

Original Research Article

COMPARATIVE PROXIMATE, VITAMINS AND MINERAL COMPOSITION OF LEAVES OF FOUR SELECTED TROPICAL NUTRITIONAL 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 nutritional 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, 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%), ash (1.81-14.82%) and fiber (2.92-7.53%) in that order. *G. latifolium* had the highest carbohydrate (64.09% dry weight) and protein (12.53%) composition while *V. amygdalina* had the highest fat (29.43%) composition. Results of Ash analysis of the four leaves showed *P. guineense* to have the highest total mineral content (14.82%) followed by *V. amygdalina* (10.75%), *O. gratissimum* (4.60%) and *G. latifolium* (1.87%) in that order. *O. gratissimum* and *P. guineense* had the highest composition of fiber (7.53% and 7.22% respectively) closely followed by *G. latifolium* (6.03%) and *V. amygdalina* (2.92%). Vitamin analysis revealed that leaves of the four vegetable plants contained high levels of vitamin C (range 18.1-45.4 mg/100g) and appreciable quantities of vitamins A (0.3-1.2mg/100g) and E (0.67-0.9 mg/100g). *V. amygdalina* leaf contained the highest concentration of vitamin C (45.4 mg/100g) and A (1.2 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)(3.6-24.8 mg/100g), Phosphorus (P) (2.8-34.3), Calcium (Ca) (12.1-19.0) and copper (Cu) (5.8-18.5) relative to their Zinc (Zn) (1.1-2.1), Potassium (K) (2.1-6.9) and Sodium (Na) (4.3-8.1) 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-spices were characterized as rich in were particularly rich in vitamins and minerals with antioxidant properties and 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*.

Comment [JFR Rev1]: Selected because of what? High availability? High demand in market? Local interest?

.... The selection of these 4 spices is based on....?

Comment [JFR Rev2]: spices

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Comment [JFR Rev3]: Nutritional? Or just spices? Why "Nutritional"?

Comment [JFR Rev4]: Just speculative, not tested, not studied in this work.

What is the take-home lesson from this work?

Why these spices are interesting for international purposes (consumption, wellness, etc.)?

How relevant (from the perspective of the consumer) is going to be the intake of these spices to help in the nutritional balance of the diet?

As spices are consumed in very small amounts (a few grams of dried material), How relevant is going to be this intake if compared with eating other fresh vegetables or fruits to get those minerals and vitamins?

If these points are not reasonable explained, the interest/novelty of the work is not defended, to be suitable for publication.

54 1. INTRODUCTION

55
56 | Plants since prehistoric times, have been used as spices, food and medicine in all cultures
57 [1-3]. More than 70% of people in developing countries depend on plants (vegetables and
58 fruits) for regular dietary needs [4]. It is well known that apart from energy needs, many
59 plants and foods are ingested because of their perceived medicinal and health benefit.
60 Indeed a significant amount of research has shown a correlation between a healthy diet and
61 lifestyle and significant reductions in diseases and associated chronic conditions [5]. Plants
62 are also a rich source of vitamins and minerals. Studies have identified a vast majority of
63 vitamins with antioxidant properties from vegetable plants like vitamins A, C and E [6].
64 Selective intake of food containing these vitamins, minerals and phytochemicals can prevent
65 the onset of degenerative diseases like cardiovascular diseases, cancer and diabetes.

Comment [JFR Rev5]: More correct to name the species under study as "spices" not "Nutritional plants".

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

80
81 **O.** *gratissimum* commonly called African basil and belonging to the family *Lamiaceae*, is a
82 herbaceous perennial flowering plant which is woody at its base. The leaf is called scent leaf
83 because it possesses a pleasant aroma which is responsible for its use as spice and
84 condiments in cooking. It is widely distributed in tropical Africa and Asia, especially India.
85 The plant is economically important for its food flavoring (as spice and condiments) [8] and
86 essential oil which has been widely used in food industries [9,10].

Comment [JFR Rev6]: Spice, therefore, intake is in small amounts

87
88 **P.** *guineense* (family *Piperaceae*) is a climbing perennial plant native to the tropical regions
89 of Central and Western Africa. It is commonly referred to as Ashanti pepper, West African
90 pepper or African black pepper. **P.** *guineense* is economically important for its culinary uses
91 as well as medicinal, cosmetic and insecticidal uses [11]. It is a highly spicy plant and the
92 leaves have a pungent taste and pleasant aroma when crushed. It thus imparts "heat",
93 "pungency" and a spicy aroma to classic West African soups (stews). The plant oils is used
94 as aromatics in the drink industry [12].

Comment [JFR Rev7]: Spice, therefore, intake is in small amounts

95
96 **G.** *latifolium*, commonly called "utazi," "aroke" in the South Eastern and South Western
97 parts of Nigeria respectively, belongs to the family *Asclepiadaceae*. It is primarily used as
98 spice and vegetable for cooking and in traditional medicine [13]. A non-wood forest plant, it
99 is native to West Africa and widely distributed elsewhere in tropical Africa and subtropical
100 Asia.

Comment [JFR Rev8]: Spice, therefore, intake is in small amounts

101
102 **V.** *amygdalina*, popularly called bitter leaf, belongs to the family *Asteraceae*. It is widely used
103 in the West African sub-region for a number of medicinal and nutritional purposes [14,15]. It
104 has also been employed as a digestive tonic and appetizer [16].

Comment [JFR Rev9]: Spice, therefore, intake is in small amounts

Which compounds are giving the "bitter" taste? Antinutrients? Tannins? Alkaloids? Maybe these plants are not that "nutritional" after all.... Please, explain.

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108 **2. MATERIALS AND METHOD**

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110 **2.1 Plant Materials**

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112 Mature leaf samples of *O. gratissimum*, *P. guineense*, *G. latifolium* and *V. amygdalina* were
113 harvested from local farms in Cross River State, South-south Nigeria.

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115 **2.2 Methods**

116 **2.2.1 Extraction Procedure**

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118 Fresh leaves of each plant were washed and air dried at room temperature (25°C) for two
119 weeks. The dried leaves were pulverized using a mechanical grinder. A weighed quantity,
120 200g, of each plant material was extracted by cold maceration in absolute ethanol for 48
121 hours. The extracts were double filtered, first with a white muslin cloth then with Whatman
122 no.1 filter paper. The resulting ethanol leaf extracts were concentrated in vacuum using a
123 rotary evaporator (at temperatures between 40°C and 45°C) to obtain a semi-solid mass.
124 Weighed quantities of each extract were used in the macro and micro nutrient analysis
125 according to experimental protocol.

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127 **2.2.2 Proximate Analysis**

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

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133 **2.2.3 Determination of Mineral Composition**

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

140

141 **2.2.4 Determination of Vitamins A, C and Es**

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

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146 **2.2.5 Statistical Analysis**

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148 Data was presented as mean ±standard error of mean. Quantitative data generated were
149 analyzed by Anova to test the significance of the data at 5% confidence limit (p<0.05)

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151 **3. RESULTS & DISCUSSIONS**

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153 **3.1 Proximate Analysis**

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155 The result of the proximate composition of the fresh leaves of the four plants is shown in
156 Figure 1. For all four plants, carbohydrates was the major macronutrient constituents (range
157 49.61-64.09% dry wt.) followed by fats (15.06-29.43%), proteins (7.28-12.53%), ash (1.81-
158 14.82%) and fiber (2.92-7.53%) in that order.

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160 *G. latifolium* had the highest carbohydrate composition (64.09% dry weight) followed by *O.*
161 *gratissimum* (60.19%), *P. guineense* (59.04%) and finally *V. amygdalina* (49.61%). A report

Comment [JFR Rev10]: Software? Version?
Supplier? Country?

162 by Asaolu *et al.* [23], on the proximate and mineral composition of Nigerian leafy vegetables,
163 puts a range of 1.22-8.65% dry weight for the three plants *O. gratissimum*, *V. amygdalina*
164 and *G. latifolium*. It is worth noting that while carbohydrates was the major constituent in our
165 study, protein was the major constituent in the report by Asaolu *et al.* [23]. The variation in
166 composition may be as a result of variation in soil nutrient, environmental factors, age of
167 plant at harvest, geographic location, diurnal and seasonal variations, method of cultivation,
168 time of harvesting and procedures in extraction and preparation. Dietary carbohydrate is a
169 major macronutrient for both humans and omnivorous animals; human adults in the Western
170 countries obtain approximately half their daily caloric requirements from dietary carbohydrate
171 while it is the major source of energy in other countries [24]. Carbohydrate is stored as
172 glycogen, and although it is important for short-term energy needs, it is of very limited
173 capacity for providing for energy needs beyond a few hours.
174

175 Fats, the second highest macro nutrient in the four plants (15.06-29.43% dry wt.), constitute
176 the highest energy in humans. *V. amygdalina* had the highest fat composition followed by *O.*
177 *gratissimum*, *G. latifolium* and *P.guineense*. Asaolu *et al.* [23] reported a range of (3.51-
178 9.05%) while Okafor [25] reported a range of 4.5-18.77% for the three plants, *P.guineense*,
179 *G. latifolium* and *V. amygdalina*.
180

181 Protein is the second largest store of energy in the body after adipose tissue fat stores [26].
182 The result of macronutrient analysis revealed that all the four plants were a fairly rich source
183 of protein (7.28-12.53% dry wt.) and may be used as a protein supplement for patients with
184 protein deficiency diseases. *G. latifolium* had the highest protein composition followed by
185 *P.guineense*, *O. gratissimum* and *V. amygdalina* in that order. A similar report by Asaolu *et*
186 *al.* [23] and Okafor [25] put the range at 50.94-66.71% and 18.54-62.66% dry wt.
187 respectively.
188

189 Ash, which refers to the inorganic residue remaining after ignition or complete oxidation of
190 organic matter in a food sample, is a measure of the total amount of minerals present within
191 the food [27]. Results of Ash analysis (1.81-14.82%) of the four leaves shows *P.guineense*
192 to have the highest total mineral content followed by *V. amygdalina*, *O. gratissimum* and *G.*
193 *latifolium* in that order. Asaolu *et al.* [23] and Okafor [25] reported a range of 9.01-13.01%
194 and 10.13-15.56% respectively.
195

196 Fibre is a measure of the quantity of indigestible cellulose, pentosans, lignin and other like
197 components in foods. Insoluble fibers can help promote bowel health and regularity. It also
198 support insulin sensitivity and may help reduce the risk of diabetes. The fibre content in this
199 study ranged from 2.92-7.53%. *O. gratissimum* and *P.guineense* had the highest
200 composition of crude fiber closely followed by *G. latifolium* and *V. amygdalina* (Figure 1).
201 The range for three of these plants as reported by Asaolu *et al.* [23] was 4.02-12.08% dry wt.
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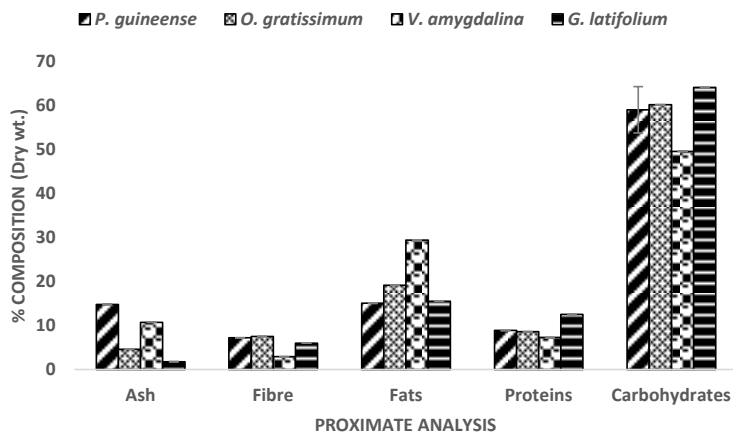


Fig 1: Proximate Analysis of crude leaf extracts of *P.guineense*, *O.gratissimum*, and *V. amygdalina* and *G.latifolium*. Values (% dry wt.) are expressed as mean \pm SEM.

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 [28-30]. The results of this study (Figure 2) revealed that leaves of the four vegetable plants contain appreciable concentration of vitamin C (range 18.1-45.4 mg/100g), vitamin E (0.67-0.9mg/100g) and beta-carotene (vitamin A)(0.3-1.2mg/100g). *V. amygdalina* leaf contained the highest concentration of vitamin C (45.4mg/100g) and vitamin A (1.2mg/100g). Other reports have also shown the plant to be rich in Vitamin C and A [30,31](13.41 and 197.5 mg/100g respectively for vitamin C respectively and a carotenoid value of 30mg/100g [31]). Odukoya *et al.* [30] also reported a Vitamin C value of 187.11mg/100g for *G.latifolium* [30]. 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 [32]. 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 [33]. Vitamin C has also been shown to facilitate iron absorption by its ability to reduce inorganic ferric ion to the ferrous form [34]. 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 [35]. It also limits the oxidation of LDL cholesterol and may help prevent or delay the development of atherosclerosis and/or coronary heart disease (CHD) [36]. 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 [37].

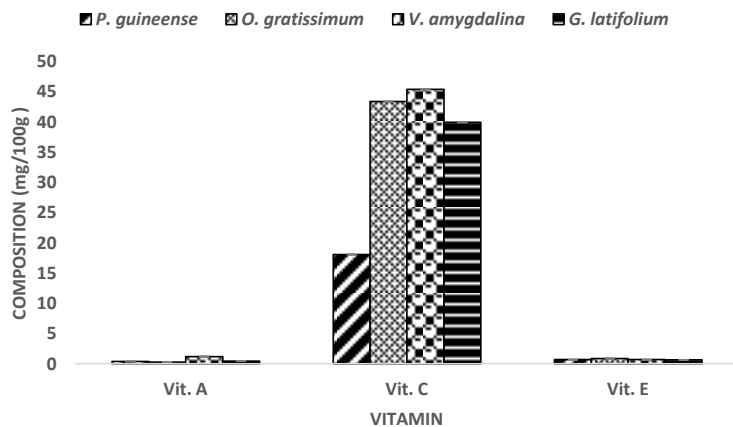


Fig 2: Quantitative Analysis of some Vitamins in *P.guineense*, *O.gratissimum*, and *V. amygdalina* and *G.latifolium*. Values are expressed as mean \pm SEM.

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) and Calcium (Ca)(12.1-19.0) and copper (Cu)(5.8-18.5), relative to their Zinc (Zn)(1.1-2.1), Potassium (K)(2.1-6.9) and Sodium (Na)(4.3-8.1) contents. A similar report by Asaolu *et al.* [23] 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 [25] 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 [38]. 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 [39]. 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 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 [40]. 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.

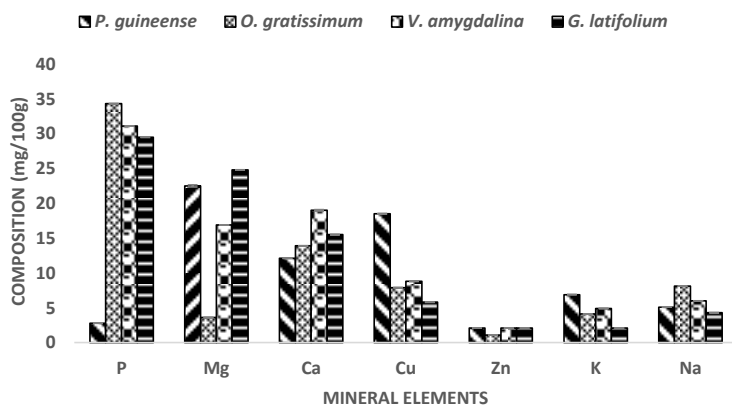
276 **Comparatively**, *G.latifolium* had the highest Mg content with *O.gratissimum* having the
 277 lowest. *G.latifolium* is thus the plant of choice to address Mg deficiency. Mg plays an
 278 essential role in a wide range of fundamental biological reactions. Apart from its cofactor
 279 role, it is involved in bone mineralization, the building of proteins, muscle contraction, nerve
 280 transmission and immune system health [4,41]
 281

282 Calcium (Ca) is the most tightly regulated ion in the extracellular fluid (ECF). In higher
 283 mammals, the most obvious role of calcium is structural or mechanical being responsible for
 284 the mass, hardness, and strength of the bones and teeth [42]. Calcium is also involved in
 285 cell movement, muscle contraction, nerve transmission, glandular secretion, and even cell
 286 division where it acts as both a signal transmitter from the outside of the cell to the inside
 287 and as an activator or stabilizer of the functional proteins involved. Calcium also plays a role
 288 in the regulatory activities of parathyroid hormone [PTH], calcitonin [CT], and a key activity of
 289 vitamin D. Ca was more predominant in *V. amygdalina* followed by *G.latifolium*,
 290 *O.gratissimum* and *P.guineense* in that order.
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292 Copper is a constituent of many enzymes including superoxide dismutase. It is also required
 293 for iron metabolism [4,43]. It was more prevalent in *P.guineense*.
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295 Zinc plays a catalytic, structural, and regulatory role in the body [44]. Zinc is essential for
 296 general growth and proper development of the reproductive organs and for normal
 297 functioning of the prostate gland. Apart from SOD, Zinc is a co-factor of over 300 enzymes
 298 including carbonic anhydrase, which is crucial to maintenance of acid-base balance in the
 299 blood, and alcohol dehydrogenase that break down alcohol. It is also a component of insulin
 300 and plays a role in its processing, storage, secretion and action [45]. The Zinc content of *P.*
 301 *guineense* may be responsible for the observed stimulated sexual behaviors of mature male
 302 rats fed with extract of *P. guineense* [46]. The level of the mineral was pretty much the same
 303 in *V. amygdalina*, *G.latifolium* and *P. guineense* (2.1 mg/100g). *O.gratissimum* had the
 304 lowest level of the mineral (1.1 mg/100g).
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306 Sodium (Na) and potassium (K) (and chloride ions Cl⁻) are the major electrolytes located in
 307 all body fluids. While sodium is extracellular, potassium is intracellular. They are responsible
 308 for the maintenance of acid/base balance, nerve transmission, muscle contraction and
 309 regulation of fluid movement in and out of cells [47]. *P. guineense* had the highest amount
 310 of potassium while *O.gratissimum* had the highest level of sodium.



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

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4. 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 rich in vitamins and mineral. ~~Taken together with 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.

COMPETING INTERESTS

The Authors declare that no competing interests exist.

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Comment [JFR Rev11]: Delete this paragraph, is out of the objective of this work, the data are not presented (not about phytochemicals, not about any Biological activity) and this conclusion is just speculative, not based in the results.

Comment [JFR Rev12]: How much?
Daily?
Portion size?

All these plants are used as spices (small amounts for improving taste)
How relevant at low dosage is the improvement in the nutritional intake?

Is it possible to eat a high amount of these leaves and spices without inducing a digestive problem?

Comment [JFR Rev13]: Which ones?

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