



Evaluation of heavy metal and microbial content of a multicomponent herbal preparation

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Abstract

Herbal mixtures are extensively consumed globally for medicinal use due to the belief that they are safe and readily accessible. The herbal preparation (SHM), evaluated in this study, is made up of 7 plants found in Nigeria and it is used internally and externally for varieties of disease conditions. The heavy metals and microbial content of SHM was evaluated. Three batches of the SMH were analyzed for presence of heavy metals using Atomic Absorption Spectrophotometer. The microbial load was also evaluated by determining the total viable yeast, mould and coliform bacteria count. The metal and microbial content of the samples were compared with WHO permissible limits and differences in concentration among the batches were determined. Fe (0.010-0.100 mg/L) and Zn (0.010-0.030 mg/L) detected were significantly ($P < 0.05$) lower than the permissible limits while Cu, Pb and Cd were absent. A batch had total yeast and mould count (4.95×10^3 Cfu/mL) above the permissible limit whereas the other batches were below the limit. Microorganisms (yeasts, mould like *Aspergillus flavus* and *Rhizoctonia solani*) were present in all the batches and hence SHM may not be safe for internal use.

Keywords: Herbal mixtures, Heavy metals, Microbial load, Atomic absorption spectrophotometer

INTRODUCTION

The use of herbal mixtures around the world is on the rise (about 80% of the world, as at 2013) [1]. This increase is spurred by the belief that herbal products are safe and readily accessible as compared to synthetic drugs that are not only costly but also have adverse effects [2]. However, recent studies have shown fluctuating amounts of contaminations ranging from heavy metals, micro-organisms and other adulterants in herbs and herbal products from different parts of the world,

indicating need for continuous evaluation of the herbal products [2-4]. Some of these studies reported microbial and metal content beyond the WHO permissible limits [5].

The acceptance of medicinal herbal products for improving health is on the increase due to their availability and affordability. Therefore, analyzing these products for their heavy metal content and microbial load has become imperative. The level of heavy metal and microbial concentration has implications on the general

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health of humans as they can cause systemic damage where they are above approved limits and even where in low limits, their bio accumulative property may render them detrimental [2,5].

Though oral medicines are not required to be absolutely sterile, there is a limit to the number of microorganisms and heavy metal contaminants these preparations should contain. The presence of high microbial count in any preparation can lead to the proliferation of such organisms within the preparation leading to spoilage and importantly they can cause infections in both healthy and immune-compromised people [5]. Although heavy metals are naturally occurring elements, continued exposure of herbal products to their constituents could lead to the birth of a variety of ailments due to their wide and prolonged use [6].

The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) have jointly proposed acceptable levels of toxic substances that can be ingested on a weekly basis called the Provisional Tolerable Weekly Intake (PTWI), generally used for contaminants that may accumulate in the body [3]. Despite this, most studies have reported the concentration of heavy metals and presence of microbial loads beyond the permissible ranges [7-8]. This may be due in part to lack of enough knowledge by traditional practitioners of the guidelines for quality assurance and control of herbal medicine that have been put in place by WHO, resulting in medications with various types of heavy metal and microbial contamination [9]. Consequently, there are many cases of heavy metal poisoning and microbial contamination most of which are, sadly, underreported or not reported. Thus there is a continuous need for constant evaluation and investigation of herbal products to validate the quality and safety for human consumption.

This present study evaluated a commonly used herbal preparation in Nigeria

represented as SMH for the purpose of this study. SMH is indicated for treatment of several conditions like; chicken pox, small pox and measles, viral infections, malaria, diarrhea, ulcers, liver inflammation, bacterial infections, inhibition of platelet aggregation, stomach cancer, aphrodisiac, obesity, arthritis, diabetes, stomach ache, pile, warts, sores, laxative among others. The herbal product contains 7 herbal constituents (*Allium sativum*, *Combretum misrantom*, *Ficus carica*, *Nauclea latifolia*, *Sterculia urens*, *Tetrapleura tetraptera*, *Xylopiya aromatica*); the various indications are believed to be due to the individual or synergistic activities of the different constituents that make up the herbal preparation. The product is indicated for use in both adults and children, consumed either internally or applied externally. However, reports on the microbial and heavy metal content of SMH herbal mixture are not available in literature. This study therefore evaluated the heavy metal content and microbial content of SHM.

EXPERIMENTAL METHODS

Materials. Buck Accusys model 211 Atomic Absorption Spectrophotometer (AAS) equipped with corresponding hollow cathode lamp (Lead, Cadmium and Iron) at the time of analysis, De-ionized water, Muller Hilton Agar, Sabouraud dextrose agar, Microscope (LABOMED USA LX 300), Autoclave, Vortex mixer and incubator. All reagents used were supplied by the laboratory and of analytical grade.

Sample collection. Based on availability, using convenience sampling, three batches of SMH were purchased from registered Pharmacies in different locations of Lagos State as supplied by manufacturer in original packages. Manufacturing date, expiry date, batch number and National Agency for Food and Drug Administration and Control (NAFDAC) registration numbers were examined. Ethical clearance was obtained

from the Ethical Review Committee of the University of Ilorin, Ilorin, Nigeria. The experiment was given an approval number UERC/ASN/2019/1859.

Preparation of calibration curve. Deionized water was used in preparing solutions of each of the metals to be tested for. Stock solution was prepared by dissolving each solid metal sample 1.0 g in 10 mL 1:1 nitric acid solution, which was transferred into 1000 mL volumetric flask and made up with deionized water. Standard solutions were made from each metal stock solution of 1000 mg/L. Calibration curve was prepared by successive serial dilution of the stock solution. Working standard solutions of iron (Fe), lead (Pb), cadmium (Cd), zinc (Zn) and copper (Cu) were used for comparison. A 100 mL quantity of standard solution of each metal was adjusted to pH 2.5 by adding 1 M nitric acid. Each blank and standard solution was transferred into individual 250 mL separating funnel. Ammonium pyrrolidine dithiocarbamate (1 mL) was added followed by 10 mL methyl isobutyl ketone then solution was shaken vigorously for 2 minutes and allowed to settle. Aqueous layer was discarded while the organic layer was aspirated directly into the flame and absorbance was recorded using atomic absorption spectrophotometer BUCK ACCUSYS 211 model equipped with each hollow cathode lamp at 283.2 nm, 248.3 nm, 228.8 nm, 327.4 nm, and 213.9 nm wavelengths for lead, iron, cadmium, copper and zinc respectively. The nebulizer, atomizer and burner were flushed each time with deionized water and after every sample, aspirated before the next. Stability of equipment was checked at intervals by introducing highest working standard solution and blank [10-11].

Sample pretreatment (aqua-regia hot plate method). A quantity of 3 mL of SMH was measured into a digestion flask and digested with 12 mL of aqua-regia (HNO₃: HCl) in the ratio 1:3 on a hot plate under a fume cupboard

at 80°C until dense white fumes appeared. The flask was allowed to cool then little quantity of deionized water was added. The herbal mixture was digested again until clear solution was obtained. This solution was left to cool and filtered into 100 mL volumetric flask then made up to mark with deionized water. This procedure was repeated three times [12-13]. Same procedure as above was carried out on the other batches.

Sample analysis. Digested samples were analyzed using atomic absorption spectrophotometer BUCK ACCUSYS 211 model equipped with each hollow cathode lamp at 283.2 nm, 248.3 nm, 228.8 nm, 327.4 nm, and 213.9 nm wavelengths for Pb, Fe, Cd, Cu, and Zn respectively. Absorbance for each metal in each digested sample was recorded. Concentration of each metal from sample was determined from calibration curve.

Microbial analysis. SHM was shaken properly to ensure a uniform distribution of the content. One milliliter of the suspension was homogenized with 9 ml of distilled water and shaken vigorously. One milliliter of this suspension was then transferred to another tube until a tenfold serial dilution was obtained. Molten nutrient agar was sterilized at 121°C for 15 minutes and cooled to about 45°C for bacterial count and Sabouraud dextrose agar for fungal count. The mixture solidified at room temperature. The plates were incubated at 37°C for 24 hours and 25°C for 3-5 days for bacterial and fungal cells respectively. Colonies were counted using a digital colony counter and plates having between 25 and 250 colonies were counted as colony forming units per milliliter (cfu/ml) [14]. Morphological characteristics and staining with microscopic identification were carried out on the fungal isolates. Microbial load was translated as Cfu/mL of sample [5].

Statistical analysis. Results were presented as the mean ± S.E.M. Data were analyzed using Graphpad version 8.01. Statistical analysis was

carried out using ANOVA and statistical significance was taken at $P < 0.05$.

RESULTS

Heavy metal content. Traces of heavy metals (Fe and Zn) were found in samples of the herbal mixture investigated in this study. The quantitative analysis revealed that copper, lead and cadmium were undetectable by the atomic absorption spectrophotometer (AAS) in all batches of SHM. Iron (Fe) and Zinc (Zn) were present at varying concentrations across the batches analyzed. The concentration of Fe ranged between 0.010-0.100 mg/L while Zn was between 0.010-0.030 mg/L across the three batches. Batch C had the highest concentration of Fe and Batch B the highest for Zn while Batch A recorded the least concentration of both Fe and Zn. (Table 1) The concentrations of heavy metals obtained from each sample were compared with the

WHO permissible limits of 0.1 mg/L, 20 mg/L, 0.3 mg/L, 50.0 mg/L, and 10 mg/L for Fe, Cu, Cd, Zn and Pb respectively (WHO, 2007). Fe and Zn were present but in ranges within the WHO permissible limits. The highest concentration of Fe recorded was 0.1 mg/kg which is not significantly ($P < 0.05$) when compared to the permissible limit of 0.1 mg/L, Zn had the highest concentration of 0.030 mg/L with a permissible limit of 50 mg/L. Cu, Cd and Pb were absent in the entire sample as presented in tables 2, 3 and 4.

Microbial content. Total viable count and combined total yeast and mould count were determined (Table 5). After incubation, all plates containing 25-250 colonies were considered and their colony forming units per milliliter calculated. Counts above 250 are considered too numerous to count largely because it may be impossible to tell if colonies are separated [15].

Table 1: Concentration of Fe, Cu, Zn, Pb and Cd in Batches 1, 2, and 3 SHM samples

Samples	Concentration (mg/L)				
	(Fe)	(Zn)	(Cu)	(Pb)	(Cd)
A1	0.015 ± 0.005	0.020 ± 0.000	0.000	0.000	0.000
A2	0.030 ± 0.010	0.020 ± 0.000	0.000	0.000	0.000
A3	0.030 ± 0.000	0.020 ± 0.000	0.000	0.000	0.000
B1	0.060 ± 0.010	0.020 ± 0.000	0.000	0.000	0.000
B2	0.025 ± 0.005	0.030 ± 0.000	0.000	0.000	0.000
B3	0.015 ± 0.005	0.010 ± 0.000	0.000	0.000	0.000
C1	0.100 ± 0.000	0.010 ± 0.000	0.000	0.000	0.000
C2	0.055 ± 0.005	0.025 ± 0.005	0.000	0.000	0.000
C3	0.010 ± 0.000	0.020 ± 0.000	0.000	0.000	0.000

Values mean ± S.E.M (n=3)

Table 2: Comparison of concentrations of Fe, Cu, Zn, Pb and Cd present in the SHM with the Permissible Limits for Batch 1 samples

Samples	Concentrations (mg/L)/Permissible limits (mg/L)				
	Fe	Cu	Zn	Pb	Cd
A1	0.015 ± 0.005/0.100	0.000/20.000	0.020 ± 0.000/50.000	0.000/10.000	0.000/0.300
A2	0.060 ± 0.010/0.100	0.000/20.000	0.020 ± 0.000/50.000	0.000/10.000	0.000/0.300
A3	0.100 ± 0.000/0.100	0.000/20.000	0.010 ± 0.000/50.000	0.000/10.000	0.000/0.300

Values mean ± S.E.M (n=3)

Table 3: Comparison of concentrations of Fe, Cu, Zn, Pb and Cd present in the SHM with the Permissible Limits for Batch 2 samples

Samples	Concentrations (mg/L)/Permissible limits (mg/L)				
	Fe	Cu	Zn	Pb	Cd
B1	0.030 ± 0.010/0.100	0.000/20.000	0.020 ± 0.000/50.000	0.000/10.000	0.000/0.300
B2	0.025 ± 0.005/0.100	0.000/20.000	0.030 ± 0.000/50.000	0.000/10.000	0.000/0.300
B3	0.055 ± 0.005/0.100	0.000/20.000	0.025 ± 0.005/50.000	0.000/10.000	0.000/0.300

Values mean ± S.E.M (n=3)

Table 4: Comparison of concentrations of Fe, Cu, Zn, Pb, and Cd with permissible limits for Batch 3 samples

Samples	Concentrations (mg/L)/Permissible limits (mg/L)				
	Fe	Cu	Zn	Pb	Cd
A1	0.030 ± 0.000/0.100	0.000/20.000	0.020 ± 0.000/50.000	0.000/10.000	0.000/0.300
B1	0.015 ± 0.005/0.100	0.000/20.000	0.010 ± 0.000/50.000	0.000/10.000	0.000/0.300
C1	0.010 ± 0.000/0.100	0.000/20.000	0.020 ± 0.000/50.000	0.000/10.000	0.000/0.300

Values mean ± S.E.M (n=3)

Table 5: Table showing microbial count in the Batches of SMH

Batch	Total viable count (cfu/ml)	Combined total yeast and mould count (cfu/ml)
A	Nil	4.95 x 10 ³
B	Nil	Nil
C	Nil	Nil
AHPA Limits	10 ⁴	10 ³
WHO limits for herbal material for internal use	10 ⁵	10 ³
Safety USP limits for products containing botanical ingredients	10 ⁴	10 ³

DISCUSSION

Microbial and chemical contamination may render herbal mixtures unsafe; these contaminations may result from handling/production process, cross contamination from other materials and/or the soil in which the herbs are planted [16].

Quantitative analysis of samples of SMH in the present study revealed that all the batches examined had Iron present; the highest concentration of 0.10 mg/L was found in Batch C. This range was lower than the permissible limits set by WHO as well as AHPA standard [9, 17]. Although iron was present in all the batches of SHM analyzed but not at a concentration that will manifest immediate toxicity. Previous studies have recorded iron concentration below the standard permissible limits [18-19]. However, heavy metal concentrations could reach levels with potentially hazardous effects in humans due to their cumulative properties. [20]. Iron is a known biologically active metal with vital functions in the human body including oxygen supply, blood formation and immunity. However when taken at high concentration adverse reactions like dizziness, nausea and vomiting, diarrhea, joints pain, shock, and liver damage may occur [21]. Iron toxicity has been reported to have an adverse effect on various

metabolic functions and cardiovascular system [22].

Zinc was also found in all batches of SHM at a concentration range of 0.010- 0.030 mg/L which was lower than the WHO permissible limit of 50.0 mg/L [9]. This corroborates similar studies that recorded Zn level below permissible limits in investigated herbal product [4, 23]. Zinc is an essential trace element necessary for proper growth, blood clotting, thyroid function, and protein and DNA synthesis [4]. Little information is available on Zn toxicity; high zinc intake beyond permissible limits produces both acute and cumulative toxic effects in human [24]. Copper, lead and cadmium were undetectable in all batches of SHM.

The analysis of microbial load of SHM showed no bacterial growth in all batches examined; this may be attributed to the antimicrobial properties of four out of the 7 herbal components of SHM which include: *Allium sativum* [25], *Combretum misrantom*[26], *Nauclea latifolia* [27]and *Sterculia uren* [28]. The individual or synergistic effect(s) of these herbs may have inhibited the growth of bacteria. More so, SHM itself is indicated for the treatment of bacterial infections. All three batches had less than 25 bacteria colonies per plate which means they are too low to be significant and

fall within the safety limit set by all regulatory bodies referenced. However, the combined total yeast and mould count for batch A displayed a value higher than the safety limits for WHO, AHPA and USP while batches B and C had very low counts thereby falling below the safety limits for herbal products [9, 29-31]. Morphological characteristics, staining and microscopic identification of the isolated fungal cells showed that 100% of the cells are yeast cells. Yeast contamination in an herbal product just like any pharmaceutical preparation; represent a potential hazard because it may cause product spoilage that may affect therapeutic properties of the said product. It may also possess some enzymes which may be of a health hazard to the final users of the product [32].

Some reports on herbal medicines in Nigeria have documented only the presence of bacteria in the product which was devoid of fungal/ yeast cells [20] while others have documented the presence of bacteria, fungi/ yeast and even parasites in the tested herbs [33], the present research shows only contamination by yeast cells. These molds when present in herbal products may points to improper collection, storage and transportation processes.

The results of this study corroborate those of previous reports that have shown fluctuating amounts of heavy metals and presence of microorganisms in herbs and herbal products from different parts of the world. There is need for adoption of good manufacturing process during collection and production of herbal medicines to reduce contamination [2,4,5,16].

Conclusion. The results of this study indicate the presence of zinc and iron in all batches of SHM analyzed. However, the concentrations did not exceed the permissible limits despite the significant batch-to-batch variations. The total viable count for all the three batches of SMH fall below set limits. Precautions should be taken when SMH is to be administered orally.

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