Quality and labeling information of *Moringa oleifera* products marketed for HIV-infected people in Zimbabwe

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Abstract

Labeling information and quality of marketed *Moringa oleifera* products were assessed. Personnel in 60 pharmacies and 11 herbal shops were interviewed about the sources, dosages, indications and counseling information of *Moringa oleifera* products. Content analysis of written information provided on *Moringa oleifera* products was also done. Three samples of *Moringa* from popular sources were acquired to determine heavy metal content and microbial contamination. The results were compared to specified limits in the European and Chinese pharmacopeia, World Health Organization guidelines and Bureau of Indian Standards. *Moringa* was available as capsules or powder in 73% of the premises. *Moringa* was recommended for seven different disease conditions. Four different dosage regimens were prescribed. The main references cited for the counseling information were unscientific literature (62%). The selected *Moringa* samples were contaminated with bacteria and fungi above the European Pharmacopeia specified limits. *Escherichia coli* and *Salmonella species* were present in all three samples. All three samples contained arsenic, nickel and cadmium above the permissible limits. *Moringa oleifera* with variable labeling information and poor microbial and heavy metal quality is widely available in Zimbabwe.

Introduction

When herbal medicines are unregulated, consumers are potentially exposed to unsafe products. Safety issues may arise from circulation of inconsistent or unsubstantiated drug information, heavy metal residues, microbial contamination or adulteration.[1,2] Heavy metal and microbial contamination is particularly of concern with HIV-infected individuals. Heavy metals could exacerbate the risk of liver and kidney damage associated with HIV-infection and treatment, while the microbial contamination may increase the risk of opportunistic infections due to a compromised immune system of HIV-infected people.[3]

In Zimbabwe, like many developing countries, regulation of the sale of herbal medicines is still in its infancy. The relevant statutory instrument was only gazette in September 2015. In addition, the national drug regulatory authority granted a transition period of one year before it would fully enforce the regulations. As a result, the impact of regulation is yet to be realized and unsafe herbal products may still be on the market. Very few studies have been conducted to assess potential safety issues with herbal products available on the market in Zimbabwe.

Assessing potential safety issues of commonly marketed herbal products would provide data to enable risk profiling of the herbs. The data would assist the drug regulatory agency when assessing herbal products for approval, to focus any analysis on relevant safety issues. The data would also serve clinicians as they counsel patients on herbal medicine use.

Moringa oleifera (drumstick/horseradish tree/moringa) is a herb commonly used as a nutritional supplement and immune enhancer by HIV-infected people in Zimbabwe. It is rich in nutrients including beta carotene, ascorbic acid, calcium, iron, proteins and carbohydrates and purported to have hypoglycaemic, hypotensive, hypocholesterolemic, anti-ulcer, antibacterial and anti-inflammatory activity.[4,5] While there is some evidence to support the health benefits of *Moringa*, very little is known about the safety of marketed Moringa products. This study was therefore undertaken to assess the labeling information as well as the heavy metal and microbial content of *Moringa oleifera* products in Zimbabwe.

Materials and Methods

Study design and ethical considerations

The study was a cross-sectional observational study incorporating laboratory assessments. The research protocol was reviewed and approved by the Joint Parirenyatwa Hospital and College of Health Sciences Research Ethics Committee (Harare, Zimbabwe). Oral and written informed consent was obtained from supervising personnel at each of the premises after assurance of confidentiality.

Sampling

A convenience sample of 60 pharmacies and 11 herbal shops was selected. Three sam-

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ples of *Moringa* were purchased for determination of microbial and heavy metal contamination. One sample was from a pharmacy, another from a herbal shop and the third from an open market in Harare. Selection was based on the premises that had the highest reported monthly *Moringa* sales.

Assessment of herbal medicine information

Personnel were interviewed about the sources, dosage regimen, indications and counseling information of *Moringa oleifera* using a previously piloted interview script. Labels and available package inserts from *Moringa* products stocked in the premises were reviewed and data on indications, dosage regimen and cautionary messages were captured.

Determination of microbial contamination

The examination of microbial contamination was performed according to the harmonized microbial enumeration tests in the European Pharmacopeia. Enumeration of bacteria was carried out on tryptone soya agar, while that of fungi was done on sabouraud dextrose agar. All samples were diluted with buffered sodium chloride-peptone water, pH 7.0 to the concentration of 10^{-5}. Subsequently, 1ml of each dilution was added to two sterile petri dishes of 10 cm diameter. For bacteria, tryptone soya agar was promptly added into each dish, mixed and the agar was allowed to set. After setting of the agar, the plates were incubated (Jeio Tech™ incubator; Jeio Tech Co., Ltd., Daejeon, Korea) at 30-35°C for three days. For fungi, Sabouraud dextrose agar medium was added to each dish, mixed and the content allowed to solidify. The plates were then incubated at 20-25°C for five days. The number of colonies for both bacteria and fungi was counted using a TRINITY V3™ automated zone reader and colony counter (Giles Scientific Inc., Santa Barbara, CA, USA). All tests were carried out in duplicate. A negative control was performed for all tests with sterile peptone water pH 7.0 used in place of the test preparation to verify testing conditions.

Determination of specific microorganisms

To determine contamination with enterobacteria in each sample, 10 g of the sample (weighed using Mettler PM 600 top loading balance) were added to 90 mL of Tryptone soya broth and mixed. After mixing, the material was incubated at 20-25 °C for 2 hours. Nine mL of enterobacteria enrichment broth-Mossel were inoculated with 1 mL quantities of the product to be examined. The four resultant dilutions of the preparation which contained 0.1 g, 0.01 g, 0.001 g and 0.0001 g of the product were incubated at 30-35°C for 24 hours. Each of the cultures was sub-cultured on a plate of violet red bile glucose agar and incubated at 30-35°C for 24 hours. Growth of colonies was examined. The smallest quantity of product that gave a positive result and the largest quantity that gave a negative result were noted. These results were used to determine the probable number of bacteria.

To determine contamination with *Escherichia coli*, 10 g of each sample was added to 90 mL buffered peptone water. Ten mL of the preparation was used to inoculate 90 mL of Tryptone soya broth, mixed and incubated at 30-35°C for 24 hours. The container with the material was shaken and 1 mL of tryptone soya broth was transferred to 100 mL of MacConkey broth and incubated at 42-44°C for 24 hours. The preparation was subcultured on a plate of MacConkey agar at 30-35°C for 24 hours. Growth of colonies was examined. To determine contamination with *Salmonella*, 25 g of each sample was added to 225 mL of buffered peptone medium, mixed and incubated at 30-35°C for 24 hours. After incubation, 0.1 mL of buffered peptone water were transferred to 10 mL of Rappaport *Vassiliadis Salmonella* enrichment broth and incubated at 30-35°C for 24 hours. The material was subcultured on plates of xylose, lysine and deoxycholate agar. This was incubated at 30-35°C for 24 hours. Growth of bacteria was examined.

Determination of heavy metal contamination

Herbal medicine samples were digested through wet digestion.[6] For all the samples, 5 g of the powdered sample was placed in a flask. Twenty mL of concentrated HNO_3 60% was added and heated on hot plate until product stopped producing brown fumes. Thirty mL of 1:1 solution of HNO_3 60% and perchloric acid 70% were added and heated until a suspension of approximately 1 mL was left in the flask. The residue was cooled and 5 mL of 0.5M HCL was added. Material was diluted with distilled water up to 25 mL and filtered through Whatman filter paper no. 42. The sample was then analyzed using the atomic absorption spectrometer (AAS) for chromium, cadmium, copper, lead, nickel, arsenic and zinc. The results were expressed in parts per million (ppm). The AAS operating parameters used were as shown below in Table 1.

Statistical analysis

The data were analyzed qualitatively and quantitatively using Stata®11.

Results

Source of Moringa oleifera products

Moringa oleifera was sold in 73% of the premises. The *Moringa* was supplied by local farmers in 94% of the cases. One local open market were the farmers operate from was commonly cited by participants (90%). A small proportion of the proprietors (4%) sold their own cultivated supplies. Occasionally (10%) *Moringa* was imported, mainly from a single South African phyto-pharmaceutical company.

Labeling information available with *Moringa oleifera* products sold in Zimbabwe

Moringa was recommended for seven different disease conditions including HIV infection, diabetes, hypertension, joint pain, prostate disorders, tuberculosis and arthritis. Four different dosage regimens were prescribed. The regimen prescribed did not vary with the conditions indicated and did not include the course duration. The main references cited for the counseling information were unscientific literature that included books, magazines and newspapers (Table 2).

Microbial counts of *Moringa* samples

All three samples were contaminated with aerobic bacteria and fungi above the European Pharmacopeia limits. *Moringa* from the herbal shop had the highest bacterial count while that from the pharmacy had the highest fungal count. The results are shown below in Table 3.

Presence of *Salmonella species*, *Escherichia coli* and enterobacteria

All three samples were contaminated with both *Salmonella species* and *Escherichia coli.* None of the samples were contaminated with bile tolerant gram-negative enterobacteria.

Table 1. Instrumental parameters for heavy metal analysis.

Element	Cr	Cd	Cu	Ni	Pb	Zn	Ar
Wavelength (nm)	357.9	228.8	324.8	232	217.3	213.9	193.7
Slit width (nm)	0.2	0.5	0.5	0.2	1	1	1
Lamp current (mA)	7	3.5	3.5	3.5	EDL	5	EDL

EDL, electrodeless discharge lamp. Flame type was air-acetylene (BOC, South Africa).

Heavy metal contamination

All three samples contained Arsenic, Nickel and Cadmium above the permissible limits. Lead, copper and zinc where within permissible limits. The results are shown in Figure 1A-F.

Discussion

Herbal therapy is rapidly developing within different cultures and regions of the world. As a result, there is likely to be variability in the composition, manufacturing processes and clinical application of products of the same herb.[7] In order to reduce the risk of adverse events attributable to unsafe herbal medicines, the WHO recommends the provision of minimum labeling information as well as assessment for contaminants of herbal products.[8,9]

Consistent with findings from previous studies, the labels reviewed in this study consisted of variable labeling information and generally lacked cautionary messages.[10] The information is also likely to be biased given that the references were predominantly unscientific and were provided by the supplier in many of the cases. It was also interesting to note that traditional knowledge contributed to the information supplied on herbal products. This provides further evidence that opinions of traditional practitioners still have the potential to influence medication choices among Zimbabweans.[11] Ideally, indications, dosage regimen and cautionary labels should be based on systematic reviews of rigorous clinical trials or documented history of clinical use in the case of traditional practices.[12] A positive observation was that most of the labels indicated the parts of the plant that was sold. This is likely because of the ease of differentiation by color between plant part powders.

The three samples assessed in this study all had microbial loads above the recommended limits. This is a common finding in similar studies.[13,14] In our case, since *Moringa* is a cultivated species in Zimbabwe, the herbal products may have been contaminated by microbes carried over from the soil or animal manure and waste used as fertilizers. Given that the herb was mainly supplied by local farmers who may not be trained in hygienic processing of herbs and operating from an open market, the contamination could also have been transferred from personnel during harvesting and processing of the plant material. Additionally, growth of microbes may have resulted from poor moisture control during drying or post-harvest storage of plant material.[15] Microbial contamination of *Moringa* products poses a significant risk for opportunistic infections in HIV-infected people because they often present late for antiretroviral therapy when their immune system is already severely compromised. Arsenic, cadmium and nickel levels in the samples evaluated were above the recommended limits. The findings are consistent with those from some previous studies but not others.[16,17] The variation may be due to differences in cultivation environments since *Moringa* is known to absorb heavy metals from environments such as industrial and waste dumps during cultivation.[18] Chronic exposure to arsenic can cause irritation of the stomach and intestines and decreased production of red and white blood cells. In addition, contamination with large amounts of arsenic can increase the chances of development of various cancers.[19] Cadmium may be introduced into the environment through the use of fertilizers and sludge that contain cadmium. It has the potential to accumulate in the body due to its slow elimination (half-life of 20-30 years). Cadmium is associated with kidney toxicity and reduced bone mineral density.[20] *Moringa oleifera* products contaminated with arsenic and cadmium could therefore potentially exacerbate problems associated with HIV-infection and treatment such as kidney, gastrointestinal and immune function as well as anemia and the risk of certain cancers. The *Moringa* samples analyzed had nickel levels marginally above the recommended limit. Nickel is relatively non-toxic in comparison with other metals. As such risk to nickel poisoning from *Moringa* consumption is considered minimal.

Table 2. Labeling information available with *Moringa oleifera* products sold in Zimbabwe.

Description	Frequency (%)
Formulation sold	
Tablet	0
Capsule	8
Powder	92
Decoction	0
Part plant sold	
Bark	44
Leaves	77
Roots	2
Unknown	8
Indications	
Chronic	71
Acute	29
Recommended dosage regimen	
1 tsp daily	31
1 tsp 3 × daily	50
1 cap daily	6
1 tbsp 3 × daily	19
Counseling messages	
Take with food	52
Take after food	6
Avoid when pregnant	2
None	40
Herbal drug information references	
Traditional knowledge	10
Unscientific literature	62
Supplier	46
Healthcare professional	0

Conclusions

Moringa oleifera products assessed contain variable, unsubstantiated labeling information and were contaminated with bacteria, fungi, cadmium, arsenic and nickel above recommended limits. The contaminants may potentially exacerbate problems associated with HIV infection and treatment. Future regulatory assessments for approval of sale of *Moringa oleifera* products should priorities assessment of microbial burden as well as cadmium and arsenic levels. The contaminants may potentially exacerbate problems associated with HIV infection and treatment.

Table 3. Microbial counts in *Moringa* samples.

Test	Source	Total aerobic microbial count (cfu/g)	Reference limit*	Comment
Total aerobic microbial count of samples	Pharmacy	1×10^7	5×10^4	Above limit
	Herbal shop	3×10^7	5×10^4	Above limit
	Open market	3×10^6	5×10^4	Above limit
Total fungal (yeasts and molds) count	Pharmacy	7×10^6	5×10^2	Above limit
	Herbal shop	5×10^6	5×10^2	Above limit
	Open market	4×10^6	5×10^2	Above limit

*European Pharmacopoeia.

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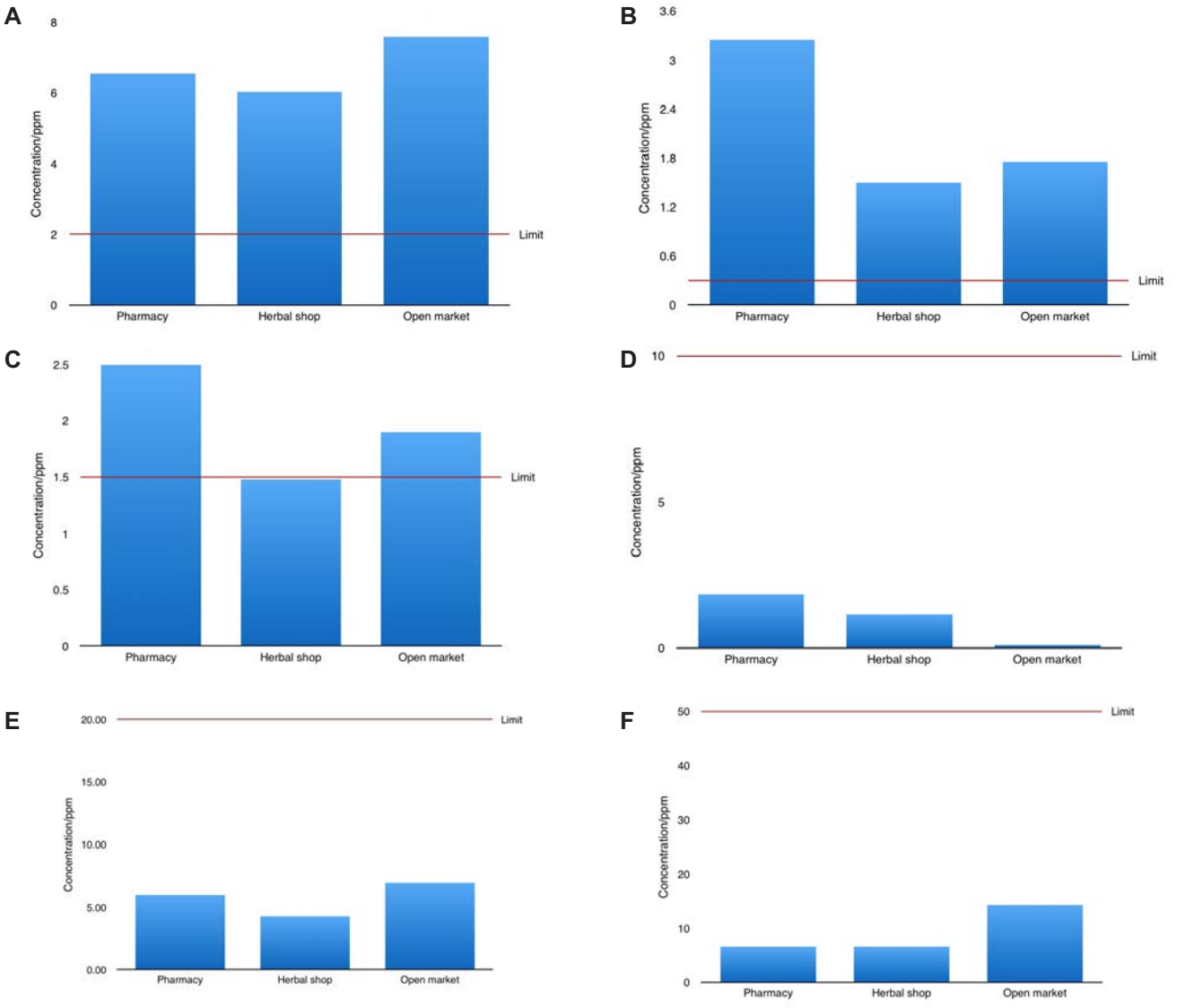

Figure 1. Concentration of Ar (A), Cd (B), Ni (C), Pb (D), Cu (E), Zn (F) detected in samples.

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