



EVALUATION OF ANTIOXIDANT POTENTIALS OF METHANOLIC EXTRACTS OF *OCIMUM GRATISSIMUM* AND *VERNONIA AMYGDALINA*

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ABSTRACT

Oxidative stress has been identified as the root cause of the development and progression of several diseases. Supplementation of exogenous antioxidants or boosting endogenous antioxidant defenses of the body is a promising way of combating the undesirable effects of reactive oxygen species (ROS) induced oxidative damage. Plants have an innate ability to biosynthesize a wide range of non-enzymatic antioxidants capable of attenuating ROS- induced oxidative damage. The antioxidant properties of methanolic extracts of two commonly consumed plants (*Ocimum gratissimum* and *Vernonia amygdalina*) in Nigeria were evaluated comparatively by in vitro method. This was to provide more scientific basis for increased domestic harnesses of these plants. The study indicated that the two plants have antioxidant properties and their activities were dose dependent. *O. gratissimum* had a mean scavenging activity of 75.150 ± 7.080 while *V. amygdalina* had 72.78 ± 7.654 . Ascorbic acid used as reference compound showed the highest antioxidant activity, with the mean free radical scavenging activity of 92.28 ± 0.6897 and 88.333 ± 0.4381 for tablets and injection respectively. As observed, the DPPH radical scavenging activities of the two plants were not significantly different from that of standard ascorbic acid. The result thus confirms that *O. gratissimum* and *V. amygdalina* extracts possess appreciable natural antioxidant potentials and this provide good scientific justification for their increased domestication. Consumption of these vegetables is thereby encouraged to help militate against deleterious effect of reactive species known as free radicals.

KEYWORDS: Antioxidants, *Ocimum gratissimum*, *Vernonia amygdalina*, Oxidative stress, Reactive oxygen species, DPPH, Ascorbic acid.

1.0 INTRODUCTION

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) play important roles in many biological processes and are involved in host defense (Eze *et al.*, 2000; Eze, 2006). Over-production of these species such as hydroxyl radical ($\cdot\text{OH}$), hydrogen peroxide (H_2O_2), superoxide anions (O_2^-), and nitric oxide (NO), as well as peroxyl nitrite contributes to the immunopathology of a vast variety of conditions such as inflammatory diseases, cancer, atherosclerosis, diabetes mellitus, hypertension, AIDS, and aging (Darley-Usmar *et al.*, 1995; Lee *et al.*, 2000). Current studies on the formation of reactive oxygen species (ROS) have shown that normal body metabolism cannot function well without these species. Oxidation is an essential process in all organisms which involve redox reactions. These reactions normally generate reactive oxygen species (ROS) or highly reactive free radicals that can react virtually with all cell components to cause tissue injury (Collier *et al.*, 1992).

Exogenous sources of free radicals include tobacco smoke, ionization radiation, certain pollutants such as cadmium, vanadium, crude oil, organic solvents and pesticides (Robinson *et al.*, 1997). They are useful to the body when moderately produced or strictly regulated (Manoj *et al.*, 2009).

Similarly, antioxidation is the reversal of the above described process (that is oxidation) mediated through scavenging of the free radicals, by dismutase enzyme systems, non-enzyme system like vitamin C, and vitamin E and phenolic-rich compounds such as flavonoids (Sardesai 1995; Rice-Evans *et al.*, 1996). The body has evolved a number of interrelated antioxidant mechanisms to maintain redox homeostasis (Toyokuni *et al.*, 1999). Antioxidants are substances that neutralize free radicals or their actions (Sies, 1999). Normally, all animal cells contain antioxidants that prevent damage due to the action of reactive oxygen species (ROS) and reactive

nitrogen species (RNS). Those mechanisms include endogenous enzymes like catalase, superoxide dismutase, thioredoxin and glutathione peroxidase (Aloh and Ozougwu, 2010). When functioning properly, the antioxidant system suppresses and control excessive free radicals production, allowing control oxidative energy metabolism to proceed normally without cellular or molecular damage (Njoku *et al.*, 2011).

Thus, to sustain life, some enzymes in the human system like catalase, superoxide dismutase (SOD), glutathione systems helps to regulate and control the escalation of free radicals in the body, a situation known as oxidative stress. Oxidative stress is a disturbance in the balance between the production of reactive oxygen species or free radicals and antioxidant defense, which may lead to tissue injury. Complementary to these enzymes are some plant species that have been found useful in controlling or regulating the over-production of these free radicals in the body (Manoj *et al.*, 2009).

There has been a great deal of interest recently in the role of complementary and alternative medicines in the treatment of various acute and chronic diseases. For decades, the screening of medicinal plant materials for their therapeutic values has continued to represent potential sources of new effective medicines. Besides, evidences from epidemiological studies suggest that high consumption of fruits and vegetables is linked to reduced risk of developing most oxidative stress – induced diseases (Dani *et al.*, 2008; Wasson *et al.*, 2008; Atrooz, 2009). Examples of such diseases include cancer, diabetes mellitus, protein energy malnutrition (PEM), cataract, infections and other degenerative diseases of aging (Dani *et al.*, 2008; Wasson *et al.*, 2008; Atrooz, 2009; Omoregie and Osagie, 2011; Dhanasekaran and Ganapathy, 2011). Previous studies have shown that increased production of reactive oxygen species (ROS) may be one of the underlying causes of these diseases (Bender, 2006; Adesegun *et al.*, 2008; Wasson *et al.*, 2008; Martin and Appel, 2010). The long term effects of increased ROS level include damages to important cellular components especially proteins, nucleic acids and polyunsaturated fatty acids in cell membranes and plasma lipoproteins (Bender, 2006; Kasote *et al.*, 2011). Reports abound on the antioxidant activities of phytochemical constituents of medicinal plants (e.g. carotenoids, flavonoids, phenolics, vitamins C and E). These phytochemicals act as antioxidants by preventing damages to cell membrane due to cellular oxidative processes that may result in diseases (Obboh and Rocha, 2008; Wasson *et al.*, 2008; Ebrahimzadeh *et al.*, 2009; Atrooz, 2009; Omoregie and Osagie, 2011; Kasote *et al.*, 2011). For instance, natural polyphenols from plant vegetables have been found to exert their beneficial effect by removing free radicals, chelating metal catalyst, activating antioxidant enzymes, etc (Atrooz *et al.*, 2009; Obboh *et al.*, 2009).

The knowledge of the antioxidant properties of many medicinal plants have led to preparation of many herbal products like Yoyo bitter, Swedish bitter etc. Emphasis will be led on the two African widely used plants; *Vernonia amygdalina* (bitter leaf) *Ocimum gratissimum* (scent leaf).

Vernonia amygdalina, commonly known as bitter leaf, is a shrub that grows up to 3 meters high in the African tropics and other parts of Africa, particularly, Nigeria, Cameroon and Zimbabwe. It is known as 'Etidot' in Akwalbom State and Cross River State. In Yoruba, it is called 'Efo Ewuro' in Igbo and Hausa, it is known as 'Onugbu' and Shiwaka respectively. Among the Itsekiri, Tiv, And Edo, it is called 'Orugbo', 'Ityuna' and 'Oriwo' respectively. It is reported to have several health benefits, effective against amoebic dysentery (Moundipa *et al.*, 2000), gastrointestinal disorders, and has antimicrobial and antiparasitic activities (Hladik *et al.*, 2005). The biologically-active compounds of *Vernonia amygdalina* are saponins and alkaloids (Muraina *et al.*, 2010), terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans, xanthenes and anthraquinone (Cimanga *et al.*, 2004), and edotides (Izevbigie, 2003). Sesquiterpenes were discovered some years back by Kupchan and his co-workers (Kupchan *et al.*, 1969).

Ocimum gratissimum popularly known as scent leaf is another plant which is commonly used traditionally to treat many ailments. It is an aromatic and perennial woody shrub, 1-3m tall, and has erected stem. It is also known as Clove Basil and African Basil. *Ocimum gratissimum* is native to Africa, Madagascar, Southern Asia, and the Bismark Archipelago. Local names are 'Ntonng' in Ibibio 'Efinrin' in Yoruba, 'Daidoya' in Hausa, 'Nchuawu' in Igbo, and 'Kunudiri' in Okrika (Odukoya *et al.*, 2005).

Ocimum gratissimum is a vegetable commonly used as spice in its unprocessed form in most West African dishes because of its very intense and dominant flavour (Odukoya *et al.*, 2005). It has also been reported to possess antioxidant property (Odukoya *et al.*, 2005; Leal *et al.*, 2006; Afolabiet *et al.*, 2007) which may be due to the presence of volatile oils which contain phenolics, eugenols, thymol, flavonoids, phenylpropanoids, linalool, citral *et.c.* (Jedlickova *et al.*, 1992; Leal *et al.*, 2006). Apart from its antioxidant property, *Ocimum gratissimum* also have chemotherapeutic, antispasmodic and analgesic properties (Ilori *et al.*, 1996; Nakamura *et al.*, 1999; Aziba *et al.*, 1999; Rabelo *et al.*, 2003). Many phytochemicals have been identified as components of food and medicine and more are still being discovered (Ajali, 2004).

Vitamin C (Ascorbic acid) which is used as a standard drug for this work is widely used and a known antioxidant (Lawrence, 2013). It is an electron donor, and this property accounts for all its known functions. As an electron donor, vitamin C is a potent water-soluble

antioxidant in humans (Sebastian *et al.*, 2003). Its antioxidant effects have been demonstrated in many in vitro experiments. Epidemiological studies show that diets high in fruits and vegetables are associated with lower risk of cardiovascular disease, stroke and cancer, and with increased longevity (Sebastian *et al.*, 2003).

2.0 MATERIALS AND METHODS

2.1. Collection and Identification of Plant Materials

Fresh leaves of *Ocimum gratissimum* were procured from Itam market in Itu-Uyo, Nigeria while that of *Vernonia amygdalina* were obtained from a farmland, also in Itu-Uyo, Nigeria both in March, 2016. All the plant leaves were identified and authenticated by Dr (Mrs.) U. E. Essiet, a plant Taxonomist of Botany and Ecological Studies Department, Faculty of Science, University of Uyo, Nigeria. The plant leaves were deposited in the University of Uyo Herbarium, Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, Nigeria. *Ocimum gratissimum* had a voucher number of UUPH 10(j), while that of *Vernonia amygdalina* was UUPH 38(a).

2.2 Preparation of Plant Extracts

The plant materials were rinsed with water to remove dirt and then dried at room temperature. The dried plant materials were pulverized mechanically. 109g of pulverized *Ocimum gratissimum* leaves was extracted by soaking in 1500ml of pure methanol for 72 hours at room temperature with intermittent shaking. For *Vernonia amygdalina* powdered leaves, 240g was soaked in 2500ml of pure methanol. The mixtures were subsequently filtered at the end of the extraction period and concentrated in a water bath at a temperature of 50-60°C. The concentrates were then stored at 4°C for subsequent analysis.

2.3 Chemicals

DPPH (2,2-diphenyl-1-picrylhydrazyl) was obtained from the Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, University of Uyo, Nigeria. Methanol was a product of Kermel Chemicals purchased in Uyo, Nigeria. Vitamin C tablet used was a product of Emzor pharmaceutical industries while the injection was a product of Hubei Tianyao pharmaceutical marketed by Chupet pharm. co. ltd. They were purchased from Siban pharmacy, Uyo, Nigeria.

2.4 Antioxidant Assay

Estimation of DPPH Radical Scavenging Activity

Antioxidant activities of the methanolic extracts of *Ocimum gratissimum* and *Vernonia amygdalina* were measured as their ability to scavenging stable DPPH radicals. The scavenging activity of the plant extracts and standard vitamin C were determined using the modified Blois method (Blois, 1958). Four different concentrations (500, 250, 125, and 62.5µg/ml) of extracts were prepared in methanol using different test tubes. To 2ml solution of each extract and standard vitamin C solution, 1ml of 0.3mM of freshly prepared DPPH

solution in methanol was added. The mixtures were allowed to react in the dark at room temperature for 30minutes. The absorbances of the resulting solutions were measured at 517nm using spectrophotometer against DPPH control containing only 2ml of methanol in place of the extracts. That is, 1ml of 0.3mM DPPH solution added to 2ml of methanol served as a negative control. Ascorbic acid prepared in the same concentrations as the test extracts was used as the reference standard for comparison. The absorbances were measured in triplicates and the scavenging activities in percentage were calculated. The percentage scavenging activity for each concentration represents the mean of three values obtained from triplicate determination.

Percentage DPPH scavenging activity of the extracts and reference drug were determined using the formula:

$$\text{Percentage Scavenging activity} = \frac{(\text{Absorbance of Control} - \text{Absorbance of sample}) \times 100\%}{\text{Absorbance of control}}$$

2.5 Statistical Analysis

Data were expressed as means $\pm$ Standard Error of Mean (SEM). The data analysis was done using the Graph Pad Instat Computer Software Version 3.10. One-way analysis of variance (ANOVA) was used to compare the means of the four groups. P value > 0.05 was regarded as insignificant. Graphs were plotted using Excel 2003 (version 11.0) and Excel 2007 (version 12.0).

3.0 RESULTS

The two extracts possessed high mean scavenging activities which were 75.15 ± 7.0815 and 72.78 ± 7.6541 for *Ocimum gratissimum* and *Vernonia amygdalina* respectively. Comparing the mean scavenging activities of these extracts with that of the standard vitamin C tablet and injection, the differences observed were not significant ($P > 0.05$). That is, for the mean scavenging activity of *Ocimum gratissimum* (75.15 ± 7.080), compared to that of vitamin C tablet (92.28 ± 0.6897) and injection (88.333 ± 0.4381), no significant difference ($P > 0.05$) was observed. Also, there was no significant difference ($P > 0.05$) between the mean scavenging activity of *Vernonia amygdalina* (72.78 ± 7.654) and that of vitamin C tablet and injection.

It has also been observed that *Ocimum gratissimum* possess higher antioxidant potential than *Vernonia amygdalina* but vitamin C exhibited the highest.

The DPPH scavenging activities of different concentrations of methanolic extracts of the samples used and their IC_{50} are presented in table next page:

Considering the IC_{50} , The standard ascorbic acid had the lowest IC_{50} , which was 30µg/ml for tablet and 35.5µg/ml for the injection. The IC_{50} values of *Ocimum gratissimum* and *Vernonia amygdalina* were 50.0µg/ml and 55.0µg/ml respectively.

Table 1: The Scavenging activities and IC₅₀ of vitamin C tablet, vitamin C injection, *Ocimum gratissimum*, and *Vernonia amygdalina*.

	Sample	Concentration (µg/ml)	Scavenging Activity (%)	Mean Scavenging Activity (%)	IC ₅₀ (µg/ml)
1.	Vitamin C tablet	62.5	90.69±0.0038	92.28±0.6897	30.0
		125.0	91.57±0.1559		
		250.0	93.26±0.1184		
		500.0	93.59±0.0779		
2.	Vitamin C injection	62.5	87.32±0.3868	88.33±0.4381	35.5
		125.0	88.06±0.1559		
		250.0	88.54±0.0078		
		500.0	89.41±0.7794		
3.	<i>Ocimum gratissimum</i>	62.5	56.30±0.1184	75.15±7.0815	50.0
		125.0	73.43±0.3897		
		250.0	81.46±0.0779		
		500.0	89.41±0.1184		
4.	<i>Vernonia amygdalina</i>	62.5	51.99±0.0779	72.78±7.6541	55.0
		125.0	71.48±0.0376		
		250.0	80.24±0.0376		
		500.0	87.39±0.1155		

Absorbance of the blank (negative control) at 517.0nm = 1.483

The scavenging activities for each concentration are means of triplicate determinations ± SEM

MSA of *Ocimum gratissimum* versus MSA of Vitamin C tablet and injection – NSD (P>0.05)

MSA of *Vernonia amygdalina* versus MSA of Vitamin C tablet and injection – NSD (P>0.05).

MSA = Mean Scavenging activity

NSD = No Significant Difference

The graphs showing the scavenging activity of each of the samples are given below:

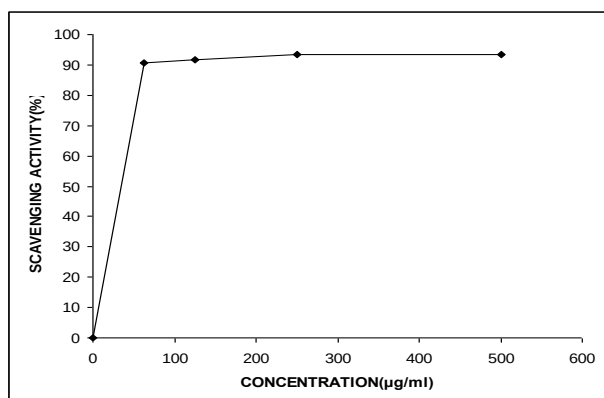


Figure 1: Scavenging activity of vitamin c tablet.

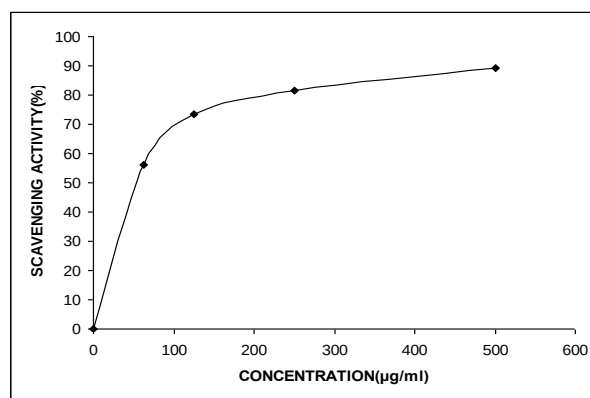


Figure 3: Scavenging activity of *ocimum gratissimum*.

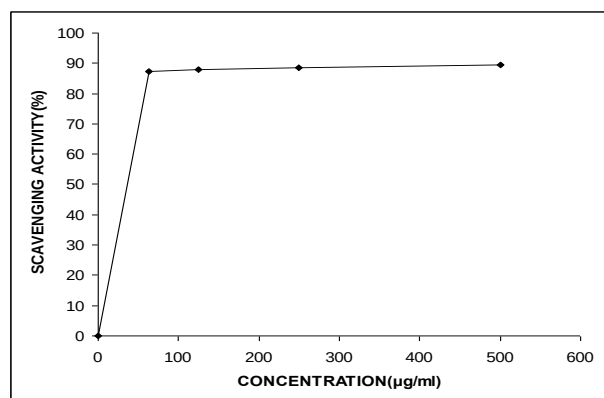


Figure 2: Scavenging activity of vitamin c injection.

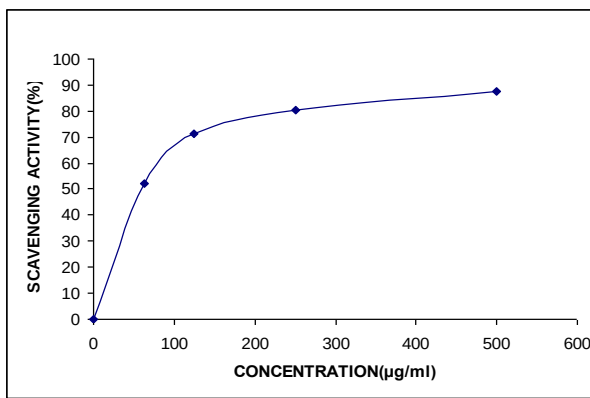
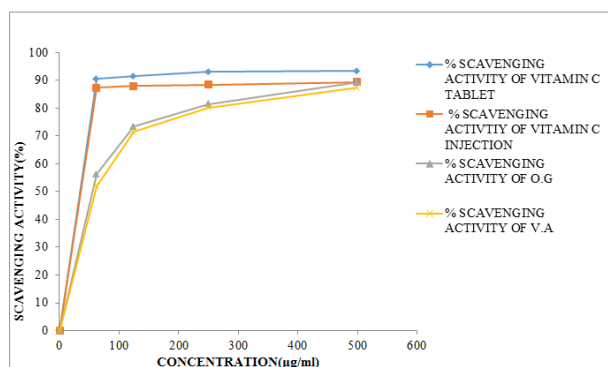


Figure 4: Scavenging activity of *vernonia amygdalina*.



O.G = *Ocimum gratissimum* V.A = *Vernonia amygdalina*

Figure 5: Scavenging Activity of *ocimum gratissimum* and *Vernonia amygdalina* compared to vitamin C.

4.0 DISCUSSION

From this work, it has been discovered that the two widely used medicinal plants, *Ocimum gratissimum* and *Vernonia amygdalina* have antioxidant properties. These activities were found to increase as the concentrations increased. For example, the antioxidant activities exhibited by the extracts at 125 µg/ml were higher than the activities at 62.5 µg/ml. Also, the activities at 500 µg/ml were higher than the activities at 250 µg/ml. That is to say, with increased concentration, the DPPH radical scavenging activity increased. Despite the fact that similar concentrations were used for both vitamin C tablets and injections, the tablet exhibited the highest DPPH scavenging activity, with a mean value of 92.28 ± 0.6897 . That of the injection was 88.333 ± 0.4381 . This might have been due to brand variation. Storage condition can also be a factor. Under normal storage conditions, commercial type ascorbic acid tablets are stable for over five years (greater than 95% potency retention) (Rubin *et al.*, 2007). The injection is more prone to oxidation reaction in the presence of light and high temperature. This might contribute to reduction in antioxidant effect. The solvent used for formulation is another factor.

The IC_{50} is an important parameter which portrays the potencies of the samples evaluated and it expresses the amount of antioxidant required to decrease the DPPH radical concentration by 50% (Chanda *et al.*, 2011). A lower IC_{50} corresponds with a higher antioxidant power. Comparing the IC_{50} of the two extracts with that of vitamin C, it can be inferred that the potencies of vitamin C tablet and injection as free radical scavengers are greater than that of *Ocimum gratissimum* and *vernonia amygdalina* but the plants also possess significant potencies.

From various studies, it has been observed that phenolic substances and flavonoids are associated with antioxidant activities of medicinal herbs and they play important role in stabilizing lipid peroxidation (Joyce, 1987; Yeh-Lin *et al.*, 2012). This is by absorbing and neutralizing free radicals, quenching singlet and triplet

oxygen, and decomposing peroxides (Dorman *et al.*, 2003). Phenolic compounds are classified as simple phenols and polyphenols based on the number of phenol units in the molecule (Amorati and Valgimigli, 2012). The antioxidant potential of the phenolic compounds is believed to be conferred on them by their hydroxyl group (-OH), which is bonded directly to an aromatic hydrocarbon (phenyl) ring. This makes them donate electrons easily to electron seeking free radicals, thus down regulating their menace in living cells. The antioxidant capacity of these phenolic compounds is mainly attributed to their redox potential (Demiray *et al.*, 2009; Premanath and Lakshmidiri, 2010).

In many phytochemical studies, *Ocimum gratissimum* and *Vernonia amygdalina* have been found to contain high amount of flavonoid and phenolic compounds. These phytochemicals are could be responsible for their antioxidant activities.

This study corroborated with previous researches which reported this activity (antioxidant activity) in these plants. In one study, *Ocimum gratissimum* was found to possess a very high antioxidant capacity (Omale *et al.*, 2008). In another study, it was found to contain higher amounts of total phenolics and flavonoids than *Vernonia amygdalina* (Kelly *et al.*, 2013). These compounds impacted on it a higher DPPH radical scavenging activity than *Vernonia amygdalina*. In line with the results of this work, a lower IC_{50} was also recorded for *Ocimum gratissimum*. *Ocimum gratissimum* had the IC_{50} of 105.7 µg/ml while *Vernonia amygdalina* had 111.8 µg/ml. Vitamin C had the lowest IC_{50} of 91.0 µg/ml (Kelly *et al.*, 2013). In another study where commonly eaten vegetables in Akwa Ibom State of Nigeria were evaluated for their antioxidant properties, *Ocimum gratissimum* was also found to possess a higher free radical scavenging activity than other vegetables including *Vernonia amygdalina* (Eseyin *et al.*, 2015).

The lower IC_{50} values recorded in Kelly's work and in this study for *Ocimum gratissimum* extract suggest that it has a higher antioxidant potential than *Vernonia amygdalina*. The two plant extracts exhibited comparable DPPH free radical scavenging activity in a dose-dependent manner.

4.1 CONCLUSION AND RECOMMENDATION

The antioxidant effects of these plants explain their wide acceptability in folkloric medicines to prevent, manage, and slow down the progression of various oxidative stress-related diseases. The result of this study shows that the widely used *Ocimum gratissimum* and *Vernonia amygdalina* have appreciably potent antioxidant activities that cannot be neglected. The study reveals that these plants are reservoirs of natural antioxidants which can be utilized nutritionally and medicinally. Hence, increased domestication of these plants could provide raw materials which are affordable and easily accessible. Increased consumption of these vegetables is thereby

recommended to help militate against deleterious effect of reactive species known as free radicals.

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