ASSESSMENT OF HEAVY METALS AND PHYTOCHEMICAL COMPOSITION OF *PIPER GUINEENSE* (THONNING, 1827) LEAVES COLLECTED FROM THREE MARKETS IN LAGOS, NIGERIA

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ABSTRACT

The study examined the phytochemical composition and heavy metals (Cu, Zn, As, Ni, Pb, Cd, Cr) present in *Piper guineense* from three markets in Lagos, Nigeria. The qualitative and quantitative analyses were carried out on both wet and dry samples using standard procedure. The results revealed the presence of bioactive compounds namely Alkaloids (53.12 ± 0.14 and 37.66 ± 0.00 mg/100g), Tannins (6.21 ± 0.13 and 4.12 ± 0.00 mg/100g), Flavonoids (67.99 ± 0.17 and 23.98 ± 0.00 mg/100g), Terpenes (14.63 ± 0.18 and 8.95 ± 0.00 mg/100g), Saponins (5.99 ± 0.14 and 4.76 ± 0.00 mg/100g), Phenols (6.02 ± 0.11 and 2.97 ± 0.00 mg/100g) for dry and wet samples respectively from *P. guineense* collected from Igando while from Ikotun market the values are: Alkaloids (4.88 ± 0.00 and 29.87 ± 0.02 mg/100g), Tannins (4.44 ± 0.00 and 2.97 ± 0.02 mg/100g), Flavonoids (58.87 ± 35.36 and 12.65 ± 0.01 mg/100g), Terpenes (6.98 ± 0.00 and 4.96 ± 0.02 mg/100g), Saponins (2.67 ± 0.00 and 2.02 ± 0.01 mg/100g), Phenols (4.55 ± 0.00 and 1.88 ± 0.02 mg/100g). Egbeda had Alkaloids (40.43 ± 18.08 and 33.21 ± 0.01), Tannins (9.06 ± 4.17 and 4.08 ± 0.00), Flavonoids (49.77 ± 25.60 and 23.88 ± 0.00), Terpenes (8.86 ± 8.34 and 6.16 ± 0.01 mg/100 g), Saponins (3.11 ± 3.94 and 2.14 ± 0.00 mg/100g) and Phenols (5.13 ± 1.37 and 3.45 ± 0.02 mg/100g). Values of phytochemicals (dry and wet weight) were significantly ($p < 0.05$) higher in samples from Igando than samples of other markets. However, there was no significant difference among heavy metals detected in *Piper guineense* across the three markets. All the metal in the *P. guineense* were below WHO maximum permissible limit. The findings indicated that *P. guineense* collected from the markets are safe for consumption and good for medicinal use. However, efforts should be made by farmers planting *P. guineense* and seller of the vegetable to avoid further contamination either through soil or direct exposure to metals compounds.

Keywords: *P. guineense*, Markets, Consumption, Metals, Bioactive compound.

Introduction

Plants are rich sources of bioactive compounds which have many health promoting effects like antioxidant, antibacterial, antihypertensive and anti-inflammatory etc. (Asgarpanah and Haghighat, 2012). Phytochemicals such as terpenoids, phenolic metabolites, tannins and alkaloid are examples of secondary metabolites produced by plants, from which the plants are thought to get their healing properties (Harborne, 1999). According to the World Health Organization, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs and so it is extremely important to use the most appropriate methods to extract the active components from plant materials (Bennerman and Chen, 1983).

Medicinal plants have been cited as a potential source of heavy metal contamination during cultivation or processing (Adeyolanu *et al.*, 2016). Heavy metals such as lead (Pb), cadmium (Cd) and arsenic (As) have toxic effects on human health. Toxic metals can accumulate persistently in the body over a lifetime. Although, some heavy metals such as Cu, Fe, Mg, Zn and Co are essential elements for normal growth, development and metabolism of plant, however, high concentrations of these elements could lead to physiological disorders in plants (Dwivedi and Dey, 2002).

Piper guineense is an evergreen climbing shrub producing woody stems from 4-20 metres tall. The plant supports itself on other plants by means of adventitious roots which are produced along the stems. The plant is regionally known as ashanti pepper, Benin pepper, false cubeb, Guinea cubeb and is a popular spice in Africa that are semi- cultivated in countries such as Nigeria where the leaves, known as uziza, are used as a flavouring for stews and also as a medicine (Ruffo *et al.*, 2002). Despite the importance of *P. guineense* leaf, its properties as regards bioactive ingredients and heavy metals concentration has often been neglected. Therefore, the purpose of this study is to assess the contents of phytochemical and heavy metals in *P. guineense* from the selected markets (Igando, Ikotun and Egbeda) in Lagos State, Nigeria.

MATERIALS AND METHOD

Collection of Samples

Fresh leaves of *Piper guineense* (**Plate 1**) was collected between July and September 2019, from three different markets (Igando, Ikotun and Egbeda) located in Alimosho Local Government Area of Lagos State.

Pre-treatment Methods for Plant Samples

The leaves of the *P. guineense* were separately detached from the stems and cleaned by gentle washing with distilled water. Thereafter, the samples were separately air-dried, for three days, and then pulverized using pestle and mortar, and then saved for analysis.

Sample Extraction

20 g each of the pulverized leave samples was weighed into labeled sample vials, macerated, and moistened with 100 ml of 70% methanol. The vials were capped and the mixture was allowed to stand for 24 hours. The extracts were placed on a water-bath at 40°C to evaporate the methanol. Thereafter, the residues were saved as crude extracts for phytochemical analysis.

Plate 1: Leaves of *Piper guineense*

Quantitative Determination of Phytochemicals

Tannins

25ml of 80% methanol was added to 0.50g of the pre-treated sample, warmed for 30 min while the solution was filtered through Whatman #1 filter paper, and diluted with 50ml of 80% methanol. 1ml of the extract was mixed with 0.6ml glacial acetic acid, 10ml pyridine (20%) and 2.5 ml aluminium chloride (10%), and then diluted with 25ml distilled water. The mixture was allowed to stand for 30 min at 25°C. Total tannins was determined by measurement of the absorbance at 420nm. A calibration standard (0.1- 0.5 mg/L) prepared using tannic acid, was carried out through the procedure.

Terpenes

The total terpenoid content of the plant extracts was determined based on an assay using linalool as the standard for estimation. An aliquot of the reaction mixture obtained with Salkowski reagents was transferred to colorimetric cuvette. The absorbance was measured at 538nm against a blank (95% (v/v) methanol). For the standard calibration curve, 0.2ml of linalool solution in methanol was prepared and diluted to give a calibration standard in the range 0.2 -1.0mg/L. Calibration curve of linalool was plotted and the total terpenoid contents expressed as milligrams of linalool equivalents per gram (mg linalool/g) of plant material was determined using the regression equation of the calibration curve.

Saponin

The basic principle of this method is the reaction of oxidized saponin with vanillin. Sulfuric acid is used as oxidants and the distinctive color of this reaction is purple and is measured in 430nm. 0.5g of the homogenized plant material was extracted in 50ml of methanol by sonication, in an ultrasonic bath, for 30 min. The crude extract was mixed with 1ml of vanillin-sulfuric acid reagent, in a reaction vial. The vial was heated on a water bath at 60°C for 10 min

to allow full color development. Thereafter, the mixture was cool for 10 min at room temperature before taking measurement at 430nm using a spectrophotometer. Diosgenin was used as calibration standard.

Phenol

100mg each of the extract of the leaves was dissolved in 100ml of distilled water. Then 1ml of this solution was transferred to a test tube. Thereafter, 0.5ml of 2N Folin- Ciocalteu reagent and 1.5 ml of 20% Na₂CO₃ solution was added. The final volume was made up to 8ml with distilled water. The mixture was shaken vigorously, and then allowed to stand for 2 hours, at 25°C. Thereafter, the absorbance was taken at 765nm. The data was use to estimate the total phenolic content using a calibration curve obtained from various diluted concentration of gallic acid.

Alkaloids

2g of extract residue was dissolved in 5ml of 2M HCl and then filtered through Whatman #1 filter paper. 1ml of the solution was transferred to a separating funnel and washed 3 times with 10ml portions of chloroform. The pH of this solution was adjusted to 7.0, with 0.1 N NaOH, followed with addition of 5ml of BCG solution and 5ml of phosphate buffer solution. The extract was diluted to 10ml with chloroform, in a volumetric flask. Atropine (0.25, 0.50 and 1.0 mg/ml) was used as standard for the calibration curve, and was carried through the above procedure. The absorbance of the complex in chloroform was measured at 470nm with the spectrophotometer (Spectrumlab 352s), against the blank prepared as above but without Atropine.

Flavonoid

0.5g of the homogenized plant material was extracted in 50ml of methanol by sonication, in an ultrasonic bath, for 30 min. 1ml of the extract in methanol was mixed with 1ml of methanol, 0.5 ml of 1.2% aluminium chloride solution, and 0.5ml potassium acetate (120 nm). The mixture was allowed to stand for 30 min at room temperature for color development. Thereafter, the absorbance was measured at 415nm. Quercetin was used as standard, and was carried through the procedure. The flavonoid content is expressed in terms of quercetin equivalent (mg/kg) of extracted compound.

Qualitative Determination of Phytochemicals

Test for Phenol

5ml of the extract was pipetted into a 3ml test tube, then 10ml of distilled water was added. 2ml of ammonium hydroxide solution and 5ml of concentrated amyl alcohol were also added and left to react for 30mins. Development of bluish green color was taken as a positive presence of phenol.

Test for Tannin

0.5g of the sample was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

Test for Terpenes

5ml of each extract was mixed in 2ml of chloroform, followed by 3ml of concentrated H₂SO₄ to form a layer. A reddish brown coloration of the interface was formed to show positive results for the presence of terpenes.

Test for Alkaloid

A 5mg sample of the extract dissolved in 3ml of acidified ethanol was warmed slightly and then filtered. Few drops of Mayer reagent and 1ml of Dragendroff reagents were added to 1ml of the filtrate while turbidity was observed.

Test for Saponin

2g of the sample was boiled in 20ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously until the formation of emulsion is observed.

Test for Flavonoid

5ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H₂SO₄.

Digestion and Measurement of Heavy metal (Cd, Cu, Cr, Pb, Zn, As, Ni)

Heavy metals were digested and measured following the method described by Adu *et al.* (2020).

Statistical Analysis

Data were computed using statistical package for social sciences (SPSS, version 20). The differences in the means were tested using one-way analysis of variance (ANOVA) while the significant level was calculated as $p < 0.05$.

RESULTS

The qualitative results of the phytochemicals of *Piper guineense* (dry and wet samples) from Igando, Ikotun and Egbeda markets in Lagos, Nigeria, revealed the presence of Alkaloids, Tannins, Flavonoids, Terpenes, Saponins and Phenols. While Table I showed the quantitative phytochemicals of *P. guineense*, the concentration of heavy metals (dry and wet sample) in *P. guineense* from the three markets are presented in Table II. On the other hand, the World Health Organization (WHO) heavy metal's recommended limits for cultivated plants and soil is shown in Table III.

The amount of alkaloids in the dry samples from the three markets ranged from 40.43 ± 18.08 - 53.12 ± 0.14 mg/ 100g and the wet samples (29.87 ± 0.02 - 37.66 ± 0.00 mg/ 100g). Alkaloids (dry and wet weight) in *P. guineense* gotten from Igando market was significantly ($p < 0.05$) higher than the values recorded from other markets. The concentrations of tannins in the dry samples from the three markets ranged from 4.44 ± 0.01 - 9.06 ± 4.17 mg/ 100g while the wet samples varied from 2.97 ± 0.02 to 4.12 ± 0.00 mg/ 100g. The highest tannins recorded in *P. guineense* was gotten from Egbeda market in comparison with that from Ikotun and Igando.

Flavonoids contents in the dry samples from Igando, Ikotun and Egbeda markets ranged from 49.77 ± 25.60 - 67.99 ± 0.17 mg/100 g, while 12.65 ± 0.01 - 23.98 ± 0.00 mg/100 g was recorded in the wet samples. The contents of Flavonoids in *P. guineense* from Igando market was

significantly higher than flavonoids obtained in other markets. The quantity of terpenes in the dry samples from the three markets ranged from 6.98 ± 0.00 - 14.63 ± 0.18 mg/ 100 g while in the wet samples, 4.96 ± 0.02 - 8.95 ± 0.00 mg/100 g was obtained. Terpenes in *Piper guineense* gotten from Igando market was the highest among the markets. The amount of saponins in the dry and wet samples respectively were 2.67 ± 0.00 - 5.99 ± 0.14 mg/100g and 2.02 ± 0.01 - 4.76 ± 0.00 mg/100 g. The amount of phenols in the dry samples ranged from 4.55 ± 0.00 to 6.02 ± 0.11 mg/100g while in the wet samples, the values are 1.88 ± 0.02 to 3.45 ± 0.02 mg/ kg. Samples from Igando market had the peak saponin and phenol contents.

Table I: Phytochemicals (dry and wet Samples) of *Piper guineense* from three markets in Lagos, Nigeria

Locations						
Igando			Ikotun		Egbeda	
	Dry weight	Wet weight	Dry weight	Wet weight	Dry weight	Wet weight
Alkaloids	53.12 ± 0.1^{ab}	37.66 ± 0.00^a	49.88 ± 0.00^b	29.87 ± 0.02^{bb}	40.43 ± 18.08^{bc}	33.21 ± 0.01^c
Tannins	6.21 ± 0.13^a	4.12 ± 0.00^b	4.44 ± 0.01^b	2.97 ± 0.02^{aa}	9.06 ± 4.17^c	4.08 ± 0.00^b
Flavonoids	67.99 ± 0.17^a	23.98 ± 0.00^{bb}	58.87 ± 35.3^b	12.65 ± 0.01^{ab}	49.77 ± 25.60^{bc}	23.88 ± 0.00^c
Terpenes	14.63 ± 0.18^a	8.95 ± 0.00^{ab}	6.98 ± 0.00^{bb}	4.96 ± 0.02^b	8.86 ± 8.34^{ac}	6.16 ± 0.01^b
Saponins	5.99 ± 0.14^a	4.76 ± 0.00^{aa}	2.67 ± 0.00^b	2.02 ± 0.01^{ab}	3.11 ± 3.94^b	2.14 ± 0.00^{ab}
Phenols	6.02 ± 0.11^a	2.97 ± 0.00^{aa}	4.55 ± 0.00^b	1.88 ± 0.02^{ab}	5.13 ± 1.37^b	3.45 ± 0.02^{ab}

Mean $\pm$ SD (mg/100g) with difference superscript in the row = significant different ($p < 0.05$)

As presented in Table II, the level of metals(mg/kg) are as follows: Arsenic in *Piper guineense* (dry and wet) from Igando, Ikotun and Egbeda markets respectively are: (0.05 ± 0.01 and 0.04 ± 0.02), (0.05 ± 0.03 and 0.04 ± 0.01) and (0.05 ± 0.07 and 0.05 ± 0.02). The content of Cadmium in *P. guineense* (dry and wet weight) from Igando, Ikotun and Egbeda was (0.05 ± 0.04 and 0.04 ± 0.00), (0.05 ± 0.03 and 0.04 ± 0.01) and (0.04 ± 0.02 and 0.03 ± 0.02) respectively. Copper content in *P. guineense* (dry and wet) from Igando, Ikotun and Egbeda was (0.05 ± 0.01 and 0.04 ± 0.02), (0.05 ± 0.03 and 0.04 ± 0.01) and (0.05 ± 0.07 and 0.05 ± 0.02) respectively. The amount of chromium in *P. guineense* (dry to wet) from Igando, Ikotun and Egbeda was (0.04 ± 0.01 , 0.04 ± 0.02), (0.05 ± 0.01 , 0.03 ± 0.00) and (0.04 ± 0.01 , 0.02 ± 0.02) respectively. Lead concentration in *P. guineense* (dry to wet) from Igando, Ikotun and Egbeda was (0.03 ± 0.00 , 0.03 ± 0.03), (0.04 ± 0.00 , 0.02 ± 0.03) and (0.05 ± 0.01 , 0.04 ± 0.03) respectively. The amount of nickel in *P. guineense* (dry and wet weight) from Igando, Ikotun and Egbeda was (0.05 ± 0.01 , 0.02 ± 0.01), (0.05 ± 0.00 , 0.03 ± 0.01) and (0.05 ± 0.01 , 0.05 ± 0.02) respectively. The amount of zinc in *P. guineense* (dry and wet weight) from Igando, Ikotun and Egbeda respectively was (0.37 ± 0.02 , 0.26 ± 0.00), (0.29 ± 0.00 , 0.14 ± 0.00) and (0.31 ± 0.10 , 0.19 ± 0.00). There was no significant difference ($p > 0.05$) in contents of all heavy metals detected in *P. guineense* across the three markets.

Table II: Heavy metals in *Piper guineense* (Dry and wet weight) from three markets in Lagos, Nigeria

	Locations					
	Igando		Ikotun		Egbeda	
	Dry weight	Wet weight	Dry weight	Wet weight	Dry weight	Wet weight
Arsenic	0.05± 0.01 ^a	0.04 ± 0.02 ^a	0.05 ± 0.03 ^a	0.04 ± 0.01 ^a	0.05± 0.07 ^a	0.05 ± 0.02 ^a
Cadmium	0.05± 0.04 ^a	0.04 ± 0.00 ^a	0.05 ± 0.01 ^a	0.04± 0.01 ^a	0.04± 0.02 ^a	0.03± 0.02 ^a
Copper	0.12± 0.00 ^a	0.08 ± 0.00 ^a	0.19 ± 0.00 ^a	0.10 ± 0.00 ^a	0.08± 0.06 ^a	0.04± 0.00 ^a
Chromium	0.04± 0.01 ^a	0.04± 0.02 ^a	0.05 ± 0.01 ^a	0.03 ± 0.00 ^a	0.04± 0.01 ^a	0.02± 0.02 ^a
Lead	0.03± 0.00 ^a	0.03± 0.03 ^a	0.04 ± 0.00 ^a	0.02 ± 0.03 ^a	0.05± 0.01 ^a	0.04 ± 0.03 ^a
Nickel	0.05± 0.01 ^a	0.02± 0.01 ^a	0.05 ± 0.00 ^a	0.03 ± 0.01 ^a	0.05± 0.03 ^a	0.05 ± 0.03 ^a
Zinc	0.37 ± 0.02 ^a	0.26 ± 0.00 ^a	0.29 ± 0.00 ^a	0.14 ± 0.00 ^a	0.19± 0.00 ^a	0.19± 0.00 ^a

Mean ±SD(mg/kg) with difference superscript in the row = significant different (p<0.05)

Table III: WHO permissible limits for heavy metals in plants and soil

Elements	Targets value of soil (mg/kg)	Permissible value of plant (mg/kg)
Cd	0.8	0.002
Zn	50	0.60
Cu	36	10
Cr	100	1.30
Pb	85	2
Ni	35	10

Target values are specified to indicate desirable maximum levels of elements in unpolluted soils Source: WHO (1996)

The frequency distributions of the phytochemicals in *P. guineense* for dry and wet weight respectively are shown in Figure 1 and 2. For the leaf dry weight, most frequent and least phytochemicals respectively across the markets are: flavonoid and saponin(Igando market), alkaloid and saponin (Ikotun market) and flavonoid and saponin(Egbeda), while for the wet weight, the most frequent and least phytochemicals are: alkaloid and phenol (Igando market), alkaloid and phenol(Ikotun market), alkaloid and saponin (Egbeda market) .

Figure 1: Dry Weight of phytochemical compound in *P. guineense* leaves

Figure 2: Wet Weight of phytochemical compound in *P. guineense* leaves

On the other hand, the frequency distributions of heavy metals in *P. guineense* for dry and wet weight respectively are shown in Figure 3 and 4. For the dry weight, most frequent and least metals respectively are: Igando market (zinc and lead), Ikotun market (zinc and lead) and Egbeda market (copper and cadmium) while for the wet weight, the highest and least heavy metals are: Igando (zinc and nickel), Ikotun (zinc and lead) and Egbeda (zinc and chromium) .

Figure 3: Dry Weight of heavy metals composition in *Piper guineense* leaves

Fig 4: Wet Weight of heavy metal composition in wet *Piper guineense* leaves

Discussion

Phytochemical analysis is very useful in the evaluation of some active biological components of some vegetables. The phytochemical composition of *Piper guineense* (Uziza leave) recorded in this study which revealed the presence of alkaloids, tannins, saponins, flavonoids, terpene, and phenols was similar to that examined at Osaa-Oke in Obingwa L.G.A, Aba, Abia State (Chiwendu *et al.*, 2016). Dibolu *et al.*(2017) reported that leaf extracts of *P. guineense* has

strong or intense positivity for alkaloids, moderate positivity for flavonoids, steroids, tannins, terpenoids, cardiac glycosides and total phenols, and weak positivity for saponins. On the other hand, Besong *et al.* (2016) reported that *P. guineense* contains Alkaloids (piperine, wisamine, dihydrowasamine, dihydropiperine), flavonoids, saponins, tannins, resins and essential oil (dillapiole, elemicin, cardiac glycosides).

In this study, the dry samples showed higher level of bioactive compounds than the wet samples. The reason may be that the bioactive compounds are not volatile compounds and hence have a high dried weight. This result is in correlation with the findings of Aja *et al.*, (2010). High level of flavonoids which ranged from 67.99 ± 0.17 mg/100g in the examined dry samples to 12.65 ± 0.1 mg/ 100g in the wet samples implied that *P. guineense* is good in the control of inflammation and cancer prevention as opined by Winkel-shirley (2001). Flavonoids may help provide protection against this disease by contributing along with antioxidants vitamins and enzymes, to the total antioxidant defense system to the human body. The results of the present study also showed low level of tannins, saponins and phenols. Since, the values are low, it could indicate that these phytochemicals found in *P. guineense* are not high enough to constitute human poison. Similar low concentrations of phytochemicals have been reported by Chinwendu *et al.* (2016).

Heavy metal is not toxic per se but become toxic only when its concentration in the plant and animal exceeds a certain threshold, that is the dose makes the poison. Heavy metals are significant environmental pollutants and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons (Jaishankar *et al.*, 2014). The level of cadmium reported in *P. guineense* in this study from the three markets was not higher than the maximum permissible limit (0.002 mg/kg) recommended by World Health Organization (WHO). Similarly, the concentration of zinc and copper were lower than the standard permissible limit of 10mg/kg. The levels of arsenic, chromium, lead and nickel in *P. guineense* from the three markets are all lower than the permissible limit recommended by WHO as shown in Table 3.

Conclusion

The study has shown that *Piper guineense* is a potential nutritive and medicinal vegetable. The phytochemical analysis of *P. guineense* serves as a good complement for food and also contains some considerable amount of anti-oxidant such as alkaloid, flavonoids and terpenes which could contribute some health benefit to its consumers. *P. guineense* from Igando had the highest alkaloids, flavonoids, saponins, terpenes and phenol; while the highest tannin content was found in *P. guineense* from Egbeda. All the metals in the *P. guineense* were below WHO maximum permissible limit. The findings therefore indicated that *P. guineense* collected from the markets are safe for consumption and good for medicinal use.

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