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Phytochemical and heavy metal contents in *Gnetum africanum* (Welw. 1984) collected from three local markets in Lagos, Nigeria

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Abstract
Gnetum africanum (Okazi leave) is an evergreen climbing vegetable which is highly priced in most regional markets in Nigeria. The present study examined the composition of phytochemicals (Alkaloids, Tannins, Flavonoids, Terpenes, Saponins, and Phenols) and heavy metals (Zinc, Lead, Copper, Cadmium, Chromium, Nickel and Arsenic) in *G. africanum* collected from three different markets (Iyana-Iba, Igando and Ikotun markets) in Lagos state, Nigeria. All analyses were done using standard analytical procedures. The highest dry weight of alkaloid (83.89±0.03mg/100g), flavonoid(108.75±1.11 mg/100g) and phenol(10.07±0.06 mg/100g) were obtained in *G. africanum* from Igando market; the peak tannins and saponins with values of 14.61±0.01 mg/100g and 4.08±0.00 mg/100g respectively were recorded in *G. africanum* from Ikotun market, while the highest dry weight for terpenes (23.64±0.03 mg/100g) was recorded in samples from Iyana-Iba market. On the contrary, the least dry weight for alkaloid(62.12±0.01mg/100g), phenol (6.22±0.00mg/100g), flavonoids (81.66±0.04 mg/100g) and terpenes (9.64±0.01 mg/100g) were recorded in samples from Ikotun market, while the least saponin (1.22±0.01 mg/100g), and tannins (7.95±0.03 mg/100g) were obtained from samples in Igando market. G**.** *africanum* from Iyana-Iba market possessed the highest wet weight value of alkaloids (62.07±0.01 mg/100g) and terpenes (15.99±0.03 mg/100g); samples of Ikotun market had highest wet weight in tannins (9.95±0.01 mg/100g) and saponin(3.33±0.03 mg/100g), while samples at Igando market had peak flavonoid and phenol (77.23±0.01 mg/100g, 7.12±0.01 mg/100g) wet weight respectively. All the levels of heavy metals (dry and wet weights) determined in the samples were not significantly different across the three markets ($p>0.05$). In conclusion, the secondary metabolites recorded in the leaves of *Gnetum africanum* indicated a good medicinal status while the heavy metal content in the leaves being below permissible limits indicated that the plants from the three markets is safe for consumption.

Keywords: Metabolites, metals, *Gnetum africanum*, edible, safety, medicinal

1. Introduction
Vegetables constitute an important part of the human diet since they contain carbohydrates, proteins, vitamins, minerals as well as trace elements. Several varieties of leafy vegetables either in the wild or under cultivation in the rural areas have been cited as a potential source of micronutrients [1-2]. Often time, many vegetable particularly the leafy vegetables are mainly consumed for their nutritional values without much consideration for their medicinal importance. *Gnetum africanum* commonly called "Okazi" by Igbos and "Afang" by Efik of Nigeria is one of the most popular green leafy vegetables in Nigeria and has gained popularity as a delicious food leaf in other African countries such as Cameroon, Gabon, Congo and Angola. *G. africanum* leaves are widely consumed in the South Eastern Nigeria due to its palatability and taste and often cooked with *Talinium triangulare* (water leaf) to give the soup a special flavor [2].

The vegetable grows as a wild evergreen climbing vegetable in the rain forest of Nigeria but recently has been cultivated successfully in farms. Different authors have reported on phytochemicals, anti-nutritional, mineral composition and other essential contents in *G. africanum.* For instance, the chemical composition of both wild and domestic species of *G. africanum* collected from Urua, Akpan, Ndem in Uyo and AkwaIbom State University, Obio Akpa Campus Teaching and Research Farms respectively, had been reported [3]. Due to large consumption rate of leafy vegetables in Nigeria, frequently assessment of their safety status for consumption cannot be over emphasized. The aim of this study is to access the phytochemical and heavy metals in *Gnetum africanium* from three [3] selected markets in Lagos State: Iyana-Iba, Igando and Ikotun.

2. Materials and Methods

2.1 Sampling Sites

Samples were collected between November and December 2019 from three major markets- Iyana-Iba, Igando and Ikotun in Lagos State, Nigeria. Igando and Ikotun markets are located in Alimosho Local Government Area (LGA) while Iyana-Iba market is under Ojo LGA.

2.2 Pre-Treatment Methods for Plant Samples

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

2.3 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.

2.4 Qualitative determination of phytochemicals

2.4.1 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.

2.4.2 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.

2.4.3 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 H_2SO_4.

2.4.4 Test for Terpenes

(Salkowski test)5ml of each extract was mixed in 2ml of chloroform, followed by 3ml of concentrated H_2SO_4 to form a layer. A reddish brown coloration of the interface was formed to show positive results for the presence of terpenes.

2.4.5 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's reagent and 1ml of Dragendroff's reagents were added to 1ml of the filtrate while turbidity was observed.

2.4.6 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.

2.5 Quantitative Determination of Phytochemicals

2.5.1 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.

2.5.2 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 through the procedure.

2.5.3 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.

2.5.4 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.

2.5.5 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.

2.5.6 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.

2.6 Heavy metal Digestion and Measurement
Heavy metals was digested and measured following the method described in literature [4].

2.7 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 $p< 0.05$.

3. Results
The phytochemicals (alkaloids, tannins, flavonoids, terpenes, saponins and phenols) and heavy metals (arsenic, cadmium, copper, chromium, lead, nickel and zinc) were detected in all the samples collected from the three markets. The results of the quantitative phytochemical composition (in mg/100g) and heavy metals (in mg/kg) in *G. africanum* are presented in Tables 1 and 2 respectively. While the highest dry weight of alkaloid (83.89±0.03), flavonoid (108.75±1.11) and phenol (10.07±0.06) were obtained in *G. africanum* from Igando market, the peak tannins and saponins with values of 14.61±0.01 and 4.08±0.00 respectively were recorded in *G. africanum* from Ikotun market. However, highest dry weight for terpenes (23.64±0.03) was recorded in samples from Iyana-Iba market. On the contrary, the least dry weight for alkaloid(62.12±0.01), phenol (6.22±0.00), flavonoids (81.66±0.04) and terpenes (9.64±0.01) were recorded in samples from Ikotun market, while the least saponin (1.22±0.01), and tannins (7.95±0.03) were obtained from samples in Igando market.

G**.** *africanum* from Iyana-Iba market possessed the highest wet weight value of alkaloids(62.07±0.01) and terpenes (15.99±0.03); samples of Ikotun market had highest wet weight in tannins (9.95±0.01) and saponin(3.33±0.03), while samples at Igando market had peak flavonoid and phenol (77.23±0.01, 7.12±0.01) wet weight respectively. Figures 1 and 2 showed the distribution of the phytochemicals in term of wet and dry weight respectively in G**.** *africanum* from the three markets. Flavonoid was the most abundant, followed by alkaloids across the markets.

The levels of all heavy metals (dry and wet weights) determined in the samples as presented in Table 2 were not significantly different across the three markets. Although zinc had the highest wet and dry weights respectively (0.14±0.03mg/kg, 0.19±0.00mg/kg) in the examined G. *africanum* and these values were recorded in samples from Iyana-Iba. The plot of frequency distribution of the metals for the wet weight (Figure 3) indicated that Zn, Cu and Zn respectively had the highest values in samples of *G. africanum* from Iyana-Iba, Igando and Ikotun. On the other hand, the metals with highest concentration in samples of *G. africanum*(dry weight) from Igando and Ikotun was Cu, while Zn concentration was highest in samples from Iyana-Iba(Figure 4).

Table 1: Quantification of Phytochemicals in G. *africanum* from three markets in Lagos State, Nigeria

	Locations					
	Iyana-Iba		Igando		Ikotun	
Phytochemicals	**Wet weight (mg/100g)**	**Dry weight (mg/100g)**	**Wet weight (mg/100g)**	**Dry weight (mg/100g)**	**Wet weight (mg/100g)**	**Dry weight (mg/100g)**
Alkaloids	62.07±0.01^{a}	78.54±0.01^{ab}	51.12±0.00^{b}	83.89±0.03^{ac}	34.97±0.04^{c}	62.12±0.01^{bb}
Tannins	7.66±0.00^{a}	11.65±0.01^{b}	8.43±0.01^{a}	7.95±0.03^{c}	9.95±0.01^{ab}	14.61±0.01^{bc}
Flavonoids	58.87±0.01^{a}	92.63±0.00^{ab}	77.23±0.01^{b}	108.75±1.11^{ac}	49.86±0.01^{c}	81.66±0.04^{bc}
Terpenes	15.99±0.03^{a}	23.64±0.03^{ab}	11.01±0.01^{b}	14.08±0.01^{bb}	6.87±0.01^{c}	9.64±0.01^{aa}
Saponins	1.78±0.01^{a}	3.96±0.04^{ab}	1.02±0.01^{a}	1.22±0.01^{bb}	3.33±0.03^{b}	4.08±0.00^{ab}
Phenols	5.87±0.01^{ab}	7.75±0.01^{b}	7.12±0.01^{ab}	10.07±0.06^{bb}	5.04±0.01^{ab}	6.22±0.00^{ac}

Mean ±SD with different superscript in the row = significant different ($p< 0.05$)

Table 2: Heavy metals content in G. *africanum* from three markets in Lagos State, Nigeria

	Locations					
	Iyana-Iba		Igando		Ikotun	
Parameters	**Wet weight (mg/kg)**	**Dry weight (mg/kg)**	**Wet weight (mg/kg)**	**Dry weight (mg/kg)**	**Wet weight (mg/kg)**	**Dry weight (mg/kg)**
Arsenic	0.02± 0.01	0.03±0.01	0.01±0.00	0.03±0.03	0.03±0.01	0.03±0.00
Cadmium	0.04±0.03	0.03±0.01	0.03±0.01	0.03±0.02	0.03±0.01	0.04±0.01
Copper	0.06±0.01	0.09±0.01	0.08±0.01	0.15±0.01	0.05±0.00	0.06±0.01
Chromium	0.03±0.02	0.04±0.01	0.04±0.02	0.02±0.00	0.03±0.02	0.04±0.04
Lead	0.04±0.01	0.05±0.03	0.03±0.01	0.03±0.00	0.01±0.00	0.02±0.00
Nickel	0.03±0.01	0.03±0.01	0.02±0.00	0.03±0.02	0.02±0.01	0.04±0.01
Zinc	0.14±0.03	0.19±0.00	0.05±0.00	0.07±0.01	0.02±0.01	0.07±0.01

Mean ±SD with no superscript in the row = non -significant different ($p>0.05$)

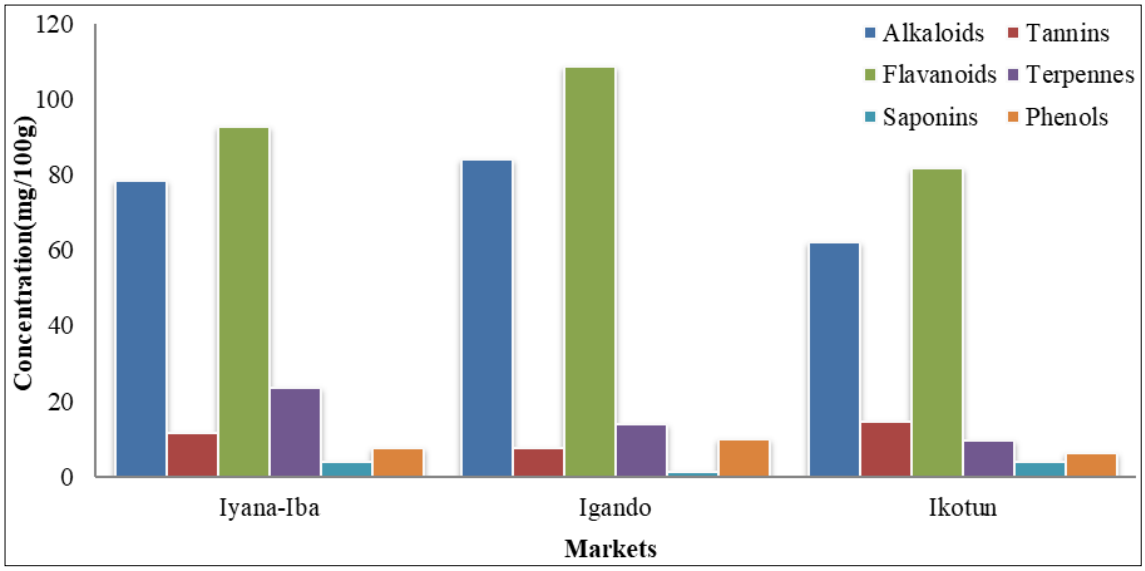

Fig 1: Wet weight of Phytochemicals on dry G. *africanum* from the sampled markets.

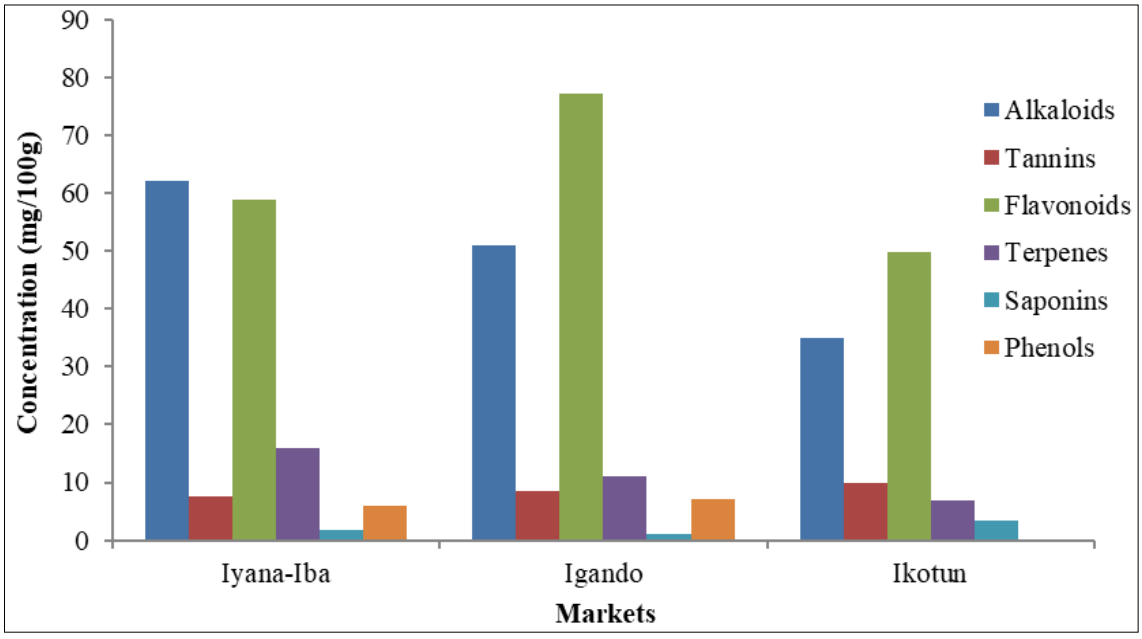

Fig 2: Dry weight of Phytochemicals on wet G. *africanum* from the sampled markets

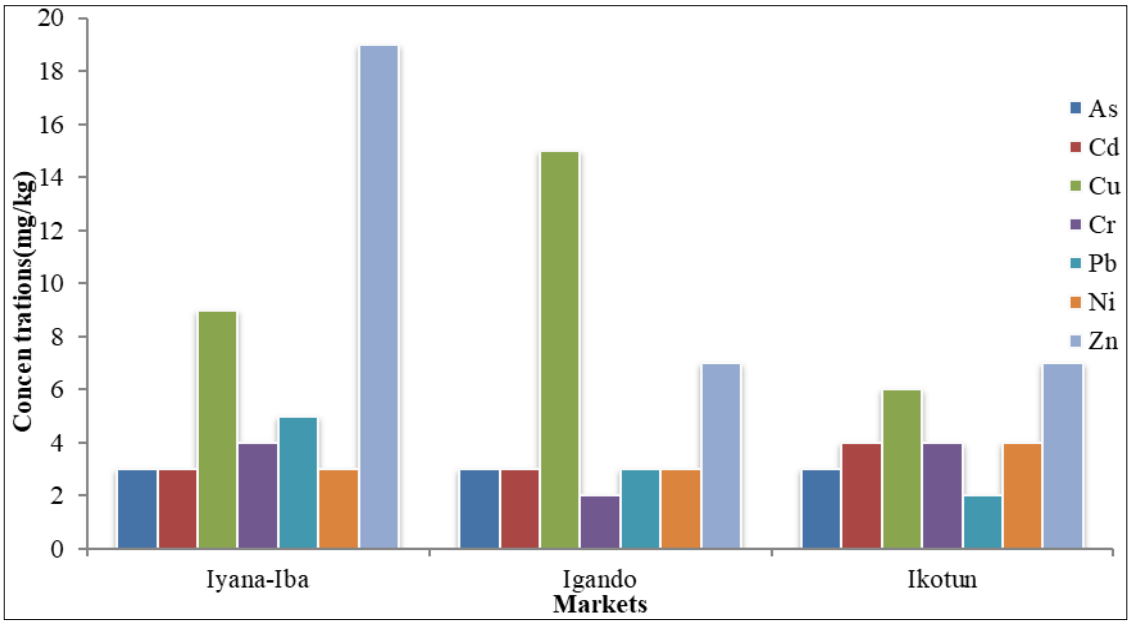

Fig 3: Wet Weight of heavy metals on dry G. *africanum* from the sampled markets

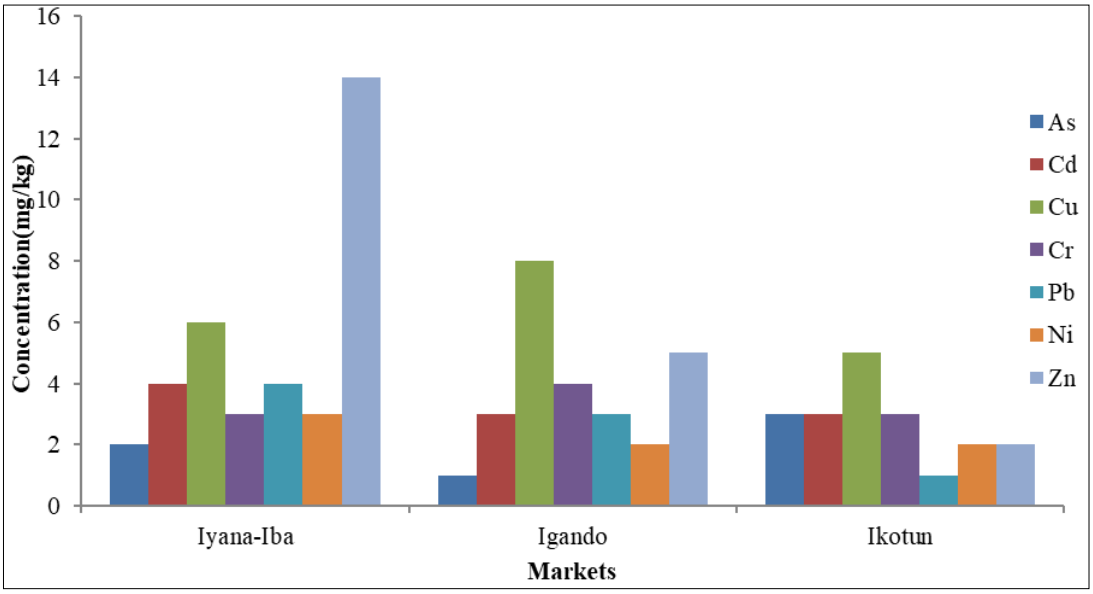

Fig 4: Dry Weight of heavy metals on wet G. *africanum* from the sampled markets

4. Discussion

In this study, the qualitative analysis for both wet and dry samples of *Gnetum africanum* revealed the presence of phytochemicals (secondary metabolites): alkaloids, tannins, flavonoids, terpenes, saponins and phenols, although flavonoid was the most abundant, followed by alkaloids across the markets. This observation was similar to previous findings on the same plant species [5]. The concentrations of all the phytochemicals recorded in this study were higher than that reported on *Talinum triangulare* [6]. Tannins help in healing wounds and inflamed mucous membrane [7] while flavonoids are known for its antioxidant activities and act as detoxifiers with the ability to modify a cell's reaction to carcinogens, viruses and allergens [5, 8]. Most saponins which readily dissolve in water are poisonous to fish [9]. However, saponins may serve as anti-feedants but due to its bitter taste, can reduce plant palatability in livestock feed or even induce them with life threatening animal toxicity [10]. The medicinal properties of most secondary metabolites in *G. africanum* have been reported in Nigeria [11].

The significant differences obtained in the concentration of phytochemicals in *G. africanum* across the three markets buttress the report that environmental factors: climate, soil pH, soil type etc. could result in change in the intrinsic properties of a plant [6]. In the present study, *G. africanum* from Igando market had an edge over samples from other markets in term of phytochemical content. However, all the samples obtained from the three (3) markets are considered edible with moderate concentrations of the secondary metabolites. All the heavy metals recorded in *G. africanum* from the three markets were below the standard maximum permissible limits for metals in plant. All the concentrations of metals in this study were lower than that reported in *Spinacia oleracea* [12]. The low level of metals obtained in *G. africanum* could imply that the three markets as well as sources of cultivation of the plant are less contaminated with metals. Similarly, low heavy metals concentration in *G. africanum* from Uyo, AkwaIbom in Nigeria has been reported [14]. However, an increased levels of heavy metals was reported for leafy vegetables selected from markets in Onyana and this increase was attributed to atmospheric deposits at the market [15]. Generally, the values of metals recorded in this study revealed drastic decrease from wet to dry samples. This could imply that the quantity of heavy metal is higher when freshly harvested than when in dry form.

5. Conclusion

The samples of *G.africanum* collected from the three markets possess high medicinal values as shown in their phytochemical contents. Also, all heavy metals concentration recorded for the study were below maximum standard recommended permissible limit, hence the plants are considered safe for human consumption.

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