Original Research Article

COMPARATIVE PROXIMATE, VITAMIN AND MINERAL COMPOSITION OF LEAVES OF FOUR SELECTED TROPICAL VEGETABLE 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, vitamin and mineral composition of the leaves of four selected tropical vegetable plants namely: *Ocimum gratissimum*, *Piper guineense*, *Gongronema latifolium* and *Vernonia amygdalina*. **Methodology**: Fresh leaves of each vegetable were washed and air dried at room temperature for two weeks. The dried leaves were pulverized using a mechanical grinder. Measured amounts were subjected to quantitative proximate, vitamin and mineral analysis. **Results**: For all four plants, carbohydrates was the major macronutrient constituents (range 49.61-64.09% dry wt.) followed by fats (15.06-29.43%), Protein (7.28-12.53%), ash (1.81-14.82%) and fiber (2.92-7.53%) in that order. *G. latifolium* had the highest carbohydrate (64.09±0.09% dry weight) and protein (12.53±0.10%) composition while *V. amygdalina* had the highest fat (29.43±0.03%) composition. Results of Ash analysis of the four leaves showed *P.guineense* to have the highest total mineral content (14.82±0.12% dry wt.) followed by *V. amygdalina* (10.75±0.01%), *O. gratissimum* (4.60±0.04%) and *G. latifolium* (1.81±0.01%) in that order. *O. gratissimum* and *P.guineense* had the highest composition of fiber (7.53±0.02% and 7.22±0.02% respectively) closely followed by *G. latifolium* (6.03±0.02%) and *V. amygdalina* (2.92±0.02%). Vitamin analysis revealed that leaves of the four vegetable plants contained high levels of vitamin C (range 18.1-43.4mg/100g) and appreciable quantities of vitamins A (0.3-1.2mg/100g) and E (0.67-0.9mg/100g). *V. amygdalina* leaf contained the highest concentration of vitamin C (43.4±0.01 mg/100g) and A (1.2±0.9 mg/100g) while *O. gratissimum* had the highest vitamin E content (0.9 mg/100g). The mineral assay indicated that the leaves of the plants contain high levels of Magnesium (Mg)(3.6-24.8mg/100g), Phosphorus (P) (2.8±-34.3mg/100g), Calcium (Ca) (12.1-19.0 mg/100g) and copper (Cu))(5.8-18.5 mg/100g) relative to their Zinc (Zn) (1.1-2.1 mg/100g), Potassium (K) (2.1-6.9mg/100g) and Sodium (Na) (4.3-8.1 mg/100g) contents. **Conclusion**: In conclusion, these plants were shown to be rich in carbohydrates, proteins and fats, vitamins and minerals justifying their use in diets. That the plants were particularly rich in vitamins and mineral with antioxidant properties could explain 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.

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

1. INTRODUCTION

Plants since 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 vitamins with antioxidant properties from vegetable plants like vitamins A, C and E [6]. Selective intake of food containing these 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, vitamin 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 are also commonly employed in ethno pharmacology for the treatment of various diseases. A comparative analysis of the proximate, vitamin and mineral 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].

O. 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].

P. 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. *P. 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 a pungent taste and 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].

G. 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.

V. 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 *O. gratissimum, P. guineense, G. latifolium and V. amygdalina* were harvested from local farms in Cross River State, South-south 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. The powdered samples were then stored at room temperature in separate tightly corked containers until required for use. Weighed quantities of each extract were used in the macro and micro nutrient analysis according to experimental protocol.

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 (Jenway Flame Photometer model PFP7) while iron, copper, zinc, calcium and magnesium were determined by Atomic absorption spectrophotometric method (Pelkin Elmer 2380 atomic absorption spectrophotometer) as described by James [19] and the Association of Official Analytical Chemists, AOAC [20]. Phosphorus was determined spectrophotometrically (Spectrophotometer SEAC, Italy) by the vanadomolybdate yellow method [21].

2.2.4 Determination of Vitamins

Vitamin A and E concentration was determined by the spectrophotometric method (Spectrophotometer SEAC, Italy) as described by Pearson [22].

For Vitamin A, to 1g of plant sample was added ethanol (3ml) to precipitate the proteins before extraction with heptane (5ml). After vigorous shaking the heptane layer was separated and absorbance read at 450nm against a heptane blank using a UV/Vis spectrophotometer (SEAC, Italy). A standard was also prepared and read at 450nm. Vitamin A from sample was calculated from the known concentration of standard.

For the determination of Vitamin E, a weighed sample (1g) was macerated with petroleum ether (20ml), filtered and the filtrate evaporated to dryness and re-dissolved in ethanol (2ml). To the re-dissolved sample was then added 1 ml each of 0.2% Ferric chloride and 0.2% dipyridyl (both dissolved in ethanol) and the mixture made up to 5ml with ethanol. The solution was mixed thoroughly and absorbance taken at 520nm using a UV/VIS spectrophotometer (SEAC, Italy) against a corresponding blank. A standard was also prepared and read at 520nm. Vitamin E from sample was calculated from the concentration of the standard.

Vitamin C was determined by the method of AOAC [23].

2.2.5 Statistical Analysis

Data was presented as mean ± standard error of mean. Quantitative data generated were analyzed by one way Anova, with the help of a statistical package SPSS version 18.0 for Windows, to test the significance of the data at 5% confidence limit ($p<0.05$).

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. For all four plants, carbohydrates was the major macronutrient constituents (range 49.61-64.09% dry wt.) followed by fats (15.06-29.43%), proteins (7.28-12.53%), ash (1.81-14.82%) and fiber (2.92-7.53%) in that order.

G. latifolium had the highest carbohydrate composition 64.09±0.09% dry weight) followed by *O. gratissimum* (60.19±0.04%), *P.guineense* (59.04±5.27%) and finally *V. amygdalina* (49.61±0.01%). A report by Asaolu *et al*. [24], on the proximate and mineral composition of Nigerian leafy vegetables, puts a range of 1.22-8.65% dry weight for the three plants *O. gratissimum*, *V. amygdalina* and *G. latifolium*. It is worth noting that while carbohydrates was the major constituent in our study, protein was the major constituent in the report by Asaolu *et al*. [24]. The variation in composition may be as a result of variation in soil nutrient, environmental factors, age of plant at harvest, geographic location, diurnal and seasonal variations, method of cultivation, time of harvesting and procedures in extraction and preparation. 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 [25]. 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 (15.06-29.43% dry wt.), constitute the highest energy in humans. *V. amygdalina* (29.43±0.03%) had the highest fat composition followed by *O. gratissimum* (19.14±0.01%), *G. latifolium* (15.56±0.02%) and *P.guineense* (15.06±0.05). Asaolu *et al*. [23] reported a range of (3.51-9.05%) while Okafor [26] reported a range of 4.5-18.77% for the three plants, *P.guineense, G. latifolium* and *V. amygdalina*.

Protein is the second largest store of energy in the body after adipose tissue fat stores [27]. The result of macronutrient analysis revealed that all the four plants were a fairly rich source of protein (7.28±0.02-12.53±0.10% dry wt.) and may be used as a protein supplement for patients with protein deficiency diseases. *G. latifolium* had the highest protein composition (12.53±0.10%) followed by *P.guineense* (8.88±0.08%), *O. gratissimum* (8.60±0.05%) and *V. amygdalina* (7.28±0.02%) in that order. A similar report by Asaolu *et al*. [24] and Okafor [26] put the range at 50.94-66.71% and 18.54-62.66% dry wt. respectively.

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 [28]. Results of Ash analysis of the four leaves (range 1.81-14.82% dry wt) shows *P.guineense* to have the highest total mineral content (14.82±0.12%) followed by *V. amygdalina* (10.75±0.01%), *O. gratissimum* (4.60±0.04%) and *G. latifolium* (1.81±0.01%) in that order. Asaolu *et al*. [24] and Okafor [26] reported a range of 9.01-13.01% and 10.13-15.56% respectively.

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 supports insulin sensitivity and may help reduce the risk of diabetes. The fibre content in this study ranged from (2.92-7.53% dry wt. *O. gratissimum* (7.53±0.02%) and *P.guineense* (7.22±0.02%) had the highest composition of crude fiber closely followed by *G. latifolium* (6.03±0.2%) and *V. amygdalina* (2.92±0.02%) (Figure 1). The range for three of these plants as reported by Asaolu *et al*. [23] was 4.02-12.08% dry wt.

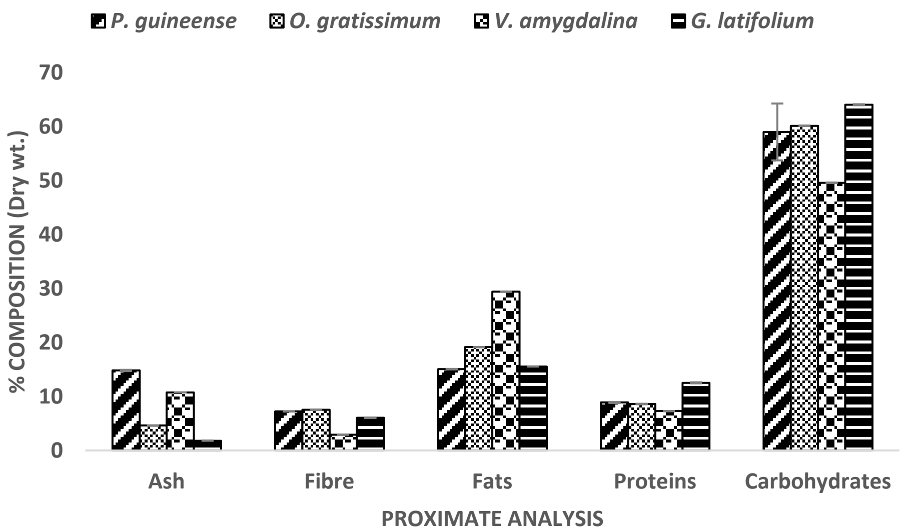

Fig 1: Proximate Analysis of crude leaf extracts of *P.guineense, O.gratissimum,* and *V. amygdalina* and *G.latifolium*. Values (% dry wt.) are expressed as mean ± SEM.

3.2 Vitamins

The protective action of fruit and vegetables has been attributed to the presence of antioxidants, especially vitamins known to have antioxidant properties like ascorbic acid, α-tocopherol and beta-carotene [28-31]. The results of this study (Figure 2) revealed that leaves of the four vegetable plants contain appreciable concentration of vitamin C (range 18.1-43.4 mg/100g), vitamin E (0.67-0.9 mg/100g) and beta-carotene (vitamin A)(0.3-1.2mg/100g). *V. amygdalina* leaf contained the highest concentration of vitamin C (43.4±0.10 mg/100g) and vitamin A (1.2±0.9 mg/100g). Other reports have also shown the plant to be rich in Vitamin C and A [31,32](13.41 and 197.5 mg/100g respectively for vitamin C respectively and a carotenoid value of 30mg/100g [32]). Odukoya *et al*. [31] also reported a Vitamin C value of 187.11mg/100g for *G.latifolium*. These results seem to suggest that fresh leaves of the plants are good sources of vitamins with antioxidant activities. Vegetable leaves/ Spices provide a variety of vitamins and minerals as well as macronutrients to the diet [33]. These vitamins with antioxidant properties may 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 [34]. Vitamin C has also been shown to facilitate iron absorption by its ability to reduce inorganic ferric ion to the ferrous form [35]. 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 [36]. It also limits the oxidation of LDL cholesterol and may help prevent or delay the development of atherosclerosis and/or coronary heart disease (CHD) [37]. 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 [38].

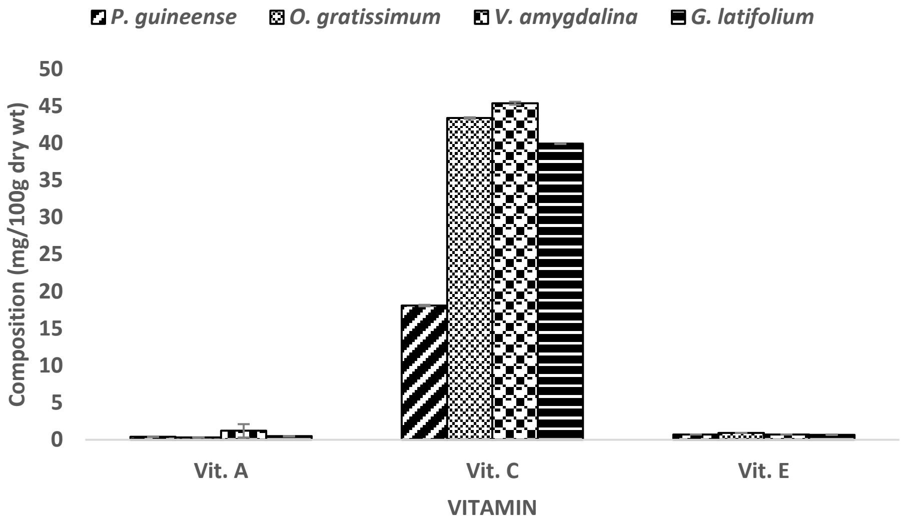

Fig 2: Quantitative Analysis of some Vitamins in *P.guineense, O.gratissimum,* and *V. amygdalina* and *G.latifolium*. Values are expressed as mean ± SEM.

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)(3.6-24.8 mg/100g), Phosphorus (P)(2.8-34.3 mg/100g), Calcium (Ca)(12.1-19.0mg/100g) and copper (Cu)(5.8-18.5mg/100g), relative to their Zinc (Zn)(1.1-2.1mg/100g), Potassium (K)(2.1-6.9mg/100g) and Sodium (Na)(4.3-8.1mg/100g) contents. A similar report by Asaolu *et al*. [24] for the three plants *O. gratissimum*, *V. amygdalina* and *G. latifolium* gave the range as Mg (61.08-92.51 mg/100g), P (12.52-29.42), Ca (64.8-72.65), Cu (ND-5.69), Zn (6.85-18.15), K (72.25-99.01) and Na (32.97-84.10) mg/100g. The report by Okafor [26] puts the range (converted from parts per million) for three of the plants, *P.guineense, G. latifolium* and *V. amygdalina* as Mg (5.6-14.7), Ca (0.71-46.0), Cu (0.01-0.015), Zn (0.081-0.205), K (30.4-33.6) and Na (3.5-5.8) mg/100g. In addition to the numerous biological roles these minerals play, they also serve as co-factors 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 [39]. 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 [40]. 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.

Except for *P.guineense*, Phosphorus (P) was the major constituents of the mineral elements assayed. *O.gratissimum* had the highest phosphorus content (34.3±0.3 mg/100g) closely followed by *V. amygdalina* (31.1±0.1 mg/100g) and *G.latifolium* (29.5±0.5 mg/100g) 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 [41]. 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.

Comparatively, *G.latifolium* had the highest Mg content (24.8±0.2mg/100g) with *O.gratissimum* (3.6±0.1 mg/100g) 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 [4,42]

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 [43]. Calcium is also involved in cell movement, muscle contraction, 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* (19.0±1.0mg/100g) followed by *G.latifolium* (15.5±0.5mg/100g), *O.gratissimum* (13.9±0.1mg/100g) and *P.guineense* (12.1±0.1mg/100g) in that order.

Copper is a constituent of many enzymes including superoxide dismutase. It is also required for iron metabolism [4,44]. It was more prevalent in *P.guineense* (18.5±0.5 mg/100g).

Zinc plays a catalytic, structural, and regulatory role in the body [45]. 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 [46]. 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* [47]. The level of the mineral was pretty much the same in *V. amygdalina*, *G.latifolium* and *P. guineense* (2.1mg/100g). *O.gratissimum* had the lowest level of the mineral (1.1±0.1 mg/100g).

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, muscle contraction and regulation of fluid movement in and out of cells [48]. *P. guineense* had the highest amount of potassium (6.9±0.1mg/100g) while *O.gratissimum* had the highest level of sodium (8.1±0.1 mg/100g).

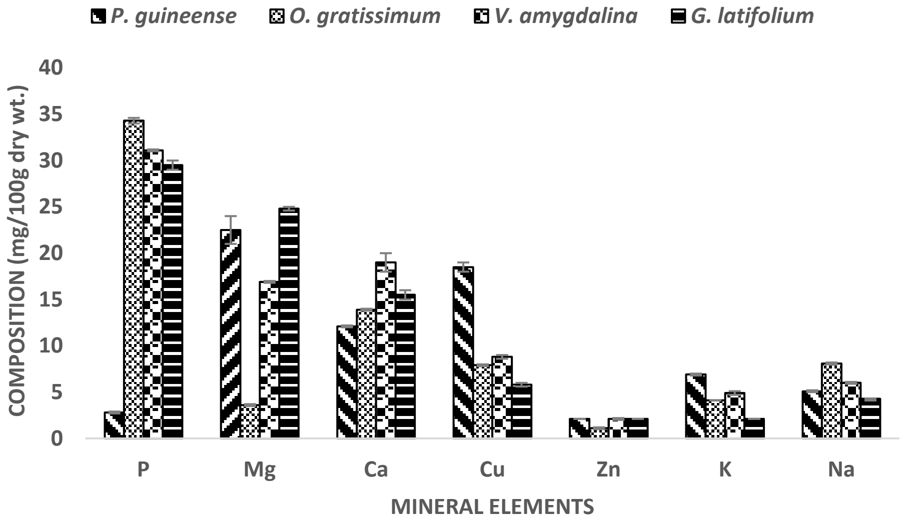

Fig 3: Quantitative Analysis of some Minerals in *P.guineense, O.gratissimum, V. amygdalina* and *G.latifolium*. Values are expressed as mean + SEM.

4. Conclusion

In summary, the leaves of 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. Taken together with earlier work on the comparative phytochemical analysis of the leaves of these four plants [7], the findings may explain 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.

COMPETING INTERESTS

The Authors declare that no competing interests exist.

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Fig 4: Serving of Vernonia