International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 16, No. 3, p. 389-396, 2020

INNSPUB

RESEARCH PAPER OPEN ACCESS

Determination of the ascorbic acid content, mineral and heavy metal levels of some common leafy vegetables of Jos, Plateau State (North Central Nigeria)

Dalen Gwatau Dafam[1*], Abdulkarim Agunu[2], Adama Dénou[1,4], Daniel Christopher Kagaru[1], Temitayo Lucia Ohemu[1], Ukpe Ajima[3], Josephine Nanle Damos[1], Victoria Akpidi Okwori[1]

[1]*Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, University of Jos, Jos, Nigeria*

[2]*Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria*

[3]*Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Jos, Jos, Nigeria*

[4]*Département des Sciences Pharmaceutiques, Faculté de Pharmacie, Université des Sciences, des Techniques et des Technologies de Bamako, Bamako, Mali*

Key words: Leafy vegetables, Vitamin C, Elemental and heavy metal, Nigeria.

http://dx.doi.org/10.12692/ijb/16.3.389-396 Article published on March 29, 2020

Abstract

Vitamin C (Ascorbic acid) is the most important vitamin in fruits and vegetables. It is required for the prevention of scurvy and maintenance of healthy skin, gums and blood vessels. It functions in absorption of inorganic iron, reduction of plasma cholesterol level, inhibition of nitrosamine formation, enhancement of the immune system, and reaction with free radicals. Therefore Vitamin C and elemental analysis of some local leafy vegetables namely; Pumpkin leaves, Jute leaves, Bitter leaf, Cubeb leaves, Bush buck leaves, Clove basil leaves, Curry leaf, Water leaf, African Eggplant and Moringa leaves were performed. Vitamin C determination was carried out on fresh leaves using UV spectrophotometry. Element analysis was performed on dried samples using atomic spectrophotometer for readings. The vitamin C content of the samples ranged from 5.70 mg/100 ml to 815.00 mg/100 ml. Elemental analysis showed that the leafy vegetables possessed calcium content ranging from 11.50 mg/100 ml to 830.00 mg/100 ml; potassium content ranged from 2.26 mg/100 ml to 2814.15 mg/100 ml; magnesium content ranged from 29.46 mg/100 ml to 677.0 mg/100 ml; sodium content ranged from 0.21 mg/100 ml to 370.0 mg/100ml and phosphorus content ranged from 5.00 mg/100mlto 600.00 mg/100 ml. Lead was absent in the samples but some contained traces of cadmium. These findings revealed that Nigerian leafy vegetables contain an appreciable amount of vitamin C and minerals with traces of toxic elements. However, they should be included in diets to supplement the daily requirement needed for sustainable health.

* **Corresponding Author:** Dalen Gwatau Dafam ✉ dalendafam@gmail.com

Introduction

Green leafy vegetables occupy an important place among the food crops as they provide adequate amounts of many vitamins and minerals for humans. They are rich sources of oil, carbohydrates, carotene, ascorbic acid, retinol, riboflavin, folic acid and minerals like calcium, iron, zinc, magnesium, manganese and selenium depending on the vegetable consumed (Ihekoronye and Ngoddy, 1985; Fasuyi, 2006). Vegetable fats and oil lower blood lipids thereby reducing occurrence of diseases associated with damage of coronary artery (Ononugbu, 2002). Green leafy vegetables constitute an indispensable constituent of human diet in Africa generally and West Africa in particular. Apart from the variety which they add to the menu (Mepha *et al.*, 2007; Sobukola *et al.*, 2007), they are valuable sources of nutrients especially in rural areas where they contribute substantially to protein, minerals, vitamins, fibers and other nutrients which are usually in short supply in daily diets (Mohammed and Sharif, 2011). In addition, green leafy vegetables are used in the diet of postpartum women during which time it is claimed that they aid the contraction of the uterus. However low consumption of green leafy vegetables in diet is one of the major factors which lead to deficiency of vitamins and iron. Minerals and vitamins cannot be synthesized by animals and must be provided from plants or vitamins and mineral-rich water. Vitamins belong to a group of potent, non-protein, organic compounds required in minute amounts for proper body function and good health (Fenell, 2004). Some vitamins such as A, D, E and K are fat soluble while others (the B vitamins and vitamin C) are water soluble. Vitamin C (Ascorbic acid) is the most important vitamin in fruits and vegetables. Except human and other primates, most of the phylogenetically higher animals can synthesize vitamin C (*L*-ascorbate) (Rekha *et al.,* 2012). More than 90 % of the vitamin C in human diets is supplied by fruits and vegetables (including potatoes). Ascorbic acid is the principal biologically active form but *L*-dehydroascorbic acid, an oxidation product, also exhibits biological activity (Choi *et al.,* 2007). Vitamin C is required for the prevention of scurvy and maintenance of healthy skin, gums and blood vessels. It functions in collagen formation, absorption of inorganic iron, reduction of plasma cholesterol level, inhibition of nitrosamine formation, enhancement of the immune system, and reaction with singlet oxygen and other free radicals (Kaviarasan *et al.*, 2007). As an antioxidant, it reportedly reduces the risk of arteriosclerosis, cardiovascular diseases and some forms of cancer (Yen *et al.*, 2005).

On the other hand, heavy metals are among the major contaminants of food supply and may be considered the most important problem to our environment (Zaidi *et al.*, 2005) that can reduce both the productivity of plants and endanger the safety of plant products such as foods and feeds (Zheljazkov *et al.*, 2006). Heavy metals are not biodegradable, possess long biological half-lives and have the potential to accumulate in the different body organs leading to undesirable effects (Radwan and Salama, 2006; Sathawara *et al.*, 2004). Heavy metal contamination may occur due to factors including irrigation with contaminated water, the addition of fertilizers and metal-based pesticides, industrial emissions, transportation, harvesting process, storage and/or sale (Radwan and Salama, 2006; Duran *et al.*, 2007; Tuzen and Soylak, 2007). Lead and cadmium are among the most abundant heavy metals and are particularly toxic. Consumption of excessive amount of these metals in food is associated with etiology of a number of diseases, especially with cardiovascular, kidney, nervous as well as bone diseases (Sanchez-Castillo *et al.*, 1998; Steenland and Boffetta, 2000).

Some chronic effects of lead poisoning are colic, constipation and anemia (Bolger *et al.*, 2000). Other metals such as copper and zinc are essential for important biochemical and physiological functions and necessary for maintaining good health throughout life (Radwan and Salama, 2006). Copper is necessary for body pigmentation in addition to iron, for the maintenance of a healthy central nervous system, for prevention of anemia, and is interrelated with the function of zinc and iron in the body (Goldhaber, 2003; Duran *et al.*, 2007).

Therefore the present study was conducted to determine the Vitamin C and minerals of some local leafy vegetables namely; Pumpkin leaves, Jute leaves, Bitter leaf, Cubeb leaves, Bush buck leaves, Clove basil leaves, Curry leaf, Water leaf, African Eggplant and Moringa leaves.

Materials and methods

Plant material collection and identification

The plant material consisted of ten vegetables from *Telfairia occidentalis*, *Corchorus olitorius*, *Vernonia amygdalina*, *Piper guineense*, *Gongronema latifolium*, *Ocimum gratissimum*, *Murraya koenigii*, *Talinum triangulare*, *Solanum aethiopicum* and *Moringa oleifera* known locally as Pumpkin leaves, Jute leaves, Bitter leaf, Cubeb leaves, Bush buck leaves, Clove basil leaves, Curry leaf, Water leaf, African Eggplant and Moringa leaves respectively.

All the ten investigated vegetables were obtained from the market of Farin-gada, Jos North Local Government and identified by a plant taxonomist at the herbarium unit of the Federal college of forestry, Jos. The samples were washed then used fresh or dry according to the type of analysis.

Determination of Vitamin Content

Preparation of extract: The blended sample (10 g) was homogenized with about 50 ml of 5 % metaphosphoric acid-10 % acetic acid solution. It was then quantitatively transferred into a 100 ml volumetric flask and shaken gently until a homogeneous dispersion was obtained.

The homogenate was diluted up to the mark with the 5 % metaphosphoric acid-10 % acetic acid solution. The solution was then filtered and the clear filtrate collected for the determination of vitamin C in that sample.

Procedure: Bromine water was added to the filtered sample solution to oxidize the ascorbic acid to dehydroascorbic acid. Then a few drops of thiourea were added to it to remove the excess bromine and thus the clear solution was obtained.

Standard solutions of ascorbic acid (0.005 mg/ml, 0.010 mg/ml, 0.015 mg/ml, 0.020 mg/ml and 0.025 mg/ml) were prepared from 0.5 mg/ml stock solution of ascorbic acid by proper dilution. Then 1 ml of 2,4-Dinitrophenylhydrazine solution was added thoroughly with all standards and also with the oxidized ascorbic acid. For the completion of the reaction, all the standards, samples and blank solution were kept at 37°C temperature for 3 hours in a water bath (thermostatic).

After this incubation all of those were cooled in an ice bath and treated with 5 ml of 85 % H_2SO_4 with constant stirring. As a result, a colored solution was obtained whose absorbance was taken at 521 nm using the UV- spectrophotometer.

A cuvette containing the prepared blank solution was used to calibrate the spectrophotometer to the point zero. Samples of the extract were placed into the cuvette and readings were taken when the figure in the display window became steady. The spectrophotometer was blanked each time with the prepared blank solution before the readings were taken. The operation was repeated three times for each sample and the average readings were recorded. The absorbance obtained was extrapolated on a vitamin C standard curve. Each value of the vitamin C obtained from the standard curve was put into the equation below to determine the final concentration.

$$C = [(A \times 1000)/m(V_1/V_2) \times 1000] \times 50$$

Where

C= concentration of the vitamin C in the sample.

A = concentrations extrapolated from the standard curve.

m = mass of the sample taken

V_2=volume of the extract taken after the filtration (10 ml).

V_1=volume of acid used to extract the vitamin C before the filtration (50 ml).

Mineral analysis

The minerals in the leafy vegetables were analyzed from solution obtained when 5 g of the samples were digested with 10 ml of 5N concentrated hydrochloride. The mixtures were placed on a water bath and evaporated almost to dryness. The solution was cooled and filtered into 100 ml standard flask and diluted to volume with distilled water. Atomic absorption spectrophotometer was used to analyze the minerals separately after acid digestion of the sample.

Determination of Calcium (Ca) Content

About 1 ml of the sample was pipette into a test tube in duplicate. Then 3 ml of calcium working reagent was added and absorbance at 512 nm was read against the blank.

Determination of Potassium (K) Content

About 5 ml of the sample was pipette into a test tube in duplicate. Then 2 ml of cobalt nitrite was added, shaken vigorously and allowed to stand for 45 minutes and centrifuged for 15 minutes. The supernatant was drained-off and 2 ml of ethanol was added to the residue. The solution was shaken vigorously and centrifuged for another 15 minutes. The supernatant was drained off and 2 ml of distilled water was added to the residue. The solution was boiled for 10 minutes with frequent shaking to dissolve the precipitate. About 1ml of 1% choline hydrochloride and 1ml of 2% sodium ferric cyanide was added. Then 2ml of distilled water was also added and the solution was shaken to mix well. The absorbance was taken at 620 nm against the blank.

Determination of Magnesium (Mg) Content

About 5ml of the sample was pipette into a test tube in duplicate. Then 1 ml of 0.67 N sulphuric acid (H_2SO_4) was added and 1ml of 0.05 % titan yellow was also added.

Then 1 ml of 0.01 % gum acacia was added and 2 ml of 10 % sodium hydroxide (NaOH) were also added. The solution was mixed and the absorbance was taken at 520nm against the blank.

Determination of Sodium (Na) Content

About 5 ml of the sample were pipette into a test tube in duplicate and 0.1 ml of concentrated hydrochloric acid (HCl) was added and boiled for 1 hour in a boiling water bath. Then 2 ml of distilled water were also added and the solution was shaken to mix well. The absorbance was taken at 520 nm against the blank.

Determination of Phosphorus (P)

About 5 ml of the sample were pipette into a test tube in duplicate and 0.25 ml of concentrated sulphuric acid (H_2SO_4) was added and boiled for 1 hour in a boiling water bath. A spatula tip filled with sodium periodate was added and heated for another 10 minutes, collected and the absorbance was taken at 520 nm against the blanks.

Analysis of heavy metals

Heavy metals like Lead, Cadmium, Nickel, Copper and Zinc analyses were done for the ten samples. Heavy metals analysis was carried out on dried samples and BUSK Scientific 205 model Atomic spectrophotometer used to measure readings.

Calibration curve

After determination of the λ_{max} of the colored complex (521 nm) using a UV- spectrophotometer the absorbance of the all standards (converted to colored complex) were taken to construct a calibration curve. The calibration curve was constructed by plotting the concentration versus the corresponding absorbance. Molar absorptivity was found 0.0328 L mol^{-1} cm^{-1} using Beer - Lambert plots.

Statistical analysis

The data were statistically analysed and were expressed in mean ± Standard deviation. Values of $p < 0.05$ were considered as significant.

Results and discussion

The results of the Vitamin C (ascorbic acid) composition of the analyzed vegetables are shown in Table 1. The vitamin C content of the ten fresh leafy vegetables ranged from 5.7 ± 0.83 mg/100ml in *Moringa oleifera* to 815.00 ± 0.81 mg/100ml in *Murraya koenigii* (Curry Leaf).

Table 1. Concentration of ascorbic acid in the leafy vegetables.

SNo	Vegetables	Amount of Ascorbic Acid (mg/100ml)
1	*Telfairia occidentalis* (Pumpkin Leaves)	137.82 ± 3.11
2	*Corchorus olitorius* L. (Jute Leaf)	153.63 ± 1.22
3	*Vernonia amygdalina* (Bitter Leaf)	202.40 ± 5.08
4	*Piper guineense* (Cubeb Leaf)	512.18 ± 0.20
5	*Gongronema latifolium* (Bush Buck Leaf)	299.20 ± 0.51
6	*Ocimum gratissimum* (Clove Basil Leaf)	62.75 ± 6.75
7	*Murraya koenigii* (Curry Leaf)	815.00 ± 0.81
8	*Talinum triangulare* (Waterleaf)	116.35 ± 1.08
9	*Solanum aethiopicum* (African Eggplant)	406.13 ± 1.41
10	*Moringa oleifera* (Moringa leaves)	5.7 ± 0.83

Values are means ± standard deviation of triplicate determinations.

Previous research in south east Nigeria on leaf vegetables estimated that vitamin C composition varied from 0.08 to 3.18 mg/100ml (Achikanu *et al.*, 2013).This great difference could be attributed to climate, soil and season of collection differences. Vitamin C is a potent antioxidant that facilitates the transport and uptake of non-heme iron at the mucosa, the reduction of folic acid intermediates and the synthesis of cortisol. Its deficiency includes fragility to blood capillaries gum decay, scurvy (Bender, 2009). The United States National Academy of Sciences recommends 60 – 95 milligrams per day and no more than 2 grams (2,000 milligrams) per day (USNAS, 2013). All the vegetables fall within this and are good sources of vitamin C except for *Moringa oleifera* (5.7 ± 0.83 mg/100ml). Vitamin C content in *Moringa oleifera* as seen in other research works shows that the value obtained is remarkably low.

Factors responsible for this include climate, soil type, time of plant collection, storage condition of the fresh leaves prior to experiment.

Table 2. Elemental concentration in the leafy vegetables.

SNo	Vegetables	Calcium (mg/100ml)	Potassium (mg/100ml)	Magnesium (mg/100ml)	Sodium (mg/100ml)	Phosphorus (mg/100ml)
1	*Telfairia occidentalis* (Pumpkin Leaves)	27.48 ± 0.04	2760.05 ± 0.02	76.46 ± 0.02	47.81 ± 1.23	13.02 ± 0.08
2	*Corchorus olitorius* L. (Jute Leaf)	30.55 ± 0.05	2814.15 ± 8.08	76.69 ± 0.13	54.56 ± 0.42	6.68 ± 0.02
3	*Vernonia amygdalina* (Bitter Leaf)	151.60 ± 1.40	61.50 ± 0.38	96.50 ± 0.96	57.50 ± 0.34	61.55 ± 0.60
4	*Piper guineense* (Cubeb Leaf)	179.52 ± 0.11	98.52 ± 0.10	35.54 ± 0.36	20.87 ± 0.04	217.70 ± 0.41
5	*Gongronema latifolium* (Bush Buck Leaf)	11.50 ± 5.24	33.20 ± 4.72	54.00 ±1.83	11.00 ± 0.25	12.50 ±1.09
6	*Ocimum gratissimum*, (Clove Basil Leaf)	51.44 ± 2.64	47.01 ± 1.76	31.84 ± 0.84	39.38 ± 3.71	68.31 ± 1.18
7	*Murraya koenigii* (Curry Leaf)	830.00 ± 4.72	811.00 ± 2.81	216.0 ± 3.19	370.0 ± 1.83	600.00 ± 2.16
8	*Talinumtriangulare* (Waterleaf)	71.03 ± 1.92	82.11 ± 1.38	87.41 ± 0.95	83.68 ± 1.49	184.86 ± 0.84
9	*Solanum aethiopicum* (African Eggplant)	498.47 ± 2.14	2.26 ± 0.55	29.46 ± 0.40	0.21 ± 0.00	35.50 ± 3.30
10	*Moringa oleifera*(Moringa leaves)	728.00 ± 0.04	23.20 ± 0.31	677.0 ± 0.16	214.00 ± 0.02	5.00 ± 0.12

Values are means ± standard deviation of triplicate determinations.

It is pertinent to relate the vitamin C content of these vegetables samples to the claimed curative uses in herbal medicine. Several of these samples are claimed to be useful in the treatment of sores, cuts, wounds, abscesses, malaria, and skin diseases and cold (Odugbemi, 2008). Vitamin C has been reported to be protective and therapeutic in cardiovascular diseases (Kurl *et al.*, 2002) and some of these plants are claimed to be useful as diuretic or antihypertensive (Odugbemi, 2008).

Several of these vegetables have high vitamin C content. Raised blood pressure is a major cause of stroke and studies have shown that high intake of fruits and vegetables are associated with reduced risk of ischemic stroke (Johnson *et al.*, 2003).

Table 3. Heavy elements concentration in some vegetables.

Vegetables	Lead (ppm)	Cadmium (ppm)	Nickel (ppm)	Copper (ppm)	Zinc (ppm)
Telfaria occidentalis (Pumkin leaf)	N.D	N.D	137.5	N.D	N.D
Corchorusolitorius L. (Jute leaf)	N.D	N.D	118.0	N.D	N.D
Piper guineense (Cubeb leaf)	N.D	N.D	N.D	N.D	N.D
Vernonia amygdalina (Bitter leaf)	N.D	0.021	59.0	N.D	N.D
GongronemaLatifolium (Bush buck leaf)	N.D	0.019	78.5	125.0	N.D
Occimumgratissimum (Clove Basil leaf)	N.D	N.D	177.0	N.D	N.D
Murrayakoenigii (Curry leaf)	N.D	0.021	78.5	N.D	N.D
Talinumtriangulare (Water leaf)	N.D	N.D	N.D	N.D	N.D
Solanumaethiopicum (African Eggplant leaf)	N.D	N.D	88.5	N.D	N.D
Moringaoleifera (Moringa leaves)	N.D	N.D	118.0	N.D	N.D

N.D "Denotes not detectable".

Vitamin C has also been reported to be protective and therapeutic in cancer (Padayatty *et al.*, 2003). *Telfairia occidentalis* (Pumpkin Leaves) (137.82 ± 3.11 mg/100ml) is claimed to be useful in the herbal treatment of cancer and is rich in ascorbic acid. Vitamin C is also reported to be protective and therapeutic in eye diseases (Padayatty *et al.*, 2003).

Table 2 shows some elemental composition including calcium, potassium, magnesium, sodium and phosphorus of the analyzed vegetables. Among these vegetables, calcium content ranged from 830.00 ± 4.72 mg/100ml in *Murraya koenigii* (Curry Leaf) to 11.50 ± 5.24 mg/100ml in *Gongronema latifolium* (Bush Buck Leaf). Potassium content ranged from 2814.15 ± 8.08 mg/100ml in *Corchorus olitorius* L. (Jute Leaf) to 2.26 ± 0.55 mg/100ml in *Solanum aethiopicum* (African Eggplant). Magnesium content ranged from 677.0 ± 0.16 mg/100ml in *Moringa oleifera* to 29.46 ± 0.40 mg/100ml in *Solanum aethiopicum* (African Eggplant). Sodium content ranged from 370.0 ± 1.83 mg/100ml in *Murraya koenigii* (Curry Leaf) to 0.21 ± 0.00 mg/100ml in *Solanum aethiopicum* (African Eggplant).

Table 4. Estimated daily mineral intakes of humans according to Milton in 2003.

SNo	Elements	Recommended Daily Intake (mg/day)
1	Calcium	800
2	Potassium	1600-2000
3	Magnesium	350
4	Sodium	500
5	Phosphorus	800

Phosphorus content ranged from 600.00 ± 2.16 mg/100ml in *Murraya koenigii* (Curry Leaf) to 5.00 ± 0.12 mg/100ml in *Moringa oleifera*. Calcium and Phosphurus are the most represented minerals. Phosphorus is a mineral that takes 1% of a total body weight (El-Bassel and El-Gazzar, 2019).

Table 3 shows the vegetables that contain heavy metals. Cadmium was detected in Bush buck leaf (0.019 mg/kg) while Bitter leaf and curry leaf contain the same amount (0.021 mg/kg). Copper was detected in Moringa leaf (75 mg/kg) and Bush buck leaf (125 mg/kg) only. Nickel was detected in all the vegetable samples analyzed except Water leaf.

The lowest concentration of Nickel is 59.0 mg/kg in Bitter leaf and the highest concentration of 196.5 mg/kg in Cubeb leaf. Lead and Zinc were not detected in all the ten local leafy vegetables analyzed.

Among all the samples analyzed, only Water leaf was found not to contain any of the heavy metals assayed.

These findings are similar to those of the team of Sobukola where the levels of Lead, Cadmium, Copper, Zinc, Cobalt and Nickel for the leafy vegetables were low (Sobukola *et al.*, 2010). However, Achikanu and coworkers (2013) revealed that Moringa leaves and African eggplant leaves contain more than 60 mg/ 100 ml of Lead. The results obtained show that most of the vegetables analyzed fell within safe range. Table 4 shows the daily requirements of each element estimated by Milton in 2003. From this table, the vegetables have to be taken more than 100g a day to meet the daily requirements for calcium, potassium and sodium. Although, 100g of *Moringa oleifera* (677.0 ± 0.16 mg/100ml) can deliver the daily requirements for magnesium while 100g of *Murraya koenigii* (Curry Leaf) (600.00 ± 2.16 mg/100ml) can deliver the daily requirements for phosphorus.

Conclusion

The present study has shown that the Nigerian vegetables examined have an appreciable content of vitamin C. The vegetables also contained good minerals with calcium and potassium being most abundant while sodium was least. The results suggest that the vegetables if consumed in sufficient amount would contribute greatly towards meeting human nutritional requirement for normal growth and adequate protection against diseases arising from malnutrition.

References

Achikanu CE, Eze-Steven PE, Ude CM, Ug OC. 2013. Determination of the vitamin and mineral composition of common leafy vegetables in south eastern Nigeria. International Journal of Current Microbiology and Applied Sciences **2(11),** 347-353.

Bender DA. 2009. Nutritional biochemistry of vitamins. 2nd edition. Cambridge University Press. https://doi.org/10.1017/CBO9780511615191

Bolger M, Carrington C, Larsen JC, Petersen B. 2000. Safety evaluation of certain food additives and contaminants. Lead. WHO Food Additive Series **44,** 212–273.

Choi CJ, Kanthasamy A, Anantharam V, Kanthasamy AG. 2007. Interaction of metals with prion protein: possible role of divalent cations in the pathogenesis of prion diseases. Neurotoxicology **27,** 777-87.

Duran A, Tuzen M, Soylak M. 2007. Trace element levels in some dried fruit samples from Turkey. International Journal of Food Science and Nutrition **59,** 581–589.

El-Bassel HA, El-Gazzar HH. 2019. Comparable study between organic and nonorganic vegetables in their contents of some nutritive components. Journal of Medicine in Scientific Research **2,** 204-8.

Fasuyi AO. 2006. Nutritional potentials of some tropical vegetables meals. Chemical characterization and functional properties. African Journal of Biotechnology **5(1),** 49-53.

Fenell D. 2004. Determinants of Supplement Usage. Preventive Medicine **5**, 932-939.

Goldhaber SB. 2003. Trace element risk assessment: essentially vs. toxicity. Regulatory Toxicology and Pharmacology **38,** 232–242.

Ihekoronye AI, Ngoddy PO. 1985. Integrated Food Science and Technology for the Tropics. Macmillan Publishers, New York, 296-301.

Johnson LJ, Susan M, Laura JK. 2003. The Antioxidants-Vitamin C, Vitamin E, Selenium, and Carotenoids. Journal of Agromedicine **9(1),** 65-82.

Kaviarasan S, Ramamurty N, Gunasekaran P, Varalakshmi E, Anuradha CV. 2007. Fenugreek (Trigonellafoenumgraecum) seed extract prevents ethanol-induced toxicity and apoptosis in Chang liver cells. Alcohol & Alcoholism **41(3),** 267-73.

Kurl S, Tuomainen TP, Laukkanen JA. 2002. Plasma vitamin C modifies the association between hypertension and risk of stroke. Stroke **33,** 1568–73.

Mepha HD, Eboh L, Banigo DEB. 2007. Effects of processing treatments on the nutritive composition and consumer acceptance of some Nigeria edible leafy vegetables. African Journal of food Agriculture, Nutrition and Development **7(1)**, 1-18.

Milton K. 2003. Micronutrient intakes of wild primates: are humans different. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology **136(1),** 47-59.

Mohammed MI, Sharif N. 2011. Mineral composition of some leafy vegetables consumed in Kano, Nigeria. Nigerian Journal of Basic and Applied Sciences **19(2)**, 208-211.

Odugbemi T. 2008. A Textbook of Medicinal Plants from Nigeria. University of Lagos Press, Lagos, 542.

Ononugbu IC. 2002. Lipids in Human Existence. 1st Edition. Express Publishing Company, Nsukka, Nigeria, 115.

Padayatty SJ, Katz A, Wang Y, Eck P, Kwon O, Lee JH, Chen S, Corpe C, Dutta A, Dutta SK, Levine M. 2003. "Vitamin C as an antioxidant: evaluation of its role in disease prevention". Journal of the American College of Nutrition **22(1),** 18–35.

Radwan MA, Salama AK. 2006. Market basket survey for some heavymetals in Egyptian fruits and vegetables. Food and Chemical Toxicology **44,** 1 273-1 278.

Rekha C, Poornima G, Manasa M, Abhipsa V, Pavithradevi J, Vijay kumar HT, Prashith KTR. 2012. Ascorbic Acid, Total Phenol Content and Antioxidant Activity of Fresh Juices of Four Ripe and Unripe Citrus Fruits.Chemical Science Transactions **1(2),** 303-310.

Sanchez-Castillo CP, Dewey PJS, Aguirre A, Lara JS, Vaca R, de la Barra PL. 1998. The mineral content of Mexican fruits and vegetables. Journal of Food Composition and Analysis **11,** 340–356.

Sathawara NG, Parikh DJ, Agarwal YK. 2004. Essential heavy metals in environmental samples from western India. Bulletin of Environmental Contamination and Toxicology **73,** 756–761.

Sobukola OP, Dairo OU, Odunewu AV, Fafiolu BO. 2007. Thin layer drying process of some leafy vegetables under open sun. Journal of Food Science and Technology **13(1),** 35-40.

Sobukola OP, Adeniran OM, Odedairo AA, Kaj OE. 2010. Heavy metal levels of some fruits and leafy vegetablesfrom selected markets in Lagos, Nigeria. African Journal of Food Science **4(2),** 389 – 393.

Steenland K, Boffetta P. 2000. Lead and cancer in humans: where are we now? American Journal of Industrial Medicine **38,** 295–299.

Tuzen M, Soylak M. 2007. Evaluation of trace element contents in canned foods marketed from Turkey. Food Chemistry **102,** 1089–1095.

United States National Academy of Sciences (USNAS). 2013. http://nasonline.org/pub/2013/rda.

Yen JL, Su NY, Kaiser P. 2005. The yeast ubiquitin ligase SCFMet30 regulates heavy metalresponse. Molecular Biology of the Cell **16(4),** 1872-82.

Zaidi MI, Asrar A, Mansoor A, Farooqui MA. 2005. The heavy metal concentrations along road side's trees of Quetta and its effects on public health. Journal of Applied Sciences **5(4),** 708-711.

Zheljazkov VD, Craker LE, Xing B. 2006. Effects of Cd, Pb, and Cu on growth and essential oil contents in dill, peppermint, and basil. Environmental and Experimental Botany **58,** 9–16.