Health Risk Assessment and Determination of Some Heavy Metals in Commonly Consumed Traditional Herbal Preparations in Northeast Ethiopia

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Background. Most traditional medicines were prepared from plant origins. These plants could be contaminated by heavy metals, pesticides, and/or toxins. Therefore, the aim of the present study was to determine the level of heavy metals such as lead (Pb), cadmium (Cd), chromium (Cr), and copper (Cu) in frequently used traditional herbal preparations sold in Northeast Ethiopia and to estimate their health risks associated with their daily intake. Methods. A total of 6 traditional herbal preparations were randomly collected from local herbal shops of Dessie and Kombolcha town, Northeast Ethiopia. The samples were prepared for analysis by wet digestion method using nitric acid and hydrochloric acid treatment. The accuracy of the method was analyzed by the spike recovery test. Determination of Pb, Cd, Cr, and Cu by microwave plasma atomic emission spectroscopy was made in herbal preparations traditionally used in Dessie and Kombolcha town, Northeast Ethiopia. By calculating estimated daily intake (EDI), hazard quotients (HQ), and Hazard Index (HI) of metals, the health risk associated with the consumption of the analyzed herbal preparations was also evaluated. Results. The levels of heavy metals were in the range of 3.0–3.92 mg/kg for Pb, 5.35–10.7 mg/kg for Cr, and 0.815–12.3 mg/kg for Cu. However, cadmium was not detected in any of the traditional herbal preparations. This study revealed that the level of Pb and Cu in all analyzed samples was within the WHO maximum permissible limit of 10 mg/kg and 40 mg/kg, respectively. The level of Cr in all traditional herbal preparations was beyond the WHO maximum permissible limit (2 mg/kg). From the health point of view, the HQ value of Cr for KD-03 and KD-04 is greater than 1, suggesting potential health risk. Furthermore, the HI value had revealed that the consumption of KD-02, KD-03, and KD-04 samples had the potential of posing health risks to consumers over long-term consumption of herbal preparations. Conclusion. This study showed that most of the metal concentration levels in the herbal products were within the WHO maximum permissible limits. However, all samples had Cr levels above the WHO maximum permissible limit. Based on the results of this study, there would be a noncarcinogenic health risk to the consumer associated with the consumption of some herbal preparations marketed in Northeast Ethiopia.

1. Background

It is estimated that about 70–80% of the world’s population relies on nonconventional medicine, mainly of herbal origin. However; owing to the nature and sources of herbal medicines, they are sometimes contaminated with arsenic, heavy metals (lead, mercury, cadmium, and chromium), pesticides, and/or toxins, resulting in multiorgan damage to the consumers [1].

Traditional and complementary medicine is an important and often underestimated health resource with many applications, especially in the prevention and management of chronic diseases and in meeting the health needs of aging populations [2]. Ethiopia is known for its rich tradition of herbal medicine. Herbs are traditionally used for the treatment and prevention of diseases such as stomach pain, headache, diabetes, hypertension, rheumatism, and many others [3].
2. Materials and Methods

2.1. Materials

2.1.1. Instruments and Apparatus. Microwave plasma atomic emission spectroscopy (Agilent MP-AES 4210, USA), vacuum oven (digital heat j.p. Selecta, Spain), hotplate (digital timer function hotplate, Thermo Scientific, USA), digital analytical balance (Mettler Toledo Me204, Switzerland), Whatman filter paper (Whatman TM No. 41, 150 mm diameter, GE Healthcare UK Limited), porcelain/ceramic mortar and pestle, grade A volumetric flasks of 50 ml, 100 ml, 500 ml, and 1000 ml (Pyrex, USA), grade A volumetric pipette of 0.5 ml, 1 ml, and 5 ml and a beaker of different sizes (Pyrex, USA), Teflon beaker (plastic wear PTFE beaker, UK), and vortex shaker (Mfr, S1-S1-p236, USA) were used for a multielemental analysis of heavy metals.

2.1.2. Chemicals and Reagents. Analytical grade chemicals and reagents were used throughout the experiment. Chemicals and reagents used for this particular experiment were hydrochloric acid (37%, Sigma Aldrich product of USA), nitric acid (70%, Sigma Aldrich product of USA), and ultrapure water (Milli-Q TM water system Darmstadt, Germany) used for standard solution preparation. Distilled water and stock solution of standard Pb, Cd, Cr, and Cu (BDH, Chemicals, Ltd., Merck, England) were obtained from the Ethiopian Public Health Institute (EPHI) laboratory, Addis Ababa, Ethiopia.

2.2. Methods

2.2.1. Study Area and Period. The study was conducted in Dessie and Kombolcha town, South Wollo zone, Amhara region, Northeast Ethiopia, from October 2019 to July 2020. South Wollo zone is one of the six zones of the Amhara region, Northeast Ethiopia. Dessie, the capital city of South Wollo zone, is 401 km far from Addis Ababa, the capital city of Ethiopia, and 540 km far from Bahir Dar, the capital city of Amhara regional state. Kombolcha is an industrial town in the east Amhara region, Northeast Ethiopia, and is located 18 km far from Dessie town. The major natural vegetation of the study areas is characterized by grassland (grazing areas) and small forests containing mainly acacia trees and shrub species. A large part of the study area is covered with agricultural farm areas. The study areas were selected because traditional herbal products were commonly used by the community to treat chronic diseases such as hypertension, diabetes mellitus, and cancer. According to the city administrative health office report, there are 29 officially
licensed traditional practitioners in Dessie and Kombolcha town during this study.

2.2.2. Sample Collection. A total of six traditional herbal preparations frequently used in the treatment of hypertension, diabetes mellitus, malaria, sexual dysfunction, kidney, and GI problems in Dessie and Kombolcha town, Northeast Ethiopia, were included in this study. Herbal samples were commercially available in powder form in plastic containers. About 150 g of each herbal preparation was purchased randomly from six different herbal shops of Dessie and Kombolcha town from February 1 to 21, 2020. Each collected sample was randomly coded from KD-01 to KD-02 and was stored in polyethylene bags for further analysis [15]. The experimental work for the determination of heavy metals was conducted at the food and drug quality control laboratory of the Ethiopian Public Health Institution (EPHI), Addis Ababa, Ethiopia. Sample code, the composition of herbal preparations, their traditional use, and collection areas are summarized in Table 1.

2.2.3. Sample Digestion and Analysis. About 150 g of each sample of traditional herbal products was accurately weighed and powdered and homogenized using mortar and pestle. Pyrex grade A volumetric flask glass with standard taper stopper was used for preserving homogenized sample. The six samples were analyzed for their toxic metals concentrations. The digestion of the samples was carried out using the wet digestion method described by Alhusban et al. [16]. From the homogenized powder of herbal preparations, about 1 g of each sample was accurately weighed and transferred to a 50.0 mL Teflon beaker. The sample solution was heated for 1 h at 110°C using a vacuum oven. Then, 10 mL of aqua regia solution (a mixture of HNO₃ (70%) and HCl (37%) in (1 : 3) v/v ratio) was added to a beaker, and the sample solution was heated for 2 h at 240°C until all the samples dissolved completely and a clear and colorless solution was obtained. After digestion was complete, the sample solution was allowed to cool in the air, and 30 mL of distilled water was added. Then, the solution was filtered using Whatman filter paper and diluted to 50.0 mL with distilled water. Finally, the concentration of Pb, Cd, Cr, and Cu in each sample was determined using microwave plasma atomic emission spectroscopy. All experiments were carried out in triplicate for precision and accuracy of the results, and blank solutions (containing the only mixture of HNO₃ and HCl) were also prepared following the same procedure used for the samples.

2.2.4. Calibration Curve Procedure. A pure analytical grade reference standard metal containing 1000 mg/L stock solution of Pb, Cd, Cr, and Cu was obtained from the Ethiopian Public Health Institution. Then, 10 mL of stock solution was diluted to 100 mL with water for each metal. Finally, from this intermediate solution (100 mg/L), five working standard solutions of 1, 5, 10, 15, and 20 mg/L were prepared. The calibration curve was constructed by plotting the standard concentration of each heavy metal against the emission intensity of heavy metals. Calibration curve regression equation and their respective correlation coefficients ($R^2$) are presented in Table 2. The concentration of the heavy metals in each sample was determined using linear regression equations obtained from the calibration curve. The concentration of heavy metals in 1 g of each sample in terms of mg/kg was determined using the following equation:

$$\text{Concentration in dry weight (mg/kg)} = \frac{\text{conc. of metal (mg/L)} \times V (L)}{\text{weight of the sample (kg)}}$$

where $V$ is the final volume (50 mL) of the digested sample solution and $W_t$ is the initial weight (1 g) of the sample measured for analysis.

2.2.5. Recovery Test. The accuracy of the method was assessed by spiking herbal samples (1 g) with known amounts of standard metals (1 ppm), and the percentage recovery was calculated. The results were obtained as the average of three replicates of each sample. The results showed the validity of the employed methods and good repeatability for the analysis of herbal samples. As shown in Table 2, the percentage of recovery values ranged from 81.8% to 117.5%. The percent recovery was determined according to the following equation:

$$\text{Percent recovery} = \frac{\text{concentration of spiked sample} - \text{concentration of unspiked sample}}{\text{conc. of known spike added}} \times 100.$$  

2.2.6. Health Risk Assessment of Heavy Metals. The risk of intake of heavy metal contaminated herbal preparations to human health was evaluated based on estimated daily intake (EDI) of metal, hazard quotient (HQ), and Hazard Index (HI). The daily intake dose (EDI) was calculated to estimate the average daily metals loading into the body system of a specified bodyweight of a consumer. The value of EDI was determined by the following equation [17–19]:

$$\text{EDI} = \frac{\text{concentration of metal (mg/kg)}}{\text{bodyweight (kg)}} \times \text{intake (g/kg)}.$$
Table 1: List of traditional herbal preparations commonly used by the community of Dessie and Kombolcha town, Northeast Ethiopia.

| Sample code | Composition of herbal preparations | Traditional use | Collection area |
|-------------|------------------------------------|-----------------|-----------------|
| KD-01       | Aloe Percrassa, Verbascum sinaiticum | Sexual impotency | Kombolcha        |
| KD-02       | Chenopodium murale                 | Kidney problem  | Dessie          |
| KD-03       | Urtica simensis, Trigonella Foenum-graceum, Calpurnia aurea | Peptic ulcer disease | Dessie          |
| KD-04       | Verbena officinalis, Dodonaea angustifolia, Calpurnia aurea | Gastrointestinal problem | Dessie          |
| KD-05       | Carica papaya, Dodonaea angustifolia | Malaria and diabetes mellitus | Kombolcha        |
| KD-06       | Rumex abyssinicus, Trigonella Foenum-graceum, Thymus vulgaris | Hypertension | Dessie          |

Table 2: Recovery test results and calibration curve equations ($Y = mx + b$) for the determination of heavy metals in herbal preparations.

| Analyte | % recovery (%) | Regression equation | $R^2$ | Wavelength (nm) |
|---------|----------------|---------------------|-------|-----------------|
| Pb      | 85.5–116.4     | $Y = 1899.4x + 200.23$ | 0.9976 | 405.7           |
| Cd      | 81.8–112.6     | $Y = 15305X + 16275$  | 0.9975 | 228.8           |
| Cr      | 86.6–114.8     | $Y = 13969X + 3289.8$ | 0.9973 | 425.4           |
| Cu      | 83.8–117.5     | $Y = 103051X + 34163$ | 0.9966 | 324.7           |

$\text{EDI} = \frac{C_{\text{metal}} \times IR}{BW}$,  

where $C_{\text{metal}}$ (mg/kg) is an average weighted heavy metal content in traditional herbal preparations, IR (ingestion rate) is the average daily consumption of herbal preparations (ram/day person), and BW is the average body weight (kg). Average daily consumption of herbal preparations (KD-02-KD-06) for an adult is 20 g/person/day while for KD-01 samples it was 10 g/person/day of dry weight. The average bodyweights of adults were considered 65 kg [20].

Hazard Quotient (HQ) is used to assess the noncarcinogenic risk to humans from long-term exposure to heavy metals from vegetables, medicinal plants, and fruits. If HQ is <1, there is no potential health effects are expected from exposure, while HQ > 1 signifies that there are potential health risks due to exposure. The HQ is calculated as a fraction of the determined dose to the reference dose as shown in the following equation [18,21–23]:

$$\text{HQ} = \frac{\text{EDI}}{\text{RfD}}$$

where EDI is the average herbal preparations intake per day (mg/kg/day) and RfD is the oral reference dose of the metal (mg/kg/day). RfD is an approximation of daily tolerable exposure to which a person is expected to have without any significant risk of harmful effects during a lifetime. RfD for Pb, Cu, Cd, and Cr is 0.004 mg/kg/day, 0.04 mg/kg/day, 0.001 mg/kg/day, and 0.003 mg/kg/day, respectively (14).

Hazard Index (HI) helps to evaluate the overall noncarcinogenic risk to human health through more than one heavy metal. Exposure to more than one pollutant results in additive effects. It is the sum of the hazardous quotient (HQ) of all heavy metals as described in the following equation: [18,19,22–24].

$$\text{HI} = \Sigma \text{HQpb} + \text{HQCu} + \text{HQCr} + \text{HQCd}$$

If $\Sigma \text{HQ} < 1$, there is a possibility of adverse effects on human health [23,24].

2.2.7. Data Analysis. All quality control measures were taken including calibration check measures, determination of recovery test, and replicate analysis of samples. The data were analyzed using SPSS version 23. The results were expressed as mean ± standard deviation (mg/kg, dry weight) of triplicate analysis.

3. Results and Discussion

3.1. Determination of Heavy Metals in Herbal Preparations. The results of this study showed that heavy metals (Pb, Cr, and Cu) were present in varying concentrations in the six herbal preparations commonly used in Northeast Ethiopia. The average concentration of heavy metals in traditional herbal preparations was shown in Table 3.

The concentration of lead in herbal samples ranged from 3.30 ± 0.8 to 3.92 ± 0.8 mg/kg (Table 3). The highest and the lowest concentrations were found in KD-06 and KD-01 samples which were used by traditional healers to treat hypertension and sexual impotency, respectively. The WHO maximum permissible limit for lead in traditional herbal preparations is 10 mg/kg [25]. Hence, all samples analyzed were within this permissible limit. Maghrabi reported similar results from different samples of herbal drugs marketed in Saudi Arabia, with the levels of Pb below the WHO permissible limit [26]. However, contrary to this finding, the concentration of lead above the WHO maximum permissible limit was reported in previous studies [12,27–30]. Lead is one of the most toxic heavy metals and progressive exposure may cause poor muscle coordination, gastrointestinal symptoms, brain and kidney damage, hearing and vision impairments, and reproductive defects. Lead bioaccumulates in biological tissues; patients who use medicinal herbs with even low concentrations of Pb over a long period of time might be at risk of chronic Pb toxicity and should be monitored for any signs of lead poisoning [3,8,31].

The WHO maximum permissible limit of cadmium in traditional herbal products is 0.3 mg/kg [25]. Cadmium was not detected in any of traditional herbal preparations, i.e.,
below the detection limit of the instrument (0.00028 mg/L) (Table 3). Similar findings have been reported in Jordan [16] and Bangladesh [32]. However, high levels of cadmium above the WHO permissible limit have been reported in South Africa [29], Iran [28], and Nigeria [12,33]. An intake of high levels of cadmium is known to be very toxic and carcinogenic. Its ingestion at lower levels can accumulate in the body and result in renal tract impairment, lung damage, and fragile bones [6,9,29].

In this study, the concentration of chromium was found in the range of 0.81 ± 0.08–12.3 ± 0.33 mg/kg as shown in Table 3. The maximum concentration was reported in the KD-01 sample while the minimum amount was reported in the KD-05 sample. The level of chromium in herbal products was above the WHO recommended permissible limits (2 mg/kg) [25]. This might be due to contamination of the raw materials used in manufacturing and contamination during the harvesting and manufacturing process of herbal plants. Similar results of high levels of chromium have been reported in a study conducted in South Africa. Exposure to high levels of chromium can result in respiratory tract problems, lung cancer, dermatitis, and permanent nose damage [29]. The health risk due to metal contamination, in general, depends on the average daily dietary intake. Chromium is lethal and toxic to humans even at lower concentrations.

The concentration range of copper in tested samples was 0.81 ± 0.08–12.3 ± 0.33 mg/kg as shown in Table 3. The maximum concentration was reported in the KD-01 sample while the minimum amount was reported in the KD-06 sample preparation. This result revealed that the concentration of copper in all traditional herbal products was found below the WHO permissible limit (40 mg/kg) [25]. Similar results were reported in Brazil [24], Nigeria [9], and Ghana [27]. Copper is an essential element for the human metabolic system. It regulates various biological processes like redox reactions, energy production, connective tissue formation, iron metabolism, and synthesis of neurotransmitters [9]. However, chronic exposure to a high concentration of copper causes irritation of nasal mucosa, vomiting, nausea, diarrhea, kidney, and liver damage [9,30].

### 3.2. Health Risk Assessment of Heavy Metals

Health risk estimation based on the estimated daily intake (EDI) of the heavy metal contaminant is one of the vital health risk assessment tools. It takes into account the frequency and duration of exposure and the bodyweight of the exposed persons. In general, health risk due to metal contamination depends on the average daily dietary intake [17]. The EDI for Cd, Pb, Cr, and Cu was within the tolerable daily intake reference limits for KD-01, KD-02, KD-05, and KD-06. The EDI for Cr was determined to be higher than the tolerable daily intake reference limit for KD-03 and KD-04 (Table 4).

The noncarcinogenic risk of heavy metals in the herbal preparations was calculated by the HQ, and the results are shown in Table 5. HQ for noncarcinogenic effects measures the long-term exposure of the heavy metal contaminants present in the herbal preparations. If the HQ value is less than 1, then the exposed consumers are assumed to be safe, and if the HQ value is equal to or higher than 1, it is considered as a level of concern or poses a health risk [18, 19, 34]. The findings showed that the HQ values for Cd, Pb, Cr, and Cu were all less than 1, suggesting the consumption of these herbal preparations (KD-01–KD-06) poses no health risk due to these metals. However, the HQ of Cd for KD-01 (1.08) and KD-04 (1.04) is greater than 1, suggesting that the long-term exposure to KD-03 and KD-04 samples poses a health risk due to overexposure to chromium (VI) (Table 5).

If the HI value of any heavy metals in herbal preparations is less than 1, it implies that the exposed population is unlikely to experience any adverse health effect in their lifetime. However, if the HQ is equal to or higher than 1, there is a potential health risk to the exposed population [22,35,36]. The HI values for KD-01, KD-05, and KD-06 were less than 1. This indicates that the combined effects of the heavy metal contaminants present in a particular herbal preparation pose no health risk in the long term for both adults. The HI for KD-02, KD-03, and KD-04 was higher than 1 probably due to a high daily intake of Cr in this herbal preparation. This indicates that long-term exposure to KD-02, KD-03, and KD-04 samples poses potential noncarcinogenic adverse health effects. Chromium (VI) is lethal and toxic to humans even at lower concentrations.

### Table 3: Mean concentration of heavy metals in traditional herbal medicinal preparations (mean ± SD, n = 3, mg/kg dry weight).

| Sample code | Pb     | Cd     | Cr     | Cu     |
|-------------|--------|--------|--------|--------|
| KD-01       | 3.30 ± 0.80 | ND     | 10.70 ± 0.27 | 12.3 ± 0.33 |
| KD-02       | 3.75 ± 0.13  | ND     | 8.45 ± 0.13  | 10.5 ± 1.14 |
| KD-03       | 4.00 ± 0.55  | ND     | 10.60 ± 0.30 | 11.05 ± 0.32 |
| KD-04       | 4.00 ± 0.36  | ND     | 10.15 ± 0.2  | 11.85 ± 0.35 |
| KD-05       | 3.00 ± 0.26  | ND     | 5.35 ± 0.65  | 0.81 ± 0.08  |
| KD-06       | 3.92 ± 0.8   | ND     | 5.60 ± 0.76  | 0.86 ± 0.11  |

*ND = Not Detected.*
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