Heavy metal analysis of polyherbal formulations marketed in Ilorin

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Abstract

Globally, consumption of herbal preparations is on the increase with corresponding increase in the numbers of pharmaceutical industries engaged in herbal production. There is need for regular assessment of the quality of these herbal products to safeguard the health of the consumers of herbal drugs. Besides other quality parameters for evaluation of herbal products, heavy metals analysis is essential due to the potential health hazards implicated in their consumptions. Therefore, the study evaluates the heavy metal contents of two polyherbal liquid preparations marketed in Ilorin, North Central, Nigeria. Samples of the two herbal products (AMO and ZAK herbal bitters) were pre-treated and analyzed for the presence of iron, lead, cadmium, Copper, manganese and zinc using a validated Atomic Absorption Spectrophotometric (AAS) method. The analysis was done in triplicate and International Conference on Harmonization (ICH) guideline followed.
Validation results showed linearity between 5-50 mg/L, 0.1-0.4 mg/L, 1-4 mg/L, 0.02-1.0 mg/L, 0.2-1.6 mg/L and 1-4 mg/L for iron, manganese, lead, copper, zinc and cadmium respectively, while the limit of detection was 0.001 mg/L, 0.001 mg/L, 0.002 mg/L, 0.005 mg/L, 0.025 mg/L, and 0.002 mg/L, for copper, manganese, cadmium, iron, lead, and zinc respectively. The findings showed higher concentrations of iron above the World Health Organization (WHO) permissible limit in the two preparations at 15.1 mg/L and 42.6 mg/L for AMO and ZAK herbal bitters respectively. However, lead, cadmium, and zinc were undetectable in both samples and the observed amount for copper and manganese were below the WHO limit. Our findings revealed the presence of iron at concentrations exceeding WHO permissible limits in both polyherbal preparations evaluated. This indicates a potential risk for iron poisoning with long term consumption of these products. Intensive effort by regulatory agencies to ensure the safety and quality of polyherbal formulations is recommended.

Keywords: polyherbal, heavy metals, atomic absorption spectrophotometry, WHO

INTRODUCTION

Global interest in the use of herbal preparations for therapeutic purposes has increased rapidly in the last three decades[1]. Over these periods, this growing interest has been attributed, in part, to its presumed wider margin of safety, cost effectiveness, and easy

accessibility over conventional medicines. In Africa, the use of herbal medicines has been fully integrated into the health care system, partly, as a result of the unevenness between the ratio of traditional health care providers and orthodox health practitioners, relative to the whole population[2]. Previous studies have demonstrated the use of herbal medicines amongst Nigerians with and without any form of chronic illness[3-5], as well as during pregnancy[6].

Contamination of herbal preparation with heavy metals is known to be associated with serious health hazards. Some of these metals, including lead, have been reported to be carcinogenic, as a result of their inherent ability to form bonds with sulphydryl groups of proteins and depletion of glutathione[7,8], while others generate reactive radicals which may alter DNA bases as well as calcium and sulphydryl homeostasiss[7]. This implies that consumptions of herbal preparation contaminated with these metals at concentrations beyond safety limits may be detrimental to human health. Besides, these metals are non-biodegradable and long term exposure may result in bioaccumulation and still elicit effects which are detrimental to human health[9]. Increasing acceptability and use of herbal medicines has spurred the introduction of newer herbal formulations into the market. While a number of these products have safety and quality endorsement, some remain untested and their use are not monitored[10]. This limit the understanding of the risk associated with their usage. Therefore, quality and safety assessment of products, to determine the presence and concentration of heavy metals, which pose significant health risk to people is crucial. This has been previously emphasized by the WHO in her guideline on safety monitoring of herbal medicines in pharmacovigilance systems[11].

In modern medicine, the use of combination therapy in disease management is well established, mainly because of its superiority over the use of a single therapy[12]. Combination therapies, which is known to improve medication adherence, reduce drug resistance and provide synergistic therapeutic effect, has offered new hopes for treatment of various diseases such as hypertension [12] and cancer immunology[13]. Similarly, the use of combination of medicinal plants to achieve enhanced and synergistic therapeutic effect is a known and well-practiced culture. For instance, Okudiabet, a polyherbal formulation consisting of *Stachytarpheta angustifolia, Alstonia congensis bark and Xylopia aethiopica fruits extract* is indicated for the treatment of diabetes[14] , while Mama decoction, a polyherbal preparation consisting of the leaves of *Mangifera indica*, *Alstonia boonei*, *Morinda lucida* and *Azadirachta indica*, is indicated for the treatment of malaria infection[15] . Even though, polyherbal formulations are generally accepted to possess excellent therapeutic potentials, their safety and quality assessment are generally not as comprehensive as those of conventional medicines. Several reports in the literature have demonstrated the presence of heavy metals in herbal medicines at concentrations higher than acceptable limits set by the WHO[16-18]. These emphasize the need to continuously screen herbal formulations for heavy metals contamination, so that information on the risk associated with their usage can be available to regulatory bodies and the public. This paper reports

the heavy metal contents of two newly marketed polyherbal formulations (AMO and ZAK herbal bitters) in Ilorin metropolis using a validated AAS method.

MATERIALS AND METHODS

Polyherbal Formulations

Two polyherbal formulations (AMO and ZAK herbal bitters) were evaluated in this study. The herbal bitters were purchased from a registered pharmacy in Ilorin. The two formulations were selected for evaluation because of its high level of prevalence in the study area. Other inclusion criteria include: registration with National Agency for Food and Drug Administration and Control (NAFDAC), non-expiration, and good physical state.

Materials

Reference heavy metal standards; iron (Fe), lead (Pb), copper (Cu), cadmium (Cd), manganese (Mn) and zinc (Zn) were all obtained from Merck-KGaA (Darmstadt, Germany) and donated by the Central Research Laboratory (CRL), Ile-Ife, Nigeria for the study. All other reagents were of analytical grades. Heavy metal analysis was carried out using an Atomic Absorption Spectrophotometer (Buck Accusys model 211)

Preparation of stock solutions and calibration standards

Reference solutions of the heavy metals were prepared from their respective reference standards by dissolving 1000 mg in 10 mL of nitric acid solution and made to volume with de-ionised water to obtain a final concentration of 1 mg/ml. Working solutions were prepared from the 1 mg/ml stock solution and was subsequently used in the preparation of calibration standards for each metal. A 100 mL quantity of each of the standard metal solution was adjusted to pH of 2.5 by adding two drops of 1 M nitric acid solution and then subjected to further treatment as previously described[19]. The absorbance of the resultant solution was subsequently measured using an Atomic Absorption Spectrophotometer (AAS), each equipped with corresponding hollow cathode lamp at wavelengths 248.3, 279.48, 283.31, 213.86, 357.87, and 324.75nm for iron (Fe), manganese (Mn), lead (Pb), zinc (Zn), cadmium (Cr) and copper (Cu) respectively. The nebulizer, atomizer and burner were flushed each time with de-ionized water after each sample was aspirated before the next. The stability of the equipment was monitored at intervals during analysis as previously described (19). Calibration curves for each metal was constructed using a linear regression equation for nominal concentration against absorbance[19]

Sample preparation and digestion

Samples were digested using 10 mL of a mixture of concentrated hydrochloric and nitric acid (1:3) to 25 mL volume of the herbal formulation and heated at a temperature of 115^{o}C until a clear solution was obtained. The solution was thereafter left to cool and made up to 50 mL with distilled water. Lanthanum was added to these solutions to prevent potential anionic inference.

Sample Analysis

Absorbance for each metal was taken at wavelengths 248.3, 279.48, 283.31, 213.86, 228.80, and 324.75nm for iron (Fe), manganese (Mn), lead (Pb), zinc (Zn), cadmium (Cd) and copper (Cu) respectively after digestion of the samples. This was done in triplicate using a validated Atomic AAS method. Metal concentration in each of the sample was extrapolated from the regression equation obtained from the respective calibration curve generated for each metal.

Method Validation

Linearity and range

Five different concentrations of the reference samples of each of the heavy metals were prepared and the absorbance were measured at specified wavelengths. Linearity was evaluated by determining the correlation coefficient (r^2) which correspond to the linear regression equation obtained from the plot of nominal concentrations of heavy metals against absorbance. The sensitivity of the method was assessed using the limit of detection (LOD) values, calculated using the formula 3.3SD/b, where SD is the standard deviation of analytical responses and b is the slope of the calibration curves for each of the metal investigated.

Precision studies

The inter and intra-day precision analysis was carried out by spiking ten blank samples with a known concentration of the reference heavy metal sample (0.4 µg/ml for copper and zinc, 2 µg/ml for iron, manganese, cadmium and lead) three times within the same day and on three consecutive days. Level of precision was thereafter determined by calculating the relative standard deviation (RSD) values for each heavy metal and compared with standard values in the literature.

Accuracy

For recovery studies, pre-analyzed samples of the two formulations were spiked with known concentration of the reference standards of each heavy metal and subsequent solutions were reanalyzed. The concentrations of each of the heavy metal was determined and compared to known values of heavy metal added.

Statistical Analysis

The results were expressed as the mean ± S.D. Data was analyzed using Excel 2016. Statistical analysis was carried out using Student's t-test to compare heavy metal content in the polyherbal formulations with standard limits. Probability values lesser than 0.05 was considered statistically significant.

Results

Results obtained from linearity studies is shown in Table 1 below. The analytical method was linear over varying concentration ranges with the mean correlation coefficient ranging between 0.988 and 1.000. The limit of detection was 0.001 mg/L, 0.001 mg/L, 0.002 mg/L, 0.005 mg/L, 0.025 mg/L, and 0.002 mg/L for copper, manganese, cadmium, iron, lead, and zinc respectively. Inter and intra-day precision ranges from 0.34-3.69% and 0.25-6.25% for AMO bitters and 1.03-5.57% and 0.97-14.11% for ZAK herbal bitters respectively. Mean percentage recovery was between 92-105% for both formulations Precision and recovery results are summarized in Tables 2 and 3 respectively.

Table 1: Linearity studies

Parameters	Fe	Zn	Mn	Cd	Pb	Cu
Linear Range (mg/L)	5-50	0.2-1.6	0.1-0.4	1-4	1-4	0.02-1.0
R^2	0.993	0.991	0.998	0.988	0.994	1.000
Slope	41.821	7.1864	15.376	12.586	0.0088	15.411
Intercept	0.0513	0.0317	0.0586	-0.1700	-0.0004	0.0039
LOD (mg/L)	0.005	0.002	0.001	0.078	0.025	0.001

Table 2: Precision studies

Sample	Heavy metals	Concentration mg/L	Intra-day Precision Mean(SD)	Intra-day Precision(%CV)	Inter-day Precision Mean(SD)	Inter-day Precision(%CV)
AMO bitters	Pb	2.00	1.98±0.025	1.26	2.05±0.089	4.38
	Fe	2.00	1.97±0.007	1.34	1.98±0.005	0.25
	Cu	0.40	0.42±0.003	0.81	0.39±0.056	5.59
	Cd	2.00	2.04±0.057	2.79	1.98±0.128	6.45
	Zn	0.40	0.39±0.014	3.69	0.41±0.054	5.37
	Mn	2.00	1.99±0.048	2.41	2.04±0.097	4.75
ZAK herbal bitters	Pb	2.00	2.01±0.039	1.96	1.97±0.086	4.38
	Fe	2.00	1.99±0.021	1.03	1.98±0.102	5.13
	Cu	0.40	0.39±0.023	5.97	0.38±0.073	19.11
	Cd	2.00	2.04±0.051	2.48	1.99±0.114	5.74
	Zn	0.40	0.41±0.018	4.37	0.38±0.023	6.13
	Mn	2.00	1.97±0.043	2.18	2.00±0.035	1.74

Table 3: Recovery studies

	Heavy Metal	% recovery	%CV
AMO Bitters	Lead	91.9	1.24
	Iron	101.4	2.07
	Copper	104.6	0.86
	Cadmium	95.4	0.88
	Zinc	92.7	1.65
	Manganese	98.7	0.74
ZAK Herbal Bitters	Lead	93.3	1.43
	Iron	98.8	1.77
	Copper	99.7	2.57
	Cadmium	97.6	1.51
	Zinc	96.4	1.29
	Manganese	95.3	1.32

Lead, zinc and cadmium were undetectable in both AMO and ZAK herbal bitters while the presence of iron, copper and manganese were observed in varied concentrations (Table 4). The mean±SD concentration of iron in AMO and ZAK herbal bitters were 15.0±0.04 mg/mL and 42.75±0.07 mg/mL respectively, the values exceeded WHO safety limits for iron in local drinks[20].

Table 4: Mean±SD concentrations of heavy metals present in AMO bitters and ZAK herbal bitters in mg/L

Sample	Pb	Fe	Cd	Cu	Zn	Mn
AMO bitters	0.00±0.00	15.0±0.04	0.00±0.00	0.03±0.006	0.00±0.00	0.16±0.008
ZAK herbal	0.00±0.00	42.75±0.07	0.00±0.00	0.026±0.005	0.00±0.00	0.351±0.006
WHO limit (mg/kg)	10.00	0.1	0.3	NE	50	NE
Singapore (mg/L)				150		

NE: Not Established; WHO: World Health Organization.

Discussion

The determination of the presence and concentration of heavy metals in herbal preparations is critical to ensure optimal quality standards and establish the risk associated with their consumption. The WHO recognizes the need to ensure adequate safety and quality of all herbal preparations and set guidelines for quality control of these products[11]. However, most manufacturers are unaware and majority of their product gain assess into the open market without been subjected to strict quality assessment. In this paper, we report the contents of selected heavy metals in two polyherbal liquid preparations newly marketed and increasingly consumed in Ilorin metropolis using a validated AAS method. While a number of studies have evaluated the heavy metal contents of herbal medicines consumed in Nigeria, the validation of the method employed in most of these studies were not reported[16,18] . The validation of the AAS method described is desirable to build confidence and guarantee reproducibility of the analysis of the heavy metal contents presented in this paper.

The analysis was linear over varying concentrations range, with the correlation coefficient (r^2) exceeding 0.98 for all metals investigated in both preparations. The observed inter and intraday precision did not exceed 15% and the accuracy was above 90%, indicating an accurate, precise and robust assay. Cadmium, lead and zinc were absent in both polyherbal formulations, while variable concentrations of iron, manganese and copper were observed. Both polyherbal preparations contained high levels of iron (15.0 and 42.6mg/L for AMO and ZAK herbal bitters respectively). The findings in this paper revealed a significantly ($p<0.05$) higher Amount of iron when compared to 0.1 mg/L set by the WHO standard[11]. The presence of iron above WHO safety limits in some other herbal preparations consumed in Nigeria have previously reported. For instance, Ayanniyi *et al*, 2016 reported observed iron concentrations up to 0.27mg/L in some herbal bitters consumed in Ilorin. While iron may be of therapeutic value such as in the treatment of iron deficiency anaemia, excessive intake could be detrimental to human health, causing gastrointestinal irritation, characterized by diarrhoea, abdominal pain and vomiting[21]. When compared with AMO bitters, the iron contents of ZAK herbal bitter is significantly higher. This may be explained by the differences in the

individual plant composition of the formulations as well as the sources of collection of plant materials used in the production of the polyherbal preparations. The high level of iron observed in the samples evaluated raises safety concerns and suggest the need for periodic evaluation of the elemental profile of polyherbal preparation

The concentration of manganese in AMO bitters was well below those found in ZAK herbal bitters. This may be attributable to the differences in the composition of plant materials in the two preparations as well as the location where they were obtained. The observed concentrations of manganese in both liquid polyherbals were higher than those previously reported in five herbal liquid preparations commonly consumed in Kano state, North Western part of Nigeria[22]. It is however important to mention that relatively high level of manganese in herbal medicines has been previously reported elsewhere[17,23]. While manganese may be an essential co-enzyme in oxidative processes, extremely high levels can be detrimental[24]. Although, there is no permissible limits set by WHO regarding the manganese contents of herbal medicines, it is important to keep the level of these essential metal within safety limits. The copper contents in the two polyherbal formulations investigated were comparable, though lower compared to those reported present in selected medicinal herbs in Kenya, Nigeria and India[22,25]. Although, the ingestion of copper within safe limits play essential physiological roles, including detoxification of free radicals and tissue development[26], excessive consumption may be associated with harmful effects such as skin infection and irritation of the respiratory tract[25]. While it is important to point out that the WHO has not yet established permissible limits for copper in herbal medicines, the level of copper reported in the two polyherbal liquid formulations was significantly below the national limits of 150 mg/L set by Singapore[27]. The high level of iron noted in this study raises safety concerns and highlight the need for occasional and comprehensive assessment of metal contents of polyherbal formulations to ascertain its suitability for safe human consumption.

We screened only two polyherbal formulations which have been recently introduced into the open market and increasingly consumed in Ilorin. Thus, findings reported here may not be sufficient to make a general statement regarding the quality of other herbal mixtures marketed and consumed in other part of Nigeria. However, this finding is desirable to emphasize the need to monitor the quality and safety profile of herbal mixtures sold to the public and ensure strict compliance to standard specifications set by the regulatory bodies to safeguard the health of the populace who regularly consumed these herbal products.

Conclusions

Our result revealed the presence of iron at concentrations exceeding WHO permissible limits in both polyherbal preparations evaluated. This indicates a potential risk for iron poisoning with long term consumption of these products. Intensive effort by regulatory agencies to ensure the safety and quality of polyherbal formulations is recommended.

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