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 Kasa 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, 18.49±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 Deutereium 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, Kasa 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 geoclimatic conditions and environmental pollution through industrial activities, 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/cm3. 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 & Scerbaf, 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 Kasa 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 Kasa 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 numbers**

<table>
<thead>
<tr>
<th>S/N</th>
<th>Scientific Name</th>
<th>Hausa Name</th>
<th>Sample Code</th>
<th>Plant Part used</th>
<th>Voucher No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Cassia singueana</td>
<td>Runhu</td>
<td>RH</td>
<td>Leaves</td>
<td>PCG/UDUS/Legu/0001</td>
</tr>
<tr>
<td>02</td>
<td>Guiera senegalensis</td>
<td>Sabara</td>
<td>SB</td>
<td>Leaves</td>
<td>PCG/UDUS/Comb/0002</td>
</tr>
<tr>
<td>03</td>
<td>Combretum micranthum</td>
<td>Geza</td>
<td>GZ</td>
<td>Leaves</td>
<td>PCG/UDUS/Legu/0002</td>
</tr>
<tr>
<td>04</td>
<td>Senega italic</td>
<td>Fulasko</td>
<td>FK</td>
<td>Leaves</td>
<td>PCG/UDUS/Case/0002</td>
</tr>
<tr>
<td>05</td>
<td>Anogeissus leicoparus</td>
<td>Marke</td>
<td>MK-L</td>
<td>Leaves</td>
<td>PCG/UDUS/Comb/0001</td>
</tr>
<tr>
<td>06</td>
<td>Boswellia dalzielle</td>
<td>Hanu</td>
<td>LN</td>
<td>Bark</td>
<td>PCG/UDUS/Burs/0001</td>
</tr>
<tr>
<td>07</td>
<td>Cassia aereh</td>
<td>Malga</td>
<td>MG</td>
<td>Bark</td>
<td>PCG/UDUS/Case/0001</td>
</tr>
<tr>
<td>08</td>
<td>Prospolis Africana</td>
<td>Kirtya</td>
<td>KY</td>
<td>Bark</td>
<td>PCG/UDUS/Legu/0003</td>
</tr>
<tr>
<td>09</td>
<td>Anogeissus leicoparus</td>
<td>Marke</td>
<td>MK-B</td>
<td>Bark</td>
<td>PCG/UDUS/Comb/0001</td>
</tr>
<tr>
<td>10</td>
<td>Detarium microcarpum</td>
<td>Taura</td>
<td>TR</td>
<td>Bark</td>
<td>PCG/UDUS/Legu/0004</td>
</tr>
</tbody>
</table>

**Sample preparation**

All the samples were collected in coarse and powdered forms. The samples were each pulverized in a clean and acid rinsed (10% HNO₃) 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 (HNO₃), sulphuric acid (H₂SO₄) were M&B product and perchloric acid (HClO₄), 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°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°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):

\[
\%\text{ moisture content} = \frac{W_1 - W_0}{W_1 - W_2} \times 100 \ldots 1
\]

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°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).

\[
\%\text{ moisture content} = \frac{W_2 - W_0}{W_1 - W_0} \times 100 \ldots 2
\]

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 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 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-Illi 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 cm³ of 69.5% concentrated HNO₃ acid was added and heated in a teacot 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 digestes were each diluted with about 20 cm³ of double distilled water and boiled for another 15 min. 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 (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. In the results of 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>
<thead>
<tr>
<th>Samples</th>
<th>Cr (mg/kg)</th>
<th>Cd (mg/kg)</th>
<th>Mn (mg/kg)</th>
<th>Cu (mg/kg)</th>
<th>Fe (mg/kg)</th>
<th>Pb (mg/kg)</th>
<th>Zn (mg/kg)</th>
<th>Ni (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Runhu (leaves)</td>
<td>0.15±0.001</td>
<td>0.10±0.005</td>
<td>4.60±0.001</td>
<td>0.27±0.003</td>
<td>4.43±0.003</td>
<td>1.13±0.001</td>
<td>1.88±0.001</td>
<td>0.34±0.003</td>
</tr>
<tr>
<td>Sabara (leaves)</td>
<td>0.02±0.001</td>
<td>0.02±0.001</td>
<td>10.67±0.001</td>
<td>0.23±0.001</td>
<td>9.82±0.001</td>
<td>0.64±0.001</td>
<td>2.05±0.001</td>
<td>0.17±0.001</td>
</tr>
<tr>
<td>Geza (leaves)</td>
<td>0.01±0.001</td>
<td>0.01±0.001</td>
<td>19.80±0.001</td>
<td>0.16±0.001</td>
<td>7.37±0.001</td>
<td>0.39±0.001</td>
<td>0.75±0.001</td>
<td>0.14±0.001</td>
</tr>
<tr>
<td>Fulasko (leaves)</td>
<td>0.05±0.007</td>
<td>0.05±0.007</td>
<td>1.90±0.001</td>
<td>0.12±0.001</td>
<td>18.49±0.001</td>
<td>0.37±0.001</td>
<td>0.69±0.001</td>
<td>0.08±0.001</td>
</tr>
<tr>
<td>Marke (leaves)</td>
<td>0.06±0.001</td>
<td>0.06±0.001</td>
<td>4.78±0.001</td>
<td>0.11±0.001</td>
<td>3.49±0.002</td>
<td>0.38±0.001</td>
<td>1.17±0.001</td>
<td>0.07±0.001</td>
</tr>
<tr>
<td>Hanu (bark)</td>
<td>0.01±0.001</td>
<td>0.01±0.001</td>
<td>8.70±0.001</td>
<td>0.04±0.001</td>
<td>3.69±0.001</td>
<td>0.05±0.001</td>
<td>0.05±0.001</td>
<td>0.05±0.001</td>
</tr>
<tr>
<td>Malga (bark)</td>
<td>0.01±0.001</td>
<td>0.01±0.001</td>
<td>5.38±0.001</td>
<td>0.04±0.001</td>
<td>4.21±0.001</td>
<td>0.54±0.001</td>
<td>0.61±0.001</td>
<td>0.03±0.001</td>
</tr>
<tr>
<td>Kinya (bark)</td>
<td>0.01±0.001</td>
<td>0.01±0.001</td>
<td>5.45±0.001</td>
<td>0.03±0.001</td>
<td>2.17±0.002</td>
<td>0.62±0.001</td>
<td>1.65±0.001</td>
<td>0.07±0.001</td>
</tr>
<tr>
<td>Runhu (bark)</td>
<td>0.01±0.001</td>
<td>0.01±0.001</td>
<td>3.09±0.001</td>
<td>0.03±0.001</td>
<td>2.81±0.001</td>
<td>0.65±0.001</td>
<td>0.43±0.001</td>
<td>0.04±0.001</td>
</tr>
<tr>
<td>Taura (bark)</td>
<td>0.02±0.001</td>
<td>0.02±0.001</td>
<td>4.90±0.002</td>
<td>0.08±0.001</td>
<td>5.88±0.003</td>
<td>0.54±0.001</td>
<td>0.64±0.001</td>
<td>0.07±0.001</td>
</tr>
</tbody>
</table>

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 (0.01±0.003 mg/kg) in Senna italic (leaves). In this study, the concentrations of Cr, 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 sinueana (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 Igiewe 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. The 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.
Determination of Heavy Metals Concentration in Selected Crude Drugs Sold in Sokoto

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, myoglobin, 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

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

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 (bark) 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 amount s as 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 bark 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 preserved 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.

References


Determination of Heavy Metals Concentration in Selected Crude Drugs Sold in Sokoto


Sebastiani L & Scebbaf T 2004 Heavy Metal Accumulation and Growth Responses in Poplar Clones Eridano (Populus deltoids × maximowiczii) and I-214 (P. × euramerica) exposed to industrial waste. Envt. Exp. Bot., pp. 52-79.


Appendix

Appendix 1: Position of the study area in Sokoto Metropolis