ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 11, Issue, 03, pp.1839-1844, March, 2019

DOI: https://doi.org/10.24941/ijcr.34624.03.2019

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

DETERMINATION OF LEVELS OF SOME METALS IN SELECTED TRADITIONAL MEDICINAL PLANTS IN WOLAITA ZONE, SOUTHERN ETHIOPIA

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ARTICLE INFO

Article History:
Received 12th December, 2018
Received in revised form
15th January, 2019
Accepted 24th February, 2019
Published online 31st March, 2019

Key Words:
Elemental analysis, Medicinal plants, Flame Atomic Absorption Spectroscopy.

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ABSTRACT

The purpose of the current study was to determine the concentration of selected essential and nonessential metals; Na, Ca, Cu, Fe, Zn, Mn, Cr, Ni, Cd, and Pb in traditional medicinal plants; Artemisia afra (ariti), Hagenia abyssinica (kosso enchet), Foeniculum vulgare (Ensilal), Echinops kebericho (qeberecho) grown in Wolaita Zone, southern Ethiopia. A wet digestion procedure involving the use of mixtures of (69-72%) HNO3 and (70%) HClO4 at an optimum temperature and time duration were used to isolate metals from the medicinal plants by using FAAS. Based on the results, the concentration of Ca ranged from 1.75 mg/kg to 4.98 mg/kg, the concentration of Mg ranged from 1.35 mg/kg to 2.22 mg/kg, the concentration of Na ranged from 1.29 mg/kg to1.80mg/kg, Mn ranged from 0.09 mg/kg to 1.21 mg/kg and that of Fe lied in range of 0.23 mg/kg to 0.78 mg/kg in the plants studied. Among the toxic heavy metals, the concentration of Pb was in the least range (0.08 mg/kg to 0.11 mg/kg) and the levels of remaining trace metals were in the ranges of 0.54-0.97 mg/kg, 0.25-0.29 mg/kg and 0.20-0.33 in Zn, Cd and Cu respectively. Further studies will be continued on the screening of phytochemical activities of the plants under study.

Citation: Alemayehu Hailemariam and Mesfin Bibiso, **2019.** "Determination of levels of some metals in selected traditional medicinal plants in Wolaita zone, southern Ethiopia", *International Journal of Current Research*, 11, (03), 1839-1844

INTRODUCTION

Medicinal plants are plants, either growing wild or cultivated and used for medicinal purposes. Traditional medicines include herbal medicines composed of herbs, herbal materials, herbal preparations, and finished herbal products, that contain as active ingredients parts of plants, or other plant materials, or combinations of all. Traditional medicines may also use animal parts and/or minerals (WHO Traditional medicine strategy 2002–2005). Literatures on medicinal plant tells that at least 25% of all modern medicines are derived, either directly or indirectly, from medicinal plants, primarily through the application of modern technology to traditional knowledge. In the case of certain classes of pharmaceuticals, such as antitumor and antimicrobial medicines, this percentage may be as high as 60% (WHO Traditional medicine strategy 2002–2005). Traditional medicines have always played a key role in world health and continue to be used to treat a vast array of conditions and complaints. Many countries in Africa, Asia and Latin America use traditional medicine (TM) to meet some of their primary health care needs. In Africa, up to 80% of the population uses traditional medicine for primary health care. Traditional medicine has maintained its popularity in all regions of the developing world and its use is rapidly spreading in the industrialized countries (Kassaye *et al.,* 2006). Studies show that the use of plants as sources of both preventive and curative traditional medicine preparations for human beings and livestock was dated beyond recorded history perhaps as old as the history of mankind. Historical accounts confirm that traditional medicinal plants were in use as early as 5000 to 4000 B.C. in China, and 1600 B.C. by Syrians, Babylonians, Hebrews and Egyptians. Considerable indigenous knowledge, from the earliest times, is linked to the use of traditional medicine in different countries. Evidence obtained from observations of animals shows that even chimpanzees use of plant species for their medicinal value (Farnsworth, 1994). Plants in general and medicinal plants particularly in the case of this study can easily be contaminated by metals in the course of cultivation or later during the processing stage and therefore determining the content of the metals accumulated is of high importance. The human body requires both the metallic and the non-metallic elements within certain permissible limits for growth and good health. Therefore, the determination of element compositions in food and related products is essential for understanding their nutritive importance (Khan *et al.,* 2015). On other hand, the presence of some heavy metals in large quantities in the body may have a toxic effect (Khan *et al.,* 2015; Sharma *et al.,* 2009; WHO, 2005). Concentration of essential and non-essential heavy metals in medicinal plants beyond permissible limit is a matter of great concern to public safety all over the world (Shad *et al.,* 2016). The problem is rather more serious in Ethiopia, because medicinal products used by the society are neither controlled nor properly regulated by quality assurance parameters. World Health Organization recommends that medicinal plants which form the raw materials for the finished products may be checked for

the presence of heavy metals, further it regulates maximum permissible limits of toxic metals like arsenic, cadmium and lead, which amount to 1.0, 0.3 and 10 ppm, respectively (WHO, 1998). Thus, the current study aims at determining the level of the essential, non-essential and toxic elements that can be accumulated in the stated plant species which were grown in different localities of Wolaita zone in order to ensure individuals health status. Furthermore, the result of this study may help to propose the maximum dosage of the plant for normal body function in terms of trace metal content. Based on this finding the local expertise will try to manage the normal dosage by integrating their experience with the optimum quantity which is going to be reported by this study.

MATERIALS AND METHODS

Chemicals and reagents: Reagents that were used in the analysis were all analytical grade (BDH). 69-70% HNO_3 (Supreme Enterprises Cantt, India) and 70% $HClO_4$ (A.C.S. Reagent, Aldrich, UK) were used for the digestion of the plant samples. Stock standard solution of concentration 1000 mg/L in 2% HNO_3 of the metals of reagent grade (Buck Scientific Puro-Graphic) salts of; Na, Mg, Ca, Mn, Fe, Zn, Cu, Cr, Ni, Zn, Cd, and Pb from which 100 mg/L of intermediate standard obtained were used for the preparation of the calibration standards of each metal. Working standards were prepared from intermediate standards of each metal. Deionized water was used for sample preparation, dilution, and rinsing apparatus prior to analysis.

Instrument and apparatuses: Stainless steel axe and Teflon (SSAT) knife were used to cut the plant species while air circulating oven were for drying the samples placed on porcelain. Blending device, ceramic pestle and mortal were used for grinding and homogenizing the samples. Digital analytical balance was used for weighing the samples. Round bottom flasks with grounded glass (100 mL) fitted with reflux condenser were employed in digesting the sample on Kjeldahl heating apparatus (Gallenhamp, England). Borosilicate volumetric flasks (50, 100 and 250 mL) were used during dilution of sample and preparation of metal standard and infusion solutions. Measuring cylinders, pipettes, micropipettes (Dragon med, 1-10 µL, 100-1000 µL) were used during measuring different quantities of volumes of sample solution, acid reagents and metal standard solutions. Metals' concentration determination was done by flame atomic absorption Spectrophotometer (Buck Scientific Model 210GP) (FAAS) equipped with deuterium background corrector and hollow cathode lamps with air-acetylene flame.

Experimental procedures

Cleaning apparatus: Apparatus such as glassware, plastic containers and polyethylene bags were washed with tap water using detergent followed by rinsing with deionized water. The apparatuses will then be soaked in about 10% (v/v) nitric acid for 24 hr. followed by rinsing with deionized water several times. Then, the apparatus was dried in oven and kept in dust free place until further use.

Optimization of the working procedure: It is important to develop an optimum working procedure in order to get a reliable result from an analytical experiment. Thus, to prepare a clear and colorless sample solution that is suitable for the analysis using FAAS, different working procedures for the digestion of plant samples were assessed using mixtures of HNO3 and HClO4 acids by varying parameters such as volume of the acids mixture, digestion time and digestion temperature. By examining the nature of the final digests obtained by varying the above parameters, the optimized procedure was selected depending up on the clearness of the digests, less digestion time, less reagent volume consumption and simplicity.

Sample collection and preparation: The plant samples were taken from the medicinal plant garden for *Artemisia afra, Foeniculum vulgare* and *Echinops kebericho* which was located in Damot sore woreda, Bolola Chawkare kebele. *Hagenia abyssinica sample* was taken from the seed of kosso inchet tree grown at Damot Mountain. The parts of plant used for analysis purpose are leaf and steam for *Artemisia afra* and *Foeniculum vulgare*. Root part was taken for analysis of *Echinops kebericho* and seed was taken for analysis of *Hagenia abyssinica.* Depending on the availability of the plants, convenient amount of leaves, seeds, roots, flowers and fruits were collected from garden of traditional healers and packed into polyethylene plastic bags, labeled and transported to the laboratory for further treatment. The collected herbs of the selected plants were washed with a tap water and detergent so as to eliminate dirt, rinsed with distilled water and air dried. Plant samples were crushed and powdered by blending device and specified quantity were taken in an evaporating dish and heated in an oven at 105oC to remove moisture. Then the sample were cooled, ground, sieved and placed in cleaned screw capped polyethylene container and were stored in desiccators till digestion.

Sample digestion: Precisely 0.5 g of the crushed, powdered and sieved portion of the plant samples were accurately weighed on a digital analytical balance and quantitatively transferred into digestion tubes. An optimized amount of freshly prepared mixture of 70%(v/v) of conc. HNO_3 and 70% of conc. $HClO_4$ were added to each of plant samples according to optimized digestion procedures mentioned in Appendix 1 and 2 for each of plant samples. The digested solutions were allowed to cool for 30 minutes. To the cooled solutions, two 5 mL portions of distilled de ionized water were added to dissolve the precipitate formed on cooling and gently swirled. The resulting solutions were filtered into a 50mL volumetric flask with a Watchman filter paper number 41 to remove any suspended and turbid matter. Subsequent rinsing of the filtrate with 5 mL distilled deionized water was followed until the volume reached the mark. For each blank sample, triplicate digestions were carried out. The digested and diluted sample solutions were stored in volumetric flask and were kept in refrigerator until analysis time.

Chemical analysis

Operating conditions: Intermediate standard solutions were prepared from the atomic absorption spectroscopy standard stock solutions containing 1000 mg/L. These intermediate standards were diluted with distilled water to obtain working standards for each metal of interest. Parameters (burner and lamp alignment, slit width and wavelength adjustment) were optimized for maximum signal intensity of the instrument based on the instrument instruction. Three replicate determinations were carried out on each plant and soil samples. Hallow cathode lamp for each metal operated at the manufacturer's recommended conditions were used at its

respective primary line source. The acetylene and air flow rates were managed to ensure suitable flame conditions. All the eleven metals (Ca, Mg, Na, Fe, Mn, Zn, Cu, Cr, Ni, Cd and Pb) were analyzed by the absorption mode of the instrument. Three readings were recorded for each digest by optimizing the different FAAS conditions shown in Table to give the maximum signal intensity.

Instrument calibration: Calibration curves were prepared to determine the concentration of each metal in the sample solutions. The instrument was calibrated using series of working standards. The working standard solutions of each metal were prepared from intermediate standard solutions of the respective metals. Different parameters such as; wavelengths, concentration of the intermediate standards, working standard solutions and the correlation coefficients of the calibration curves of each metal for the plant samples are presented in: Table and the graph of calibration curves of each metals of interest are shown in Table.

Method validation

Recovery test: Method validation is the process of providing that analytical method is acceptable for its intended purpose. Because of the absence of certified reference material for the samples in the laboratory, the validity of the optimized digestion procedure was assured by spiking the samples with a standard of known concentration of the analyte metals. Thus, the efficiency of the optimized procedure was checked. The spiked samples were digested in triplicate following the same digestion procedure developed previously for plant samples. The digested spiked samples were analyzed for their respective metals using FAAS. Finally, % recovery was determined by using recovery formula;

% recovery = amount of analyte recovered /amount of metal ion added * 100

Method detection limit*:* Method detection limit is defined as the minimum concentration of analyte that can be measured and reported with 99% confidence that the analyte concentration is greater than zero. In other words, it is the lowest analyte concentration that can be distinguished from statistical fluctuations in a blank, which usually correspond to the signal of blank and three times the standard deviation of the blank (Limit of detection = yB + 3SB, where SB = standard deviation of the blanks) (Miller and Miller, 2005).

Statistical analysis: Data entry management and preliminary summaries were done on Microsoft Excel spreadsheet. The means, and standard deviations of the data collected was determined using Microsoft Excel. Experimental treatment significant differences ($p < 0.05$) were determined using one-way ANOVA for equality of means. Linear correlations were determined using the Pearson product-moment correlation. All statistical analyses were performed using SAS 9.13 for windows version software program.

RESULTS AND DISCUSSION

The result Showed that, the concentration of the eleven metals, essential (Ca, Mg, Mn, Fe, Cu, Zn, Co, Cr, Ni) and non-essential (Pb, Cd) in the four medicinal plants were determined. The results indicated that the samples had variable composition of each analyte metals with different concentration ranges among different plant species and within a given plant except for Cr and Ni which were below detection limit as shown in the table below.

Calcium status: The concentration of Calcium in the studied medicinal plants ranged from 1.80±03 mg/kg to 4.98±0.14 mg/kg. The pattern of concentration Ca in studied medicinal plants were in the order; *Foeniculum vulgare* > A*rtemisiaafra* >*Echinops kebericho* > *Hageniaabyssinica.* The concentration of calcium was relatively higher than other metals in all plant species under study except sodium in *Hageniaabyssinica*. According to the study conducted in Nigeria the levels of Ca reported to be 248.6 µg/g, and in Ethiopia it was reported 170-320 µg/g in medicinal plants (Oloyede, 2005). However, the result of this study showed that the concentration of calcium was below the above literature values. This could be attributed to different plant species had varying abilities to take up and accumulate metals (Remon *et al.,* 2005).

Sodium status: The concentration of Sodium ranged from 1.29 ±00 mg/kg to 1.80 ±.03 mg/kg in studied medicinal plants. The pattern of sodium level in plant species was in the order of; *Hagenia abyssinica> Foeniculum vulgare> Echinops kebericho > Artemisia afra*. According to the study in Pakistan the level of sodium ranged from 113.49 ± 46.42 mg/kg to 2176.36 ± 19.32 mg/kg in different traditional medicinal plants.

Iron status: The highest concentration of Iron was detected in *Hagenia abyssinica* (0.78 ± 0.01 mg/Kg) followed by *Artemisiaafra*, *Foeniculum vulgare*, and *Echinopskebericho* with concentrations of 0.61 ± 0.02, 0.31± 0.07, 0.23 ± 0.00 mg/ kg respectively. Although the limits for iron in medicinal plants have yet not been established (WHO, 2005). The permissible limit set by FAO/WHO in edible plants was 20 ppm (Farnsworth, 1994). Comparing the level of iron in this study with the above values, it was much lower than permissible level of iron in edible plants.

Magnesium status: The concentration of Magnesium was found to be higher in *Foeniculum vulgare* (2.22±.08mg/kg) and its concentration in other plant species was found in the order of 1.96 ± 0.19 mg/kg, 1.53 ± 0.24 mg/kg, and 1.35 ± 0.07 mg/kg for *Artemisia afra, Hagenia abyssinica and Echinops keberich,* respectively. In the study conducted in Pakistan the content of Mg ranged between 2241.88 ppm to 6350.63 ppm in different medicinal plants which also matches with the previous findings by Chizzola and Shazia (2011). However, the level of Mg in this study was below literature values and as a macro nutrient it is below recommended level for plants.

Manganese status: The highest concentration of manganase was measured in Artemisia afra (1.42±.06mg/kg) followed by Foeniculum vulgare, Hagenia abyssinica and Echinop skebericho with their respective mean concentration of 1.36 ± 05 ,1.37±07, and0.09±00 mg/kg.Plants from all sites of this study accumulated manganese bellow the limits proposed by FAO/WHO in edible plants (2 mg/kg). However, for medicinal plants the WHO (2005) limit is not yet established for manganese. Sheded *et al.* (2006) investigated that the range of manganese in their study was between 44.6 to 339 mg/kg in selected medicinal plants of Egypt. Amare (2010) reported that the range of manganese in his study was 157 ± 7.5 mg/kg to 421 ± 9.0 mg/kg in Croton leaves of Ethiopian medicinal plant. The concentration of manganese in Artemisia afra was reported to be 52.94 mg/kg, (Shad *et al.,* 2008).

Table 1.Optimization of digestion procedures

No	Wt (g)	Reagent in Volume			Maximum Temp. (^oC)	Time (min)	Result
		HNO_3	$HClO_4$	Total			
1	0.5	5	2	7	300	180	Colorless but turbid
2	0.5	3	2	5	300	180	Clear and Colorless
3	0.5	4	1	5	300	180	Brown yellowish
4	0.5	2	2	4	300	180	Clear but turbid
5	0.5	3	1	4	300	180	Brown yellowish
6	0.5	3	2	5	270	150	Brown yellowish
7	0.5	3	1	4	270	150	Brown yellowish
8	0.5	2	2	4	270	150	Clear and yellowish
9	0.5*	2 *	2*	4*	270*	120*	Clear and Colorless
10	0.5	2	2	4	240	180	Clear and light yellow

Table 2. Instrumental operating conditions for the analysis of metals in samples of plants

Element	Wavelength (nm)	Slit width (nm)	Energy (eV)	IDL*(mg/kg)
Cu	324.7	0.7	3.342	0.02
Cd	228.9	0.7	2.843	0.005
Pb	283.2	0.7	3.114	0.1
Ca	422.7	0.7	3.333	0.01
Fe	248.3	0.2	3.256	0.03
Mn	279.5	0.7	3.229	0.001
Zn	213.9	0.7	3.047	0.005
Na	589.0	0.2	3.333	0.1
Cr	357.9	0.7	3.256	0.01
Ni	232.0	0.2	3.229	0.03

Table 3. Distribution of selected metals in the plant species studied

Plants	Na	Fe	Ca	Mg	Mn	Zn	Pb	Cd	Cu
Foeniculumvulgare	1.49^b	0.31^c	4.98^a	2.22^a	1.21^b	0.97^a	0.08^a	0.27^a	0.25^b
A*rtemisiaafra*	1.29^d	0.61^b	4.07^b	1.96^a	1.42^a	0.93^a	0.11^a	0.27^a	0.33^a
Hageniaabyssinica	1.80^a	0.78^a	1.75^c	1.53^b	1.37^a	0.63^b	0.11^a	0.29^a	0.21^b
Echinopskebericho	1.36^c	0.23^d	1.80^c	1.35^b	0.09^c	0.54^b	0.11^a	0.25^b	0.27^{ab}
LSD	0.06	0.07	0.13	0.31	0.09	0.09	0.09	0.02	0.02
CV	2.15	8.26	2.32	9.26	5.13	6.3	46.8	4.5	4.5

*Means with the same letter are not significant; LSD least significant difference CV; coefficient of variation

Table 4. Concentrations of the working standard solutions and correlation coefficients of the calibration curve for analysis for plant samples

Metal	Conc. of working standard (mg/L)	Correlation coefficient
Cu	0.15,0.25,0.45,0.55	0.9985
Pb	0.1,0.2,0.3,0.4,0.5	0.9949
Zn	0.5,1,1.5,2,2.5	0.995
Cd	0.15,0.25,0.45,0.55	0.9985
Ca	2,4,6,8	0.9983
Na	1,1.5,2,2.5	0.9979
Fe	0.25,0.5,0.75,1	0.9932
Mn	2,4,6,8	0.9985
Mg	0.5,1,1.5,2	0.9995

Serial solutions for each element to be analyzed were prepared from intermediate standard solutions as shown in Table 4. The calibration curves were drawn from the absorbance of these serial solutions.

Table 5. Concentrations of metals (mg/kg) in studied medicinal plants

Metals	*Foeniculum vulgare*	*Artemisia afra*	*Hagenia abyssinica*	*Echinops kebericho*
Ca	4.98 ± 0.14	4.07 ± 0.01	1.75 ± 0.02	1.81 ± 0.03
Na	1.49 ± 0.01	1.29 ± 0.00	1.80 ± 0.03	1.36 ± 0.05
Fe	0.31 ± 0.07	0.61 ± 0.02	0.78 ± 0.01	0.23 ± 0.00
Zn	0.97 ± 0.07	0.93 ± 0.03	0.63 ± 0.05	0.57 ± 0.01
Mn	1.21 ± 0.01	1.42 ± 0.01	1.37 ± 0.07	0.09 ± 0.01
Mg	2.22 ± 0.08	1.96 ± 0.19	1.53 ± 0.24	1.35 ± 0.07
Cu	0.25 ± 0.03	0.33 ± 0.05	0.20 ± 0.00	0.27 ± 0.02
Cd	0.27 ± 0.01	0.27 ± 0.02	0.29 ± 0.00	0.25 ± 0.00
Pb	0.08 ± 0.05	0.11 ± 0.08	0.11 ± 0.02	0.11 ± 0.02
Cr	ND	ND	ND	ND
Ni	ND	ND	ND	ND

The concentration of manganese in the medicinal plants of this study was lower than some of the reported data for other medicinal plants. This might be due to geographical and geological differences of the sites, plants ability to take up the metal, environmental difference, etc.

Zinc status: The higher concentration of zinc was observed in *Foeniculum vulgare* (0.97±.07 mg/Kg) followed by *Artemisia afra, Hagenia abyssinica* and *Echinops kebericho* with concentrations of 0.93 ± 0.03,0.63 ± 0.05 and 0.54 ± 0.01mg/kg respectively.The permissible zinc limit set by FAO/WHO in edible plants was 27.4 mg/kg. After comparison, metal limit in the studied medicinal plants with those proposed by FAO/WHO, was found that Zn is bellow this limit for edible plants. According to WHO (2005) limits were not established for zinc. The data of the present study are consistent with the concentrations of zinc in other medicinal plants reported (Amare Getahun, 1976).

Lead status: The concentration of lead was found to be almost the same in Foeniculum vulgare, Artemisia afra, Hagenia abyssinica and Echinops kebericho with concentrations of 0.11 ± 0.02, 0.11 ±0.08, 0.11 ±0.02, 0.08 ±.05 mg/kg, respectively.The content of lead in the analyzed medicinal plants was below the permissible limit for medicinal plants set by China, Malaysia, Thailand and WHO (2005) which is (10 mg/kg).

Cadmium status: Concentrations of cadmium were measured to give 0.27 ±.01, 0.27 ±.02, 0.29 ±00, and 0.25 ± 00 mg/kg in *Foeniculum vulgare, Artemisia afra, Hagenia abyssinica*, and Echinops kebericho, respectively.The permissible limit for cadmium set by FAO/WHO, (1984) in edible plants was 0.2 mg/kg.However, for medicinal plants the permissible limit for Cd set by WHO, (2005), China andThailand were 0.3 mg/kg in finished herbal products (WHO, 2005). These results are well comparable with results reported for other medicinal plants like Artemisia ncisa had 0.95 mg/kg [12],[13]. The concentration of cadmium in this study was well comparable with the above literature values and standards.

Copper status: The highest concentration of Copper was detected in *Artemisia afra* (0.33±.05 mg/Kg) followed by, *Echinops kebericho*, *Foeniculum vulgare,* and *Hagenia abyssinica* with concentrations of 0.27 ± 0.02, 0.25 ± 0.03 and 0.20 ± 00, mg/ kg, respectively.The results indicated that concentration of copper in all medicinal plants was below the permissible limit set by FAO/WHO and permissible limit of copper set by China and Singapore in medicinal plants, which were 20 mg/kg and 150 mg/kg respectively (Khan *et al.,* 2015). The result Showed that, the concentration of the eleven metals, essential (Ca, Mg, Mn, Fe, Cu, Zn, Co, Cr, Ni) and non-essential (Pb, Cd) in the four medicinal plants were determined. The results indicated that the samples had variable composition of each analyte metals with different concentration ranges among different plant species and within a given plant except for Cr and Ni which were below detection limit.

Conclusion

In traditional medicinal plants under study, the concentration of calcium is higher than other elements in *Foeniculum vulgare, Artemisia afra* and*, Echinopskebericho*. While its level in *Hagenia abyssinica* was exceeded by sodium. The distribution of metals in *Foeniculum vulgare* was found to be in the order of; calcium > Magnesium > Sodium > Manganese > zinc > iron > Cadmium > copper > lead. The distribution of metals in *Artemisia afra* was in order of: calcium > magnesium > manganese > Sodium > iron > zinc > copper > cadmium > lead. The level of metals in *Hagenia abyssinica* were found in the order of sodium > calcium > magnesium > manganese > iron > zinc > cadmium > copper > lead. The distribution pattern of metals in *Echinopskebericho* was in the order of calcium > Sodium > Magnesium > zinc, copper > Cadmium > iron > lead > Manganese. All the non-essential toxic metals analyzed in this study were below the permissible ranges presented by FAO/WHO standards revealing that the plants are safe for dietary and medicinal uses. All essential elements analyzed were below the optimum required level. Therefore, plants under study should not supplement essential metals. The results of study suggest that these plants are safe to be utilized as herbal drugs, since the concentration of heavy metals is within the recommended limits. The concentration of trace nutrients plays a key role in secondary metabolite production in the plants which further decides the quality of herbal raw material.

Conflict of interests: The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This research is supported by the research fund of Wolaitasodo University. The authors thank staff members of Chemistry department of Wolaita Sodo University for their technological assistances.

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