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# Nutritional composition and microbial load of *Eugenia caryophyllus* and *Piper guineense* spices

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**Abstract**

This study determined the proximate composition, mineral content and microbial load of *Eugenia caryophyllus* (EC) and *Piper guineense* (PG) obtained from Garki (gk), Wuse (ws) and Karmo (km) markets of FCT, Abuja in order to ascertain the nutritional value and their safety from being contaminated with heavy metals or microbes. The results obtained for proximate (moisture ash, fibre, protein, lipid and carbohydrates) analysis, mineral content and microbial load were within the following ranges 6.14-9.07% and 6.00-7.00% (moisture), 5.79-8.04% and 5.28-7.64% (ash), 5.44-8.62% and 6.32-8.28% (fibre),1.02-1.93% and 1.37-2.54% (protein), 2.33-7.78% and 5.33-7.34% (lipid),67.53-73.84% and 69.20-75.02% (carbohydrates); 0.1-6.5µg/g and 3.05-5.85µ g/g (Energy value); 2.006-2.6672kcal/100g (Cr), 0- 868.4 µg/g and 244-326.4µg/g (Fe), 43.45-46.75 µg/g and 9.65-81.45µg/g (Mn), 57.45-115.35 µg/g and 54-96.9 µg/g (Mg), 233.05-261µg/g and 216.15-234.65µg/g (K), 0.25-6.80µg/g and 15.65-20.10µg/g (Zn) and 12.15-62.90µg/g and 19.4-29.90µg/g (Pb). ANOVA results of the parameters analyzed range from significant difference (<0.0001) to no significance difference (0.1862) among the samples. The microbial load indicated total viable count range of $1.41\times10^2$ cfu - $2.04\times10^2$ cfu for *Piper guineense* and $0.19\times10^2$ cfu - 0.69 x $10^2$ cfu for *Eugenia caryophyllus*, while yeast/mould, coliform and pathogenic organisms were not detected. Both spices have low level of most of the proximate values with the exception of carbohydrate; but they are rich in minerals. All the samples contained lead above WHO limits (10µg/g) established for herbal medicines.

*Keywords*: Spices; Nutritional value; Mineral content; Microbial load

## INTRODUCTION

Spices are seeds, fruits, roots, barks, berry, buds or other vegetable substance primarily used for flavouring, colouring or preserving food. Spices are distinguished from herbs, which are parts of leafy green plants used for flavouring or as a garnish. Many spices have antimicrobial properties. This may explain why spices are commonly used in warmer climates, which have more infectious diseases, and why the use of spices is prominent in meat, which is particularly susceptible to spoiling [1]. Spices are sometimes used in medicine, religious rituals, cosmetics or perfume production, or as a vegetable. They have pungent or aromatic substances of vegetable origin that yield no significant nutritive value [1]. Spices can be added to foods in several forms: as whole spices, as ground spices, as essential oils, as oleoresins or as prepared and filtered vinegar infusions. Herbs and spices are not just

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valuable in adding flavour to foods, but also help to preserve foods from oxidative deterioration by their antioxidant activity and also increase their shelf life [2]. Several classes of spices have been reported among which are garlic, ginger, turmeric, onions, nutmeg, cloves, *Piper guineense and* others [2].

Clove is aromatic dried flower buds of a tree which come from the Myrtaceae family, and are native to India and Indonesia. Its Latin name is *Eugenia aromaticum* or *Eugenia caryophyllata*. The plant has been used globally as a common spice for food and medicine with major chemical constituents as eugenol and eugenyl acetate, with a lower amount of methyl salicylate, pinene, vanillin and tannins [3]. The common medicinal uses are related to its antiseptic and analgesic properties. Its oil has been widely used for preventing tooth decay and toothaches and cure for nausea, hernia, intestinal gas, diarrhoea and Athlete's foot and as ointment to alleviate muscle spasms, acne, skin ulcers and sty in the eyes [3].

*Piper guineense* (African black pepper) commonly referred to as Ashanti pepper and "*Masoro*" by the Hausas, is very similar to *Piper nigrum*, which is the true pepper of commerce from which "black and white" peppers are processed. *Piper guineense* as a plant has been fully described to have medicinal cosmetics and insecticidal properties [4]. Black pepper can be produced from *Piper guineense*, its fruit is called peppercorn and is produced from unripe fully developed berries [5]. African black pepper stimulates the taste buds in such a way that an alert is sent to the stomach to increase hydrochloric acid secretion, thereby improving digestion. It has long been recognized as a carminative (a substance that helps prevent the formation of intestinal gas. In addition, black pepper has diaphoretic (promotes sweating) and diuretic (promotes urination) properties [5].

The popular use of these spices as flavours in food and for medicinal purposes prompted the need for this study; to evaluate the nutritional value, mineral content and microbial activities of *Eugenia caryophyllus* and *Piper Guineense* collected from three popular markets (Garki, Wuse and Karmo) in the Federal Capital Territory, Abuja Nigeria. Thus, their nutrients contribution and safety for use will be ascertained.

## EXPERIMENTAL

**Reagents/apparatus used.** All the reagents used were of analar grades purity; standard and appropriate sizes of glassware used were properly washed using detergents and rinsed with double-distilled deionised water. Other equipment used are Kjedahl apparatus (S.W. Germany), Muffle furnace (Korl-Kolb, Germany), Moisture balance (OHAUS/ MB200, England), AAS (GBC Avanta GF 300, Switzerland), weighing balance (OHAUS Analytical plus, England)

**Sampling and sample preparation.** The dried samples of *Eugenia caryophyllus* and *Piper guineense* were purchased in triplicate, as a normal customer, from the three markets (Wuse, Garki and Karmo). They were packaged and coded as ECgk (*Eugenia caryophyllus* from Garki market), ECws (*Eugenia caryophyllus* from Wuse market), ECkm (*Eugenia caryophyllus* from Karmo market), PGgk (*Piper guineense* from Garki market), PGws (*Piper guineense* from Wuse market), and PGkm (*Piper guineense* from Karmo market). These were taken to the laboratory for subsequent analysis. The samples were sorted-out from foreign matter and powdered using mortar and pestle and packaged in an airtight plastic container.

**Proximate analysis.** The recommended methods of the Association of Official Analytical Chemists [6-11] were used for the determination of moisture, ash, crude protein, and crude lipid and crude fibre content.

Moisture content determination: One (1.0g) gram of each sample was weighed, dried in an oven (Gallenkamp, UK) at 105°C for 24 hours, cooled in a desiccator and weighed until constant weight was obtained. The percentage loss in weight was expressed as percentage moisture content. Ash content was determined by the incineration of one gram (1.0 g) of each sample in a muffle furnace thermostat at 550°C for 3 hours. The percentage residue weighed was expressed as ash content. The Nitrogen content was estimated by micro-Kjeldahl method based on the assumption that plant proteins contain 16% nitrogen, the protein content of each sample was calculated using the formula, $Protein = percentage\ nitrogen \times 6.25$. Crude lipid was assayed by exhaustively extracting one grams of each sample for 3 hours with petroleum ether in a Soxhlet apparatus. Crude fibre was estimated by acid and alkaline digestion methods with 1.25% $H_2SO_4$ (W/V) and 1.25% NaOH (W/V) solutions. Available carbohydrates were calculated by subtracting the total of the percentages of moisture, ash, crude protein, crude lipid and crude fibre from 100% moisture free samples [11]. Calorific value (CV) was determined using the following equations: CV (kcal/100 g) = (CHO ×4) × (CL × 9) × (CP × 4) [12].

**Sample preparation for elemental analysis.** The ash samples were digested with a mixture of concentrated nitric acid and hydrochloric acid in the ratio of 1:10 and filtered through Whatman filter paper into 50 $cm^3$ volumetric flask, made-up to mark with deionized and transferred into caped plastic bottle. The same digestion process was used for blank sample preparation followed by analysis.

**Analytical procedure for the elemental content.** This study analyzed *Eugenia caryophyllus* and *Piper guineense* samples for Manganese (Mn), Iron (Fe), Zinc (Zn), Lead (Pb), Chromium (Cr), Magnesium (Mg) and Potassium (K) using GBC Avanta version 2.0 Model of Atomic Absorption Spectrometer (AAS). The AAS was equipped with appropriate hallow cathode lamps, deuterium lamp for background correction and flame type sample atomizer and optimized based on the operating conditions spelt in Table 1 and calibrated with prepared standard solutions of suitable concentrations for each element of interest after which the reagent blank and the sample were analyzed consecutively. The concentrations were determined in the samples of interest and estimated using the relation:

$$Metal\ (\mu\frac{g}{g}) = \frac{C \times V \times d.f}{W}$$

Where C is the concentration of the sample solution in µg/mL; V is the volume of the prepared sample solutions in mL; W is the weight of the sample in grams used and d.f is the dilution factor, if used [13].

**Microbial analysis.** One gram (1.0 g) of each sample was aseptically dissolved in 10 ml of sterile water and mixed properly to enhanced homogeneity. A known volume of each was added to different media prepared for the analysis using the agar dilution method. The plates containing the media and the samples in duplicate were incubated accordingly for 24-48 hours. The Saboraud dextrose post plate were incubated at 25-28°C for fungi growth. The colonies were observed post incubation, sub-cultured on nutrient agar for purity and identification [14]. The microbial isolates were identified using standard biochemical test [15]. The total viable aerobic bacteria (TVABS), fungal count (FC) were calculated from the number of colonies that appeared on the plates [16].

**Statistical analysis.** The data generated for the different parameters were subjected to a statistical analysis using Prism GraphPad statistical software for correlation and one-way ANOVA. Statistical significance between the mean were tested at 0.05 level of probability as suggested by [17].

## RESULTS AND DISCUSSION

Results of the proximate composition (% dry matter, DM), elemental and microbial load obtained from the study of *Eugenia caryophyllus* (EC) and *Piper guineense* (PG) were presented in figures. Figures 1, 2 and 3 depicted proximate parameters (percentage moisture, ash, fibre, protein, lipid and carbohydrate) and mineral contents.

**Table 1:** Instrumental Operating Condition for Determination of the Heavy Metals

| Element | Wavelength (nm) | Detection limit (µg/ml) | Slit width (nm) | Lamp Current (mA) | Nebulizer uptake | Gas flow Air/acetylene) |
|---|---|---|---|---|---|---|
| K | 324.7 | 0.025 | 0.5 | 3.0 | 5ml/min | 13.5/2.0 |
| Fe | 372.0 | 0.45 | 0.2 | 7.0 | 5ml/min | 13.5/2.0 |
| Pb | 217.0 | 0.06 | 1.0 | 5.0 | 5ml/min | 13.5/2.0 |
| Zn | 213.9 | 0.008 | 0.5 | 5.0 | 5ml/min | 13.5/2.0 |
| Cr | 357.9 | 0.05 | 0.2 | 6.0 | 5ml/min | 13.5/2.0 |
| Mn | 279.5 | 0.45 | 0.2 | 7.0 | 5ml/min | 13.5/2.0 |

Table: 3:2: Microbial load determination of *Eugenia caryophyllus* and *Piper guineense*

| | ECgk | ECws | ECkm | PGgk | PGws | PGkm |
|---|---|---|---|---|---|---|
| Total Viable Count | $0.69 \times 10^2$ cfu | $0.19\times10^2$ cfu | $0.41\times10^2$ cfu | $1.96\times10^2$ cfu | $2.04\times10^2$ cfu | $1.41\times10^2$ cfu |
| Total Yeast/mould count | Absent | Absent | Absent | Absent | Absent | Absent |
| Coliform Count | Absent | Absent | Absent | Absent | Absent | Absent |
| Pathogenic Organisms | Absent | Absent | Absent | Absent | Absent | Absent |

Key: cfu= colony forming unit.

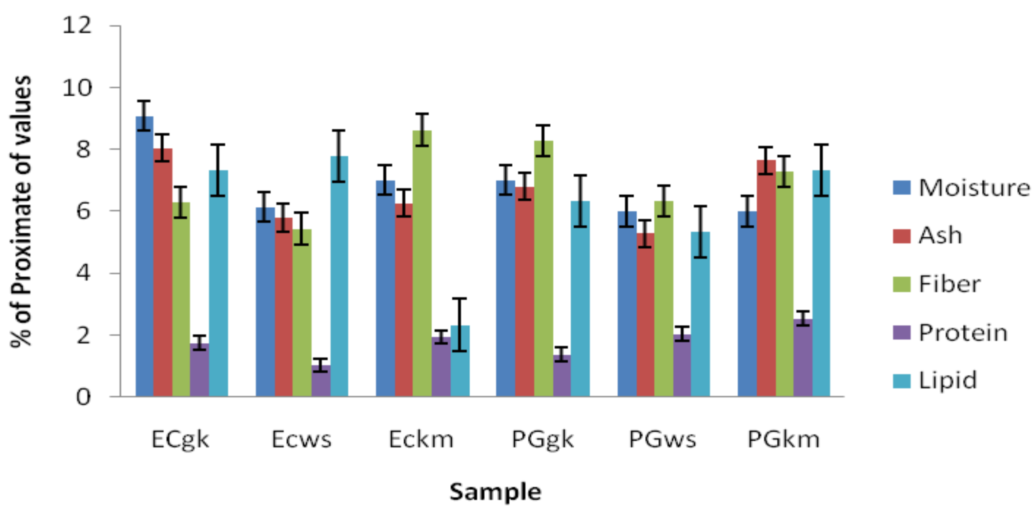


**Figure 1: Percentage of Proximate Content of the Samples**

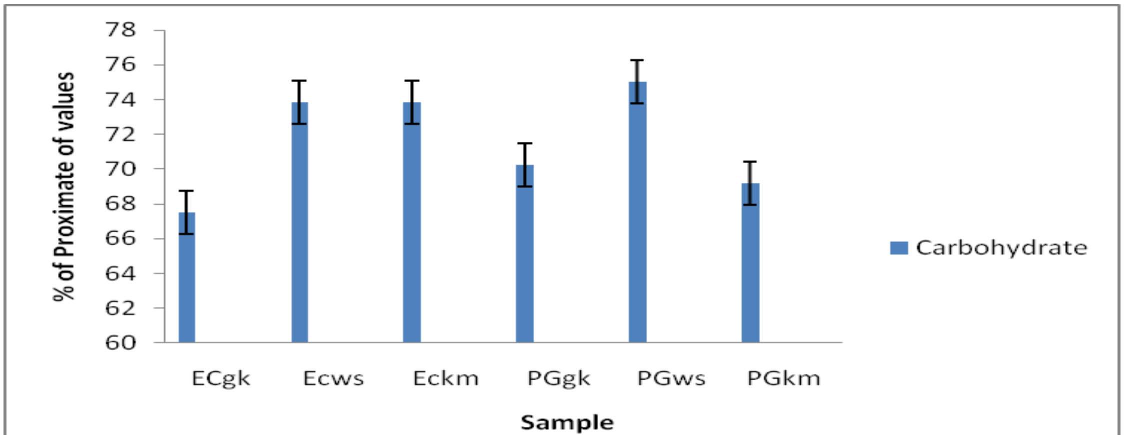


**Figure 2: Percentage of Carbohydrate Content of the Samples**

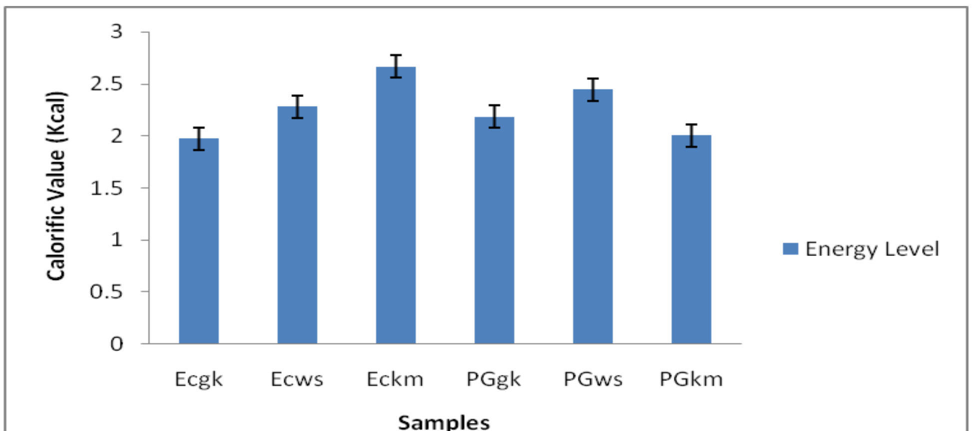


**Figure 3: Calorific Value of the Samples**

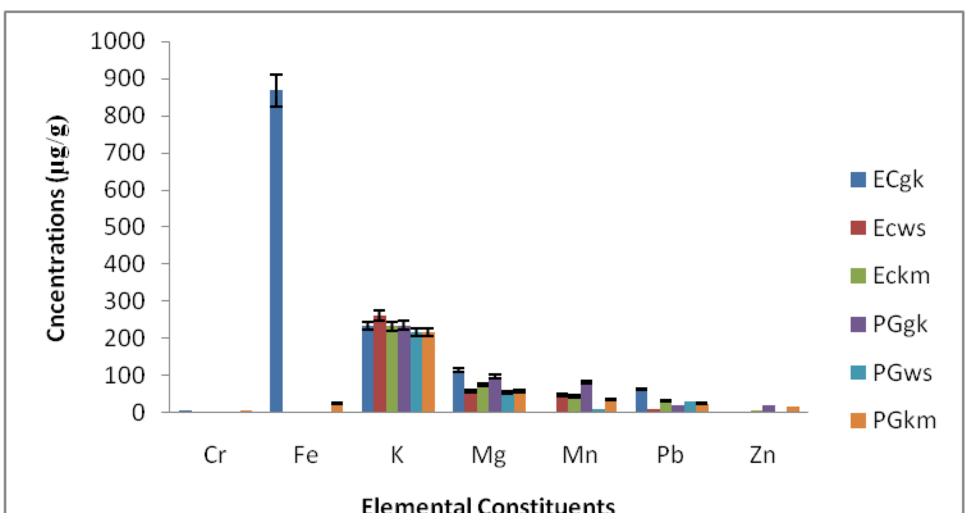


**Figure 4: Mean Concentrations of the Mineral Content**

The result indicated higher percentage of moisture in ECgk (9.07 ± 0.09%) as compared to ECkm (7.00 ± 0.00%), ECws (6.14 ± 0.24%), PGgk (7.00± 0.00%), PGws (6.00± 0.00%) and PGkm (6.00 ± 0.00%). Analysis of variance (ANOVA) of the moisture content among the samples from the different sampling sites indicated p-values as p = <0.0001<0.05. This means there is significant difference among the *Eugenia caryophyllus*, while that of *Piper guineense* indicated no significant difference (P = 0.128>0.05). The differences in moisture content could be associated with different storage conditions, which those samples were subjected or the way the samples were handled during processing, transportation, or packaged. The percentage moisture in all the samples analyzed falls within the WHO maximum permissible limit of moisture for food which is <15%, this indicated that all the samples are less susceptible to deterioration which could result from bacterial or fungal growth, giving these spices a longer shelf life (AOAC, 2006).

Ash is one of the components in the proximate analysis of biological and plant materials, consisting mainly of salty, inorganic constituents. It is a measure of the total amount of minerals present within a sample, whereas the "mineral content" is a measure of the amount of specific inorganic components present within a sample. Determination of the ash and mineral content of sample is important for nutritional labelling and food quality, which depends on taste, appearance, texture and stability. The total ash determined in the *Eugenia caryophyllus* and the *Piper guineense* samples were pictorially presented which indicated sample ECgk with higher ash content, followed by PGkm, PGgk, ECkm, ECws and PGws as 8.04 ± 0.05%, 7.64 ± 0.01%, 6.80± 0.17% , 6.26 ± 0.01%, 5.79 ± 0.00% and 5.28 ± 0.13% respectively. The percentage ash obtained for all the samples falls within the specified limit for spices, which is 5.00-8.70% [18] indicating non-contamination of the samples with silicates and other inorganic matter. The ANOVA result for percentage ash indicated p-values as p = <0.0001<0.05 which indicated a significant difference of means observed for all the samples of *Eugenia caryophyllus* and *Piper guineense* obtained at all the locations.

Fibre exhibits some health benefits such as prevention of colon cancer, reduction of the incidence of heart disease, lowering of cholesterol, blood pressure, glucose levels, inflammation, and even weight loss [19]. Barminas reported that, high fibre content of food could inhibit the intestinal absorption of nutrient especially the trace element [20]. In this study, the percentages of fibre content of *Eugenia caryophyllus* and *Piper guineense* obtained were 8.62 ± 0.11% (ECkm), 6.28 ± 0.06% (ECgk), 5.44 ± 0.09% (ECws) and 8.28 ± 0.06% (PGgk), 7.28 ± 0.04% (PGkm), 6.32 ± 0.18% (PGws). The analysis of variance of percentage means of fibre between both *Eugenia caryophyllus* (EC) and *Piper guineense* (PG) indicated p-values at p<0.05 as p<0.0001, which means there is significant difference of means observed for all the samples obtained at the various locations. This result was in agreement with the findings of Murphy who reported high fibre content of spices [21]. The necessity of fibre for normal body function is known but, for now, a limit has not been established for its consumption.

Proteins participate in virtually every process within cells. Many proteins are enzymes that catalyse biochemical reactions and are vital for metabolism. Proteins are needed in the diet to provide the essential amino acids that cannot be synthesized by the body [22]. The percentage protein obtained were 1.02± 0.12% (ECws), 1.75± 0.00% (ECgk), 1.93 ± 0.00% (ECkm), 1.37 ± 0.05% (PGws), 2.04 ± 0.05% (PGws) and 2.54 ± 0.00% (PGkm). The results obtained in this study indicated low protein content in all the samples analyzed (Figure 1) as compared to the result of similar study [23], in that study higher percentage of protein in *P. africana* and *X. aethiopica* (12.45%) spices was reported. The low protein content of the spices studied indicated that, they cannot be solely relied upon as a source of protein supplement. Analysis of variance indicated p-values at $p<0.05$ for both *Eugenia caryophyllus* and *Piper guineense* as $p<0.0001$, which indicated significant differences of mean of the percentage protein among all the samples analyzed.

Lipids are fatty acids and natural occurring derivatives compounds that are related biosynthetically [24]. The result obtained in this study indicated the percentage lipid of *Eugenia caryophyllus* (EC) and *Piper guineense* as 7.78±0.39 % (ECws), 7.33±0.58 % (ECgk), 2.33±0.58 % (ECkm) and 7.34 ± 1.16 % (PGkm), 6.33±1.16 % (PGgk) and 5.33 ± 1.16 % (PGws). These values were lower than results of similar study of “Kimba” (*Xylopia aethiopica*) (99.58%) as reported by Barminas [25]. The results of the analysis of variance for the percentage lipids indicated p-values at $p<0.05$ as $p<0.0001$ for *Eugenia caryophyllus* and $p<0.1862$ for *Piper guineense* which indicated significant difference of means among the *Eugenia caryophyllus* and insignificance difference of mean among the *Piper guineense* at the three locations.

Carbohydrate is a class of foods that provide the body with glucose, which is converted to energy used to support bodily functions and physical activity. It promotes good health by delivering vitamins, minerals, fibre, and a host of important phytonutrients. The healthiest sources of carbohydrates are unprocessed or minimally processed whole grains, vegetables, fruits and beans [26]. In this study, the mean percentage carbohydrate content of *Eugenia caryophyllus* and *Piper guineense* analyzed were graphically presented in figure 2. The result indicated carbohydrate as the proximate parameter of the highest percentage in the samples; PGws **(**75.02±1.32%), ECkm (73.86±0.56%), ECws (73.84±0.72%), PGgk (70.23±1.16%), PGkm (69.20±1.16%) and ECgk (67.53±0.57%). The ANOVA result for percentage carbohydrate for all the samples at $p<0.05$ were $p<0.0001$, which means there is significant difference observed among all the samples obtained at the three locations. The samples analyzed contained appreciable level of carbohydrate, which can be compared with other Nigerian foods, which are sources of carbohydrate. The energy value of sample ECkm (2.6672kcal/100g) appeared to be higher than samples ECws (2.2797kcal/100g), PGws (2.4464kcal/100g), PGgk (2.1844kcal/100g), PGkm (2.006kcal/100g), and ECgk (1.9712kcal/100 g) as graphically presented in figure 3. The report of Sharma showed that samples with higher energy value may contribute in giving energy; i.e. the energy value of food is a measure of the heat energy available by the complete combustion of a stated weight of the food [27].

**Mineral composition.** The result of mineral content of *Eugenia caryophyllus* (EC) and *Piper guineense* (PG) for all the locations were depicted in figure 3. The results indicated Potassium, Magnesium and Lead in all the samples (ECgk, ECws, ECkm, PGgk, PGws and PGkm), Manganese and Zinc in five samples (ECws, ECkm, PGgk, PGws and PGkm), chromium in four (ECgk, ECws, PGgk and PGkm ) and Iron in three samples (ECgk, PGgk and PGkm).

The ranges of mean concentration of iron in the samples were 24.4-868.4µg/g and the ANOVA result at $p<0.05$ was 0.0825, which indicated no significant difference. Iron is required for production of optimum hemoglobin concentration needed for blood volume expansion during growth phase, menstrual loss and normal hemoglobin concentration for different age and gender groups [28]. Iron overload typically damages cells in the heart, liver and elsewhere, which can cause significant adverse effects, including coma, metabolic acidosis, shock, liver failure, coagulopathy, adult respiratory distress syndrome, long-term organ damage, and even death [29]. The iron content of one of the samples (ECgk) was higher followed by potassium, magnesium, manganese, lead, zinc and chromium.

Potassium is crucial to heart function and plays a key role in skeletal and smooth muscle contraction, making it important for normal digestive and muscular function. However, consumption of too much of potassium can be harmful to those whose kidneys are not fully functional to remove its excess from the blood [30]. The result of this study, which indicated potassium as the mineral with high concentration following iron in the samples analyzed, is in agreement with the findings reported by other researchers [25,31], which indicated that, plant foods usually have a characteristic of high potassium content. The mean concentration of potassium obtained ranges from 216.15 - 261 µg/g. The ANOVA results at $p<0.05$ gave $p<0.0001$, which indicated significant difference of means among the samples.

Magnesium is another important macro element needed for calcium metabolism in bones. It helps prevent diseases of the circulatory system [32]. The mean concentration of magnesium obtained from the samples analyzed ranges from 54 - 115.35µg/g. The analysis of variance of mean among the samples at $p<0.05$ gave 0.0015, which means there is significant difference among observed values.

Manganese is an important mineral for human health, being absolutely necessary for development, metabolism, and the antioxidant system. However, excessive exposure or intake may lead to a condition known as manganism, a neurodegenerative disorder that causes dopaminergic neuronal death and parkinsonian-like symptoms [33,34]. The concentration ranges of manganese obtained in the analyzed samples were 9.65-81.45µg/g and the ANOVA results of p-value at 95% CI was $p=0.6273>0.050$ which means there is no significance difference of mean among the samples.

The mean concentrations of zinc obtained in the samples were shown in figure 3 and were within the ranges of 0.25-20.1µg/g and the ANOVA results of p-value at 95% CI was $p=0.2434>0.050$ which means there is no significance difference of mean among the samples. These concentrations were below WHO permissible limit for zinc in herbals [35]. Zinc is an essential microelement needed for prevention of diseases [36].The concentration of zinc in blood plasma stays relatively constant regardless of zinc intake, cells in the salivary gland, prostate, immune system and intestine use zinc signalling as one way to communicate with other cells in the body [37]. Its deficiency has been known to cause growth retardation in children, delay sexual maturation, infection susceptibility, and diarrhea, as well as affect bone metabolism [38].

Lead is a trace metal which does not have any beneficial value in either human's body or plants, its toxicity is apparent even at low concentration. The typical symptoms of lead poisoning are colic, anaemia, headache, convulsions and chronic nephritis of the kidneys, brain damage and central nervous system disorders [39]. The mean concentrations of lead obtained in the samples were shown in figure 3 and were within the ranges of 12.15-62.9µg/g and the ANOVA results of p-value at 95% CI was $p= 0.2633>0.050$ which means there is no significance difference of mean among the samples. The results indicated all the samples contained lead above WHO specified limit for lead in edible plants.

Chromium is an important element for the insulin activity and DNA transcription.

However, an intake below 0.02 mg per day could reduce cellular responses to insulin [40] but chronic exposure to it may result in liver, kidney, lung damage and others such as skin rash, nose irritations, nasal itch and lungs cancer [39]. In this study, the mean concentration of chromium obtained in the samples were shown in figure 3 and were within the ranges of 0.1-6.5µg/g. The ANOVA results of p-value at 95% CI was p= 0.3693>0.050 which means there is no significance difference of mean among the samples from all the locations.

**Microbial load composition.** The results of the microbial load analysis are represented in table 3:2. From the results, the total viable count for PGgk was $1.96\times10^2$ cfu, that of PGws was $2.04\times10^2$ cfu and that of PGkm was $1.41\times10^2$ cfu. In the same vein, the total viable counts for the ECgk, ECws and ECkm were $0.69 \times 10^2$ cfu, $0.19\times10^2$ cfu and $0.41\times10^2$ cfu respectively. Yeast/ mould, coliform and pathogenic organisms were not isolated from the samples analyzed for their microbial load.

According to the United States Pharmacopeia, the presence of some microorganisms can be allowed in non-sterile pharmaceutical products as long as they fall within an acceptable limit [16]. Limits for total viable bacteria count, yeast count and absence of *Escherichia coli* and *Salmonella typhi* were recommended as $10^2$ cfu/ml, $10^2$ cfu/ml and absence [16]. In this study, the results obtained indicated the absence of Total Yeast/mould count, Coliform Count and Pathogenic Organisms, while growth of *Bacillus species* was observed in total viable count plates but were within acceptable limits for non- sterile pharmaceutical products. The presence of *Staphylococcus aureus* in any preparation is indicative of poor hygienic practice during manufacturing or distribution. However, based on the USP recommendation, *Eugenia caryophyllus* and *Piper guineense* obtained from Garki, Wuse and Karmo markets in FCT, Abuja, Nigeria has satisfied the required standards for microbiological quality and were fit for human consumption.

**Conclusion.** The results of nutritional analysis shows that *Eugenia caryophyllus* and *Piper guineense* are good source of carbohydrate but poor source of other macronutrient particularly protein. They are rich in potassium, magnesium, manganese and fairly contained iron and zinc, which are necessarily required by the body but are also contaminated with lead, which pose health hazard to the body system. The source of the contamination therefore needs to be traced in order to avoid over-accumulation in the body of the consumers.

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