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Antioxidant properties, Macro and Micro Elements of Selected Edible Vegetables

*Omonike O. Ogbole^{ABD1}, O. O Abiodun^{CE2}, E. O. Ajaiyeoba^{EF1},

¹Department of Pharmacognosy, University of Ibadan, Ibadan, Nigeria

²Department of Pharmacology and Therapeutics, University of Ibadan, Ibadan, Nigeria

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: Many green leafy vegetables (GLV) have been shown to have significant antioxidant activity which may be an important property of medicinal plants associated with the treatment of several diseases. Trace element plays a crucial role in the medicinal value of a plant, in health and to cure disease. They play a nutritive, catalytic and balancing function in plants and animals, since animals easily consume these minerals through food.

Objective: The aim of this work is to quantify the macro and trace elements present in these vegetables in order to determine their contribution to the daily dietary requirement and also determine those element that are present in excess of their maximum dietary allowance.

Methodology: The elemental composition and antioxidant activities of ten common edible vegetables obtain from a market in Ibadan, Nigeria were analyzed using atomic absorption spectroscopy and DPPH radical scavenging assay.

Result: Three macro elements and seven trace elements were determined. The vegetables samples were found to contain trace elements such as manganese, iron, copper, cobalt, chromium (Mn, Fe, Cu, Zn, Co, Cr). *Telfaria occidentalis*, one of the most widely eaten vegetables has the best profile for macro elements with a concentration of 364.2 ± 1.1 mg/g for Ca, 55.4 ± 1.3 mg/g for Mn and 365.6 ± 0.3 mg/g for K. It also had a concentration of 1185.0 ± 0.8 mg/g for Fe.

Conclusion: Only one of the vegetables was found to accumulate lead in excess of maximum daily intake. All vegetables were found to be rich in many of the essential elements and all vegetables exhibited varying degree of antioxidant capacity with *Gnetum africanum* (Gnetaceae) having the highest radical scavenging activity of 14.58 ± 1.8 μ g/mL.

Keywords: Macro elements, Trace elements, DPPH radical scavenging, Edible vegetable

INTRODUCTION

Green leafy vegetables (GLV) are important sources of macro and micro elements which are necessary for the maintenance of good health and prevention of diseases. In most tropical African countries, the daily diet is dominated by staple foods rich in carbohydrates. In Nigeria, vegetables are the cheapest and most readily available sources of important proteins, vitamins, minerals, and essential amino acids. These vegetables are rich sources of carotene, ascorbic acid, riboflavin, folic acid, and minerals elements like calcium, iron and phosphorous and potassium (Sheela *et al.*, 2004; Okafor, 1983; Nnamani *et al.*, 2007). Vegetables are widely produced in Nigeria for local consumption.

Macro- and microelements influence biochemical processes in the human body and a number of mineral

elements play important roles in the metabolic process. An element is considered essential when reduction of its exposure below certain limit results consistently in a reduction in a physiologically important function, or when the element is an integral part of an organic structure performing a vital function (Armah *et al.*, 2001). It should be noted that for many elements, there is a very narrow range between deficiency and toxicity for the human body, thus, it is of considerable importance to determine their concentrations in plants. Plants have been noted to accumulate metals such as Pb, Cd, Ni, Cr, Co and Ag,

for which no direct benefit and no significant physiological role for plants have been established (Toaspern *et al.*, 2000; Mesjasz-Przybylowicz and Przybylowicz (2002). They could also be hazardous for human health when ingested with vegetables and medicinal plants (Jacob, 1994). It has been suggested that there is a close relationship between instances of declining reproductive health and environmental pollutants like lead (Bonde *et al.*, 2002) and low levels of Zn can induce the pathogenesis of lung cancer (Cobanoglu *et al.*, 2010) Determination of metal content in medicinal plants is a part of quality control to establish their purity, safety and efficacy according to the World Health Organization (WHO, 1992).

There is an increasing interest in the antioxidant effects of compounds derived from GLVs that could be relevant in relation to their nutritional effects and their role in health and disease (Sarkar and Bhaduri, 2001). Recent studies have shown the importance of vegetables in a healthy diet and prevention of degenerative diseases caused by oxidative stress (Sreeramulu and Raghunath, 2010). Vitamins and phytochemicals, such as ascorbic acid, carotenoids, polyphenols, and fiber have been regarded as the bioactive constituents responsible for these effects (Szeto *et al.*, 2004). Studies have shown a relationship between the consumption of diets rich in fresh fruits and vegetables and decreased risk of cancer and other diseases (Block, 1992). In recognition of the important role that major and trace elements play in health in humans, and in order to establish the antioxidant potential of commonly consumed vegetables from Nigerian market, this study presents the elemental composition and the free radical scavenging activity of ten edible vegetables.

MATERIALS AND METHODS

Plant Collection and Authentication

The plants used in this study were purchased in the month of May (2012) from Bodija market, a major market in Ibadan metropolis in Nigeria (the vegetable sellers reported to have obtained the vegetables from farms settlements spread across the city). Plant samples were authenticated by Mr. Femi Afilaka at the Herbarium of Forest Research Institute of Nigeria (FRIN), Samples were deposited at the herbarium under various voucher numbers: *Corchorus olitorius*, FHI109925, *Vernonia amygdalina* FHI 109926, *Piper guineense* FHI 109927, *Amaranthus hybridus* FHI109928, *Gongronema latifolium* FHI 109929, *Telfairea occidentalis* FHI109930, *Celosia argentea* FHI 109931, *Talinum triangulare* FHI 109932, *Amaranthus viridis* FHI 109933

Plant Extraction

Plant materials were air-dried at room temperature (RT, 29-32 °C) and pulverized using a dry mill blender. Forty gram (40 g) of each dried powdered materials was extracted by maceration in redistilled ethanol (200ml) for 72h at room temperature (RT).

Elemental Analysis

The amount of the macro elements; Calcium (**Ca**), Magnesium (**Mg**) Potassium (**K**), Manganese (**Mn**) and micro elements; Cobalt (**Co**), Copper (**Cu**), Chromium (**Cr**), Iron (**Fe**), Lead (**Pb**) and Zinc (**Zn**) were determined in the leaves of the vegetables used in this study using the modified methods Barthakur *et al.*, (1995). briefly, one g of dried samples was ashed at 600°C for 3 h. After which ashed samples were cooled to 120°C and placed in a desiccator for 1 h to cool before weighing. The process was repeated until a constant weight was obtained. Ashed samples were digested with 5 mL each of concentrated trioxonitrate (V) acid (HNO₃) and perchloric acid (HClO₄) at 150°C for 1 h, filtered with filtered paper and the filtrate was suitably diluted with deionized water (5:1). The resulting solution was used for the elemental analysis using atomic absorption spectrophotometer (AAS; Analyst 400 Model).

Antioxidant Assay Measurement of DPPH Scavenging Ability

Crude ethanol extracts of the 10 vegetables were solubilised in ethanol such that each of them had a concentration of 1.0 mg/mL. Complete dissolution was achieved by sonicating for 30 min in an ultrasonicator (Branson, Shelton Conn., USA). Freshly prepared DPPH solution was used for the assay. One milligram (1 mg) of DPPH was dissolved in 25 mL of ethanol. The solution was kept in the dark until needed for use. The stock solution of plant extracts 1mg/mL was diluted further in ethanol to obtain working concentrations of 400 µg/mL for plant extracts and 40 µg/mL of standard antioxidant compound (ascorbic acid). Serial two-fold dilution of each plant extracts or standard drug in triplicates were made in a 96-well plates, this was followed by the addition of 150 µL of DPPH solution to each well. Control wells without the addition of test extracts were also made. Test plates were incubated in the dark for 30 mins. The change in colour (from deep-violet to yellow) was measured at a wavelength of 517 nm after incubation for 30 min at RT (26 - 32°C). Thereafter, the free radical scavenging ability of the plant extracts on the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was estimated by measuring the absorbance of reduced product at 517nm using a multiwell spectrophotometer (Spectramax Gemini XS, Molecular Devices, USA). The experiment was carried out in triplicate.

Data Analysis

Optical density (OD) values were expressed as the percentage of that of the control. Percentage scavenging ability of plant extracts or standard drug in respect to the negative control well was calculated by the following formula:

$$\text{Percentage scavenging} = 100 - \frac{(\text{Absorbance of test sample}) \times 100}{\text{Absorbance of control}}$$

Fifty percent inhibitory concentration of plant extract/standard drug (IC₅₀) was determined using non-linear regression in a statistical package, Microcal Origin®.

Statistical analysis

The mean IC₅₀ values obtained for the plant extract were compared to the IC₅₀ value obtained for ascorbic acid (the standard drug) using the student t-test of a Graphpad package. Values with $p < 0.05$ were considered significant

RESULTS AND DISCUSSION

Most plants and animals require certain mineral ions in order to live; these are classified as either macroelements or microelements. Macroelements refer to elements needed in appreciable amounts for sustenance while those that are needed in small quantities to orchestrate a range of physiological functions are known as microelements. With plants, these elements must be dissolved in the soil in order to be consumed (Canadian UNICEF Committee, 2006). Many of the microelements are enzyme cofactors, which are easily supplied through the soil since only trace amounts are needed.

The present study was conducted to increase the knowledge on nutritional benefits of vegetables and focused on investigating their elemental composition and antioxidant properties. The total concentrations of 10 elements (macro and traces), in ten different vegetables sold in Bodija market, Ibadan, Nigeria were evaluated as presented in Table 1. The presence of macro and trace elements indicates the biochemical value of the vegetables. Sometimes different combinations of the elements present in vegetables influence biochemical processes and help to treat ailments. The three macro elements analyzed were present in all the vegetables in varying concentration. *Telfairia occidentalis* had the highest concentration of Calcium (364.2 mg/100 g) while the lowest concentration was found in *Piper guineense* (54.1 mg/100 g). Calcium is an essential component of bone, cartilage and the crustacean exoskeleton. It is essential for the normal clotting of blood, an activator for several key enzymes, through its role in enzyme activation, The recommended daily allowance (RDA) is 1000 mg/day (NIH, 2011). Calcium must be ingested daily and absorbed effectively in order to maintain optimal health. Dietary calcium deficiency is a condition in which there is an inadequate calcium intake, which can lead to depleted calcium stored in the bones, thinning and weakening of the bones, and osteoporosis (Haines, 2014). Vegetable such as *Telfairia occidentalis* can easily provide a great part of the RDA.

Diet rich in magnesium is essential for activation of several key enzyme systems, including kinases, muscle ATPases, and several others. Through its role in enzyme activation, magnesium (like calcium) stimulates muscle and nerve irritability, it is involved in the regulation of intracellular acid-base balance, and plays an important role in carbohydrate, protein and lipid

metabolism. All the vegetables analyzed contain magnesium in different concentration, the highest concentration of 155.5 mg/100 g was found in both *Celosia argentea* and *Amaranthus hybridus* while the lowest was found in *Vernonia amygdalina* (30 mg/100 g) as presented in Table 1. The highest potassium concentration was found in *Amaranthus viridis* (521 mg/100 g) while *Gnetum africanum* had the lowest concentration (191 mg/100 g) all other vegetables had fairly good concentration of potassium. A potassium intake sufficient to support life can in general be guaranteed by eating a variety of foods. It is present in sufficient quantities in most fruits, vegetables, meat and fish (Potassium Food Charts, 2011).

Telfairia occidentalis, *A. hybridus* and *A. viridis*, all had a fairly good distribution of the analysed elements. In *T. occidentalis*. micro elements such as Mn, Fe, Cu, Zn were present in adequate quantity, with *T. occidentalis* and *A. viridis* having highest concentration of iron 1185.0 and 1810.0 mg/kg respectively. *Telfairia occidentalis* has been used extensively in West Africa for the treatment of iron deficiency anaemia. Studies have reported a number of haemoglobin (Hb) regeneration effects of the vegetable and the antianaemic activity of both *Telfairia occidentalis* and *Amaranthus hybridus* (Alada, 2000; Dina *et al.*, 2006; Ogbe *et al.*, 2010). The administration of *T. occidentalis* leaves was reported to have increased haemoglobin (Hb) levels in anaemic mice comparable to the Hb repletion in anaemic mice given FeSO₄ (Hamlin and Latunde-Dada, 2011). The studies further strengthened the fact that both plants are good sources of dietary iron. The iron content of most vegetables reported in this study (Table 1) is much higher than the earlier reported values (Onwordi *et al.*, 2009; Chandra, 1990). Iron is an important constituent of succinate dehydrogenase as well as a part of the haeme of haemoglobin (Hb), myoglobin and the cytochromes. Iron is required for proper myelination of spinal cord and white matter of cerebellar folds in brain and is a cofactor for a number of enzymes involved in neurotransmitter synthesis (Larkin and Rao, 1990). Trace elements such as cobalt, chromium and lead were completely absent in the vegetables except for *Piper guineense* that has 285 mg/kg of lead. While it has been established in literature, that lead naturally does not concentrate or accumulate in the edible parts of fruit and fruiting vegetable plants (e.g. tomatoes, peppers, beans, zucchini), it certainly accumulated in pepper sample screened in this study. It may be possible that the *P. guineense* evaluated in this study was cultivated in a lead contaminated soil. Plants grown in lead contaminated soils can accumulate lead from the adherence of dust and translocation into the plant tissue (Finster *et al.*, 2004). The global growth in the automobile industry in the last decades (Paoliello and Chasin, 2001), as well as the expansion of the market for batteries, makes the use and recycling of batteries one of the principal forms of lead contamination in soils. Lead is a physiological and neurological toxin that can affect almost every organ or system in the human body. It can reduce cognitive development and

intellectual performance in children, damage kidneys and the reproductive system (Qin and Chen, 2010). It is worthy of note that the variation in elemental concentration can be attributed to structural differences in botanical composition, as well as in the mineral constituents of the soil on which the vegetables were cultivated (Lokhande *et al.*, 2010). In recent times, epidemiological analyses have shown that consumption of fruits and vegetables is associated with reduced risk of chronic diseases such as infection, inflammation, diabetes mellitus, acquired immune deficiency syndrome (AIDS), cardiovascular events, cancer and other degenerative diseases of aging. The generation of free radical is a vital phenomenon of the normal metabolism of the human body. Varieties of free radicals are generated as a by-product of cellular functions. Normally, these free radicals are neutralized by the enzymatic and non enzymatic antioxidants present as an inbuilt antioxidant mechanism in the body and also by the dietary antioxidant supplement in the daily diet (Jang *et al.*, 2008).

Vegetables are rich sources of nutrients which form a major part of nature anti-aging activity (Gupta and Prakash, 2008). The 50% inhibitory concentration (IC_{50}) of the extract of the vegetables and ascorbic acid are presented on Table 2. The IC_{50} of the vegetable extracts ranged from 14.58 ± 1.8 to $> 400 \mu\text{g/mL}$. The extract of *G. africanum* was observed to have the lowest IC_{50} value of $14.58 \mu\text{g/mL}$ followed by extract of *C. olerius* with IC_{50} value of $33.86 \mu\text{g/mL}$. However the antioxidant activities of *G. africanum* and *C. olerius* was significantly lower than the that of ascorbic acid ($P=0.005$ & 0.0001).

In summary, *Gongronema latifolium* was most effective as a radical scavenger while *Gnetum africanum* had the lowest scavenging ability. *Vernonia amygdalina*, *Corchorus olerius* and *Celosia argentea* also had high percentage scavenging properties (80%, 77% and 79% respectively). The scavenging activities of *Piper guineense*, *Gnetum africanum* and *Amaranthus viridis* were low.

The presence of antioxidant activities in plant species is very essential and because it is readily available and affordable in Nigeria for the common man, who might not be able to afford the cost of antioxidants such as α -tocopherol. As a result, it is imperative for diets to contain adequate amounts of these antioxidants in order to prevent disease and infections due to free radical scavengers. Mineral elements are found in considerable amount which may be directly or indirectly helpful in the management of many diseases.

CONCLUSION AND RECOMMENDATION

The elemental composition and antioxidant activities of ten common edible Nigerian vegetables were analysed, the results further expand the scope of knowledge on plants in these aspects. The importance of the essential elements and antioxidants in the nutrition of plants, animals and humans, their metabolic inter-relationships cannot be over emphasized. They influence other vital factors needed for the survival of living organisms like enzymes, anti-oxidants and vitamins. It is important to regularly obtain updates on the mineral content of the commonly consumed plant foods used as human and animal foods and feeds, respectively.

TABLE 1: Elemental analysis of ten edible vegetables

S/N	Vegetable	Macro elements (mg/g)										Micro elements (mg/Kg)				
		Ca	Mg	K	Co	Cr	Cu	Fe	Mn	Pb	Zn					
1	<i>Amaranthus hybridus</i>	157.23±2	155.4±1.5	381.4±1.4	0.00	0.00	1.61±1.1	282.0±1.5	74.2±0.6	0.00	57.5±1.0					
2	<i>Amaranthus viridis</i>	105.1±1.1	152.1±2.5	521.3±1.8	0.00	0.00	1.10±0.9	1810.0±1.4	162.5±0.4	0.00	67.5±2.0					
3	<i>Celosia argentea</i>	274.3±1.6	155.3±1.2	340.1±1.5	0.00	0.00	23.4±2.0	352.0±1.4	80.0±0.3	0.00	209.5±1.3					
4	<i>Choc horus oltorius</i>	147.1±1	83.2±0.4	365.2±1.5	0.00	0.00	18.0±3.0	1030.0±1.3	130.0±1.4	0.00	34.8±3.0					
5	<i>Gnatum africanum</i>	91.2±0.3	34.1±1.6	191.7±1.3	0.00	0.00	8.5±2.0	240.0±1.6	267.5±2.0	0.00	15.3±1.8					
6	<i>Gongronema latifolium</i>	144.7±1	58.9±2.0	225.3±1.5	0.00	0.00	19.8±3.1	319.5±1.4	127.5±1.7	0.00	23.9±1.4					
7	<i>Piper guineensis</i>	54.2±1.4	150.9±0.6	283.9±0.4	0.00	0.00	1.60±1.1	292.5±1.2	123.0±1.3	281.5±1.6	49.2±2.0					
8	<i>Talinum triangulare</i>	215.8±2.1	111.1±1.48	248.8±1.3	0.00	0.00	1.68±1.4	585.0±1.6	518.5±1.5	0.00	40.8±2.0					
9	<i>Telfairea occidentalis</i>	364.2±1.1	55.4±1.3	365.6±0.3	0.00	0.00	1.60±3.1	1185.0±0.8	65.0±2.3	0.00	53.4±3.1					
10	<i>Vernonia amygdalina</i>	118.9±2.0	30.0±1.4	334.0±1.5	0.00	0.00	2.45±0.5	282.5±1.8	880.0±2.7	0.00	37.9±1.6					

TABLE 2: DPPH radical scavenging activities of ten vegetables

Plant names (Family)	50% Inhibitory concentration (IC ₅₀) µg/mL
<i>Amaranthus viridis</i> (Amaranthaceae)	>400
<i>Talinum triangulare</i> (Portulacaceae)	272.35 ± 3.1
<i>Telfairea occidentalis</i> (Cucurbitaceae)	245.56 ± 2.0
<i>Amaranthus hybridus</i> (Amaranthaceae)	210.23 ± 2.0
<i>Celosia argentea</i> (Amaranthaceae)	83.84 ± 1.48
<i>Gongronema latifolium</i> (Asclepiadaceae)	49.95 ± 2.7
<i>Piper guineense</i> (Piperaceae)	48.03 ± 0.6
<i>Vernonia amygdalina</i> (Asteraceae)	40.61 ± 2.1
<i>Corchorus olitorius</i> (Tiliaceae)	33.86 ± 1.1
<i>Gnetum africanum</i> (Gnetaceae)	14.58 ± 1.8
Ascorbic acid	4.14 ± 0.26

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Address for correspondence:

Omonike Ogbole
Department of Pharmacognosy, Faculty of Pharmacy,
University of Ibadan, Ibadan, Oyo State, Nigeria
E-mail: nikeoa@yahoo.com
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