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Variability of Heavy Metal Content of Phytomedicines from Foreign and Local Sources Sold in Nigeria.

[1]Samali, A; [2]Florence D. Tarfa; [1]Mustapha K.B; [1]Ibekwe Nneka, N and Adigwe[2] O.P,
[1]Department of Medicinal Chemistry and Quality Control, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja Nigeria
[2]Diretor-General Office, National Institute for Pharmaceutical Research and Development, (NIPRD), Abuja Nigeria

ABSTRACT

The popularity of herbal products has been on increase throughout the world; but one major challenge facing it is lack of guaranteed safety with respect to heavy metals contamination. In this study, Chinese, Indian and Nigerian phytomedicine were evaluated for Copper (Cu), Cadmium (Cd), Lead (Pb) and Zinc (Zn) in order to ascertain and compare their safety or level of contamination. The samples were purchased, processed and analyzed using Flame Atomic Absorption Spectrometer (FAAS). The results obtained were presented as mean concentrations of Cu, Cd, Pb and Zn in the Nigerian, Chinese and Indian phytomedicine as 1.23±0.03µg/g, 5.46±0.04µg/g, 5.16±0.01µg/g (Cu), 2.07±0.04µg/g, 9±0.06µg/g, 6.28±0.03µg/g (Cd), 4.42±0.18µg/g, 7.74±0.07µg/g, 5.05±0.06µg/g (Pb) and 2.90±0.04µg/g, 6.40±0.04µg/g, 2.53±0.04µg/g (Zn) respectively. The results indicated all the Nigerian herbal remedies analyzed and 33.33% of the Chinese and Indian herbal remedies contained cadmium above WHO permissible limit (0.3 µg/g) for herbals. The analysis of variance (ANOVA) results obtained at p-value 95% ($p < 0.050$) ranges from significant to non-significant difference of means among the three countries. The correlation coefficient (r) also ranges from negative to strong degree of association. The variability of the heavy metals content could be associated to geographical, environmental, anthropogenic factors and heavy metals absorption potentials of the medicinal plants used. Environmental monitoring of herbal materials is required in order to ensure their safety from chemical pollutants that could lead to bio-accumulation of heavy metals in herbal materials.

Keywords: Phytomedicines, Heavy metals, India, China, Nigeria

Corresponding author: ayubasamali8@gmail.com

Introduction

The uses of phytomedicine medicines for treatment, cure or management of several disease conditions such as breast, cervical and prostate cancers, asthma, skin infections, jaundice, scabies, eczema, typhoid, erectile dysfunctions, snakebite, gastric ulcer, cardiovascular disorders and others are well known [1, 2]. However, toxicities and poisoning effect resulting from heavy metal contamination and variable constituents of complex nature of the medicine have been under estimated, and as a result several consequences have befallen human health [3, 4].

Standard guidelines and the regulations such as WHO have been violated severally due to crave for potency, efficacy and effectiveness, leaving out safety [5]. The hazards associated to these can be minimize or avoided by adhering to scientific measures such as current good manufacturing practice (CGMP) and application of modern analytical techniques that will lead to less hazardous products.

The aim of the study is to evaluate heavy metals content of local (Nigerian) and foreign (Chinese and Indian) phytomedicine sold in Nigeria in order to ascertain, compare and draw a conclusive statement with respect to their fitness for human use.

Materials and Methods

Materials

The reagents used were of analar-R grade and appropriate type and sizes of glassware were used. Agilent Technologies (www.Agilent.com) supplied stocked calibration solutions of Cd, Cu, Pb and Zn at 1000µg/mL in 5% HNO_3 was used. Concentrated solutions of HNO_3 (Purity of 70%, ACS reagent grade for general purpose) and $HClO_4$ (Purity of 70%, PCA reagent grade for trace metal basis) used for the sample digestion were obtained from Sigma-Aldrich. Filter paper (Whatman No.1 Size 110mm), Micropipette (Model: perfect choice, sizes 0-200µl and 0-1000µl), Plastic sample bottles ($100cm^3$) and Atomic Absorption Spectrometer (Agilent Technologies 200 series AA, model 240 FSAA, SPECTRAA version 5.2 (38) 2013 was used for the analysis.

Samples and Sampling

Samples of herbal remedies for different disease conditions from China, India and Nigeria were used in this study. The samples of Chinese and Indian origin were purchased from the Companies' representative in Nigeria, while the local samples were purchased at Kurmi market, Kano, Nigeria. The samples were labeled and coded as C1, C2, C3, C4, C5 (Chinese), I1, I2, I3, I4, I5 (Indian) and N1, N2, N3, N4, N5 (Nigerian).

Sample Digestion into Solutions

The powdered samples were weighed out 1.0g into beakers and added 20 cm^3 of concentrated nitric acid (S.G. 1.4; 69%W/V) and kept for 24 hours. The samples were carefully heated at 150^oC on a powered electrically connected sand-bath in fume hood chamber with periodic addition of 15 cm^3 concentrated nitric acid until the production of red nitrous oxide (NO_2) ceased. The samples were cooled and 3cm^3 of perchloric acid (S.G; 70%) was added and further heated until cleared sample solution was obtained. The samples were diluted with deionized water, filtered into 100cm^3 volumetric flasks, made up to mark and transferred into a capped labeled plastic bottle and kept at room temperature prior to analysis. The sample analyte procedure was followed for blank sample preparation, only that sample analyte was not introduced in the process [4 - 5].

Preparation of Calibration Standard Solutions

The working standard of cadmium, copper, lead and zinc solutions were prepared

from stock standard solutions of concentrations 1000 µg/mL by pipetting appropriate volume into 50 cm^3 volumetric flasks, made-up to mark with deionized water and serially diluted to appropriate concentration within the specified working range of the selected wavelength.

Procedure for Heavy Metals Analysis

The concentrations of some heavy metals (Cu, Cd, Pb and Zn) in the herbal remedies were determined using SpectrAA 240 Model of Fast Sequential Atomic Absorption Spectrometer. The Atomic Absorption Spectrometer equipped with all the necessary components was switched on and optimized based on the operating conditions presented in Table 1.0 and calibrated using the prepared standard solutions of the metals of interest followed by running the prepared blank solution and the sample analyte consecutively. The data obtained were processed by calculation the actual concentrations in the samples analyte using the relation:

$$\text{Metal (µg/g)} = \frac{C \times V \times D.f}{W}$$

Where C is the concentration obtained from the AAS machine (mg/L); V is the volume of the ample solutions in mL; W is the sample's weight in grams and d.f is the dilution factor, if used [4][7].

Table 1.0 Equipment Operating Condition for Determination of the Heavy Metals

Element	wavelength (nm)	Detection limit (µg/ml)	Slit width (nm)	Lamp Current (mA)	Nebulizer uptake	Gas mix (Air/acetylene)
Cu	324.7	0.02	0.5	3.0	5ml/min	13.5/2.0
Cd	228.8	0.002	0.5	3.0	5ml/min	13.5/2.0
Pb	217.0	0.06	1.0	5.0	5ml/min	13.5/2.0
Zn	213.9	0.008	0.5	5.0	5ml/min	13.5/2.0

Statistical Data Analysis

All the data obtained were subjected to statistical analysis using PRISM Graphad Software version 5. One-way analysis of variance (ANOVA) and Pearson correlation analysis was performed on the data obtained, where Mean, range, standard deviation, p-value and correlation coefficients (r) were obtained. The level of statistical significance was set at $p<0.05$ and correlation coefficient was tested on the samples and the results were interpreted according to the Rule of Thumb Interpretation Guideline [8 - 10]

Results and Discussion

The study analyzed herbal remedies from China, India and Nigeria for heavy metals content and the results of the concentrations obtained were reported in Table 3.0 and statistically analyzed using ANOVA and correlation coefficient methods.

Table 2.0: Concentrations of Heavy Metals in Chinese, Indian and Nigerian Herbal Remedies

Countries	Heavy Metal Concentrations (µg/g)			
	Cu	Cd	Pb	Zn
Nigeria	0.86±0.06	2.23±0.03	7.05±0.52	8.56±0.00
	0.97±0.00	1.61±0.07	ND	0.63±0.06
	1.76±0.05	2.46±0.03	1.96±0.01	2.16±.0.06
	1.01±0.04	1.34±0.04	7.50±0.15	2.74±0.02
	1.22±0.00	2.85±0.01	ND	2.45±0.05
	1.54±0.01	1.94±0.06	1.16±0.04	0.88±0.07
China	5.12±0.02	ND	6.78 ±0.04	0.44±0.03
	6.29±0.02	ND	8.79±0.10	1.61±0.03
	4.62±0.03	ND	7.00±0.02	6.96±0.07
	6.86±0.02	ND	9.51±0.05	11.06±0.03
	4.27±0.03	9.04±0.06	9.22±0.05	2.99±0.01
	5.58±0.01	8.96±0.06	5.11±0.01	15.35±0.05
India	4.62±0.03	ND	6.99±0.04	4.22±0.00
	3.95±0.03	ND	7.05±0.04	2.80±0.10
	7.51±0.01	ND	6.26±0.04	2.62±0.09
	6.85±0.00	6.28±0.03	4.64±0.05	1.46±0.00
	4.99±0.00	6.28±0.02	1.21±0.06	1.54±0.02
	3.01±0.00	ND	4.22±0.10	ND
WHO Limit	20	3	10	50

Key: ND means below detection limit of Cu (<0.02), Cd (<0.002), Pb (<0.06) and Zn (<0.008)

The concentrations of the heavy metals presented in table 2.0 indicated the concentration ranges of Cu, Cd, Pb and Zn in Nigerian herbal remedies as 0.86-1.54 µg/g (Cu), 1.34-2.85 µg/g (Cd), 1.16-7.50 µg/g (Pb) and 0.63-8.56 µg/g (Zn), in Chinese herbal remedies as 4.27-6.86µg/g (Cu), 8.96-8.96 g/g (Cd), 5.11-9.51g/g (Pb) and 0.44 -15.35µg/g (Zn) and Indian herbal remedies as 3.01-7.51µg/g (Cu), 6.28 µg/g (Cd), 1.21-7.05 µg/g (Pb) and 1.46-4.22µg/g (Zn). The concentrations of Cu, Pb and Zn obtained in Nigerian, Chinese and Indian herbal remedies were below WHO permissible limits (Cu, 20 µg/g, Pb, 10 µg/g and Zn, 50µg/g), while all the Nigerian herbal remedies analyzed and 33.33% of the Chinese and Indian herbal remedies each contained Cd above WHO permissible limit (0.3 µg/g) [5]. Previous studies of Chinese, Indian and Nigerian herbal remedies have reported contamination with heavy metals [3, 11 – 14].

Conclusion

Phytomedicine from the three countries indicated variable concentration of heavy metals content. The variability of the heavy metal concentration could be attributed to regional, environmental or anthropogenic factors. The need to employ harmonized standards, modern professionalism, awareness and enforcement of law for compliance with standard requirement is necessary in order to safeguard the health of the citizens.

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