Original Research Article

COMPARATIVE PROXIMATE, ANTIOXIDANT VITAMINS AND MINERAL COMPOSITION OF FOUR SELECTED TROPICAL NUTRITIONAL/ MEDICINAL PLANTS NAMELY: *Ocimum gratissimum*, *Piper guineense*, *Gongronema latifolium* and *Vernonia amygdalina*.

ABSTRACT

Aim: The aim of the study was to carry out a comparative analysis of the proximate, antioxidant vitamins and mineral composition of the leaves of four selected tropical nutritional/ medicinal plants namely: *Ocimum gratissimum*, *Piper guineense*, *Gongronema latifolium* and *Vernonia amygdalina*. **Methodology**: The macro and micronutrients in the plant leaves were extracted by cold maceration in ethanol and subjected to quantitative proximate, antioxidant vitamins and minerals analysis. **Results**: For all four plants, carbohydrates was the major macronutrient constituents followed by crude fats, crude proteins, ash and fiber in that other. *G. latifolium* had the highest carbohydrate and protein composition while *V. amygdalina* had the highest crude fat composition. Results of Ash analysis of the four leaves showed *P.guineense* to have the highest total mineral content followed by *V. amygdalina, O. gratissimum* and *G. latifolium* in that order. *O. gratissimum* and *P.guineense* had the highest composition of crude fiber closely followed by *G. latifolium* and *V. amygdalina*. Vitamin analysis revealed that leaves of the four vegetable plants contained high levels of vitamin C and appreciable quantities of vitamins A and E. *V. amygdalina* leaf contained the highest concentration of vitamin C and A while vitamin E was pretty much the same for the four plants. The mineral assay indicated that the leaves of the plants contain high levels of Magnesium (Mg), Phosphorus (P) and Calcium (Ca) relative to their copper (Cu), Zinc (Zn), Potassium (K) and Sodium (Na) contents. **Conclusion**: In conclusion, these plants were shown to be rich in carbohydrates, proteins and fats, vitamins and minerals justifying their use in diets. The plants were particularly reach in antioxidant vitamins and mineral justifying the therapeutic uses of various preparations of these leafy vegetables in traditional medicine, for the treatment and management of diseases that have their etiology and pathophysiology in free radical generation and oxidative stress.

Key words: Proximate, Vitamins, Minerals, Tropical nutritional plants, *Ocimum gratissimum*, *Piper guineense*, *Gongronema latifolium* and *Vernonia amygdalina*.

1. INTRODUCTION

Plants, from prehistoric times, have been used as spices, food and medicine in all cultures [1-3]. More than 70% of people in developing countries depend on plants (vegetables and fruits) for regular dietary needs [4]. It is well known that apart from energy needs, many plants and foods are ingested because of their perceived medicinal and health benefit. Indeed a significant amount of research has shown a correlation between a healthy diet and lifestyle and significant reductions in diseases and associated chronic conditions [5]. Plants are also a rich source of vitamins and minerals. Studies have identified a vast majority of antioxidant vitamins from vegetable plants like vitamins A, C and E [6]. Selective intake of foods containing these antioxidant vitamins, minerals and phytochemicals can prevent the onset of degenerative diseases like cardiovascular diseases, cancer and diabetes.

Given the plethora of vegetable plants available it becomes difficult to identify which plant should be added to our diet to address particular nutrient deficiency or ameliorate particular ailments. Moreover, some plants may contain appreciable levels of anti-nutrients rendering them unsafe for human consumption. It is with a view to establishing the relative proximate, antioxidant vitamins and mineral composition in some commonly used vegetable leafs in the southern region of Nigeria namely *Ocimum gratissimum*, *Piper guineense*, *Gongronema latifolium* and *Vernonia amygdalina*, that the current study is being carried out. The focus on the four plants stems from their common use as vegetables and spices in soups in the southern part of Nigeria. The plants have also been employed in ethnobotany for the treatment of various diseases. A comparative analysis of the proximate, antioxidant vitamins and minerals composition of the four plants will provide a bio-rational basis for the choice of the plants for addressing some nutrient deficiency. Earlier work in our laboratory had carried out a comparative analysis of the phytochemical composition of the four plants [7].

Ocimum gratissimum commonly called African basil and belonging to the family *Lamiaceae*, is a herbaceous perennial flowering plant which is woody at its base. The leaf is called scent leaf because it possesses a pleasant aroma which is responsible for its use as spice and condiments in cooking. It is widely distributed in tropical Africa and Asia, especially India. The plant is economically important for its food flavoring (as spice and condiments) [8] and essential oil which has been widely used in food industries [9,10].

Piper guineense (family *Piperaceace*) is a climbing perennial plant native to the tropical regions of Central and Western Africa. It is commonly referred to as Ashanti pepper, West African pepper or African black pepper. *Piper guineense* is economically important for its culinary uses as well as medicinal, cosmetic and insecticidal uses [11]. It is a highly spicy plant and the leaves have pungent taste and a pleasant aroma when crushed. It thus imparts “heat”, “pungency” and a spicy aroma to classic West African soups (stews). The plant oils is used as aromatics in the drink industry [12].

Gongronema latifolium, commonly called “utazi,” “aroeke” in the South Eastern and South Western parts of Nigeria respectively, belongs to the family *Asclepiadaceae*. It is primarily used as spice and vegetable for cooking and in traditional medicine [13]. A non-wood forest plant, it is native to West Africa and widely distributed elsewhere in tropical Africa and subtropical Asia.

Vernonia amygdalina, popularly called bitter leaf, belongs to the family *Asteraceae*. It is widely used in the West African sub-region for a number of medicinal and nutritional purposes [14,15]. It has also been employed as a digestive tonic and appetizer [16].

2. MATERIALS AND METHOD

2.1 Plant Materials

Mature leaf samples of *Ocimum gratissimum, Piper guineense, Gongronema latifolium and Vernonia amygdalina* were harvested from local farms in Cross River State, Nigeria.

2.2 Methods

2.2.1 Extraction Procedure

Fresh leaves of each plant were washed and air dried at room temperature ($25^{o}C$) for two weeks. The dried leaves were pulverized using a mechanical grinder. A weighed quantity, 200g, of each plant material was extracted by cold maceration in absolute ethanol for 48 hours. The extracts were double filtered, first with a white muslin cloth then with Whatman no.1 filter paper. The resulting ethanol leaf extracts were concentrated in vacuum using a rotary evaporator (at temperatures between $40^{o}C$ and $45^{o}C$) to obtain a semi–solid mass. Weighed quantities of each extract was dissolved in 5% Tween 80 solution for use in the macro and micro nutrient analysis.

2.2.2 Proximate Analysis

Proximate composition of the leaf extracts was determined using methods prescribed by the Association of Official Analytical Chemists (AOAC) [17] and the Food and Agriculture organization (FAO) [18].

2.2.3 Determination of Mineral Composition

Potassium and sodium were determined by the Flame photometric method while iron, copper, zinc, calcium and magnesium were determined by atomic absorption spectrophotometric method as described by James [19] and the Association of Official Analytical Chemists, AOAC [20]. Phosphorus was determined spectrophotometrically by the vanadomolybdate yellow method.

2.2.4 Determination of Some Antioxidant Vitamins

Vitamin A and E concentration was determined by the spectrophotometric method as described by Pearson [21]. Vitamin C was determined by the method of AOAC [22].

3. RESULTS & DISCUSSIONS

3.1 Proximate Analysis

The result of the proximate composition of the fresh leaves of the four plants is shown in Figure 1. On balance, for all four plants, carbohydrates was the major macronutrient constituents followed by crude fats, crude proteins, ash and fiber in that other. *G. latifolium* had the highest carbohydrate composition followed by *O. gratissimum*, and then *P.guineense* and finally *V. amygdalina.* Dietary carbohydrate is a major macronutrient for both humans and omnivorous animals; human adults in the Western countries obtain approximately half their daily caloric requirements from dietary carbohydrate while it is the major source of energy in other countries [23]. Carbohydrate is stored as glycogen, and although it is important for short-term energy needs, it is of very limited capacity for providing for energy needs beyond a few hours.

Fats, the second highest macro nutrient in the four plants, constitute the highest energy in humans. *V. amygdalina* had the highest crude fat composition followed by *O. gratissimum*, *G. latifolium* and *P.guineense*.

Protein is the second largest store of energy in the body after adipose tissue fat stores [24]. The result of macronutrient analysis revealed that all the four plants were a fairly rich source of protein and may be used as a protein supplement for patients with protein deficiency diseases. *G. latifolium* had the highest protein composition followed by *P.guineense*, *O. gratissimum* and *V. amygdalina* in that order.

Ash, which refers to the inorganic residue remaining after ignition or complete oxidation of organic matter in a food sample, is a measure of the total amount of minerals present within the food [25]. Results of Ash analysis of the four leaves shows *P.guineense* to have the highest total mineral content followed by *V. amygdalina, O. gratissimum* and *G. latifolium* in that order.

Crude fibre is a measure of the quantity of indigestible cellulose, pentosans, lignin and other like components in foods. Insoluble fibers can help promote bowel health and regularity. It also support insulin sensitivity and may help reduce the risk of diabetes. *O. gratissimum* and *P.guineense* had the highest composition of crude fiber closely followed by *G. latifolium* and *V. amygdalina*.

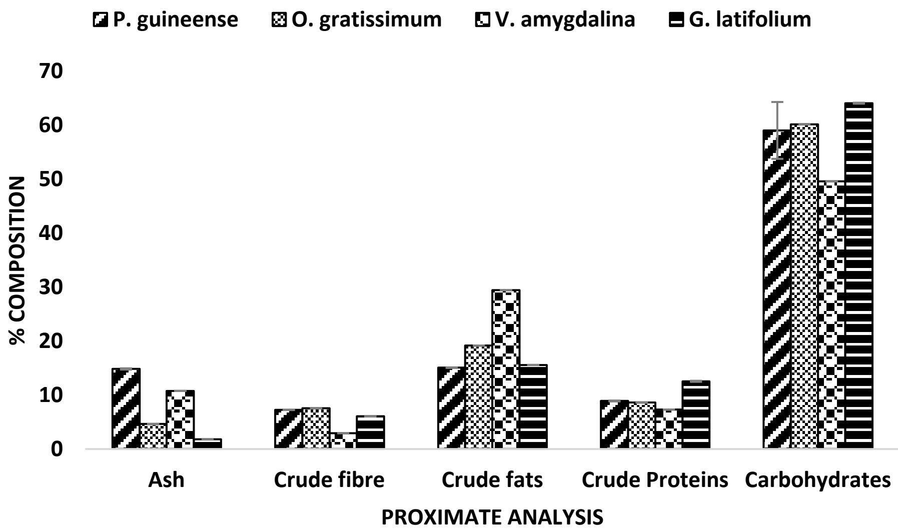

Fig 1: Proximate Analysis of crude leaf extracts of *P.guineense, O.gratissimum,* and *V. amygdalina* and *G.latifolium*. Values are expressed as mean + SEM.

3.2 Vitamins

The protective action of fruit and vegetables has been attributed to the presence of antioxidants, especially antioxidants vitamins including ascorbic acid, α-tocopherol and beta-carotene [26-28]. The results of this study (figure 2) revealed that leaves of the four vegetable plants contain appreciable concentration of vitamin C, vitamin E and beta-carotene (vitamin A). *V. amygdalina* leaf contained the highest concentration of vitamin C and vitamin A. Other reports have also shown the plant to be rich in Vitamin C and A [28,29]. The level of Vitamin E was pretty much the same for the four plants with *O.gratissimum* having a slightly higher amount. These results seem to suggest that fresh leaves of the plants are good sources of antioxidant vitamins. Vegetable leaves/ Spices provide a variety of vitamins and minerals as well as macronutrients to the diet [30]. These antioxidant vitamins appear to be partly responsible for the antioxidant properties of the leaves. Vitamin C is an antioxidant which helps to protect the body against cancer and other degenerative diseases such as arthritis and type 2 diabetes mellitus and also strengthens the immune system [31]. Vitamin C has also been shown to facilitate iron absorption by its ability to reduce inorganic ferric ion to the ferrous form [32]. This suggests that the vegetable leaves may be beneficial to people suffering from iron-deficiency anemia. Vitamin E (α-tocopherol) appears to be the most important lipid soluble antioxidant protecting membranes from lipid peroxidation by acting as a chain-breaking antioxidant [33]. It also limits the oxidation of LDL cholesterol and may help prevent or delay the development of atherosclerosis and/or coronary heart disease (CHD) [34]. This probably explains why high vitamin E intake is associated with lower rates of heart diseases. Beta-carotene is a lipid-soluble antioxidant. It is the precursor of vitamin A, so it is necessary for the production and re-synthesis of rhodopsin. High levels of beta-carotene intake have been correlated with lower risk of lung cancer, coronary heart disease, stroke and age-related eye disease [35].

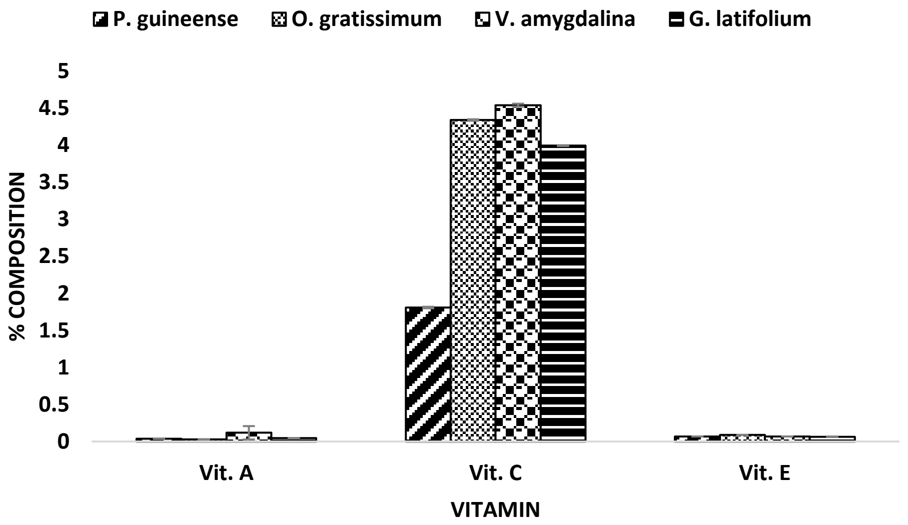

Fig 2: Quantitative Analysis of some Antioxidant Vitamins in *P.guineense, O.gratissimum,* and *V. amygdalina* and *G.latifolium*. Values are expressed as mean ± SEM.

3.3 Minerals

The results of the quantitative analysis of mineral elements (Figure 3) indicate that the leaves of the plants contain high levels of Magnesium (Mg), Phosphorus (P) and Calcium (Ca) relative to their copper (Cu), Zinc (Zn), Potassium (K) and Sodium (Na) contents. In addition to the numerous biological roles these minerals play, they also serve as co-factor in certain biochemical reactions including those involving antioxidant enzymes. Magnesium serves as a co-factor for the enzyme catalase, a primary antioxidant that detoxifies hydrogen peroxide by dismutation to water and oxygen. Similarly Copper and Zinc, are vital co-factor of the different forms of SOD found in plants and animals [36]. Superoxide dismutase (SOD) is a primary antioxidant enzyme that catalyses the dismutation or disproportion of superoxide anion radicals (O_2-) to hydrogen peroxide and molecular oxygen [37]. It is therefore suggested that these minerals contribute to the antioxidant properties of the plants probably by boosting the levels of antioxidant enzymes such as SOD and catalase.

Phosphorus: Except for *P.guineense*, Phosphorus (P) was the major constituents of the mineral elements assayed. *O.gratissimum* had the highest phosphorus content closely followed by *V. amygdalina* and *G.latifolium* in that order. Phosphorus is an ubiquitous mineral in the human body and has diverse functions ranging from the transfer of genetic information to energy utilization [38]. It forms the backbone of DNA and RNA, it is an essential component of phospholipids that form all membrane bilayers and is an integral component of the body’s key energy source, adenosine triphosphate (ATP). Phosphorus also plays a vital role in the dissociation of oxygen from hemoglobin, it is the main intracellular buffer and therefore is essential for pH regulation of the human body and is a key component of the second messenger molecules such as cyclic adenosine monophosphate (cAMP), cyclic guanine monophosphate (cGMP) and inositol polyphosphates. Taken together with the equally high level of carbohydrates, the four plants are a very good source of energy.

Magnesium (Mg): Comparatively *G.latifolium* had the highest Mg content with *O.gratissimum* having the lowest. *G.latifolium* is thus the plant of choice to address Mg deficiency. Mg plays an essential role in a wide range of fundamental biological reactions. Apart from its cofactor role, it is involved in bone mineralization, the building of proteins, muscle contraction, nerve transmission and immune system health [4,39]

Calcium (Ca) is the most tightly regulated ion in the extracellular fluid (ECF). In higher mammals, the most obvious role of calcium is structural or mechanical being responsible for the mass, hardness, and strength of the bones and teeth [40]. Calcium is also involved in cell movement and muscle contraction to nerve transmission, glandular secretion, and even cell division where it acts as both a signal transmitter from the outside of the cell to the inside and as an activator or stabilizer of the functional proteins involved. Calcium also plays a role in the regulatory activities of parathyroid hormone [PTH], calcitonin [CT], and a key activity of vitamin D. Ca was more predominant in *V. amygdalina* followed by *G.latifolium*, *O.gratissimum* and *P.guineense* in that order.

Copper is a constituent of many enzymes including superoxide dismutase. It is also required for iron metabolism [4,41]. It was more prevalent in *P.guineense.*

Zinc plays a catalytic, structural, and regulatory role in the body [42]. Zinc is essential for general growth and proper development of the reproductive organs and for normal functioning of the prostate gland. Apart from SOD, Zinc is a co-factor of over 300 enzymes including carbonic anhydrase, which is crucial to maintenance of acid-base balance in the blood, and alcohol dehydrogenase that break down alcohol. It is also a component of insulin and plays a role in its processing, storage, secretion and action [43]. The Zinc content of *P. guineense* may be responsible for the observed stimulated sexual behaviors of mature male rats fed with extract of *P. guineense* [44]. The level of the mineral was pretty much the same in *V. amygdalina*, *G.latifolium* and *P. guineense*. *O.gratissimum* had the lowest level of the mineral.

Sodium (Na) and potassium (K) (and chloride ions Cl^-) are the major electrolytes located in all body fluids. While sodium is extracellular, pottassium is intracellular. They are responsible for the maintenance of acid/base balance, nerve transmission and muscle contraction and regulation of fluid movement in and out of cells [45]. *P. guineense* had the highest amount of potassium while *O.gratissimum* had the highest level of sodium.

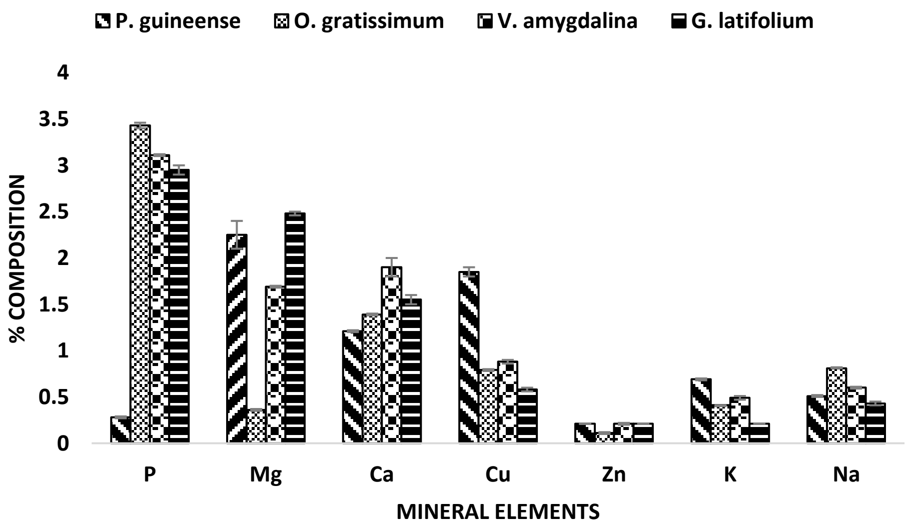

Fig 3: Quantitative Analysis of some Minerals in *P.guineense, O.gratissimum, V. amygdalina* and *G.latifolium*. Values are expressed as mean ± SEM.

4. Summary and Conclusion

In summary, the four plants, *P.guineense, O.gratissimum, V. amygdalina* and *G.latifolium*, have been shown to be rich in carbohydrates, proteins and fats, vitamins and minerals justifying their use in diets. The plants are particularly reach in antioxidant vitamins and mineral. Taken together with our earlier work on the comparative phytochemical analysis of these plants [7], the findings have good correlation with the therapeutic uses of the various preparations of these leafy vegetables in traditional medicine for the treatment and management of diseases that have their etiology and pathophysiology in free radical generation and oxidative stress like diabetes, arthritis, rheumatism, eye problems and infectious diseases such as AIDS. Increased consumption of the leaves of these plants is therefore recommended (especially as they have been shown to contain low levels of anti-nutrients [7]) for optimized health and wellness, and to boost the endogenous antioxidant system and in so doing, help prevent the development of certain free radical related diseases.

COMPETING INTERESTS

The Authors declare that no competing interests exist.

References

1. Farnsworth NR, Akerele O, Bingel AS, Soejarto DD, Guo Z. World Health Organ. 1985; 63: 965.
2. Bandaranayake, WM. Quality Control, Screening, Toxicity, and Regulation of Herbal Drugs. In: Ahmad I, Aqil F, and Owais M, editors. Modern Phytomedicine. Turning Medicinal Plants into Drugs. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim; 2006; pp25-57.
3. Odugbemi TA. A textbook of Medicinal Plants from Nigeria. Lagos: University of Lagos Press; 2008.
4. Nahak G, Suar M, Sahu RK. Antioxidant Potential and Nutritional values of vegetables: A Review. Research Journal of Medicinal Plant. 2014; 8(2): 50-81.
5. Poe K. Plant-Based diets and phytonutrients: Potential health benefits and disease prevention. Arch Med. 2017; 9(6): 7.
6. Godber JS. Nutrient bioavailability in humans and experimental animals. J Food quality. 1990; 13: 21-36.
7. Mgbeje BIA, Umoh EU and Emmanuel-Ikpeme C. Comparative Analysis of Phytochemical Composition of Four Selected Tropical Medicinal Plants Namely: *Ocimum gratissimum, Piper guineense, Gongronema latifolium* and *Vernonia amygdalina.* Journal of Complementary and Alternative Medical Research. 2019: 7(3): 1-11.
8. Akinmoladun AC, Ibukun EO, Afor ED, Obuotor EM & Farombi, EO. Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum*. Science Research Essay. 2007; 2(5): 163-166.
9. Lachowicz KJ, Jones GP, Briggs DR, Bienuenu FE, Palmer MV, Tings SST & Hunter MO. Characteristics of essential oils from basil (*Ocimum basillicum*) grown in Australia. J. Agric. Food Chem. 1996; 144: 877-881.
10. Machale KW, Niranjan KU & Pangarkar VG. Recovery of dissolved essential oils form condensate waters of basil and *Menthe arvensis* distillation. J. Chem. Tech. Biotech. 1997; 69: 362-366.

11. Okwute SK. Plants derived pesticidal and antimicrobial agents for use in Agriculture. A review of phytochemical and biological studies on some Nigeria plants. J of Agric. Science and Technology. 1992; 2(1): 62-70.
12. Rehm SS & Espig GT. The cultivated plants of the tropics and subtropics cultivation, economic value, utilization. Germany: Verlay Josef Margraf; 1991, 552 pps. ISBN 3-8236-1169-0.
13. Ugochuwku NH, Babady NF, Cobourne MN & Gasset SR. The effect of *Gongronema latifolim* extracts on serum lipid profile and oxidative stress in hepatocytes of diabetic rats. J Biosci. 2003; 28(1): 1-5.
14. Igile GO, Fafunsho M, Fasanmade A, Burda S, Jurzysta M, & Oleszek W. Toxicity of *Vernonia amygdalina* leaves, extracts and purified saponins in mice. Proc. Eurp. Food Tox. 1994; 2: 394-399.
15. Okafor JC. Conservation and use of traditional vegetable from woody forest species in south eastern Nigeria. Fame Agriculture Centre, Enugu, Nigeria; 2005. Pp 55-59. Available online at http://www.biodiversityinternational.org.
16. Iwu MM. Handbook of African medicinal plants. 1st ed. Florida: CRC Press; 1993; pp. 221-222.
17. AOAC. *Official Methods of Analysis* 14th ed. Association of Official Analytical Chemists, Washington DC; 1984.
18. FAO. Manuals of food quality control. Food analysis: general techniques, additives, contaminants and composition. FAO Food and Nutrition Paper. 1986; 14(7): 203-232.
19. James CS. *Analytical Chemistry of Food*. New York: Chapman Hall; 1995. Available at http://dx.doi.org/10.1007/978-1-4615-2165-5.
20. AOAC. Official methods of analysis. 15th ed. Association of Official Analytical Chemists Washington D.C; 1990 http://dx.doi.org/10.3923/pjn.2009.1204.1208.
21. Pearson, D. Chemical analysis of food. 7th ed. Edinburgh, New York: Churchill Livingstone; 1976, Pp. 7-9.
22. AOAC. Official Methods of Analysis. 13th ed. Association of Official Analytical Chemists, Washington D.C; 1980.
23. Keim NL, Levin RJ, and Havel PJ. Carbohydrates. In: Ross AC, Caballero B, Cousins RJ, Tucker KL, Ziegler TR, editors. Modern Nutrition in health and disease. 11th edition. Philadelphia: Lippincott Williams & Wilkins; 2014; pp 36-57.
24. Cahill GF. Starvation in man. N Engl J Med. 1970; 282:668–75.
25. Ismail BP. Ash content determination. In: Nielsen S, ed. Food Analysis Laboratory Manual. Food science Text series. Cham: Springer; 2017; Pp 117-119.
26. Cao GH, Sofic EE & Prior RL. Antioxidant capacity of tea and common vegetable. Journal of Agriculture and Food Chemistry. 1996; 44: 3426-3430.
27. Grivetti LE & Ogle BM. Value of traditional foods in meeting macro-and micro nutrients needs: the wild plant connection. Nutrition Research and Reviews. 2000; 13: 31-46.
28. Odukoya OA, Inya-Agha SI, Segun FI, Sofidiya MO & Ilori OO. (2007). Antioxidant activity of selected Nigeria green leafy vegetables. American Journal of food Technology. 2007; 2(3): 169-175.
29. Palaniswany UR, Caporuscio CX & Stuarts JD. A Chemical analysis of antioxidant Vitamins in fresh curry leaf (*Murraya Koenigil*) by reversed phase HPLC with UV detection. Acta Horticulture (ISHS). 2003; 620: 475-478. Available online at http://www.actahort.Org/books/620/62057.htm.
30. Hiza H. Availability of Spices on the rise in the U.S. food supply. US Department of Agriculture, Centre for Nutrition Policy and Promotion. Nutrition insight. 2008; 39.
31. Mensah JK, Okoli RI, Ohaju-Obodo JO & Eifediyi KB. Phytochemical, nutritional and medicinal properties of some leafy vegetable consumed by Ede people of Nigeria. African Journal of Biotechnology. 2008; 7(14): 2304-2309. Available online at http://www.academicjournals.org/AJB.
32. Charttejea MN & Shinde RQ. Textbook of medical Biochemistry. 6th Ed. New Delhi: Jaypee Brothers; 2005; Pp. 124 -132.

33. Traber MG & Atkinson JC. Vitamin E, antioxidants and nothing more. Free Ecological Biology and Medicine. 2007; 43(1): 4-15.
34. Jialal IW & Fuller CJ. Effect of vitamin E, Selenium and beta-Carotene on LDL oxidation and atherosclerosis. Canadian Journal of Cardiolog. 1995; 11: 97G-103G.
35. Johnson EJ. Aging and carotene nutritive. Age. 1993; 16: 59-66.
36. Ahmad, S. Oxidative Stress and antioxidant defenses in Biology. New York: Chapman and hall; 1995; Pp. 1-18, 238-258.
37. Zelko IB, Mariani TF & Folz RM. Superoxide dismutase multigene family: a comparison of the CU/Zn-SOD (SODI), Mn-SOD, (SOD2) and EC-SOD (SOD3) gene structures, evolution and expression. Free Radical Biology and Medicine. 2002; 33(3): 337-349.
38. O'Brien KO, Kerstetter JE, Insogna KL. Phosphorus. In: Ross AC, Caballero B, Cousins RJ, Tucker KL, Ziegler TR, editors. Modern Nutrition in health and disease. 11th edition. Philadelphia: Lippincott Williams & Wilkins; 2014; pp 150-158.
39. Rude RK. Magnesium. In: Ross AC, Caballero B, Cousins RJ, Tucker KL, Ziegler TR, editors. Modern Nutrition in health and disease. 11th edition. Philadelphia: Lippincott Williams & Wilkins; 2014; pp 159-175.
40. Weaver CM & Heany RP. Calcium. In: Ross AC, Caballero B, Cousins RJ, Tucker KL, Ziegler TR, editors. Modern Nutrition in health and disease. 11th edition. Philadelphia: Lippincott Williams & Wilkins; 2014; pp 133-149.
41. Collins JF. Copper. In: Ross AC, Caballero B, Cousins RJ, Tucker KL, Ziegler TR, editors. Modern Nutrition in health and disease. 11th edition. Philadelphia: Lippincott Williams & Wilkins; 2014; pp 206-216.
42. King JC and Cousins RJ. Zinc. In: Ross AC, Caballero B, Cousins RJ, Tucker KL, Ziegler TR, editors. Modern Nutrition in health and disease. 11th edition. Philadelphia: Lippincott Williams & Wilkins; 2014; pp 189-205.
43. Li YV. Zinc and Insulin in pancreatic beta-cells. Endocrine. 2014; 45(2): 178-189.
44. Kamtchouing P, Mbongue GYF, Dimo T, Watcho P, Jatsa HB & Sokeng SD. Effect of *Aframomum melegueta* and *Piper guineense* on sexual behavior of mate rats. Behavioural Pharmacology. 2002; 13: 243-247.
45. Vasudevan DM and Sreekumari S. Textbook of Biochemistry for Medical Students. 5th Ed. New Delhi: JP Medical Ltd; 2007; pp. 373-378.