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Determination of phytochemicals and some heavy metals in leaves of some edible vegetables marketed south eastern Nigeria

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Abstract
The heavy metal content of edible vegetables has been studied. Heavy metal toxicity enters into the human food chain through certain food stuffs. Two different vegetables (*Telfairia occidentalis* and *Talinum triangulare*) were purchased in two different markets in Aba and were analyzed for their heavy metal contents using Atomic Absorption Spectrophotometer (AAS). Iron (Fe) levels were the highest followed by Zinc (Zn) whereas Lead (Pb) and Cadmium (Cd) were not detected by our analytical method. The concentration of Iron (Fe) in *T. occidentalis* sampled at new market (357 ± 0.17mg/kg), *T. occidentalis* sampled at umungasi market (419 ± 0.14mg/kg), *T. triangulare* sampled at new market (388 ± 0.02mg/kg) and *T. triangulare* sampled at umungasi market (342 ± 0.25mg/kg) while Zn has (76.1 ± 0.02mg/kg) and (115 ± 0.44mg/kg) in *T. occidentalis* sampled at new market and umungasi market. And (266 ± 0.77mg/kg) and (58.8 ± 0.26mg/kg) in *T. triangulare* sampled at new market and umungasi market respectively. The result showed that Zn in vegetables were above the recommended maximum level. Phytochemical studies indicated Alkaloids, flavonoids, and saponins in the vegetable sampled while tannins were only detected in *T. triangulare*.

Keywords: heavy metals, vegetables, *Telfairia occidentalis*, *Talinum triangulare*, Nigeria

Introduction

Most developing countries depend on starch-based food as the main staple food for the supply of both energy and protein. This account in part, for protein deficiency which prevails among the populace as recognize by food and agricultural organization (Ladeji *et al*., 1995) [23].

In Nigeria, as in most other tropical countries of Africa where the daily diet is dominated by starchy staple foods, vegetables are the cheapest and most readily available sources of important proteins, vitamins, mineral and essential amino acids (Thompson and Kelly, 1990) [46].

Vegetables are the fresh and edible portions of herbaceous plants, which can be eaten raw or cooked (Dhellot *et al*., 2006) [10]. They contain valuable food ingredients which can be used as energy sources, body building, regulatory and protective material.

Many people are aware that eating plant based foods add much needed fiber, vitamins and minerals to the diet but what is less well known is the many benefit of the phytochemicals that these plant contain.

Phytochemicals come in a variety of forms and different vegetables have higher concentrations of a particular phytochemical than others. It is important to understand that maintaining a diet that contains variety of vegetables will combine the benefits of the phytochemicals available.

The implications associated with heavy metals contaminations are of great concern particularly in agricultural production systems (Gupta and Gupta, 1998) [17]. Diverse amount of heavy metals are found as contaminants in agricultural production especially in urban horticultural crop production. The contamination of agricultural soils is often a direct or indirect consequence of anthropogenic activities (McLaughlin *et al*., 1999) [30]. Sources of anthropogenic metal contamination in soils include urban and industrial waste, mining and smelting of non-ferrous metals and metallurgical industries. Others sources of anthropogenic contamination include the addition of manure, sewage, sludge, fertilizers and pesticides to soils (McBride, 2003) [28]. These metals, once introduced to the environment by one particular method, may spread to various environment components, which may be caused by the nature of interactions occurring in the natural systems (Dube *et al*., 2001) [11].

The uptake of these metals by crops results in the bioaccumulation of these elements in plant tissue. The kind of metal, plant species and plant parts however influence the uptake of the metals (Juste and Mench, 1992) [22].

Vegetables contain both essential and toxic elements over a wide range of concentrations (Ajewole, 1999) [1]. The concentration of these elements is a function of the concentration in the soil in which the vegetable is planted. These toxic elements are usually introduced into the vegetables from the environment. They could be in the soil, air, or nutrient solutions and include heavy metals and other trace elements.

Alloway and Davies (1971) [4] reported that plants grown on soils possessing enhanced metal concentration due to pollution have increased heavy metal ion content. One of the ways by which heavy metals are consumed is via the food chain. If the consumption of these metals through plant sources is not regulated, it may lead to accumulation in man with high potentials for health hazards.

Heavy metal contamination of vegetables cannot be under estimated as these foodstuffs are important components of human diet. Vegetables are rich sources of vitamins, minerals and fibers, and also have beneficial antioxidative effects. However, intake of heavy metal contaminated Vegetables may pose a risk to the human health. Heavy metal contamination of food is one of the most important aspects of food quality assurance. Heavy metals are non-biodegradable and persistent environmental contaminants, which may be deposited on the surfaces and then absorbed into the tissues of vegetables.

Telfairia occidentalis (pumpkin leaf) is one of the commonly consumed leafy vegetables in Nigeria. It is used in herbal preparations of African traditional medicine, it has the ability to attenuate hyper cholesterolemia.

Oyolu (1978) [40] reported that the leaves of *Telfairia occidentalis* together with the edible shoots contain moisture, crude, protein, carbohydrates, oils, ash and iron while Longe *et al*., (1983) [26] reported that the minerals namely; calcium, potassium, magnesium, iron, sodium and phosphorous are concentrated in testa, pulp and husk.

Talinum triangulare (waterleaf) is an herbaceous perennial leaf, calls cent and glabrous plant widely grown in tropical regions as a leaf vegetable (Ezekwe *et al*., 2001) [13]. Nutritionally, *Talinum triangulare* has been shown to possess the essential nutrients like beta carotene, minerals (such as calcium, potassium and magnesium), pectin, protein and vitamins (Ezekwe *et al*., 2001) [13].

Aims and Objective of the study

This study was built in the foundation that consumption of vegetables has been shown as one of the ways by which heavy metals are consumed by man. Secondly, there are reports on literature on the health hazards associated with heavy metals concentration. The objectives of this study are stated as follows; to evaluate phytochemical composition of these vegetables (*Telfairia occidentalis* and *Talinum triangulare*) and to assess the heavy metal content of the leaves of the vegetables *Telfairia occidentalis* and *Talinum triangulare* marketed in Aba.

Materials and Method

Sampling

Two vegetables (*Telfairia occidentalis* and *Talinum triangualare*) were purchased in two different markets in Aba (New market and Umungasi market) Abia State Nigeria.

Sample Preparation

The vegetable leaves were washed and air-dried. Each of the dried leaves was ground into fine powder. These powders were packaged in an air tight plastic container.

Heavy metal analysis

Powdered sample (5.0g) was transferred to a 25ml conical flask; 5ml of concentrated H_2SO_4 was added followed by 25ml of concentrated HNO_3 and 5ml of concentrated HCl. The content of the tube were heated at 200^{oc} for 2 hours in a fuming cupboard, and then cooled to room temperature. Then, 20ml of distilled water was added and the mixture was filtered using filter paper. Finally, the mixture was transferred to a 50ml volumetric flask, filled to mark, allowed to settle for at least 15 hours. The resulted filtrate was analyzed for total Cd, Fe, Zn, and Pb, by Atomic Absorption Spectrophotometer. This sample procedure was carried out for each plant sample (Jones, 1984).

Quantitative phytochemical analysis

Determination of the alkaloid content

This was done by the alkaline precipitation gravimetric method of Harbone (1973) [18]. As described by Obadoni and Ochuko, (2001) [35] and Okwu (2005) [37]. Each of the samples (5g) was weighed into a 250ml beaker and 200ml of 20% acetic acid in ethanol (1:10) was added and covered to stand for 4 hours. This was filtered and the extract was concentrated using a water bath to one quarter of its original volume by evaporation and treated with drop-wise addition of concentrated aqueous NH_40H until alkaloids was precipitated. The whole solution was allowed to settle and the precipitate was collected by filtration which was later dried and weighed. The alkaloid content was calculated and expressed as mg/100g of the weight samples analyzed. The percentage alkaloid was determined as follows:

$$\% \text{ alkaloid} = \frac{w_2 - w_1 * 100}{Ws}$$

Where W_2 = weight of filter paper + extract

W_1 = weight of filter paper

Ws = sample weight

Determination of the tannin content

Tannin content was determined by the photometric method of Harbone (1973) [18] as described by Okwu (2005) [37]. 5g of the samples were weighed into 100ml plastic bottle. 50ml of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to mark. Then 5ml of the filtrate was pipette out into a tube and mixed with 3ml of 0.1M $Fecl_3$ in 0.1N Hcl and 0.008M potassium ferrocyanide. The absorbance was measured in a digital spectrophotometer model 390 at 395nm wavelength within 10 minutes. A reagent blank sample was prepared. The reagent blank was used to calibrate the instrument to zero. A set of serial standard solutions were prepared using tannic acid to get mg/100g and measured to construct the calibration curve. The percentage tannin was calculated as shown:

$$\% \text{ tannin} = \frac{\text{Ab (test)} * \text{conc. (STD)} * 100 * \text{VF}}{\text{Ab (STD) w VA}}$$

Where Ab (test) = Absorbance of test sample

Ab (STD) = Absorbance of standard tannin

Conc. (STD) = Concentration of standard tannin

W = Sample weight

VF = Total volume of sample solution

VA = Volume of aliquot

Determination of the saponin content

This was done by the method of Harbone (1973) [18] as described by Obadoni and Ochuko (2001) [35] and Okwu (2005) [37]. Each of the plant samples (5g) were dispersed in 200ml of 20% ethanol. The suspension was heated over a hot water bath for 4 hours with continuous stirring at about 55^{oc}.

The mixture was filtered and the residue re-extracted with another 200ml of 20% ethanol. The combined extracts were reduced to 40ml over a water bath at about 90^{oc}. The concentration was transferred into a 250ml separatory funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded and the purification process was repeated of n-butanol (60ml) was subsequently added and the combined n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath and after evaporation the samples were dried in the oven to a constant weight. The saponins content was calculated and expressed as mg/100g of the weight of the sample analyzed. The percentage saponin was calculated as shown:

$$\% \text{ saponin} = \frac{w_2 - w_1 * 100}{Ws}$$

Where W_2 = weight of filter paper + extract
W_1 = weight of filter paper
Ws = sample weight

Determination of flavonoid content
The total flavonoid content of plant extract was determined according to spectrophotometric method as described by Zou *et al.*, (2004) [53]. A 5g portion of each of the samples was weighed into 100ml plastic bottle. 50ml of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to mark. The sample solution (0.5ml) was mixed with 2ml of distilled water and subsequently with 0.15ml of 5% $NaNO_2$ solution. After 6 minutes of incubation, 0.15ml of 10% $AlCl_3$ solution was added and then allowed to stand for 6 minutes. This was followed by the addition of 2ml of 4% NaOH solution and the solution was brought to 5ml by the addition of distilled water. The solution was thoroughly mixed and allowed to stand for another 15 minutes and finally read at wavelength of 510nm. A blank sample for each extract was used for background subtraction. The content of flavonoids in each extract was determined using a standard curve prepared from catechin to get mg/100g and the absorbance measured. The percentage flavonoid is calculated as shown:

$$\% \text{ flavonoid} = \frac{\text{Ab (test)} * \text{conc. (STD)} * 100 * \text{VF}}{\text{Ab (STD w VA)}}$$

Where Ab (test) = Absorbance of test sample
Ab (STD) = Absorbance of standard flavonoid
Conc. (STD) = Concentration of standard flavonoid
W = Sample weight
VF = Total volume of sample solution
VA = Volume of aliquot

Results
Heavy metals and the phytochemical results of both vegetables are given in tables 1, 2 and 3.

Table 1: Phytochemical result of *Telfairia occidentalis* and *Talinum triangulare* sampled at New market Aba South, Abia State Nigeria.

Parameters (Mg/100g)	*Telfairia occidentalis*	*Talinum triangulare*
Alkaloids	0.98 ± 0.50	1.21 ± 0.12
Flavonoids	2.00 ± 1.00	3.42 ± 0.50
Saponins	4.28 ± 0.10	6.11 ± 0.10
Tannins	< 0.01	0.14 ± 0.02

Values are Mean ± standard deviation (SD) of two determinations

Table 2: Phytochemical result of *Telfairia occidentalis* and *Talinum triangulare* sampled from Umungasi market Aba North, Abia State Nigeria.

Parameters (Mg/100g)	*Telfairia occidentalis*	*Talinum triangulare*
Alkaloids	2.09 ± 0.20	3.33 ± 0.20
Flavonoids	3.12 ± 0.30	6.27 ± 0.10
Saponins	5.23 ± 0.30	8.01 ± 0.20
Tannins	< 0.01	0.15 ± 0.10

Values are Mean ± standard deviation (SD) of two determinations

Table 3: Heavy Metal Analysis result of the vegetable leaves.

Parameters (Mg/Kg)	*Telfairia occidentalis* At New Market	*Telfairia occidentalis* At Umungasi Market	*Talinum triangulare* At New Market	*Talinum triangulare* At Umungasi Market
Iron (Fe)	357 ± 0.17	419 ± 0.14	388 ± 0.02	342 ± 0.25
Zinc (Zn)	76.1 ± 0.02	115 ± 0.44	266 ± 0.77	58.8 ± 0.26
Lead (Pb)	< 0.01	< 0.01	< 0.01	< 0.01
Cadmium (Cd)	< 0.01	< 0.01	< 0.01	< 0.01

Values are Mean ± standard deviation (SD) of two determinations

Discussion
The heavy metal compositions of two different vegetables (*Telfairia occidentalis* and *Talinum triangulare*) are given in table 3.
Iron (Fe) concentration were the highest followed by Zinc (Zn), Pb and Cadmium were not detected by our analytical method. The concentration s of fe were 357 ± 0.17mg/kg and 388 ± 0.02mg/kg in *Telfairia occidentalis* and *Talinum triangulare* respectively sampled at New market, while the Fe concentration of the vegetables sampled at Umungasi market were; 419 ± 0.14mg/kg and 342 ± 0.25mg/kg for *Telfairia occidentalis* and *Talinum triangulare* respectively.From the result, the concentration of fe across the various vegetables samples were below the 425.50mg/kg as recommended maximum level for vegetables as described by (Weigert, 1991) [51].
Zinc (zn) concentrations was highest in *Talinum triangulare* sampled at Umungasi market (266 ± 0.77mg/kg) followed by *Telfairia occidentalis* sampled at Umungasi market (115 ± 0.44mg/kg), then 76.1 ± 0.02mg/kg, and 58.8 ± 0.26mg/kg

was found in *Telfairia occidentalis* and *Talinum triangulare* sampled at New market respectively.Similarly, the concentration of zinc in vegetable sampled at Umungasi (*Telfairia occidentalis*) 115 ± 0.44mg/kg and New market (*Talinum triangulare*) 266 ± 0.77mg/kg were above the recommended maximum level for vegetables as adopted by (Weigert, 1991) [51].

Iron is thus essential but at some levels poses potential toxicity. The first indication of iron poisoning is stomach pain, as iron is corrosive to the lining of the gastrointestinal tract including the stomach. Vomiting are also common symptoms and bloody vomiting may occur as a result of metabolic acidosis which in turn damages the internal organs, particularly the brain and the liver.

Iron poisoning can cause hypovolemic shock due to iron's potential ability to dilate the blood vessels. The detrimental effects of chronic iron toxicity are due in part to iron accumulation in various organs. These include the heart, liver, brain, pancreas and joints. It interferes with zinc and copper metabolism which are needed to maintain the integrity of the joint surfaces.

Iron has an affinity for cardiac tissue and in excess, cause's damage to the heart muscle. Excessive iron antagonizes chromium needed for insulin transport and thus results in diabetes (Allen, 2002) [3].

Zinc is known to be a mobile metal (Mapandu *et al*., 2005) [27]. The zinc phytotoxicity in most leaf vegetables occurs when zinc accumulated to an average tissues concentration of 500mg/kg. This is far from the concentrations of zinc found in these vegetables.

The percentage metal uptake was the basic parameters used to assess human exposure to heavy metal through the food chain (Uwah *et al*., 2009) [48]. The parameters quantify the relative differences in bioavailability of soil metal to plant and translocation within plant, which is the function of both soil and plant properties.Zinc which is common micronutrients for the plants were found as mostly absorbed in the leaf and root tissues of the vegetables. The quantity absorbed by plants where highest in the vegetables sampled at umungasi market and their distribution was uniformed in the both species.

The present study carried out on the plant samples revealed the presence of medicinally active constituents. Alkaloids, flavonoids, saponins and tannins are present in both qualitative and quantitative analysis of phytochemical constituents.

Tannins were not detected in *Telfairia occidentalis* sampled in both new market and umungasi market. Saponins were the highest in both vegetables (*Telfairia occidentalis* and *Talinum triangulare*) and umungasi recorded the highest i.e. 5.23 ± 0.30mg/100g and 8.01 ± 0.20mg/100g respectively. Then new market followed with 4.28 ± 0.10mg/100g and 6.11 ± 0.10mg/100g respectively.Other parameters detected are alkaloids, flavonoids while tannins were not detected in *Telfairia occidentalis* but detected in *Talinum triangulare*.

Below is the order of detection;

Saponins > Flavonoids > Alkaloids; Saponins > Flavonoids > Alkaloids in *Telfairia occidentalis* sampled in both market. For *Talinum triangulare*, it follows the same trend like that of *Telfairia occidentalis* only that tannins were present.

Saponins are largely found in plants or vegetables and most plants that contain saponins can be detrimental and even toxic to animals, they are considered especially beneficial to the digestive, cardiovascular, and immune systems of humans. Ingesting saponins has been linked with a decrease in overall blood cholesterol and it is thought that they can contribute significantly to the prevention of arteriosclerosis. Saponins have been used in the treatment of a number of cardiovascular disorders and conditions, including varicose veins and thrombosis. Saponins aid the absorption of minerals such as calcium and silicon and to facilitate and ease the process of digestion in general and also contribute to the health efficiency of the immune system by binding to germs and other pathogens as they enter the body. In this scene, they function as natural antibodies (Michelle, 2013).

Flavonoids are a prominent group of secondary metabolites in vegetables that may possess biological activity and have beneficial effects on human health as antimicrobial, anti inflammatory, anti-diabetic, anti-cholesterolemic, antioxidant and anti-cancer agents (Vanamala *et al*., 2006; Huang and HO, 2010; Le *et al*., 2007) [19]. Flavonoids are reported to possess strong free radical scavenging activities based on their ability to act as hydrogen or electron donors and chelate transition metals (Hseu *et al*., 2008).

Most of the known functions of alkaloids are related to protection. For example, aporphine alkaloid produced by some plant protects it from parasitic mushrooms. Alkaloids such as serotonin, dopamine and histamine are important neuro transmitters in animals and also regulate plant growth (Hesse, 2009).

Alkaloids have been reported as one of the important group of phytoconstituents obtained from natural sources. It plays an important role in the ecology of organisms which synthesize them.They belong to β-carboline groups possess antimicrobial and anti-parasiticity activities (Bouayad *et al*., 2011). Alkaloids present in a plant shows that the plant can be effective anti malaria, since alkaloids consist of quinine which is an anti-malaria (Robinson, 1985) [43].

Tannins are water soluble polyphenols that are present in many plant foods. However, food rich in tannins are considered to be of low nutritional value because it inhibits the efficiency of concerting the obsorbed nutrients to new body substances.Incidences of certain cancer have been reported to be related to consumption of tannins rich food. Tea polyphenols and many tannins components were suggested to be anti-carcinogenic and many tannin molecules have also been shown to reduce the mutagenic activity of a number of mutagens. Many carcinogens and/or mutagens produce oxygen free radicals for interaction with cellular macromolecules (Weiburger *et al*., 1999).

The anti-carcinogenic and anti-mutagenic potentials of tannins may be related to their anti-oxidative property which is important in protecting cellular oxidative damage, including lipid per oxidation. The generation of superoxide radicals was reported to be inhibited by tannins and related compounds (Huang *et al*., 1985).

Conclusion

Heavy metal concentration in the vegetables studied may not present potential public health hazard. This is because the average amount of vegetables consumed by day by a person in most of the developing country in Africa is less than the international daily average of 50g for leafy vegetables (Itanna, 2003). It is because of this, that the intake of heavy metals from plant food constitutes much less than the theoretical maximum daily intakes which are used to express the exposure of consumers and associated health risk.

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