium* was more effective at scavenging DPPH radicals when compared to *V. amygdalina* and *O. gratissimum* as can be seen in their IC_{50}values (153.33µg/ml, 181.5µg/ml and 189.67µg/ml) respectively. The extracts favourably compared to Vitamin E, a standard free radicals scavenger with IC_{50}value of 138.88µg/ml. Vitamin E and *G. Latifolium* successfully scavenged 75% of the radicals at 400µg/ml of extracts. The DPPH radical scavenging properties of *V. amygdalina, G. latifolium, and O. gratissimum* were comparable to Vitamin E. The high free radical scavenging properties of these extracts are indicative of a high antioxidant activity mediated via electron or hydrogen ions donation, which is attributable to their rich phenolic and flavonoids contents. Earlier studies have indicated that flavonoids exert their biological effect at the cellular level by specific interaction, with molecular targets such as nucleic acid, polysaccharides, protein and nerve cells [38,39]. Flavonoids and generally plant derived phenolic compounds are electron rich and therefore could act as sink for protons and free radicals [38,39]. *G. latifolium* had a significantly ($p<0.05$) lower phenolic and flavonoid content but possessed better inhibition of hydrogen peroxide-induced peroxidation of rabbit brain. It is known that *G. latifolium* contains phytochemicals other than phenolic compounds and flavonoids. These other phytochemicals in combination with phenolic and flavonoid content may have acted in combination to bring about the synergism that produced a better antioxidant property. Wu and co-workers have shown that some other agents like carnosine, anserine and free amino acids possessed antioxidant activities [40]. Malondialdehyde (MDA) and 4-hydroxynonenalsHNE) are products of lipid peroxidation [41]. Lipid peroxides and MDA

react with thiobarbituric acid and this substance has been extensively measured as an index for assessing lipid peroxidation level in tissues [42]. The threshold inhibitory concentrations of the ethanol extracts of the plants against lipid peroxidation in rabbit brain homogenate revealed that the extracts were appreciably effective against peroxidation in a dose dependent fashion. *G. latifolium* extract had a higher level of inhibition of lipid peroxidation than *V. amygdalina* and *O. gratissimum* extracts. Production of TBARS was significantly lower ($p<0.05$) in brain homogenates treated with extracts of *G. latifolium*, (IC_{50}=362.59±15.6*μ*g/ml), than *V. amygdalina* (IC_{50}=629.5±22.72*μ*g/ml) and *O. Gratissimum* (IC_{50}not determinable, but IC_{20} =785.73±40.23*μ*g/ml). The ability of the extracts to scavenge free radicals and inhibit peroxidation in the brain homogenates could be attributed to their phenolic and flavonoid contents, the prevalence of phenolic and flavonoid-rich components in human diet have been associated with significant reduction of risk factors in chronic human pathologies like neurodegeneration, cancer [39], diabetes and cardiovascular diseases.

Plant phytochemicals such as polyphenols have been reported to be able to form complexes with metals, scavenge free radicals and exhibit strong antioxidant activity, which is mainly due to their redox properties that allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers [36], this makes free radicals unavailable for the initiation of lipid peroxidation. Antioxidants effects have been reported for flavonoids. They possess several beneficial effects, such as anti-inflammatory, anti-allergic, and anti-viral as well as an anticancer activity [39]. They have also been suggested to play a protective role in liver diseases, cataracts, and cardiovascular diseases [36]. Flavonoids such as quercetin and silybin acting as free radical scavengers have been shown to exert a protective effect in reperfusion ischemic tissue damage [43,44]. A correlation between the antioxidant activities and total phenolic contents of many species has been reported [45,46].The capability of the extract of *V. amygdalina*, *G. latifolium*, and *O. gratissimum* to inhibit lipid peroxidation is attributable to their reported rich phytochemical contents and observed free radical scavenging ability. Their polyphenolic compounds may have formed complexes with free radicals, thereby preventing them from catalysing and initiating lipid peroxidation or could have scavenged the radicals produced. The neuroprotection of the cerebellum by the methanol extract of *V. amygdalina* leaves on the gamma-irradiated brain of Wistar rats has been reported [47].

A study has shown that a number of events associated with Alzheimer's disease are capable of stimulating production of free radicals and depletion of antioxidant [48]. The oxidative breakdown of polyunsaturated fatty acids (PUFA) is a chain-reaction, which can be initiated by a number of oxidants, pro-oxidants and trace metals (Cu, Fe). The brain is rich in PUFAs and has a high oxygen demand (consumes about 20% of respiratory oxygen) [49] which predisposes it to oxidative damage. The brain has also been found to contain low level of antioxidants [50], and trace metals such as Fe and Cu which catalyzes free radicals chain reaction. The combination of these factors results in a fast depletion of antioxidants stores and production of partially reduced oxygen intermediates, thus, a tilt towards peroxidation of brain lipids, protein damage and DNA lessions. In the present study, the Fenton type reaction inhibited by the extracts in this study demonstrated their antioxidant ability and potentials to stop the initiation of the chain reactions leading to peroxidation of membrane lipids, protein damage and DNA lesions. Polyphenols are known to cross the blood-brain barrier and can exert their antioxidant and iron chelating properties in the brain. The Inhibition of lipid peroxidation in brain homogenates is analogous to neuro-protection. These extracts may be useful against the deleterious effect of free radicals mediated damage in cellular components.

5. CONCLUSION

Extracts of *G. latifolium*, *V. amygdalina*, and *O. gratissimum* have demonstrated good ability to scavenge free radicals and protect against hydrogen peroxide-induced lipid peroxidation in rabbit brain homogenates. These effects of these extracts are analogous to neuro-protection and hence they possess beneficial properties for the management of neuro-degeneration. The vegetables could serve as rich sources of antioxidants for improving nutrition and health.

Following the findings of this study, we recommend an increased awareness on the benefits of consuming these vegetables. The results obtained in the present study provided scientific support to use these plants based on its antioxidant and neuroprotective properties.

ETHICAL APPROVAL

We, hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed 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

1. Fasuyi AO. Nutritional potentials of some tropical vegetable leaf meals: Chemical characterization and functional properties. Afr. J. Biotech. 2006;5(1):49-53.
2. Okafor JO. Horticultural Promising Indigenous Wild Plant Species of the Nigeria Forest Zone. Hort.1983;123:165-176.
3. Xing-Jun W, Yi-ping W, WelW, Wei-kang S, Ya-ming, XU, Li-Jiang X. Free radical scavenging and inhibition of lipid peroxidation by magnesium lithospermate B1. Acta Pharmacol. 2000;21(9):855-858.
4. Gülçin I, Sat IG, Beydemir S, Elmastas M, Kufrevioglu OI. Comparison of antioxidant activity of clove (*Eugenia caryophylata thunb*) buds and lavender (Lavandulastoechas L.). Food Chem. 2005;87:393–400.
5. Tabassum IB, Siddiquia ZN, Rizvib JS. Protective effect of *Ocimum sanctum* on lipid peroxidation, nucleic acids and protein against restraint stress in male albino rats. Biology and Medicine. 2009;1(2):42-53.
6. Huang X, Moir RD, Tanzi RE, Bush AI, Rogers JT. Redox-active metals, oxidative stress, and Alzheimer's disease pathology. Ann. NY Acad. Sci. 2004;1012:153–163.
7. Shulman RG, Rothman DL, Behar KL, Hyder F. Energetic basis of brain activity: Implications for neuroimaging. Trends Neurosci. 2004;27(8):489-495.
8. Silva JB, Temponi VS, Gasparetto CM, Fabri RL, Aragão DM, Pinto NC, Ribeiro A Scio E, Del-Vechio-Vieira G, Sousa OV, Alves MS. *Vernonia condensata* Baker (Asteraceae): A Promising Source of Antioxidants. Oxidative Medicine and Cellular Longevity. 2013;1-9.
9. Alisi CS, Ojiako OA, Osuagwu CG, Onyeze GOC. Free radical scavenging and In-vitro Antioxidants effects of ethanol extracts of the medicinal herb *Chromolaena odorata Linn* Brit. J. Pharm. Res. 2011;1(4):141-155.

10. 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.
11. Akinmoladun AC, Ibukun EO, Afor E, Obuotor EM, Farombi EO. Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum*. Sci. Res. and Essay. 2007;2(5):163-166.
12. Shafazila TS, Pat ML, Hung LK. Inhibition of Lipid Peroxidation by Extract and Fractions of *Dendrobium soni*' RedBom'. IPCBEE. 2011;7:19-22.
13. Dubey NK, Tiwari TN, Mandin D, Andriamboavonjy H. Chaumont JP. Antifungal properties of *Ocimum gratissimum* essential oil (ethyl cinnamate chemotype). Fitoterapia. 2000;7(15):567-569.
14. Igile GO, Oleszek W, Jurzysta M, Burda S, Fafunso M, Fasanmade A. Flavonoids from *Vernonia amygdalina* and their antioxidants activities, J. Agric. Food Chem.1994;42:2455-2448.
15. Yeap SK, Wan YH, Boon KB, Woon SL, Huynh Ky, Abdul Hadi NY, Noorjahan BA. *Vernonia amygdalina*, an ethno-veterinary and ethnomedical used green vegetable with multiple bioactivities. Journal of Medicinal Plants Research. 2010;4(25):2787-2812.
16. Atangwho IJ, Ebong PE, Eyong EU, Williams IO, Eteng MU, Egbung GE. Comparative chemical composition of leaves of some antidiabetic medicinal plants: *Azadiracta indica, Vernonia amygdalina and Gongronema latifolium*. Afri. J. Biotech. 2009;8(18):4685-4689.
17. Akpaso MI, Atangwho IJ, Akpantah A, Victor AF, Anozeng OI, Ebong PE. Effect of Combined Leaf Extracts of *Vernonia amygdalina* (Bitter Leaf) and *Gongronema latifolium* (Utazi) on the Pancreatic β-Cells of Streptozotocin-Induced Diabetic Rats. Bri. J. of Med. & Med Res. 2011;1(1):24-34.
18. Molgaard P, Nielsen SB, Rasmussen DE, Drummond RB, Makaza N, Andreassen J. Anthelmintic screening of Zimbabwean plants traditionally used against schistosomiasis. J. Ethnopharmacol. 2001;74:257-264.
19. Ogbulie JN, Ogueke CC, Nwanebu FC. Antibacterial properties of *Uvariachamae, Gongronema latifolium, Garcinia kola, Vernonia amygdalina and Aframomium melegueta*. African J. Biotechnol. 2007;6:1549-1553.
20. Tona L, Cimanga RK, Mesia K, Musuamba CT, Bruyne T, Apers S, Hermans N, Van MiretS,Pieters L,Totte J, Vlietnk AJ. *In vitro* antiplasmodial activity of extracts and fractions of seven medicinal plants used in Democratic Republic of Congo. Journal of Ethnopharmacology. 2004;93:27-32.
21. Oboh G. Effect of blanching on the antioxidant properties of some tropical green leafy vegetables. Lebensm - Wiss. u. –Technol - Food Sci. Technol. 2005;38:513-517.
22. Odukoya OA, Inya-Agha SI, Segun FI, Sofidiya MO, Ilori OO. Antioxidant activity of selected Nigerian green leafy vegetables. Am. J. Food Tech. 2007;2:169-175.
23. Atangwho IJ, Ebong PE, Eyong EU, Eteng MU, Uboh FE. *Vernonia amygdalina* Del: A potential prophylactic antidiabetic agent in lipids complication. Glob. J. Pur. Appl. Sci. 2007;13(1):103-106.
24. Arhoghro EM, Ekpo KE, Anosike EO, Ibeh GO. Effect of aqueous extract of bitter leaf (*Vernonia amygdalina* Del) on carbon tetrachloride (CCl_4) induced liver damage in albino wistar rats. Eur. J. Sci. Res. 2009;26:122-130.
25. Adesanoye OA, Farombi EO. Hepatoprotective effects of *Vernonia amygdalina* (*Astereaceae*) in rats treated with carbon tetrachloride. Exp. Toxicol. Pathol. 2009;62:197-206.

26. Ugochukwu NH, Babady NE. Antioxidant effects of *Gongronema latifolium* in hepatocytes of rat models of non-insulin dependent diabetes milletus. Fitoterapia. 2002;73:612-618.
27. Nnodim J, Emejulu AA. The protective role of *Gongronema latifolium*in acetaminophen-induced hepatic toxicity in Wistar rats. Asian Pacific Journal of Tropical Biomedicine. 2011;S151-S154
28. Wettasinghe M, Shahidi F. Scavenging of reactive-oxygen species and DPPH free radicals by extracts of borage and evening primrose meals. Food Chem. 2000;70:17-26.
29. Zou YP, Lu YH, Wei DZ. Antioxidant activity of a flavonoid-rich extract of *Hypericum perforatum* L. *In vitro*. Journal of Agricultural and Food Chemistry. 2004;52:5032–5039.
30. Bondent V, Brand-Williams W, Bereset C. Kinetic and mechanism of antioxidant activity using the DPPH free radical methods. Lebensmittel Wissenschaft and Technologie.1997;30:609–615.
31. Velazquez E, Tournier HA, Mordujovich de Buschiazzo P, Saavedr G, chinella GR. Antioxidant activity of Paraguayan plant extracts. Fitoterapia. 2003;74:91–97.
32. Muralikrishnan D, Binu T, Bala VM. Antiparkinson Drug – Mucunapruriens shows Antioxidant and Metal Chelating Activity. Phyto. Res. 2008;22:6-11.
33. Liu J, Edamatsu R, Kabuto H, Mori A, Antioxidant action of Guilingji in the brain of rats with $FeCl_3$ induced epilepsy. Free Radical Biology and Medicine. 1990;9:451–454.
34. Marquardt DW. An algorithm for least squares estimation of non-linear parameters. J. Soc. Ind. Appl. Mat.1964;2:431-441.
35. Alisi CS, Ojiako AO, Osuagwu CG, Onyeze GOC. Response Pattern of Antioxidants to Lipid Peroxide Concentration in Carbon Tetrachloride-Induced Hepato - Toxicity is Tightly Logistic in Rabbits. European Journal of Medicinal Plants. 2011;1(4):118-129.
36. Oriakhi K, Oikeh EI, Ezeugwu N, Anoliefo O, Aguebor O, Sheena E. Comparative antioxidant activities of extracts of *Vernonia amygdalina* and *Ocimum gratissimum* leaves. Journal of Agricultural Science. 2014;6:13-20.
37. Audu SA, Taiwo AE, Ojuolape AR, Sani AS, Bukola AR, Mohammed IA.study review of documented phytochemistry of *Vernonia amygdalina* (Family *Asteraceae*) as the basis for pharmacologic activity of plant extract. Journal of Natural Sciences Research. 2012;2:1-9.
38. Oboh G, Puntel RL, Rocha JBT. Hot peer (Capsicum annuum, Tepin and Capsicum chinese, Habanero) prevents Fe^{2+}- induced lipid peroxidation in Brain: *In vitro*. Food Chem. 2007;102:178–185.
39. Narayana KR, Sripal RM, Chaluvadi MR, Krishna DR. Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. Indian J. of Pharm. 2001;33:2-16
40. Wu HC, Shiau CY, Chen HM, Chiou TK. Antioxidant activities of carnosine, anserine, some free amino acids and their combination. J. Food Drug Anal. 2003;11:148-153.
41. Smith KJ, Kapoor R, Felts PA. Demyelination: The role of reactive oxygen and nitrogen species. Brain Pathol. 1999;9:69–92.
42. Esterbauer H, Schaur RJ, Zollner H. Chemistry and Biochemistry of 4-Hydrononenal, Malondialdehyde and Related Aldehydes', Free radical biol. med.1991;11:81-128.
43. Santos AC, Vyemura SA, Lopes JL, Bazon JN, Mingatto FE. Effect of naturally occuring flavonoids on lipid peroxidation and membrane permeability transition in mitochondria. Free Radic Biol Med.1998;24:1455-61.

44. Fraga CG, Mactino US, Ferraro GE, Coussio JF, Boveris A. Flavonoids as antioxidants *V. amygdalina* evaluated by *In vitro* and in situ liver chemiluminescence. Biochem Med MetabolBiol.1987;36:717-20.
45. Juliani HR, Simon JE. Antioxidant activity of Basil. Trend in new crops and new uses (J. Janick and A. Whipkey (Eds.) ASHS Press, Alexandrica, VA. 2002;575-579.
46. Nantitanon W, Yotsawimonwat S, Okonogi S. Factors influencing antioxidant activities and total phenolic content of guava leaf extract. LWT - Food Sci. and Techno. 2010;43:1095-1103.
47. Owoeye O, Farombi EO, Onwuka SK. Gross morphometric reduction of rats cerebellum by gamma irradiation was mitigated by pre-treatment with *Vernonia amygdalina* leaf extract. Rom. J. Morphol. Embryol. 2011;52(1):81–88.
48. Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB. Identification of human brain tumour initiating cells. Nature. 2004;432(7015):396-401.
49. Marcello I, Sara V, Gelsomina F, Franco F. Neuroprotective Herbs and Foods from Different Traditional Medicines and Diets. Molecules. 2010;15:3517-3555.
50. Vega-Naredo B, Poeggeler V, Sierra-Sanchez B, Caballero C, Tomas-Zapico O, Alvarez-Garcia. Melatonin neutralizes neurotoxicity induced by quinolinic acid in brain tissue culture. J. Pineal. Res. 2005;39:266–275.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=561&id=32&aid=4911