DETERMINATION OF PHYSICO-CHEMICAL PARAMETERS AND SOME HEAVY METALS IN SELECTED HERBAL DRUGS SOLD IN KARA MARKET, SOKOTO-NIGERIA

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Abstract: The use of Herbal drugs for various medicaments has been on the increase in Nigeria as it is in other developing and even developed countries. 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 drugs sold in *Kara* 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 moisture was found to be 3.56 – 5.0 %, total ash, 11.7 – 19.0 % and acid insoluble ash contents 0.5 – 9.3 %. The results also revealed maximum concentrations (mg/Kg) of the heavy metals in the samples as: 0.156±0.001, 0.103±0.005, 44.900±0.002, 0.275±0.003, 18.496±0.001, 1.133±0.001, 3.649±0.001 and 0.340±0.003 for Cr, Cd, Mn, Cu, Fe, Pb, Zn and Ni, respectively. The moisture indicated an excellent preservative state of the crude drugs but insoluble ash values indicated the likely presence of silica as contaminant in the drugs. The levels of Cd, Cu, Fe, Pb, Zn and Ni were all found to be within the World Health Organization (WHO) limits for heavy metals in plant samples. While, Cr in *Cassia singueana* and *Anogeissus leiocarpus* leaves; as well as Mn in *Guiera senegalensis, Combretum micranthum* and *Deuterium microcarpum D. senegalense* exceeded the WHO limits. Prolong intake of these plants could build toxic concentration of even the metals that were below WHO limit. It is therefore suggested that the quality, safety and efficacy of these crude drugs be improved through pharmacovigilance.

Keywords: Heavy metals, herbal drugs, *Kara market*, physico-chemical parameters

Introduction

Worldwide trend towards the utilization of natural products for health benefit has created an enormous need for information about the properties and uses of these products (Vidita *et al.*, 2013). Natural products in medicine constitute a vast array of raw materials from which medicinal plants constitute an important part. There is well established recognition that these medicinal plants (herbal drug remedies) play a major role in this trend of health care sector especially in developing nations for the management of various diseases and ailments (Vidita *et al.*, 2013). "Crude herbal drugs", crude drugs or simply herbal drugs denotes plants or plant parts that have been converted into phytopharmaceuticals by means of simple process involving harvesting, drying, and storage (EMEA, 1998; Jose *et al.*, 2011). About four billion people (80% of the world's population) living in developing countries use herbal medicine as their source of primary health care (Bisset, 1994; Farnsworth, 1985; Bodeker, 2005). Thus herbal medicines have a prominent role to play in the pharmaceutical markets and health care sector of the 21st Century (Annan, 2008).

Heavy metals are widespread in soil as a result of geo-climatic conditions and environmental pollution through industrial activiies, automobile exhaust, heavy-duty electric power generators, municipal wastes, refuse burning and pesticides used in agriculture (Jarup, 2003). Therefore, their assimilation and accumulation in plants is obvious (Jarup, 2003; Orish *et al.*, 2012). Human beings, animals and plants take up these metals and other pollutants from the environment through inhalation and ingestion (Orish *et al.*, 2012). Heavy metals have the tendency to accumulate in both plants and human organs (Bayor, 2009). The accumulation of heavy metals can have middle-term and long term health risks, and strict periodical surveillance of these contaminants is therefore advisable (Abou-arab, 2001; Orish *et al.*, 2012).

Heavy metals are defined as those groups of elements that have specific weights higher than 5 g/cm^3. A number of them (Co, Fe, Mn, Mo, Ni, Zn and Cu) are essential micronutrients and are required for normal growth and other important metabolic processes. Metals which are considered non essential (Pb, Cd, Cr, Hg, etc.) are potentially highly toxic to plants (Sebastiani & Scebbaf, 2004; Rama & Prasad, 1998; Rai *et al*., 2004).

The aim of this research was to determine the physico-chemical parameters and some heavy metals in selected herbal drugs sold in Kara market, Sokoto-Nigeria and to compare the heavy metals with standard limits set by World Health Organization.

Materials and Methods

Sampling procedure

The sampling was carried out in the month of May, 2015. The samples were collected within *Kara* market, in Sokoto Town. Ten (10) samples (5 leaves and 5 barks) were collected in polyethylene bags. The parts were identified by a consultant Taxonomist (Mal. Umar Shehu Gallah) 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 (see Table 1) and deposited at the Herbarium of the Department for future reference.

Table 1: Names of the herbal drugs, identification codes used and voucher vumbers

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

Sample preparation

All the samples were collected in coarse and powdered forms. The samples were each pulverized in a clean and acid rinsed (10% HNO_3) agate mortar. The homogenized powders were stored air tight from which representative samples were quantitatively taken for physicochemical and heavy metal determinations.

Chemicals and equipment

Chemicals of analytical grade were used; nitric acid (HNO_3), sulphuric acid (H_2SO_4) were M&B product and perchloric acid ($HClO_4$), hydrochloric acid (HCl) were from BDH. Standard solutions of the metals under investigation were from AAS machine's manufacturer. The AAS machine used was Varian AA240FS, while furnace was Lanton 678X model.

Determination of moisture content

The percentage moisture lost due to drying at 105^{o}C was done in accordance with the work of James (1995) method. Three (3.00 g) of the powdered drug sample was weighed into a pre-weighed crucible and placed into hot drying oven at 105^{o}C for 24 h. The sample was then cooled in a desiccator and weighed again. This was repeated until constant weight was recorded.

The weight lost due to moisture was calculated using equation (1);

$$\%\ moisture\ content = \frac{W_1 - W_2}{W_1 - W_0}\ x\,100 \ldots\ldots 1$$

Where: W_0 = Weight of empty crucible; W_1 = Weight of fresh sample; W_2 = Weight of dried sample

Determination of total ash value

The dried sample (3.00 g) was weighed into pre-weighed crucible and placed in Lenton Furnace at 600^{0}C for three hours. The sample was cooled in desiccator and weighed. The ash in the sample was determined as percentage of the initial dry weight of the sample as shown in equation 2 (James, 1995).

$$\%\ moisture\ content = \frac{W_2 - W_0}{W_1 - W_0}\ x\,100 \ldots\ldots 2$$

Where: W_0 = Weight of empty crucible; W_1 = Weight of crucible + dry sample; W_2 = Weight of crucible + ash sample

Determination of acid insoluble ash

The total ash obtained was boiled for 5 min with 25 cm^3 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 dried sample.

Digestion of samples and analysis of heavy metals

Wet ashing technique was used for the digestion of the samples for the analysis of the drug samples (Miller-Ihli and Baker, 2000). The process was carried out by taking 1.00 g of each of the prepared drug sample into separate digestion tube. Then 20.00 cm^3 of 69.5% concentrated HNO_3 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 cm^3 of the concentrated HNO_3 and 2.00 cm^3 of 60% $HClO_4$ acids and the heating process continued until clear solutions were obtained. The digests were each diluted with about 20 cm^3 of double distilled water and boiled for another 15 min. The contents were allowed to cool and further transferred into 50 cm^3 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 (Daniel, 2003; Audu and Lawal, 2006). 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 drug samples were determined by employing the hollow cathode lamps for the respective elements at the proper wave length and slit width (0.5 nm), in atomic absorption spectrophotometer (Model No. AA240FS, Varian). The flame type used for all the elements was air-acetylene.

Results and Discussion

The results of the analyses are presented in Tables 2 and 3. The results in Table 2 showed the moisture content, total ash and acid insoluble ash values of the herbal drugs. Moisture is an intrinsic factor which must be considered in relation to drug deterioration (Williams, 2009). Air–dry–drugs can contain up to 10-12% moisture, and in some instances this may be sufficient to activate enzymes and bring about decomposition of compounds such as glycosides (Jose *et al.*, 2011). The moisture content obtained for the drugs under study, ranges from 3.56 – 5.0%. Both the upper and lower boundary values of this range are below the 10% maximum limit for moisture in powdered medicinal plants prescribed in European Pharmacopoeia (2007). Thus, the moisture composition of the herbal drugs under study is favourable for preservative purposes. Deterioration of the drugs which can occur as a result of excess moisture is limited due to minimal water available and as such, lowers the degree to which it bound to the drug material, partake in different reactions and facilitate the growth of microorganisms (Yeager & Ward, 1981). Control or limiting moisture in herbal drugs is one of the oldest preservation strategies which is achieved most effectively through prevented bacterial, fungal and yeast growth (African Pharmacopoeia, 1986; Mudau, & Ngezimana, 2014). The moisture evaluated in this work, compares favourably with moisture value of 4.26% reported by Gite *et al.* (2010) on evaluation of physicochemical standardization parameters of *Enicostemma axillare*. However, the range (5.21%–7.42%) of moisture contents of the individual drugs, marketed formulations, and in-house prepared formulations

evaluated by Harinarayan *et al*. (2011) were above the range limits obtained in this study. The excellent moisture content of our drug samples, may probably be due to, the climatic condition of the study area which is sunny, dry and less humid (Adaramola & Oyewola, 2011); and as such, serves as a contributory factor to having the herbal drugs in a very dried state despite their exposure (displayed) in an open environment at the selling outlet.

Table 2: Moisture, ash and acid insoluble ash content in the crude drugs

Samples	M C (%)	AV (%)	A I A (%)
Runhu (leaves)	4.83±0.62	15.3±0.24	9.3±1.25
Sabara (leaves)	3.56±0.09	11.7±0.25	6.2±0.21
Geza (leaves)	4.50±0.41	15.2±0.24	3.0±0.33
Fulasko (leaves)	4.83±0.62	19.0±0.82	3.6±0.14
Marke (leaves)	5.0±0.40	18.0±0.82	2.0±0.33
Hanu (bark)	4.5±0.40	18.7±1.24	3.0±0.41
Malga (bark)	4.67±0.47	18.0±0.82	0.5±0.08
Kirya (bark)	4.83±0.62	18.3±1.25	2.5±0.16
Marke (bark)	3.83±0.24	16.5±0.41	3.0±0.08
Taura (bark)	4.17±0.62	13.0±0.82	0.5±0.08

MC= Moisture Content, AV = Ash Value, AIA = Acid Insoluble Ash, Mean±SD

Ash value is useful in determining authenticity and purity of drugs and is also an important quantitative standards (Kokate, 2006). All the individual drugs studied in this work, were found to have total ash values in the range from 11.7% in *Guiera senegalensis* (leaves) to 19.0 % in *Senna italic* (leaves) (Table 2). This means that only *Guiera senegalensis* (leaves) and *Detarium microcarpum; D. Senegalense* (bark) were within the 14% maximum limit required as total ash in powdered medicinal plants (European Pharmacopoeia, 2007). The high total ash of the drugs centered more in the bark samples with an average of 16.9% than in the leaves drug samples that are having 15.84% as an average value. Also, the variation of the ash values in the individual drugs is more pronounced in the leaves when compared to the barks. In total, 80% of the drug samples lie outside the standard limit and were so found to generally have high total ash values which conversely indicate contamination. A high ash value in drug sample beyond allowable limit was shown to be an indication of contamination, substitution, adulteration, or carelessness in preparing the drug or drug combinations (Harinarayan *et al*., 2011).

The acid insoluble ash content ranges from 0.5 – 9.3% with *Cassia arereh* and *Detarium microcarpum D. Senegalense* (all barks) containing the lowest amount while *Cassia singueana* (leaves) with the highest percentage (9.3%). Unlike total extractive ash values, the drugs samples that are leaves possess higher amount of the acid insoluble ash compared to the bark drugs; this is evident from an average value of 4.82% against 1.9%, respectively. Also, only 30% of the total drug samples were in agreement with 2.0% maximum limit for acid insoluble ash in powdered medicinal plants. This result is an indication that 70% of the drug samples have silica, silicate and like materials as contaminants (Kokate, 2006; Harinarayan *et al*., 2011). Both the total ash and acid insoluble ash determined, indicated some level of contamination in the herbal drugs by different contaminants among which silica material constitute an important fraction. Considering the geographical location of the study area; being semi arid (50-150 mm rainfall), mostly bare ground and sandy soil (Valentin *et al.,* 1999), coupled with average annual wind speed of 4.0 ms^{-1} (Adaramola &. Oyewola, 2011), atmospheric deposition can be one of the possible causes for the high silica material observed in the herbal drugs.

Table 3: Concentration of heavy metals in the herbal drugs

Samples	Heavy Metal Concentration (mg/Kg)							
	Cr	Cd	Mn	Cu	Fe	Pb	Zn	Ni
Runhu (leaves)	0.156±0.001	0.103±0.005	4.600±0.001	0.275±0.003	4.433±0.003	1.133±0.001	1.881±0.001	0.340±0.003
Sabara (leaves)	0.028±0.001	0.027±0.001	10.673±0.001	0.223±0.001	9.826±0.001	0.644±0.001	2.055±0.001	0.170±0.001
Geza (leaves)	ND	0.016±0.001	19.808±0.001	0.165±0.001	7.371±0.002	0.395±0.001	0.753±0.001	0.146±0.001
Fulasko (leaves)	0.055±0.007	0.015±0.001	5.190±0.001	0.123±0.001	18.496±0.001	0.372±0.001	0.698±0.001	0.085±0.001
Marke (leaves)	0.061±0.001	0.016±0.001	4.780±0.001	0.119±0.001	3.498±0.002	0.386±0.001	1.179±0.001	0.070±0.001
Hanu (bark)	ND	0.016±0.001	8.703±0.001	0.047±0.001	3.401±0.002	0.492±0.001	3.649±0.001	0.054±0.001
Malga (bark)	ND	0.017±0.001	5.382±0.001	0.041±0.001	4.214±0.001	0.548±0.001	0.611±0.001	0.037±0.001
Kirya (bark)	ND	0.017±0.001	5.453±0.001	0.036±0.001	2.173±0.002	0.623±0.001	1.659±0.001	0.079±0.001
Marke (bark)	ND	0.019±0.001	3.090±0.001	0.034±0.001	2.819±0.001	0.651±0.001	0.432±0.001	0.048±0.001
Taura (bark)	ND	0.023±0.001	44.900±0.002	0.084±0.001	5.881±0.003	0.544±0.001	0.640±0.001	0.070±0.001

Mean±SD; ND=Not Detected

The results in Table 3 show the concentrations of Cr, Cd, Mn, Cu, Fe, Pb, Zn and Ni in the samples with Mn having an overall concentration of 44.90±0.002 mg/Kg in *Detarium microcarpum*; *D. Senegalense* (bark) and Cd the least (0.015±0.003 mg/Kg) in *Senna italic* (leaves). In this study, the concentrations of Cd, Cu, Fe, Pb, Zn and Ni in all the samples collected were within the permissible limits recommended by WHO (Table 4). Cr was detected only in four (4) drugs (all leaves), out of which three drugs viz: *Cassia singueana* (0.156 mg/kg), *Senna italic* (0.055 mg/kg) and *Anogeissus leiocarpus* (0.061 mg/kg) were out of WHO limit concentrations (0.05 mg/kg). The concentrations of Cr obtained for some drugs in this work, are similar to result of Igweze *et al.* (2012) who reported 0.0506 mg/Kg of chromium on contaminated Nigerian herbs. In this work also, all the bark drug samples were not found to contain Cr, a result that also resemble the work of Ekeanyanwu *et al.* (2013) who found no detectable Cr on analysis of some selected toxic heavy metals in some branded Nigerian Herbal Products. Also, three drugs; *Guiera senegalensis, Combretum micranthum* and *Detarium microcarpum;* with respective concentrations of 10.673 mg/kg, 19.808 mg/kg and 44.90 mg/kg (representing 30.00% of the total samples) were above the WHO limit range of 2 – 9 mg/kg for Mn. However, the manganese evaluated in this work (3.090 – 44.900 mg/Kg) is lower than 78.90 mg/Kg reported by Abdul Ghani *et al.*, (2012) on the heavy metals and nutritional composition of some selected herbal plants of Soon Valley, Khushab, Punjab, Pakistan. Our upper range limit for magnesium also is close to and similar to manganese concentration of 52.95 mg/Kg reported by Shad *et al.* (2008) on determining the profile of heavy metals in selected medicinal plants in Peshawar, Pakistan.

Though Cr and Mn are heavy metals, and their concentrations in some of the drugs were above WHO tolerable limit, it is worth noting that they are unlike Pb, Cd and Ni that are extremely toxic. But instead, they are like Cu, Fe, and Zn, in that all of them are micronutrients (Iatrou *et al.*, 2015) required for certain metabolic process in both plants and animals (Veronique & Schuyler, 1994). For example, it has been indicated that; Mn promotes growth, development and cell function, helps many body enzymes and a healthy immune system (Kabata-pendias and Pendias, 1999); Cr is required for glucose metabolism and may prevent diabetes and reduce cholesterol (Leigh & Philip, 2006); Cu is involved in respiratory and red blood cell function, healthy nerves, taste sensitivity, and healthy bone development; Fe is necessary for formation of hemoglobin, chlorophyll, mycoglobin, energy production and a healthy immune system (Cervantes *et al*., 2001); while Zn is needed for a healthy immune system among others (Rai *et al*., 2004).

Table 4: Range of Heavy metal concentration and their respective WHO standard limits

Metal	Range of conc. (mg/Kg) of Heavy metal in the samples	WHO Limits (mg/Kg)
Chromium	0.028 – 0.156	0.05
Cadmium	0.015 – 0.103	0.3
Manganese	3.090 – 44.900	2 – 9 daily intake
Copper	0.034 – 0.275	40
Iron	2.173 – 18.496	20*
Lead	0.372 – 1.133	10
Zinc	0.432 – 3.649	50
Nickel	0.037 – 0.340	8.0

20*mg/Kg for edible plants has been used because the limit of iron in medicinal plants by WHO has not been established

The cumulative heavy metals per sample was evaluated to be 52 mg/Kg in *Detarium microcarpum* (bark) followed by 28 mg/Kg in *Combretum micranthum* leaves. The high cumulative amount of the metals in *Detarium microcarpum* (bark) was essentially due to manganese concentration; which was exceptionally high. The reason for the escalated quantity of manganese in *Detarium microcarpum (*back) could not be established. Also, the summation of the concentrations of each metal in the drugs was considered, it was observed that the metals can be arrange in order of decreasing amounts as follows: Mn> Fe>Zn>Pb>Cu>Ni>Cr>Cd. This series shows Mn to be also the metal of the highest total concentration. The amount of Mn in *Detarium microcarpum*; *D.Senegalense* (44.9 mg/Kg) is the main contributor to the high total Mn recorded for all the drugs. Precisely, this amount, constituted 40% of the total cumulative weight of Mn in the samples. Fe counts the second highest cumulative amount of the metals determined in the drugs. More than 65% of this weight came from the leaves samples. The high Fe content in leaves than in the barks is not surprising because, leaves are known to contain chlorophyll pigment- an Fe- porphyrin molecule used by plants for photosynthesis (Han *et al*., 2004).

Conclusion

This study has shown that the selected herbal drugs sold at Kara market are in good preservative condition. The extractive ash values of the drug samples indicated contamination most probably with silica or silicate material. The concentrations of the heavy metals determined, except for manganese and chromium in some drugs, were all within the permissible limits recommended by WHO. Frequent pharmacovigilance on the herbal drugs sold at local markets is required for improved safety and efficacy.

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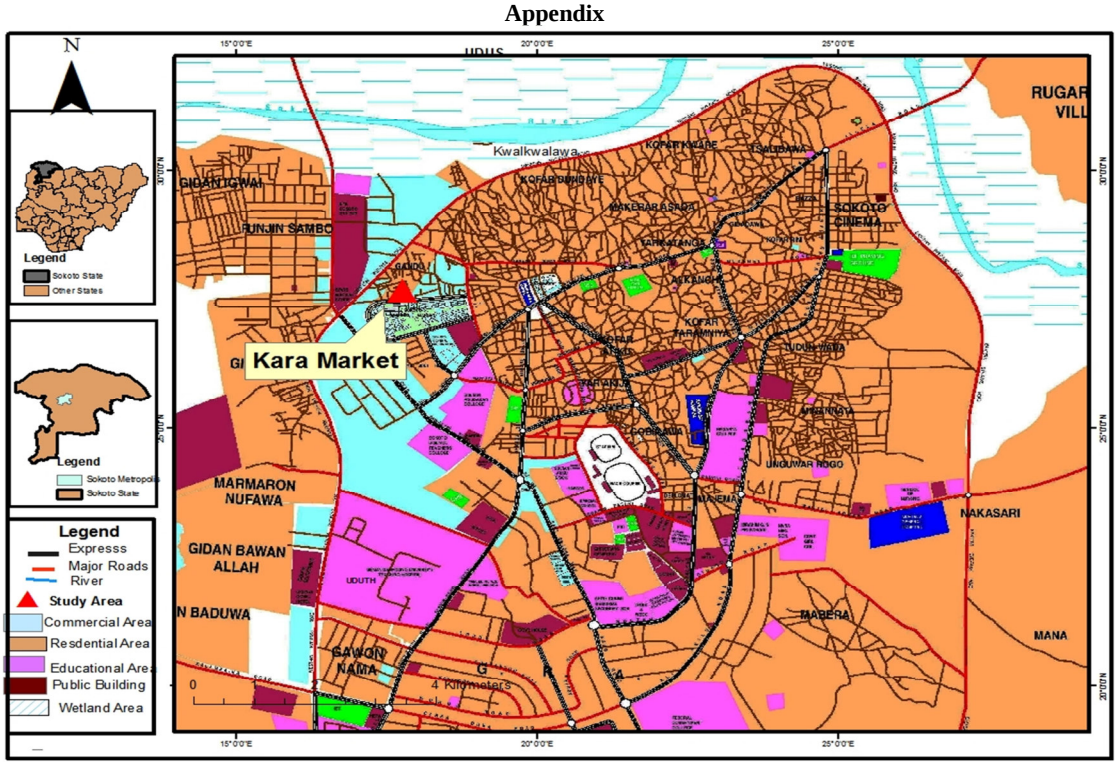
Appendix



Appendix 1: Position of the stury area in Sokoto Metropolis