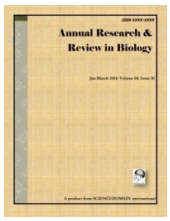

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Evaluation of the Antioxidant Properties of Some Commonly Eaten Vegetables in Akwa Ibom State of Nigeria

Olorunfemi A. Eseyin[1*], Goodnews Charlie Etiemmana[1], Monday Enobong[1], Aniekan Ebong[1], Iweh Etim[1], Sunday A. Udobre[1], Ekarika Johnson[1], Emmanuel Attih[1] and Asanga Effiong[1]

[1]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, University of Uyo, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author OAE designed and supervised the study. Authors GCE, AE and EJ did the antioxidant components evaluation. Authors ME, IE and EA did the study on antioxidant activities. Author SAU drafted the manuscript. Author Asanga Effiong performed the statistical analysis. All authors read and approved the final manuscript. Authors OAE, GCE, ME and SAU also did the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate the antioxidant components and also antioxidant activities of ten local vegetables commonly consumed in Akwa Ibom State, Nigeria.
Place and Duration of Study: Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, University of Uyo, Akwa Ibom State, Nigeria between April 2011 and July 2012.
Methodology: Standard methods were used to evaluate the concentration of total phenols, vitamin C, vitamin E, total flavonoids, total tannins, anthocyanin, β-carotene and lycopene in the leaves of *Heinsia crinata, Ocimum gratissimum, Telfaira occidentalis, Talinum triangulare, Corchorus olitorius, Amaranthus hybridus, Gnetum africana, Vernonia amygdalina, Gongronema latifolia* and *Lasianthera africana*. Ferric reducing antioxidant power (FRAP), iron chelating activity, Nitric oxide

**Corresponding author: Email: femieseyin2@yahoo.co.uk, olorunfemieseyin.pharm.lect@uniuyo.edu.ng;*

and 2,2-Diphenyl-1-Picrylhydrazyl (DPPH)-radical scavenging activities, and total antioxidant activity were also determined using standard methods.

Results: *Lasianthera africana* recorded the highest amount of Lycopene ($0.9951\pm2.23\times10^{-3}$ mg/100ml) and Anthocyanin ($0.0943\pm1.30\times10^{-3}$). *Gongronema latifolium* also recorded the highest amount of Tannin ($6.6\pm1.01\times10^{-3}$ mg/100g) and β-carotene ($1.692\pm1.31\times10^{-4}$ mg/100 ml). Similarly, *Hensia crinata, Cochorius olitorius* and *Vernonia amygdalina* recorded the highest amount of Vitamin E (24.761±7.22×10-2 µM), total phenol ($0.100\pm6.84\times10^{-5}$ mg/ml) and flavonoids ($36.784\pm1.72\times10^{-2}$ mg/ml), respectively. *Ocimum gratissimum* gave the highest % inhibition (74.0%) of DPPH at a concentration of 60 µg/ml of extracts compared to the standard drugs (Vitamin C, 92.0% and Vitamin E, 35.0%). *Vernonia amygdalina* showed highest inhibition (89.97%) of Nitric Oxide while *Ocimum gratissimum* inhibited ferric reducing antioxidant activity by 91.43%. It was found that Vitamin C correlated significantly with the antioxidant activity (r^2 =0.7072, P=0.05). Flavonoids also correlated significantly with the Ferric Reducing antioxidant Power (r^2 =0.6784, P<0.05).

Conclusion: *Ocimum gratissimum* exhibited the best antioxidant activity and should be explored further for this purpose.

Keywords: Vegetables; antioxidants; flavonoids; Nitric oxide; DPPH.

1. INTRODUCTION

Many human diseases are caused by oxidative stress which is usually initiated by free radicals such as Superoxide anions, hydrogen peroxide, hydroxyl radical and nitric oxide. These free radicals react with macromolecules such as DNA, proteins and lipids, thereby damaging them. The consequences of this damage are diseases such as diabetes, hypertension, atherosclerosis, cancer, myocardial infarction, arthritis, anemia, asthma, inflammation, neurodegenerative diseases [1]. Oxidative stress is also implicated in the progression and complications of infectious diseases such as malaria and HIV/AIDS. Endogenous antioxidants such as super oxide dismutase, catalase, glutathione reductase, ascorbic acid and tocopherol protect the body against the damaging effects of free radicals [2]. However, certain pathologic situations may disrupt the protective effects of these endogenous antioxidants. To protect man from the destructive effects of free radicals in such situations the administration of exogenous antioxidants is required. Natural products of plant origin have been found to exhibit strong antioxidant activity due mainly to the presence of antioxidant components such as flavonoids, phenols, flavonols, proanthocyanins, vitamin C, carotenoids and lycopene.

Antioxidants such as glutathione, uric acid, Lipoic acid and enzymes such as catalase, peroxidase and superoxide dismutase) are synthesized in the cells. Vitamins such as A, C and E, and minerals such as copper, zinc, manganese and selenium, as well as pigments like lycopenes and anthocyanins are derived from diets of plants and animal sources [3].

Vegetables are the herbaceous plants with edible parts which can be eaten raw or cooked [4]. Most vegetables contain valuable nutrients like minerals, vitamins, proteins, amino acids, fats and oils, carbohydrates, antioxidants and energy values [5].

Heinsia crinita (Afzel.) G. Taylor [family Rubiaceae] (bush apple*), Occimum gratisimum* Linn. [family Labiatae] (fever plant or thymol tree)*, Telfaira occidentalis* Hook f [family Cucurbitaceae] (fluted pumpkin)*, Talinum triangulare* (Jacq.) Willd. [family Portulacaceae] (water leaf)*, Corchorus olitorius* Linn. [family Tiliaceae] (bush okra), *Amaranthus hybridus* Linn. [family Amaranthaceae] (African spinach)*, Gnetum africana* [family Gnetaceae] (wild spinach)*, Vernonia amygdalina* Del. [family Compositae] (bitter leaf)*, Gongronema latifolia* K. Schum [family Asclepiadaceae] (Bush buck) *and Lasianthera africana* P. Beauv. [family Icacinaceae] (eru) are among the vegetables that are common, edible, easily cultivated, less costly to maintain and widely accepted by the Akwa Ibom people in Nigeria. Unfortunately, information in scientific literature on the antioxidant properties and components of these plants is still scanty. The objective of this study was to evaluate the total phenol, total flavonoids, carotenoids, tannins, anthocyanins, Lycopenes, Vitamin C and E in the leaf extracts of the selected vegetables and their antioxidant activity.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plants

The plants were collected from Uyo metropolis in Akwa Ibom State of Nigeria, in the month of April, 2011 and were identified by Dr (Mrs) M. E. Bassey, a Plant Taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo, Akwa Ibom State Nigeria. They were assigned the following voucher numbers: *Heinsia crinita* (UUH67I), *Ocimum gratisimum* (UUH38(aii)), *Telfairia occidentalis* (UUH28(d)), *Talinum triangulare* (UUH64(b)), *Corchorus olitorius* (UUH76(a)), *Amaranthus hybridus* (UUH2(bi)), *Gnetum africana* (UUH32(a)), *Vernonia amygdalina*(UUH10(j)), *Gongronema latifolium* (UUH9(a)) and *Lasianthera africana* (UUH36(b)).

2.2 Plant Extraction

The leaves were destalked, rinsed in water to remove dirt, dried in the sunlight for days (with regular turning of the leaves to avoid fungal growth and partial drying) and then ground to powder. The dried and powdered leaves samples were stored in air-light containers for the analysis.

The powdered leaves were weighed, and a mass of 200g to 350g was taken for each of the samples for extraction. Extraction of the leaves samples was carried out using cold maceration method in an extracting jar with 70% methanol for 72 hours. The crude liquid extract (filtrate) obtained from the mixture by filtration was concentrated in vacuo and dried with silica gel in a dessicator. The dried crude leaves extracts were then weighed and the percentage yield determined.

2.3 Phytochemical Analysis

Phytochemical screening of the dried methanolic extract of the plants was carried out using the standard method described by [6].

2.4 Evaluation of the Antioxidant Components

The antioxidant components, namely Vitamin C (ascorbic acid), vitamin E (Tocopherols), flavonoids, tannins, carotenoids, phenols, Anthocyanins, and lycopenes of the ten vegetables were evaluated using standard methods of analysis, as described below.

2.5 Determination of Phenolics

The amount of phenolics in each of the extract was determined with Folin-Ciocalteu reagent using the method of Spanos and Wrolstad [7].

2.6 Determination of Vitamin C

Vitamin C content in the extract was determined using colorimetric method developed by Klein and Perry [8].

2.7 Determination of Vitamin E

Vitamin E content of each of the extract was determined spectrophotometrically according to the method given by Rutkowski and Grzegorzyk [9].

2.8 Determination of Flavonoids

Aluminium chloride colorimetric method described by Aiyegoro and Okoh [10].

2.9 Determination of Anthocyanin

The method developed by Fuleki and Francis [11].

2.10 Determination of Tannins

The Tannin content in each of the extract was analysed using the method described by Bohm and Kocipai-Abyazan [12].

2.11 Determination of Carotenoids and Lycopene

The quantitative content of carotenoids and lycopene in the leafy extract was determined by the colorimetric method described by Nagata and Yamashita [13].

2.12 Determination of DPPH- Radical Scavenging Activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) – free radical scavenging capacity of the selected vegetable extracts, vitamin C and E were evaluated. A dose of 0.2 ml of the vegetable extracts were each added to 3.8 ml ethanol solution of DPPH radical until a final concentration of 0.1mM was obtained. The mixture was agitated vigorously for 1 min and left

to stand at room temperature for 30 minutes. The absorbance of each sample (As) was recorded on a UV at 517nm against ethanol blank. Negative control (A) was taken after adding DPPH solution to 0.2ml of the respective vegetable extracts. The percent DPPH discolouration of the sample was calculated according to the equation:

$$\% \text{ discolouration} = \frac{[\ 1-(\ As\ )}{(As)} \times 100$$

The free radical scavenging capacity of the vegetable extracts and vitamins were expressed as an equivalent of that of Trolox. The absorbance of each sample was measured in triplicate (n=3).

The results were calculated and expressed as micromoles curve of Trolox equivalents (TE) per gram of the vegetable extract using the calibration curve of Trolox [14].

2.13 Determination of Total Antioxidant Activity

The total antioxidant activity was determined by the conjugated diene method of Lingnert et al [15].

2.14 Ferrous Ion Chelating Ability

This was estimated by using the method described by Dinis et al. [16].

2.15 Reducing Power Assay

The reducing power assay was estimated by the method of Athukorala et al. [17].

2.16 Nitric Oxide (NO) Scavenging Assay

This was determined by the method of Green et al. [18].

2.17 Statistical Analysis

Data were analysed using one way ANOVA with the aid of graphpad prism (USA).

3. RESULTS AND DISCUSSION

The concentration of antioxidant components are shown in Table 1 while the results of the antioxidant activities are displayed in Figs. 1, 2, and 3.

Total phenol in the vegetables are at low concentrations, ranging from $0.100\pm6.84\times10^{-5}$ (*Corchorius olitorius*) to $0.025\pm7.35\times10^{-5}$ mg/ml (*Amaranthus hybridus*). Phenols and phenolics exert their antioxidant activity mainly by free radical scavenging. Tannins content was highest in *Gongronema latifolium* ($6.6\pm1.0\times10^{-3}$) and lowest in *Vernonia amygdalina*, ($3.5\pm5.60\times10^{-3}$ mg/100 g).

Tannin exerts its antioxidant activity by scavenging free radicals, inhibiting Lipid peroxidation and chelating of metal [19]. Flavonoids recorded its highest content in *Vernonia amygdalina* ($36.784\pm1.72\times10^{-2}$) and the lowest in *Heinsia crinata* ($0.750\pm1.27\times10^{-3}$ mg/ml). Flavonoids exert their antioxidant activity by scavenging free radicals, chelating metals and inhibiting lipid peroxidation. The –OH at C3 plays a role in chelating and scavenging activity [20].

The highest value of β- carotene was seen in *Gongronema latifolium* ($1.692\pm1.31\times10^{-4}$) and the lowest in *Talinum triangulare* ($0.144\pm2.32\times10^{-2}$ mg/100 ml). β- Carotene is the precursor of Vitamin A. It shows antioxidant activity in humans and also plays a role in vision [21].

Lycopene is one of the carotenoids. *Lasianthera africana* recorded the highest amount of lycopene ($0.995\pm2.23\times10^{-3}$ and *Hensia crinata* ($0.034\pm1.71\times10^{-3}$ mg/ml) the least. The mechanism by which carotenoids exert their antioxidant activity is by quenching the singlet oxygen and by trapping peroxyl radical [22,23].

Anthocyanin content of *Lasianthera africana* ($0.943\pm1.30\times10^{-3}$) was the highest while that of the *Corchorus olitorius* ($0.0133\pm2.27\times10^{-4}$ %w/w) was the least. The anthocyanidin exerts its antioxidant activity by scavenging the hydroxyl radicals, superoxide ions and lipid peroxyl radicals. The OH at C3 and the polyhydroxylation of the aromatic rings A and B are responsible for the antioxidant activity [20]. Anthocyanin functions in colour impartation and protection against diseases [24].

Vitamin C content in *Ocimum gratisimum* ($23.244\pm8.59\times10^{-3}$) was the highest and that of *Vernonia amygdalina* ($0.1564\pm6.43\times10^{-4}$ mg/ml) was the least. The result suggests that consumption of adequate *Ocimum gratisimum* may provide the recommended daily allowance of 60 mg (for adults) and 20 mg (for children) [25]. The body requires Vitamin C for formation

of collagen, blood and hormones. It also helps in the development of bones, teeth, prevention of scurvy and as antioxidants against free radicals [26]. Vitamin C inhibits, minimizes and terminates the propagation of the free radicals by donating hydrogen and electron thus changing its structure from ascorbic acid to dehydroascorbic acid [27].

Vitamin E content of *Heinsia crinata* was as high as $24.761 \pm 7.22 \times 10^{-2}$ µM and that of *Talinum triangulare* was as low as $0.200 \pm 6.86 \times 10^{-4}$ µM. The result suggests that consumption of *Heinsia crinata* alone cannot combat vitamin E deficiency, taking into cognizance the Recommended Daily Allowance of 15 mg for adult. Vitamin E exerts it antioxidant activity by donating the hydrogen or the hydroxyl group of its chroman ring to neutralize the free radical [28].

The result in Fig. 1 shows that at 60 µg/ml dose of the extract of *Ocimum gratisimum* inhibited DPPH radical by 74.0% compared to the standard drug-Vitamin E (35.0%), suggesting that *Ocimum gratisimum* is a better scavenger than α- Tocopherol. In Fig. 3 *Vernonia amygdalina* demonstrated the highest antioxidant power of 98.50% and also the highest inhibition of Nitric Oxide by 89.97% while *Ocimum gratisimum* inhibited Ferric Reducing Antioxidant activity by 91.43%.

Vitamin C correlated significantly with the antioxidant activity ($r^2 = 0.7072$, $P = 0.05$). Flavonoids also correlated significantly with the Ferric Reducing Antioxidant Power ($r^2 = 0.6784$, $P = 0.05$).

From the results of this work, it was observed that there is a great variation in the rate of antioxidants activity in the different plant extracts based on the model involved. Some plants showed very high antioxidant activity in one or more methods but less in another model [29].

From the methods used in this study, it was noted that the plants under considerations showed a significant property in their ability to act as natural antioxidant with *Vernonia amygdalina* and *Gnetum africana* showing the highest antioxidant activity from among the ten plants under considerations. Nitric oxide (NO) is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical that plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and antitumor activities. Suppression of released NO may be partially attributed to direct NO scavenging, as the different plant extracts decreased the amount of nitrite. The scavenging of NO by the extracts was increased in dose dependent manner (Fig. 3).

The chelation of Fe^{2+} ions was estimated by the method of Dinis [16] in which Ferrozine quantitatively formed complex with Fe^{2+}. In the presence of chelating agents, the formation of this complex is disrupted, thereby impeding the formation of red colour imparted by the complex as well. Measurement of this colour change therefore allows for the estimation of the chelating ability of the coexisting chelator.

As shown in Fig. 3, the formation of Fe^{2+} Ferrozine complex is not complete in the presence of the different plant extracts indicating that the different plant extract of which *Vernonia amygdalina* show the highest significant rate of about 86.35% inhibition, chelate the iron. Metal chelating agents reduces the concentration of the catalysing transition metal in lipid peroxidation by forming sigma bond with metals, reducing the redox potentials, thereby stabilising the oxidized form of the metal ion.

Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant. In the reducing power assay, the presence of antioxidants in the samples would result in the reducing of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{2+} complex can then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in reductive ability. Fig. 2 shows dose response curves for the reducing powers of the extract. It was found that the reducing powers of all the extracts also increased with the increase in their concentrations. All extracts showed good reducing power that was comparable with Vitamin C ($p=0.05$).

This study suggests that the antioxidant activity of all the extracts may be attributed to the reduction of free radicals, chelation of metal ions, or a combination thereof due to the presence of phytoconstituents.

Table 1. Antioxidant components of vegetables

	Plant	Phenols (mg/ml)	Tannins (mg/100g)	Flavonoids (mg/ml)	B-carotene (mg/100ml)	Lycopene (mg/100ml)	Anthocyanin (%w/w)	Vitamin C (mg/ml)	Vitamin E (µM)
1	*Heinsia crinata*	0.080± 6.6×10^{-5}	6.2993± 3.87×10^{-3}	0.750± 1.27×10^{-3}	1.496± 2.45×10^{-3}	0.034± 1.71×10^{-5}	0.0225± 2.43×10^{-4}	6.7± 2.96×10^{-3}	24.761±. 7.22×10^{-2}
2	*Occimum gratissimum*	0.077± 6.80×10^{-5}	5.7± 1.40×10^{-2}	23.244± 8.5910^{-3}	1.199± 1.22×10^{-3}	0.7791± 3.21×10^{-4}	0.0135± 1.12×10^{-2}	23.244± 8.59×10^{-3}	0.425± 1.76×10^{-3}
3	*Telfaira occidentalis*	0.06± 6.62×10^{-5}	5.8± 6.04×10^{-3}	20.124± 1.72×10^{-2}	1.221± 2.03×10^{-3}	0.7292± 2.25×10^{-5}	0.0269± 1.22×10^{-2}	2.976± 3.66×10^{-3}	0.900± $.345\times10^{-3}$
4	*Talinum triangulare*	0.005± 1.12×10^{-5}	5.1± 1.42×10^{-3}	24.17± 8.59×10^{-3}	0.144± 2.32×10^{-2}	0.0097± 1.59×10^{-5}	0.0494± 2.30×10^{-3}	1.155± 1.44×10^{-3}	0.200± 6.86×10^{-4}
5	*Corchorus olitorius*	0.100± 6.84×10^{-5}	5.3± 4.03×10^{-3}	23.416± 1.72×10^{-2}	0.865± 1.55×10^{-3}	0.1102± 1.40×10^{-4}	0.0133± 2.27×10^{-4}	2.578± 3.15×10^{-3}	0.625± 6.07×10^{-4}
6	*Amaranthus hybridus*	0.025± 7.35×10^{-5}	5.4± 2.41×10^{-3}	28.110± 2.86×10^{-2}	1.641± 1.21×10^{-3}	0.1936± 1.04×10^{-3}	0.0449± 1.34×10^{-5}	0.7619± 1.80×10^{-3}	0.325± 1.57×10^{-3}
7	*Gnetum africana*	0.034± 1.01×10^{-4}	5.5± 2.44×10^{-3}	23.701± 2.86×10^{-2}	1.339± 2.01×10^{-4}	0.2628± 2.44×10^{-5}	0.0584± 2.28×10^{-5}	0.3946± 2.82×10^{-3}	0.550± 1.92×10^{-3}
8	*Vernonia amygdalina*	0.034± 2.82×10^{-5}	3.5± 5.60×10^{-3}	36.784± 1.72×10^{-2}	1.243± 2.11×10^{-3}	0.7839± 1.05×10^{-3}	0.0135± 3.13×10^{-4}	0.1564± 6.43×10^{-4}	0.250± 8.43×10^{-4}
9	*Gongronema latifolium*	0.010± 2.84×10^{-5}	6.6± $1..01\times10^{-3}$	26.822± 1.72×10^{-2}	1.692± 1.31×10^{-4}	0.2959± 3.28×10^{-3}	0.0629± 1.22×10^{-5}	8.272± 1.96×10^{-2}	0.425± 5.27×10^{-4}
10	*Lasianthera africana*	0.038± 5.28×10^{-5}	6.3± 7.5×10^{-3}	25.248± 1.72×10^{-2}	1.201± 1.17×10^{-5}	0.9951± 2.23×10^{-3}	0.0943± 1.30×10^{-3}	8.938± $1.3\ 1\times10^{-2}$	0.450± 1.0×10^{-3}

Each value is expressed as mean ± standard deviation (n=3).Total Phenol content is expressed in milligram Tannic Acid Equivalent (mgTAE)..Total Flavonoid content is expressed in milligram Quercertin Equivalent (mgQE)

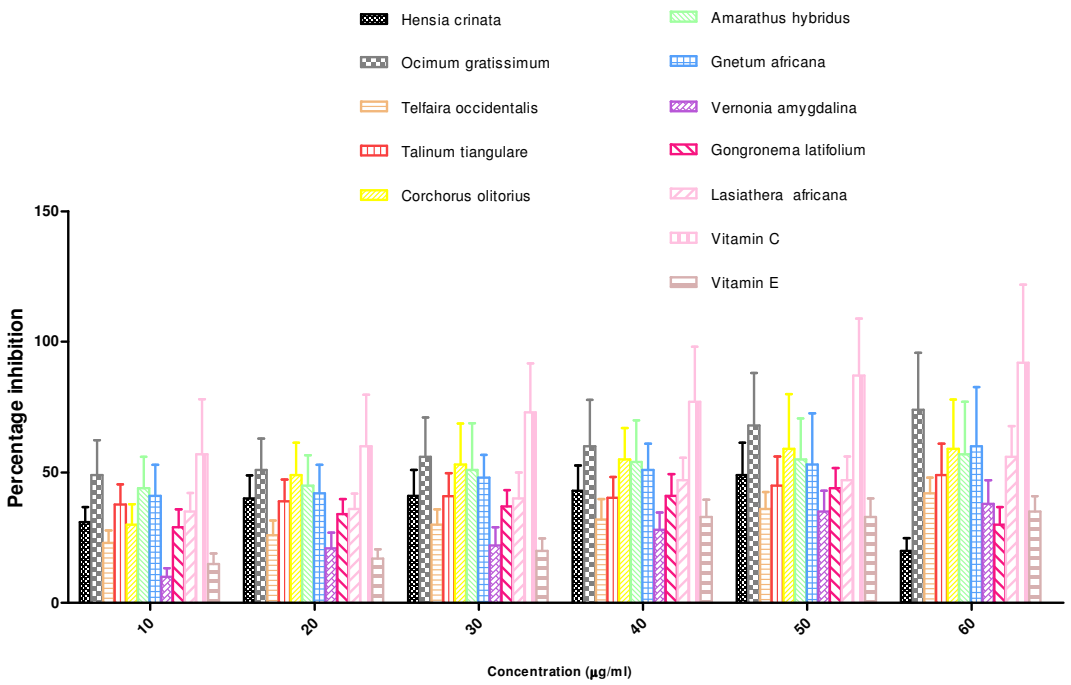

Fig. 1. Percent DPPH inhibition

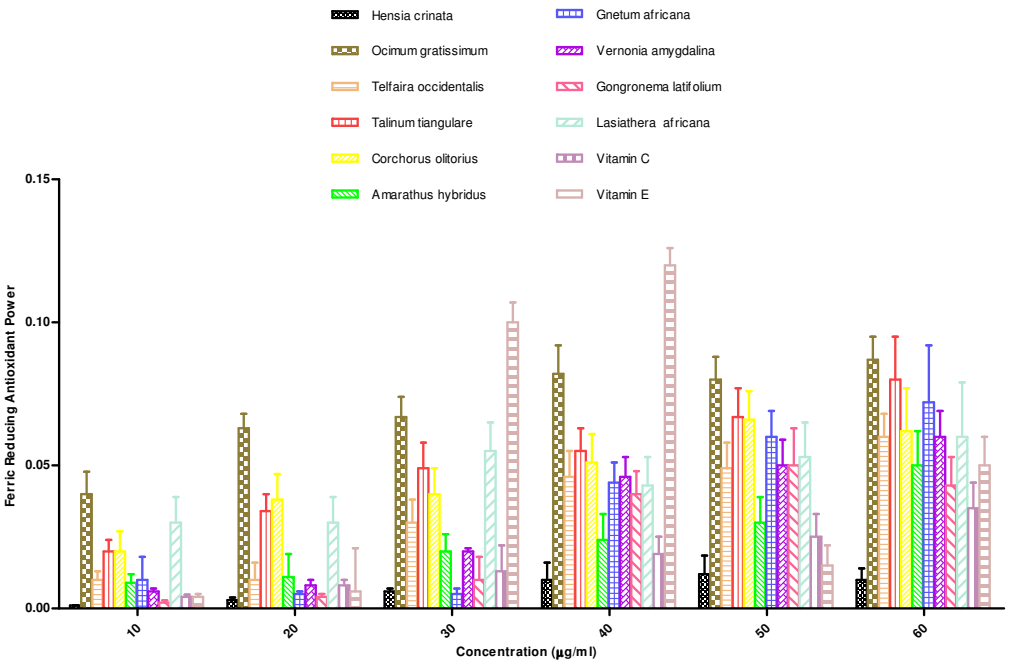

Fig. 2. Ferric reducing antioxidant power

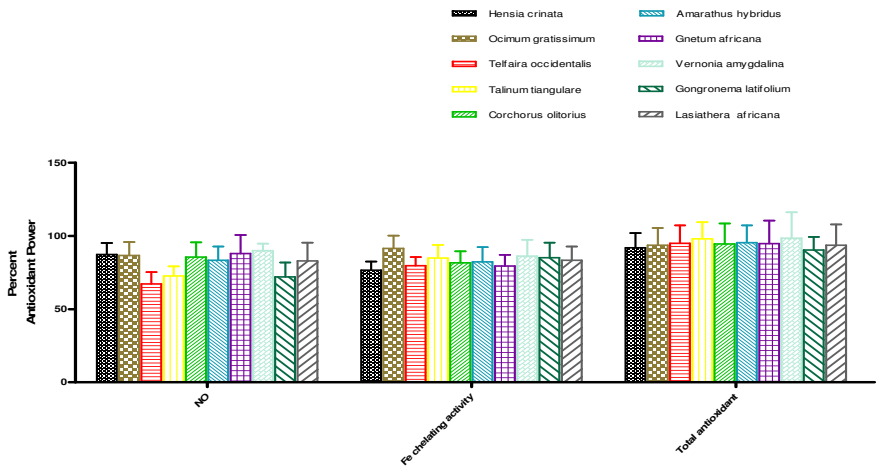

Fig. 3. Percent NO, Fe chelating and total antioxidant activity

4. CONCLUSION

Antioxidant properties of ten vegetables in Akwa Ibom, Nigeria were evaluated. The results revealed that *Corchorus olitorius* is rich in phenol, *Gongronema latifolium* in tannins and β-carotene. *Vernonia amygdalina* is rich in flavonoids, *Lasianthera africana* in lycopene and Anthocyanin. *Ocimum gratisimum* is rich in vitamin C while *Heinsia crinata* is rich in Vitamin E.

The investigation has revealed that these vegetables are potential sources of antioxidant agents. Purification, identification and structure elucidation of the phytochemical constituents could lead to the discovery of new drugs that may cure many degenerative diseases. These vegetables are therefore useful sources of the mentioned antioxidant components and can be appropriately, maximally and quantitatively applied in human diets and health management, animal farming, traditional medicinal practices, new drug development, advanced medicinal applications, profitable local and international trades, as well as economic settlement especially in these days of global economic adversity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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