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pH Variation, Mineral Composition and Selected Trace Metal Concentration in Some Liquid Herbal Products Sold in Nigeria

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Abstract: *This study investigated the levels of pH, minerals (calcium, magnesium, sodium and potassium) and trace metals (nickel, iron, zinc, lead and cobalt) in nine herbal medicines produced in Nigeria. Samples for metals were processed, digested and analyzed by atomic absorption spectrometry while phosphorus content was analyzed colorimetrically. The pH was analyzed electrometrically using pH meter with probe. The mean levels of metal in the liquid herbal medicine samples (alkaline earth metals viz: calcium, magnesium, sodium and potassium, and trace metals viz: nickel, iron, zinc, lead and cobalt). Test parameters depicted the range of 3.00 – 5.09 pH, 0.44 – 10.76 mg/l phosphorus, 2.03 – 202.68mg/l potassium, 2.04 – 26.38 magnesium, 7.04 – 9.74 mg/l sodium and 14.33 – 109.55 mg/l calcium. The trace metals were in the range of <0.001 - 0.068mg/l nickel, <0.001 - 0.024mg/l zinc, <0.001 - 0.177 mg/l cobalt and 1.302 – 27.081 mg/l iron. The occurrence of nickel, zinc, lead, iron and cobalt in the liquid herbal medicine were 22.22%, 22.22%, 0.00%, 100% and 33.33% respectively. Analysis of variance showed that there was significant variations ($p<0.05$) among the various herbal medicine products apart from lead which was not detected in all the samples. Results obtained for test parameters were within the level that could not induce significant toxicity on consumers. However, some of the products were yet to be registered with the National Association of Traditional Medicine Practitioner of Nigeria (a Nigerian agency regulating the activities of traditional medicine practitioners), and National Agency for Food, Drug and Administration and control (a Nigerian Agency regulating food and drugs). Hence, the need for a more stringent regulation and surveillance of the products become pertinent.*

Keywords: *Humans, Toxicity, Traditional Medicine, Trace metals, Minerals*

1. INTRODUCTION

Globally, the use of herbal medicine in treatment of various forms of diseases is still very high. World health organization have reported that 70 – 80% of global population relies heavily on herbal medicine for the treatment of diseases (Epidi *et al*., 2016a,b; Kigigha *et al*., 2015, 2016, 2018; Izah *et al*., 2018a-d; Izah, 2018; Izah and Aseibai, 2018; Onimisi *et al*., 2016). Most of the individuals that rely on herbal medicine for treatment are from developing nations in many rural communities. In Nigeria probably due to increased patronage there has been an increase in herbal medicine in the Nigerian market, this comprises of both imported and locally made products (Onimisi *et al*., 2016).

In Nigeria, some of the herbal medicine currently in market today have been registered with the National Agency for Drug Administration and Control (NAFDAC) - the Nigerian body responsible for the Regulation of food and drug matters (Omimisi *et al*., 2016), while several others are yet to be registered (Umar *et al*., 2016). The use of medicine from herbs has unconsciously exposed consumers to high concentrations of toxic metals (Adepoju-Bello *et al*., 2012). This is because most of the active ingredients of herbal medicines emanate from plant materials which have the tendency to take up metallic ions when they are grown in contaminated soil (Izah and Angaye, 2016). Furthermore, plants pick up heavy metals from their environment through atmospheric contamination from industries, packaging material and other mediums (Adepoju-Bello *et al*., 2012).

Typically, heavy metals are metalloids with higher specific gravity ≥ 5and molecular weight (Idris *et al*., 2013; Izah *et al*., 2016, 2017a,b; Izah *et al*., 2018e,f). Heavy metals are basically classified based on their toxicity, that is, essential (metals required by living organisms at certain concentrations) including iron, zinc, copper, manganese, chromium etc, and non-essential heavy metals (metals that do not have any biological functions in livings organisms and could induce toxicity even at low concentrations. The toxicity of these heavy metals has been widely reported in literature.

The consumption of herbal medicine in developing nations, including Nigeria, is still prevalent especially among individuals that reside in rural area. In Nigeria, National Association of Traditional Medicine Practitioner of Nigeria (a Nigerian agency regulating the activities of traditional medicine practitioners), and National Agency for Food, Drug and Administration and control (a Nigerian Agency regulating food and drugs products) regulates the activities of traditional medicine practitioners. However, some individuals that produce and sell this herbal medicine are yet to be registered with either of the agencies. As such, the safety and quality of these prepared herbal medicines are a potential source of concern for health authorities, pharmaceutical industries and the public at large (that is the end user of such remedies).

A number of elements (including macro and trace)are important to plants but portend harmful effect at high concentrations, especially to plants, animal and human that consume them. Hence, medicinal plants require special processing and treatment with regard to their elemental composition. Authors have reported that the pharmacological effects of most plants are due to the presence of the active compounds or secondary metabolites they possess (Kigigha *et al*., 2018, 2016, 2015) and are influenced by the associated macro and micro elements they possess. Therefore, there is the need to regulate by way of assessing the metallic contents of these herbal products. This study is aimed at assessing the levels of pH, minerals and some trace metals in liquid herbal medicine sold in Nigeria.

2. MATERIALS AND METHODS

2.1. Sample Collection

A total of nine (9) products of liquid herbal medicine were purchased from retail outlets in Port Harcourt, Rivers State, Nigeria. Samples with date of production and expiring date were purchased. Other information about the active ingredients, state and country of production, registration details with National Association of Traditional Medicine Practitioner of Nigeria (a Nigerian agency regulating the activities of traditional medicine practitioners), and National Agency for Food, Drug and Administration and control (a Nigerian Agency regulating food and drugs products) of the various products under study is presented in Table 1.

Sample code	Ingredients	Duration	Manufactured In:	NAFDAC registration	National Association of Traditional Medicine Practitioner of Nigeria Registration
UNC	Aloe–vera plant Ginseng plant Moringa plant	June-2017-2022	Edo state, Nigeria	-	Yes
YCB	Aloe vera *Acinos arventis* *Citrus aurantifolia* *Chenopodium murale* *Cinamomum aromatic*	December 2017 – December 2019	Lagos state, Nigeria	Yes	-
RHB	Root of *Curculigo pilosa* Stem of *Uvaria chamae* Bark of *Citrullus colocynthis*	August 2018 – August 2020	Lagos state, Nigeria	Yes	-
UAM	-	February 2017 – January 2020	Enugu, Nigeria	Yes	Yes

IGC	*Vernonia amygdalina* *Saccharum officinarum* *Allium Sativum* *Cajanus cajan* *Zingiber officinale* Caramel Water	February 2018 – February 2020	Lagos state, Nigeria	Yes	-
SHM	Herbs Roots, seeds, barks, leaves *Aloe vera*	August 2018 – November 2021	Anambra State, Nigeria	-	Yes
EFM	Selected natural herbs and roots.	January 2017 – January 2020	Enugu State, Nigeria	-	-
GCB	*Citrus aurantifolia* leaves *Mangifera Indica* leaves *Vernonia amydgalina* leaves *Saccharum officinarum* leaves *Allium sativum* Caramel *Moringa oleifera* Water	August 2017 – July 2020	Lagos state, Nigeria	Yes	-
GHM	31 roots and herbs Fruits Barks	January 2018 – January 2022	Anambra State, Nigeria		

(-) information not available in the pack

2.2. Sample Preparation and Laboratory Analysis

2.2.1. Heavy Metals and Macro Nutrient

Samples were stored in their original bottles in a cool, dry place prior to commencement of analysis. 25 ml portion of well-mixed liquid herbal extract was sub-sampled and transferred into acid-washed and water-rinsed 100 ml pyrex glass beakers, 5 ml of concentrated nitric acid (HNO_3) was added before subjecting the mixture to a slow boil and evaporation on a hot plate. The mixture was concentrated to a volume of 10 ml before precipitation. Another 5 ml of concentrated nitric acid (HNO_3) was added to aid complete digestion (which is reflected by a light colored, clear solution) after further heating on the hotplate. Afterwards, the digest was left to cool. The wall of the beaker was washed down and filtrate transferred into a 25 ml volumetric flask, adding rinsing to the flask before diluting to mark. Portions of the filtrate were aspirated for macro and trace element analysis on the GBC Avanta A6600 flame atomic absorption spectrophotometer (FAAS) (Aigberua *et al*., 2017).

2.2.2. Phosphorus (Ascorbic Acid Method)

50 ml of herbal solution was transferred into a micro-kjedahl flask. 1 ml and 5 ml portions of concentrated sulphuric acid (H_2SO_4) and nitric acid (HNO_3) were added respectively. The mixture was then digested to a volume of 1 ml and continued until solution became colorless to remove HNO_3. The digest was cooled and approximately 20 ml of distilled water added, phosphorus was determined by adding the content of one phosver-5-phosphate reagent powder pillow to the mixture, a 5-minute reaction period was allowed and the presence of phosphorus was observed as samples depicted a tinge of blue coloration. Concentration of phosphorus (in mg/l) was determined using the HACH DR 890 colorimeter (APHA, 1995).

2.2.3. pH Determination

The pH was read electrometrically using calibrated HANNA HI8314 model pH meter dipped in herbal solution (Aigberua, 2018).

2.3. Statistical Analysis

Statistical package for Social Science version 20 was used for the statistical computation, Descriptive statistics was carried out and data were expressed was mean ± standard deviation. One way analysis of variance was carried out and Waller-Duncan statistics was used to compare the means. Pearson correlation matrix was used to show the relationship between the parameters under investigation in the liquid herbal medicine products.

3. RESULTS AND DISCUSSION

Results obtained for pH, mineral and selected heavy metals concentration in liquid herbal medicine sold in Nigeria is presented in Tables 2 and 3 respectively. While the Pearson correlation matrix is presented in Table 4. The pH values ranged from 3.00 – 5.09, being significantly different ($P<0.05$) among the various samples. Also, pH showed positive significant correlation with nickel ($r=0.476$, $P<0.05$) and cobalt ($r=0.415$, $P<0.05$) and negatively correlate with zinc($r= -0.482$, $P<0.05$) and iron ($r=-0.442$, $P<0.05$). Typically, pH in this context is a measure of the acidity of stomach. Studies have shown that the pH of gastric acid is 1-3 in the human stomach lumen (Marieb and Hoehn, 2010).Beasley *et al*. (2015) reported pH of the human stomach to be around 1.5. Russell *et al*. (1993) reported median pH of 1.3 for fasted gastric pH, 4.9 for gastric pH during the meal, 6.5 for fasted duodenal pH, and duodenal pH during the meal among young and elderly men, and women. The authors also reported that following a meal, gastric pH decreased from a peak pH of 6.2 to pH 2.0 within 4 hours among most of the group studied. As such, the values obtained for this study is close to what has been reported for some liquid herbal solutions(3.55 – 8.00) sold in Nigeria (Onwordi *et al*., 2015). Typically, the acidity of the herbal solutions does not possess the tendency to cause major health effect in humans. Generally the acidity of the stomach varies according to species and can be affected by age or other treatments (Beasley *et al*., 2015).

The concentration of phosphorus ranged from 0.44 – 10.76mg/l. There was significant variations ($p<0.05$) in the various samples. Phosphorus showed positive significant relationship with sodium ($r=0.399$, $P<0.05$) and calcium ($r=0.623$, $P<0.01$), and negatively correlated with cobalt ($r=-0.397$, $P<0.05$). Typically, phosphorus is the second most abundant mineral in the body and it constitute about 1% of total body weight (MedlinePlus, 2018). The authors also reported that play essential role in the formation of bones and teeth, utilization of carbohydrates and fats in the body, synthesis of protein for the growth, maintenance, and repair of cells and tissues, and also help the body for the production of ATP (MedlinePlus, 2018). Phosphorus is also vital for the functioning of the kidney, muscle contractions, normal heartbeat and nerve signaling (MedlinePlus, 2018). Based on the importance of phosphorus in the human body, the amounts found in the herbal medicine do not pose any major health challenge among individuals that use the products under study.

The minerals under study ranged from 2.03 – 202.68mg/l, 2.04 – 26.38 mg/l, 7.04 – 9.74 mg/l and 14.33 – 109.55 mg/l for potassium, magnesium, sodium and calcium respectively. Statistically, there was significant variation ($P<0.05$) in the concentration of the various herbal solutions for each of the minerals under study. Potassium showed positive correlation with magnesium ($r=0.864$, $P<0.01$) and zinc ($r=0.432$, $P<0.05$). Magnesium negatively correlated with sodium ($r=-0.641$, $P<0.01$) while calcium negatively correlated with cobalt ($r=-0.503$, $P<0.01$).These minerals (calcium, sodium, potassium and magnesium) are essential in human and living organisms in general. For instance, sodium ion is essential in the generation of nerve impulses and maintenance of the body electrolyte and metabolic functions of some organs (Izah *et al*., 2017c; Aigberua *et al*., 2018a). According to Palacios (2006), Izah *et al*. (2017a), Aigberua *et al*. (2018a), potassium help in maintaining normal pH and promoting alkaline environment that is essential for reducing the demand for skeletal salts to balance the endogenous acid produced from acid foods. In addition, potassium is also essential in carbohydrate metabolism, protein synthesis and other cellular metabolic processes (Haas, 2011; Izah *et al*., 2017c; Aigberua *et al*., 2018a). Calcium in the body is very essential in skeletal bone formation (Palacios, 2006; Izah *et al*., 2017c), functioning of certain nerves, blood clotting and activation of certain enzymes (Izah *et al*., 2017c; Aigberua *et al*., 2018a). Like calcium, magnesium is also

essential in bone development, ATP metabolisms and is a cofactor to several enzymes, and as such they are indirectly involved in metabolism of carbohydrate, lipids and proteins and other mineralsin the body. Generally, the occurrence of these minerals are in the order; sodium< magnesium<calcium<potassium.

Table2.*pH, phosphorus and some mineral level in liquid herbal products sold in Nigeria*

Sample code	pH	Phosphorus (mg/l)	Potassium (mg/l)	Magnesium (mg/l)	Sodium (mg/l)	Calcium (mg/l)
YCB	3.17±0.00b	1.70±0.00c	9.27±0.01b	2.04±0.02a	8.26±0.03b	23.34±0.02c
UAM	5.09±0.02i	1.10±0.10b	95.74±0.01g	17.73±0.04h	7.24±0.01a	14.33±0.04a
RHB	3.00±0.00a	0.53±0.02a	202.68±1.02i	26.38±0.12i	7.08±0.03a	43.96±0.03g
GHM	3.89±0.03e	7.60±0.10e	32.48±0.43d	16.61±0.31g	7.28±0.29a	109.55±0.97h
SHM	4.36±0.01g	9.77±0.15f	107.07±1.07h	13.74±0.23f	9.74±0.00c	108.67±2.12h
UNC	4.24±0.02f	2.47±0.31d	51.27±0.26e	10.17±0.17e	9.35±0.32c	28.60±0.71d
GCH	3.73±0.03d	10.76±0.14g	2.03±0.03a	3.65±0.03b	9.54±0.52c	32.28±0.35e
EFM	4.63±0.03h	1.50±0.00c	67.81±0.21f	8.38±0.01c	9.44±0.04c	19.08±0.08b
GCB	3.28±0.02c	0.44±0.03a	20.74±0.20c	9.01±0.54d	8.67±0.01b	40.77±0.17f

Data is expressed as mean ± standard deviation; Different letters along the column indicate significant variations (P<0.05) according to Waller Duncan statistics

Table 3represents the level of selected heavy metals in liquid herbal solutions sold in Nigeria. Lead was not detected in any of the herbal medicines. The concentration of nickel was not detected in seven (7) of the nine (9) test herbal solutions. The concentration of nickel in UAM and GCH was 0.068mg/l and 0.046mg/l respectively. Statistically, there was significant variation (P<0.05) between the two samples. Nickel showed negative significant relationship with iron (r=-0.384, P<0.05) and positively correlated with cobalt (r=-0.452, P<0.05).Nickel and zinc was detected in only two products viz: RHB (0.024mg/l) and GCH (0.019 mg/l). Also, both samples (RHB and GCH) showed significant variation (P<0.05).

Cobalt was detected in YCB, UAM and UNC at concentrations of 0.102mg/l, 0.177mg/l and 0.140mg/l respectively. There was significant variation (P<0.05) between the three herbal products. Iron concentration in the herbal solutions under study ranged from 1.302 – 27.081mg/l, being significantly different (p<0.05) among the various herbal products. Plant material is the major source of the active ingredients in the herbal medicines. The concentration of iron, zinc and nickel in this study were lower than the values previously reported in edible vegetables (*Gongronema latifolium, Amaranthus hybridus, Piper guineense, Talinum triangulare, Telfairia occidentalis and Ocimum gratissimum*) sold in some markets in Nigeria (Izah and Aigberua, 2017). In addition, the values of trace metals in this study is lower than the range of 3.33 – 3.80µg/g (cobalt), 163.04 – 350.95 µg/g (iron), 9.94 – 15.63 µg/g (nickel) and 6.86 – 66.59 µg/g (zinc) in some herbal medicine sold in Kano state and used for treatment of some disease conditions (Samali *et al*., 2017). In addition, the concentration of zinc was lower than WHO limits of 50mg/l in finished herbal products. Information about the limit of cobalt, iron and nickel on finished herbal drink in Nigeria is scarce in literature.

In summary, the frequency of occurrence of nickel, zinc, lead, iron and cobalt in the liquid herbal products were 22.22%, 22.22%, 0.00%, 100% and 33.33% respectively. Iron occurred in all the samples, this could be due to the role of iron in human health. Furthermore, among the various trace metals under study iron in the most abundant, a trend that have been reported in food and environmental samples (Izah *et al*., 2017b, d; Izah and Aigberua, 2017; Aigberua *et al*., 2018b). Authors have variously reported that iron is required for many biochemical/metabolic processes including oxygen transport, deoxyribonucleic acid synthesis, electron transport chain and regulation of cell growth and differentiation (Lieu *et al*., 2001; Abbaspour *et al*., 2014; Beard, 2001; Izah *et al*., 2016, 2017a,d; Izah andAigberua, 2017). In addition, the trend of iron occurring in all the herbal medicines could be due to the fact that it is the most naturally occurring trace element among the heavy metals under study. On the other hand, zinc is vital in wound healing, cell growth development, differentiation, homeostasis processes, connective tissue growth and maintenance, DNA synthesis, RNA transcription, cell division, cell activation, and regulatory, catalytic, co-catalytic and structural roles in enzyme molecules (Chasapis *et al*., 2012; Osredkar and Sustar, 2011; Izah *et al*., 2016; 2017a, d, Izah and Aigberua, 2017). Cobalt are essential for methionine metabolism (Prashanth *et al*., 2015;

Izah *et al*., 2016, 2017a). Lead was not detected in any of the herbal products. This is worthy of note, since lead does not have any biological functions in living things including human. As such the absence of lead in the samples suggests that the possibility of lead toxicity is rare.

Table3.*Level of selected heavy metals in liquid herbal products sold in Nigeria*

Sample code	Nickel, Ni(mg/l)	Zinc, Zn(mg/l)	Lead, Pb(mg/l)	Iron, Fe(mg/l)	Cobalt, Co mg/l)
YCB	<0.001±0.000a	<0.001±0.000a	<0.001±0.000	5.784±0.015f	0.102±0.002b
UAM	0.068±0.000c	<0.001±0.000a	<0.001±0.000	1.302±0.101a	0.177±0.003d
RHB	<0.001±0.000a	0.024±0.000c	<0.001±0.000	4.648±0.179d	<0.001±0.000a
GHM	<0.001±0.000a	<0.001±0.000a	<0.001±0.000	10.126±0.124h	<0.001±0.000a
SHM	<0.001±0.000a	<0.001±0.000a	<0.001±0.000	5.054±0.054e	<0.001±0.000a
UNC	<0.001±0.000a	<0.001±0.000a	<0.001±0.000	9.121±0.128g	0.140±0.007c
GCH	0.046±0.000b	0.019±0.000b	<0.001±0.000	3.200±0.200c	<0.001±0.000a
EFM	<0.001±0.000a	<0.001±0.000a	<0.001±0.000	1.710±0.092b	<0.001±0.000a
GCB	<0.001±0.000a	<0.001±0.000a	<0.001±0.000	27.081±0.012i	<0.001±0.000a

Data is expressed as mean ± standard deviation; Different letters along the column indicate significant variations (P<0.05) according to Waller Duncan statistics

Table4.*Pearson correlation matrix of pH, minerals and selected trace metals in some liquid herbal products sold in Nigeria*

Parameters	pH	P	K	Mg	Na	Ca	Ni	Zn	Fe	Co
pH	1									
P	0.115	1								
K	0.024	-0.269	1							
Mg	0.044	-0.202	0.864**	1						
Na	0.197	0.399*	-0.379	-0.641**	1					
Ca	-0.054	0.623**	0.099	0.305	-0.023	1				
Ni	0.476*	0.135	-0.066	0.006	-0.173	-0.38	1			
Zn	-0.482*	0.144	0.432*	0.324	-0.179	-0.122	0.168	1		
Fe	-0.442*	-0.226	-0.328	-0.117	0.011	0.146	-0.384*	-0.252	1	
Co	0.415*	-0.397*	-0.064	-0.056	-0.210	-0.503**	0.452*	-0.362	-0.234	1

**. Correlation is significant at the 0.05 level (2-tailed).*

***. Correlation is significant at the 0.01 level (2-tailed).*

N=9 (n=3)

4. CONCLUSION

Generally, plant parts are the major ingredients of the different herbal drinks and plant have the tendency totake up heavy metals from their environment. Consequently,the level of heavy metals in herbal products are a reflection of the mineral found in the soil from which the plant materials are cultivated, as well as the water used for the constitution of the herbal medicines. The value of the trace metals found in this study suggests the absence of any major hazard associated with trace metals in liquid herbs produced, marketed and consumed in Nigeria. Furthermore, there is need for a more stringent enforcement of drug acts and regulations and continuous monitoring and surveillance to make all practitioners register their products with National Agency for Food, Drug Administration and Control and National Association of Traditional Medicine Practitioner of Nigeria registration.

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