Determination of Levels of Heavy Metals in Some Selected Traditional Medicinal Plants in Southern Ethiopia

Tsegaye Bojago Dado

October 17, 2021

Determination of Levels of Heavy Metals in Some Selected Traditional Medicinal Plants in Southern Ethiopia

Tsegaye Bojago Dado

Department of Physics, College of Natural & Computational Sciences, Wolaita Sodo University, Ethiopia. Tel: +251916079024, P.O.Bo.x:138, Email: tsegaye.bojago@wsu.edu.et / tsegayebojago@gmail.com

ABSTRACT

Medicinal plants have worldwide applications in the treatment of different types of human diseases. The purpose of the current study was to determine the concentration of selected essential and non-essential 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%) HNO_3 *and (70%)*$HclO_4$ *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 raged 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. None of the studied samples were found to contain Cadmium, Copper and Nickel concentrations in above WHO/FAO limits for safe human consumption (25, 40 and 5 mg/kg respectively) Further studies will be continued on the screening of phytochemical activities of the plants under study.*

Key words; Elemental analysis, Medicinal plants, Flame Atomic Absorption Spectroscopy

1. INTRODUCTION

1.1 Back Ground of the Study

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, 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, 2004).

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).

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).

Other conditions commonly addressed with traditional medicines include digestive or intestinal diseases, sickle-cell anemia, hypertension, high cholesterol, headaches, insomnia, diarrhea, microbial infections, bronchitis, diabetes, burns, rashes and menopause (Mesfin, et *al.,* 2009)

Ethiopia has a long history of traditional medicines and has developed ways to combat disease utilizing them. The ways are also as diverse as the different cultures, language and belief. In fact, in Ethiopia up to 80% of the population use traditional medicine due to the cultural acceptability of healers and local pharmacopeias, the relatively low cost of traditional medicine, difficult access to modern health facilities and due to various side effects caused by modern synthetic medicines.

Studies showed that healing of traditional medicine in Ethiopia is not only concerned with curing of diseases but also with the protection and promotion of human physical, spiritual, social, mental and material wellbeing. It is widely believed in Ethiopia that the skill of traditional health practitioners is 'given by God' and knowledge on traditional medicines is passed over orally from father to a favorite child, usually a son or is acquired by some spiritual procedures (Kebede *et al*., 2006).

Now a day the interest on phytomedicines has been increasing since it is safer and more congenial to the human body. Various drugs were synthesized from Medicinal plants singly or in combination or even are used as the principal source of raw material for the other medicines (Sharma, 2009).On other hand, the presence of some heavy metals in large quantities in the body may have a toxic effect (Khan, 2008; Sharma, 2009; and, WHO, 2005).

An element can be categorized as essential if it fulfills the following two conditions; the first one is when the absence that specific element from diet results in the development of pathological symptoms, while the second condition is if there is improvement of pathological signs and symptoms up on administering of the element to living thing (Linder, 1991).

Therefore, the assessment of elemental composition of the widely used medicinal plants is highly important. In present study an attempt will be made to determine the level of essential and non-essential elements in selected traditional medicinal plants.

1.2 Statement of the Problem

The safety and quality of herbal medicines has become increasingly important for health authorities, scientific community and the public at large (WHO, 2005). While using herbs in medicinal treatment of various illnesses, one should be aware that they can turn out to be toxic due to the presence of heavy metals and other impurities in addition to the pharmacological effect. For these reasons it is essential to control the contaminants in medicinal raw materials (Kumudhaveni *et al*., 2013).

Artemisia afra, Hagenia abyssinica, Foeniculum vulgare and Echinops kebericho are among important traditional medicines used to cure an abdominal pain and hence used by most of individuals in the Wolaita zone in a regular basis for treatment of abdominal complaints Such as in digestion, dyspepsia, crampy abdominal pain and abdominal bloating. 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., 2008). 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).

Therefore, knowing their essential and toxic heavy metal contents of these plants is very important to save the life of individuals who uses these plant products. However, information on the determination of the level of essential and toxic heavy metal in the plant parts taken is scare in the literatures.

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.

1.3 Objectives of the Study

1.3.1 General objective

- To determine the extent of essential, non-essential and toxic metals in plant species; Artemisia afra(arti), Hagenia abyssinica(kosso enchet), Foeniculum vulgare (Ensilal), Echinops kebericho (qeberecho) grown in Wolaita zone of Southern Ethiopia and to compare the levels of the metals within and between plant species.

1.3.2 Specific objectives

- To determine the level of selected metal contents (Cu, Cd, Pb, Mg, Ca, Na, Zn, Mn, Fe and Cr) in Artemisia afra (ariti), Hagenia abyssinica(kosso enchet), Foeniculum vulgare(Ensilal), Echinops kebericho(qeberecho) plant samples by flame atomic absorption spectrophotometer.
- To compare the levels of selected metals in Artemisia afra, Hagenia abyssinica,Foeniculum

vulgare, and Echinops kebericho plants

- To find out the relationship between the concentrations of selected metals among each other.
- To compare the levels of the metals (Cu, Cd, Pb, Ca, Zn, Mn, Fe and Cr) in Artemisia afra, Hagenia abyssinica, Foeniculum vulgare, and Echinops kebericho plants with permissible level of these metals for living things stated in standard references of WHO and FAO guidelines.

1.4 Significance of the Study

The significance of this study is to provide adequate information on the level of essential, nonessential and trace metals content in the identified plants so that it will ensure the health dietary safety of individuals who use this plant as source of diet and/or medicine.

The results of this study could also be utilized as baseline information need to be proposed for the level of stated metals in the plant species identified.

The findings of this study will help other researchers as a bench mark and reference to conduct researches on the same areas.

1.5 Limitation of the Study

Limitations related to time, availability of appropriate analytical grade chemicals, and highly selective and sensitive instruments in the university were areas of ambiguity to accomplish the current research work effectively in the sight of researcher.

1.6 Scope of the Study

This study is delimited to assessment of the level of essential and non-essential heavy metals (Cu, Cd, Pb, Mg, Na, Ca, Zn, Mn, and Fe) in selected traditional medicinal plants; Artemisia afra(ariti), Hagenia abyssinica(kosso enchet), Foeniculum vulgare(Insisila), Echinops kebericho(qeberecho) grown in wolaita zone ,southern Ethiopia by using Flame atomic absorption Spectrophotometer.

2. MATERIALS AND METHODS

2.1 Description of the Study Area

The study was conducted on Wolaita zone, Southern Nations, Nationalities, and Peoples' Regional State (SNNPRS). The area is found at South Central Ethiopia between 6.4^0-6.9^0N latitude and 37.4^0 -37.8^0E longitude and is located at 390 km south of Addis Ababa and 160 km from Hawassa, the capital of the Regional State. Wolaita Zone has 12 rural districts and 3 town administrations. The study area encloses three agroclimatic zones, high land (Dega), and mid land (Woina dega) and low land (Qola). The Dega is above 1800 m.a.s.l. and Woina Dega 1500-1800 m.a.s.l and the qola is below 1500m.a.s.l. Majority of these climatic zones are highly degraded because of over cultivation for long periods of time and densely populated with easily erodible landscape. The plant species were collected from different localities based on their availability and knowledge of the societies regarding their medicinal values.

2.2 Apparatus and Instruments

Stainless steel axe and Teflon (SSAT) knife were used to cut the plant pieces 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 (FAAS) equipped with deuterium background corrector and hollow cathode lamps with air-acetylene flame.

2.3 Sample collection and preparation

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 105^{o}C 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.

2.3.1 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 and 70% of conc. HClO 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 bulk 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.

2.3.2 Result and Discusion

The accuracy and precision of the methods were tested by spiking the samples with a standard of known concentration of the analyte metals. The results indicated that the concentrations of elements determined are in agreement (100 ± 10%) within the acceptable range for all metals (Miller and Miller, 2005). The following lists of tables are results from optimized laboratory methods for analysis of metals using FAAS.

Table.1 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

Table 2: Analytical recovery results obtained for the validation of the optimized procedure of plant samples (*Artemisa afra*)

Metal	Conc.in sample (µg/g)	Amount added (µg/g)	Conc. in spiked sample (µg/g)	Recovery (%)
Ca	4.98	4.96	9.94 ± 0.03	100 ± 0.01
Mg	2.21	2.20	4.42 ± 0.02	99.98 ± 0.05
Fe	0.31	0.30	0.60 ± 0.02	96.66± 0.08
Mn	1.21	1.20	2.40 ± 0.03	99.16 ± 0.08
Zn	0.97	0.95	1.90 ± 0.01	95 ± 0.06
Cu	0.25	0.23	0.48 ± 0.01	104 ± 0.03
Cd	0.27	0.25	0.521 ± 0.03	88.8 ± 0.03
Pb	0.1	0.09	0.185 ± 0.08	94.44 ± 0.015
Na	1.4	1.2	2.56 ± 0.03	96.6± 0.02
Cr	0.6	0.58	1.17 ± 0.02	98.2±0.12
Ni	0.5	0.51	1.00± 0.03	98.03± 0.07

2.3.3 Determination of Essential, Non – Essential and Toxic Metals in plant Species

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

Table 3: 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	

Table 4: Concentration of trace metals in plant Species

Plants	Manganase	Zinc	Lead	cadmium	Copper
Foeniculumvulgare	1.21^{b}	0.97^{a}	0.08^{a}	0.27^{a}	0.25^{b}
Artemisiaafra	1.42^{a}	0.93^{a}	0.11^{a}	0.27^{a}	0.33^{a}
Hageniaabyssinica	1.37^{a}	0.63^{b}	0.11^{a}	0.29^{a}	0.21^{b}
Echinopskebericho	0.09^{c}	0.54^{b}	0.11^{a}	0.25^{b}	0.27^{ab}
LSD	0.09	0.09	0.09	0.02	0.02
CV	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

One-way analysis of variance showed that the mean concentration of manganese was significantly different among four plant species, F (3,8) = 425.710, p < 0.001. Post hoc analyses using LSD criterion for significance indicated that the mean concentration of manganese in *Foeniculum vulgare* was significantly lower than mean concentration of manganese in *Artemisia afra*, and *Hagenia abyssinica* with P < 0.001.Whereas the mean concentration of manganese in *Foeniculum vulgare* was significantly higher than the mean concentration of manganese in *Echinops kebericho* with P < 0.001. The mean concentration of manganese in *Artemisia afra* was found to be significantly higher than that of *Echinops kebericho*. However, there was no significant difference in the mean concentration of manganese in, *Artemisia afra* and *Hagenia abyssinica*. The mean concentration of manganese in *Hagenia abyssinica* was significantly higher than mean concentration of Sodium in *Echinops kebericho*.

CONCLUSION

Based on the findings of this study the following conclusions were made.

The level of essential and non-essential metals in four traditional medicinal plants namely; *Artemisia afra, Foeniculum vulgare, Echinops kebericho*, and *Hagenia abyssinica*, were determined.

The concentration of eleven essential and toxic elements; copper, cadmium, lead, sodium, magnesium, calcium, zinc, manganese, and iron were analyzed by flame atomic absorption spectrophotometer (FAAS) by following optimized wet digestions in a digester heater block for the digestion of the medicinal plant powder samples.

The effectiveness of digestion methods was revealed by the excellent recoveries obtained which were found within the acceptable range for the analyzed metals.

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 content.

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> 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.

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.

REFERENCES

Abebe Girma, L.J. Dawson, G. Detweiler, T.A. Gipson, T. Sahlu, 2000. *Hagenia abyssinica*

(Kosso) for internal parasite control in goats. Langston University, Langston, OK,Debub University, Awassa, Ethiopia, 10-12 November, Merkel RC, Abebe G, Goetsch AL 190195.

Adah, C.A., Abah, J., Ubwa, S.T. and Ekele, 2013. Soil Availability and Uptake of Some Heavy Metals by three Staple Vegetables Commonly Cultivated along the South Bank of River Benue, Makurdi, Nigeria. *International Journal of Environment and Bioenergy*,8(2): 56-67.

Adeloju, S.B. and A.M. Bond, 1985. Multi element determination in biological materials Agency for Toxic Substances and Disease Registry (ATSDR), (2004) Toxicological profile for copper. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.

Amare Getahun, 1976. Some common medicinal and poisonous plants used in Ethiopian folk medicine. Addis Ababa University, Ethiopia.

Annan K, Dickson RA, Amponsah IK, Jato J, Nooni IK. 2013. Pharmacognostic evaluation and physicochemical analysis of Paullinia pinnata L. (Sapindaceae). J Pharmacogn Phytochem; 2(2): 203

Aydinalp, S. Marinova, 2003. Distribution and forms of heavy metals in some agricultural soils Polish Journal of Environmental Studies, 12 pp. 629-633

Beentje, H.J, 2005. Kenya trees, shrubs and lianas. National Museums of Kenya, Nairobi, 722.

Biruktayet Assefa, Gerhard Glatzel, Christine Buchmann, 2010. Ethnomedicinal uses of Hagenia abyssinica (Bruce) J.F. Gmel. among rural communities of Ethiopia, Journal of Ethnobiology and Ethnomedicine, 6:20

Block E. Garlic and Other Alliums: The Lore and the Science. Cambridge, UK: Royal Society of Chemistry; 2010.

Cundeva, K., G. Pavlovovska and T. Stafilov, 2007. Fe (III) heptthyldithiocarbamate as a new collector for floatation separation and preconcentrating of Cr, Cu, and Pb from fresh waters before their determination by ETAAS. Journal of Brazilian Chemical Society; 18(6), 1207-1214.

Deckelbaum, R.J., C. Palm, P. Mutno and F. Declerck, 2006. Econutrition: Implementation models from the millenium villages project in Africa, Food and Nutrition Bulletin; 7, 335-342.

Denholm, J., 2010. Complementary Medicine and Heavy Metal Toxicity in Australia; Webmed Central Toxicology; 1(9)

Desalegn Desissa and P. Binggeli, 2000. Uses and conservation status of medicinal plants used by the Shinasha people differential pulse voltammetry. Anal. Chem. 57,1387.