

Physico-chemical Parameters and Heavy Metals Determination in Selected Medicinal Plants Sold in ‘Yar Marina Market, Sokoto-Nigeria

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Abstract

The increasing prevalence of environmental pollution, especially soil contamination by heavy metals has led to their increased uptake in the human food chains through plant parts. Accumulation and magnification of heavy metals and other toxins in human tissues through consumption of herbal remedies can cause serious negative impacts on health. This study was aimed at determining the physico-chemical parameters and the levels of heavy metals (Cr, Cd, Mn, Cu, Fe, Pb, Zn and Ni) concentration in selected crude herbal plants sold in ‘Yar Marina’ Market, Sokoto-Nigeria. The physico-chemical parameters were determined using standard analytical methods and the heavy metals were determined using Atomic Absorption Spectrophotometry (AAS) after wet digestion. The results shows that moisture content is 3.67 – 4.83 %, ash value 11.0 – 18.7 % and acid insoluble ash 1.0% 8.4 %. The result also revealed the maximum concentrations (mg/Kg) of the heavy metals in the samples as: 0.034±0.001, 14.977±0.001, 0.193±0.001, 15.160±0.002, 1.715±0.001, 0.335±0.001, 0.429±0.001 for Cd, Mn, Cu, Fe, Pb, Zn and Ni respectively. While the moisture content of the samples was excellent, ash and acid insoluble ash suggested some level of contermination probably with sand. Also, the levels of all the heavy metals analysed in the samples were found to be of acceptable limits for heavy metals in plant samples. The exception was only for manganese in *Cassia singueana* and *Combretum micranthum* leaves.. It is therefore, suggested that the quality, safety and efficacy of these medicinal plants be improved through pharmacovigilance.

Keywords: Heavy Metals, Medicinal Plants, Physico-chemical and ‘Yar Marina’ market.

1.0 Introduction

Over 80% of the populations in some Asian and African countries depend on traditional medicine for primary health care (WHO, 2008). Herbal remedies often contain numerous different plant and animal-derived products that combine to act synergistically to affect a desired outcome (Yang, 2010; Xie, *et al.*, 2006). However, due to the proprietary nature of herbal remedies preparation, coupled with a lack of industry regulation, the biological origin of contents can be difficult to be determined with confidence, leading to questions regarding herbal remedies quality, efficacy and safety (Heubl, 2010; WHO, 2002). Undeclared or misidentified ingredients and adulterants can pose serious health risks to consumers (Heubl, 2010; Sakurai, 2011; Gilbert, 2011; Coghlan, 2012). Survey has shown that about 75 - 80% the global population still relies on herbal remedies especially in the developing countries (Kamboj, 2000; Crawford, 2006). Almost all are available without prescription, easy to use and are thought by the consumers as “healthier” as and safer than orthodox pharmaceutical substances (Gupta, 1998). The World Health Organization (WHO) stated that users of herbal remedies globally exceed that of conventional orthodox medicine by two to three times (Evans, 1994). A number of the Nigerian herbal formulations for remedies usually may consists of several herbs and other ingredients only known to the herbalist, which are dried and ground into powder, mixed and encapsulated as either soft or hard gelatin capsules, whereas the liquid preparation are either suspensions or filtered solution of a mixture of herbs. In many instances and most commonly, dried medicinal plants (crude drugs) are sold by individual at local markets from which purchasers are directed on how to prepare concoction of selected plants for remedies of an ailment. In either ways of preparation the herbal preparation may receive little or no attention to quality assurance procedures.

The presence of heavy metals in different herbal preparations has been reported in many literatures and the levels of these hazardous metals in herbal drugs are alarming and may pose serious health risks to consumers (Xudong *et. al* 2011). Excessive amounts of heavy metals above the WHO maximum permissible limit for hazardous metals can adversely affect metabolic process in the body.

Heavy metals in this context are referred to as the way they are referred to as in literature as metals having atomic weight greater than sodium, a density greater than 5 g/cm³ and possess some level of toxicity (WHO, 2000; Alqosouimi, 2006). The aim of this research was to determine the level of some heavy metals and physico-chemical parameters in selected medicinal plants sold in ‘Yan Marina Market, Sokoto-Nigeria and to examine the compliance of those heavy metals with standard limits by World Health Organization.

2.0 Materials and Methods

2.1 Sampling Procedure and Preparation

The sampling was carried out in the month of May, 2015. The samples were collected from *Yar Marina* Market. *Yar marina* or simply *marina* market is a historic market in Sokoto city and is as old as the city itself (see sturdy area map, Fig. 1). It is the popular market at the heart of the city where all traditional materials including medicinal plants relating to the culture of Sokoto people can be easily found. Ten (10) samples (5 leaves and 5 barks) were collected from this market in polyethylene bags. The plant parts (leaves, flowers and fruits) were collected for identification purpose. The plants were identified by a consultant Taxonomist in the Department of Pharmacognosy and Ethnopharmacy, Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. Voucher specimens of the plant samples were prepared and voucher numbers were assigned and deposited at the Herbarium of the Department for future reference.

All the samples were collected in coarse and powdered forms and were all grounded and sieved separately to very fine particles in the laboratory. Representative samples were quantitatively taken from the powdered sample for both physicochemical and heavy metal analysis.

Table 1: Medicinal plants names, parts used and their voucher numbers

S/N	Hausa Name	Code	Scientific Name	Plant Part used	Voucher No.
01	Runhu	RH	<i>Cassia singueana</i>	Leaves	PCG/UDUS/Legu/0001
02	Sabara	SB	<i>Guiera senegalensis</i>	Leaves	PCG/UDUS/Comb/0002
03	Geza	GZ	<i>Combretum micranthum</i>	Leaves	PCG/UDUS/Legu/0002
04	Fulasko	FK	<i>Senna italic</i>	Leaves	PCG/UDUS/Caes/0002
05	Marke	MK-L	<i>Anogeissus leiocarpus</i>	Leaves	PCG/UDUS/Comb/0001
06	Hanu	HN	<i>Boswellia dalzielli</i>	Bark	PCG/UDUS/Burs/0001
07	Malga	MG	<i>Cassia arereh</i>	Bark	PCG/UDUS/Caes/0001
08	Kirya	KY	<i>Prosopis africana</i>	Bark	PCG/UDUS/Legu/0003
09	Marke	MK-B	<i>Anogeissus leiocarpus</i>	Bark	PCG/UDUS/Comb/0001
10	Taura	TR	<i>Detarium microcarpum; D. Senegalense</i>	Bark	PCG/UDUS/Legu/0004

2.2 Reagents and Standards

Chemicals of analytical grade were used; nitric acid (HNO₃), sulphuric acid (H₂SO₄) from M&B and perchloric acid (HClO₄), hydrochloric acid (HCl) from BDH. Standard solutions of the metals under investigation were from AAS machine's manufacturer.

2.3 Determination of Moisture Content

This is the measure of the percentage moisture lost due to drying at a temperature of 105⁰C in accordance with James, (1995) method; 3g of the leave sample was weighed into a pre-weighed crucible and placed into hot drying oven at 105⁰C for 24 hours. The sample was later removed, cooled and placed in desiccators for some time and weighed again to constant weight.

The weight lost due to moisture was obtained using this equation

$$\% \text{ Moisture Content} = \frac{W_1 - W_2}{W_1 - W_0} \times 100 \dots\dots\dots 1$$

Where W₀ = Weight of empty crucible

W₁ = Weight of fresh sample

W₂ = Weight of dried sample

2.4 Determination of Total Ash Value

This is a measure of the residue after combustion of the dried sample in a furnace at a temperature of 600⁰C for three hours (James, 1995). The sample (3g) was weight into pre-weighed crucible and placed in Lenton Furnace at 600⁰C for three hours. The sample was cooled in desiccators and weighed. The weight of the ash was determined by the difference between the dried sample, pre-weighed crucible and the ash sample in the crucible. This is obtained as percentage of the initial dry weight of the sample. The percentage ash was calculated using this equation

$$\% \text{ Ash Value} = \frac{W_2 - W_0}{W_1 - W_0} \times 100 \dots\dots\dots 2$$

Where W₀ = Weight of empty crucible

W₁ = Weight of crucible + dry sample

W₂ = Weight of crucible + ash sample

2.5 Determination of Acid Insoluble Ash

The ash obtained described as total ash was boiled for 5 min. with 25 cm³ of 10 % dilute hydrochloric acid. The insoluble matter was collected on an ash-less filter paper and washed with hot water and ignited to constant weight. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

2.6 Digestion of Samples and Analysis of heavy metals

Wet ashing technique was used for the digestion of the samples for the analysis of specific process minerals (Miller-Ihli and Baker, 2000). The process was carried out by taking 1.00 g of each of the prepared sample into separate digestion tubes. Then 20.00 cm³ of 69.5 % concentrated HNO₃ acid was added and heated in a tecator digestion block until about one third of each of the content is left. This was followed by the addition of another 10 cm³ of the concentrated HNO₃ and 2.00 cm³ of 60 % HClO₄ acids and the heating process continued until clear solutions were obtained. The digests were each diluted with about 20 cm³ of double distilled water and boiled for another 15 minutes. The contents were allowed to cool and further transferred into 50 cm³ volumetric flasks. These were all made to their marks with double distilled water. The solutions were then filtered using Whatman No. 42 filter paper into separate screw capped polyethylene bottles (Audu and Lawal, 2006; Daniel, 2003). Similarly, the blank sample solution was prepared in the same way.

The concentrations of Cr, Cd, Mn, Cu, Fe, Pb, Zn and Ni in the digests of the medicinal plants were determined by using the hollow cathode lamps for the respective elements at the proper wave length and slit width (0.5nm) atomic absorption spectrophotometer (Model No. AA240FS, Varian). The flame type used for all the elements was air-acetylene.

2.7 Statistical data analysis

The heavy metal concentrations in the digests of the herbal plants were presented as mean ± standard deviation (SD). The data were analyzed by one-way analysis of variance (ANOVA), taking probability factor of 0.05 and the software used was Statistical Package for Social Sciences (SPSS) V.15.

3.0 Results and Discussions

Table 2: Moisture, Ash and Acid insoluble ash contents of the samples

Samples	M C (%)	A V (%)	A I A (%)
Runhu (leaves)	4.17±0.62	16.5±0.41	8.4±0.33
Sabara (leaves)	4.50±0.41	12.5±0.41	1.4±0.33
Geza (leaves)	4.00±0.82	18.5±2.80	5.5±0.08
Fulasko (leaves)	4.50±0.41	18.7±1.24	5.0±0.57
Marke (leaves)	4.50±0.41	11.0±2.16	2.2±0.74
Hanu (bark)	4.17±0.24	17.7±0.47	3.9±0.37
Malga (bark)	4.67±0.47	14.0±0.41	3.4±0.24
Kirya (bark)	4.50±0.41	15.0±0.82	2.5±0.24
Marke (bark)	3.67±0.24	15.2±0.24	4.0±0.57
Taura (bark)	4.83±0.47	10.0±0.41	1.0±0.08

Key: M C= Moisture Content, A V = Ash Value, A I A = Acid Insoluble Ash, Mean±SD

Table 3: Concentration of heavy metals in the samples

Samples	Heavy Metals Concentration (mg/Kg)							
	Cr	Cd	Mn	Cu	Fe	Pb	Zn	Ni
Runhu (leaves)	ND	0.034±0.001	14.977±0.001	0.130±0.001	7.438±0.002	0.600±0.001	0.286±0.001	0.293±0.001
Sabara (leaves)	ND	0.028±0.001	5.569±0.001	0.181±0.001	4.951±0.002	0.638±0.001	0.314±0.001	0.249±0.001
Geza (leaves)	ND	0.022±0.001	11.886±0.001	0.132±0.001	7.711±0.003	0.638±0.001	0.281±0.001	0.251±0.001
Fulasko (leaves)	ND	0.021±0.001	4.028±0.001	0.160±0.001	15.160±0.002	0.725±0.001	0.335±0.001	0.178±0.001
Marke (leaves)	ND	0.019±0.001	4.461±0.001	0.165±0.001	4.027±0.001	0.775±0.001	0.332±0.001	0.429±0.001
Hanu (bark)	ND	0.019±0.001	3.457±0.001	0.193±0.001	7.813±0.001	1.715±0.001	0.221±0.001	0.128±0.001
Malga (bark)	ND	0.021±0.001	5.884±0.001	0.054±0.001	4.182±0.001	0.650±0.001	0.307±0.001	0.172±0.001
Kirya (bark)	ND	0.029±0.001	3.098±0.001	0.065±0.001	4.540±0.001	0.796±0.001	0.099±0.001	0.116±0.001
Marke (bark)	ND	0.017±0.001	2.848±0.001	0.066±0.001	4.892±0.003	0.935±0.001	0.102±0.001	0.087±0.001
Taura (bark)	ND	0.019±0.001	3.701±0.001	0.087±0.001	3.636±0.001	0.633±0.001	0.203±0.001	0.095±0.001

Mean±SD. ND=Not Detected

The results of the analyses are presented in Tables 2 and 3. The results in Table 2 show the moisture content, total ash value and the acid insoluble ash of the samples. The moisture content ranges from 3.67 – 4.83 %, the low value of moistures content could prevent bacterial, fungal and yeast growth (African Pharmacopoeia, 1986). These values were within and in agreement with the 10 % maximum limit for moisture

content in powdered medicinal plants prescribed in European Pharmacopoeia (2007).

The total ash values of the samples range from 11.0 – 18.7 % in *Anogeissus leiocarpus* (leaves) and *Senna italic* (leaves) respectively. About four samples representing 40 % of the total samples were within the 14 % maximum limit for total ash value in powdered medicinal plants (European Pharmacopoeia, 2007). The acid insoluble ash of the samples ranges from 1.0% *Detarium microcarpum* and *D.Senegalense* (bark) to 8.4 % in *Cassia singueana* (leaves) with the highest percentage (8.4 %). Only 20 % of the total samples were below 2 % (maximum limit for acid insoluble ash in powdered medicinal plants (European Pharmacopoeia, 2007).

The total ash values in the samples in this study showed that 60 % of the samples were above the limit and the acid insoluble ash of 80 % of the total samples exceeded the limit also. These values indicated the presence of carbonates and other oxides and more particularly silica in the case of insoluble ash. Ash and acid insoluble ash are important parameters in the evaluation of purity of drugs, that is, the presence or absence of foreign matter such as metallic salts and/ or silica (Musa, 2006). The results in Table 3 show the concentrations of Cr, Cd, Mn, Cu, Fe, Pb, Zn and Ni in samples with Fe having an overall concentration of 15.160 ± 0.002 mg/Kg in *Senna italic* (leaves) and Cd the least (0.017 ± 0.001 mg/Kg) in *Anogeissus leiocarpus* (bark), while Cr concentrations were below detection level in all the samples. In this study, the concentrations of Cd, Cu, Fe, Pb, Zn and Ni in almost all the samples collected were within the permissible limits recommended by World Health Organization (WHO). The exceptions were for leaves of *Cassia singueana* and *Combretum micranthum* which were above the WHO standard for manganese.

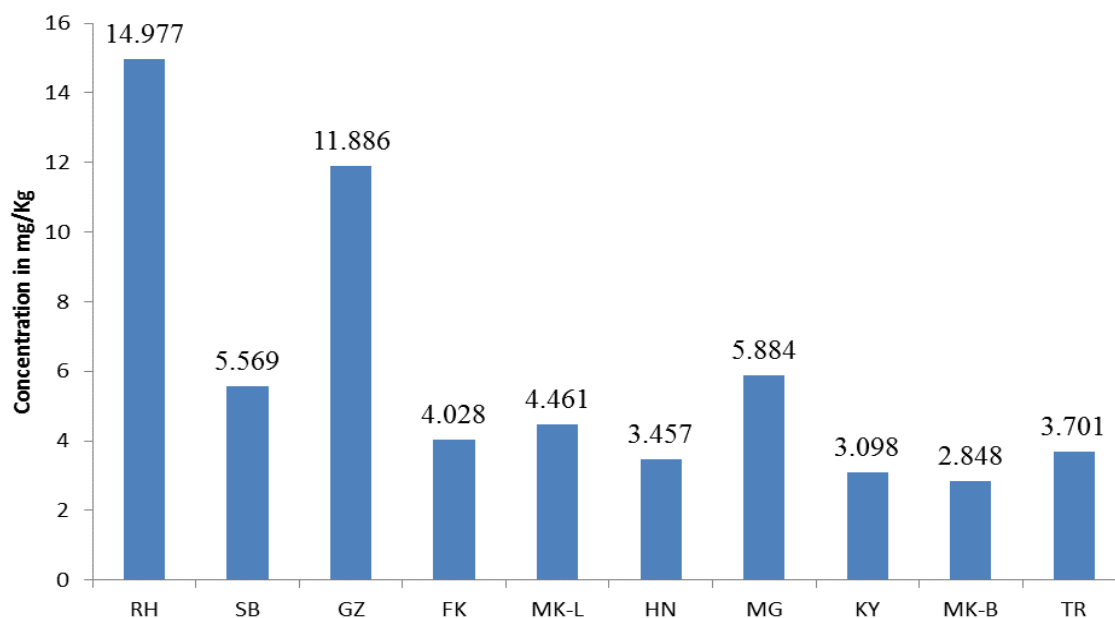


Figure: 2: Bar chart of Manganese concentrations in the samples

Fig.2. Shows the bar chart presentation of the variation of manganese in the samples. The leaves of *Cassia singueana* and *Combretum micranthum* were found to contained manganese slightly above the WHO prescribed daily allowable limits (2 – 9 mg/Kg). The range of manganese concentration in the samples was from 2.848 – 14.977 mg/Kg with *Cassia singueana* (leaves) containing the highest concentration of the metal. This was followed by *Combretum micranthum* (leaves), while the lowest concentration of the metal was in the leaves of *Anogeissus leiocarpus* and *Prosopis africana*. The concentrations of manganese in this study (2.848 – 14.977 mg/Kg) were within the range of concentration of manganese (0.227 – 38.0 mg/Kg) for the seeds and leaves respectively of *Senna Singueana* reported by Missa *et al.*, (2015). The result revealed a significant difference for manganese (Mn) at $p < 0.006$ in the plants.

The high manganese content in some samples may be attributed to the fact that the intake of manganese by plants increases in acidic soils ($pH < 5.5$) (Kabata-pendias and Pendias, 1999).

4.0 Conclusion

This study has shown that the medicinal plants being sold at marina market have moisture content appropriate for good preservation against microbial and fungal growth. This could be due to the dry nature of the area. The samples are however, to some extent likely having silica (sand) contaminations. The concentrations of heavy metals determined indicated Cd, Cu, Fe, Pb, Zn and Ni in almost all the samples were within the permissible limits recommended by WHO. The use of these herbal plants may be relatively safe, but prolong intake could be risk to health. There is therefore, need for frequent monitoring for heavy metals in the crude herbal drugs in

markets.

5.0 References

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