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PHYTOCHEMICAL AND HEAVY METALS DETERMINATION IN LEAFY VEGETABLES CONSUMED IN EASTERN NIGERIA.

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Professor, Department of Chemistry, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. **ABSTRACT**

Twelve leafy vegetables were randomly selected and analyzed for their heavy metals and anti-nutritional factors using standard analytical methods. They include *Amaranthus hybridus, Vernonia amygdalina, Telfaria occidentalis, Gnetum africanum, Pterocarpus melibreadi, Hensia critina, Cucurbita pepo, Talinum triangulare, Gougrenema latifolium, Piper guinensis, Pterocarpus santolinoides and Occimum gratissimum.* Iron (8.90-37.00 mg/100g) and zinc (1.07-22.20 mg/100g) were the dominant heavy metals present in all the leafy vegetable samples. Analysis of the phytochemicals showed high levels of alkaloids (0.84-2.42%) and Saponins (0.46-2.40%). The health implications of the heavy metal and phytochemicals determined are

discussed.

KEYWORDS: Phytochemicals, Heavy metals, Leafy vegetables.

INTRODUCTION

Good health is impossible without good nutrition, and for good nutrition you need a healthy and balanced diet, which must include fruits and vegetables (Watchtower, 2015). Vegetables generally refer to edible parts of a plant.

Vegetables can be divided into two main groups:

Below the ground vegetables.

These include roots and tubers of plants such as carrots, potatoes, onion, garlic, etc.

Above the ground vegetables.

These include:

Leaves such as spinach, lettuce, amaranthus, etc.

Flowers such as broccoli, cauliflower, etc.

Stalk such as asparagus, fennel, etc.

Pods such as peas, sweet corn, okro, etc.

Vegetable fruits like aubergine, tomato, capsicum, etc.

Vine fruits such as cucumber, pumpkin, squashes, etc.

Fungi such as a variety of mushroom.

Leaf vegetables, also called potherbs, greens, vegetables greens, leafy greens or salad greens, are plant leaves eaten as vegetables, sometimes accompanied by tender petioles and shoots. They come from a variety of plants, most shares a great deal with other leaf vegetables in nutrition and cooking methods. Leafy vegetables are typically high in phytochemicals. If leaves are cooked for food, they may be referred to as boiled greens. Leafy vegetables may be stir-fried, stewed, steamed or consumed raw. Vegetables grow on soil that may have been treated with manure, so washing these items carefully before preparing them is one vital step in making them fit for consumption. People who eat fruits and vegetables as part of their daily diet have reduced risk of many chronic diseases. USDA's MyPlate encourages making half your plate fruits and vegetables (USDA 2009).

It is obvious from the foregoing that between the extremes of optimal health and death from starvation or malnutrition, there is an array of disease states that can be caused, aggravated or alleviated by change in diet. Deficiencies, excesses and imbalances in foods we eat can produce negative impact on our health. Researchers have shown, for example, that when students are given improved diet their learning capacity also improved. Poor diet generally results to poor work and makes people more accident-prone. It quickly robs the body of a healthy appearance and natural beauty.

Leafy vegetables are important items of diet in many Nigerian homes. They have the cheapest and most abundant source of protein and add flavor, variety, taste, color and

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aesthetic appeal to diet. In Nigeria and many African countries of the tropics, vegetables are very abundant immediately after the rains but become scarce later in rainy season and more so in dry season. Understanding the importance of these traditional vegetables will affect people's attitude towards their cultivation even during periods of scarcity due to weather conditions.

The main problem in nutritional exploitation of green leafy vegetables is the presence of anti nutritional and toxic principles (Kaushalya and Wagle, 1988). The presence of a large number of inexpensive edible green leafy vegetables, their abundance and their attributive qualities coupled with diminishing usage in contemporary diet create interest to study the nutritional value as well as anti nutritional principles in selected green leafy vegetables.

The various leafy vegetables were sampled from various markets in eastern Nigeria.

Table 1: Leafy Vegetables.

S/N	IGBO/IBIBIO NAME	ENGLISH NAME	BOTANICAL NAME
1	Ugu	Fluted Pumpkin	Telfaira occidentalis
2	Uha	Africana Rose Wood	Pterocarpus melibreardi
3	Olugbu/onugbu/ikong	Bitter Leaf	Vernonia amygdalina
4	Inine Oyibo	English Amaranth	Amaranthus hybridus
5	Uziza	African pepper	Piper guinensis
6	Mmomong	Waterleaf	Talinum triangulare
7	Ukazi/Afang/Okazi		Gnetum africanum
8	Nchianwu/Ntong	Scent leaf/fever plant/tea bush	Occimium gratismum
9	Utazi/Utasi		Gongronema latifolium
10	Atama/Nkanka		Heinsia crinata
11	Ugbogoro	Pumpkin	Cucurtica pepo
12	Nturukpa		Pterocarpus santolinoides

AIM

The aim of this research is to assess phytochemical and heavy metal values of the leafy vegetables under study.

MATERIALS AND METHODS

PLANT MATERIALS

- 1. Amaranthus hybridus (Green)
- 2. *Curcubita pepo (*Ugbogoro)
- 3. *Gnetum africanum (*Ukazi)
- 4. Gougronema catifolium (Utazi)

- 5. *Hensia critina* (Atama)
- 6. *Occimum gratissimum* (Nchuanwu)
- 7. Piper guinensis (Uziza)
- 8. Pterocarpus melibreadi (Uha)
- 9. *Pterocarpus santolinoides* (Nturukpa)
- 10. Talinum triangulare (Water Leaf)
- 11. Telfaria occidentalis (Ugu)
- 12. *Vernonia amygdalina* (Bitter Leaf)

APPARATUS

- 1) Hot plate
- 2) Flame photometer
- 3) Panasonic Blender (with dry mill) 1000ml
- 4) Kjeldahl apparatus
- 5) Mechanical shaker
- 6) Incubator
- 7) Temperature regulated oven
- 8) Weighing balance
- 9) Soxhlet extractor
- 10) Atomic absorption spectrophotometer

SAMPLE COLLECTION

The twelve (12) leafy vegetables were bought from various markets in Eastern Nigeria.

SAMPLE PREPARATION

The leaves of the selected vegetables were air dried after picking and ground to powder with Panasonic blender and the required quantity taken for analysis.

DETERMINATION OF ANTI-NUTRIENTS

Alkaloid determination

1g was weighed into a 250ml beaver and 20ml of acetic acid in ethanol was added, covered and allowed to stand for 4hours at 25°c. This was filtered with filter paper No 42 and the filtrate was concentrated using a water bath to one quarter of the original volume.

Concentrated ammonium hydroxide was added drop wise to the extract until the precipitate was collected and washed with dilute NH₄OH (1% ammonia solution), then filtered with preweighed filter paper. The residue on the filter paper was the alkaloid, which was dried in the over at 105°c. The alkaloid content was calculated and expressed as a percentage of the weight of the sample analysed (Harbone, 1993; Obadonic and Ochuka, 2001).

Calculation

Determination of saponin

Exactly 1.0 gram of the ground leaf sample was put in 20% arctic acid in ethanol and allowed to stand in a water bath at 50° c for 24hours. This was filtered and the extract was concentrated using water ball to one quarter of the original volume. Concentrated NH₄OH was added drop-wise to the extract until the preciplate was collected by filtration and weighed. The saponin content was weighed and calculated in percentage (Obadoni and Ochuka, 2001).

Calculation

Determination of tannin

The Folin-Denis spectrophotometric method was used. The method was described by Pearson (1976) and further description given by Onwuka (2005). One gramme of each sample was dispersed in 10ml distilled water and shaken every 5minutes but allowed to star for 30 minutes, it was centrifuged to obtain an extract. 2.5ml of the extract was dispersed into a 50ml volumetric flask. Similarly, 2.5ml of standard tannic acid solution was dispersed into a separate 50ml flask. 10ml Folic-Denis reagent was measured into each flask, followed by 2.5ml of saturated Na₂CO₃ solution. The mixture was diluted to mark in the flask (50ml), and incubated for 90 minutes at room temperature. The absorbance was measured at 250nm.

Reading was taken with the reagent blank of zero. The tannin content was given as follows:

% Tannin =
$$\frac{An}{As} \times C \times \frac{100}{W} \times 5$$

where: W= weight of sample analyzed

An = Absorbance of sample

As = Concentration of standard thiamine solution

C = Concentration (mg/ml) of standard solution

Vf = Total volume of filtrate

Va = Volume of filtrate analyzed

Determination of Phenol

Two grammes of the sample were defatted with 100ml of petroleum ether. The defatted sample was dissolved in 5ml of Diethylether and transferred into a 50ml volumetric flask, 10ml of distilled water added, 10ml of IN potassium ferrocyanide, 2ml of ammonia solution and 5ml of amyl alcohol, then made up to the mark with distilled water (Jayaprakasha 2001). The sample was allowed to stand for about 30 minutes and absorbance read at 505nm using a ultraviolet visible spectrophotometer.

A set of working standard was prepared to contain 0ppm, 20pp, 30ppm, 40ppm and 50ppm phenol using phenolic acid reagent. The colour was developed and also read in the spectrophotometer at 505nm wavelength.

Determination of Flavonoid

Five grammes of the sample were weighed into a beaker and 100ml of 5% acetone added and this was heated at 50°C for 15 minutes. The sample was allowed to cool for about 30 minutes, the sample was filtered through a N1 filter paper into a 200ml volumetric flask, the residue was washed with plenty of distilled water and finally made up in a 200ml flask with distilled water. A clean dry crucible was weighed empty and 50ml of the extract pipette into the crucible and heated to dryness over a water bath and finally in the oven set at 80°C for 2 hours.

The sample was removed from the oven and allowed to cool and then weighed. The difference in weight was calculated as the weight of the flavonoid in the sample.

Oxalates determination by titration method

This determination had three steps of digestion, oxalate precipitation and permanganate titration (Onwuka, 2005).

Digestion

2g of the sample flour was suspended in 190ml of distilled water in a 250ml volumetric flask.

10ml of 6m Hcl was added and the suspension digested at 100^oC for one hour to 250ml mark before filtration.

Oxalate Precipitation

Duplicate portions of 125ml of the filtrate were measured into beakers and four drops of methyl was added drop wise until the test solution change colour (pH 4-4.5). Each portion was then heated to 90°C, cooled and filtered to remove precipitate containing ferrous ion. The filtrate was again heated to 90°C and 10ml of 5% Cacl2 solution added while starring constant. After heating and cooling it was allowed to stand overnight at 5°C.

The solution was then centrifuged at 2500rpm for 5 minutes. The supernatant was decanted and the precipitate completely dissolved in 10ml of 20% (v/v) H₂SO₄ solution.

Permanganate Titration

The total filtrate resulting from digestion of 2g flour was made up to 300ml. Aliquots of 125ml of the filtrate were heated until near-boiling and then titrated against 0.05m standardized persisted for 30 seconds.

The calcium oxalate content was calculated using the formula:

Where T is the titer of $KMnO_4$ (ml)g Vme is the volume mass equivalent (i.e $1cm^3$ of 0.05m $KMnO_4$ solution in equivalent dilution factor VT/A) (2.4 where V_T is the volume of titrate (300ml) and A is the aliquot used (125ml), ME is the molar equivalent of $KMnO_4$ oxalate ($KMnO_4$ redox reduction) and mg is the mass of flour used.

Phytate determination

Phytate contents were determined using the method of Young and Greaves (1940). Two grammes of each of the food samples were weighed into different 250ml conical flasks. Each samples was soaked in 100ml of 2% concentrated HCl for 3hrs. The samples were then filtered. 50ml of each filtrate was placed in 250ml beaver and 100ml distilled water added to each sample 10ml of 0.3% ammonium thiocyanate solution was added as indicator and titrate with standard iron iii chloride solution which contained 0.00195g iron per ml.

The percentage phytic acid was calculated using the formulas.

Phytic acid (%) =
$$\frac{\text{litre value} \times 0.00195 \times 1.19}{2} \times 100$$

Where 1.19 is a conversion factor.

RESULTS

Table 2 Phytochemicals.

	% Phenol	% Alkaloid	% Flavonoid	% Saponin	% HCN	% Tannins	% Phytate	% Oxalate
Amaranthus hybridus	0.04 ±0.01	2.42 ± 0.01	0.56 ± 0.00	0.86 ± 0.00	0.48 ±0.01	0.86 ± 0.01	0.13 ±0.00	0.38 ± 0.00
Vernonia amygdalina	0.09 ±0.01	1.92 ± 0.01	0.72 ± 0.00	2.40 ±0.00	0.49 ±0.01	0.65 ± 0.01	0.20 ± 0.00	0.18 ±0.00
Gnetum Africanum	0.11 ± 0.00	2.14 ± 0.01	0.58 ± 0.01	1.02 ± 0.01	0.37 ± 0.00	0.14 ±0.01	0.26 ± 0.01	0.18 ± 0.01
Gougronema catifolium	0.11 ± 0.00	2.28 ±0.01	0.86 ± 0.01	1.82 ± 0.01	0.35 ± 0.00	0.82 ±0.01	0.30 ± 0.01	0.24 ± 0.01
Telfaria occidentalis	0.05 ± 0.01	1.68 ±0.01	0.36 ± 0.01	1.98 ± 0.01	0.25 ± 0.01	0.78 ± 0.01	0.14 ± 0.01	0.20 ± 0.01
Piper guinesis	0.03 ± 0.01	0.84 ±0.01	0.84 ± 0.00	0.68 ± 0.01	0.33 ± 0.01	0.18 ±0.01	0.22 ±0.00	0.19 ±0.01
Pterocarpus santolinoides	0.02 ±0.01	1.12 ±0.01	0.22 ± 0.00	0.62 ± 0.01	0.33 ±0.01	0.08 ± 0.01	0.28 ± 0.00	0.21 ± 0.01
Pterocarpus melibreadi	0.02 ± 0.01	0.76 ± 0.02	0.32 ± 0.00	0.52 ±0.01	0.38 ± 0.01	0.11 ± 0.02	0.33 ± 0.00	0.43 ± 0.01
Occimum gratissimum	0.10 ± 0.01	1.32 ±0.01	0.74 ±0.00	1.14 ±0.01	0.24 ± 0.01	0.35 ±0.01	0.20 ± 0.00	0.29 ±0.01
Hensia critina	0.07 ± 0.01	1.88 ±0.01	0.44 ± 0.01	0.58 ± 0.00	0.23 ± 0.01	0.36 ± 0.01	0.18 ± 0.01	0.30 ± 0.00
Cucurbita pepo	0.04 ± 0.00	2.08 ± 0.03	0.3 0 ± 0.00	0.46 ±0.00	0.27 ± 0.00	0.09 ± 0.03	0.32 ± 0.00	0.23 ± 0.00
Talinum triangulare	0.03 ± 0.00	2.32 ±0.01	0.38 ±0.01	0.92 ± 0.00	0.31 ± 0.00	0.45 ± 0.01	0.07 ± 0.01	0.48 ± 0.00

Table 3: Heavy Metals(mg/100g)

	Pb	Zn	Fe	Co	Cu
Amarathus hybridus	ND	1.73 ± 0.00	14.70 ±0.00	ND	0.01 ± 0.00
Vernonia amygdalina	0.02±0.01	1.58 ± 0.01	9.30 ± 0.00	ND	0.02 ±0.00
Gnetum Africanum	0.02 ± 0.00	2.70 ±0.01	20.00 ± 0.00	ND	0.01 ± 0.01
Gougronema catifolium	0.02 ± 0.00	1.07 ±0.01	28.00 ± 0.00	ND	0.01 ± 0.01
Telfria occidentalis	ND	1.70 ± 0.01	19.00 ± 0.00	0.03 ± 0.00	ND
Piper guinesis	0.08 ± 0.01	1.80 ±0.01	19.80 ±0.00	ND	ND
Pterocarpus santolinoides	0.12±0.01	7.60 ± 0.01	22.30 ± 0.00	ND	ND
Pterocarpus melibreadi	0.16 ± 0.01	11.90 ± 0.02	37.00 ± 0.00	ND	ND
Occimum gratissimum	0.02 ± 0.01	22.20±0.01	23.30 ± 0.00	0.05 ± 0.00	ND
Hensia critini	0.12 ± 0.01	11.80 ± 0.01	11.10 ± 0.00	ND	ND
Cucurbita pepo	ND	1.52 ± 0.03	8.90 ± 0.00	ND	ND
Talinum triangulare	ND	1.48 ± 0.01	9.90 ± 0.00	0.5 ± 0.00	ND

DISCUSSION

Phytochemicals/ Antinutritional Factors

The phytochemicals/antinutritional factors in the samples are presented in Table 2. Alkaloid levels ranged between 0.76 ± 0.02 for *Pterocarpus Melibreadi* and 2.42 ± 0.01 for *Amaranthus hybridus*. Alkaloid was detected in all the vegetables with *Amaranthus hybridus* as the highest and *Pterocarpus melibreadi* the least. The levels of alkaloid in the vegetables showed that they possessed some antimicrobial properties and might contribute to medicinal values of their food (Evans, 2005).

Saponins ranged from 0.46 ± 0.00 for *Cucurbita Pepo* to 2.46 ± 0.00 for *Vernonia amygdaline*. All the twelve leafy vegetables showed significant saponins level. Saponins have been shown to possess both beneficial (cholesterol lowering) and deleterious properties (Price et al., 1987). Although some Saponins have been shown to be highly toxic under experimental conditions, acute Saponins poisoning is relatively rare both in animals and man (Osagie and Offiong, 1998). Saponins in excess also causes hypocholesterolemia because it binds cholesterol making it unavailable for absorption (Soetan and Onyewole, 2009).

Oxalate level ranged between 0.18 ± 0.00 for Vernonia amygdalina and 0.48+0.00 for Talinum triangulare. Oxalate was detected in all the vegetables, although the levels were far below the toxic level which is 25% (Oke, 1996; Munrio and Bassi, 1969). Many studies have been made with laboratory animals and human subjects showing that dietary calcium is poorly absorbed from oxalate rich foods. It has been suggested that vegetables with high oxalate level be consumed moderately since cooking in some cases, does not affect or drastically reduce content of some of these phytochemicals (Liu, 2004, Onyeka and Nwanmbekwe, 2007).

Oxalate binds to calcium present in food, thereby rendering calcium unavailable for normal physiological and biochemical roles, such as the maintenance of strong teeth and bone. The calcium oxalate which is insoluble may also precipitate around soft tissues such as kidney; causing kidney stones which are associated with blockage of renal tubules (Oke, 1969; Blood and Radosti, 1989; Ladeji, *et al.*, 2004).

Tannins ranged between 0.09±0.03 for cucurbita pepo to 0.86±0.01 for *Amaranthus hybridus*. All the vegetables contained tannin but *Amaranthus hybridus* had the highest content while

Cucurbita pepo had the least. The values agreed fairly with that reported for green leafy vegetables (Onyeka and Nwambekwe, 2007). The presence of tannin has been reported to contribute to the bitter taste of these green leaves (Onyeka and Nwambekwe, 2007). The effects of tannin vary, depending on the content and type, which in turn is dependent on contain characteristics such as type of digestive tract, feeding behavior, body size and detoxification mechanism. Levels of tannin above 5% of a diet are often lethal (Giner-Charez, 1996). Nutritional effects which have been attributed to tannins include damage to the intestinal tract, interference with the absorption of iron and possible carcinogenic effect (Osagie and Offiong 1998).

Tannin –protein complexes are insoluble and protein digestibility greatly decreased. Medicinally, polyphenols to which tannin belongs have been reported to act as antioxidant by preventing oxidative stress that causes diseases such as coronary heart diseases, some types of cancer and inflammation (Tapiero *et al.*, 2000). So drinks such as green tea that contain large amounts of these compounds might be good the health of some people despite their anti-nutrient properties (Chung *et al.*, 1998).

The values of Phytic acid ranged between 0.07 ± 0.01 for Talinum triangulare and 0.33 ± 0.00 for *Pterocarpus melibreadi*. All the leafy vegetables is investigated contained phytic acid, but the levels were reasonably low. According to Oke (1969) a phytic diet of 1-6% over a long period decreased the bioavailability of mineral elements. It also has a negative effect on amino acid digestibility, thereby posing problems to non ruminant animals due to insufficient amount of intrinsic factor phytase necessary to hydrogen the phytic acid complex (Makkar and Becker, 1998). Phytic acids have 12 replaceable hydrogen atoms with which it could form insoluble salts with minerals like Ca, Mg, Fe, and even Zn. Therefore individuals suffering from diseases associated with lack of these minerals should consume vegetables with high phytic acids moderately (Bello *et al.*, 2004; Muhammed *et al.*, 2011).

Phytochemical analysis showed the presence of flavonoids in all the twelve leafy vegetables ranging from 0.22 ± 0.00 in *Pterocarpus santilinoides* to 0.86 ± 0.01 in *Gongreneme latifolium*. Flavonoids increase endothelial nitric oxide function. Nitric oxide relaxes the smooth muscle on the wall of the artery by increasing the activity of nitric oxide synthesis, the enzyme that catalyzes the production of nitric oxide. This reduced the risk of hypertension and other cardiovascular dilatation. They prevent atherosclerosis by inhibiting the expression of the

vascular cell adhesion molecule in the endothelial cells that line the inner layer of the blood vessels and by decreasing inflammation directly.

Hydrogen cyanide content ranged from 0.23±0.01 to 0.49±0.01. *Vernonia amydalina* had the highest level of 0.49mg/100g. The level obtained are below the permissible levels in human and far below the lethal level for an adult in an 50-60mg/kg body weight (Bolhuis, 1954, Onwuka, 2005). The levels obtained fairly agreed with report of other workers (Eroarome, 2012). The knowledge of the cyanogen glycosides content of food is vital became cyanide being an effective cytochrome oxide inhibitor interfers with aerobic respiratory system. Hydrocyanic acid does not occur free, but combine with sugars to form a non-toxic compound known as cyanogenic glycoside. A lot of hydrochain at the cytochrome oxidase levels lost during soaking and cooking (Key *et al.*, 1977) so that its content in the leafy vegetables poses no danger to toxicity. The young leaves should be properly cooked in order to remove any inherent anti-nutrient effect before consumption.

Heavy Metal Content

The levels of heavy metals determined in the samples are presented in Table 3. The results revealed that iron predominant among the heavy found ranging from 8.90±0.00 (*Cucurbit Pepo*) to 37.00±0.00 *Pterocarpus melibreadi*. Iron is an essential element for human beings and animals and is an essential component of hemoglobin. It facilitate the oxidation of carbohydrate, protein and fat to control body weight (Ullah *et. al.*, 2009). Low iron content causes gastrointestinal infection, nose bleeding, and myocardial infection. Too much iron can result in hemochromatosis in those genetically vulnerable to this condition, or other potentially dangerous iron overload conditions (Manoguerra *et. al.*, 2005).

Zinc is next in abundance in this study ranging from 1.07±0.00 for *Gougronema latifolium* to 22.20±0.01 for *Occimum gratissimum*. Zinc is an essential trace element in the human body, where it is found in higher concentration in the red blood cells, as an essential part of the enzyme carbohydrate, which promotes many reactions in carbon dioxide metabolism. It helps maintain immune function, help cells divide and repair and help metabolize carbohydrates for body to use for energy. Zinc is needed for sense of taste and smell (Bowler *et. al.*, 2007). According to the National Institution of Health, USA, Zinc toxicity starts between 35-40mg daily (Dobson *et. al.*, 2004). Gastrointestinal and urinary complications are the most common side effects of zinc toxicity.

Lead (Pb) was present in eight of the twelve leafy vegetables sample analyzed. Ranging from 0.02 mg/100g to 0.16 mg/100g. These levels agreed fairly with other workers (Agbogidi and Erkenhi, 2013; Sobukola *et al.*, 2010).

Nankishore, 2014 supported increased levels of heavy metals in leafy vegetables from selected markets in Onyana due to atmospheric deposits. Leafy vegetables have the ability to absorb metals deposited on plant surfaces explored polluted environments. Although certain heavy metals (Cr, Zn, Mn, Cu and Fe) are essential components for various biological activities within the human body, elevated levels of these can cause numerous health consequences to mankind. In contrast, Pb, Cd, Hg, and As are non essential toxic element which are associated with many chronic diseases in humans. Lead can cause several unwanted effects such as disruption of the Biosynthesis of hemoglobin and anemia, a rise in blood pressure, kidney damage, miscarriages and subtle abortions, disruption of nervous systems, brain damage, decline in fertility of men through sperm damage, diminished learning abilities in children (Watt, 2009).

Copper was detected in only four of the twelve vegetables investigated. Ranges were between 0.01 mg/100g to 0.02 mg/100g Amaranthus hybridus, Gnetum africanum and Gongrenema latifolium showed 0.01 mg/100g. Vernonia amygalina showed slightly higher level of 0.02 mg/100g. In all the other vegetables investigated, Cu was not detected. RDI of Cu is 2mg. The finding, underscore the need to use organic fertilizer to fortify the soil where these vegetables are grown to boost these inadequate levels. This is because of the importance of copper. Apart from being an important biocatalyst in the body, copper is essential for body pigmentation, maintenance of the central nervous system, preventing of Fe and Zn in the body.

On the other hands, copper toxicity can induce lipid per oxidation, iron deficiency and membrane destruction within the body.

Green leafy vegetables are credited to be the richest source of cobalt (www.nutrition.com/minerals/detail.9.05am.28.07.2015). The Cobalt level detected was low in two of the three leafy vegetables analyzed ranging from 0.03mg/100g to 0.05mg/100g. Talinum triangulare had the highest level of 0.5mg/100g. The total daily intake of cobalt is variable and may be as much as milligram, but almost all will pass through the body

unabsorbed except that in vitamin B_{12} none of the vegetables had up to one milligram of cobalt which is the recommended daily intake.

Cobalt is beneficial for human because it is part of vitamin B_{12} which aids in the formation of normal red blood cells and maintains nerve tissue. Cobalt is therefore used in treating anaemia in order to help produce red blood cells. Radioactive cobalt is used in sterilizing medical equipment, irradiating food, treating cancer patients and for manufacturing plastics.

Cobalt salts, cobalt metal powder and cobalt containing dusts can be breathed in, which will cause damage to our respiratory system.

CONCLUSION

From the results and discussions of this research, it has been found that:

- i. All the leafy vegetables contained substantial anti-nutrients.
- ii. Minerals like P, Na, Cu, were very low while Ca, Zn, Fe were present in substantial quantities. Those present is very low quantities should be supplemented in diet.
- iii. Lead is the predominant toxic heavy metal present in the leafy vegetables investigated.

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