

LEVELS OF MAJOR AND TRACE METALS IN THE LEAVES AND INFUSIONS OF *CROTON MACROSTACHYUS*

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ABSTRACT. The levels of essential metals (Ca, Mg, Fe, Mn, Zn, Cu, Co, Cr, Ni) and toxic metals (Cd, Pb) were determined in the leaves of *Croton macrostachyus* (traditional medicinal plant) collected from four different regions of Ethiopia (Akaki, Abomsa, Bonga and Dilla) and also in the infusions of leaves collected from Akaki using flame atomic absorption spectrometry. An optimized digestion procedure took 2.5 h for the digestion of 0.5 g of powdered sample with 2 mL of HNO₃ and 2 mL of HClO₄ at 270 °C while 2 h were needed to digest 25 mL infusion with 4 mL of HNO₃ and 1 mL HClO₄. The recoveries of metals were in the range 92–103% for the leaves powder and 94–105% for the infusion samples. The mean concentration ranges (µg/g) were Ca (5.823–12.040), Mg (1.971–4.961), Fe (192–581), Mn (157–1,770), Zn (19.5–60.5), Cu (6.31–18.6), Co (1.97–3.45), Cr (2.13–8.75), Ni (2.15–3.80), Cd (0.75–1.08) and Pb (1.05–2.19) for the leave powders and Ca (716–1,776), Mg (16.7–80.9), Fe (1.39–3.34), Mn (2.17–3.40), Zn (0.674–7.88), Cu (1.94–2.31), Co (0.157–0.224), Cr (0.144–1.23), Ni (0.203–0.267), Cd (0.05–0.098) and Pb (0.0148–0.185) for the infusion samples. Among the quantified metals in the infusion samples, Ca showed the highest leaching rate (61.5%) in the 24 h infusion while Fe exhibit the lowest (1%) leaching rate in the 3 h infusion.

KEY WORDS: Traditional medicinal plant, *Croton macrostachyus*, Croton leaves powder, Croton leaves infusion, Major metals, Trace metals, Ethiopia

INTRODUCTION

The use of plants as treatment of diseases and as food dates beyond recorded history perhaps as old as the history of mankind. Old civilisations in China, India, Egypt and Greece had a rich knowledge and expertise in using many types of plants. It has been estimated that more than 80% of the world population utilizes plants as primary source of medicinal agents, largely due to the high cost of Western pharmaceuticals, but also because the traditional medicines are generally more acceptable from a cultural and spiritual perspective. Even in the Western world, the use of herbal medicines is steadily growing with approximately 40% of the population reporting use of herbs to treat medical illness [1, 2].

It is known that many countries in Africa, Asia and Latin America use traditional medicine 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. In China, for example, traditional herbal preparations account for 30–50% of the total medicinal consumption. In Ghana, Mali, Nigeria and Zambia, the first line of treatment for 60% of children with high fever resulting from malaria is the use of herbal medicines at home [3].

Many of the plants species used for this purpose have been found to contain therapeutic substances which can be extracted and used in preparation of drugs, but the plant itself can also be used either directly or as an extract for medication, a practice that is particularly popular in developing countries [4–7].

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Ethiopia has a long history of traditional medicine and has developed ways to combat disease through it. The ways are also as diverse as the different cultures, language and belief. In Ethiopia up to 80% of the population uses traditional medicine due to the cultural acceptability of healers and local pharmacopeias, the relatively low cost of traditional medicine and difficult access to modern health facilities [3].

In Ethiopia, traditional remedies represent not only part of the struggle of the people to fulfil their essential drug needs but also they are integral components of the cultural beliefs and attitudes [8]. Some of the common uses of the medicinal plants sold in markets include fumigation, vermifuge, pain relief and treating skin infections. Antimicrobial and wound healing plants are among some of the major medicinal plants that are commonly available in markets [6, 7, 9]. Despite its significant contribution to society, traditional medicine has received very little attention in modern research and development and less effort has been exerted to upgrade the traditional health practices in the country. But, the long history of use of medicinal plants in Ethiopia and its huge biotic riches can be of paramount importance in future research and drug discovery [10]. Even today, this huge traditional knowledge of medicinal plants is playing an important role in the development of new drugs. An example of drugs discovered based on information derived from an ethnobotanical investigation is aspirin from *Filipendula ulmar*, morphine from *Papaver somniferum*, ephedrin from *Ephedra sinica* to name a few [11].

Of the many medicinal plants in Ethiopia, *Croton macrostachyus* is the most common. *Croton macrostachyus* Hochst. ex Del. is commonly known as rush foil or broad-leaved Croton (English), Bisana (Amharic and Shinasha), Makanissa, Bakkanissa, Badessa, Alaleh, Dogoma (Oromo), Wusha, Masincho (Sidama), and Islami, Tambuk and Tambush (Tigrigna). It belongs to the Euphorbiaceae, a very large family with 300 genera and 8,000 to 10,000 species, and is the most abundant plant in the tropics [12–15].

It is native to Eritrea, Ethiopia, Kenya, Tanzania, Uganda and Nigeria. It is a medium sized deciduous tree of East Africa particularly wide spread between 200–2500 masl in mountainous forests and savannah of the tropical regions and ever green bush land areas that receive between 700-2000 mm rainfalls annually [16]. It is 3–25 m high, although more commonly 6–12 m. In Ethiopia, *C. macrostachyus* grows between 500 and 3400 masl (more frequently between 1100 and 2700 masl) [17, 18].

As *C. macrostachyus* is available almost in all parts of Ethiopia, people are using it as a medicine for treatment of snakebite, malaria, headache, internal worms, rabies, gonorrhoea, ascariasis, sexually transmitted diseases and tinea versicolor for human being in which the traditional medician ordered the patients to take unlimited dosage of the plants part like to take two, three or even more glasses of medicinal plant extract orally. The plant is also used for fever and wounds of domestic animals [18]. The leaves of *C. macrostachyus* have medicinal value including cold leaf decoction is drunk or ashes taken orally as treatment for cough; juice from fresh leaves is applied on wounds to hasten clotting.

Plants constitute is an important link to the transfer of trace elements from soil to man. The level of elements in plants varies, the content being affected by the geochemical characteristics of a soil and by the ability of plants to selectively accumulate some of these elements. Bioavailability of the elements depends on the nature of their association with the constituents of a soil. Additional sources of these elements for plants are rainfall, atmospheric dusts, plant protection agents and fertilizers that can be absorbed through the leaf blades [19–26].

Trace elements have both a curative and a preventive role in combating diseases. It is therefore of major interest to establish the levels of some metallic elements in common herbal plant because, at elevated levels, these metals can also be dangerous and toxic [27, 28]. Though they are required in very small amount, deficiency of trace elements causes diseases, whereas their presence in excess may result in toxicity to human life disturbing normal functioning of organs and central nervous system [29, 30].

The literature survey revealed that there is no report on the levels of metals in leaves of *C. macrostachyus*. Thus the objectives of this study were: (i) to determine major and trace essential (Ca, Mg, Fe, Mn, Zn, Cu, Co, Ni, Cr) and toxic metals (Cd, Pb) in leaves of *C. macrostachyus* and its infusion grown in Ethiopia by flame atomic absorption spectroscopy, (ii) to compare the levels of essential (Ca, Mg, Fe, Mn, Zn, Cu, Co, Ni, Cr) and toxic (Cd, Pb) metals in the leaves and infusions of *C. macrostachyus*, (iii) to estimate the amount of elements released from plants into the water extracts, and (iv) to look some correlation trends among the element concentrations in the different area of study by applying simple statistical tools.

EXPERIMENTAL

Apparatus and instrument

Teflon (PTFE, China) knives were used to cut the *C. macrostachyus* leaves in to pieces and an oven (Digitheat, J.P. Selecta, Spain) was used for drying the samples placed on porcelain dish. Blending device (Moulex, France) and ceramic pestle and mortar were used for grinding and homogenizing the *C. macrostachyus* leaves samples. *C. macrostachyus* samples were digested on Kjeldahl heating apparatus (Gallenkamp, England) using round bottom flasks with ground glass joint (100 mL) fitted with reflux condenser. Borosilicate volumetric flasks (25, 50 and 100 mL), pipettes (Pyrex, USA), measuring cylinders (Duran, Germany), and micropipettes (Dragonmed, 1–10 μ L, 100–1000 μ L, Shanghai, China) were used for measuring different quantities of sample solution, acid reagents and metal standard solutions. Metal concentrations were determined by flame atomic absorption spectrophotometer (Buck Scientific, Model 210VGP AAS, East Norwalk, USA) equipped with deuterium background corrector and hollow cathode lamps with air-acetylene flame.

Chemicals and reagents

All the reagents were of analytical grade. 69–70% HNO_3 (Supreme Enterprises Cantt, India) and 70% HClO_4 (Aldrich, UK) were used for the digestion of the *C. macrostachyus* leaves and infusion samples. Lanthanum nitrate hydrate, (99.9%, Aldrich, USA) was used to prevent the chemical interference on Ca and Mg in the sample solution during the analysis of *C. macrostachyus* samples. Stock standard solution of concentration 1000 mg/L in 2% HNO_3 of the metals Ca, Mg, Mn, Fe, Zn, Cu, Ni, Co, Cd and Pb (Buck Scientific Puro-Graphic[™]) from which 10 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.

Description of the study area

The samples were collected from the selected sites of four different regions namely: (i) Abomsa, a town in central Ethiopia – located in the Arsi Zone of the Oromia region, administrative center of Merti Woreda, (ii) Dilla, a market town in southern Ethiopia – the administrative center of the Gedeo Zone in the Southern Nations, Nationalities and People Region (SNNPR), and located on the main road from Addis Ababa to Nairobi, (iii) Akaki, one of the outskirts sub-cities of Addis Ababa and (iv) Bonga, the administrative center of Kefa zone in the Southern Nations, Nationalities and People Regional state. The selection of these sites was based on: firstly, availability of ethnobotanical information, secondly, the need to compare metal accumulation in this plant from industrially and agriculturally polluted areas with that of none polluted environment; thirdly, availability of the plant and its popularity in using as medicinal plant by the local peoples around. The geographical location of the sampling sites and their distance from Addis Ababa (capital city of Ethiopia) are given in Table 1.

Table 1. The geographical location of the sampling sites and their distance from Addis Ababa (capital city of Ethiopia).

Sampling site	Latitude	Longitude	Altitude (m)	Distance (km)
Abomsa	8° 35' N	39° 51' E	1438	202
Dilla	6° 24' 30'' N	38° 18' 30'' E	1570	359
Akaki	9° N	38° 45' E	2200–2800	23
Bonga	6° 15' to 8° 08' N	35° 30' to 36° 46' N	500 – 3350	440

Sample collection and treatment

Representative amount of leaves were collected from four different regions. The leaves were collected from a minimum of 16 mature plants, four plants per region and eight leaves per plant starting from the bottom to the tips by stalk position. The *C. macrostachyus* samples were put in clean plastic bags labeled according to their region and brought to the laboratory for further pretreatment. The leaves of the *C. macrostachyus* were separated with Teflon knives, washed with a running tap water so as to remove adsorbed soil particulates and then rinsed with distilled deionized water and air dried. About 300 g of the *C. macrostachyus* leaves from each region were put on acid-washed and labeled porcelain dish and dried in an oven at 80 °C for 48 h till it got brittle and crisp. After cooling to ambient temperature, the dried samples were ground in to fine powder with blending device, mortar and pestle, and sieved (1 mm). The powdered sample was then placed in pre-cleaned screw capped polyethylene container and stored in desiccators with calcium chloride to keep to constant dry weight till digestion.

The *C. macrostachyus* leaves infusion was made from *C. macrostachyus* leaves collected from Akaki site only. By varying the time, four *C. macrostachyus* leaves infusion samples were prepared. As there is no specific method of preparing the *C. macrostachyus* leaves infusion in Ethiopia, usually 25 g of fresh leaves (8 to 14 leaves) were ground in the mortar with a pestle and were added in to a 250 mL tap water (at room temperature, 25 °C). This was done on the habits of traditional medician, i.e. they keep the leaves in the water for half a day or for complete day depending on the ages of the patient. So, in this study, to compare and consider the amount of metals extracted in to the supernatant, the infusions were prepared for 3, 6, 12 and 24 hours. Finally, pure infusion sample was obtained by filtering and the decrease in volume was compensated by adding water up to the mark.

Optimization of digestion procedure

Before applying the digestion procedure for the analysis of samples, digestion time, reagent volume ratio and temperature were optimized by varying one parameter at a time for the digestion of *C. macrostachyus* leaves powder and its infusion. Thus, for a complete digestion of 0.5 g of the dry *C. macrostachyus* leaves sample, 2 mL HNO₃ (69–72%) and 2 mL HClO₄ (70%) for a total of 2.5 h at 270 °C was selected as optimized procedure while 1 mL HClO₄ (70%) and 4 mL of HNO₃ (69–72%) for a total of 2 h at 240 °C for the digestion of *C. macrostachyus* leaves infusion samples.

Digestion of the *C. macrostachyus* leaves and its infusion

Applying the optimized procedure, exactly 0.5 g of the dried and ground *C. macrostachyus* leaves sample was accurately weighed and transferred quantitatively in to a 100 mL round bottom digestion flask. To the *C. macrostachyus* leaves sample, 4 mL of freshly prepared 1:1 mixture of 70% of conc. HNO₃ and 70% of conc. HClO₄ was added. The mixture was then heated on Kjeldahl heating apparatus fitting the flask to a reflux condenser for the first 30 min by setting the temperature at 120 °C and followed by heating at 270 °C as maximum temperature for the other 2.0 h.

The digested solutions were allowed to cool for 20 min without dismantling the condenser from the flask and for an additional 10 min after removing the condenser. To the cooled solution, two 5 mL of de-ionized water was added and the solution was slowly shaken to dissolve precipitates remained on the wall of the flask and to minimize dissolution of filter paper by residue. Subsequent rinsing the flask with 5 mL portions of distilled-deionized water followed until the volume reached the mark. For each bulk samples, triplicate digestions were carried out. The digested and diluted sample solutions were stored in tightly capped polyethylene bottles and kept in refrigerator until analysis time.

To determine the amount of metals extracted from the *C. macrostachyus* leaves to the leaves infusion (to the water at room temperature, 25 °C) 25 mL of the clear infusion (after filtration) was transferred quantitatively to the 100 mL round bottom flask and heated on the hot plate to evaporate until the sample was decreased to around 3 mL. This was done to avoid dilution of the acid mixture added for digestion. The solution was allowed to cool before the addition of the acid mixture to avoid potentially explosive reactions that may occur. A 5 mL portion of 4:1 HNO₃ and HClO₄ was added and the contents swirled gently to homogenize then fitted to a reflux condenser and digested continuously for two hours on a Kjeldahl digestion block. The temperature was adjusted at 120 °C for the first 30 min and finally to the maximum temperature of 240 °C for 1.5 h. The contents were diluted to 50 mL as described above. The digestion gave a clear colorless solution. Each *C. macrostachyus* leaves infusion sample was digested in triplicate and hence a total of twelve digests were made for *C. macrostachyus* leaves infusion samples prepared by infusing for 3, 6, 12, 24 hours. All the final digest solutions were kept in a refrigerator until the analysis for the metals.

Digestion of a reagent blank was also performed in parallel with the *C. macrostachyus* samples keeping all digestion parameters the same. For the analysis of the *C. macrostachyus* samples 9 reagent blank samples were prepared. All the digested samples were stored in refrigerator until analysis. The solutions were used to determine concentration of the 11 elements by flame atomic absorption spectrometry.

Instrument calibration and method detection limit

For the present study, the pooled standard deviation of the 9 reagent blanks was calculated. The detection limits were obtained by multiplying the pooled standard deviation of the reagent blank by three. The wavelengths at which analysis was done, the correlation coefficients of the calibration curve for each of the metals and method detection limits of each metal are given in Table 2. The results clearly show that the calibration curves with good correlation coefficients and lower method detection limits were obtained during the analysis.

Table 2. Method detection limits for *C. macrostachyus* leaves, and leaves infusion samples.

Metal	Wavelength (nm)	r ²	MDL for the leaves samples (µg/g) ^a	MDL for infusion samples (µg/g) ^a
Ca	422.7	0.99994	3.1	5.2
Mg	285.2	0.99999	2.6	4.1
Fe	248.3	0.99997	0.05	0.07
Mn	279.5	0.99993	1.1	2.0
Zn	324.8	0.99952	0.4	0.5
Cu	213.9	0.99924	1.2	1.5
Co	240.7	0.99953	0.1	0.13
Cr	341.5	0.99999	0.11	0.12
Ni	357.9	0.99933	0.08	0.20
Cd	228.9	0.99981	0.30	0.40
Pb	217.0	0.99999	0.11	0.13

^aMDL = method detection limit.

Precision

The precision of the results of the present analysis is reported with corresponding pooled standard deviation of nine measurements for a bulk sample of triplicate reading per sample and relative standard deviation.

Recovery test

The efficiency of the optimized procedure was checked by adding known concentration of each metal to 0.5 g sample. The procedure was as follows: 783 µg of Ca and 0.6 µg of Co were spiked at once in to 0.5 g of *C. macrostachyus* leave powder sample and the remaining metals (58 µg of Fe, 28 µg of Mn, 6 µg of Zn, 5 µg of Cu, 0.92 µg of Cr, 0.54 µg of Cd and 0.88 µg of Pb) were spiked at once in to another round bottomed flask containing 0.5 g of *C. macrostachyus* leave powder. Similarly, 496 µg of Mg and 0.84 µg Ni were spiked at once in a flask containing 0.5 g of *C. macrostachyus* leave powder sample.

Since the concentration of metal in the *C. macrostachyus* leaves infusion is lower than that of the *C. macrostachyus* leaves powder, the recovery test for the *C. macrostachyus* leaves infusion was done by adding smaller amount of the spiked metals than that of the *C. macrostachyus* leaves powder. Hence, for spiking *C. macrostachyus* leave infusion sample, 183 µg of Ca, 0.31 µg of Cu, 0.24 µg of Co, and 0.73 µg of Cr were spiked from stock solution of each metal per gram of the sample. The remaining metals (8.50 µg of Mg, 0.98 µg of Fe, 0.34 µg of Mn, 0.17 µg of Ni, 0.11 µg of Cd and 0.16 µg of Pb) were spiked at once in to per gram of the infusion sample and 2.85 µg of Zn was spiked in another flask containing the same amount of infusion sample and the same digestion process was followed. Each sample was analyzed for their respective spiked metals by atomic absorption spectrophotometer. Each recovery test for both samples was performed in triplicates.

Conversion factor determination

To report results obtained on a dry weight basis in terms of fresh weight basis, a conversion factor was calculated by taking the weight of a known quantity of fresh *C. macrostachyus* leaves before and after drying to a constant weight. This procedure was repeated for all the analysed plant leaves from different areas. Thus 4.17, the mean value was taken as a conversion factor because 4.17 g of the wet sample (on an average) gave 1 g of dry sample.

RESULTS AND DISCUSSION

In this study, the method was validated by the spiking experiment in which known quantities of the metals standard solution were added to three samples for leaves and infusion samples. The spiked samples were then digested using the optimized procedure and calculated the percent recoveries. The percentage recovery of metals in *C. macrostachyus* leaves powder and its infusion samples were between 92% and 105% (100 ± 10), which are within the acceptable range. The results are presented in Tables 3 and 4.

Levels of major, trace and toxic metals

The accuracy and precision of the method 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 by the present FAAS method are in agreement ($100 \pm 10\%$) within the acceptable range for all metals. Thus, in the present work, the concentration of the eleven metals, essential (Ca, Mg, Mn, Fe, Cu, Zn, Co, Cr, Ni) and toxic (Pb, Cd) in the *C.*

macrostachyus leaves and their infusions were determined by FAAS. The results showed that the samples had variable composition of each analyte metals with wide concentration range. Among the identified eleven elements, all were found to be above the method detection limit except Cd not found in the samples from Dilla and Bonga sites in the *C. macrostachyus* leaves powder. On the other hand most metals were not detected in the infusion samples prepared for three and six hours. The results are given in Table 5 and 6.

Table 3. Recovery test results for *C. macrostachyus* leaves sample.

Metal	Conc. in unspiked sample ($\mu\text{g/g}$) ^a	Amount added ($\mu\text{g/g}$)	Conc. in spiked sample ($\mu\text{g/g}$) ^b	Recovery (%) ^c
Ca	12,040	1565	13,558 \pm 12	97 \pm 7
Mg	4,961	992	5,884 \pm 8	93 \pm 2
Fe	581	116	699 \pm 5	102 \pm 3.5
Mn	283	56	338 \pm 3	98 \pm 2.5
Zn	60.5	12	71.8 \pm 1.5	94 \pm 1.4
Cu	18.6	10	28.2 \pm 0.8	96 \pm 0.25
Co	2.18	1.20	3.32 \pm 4	95 \pm 4.3
Cr	8.75	1.84	10.4 \pm 5	92 \pm 8
Ni	2.62	1.68	4.27 \pm 0.3	98 \pm 5.1
Cd	1.08	1.08	2.19 \pm 0.4	103 \pm 6.2
Pb	2.19	1.75	3.84 \pm 5.1	94 \pm 2.6

^aMean concentration in un-spiked samples analyzed in triplicate. ^bMean concentration \pm SD in spiked samples, spiked and analyzed in triplicate. ^cMean percentage recovery \pm SD of triplicate analyses.

Table 4. Recovery test results for *C. macrostachyus* leaves infusion sample.

Metal	Conc. in unspiked sample ($\mu\text{g/g}$) ^a	Amount added ($\mu\text{g/g}$)	Conc. in spiked sample ($\mu\text{g/g}$) ^b	Recovery (%) ^c
Ca	1,824	365	2,182 \pm 4	98 \pm 1.3
Mg	85.1	17.0	102 \pm 3	97 \pm 3
Fe	6.50	1.95	8.33 \pm 2.8	94 \pm 1.5
Mn	3.40	0.68	4.11 \pm 5	105 \pm 5
Cu	9.48	5.69	15.1 \pm 7	99 \pm 0.8
Zn	2.49	0.62	3.08 \pm 3.5	95 \pm 4
Co	0.78	0.47	1.24 \pm 2	98 \pm 2.5
Cr	2.41	1.45	3.80 \pm 1.5	96 \pm 1.8
Ni	0.42	0.33	0.72 \pm 5	93 \pm 2
Cd	0.210	0.21	0.41 \pm 4.5	97 \pm 3.5
Pb	0.41	0.32	0.71 \pm 3.5	95 \pm 2.4

^aMean concentration in un-spiked samples analyzed in triplicate. ^bMean concentration \pm SD in spiked samples, spiked and analyzed in triplicate. ^cMean percentage recovery \pm SD of triplicate analyses.

Distribution of metals in different C. macrostachyus samples

Plants accumulate minerals essential for their growth from the environment and can also accumulate metals such as Cd, Co, Ag, and Na, which have no known direct benefit to the plant [31]. Therefore, the essential and non-essential metals in *C. macrostachyus* leaves and their infusion may be affected by different factors. Physical and chemical properties of the soil, taking medicinal samples from the farm areas (where application of natural and artificial

fertilizers taking place), increasing industrialization and associated pollution of the biosphere, storage and processing of *C. macrostachyus* samples, climatic condition of the region and other factors are the main contributors for the mineral contents of *C. macrostachyus* leaves and their infusion.

Concentration of metals in *C. macrostachyus* leaves powder

Table 5 shows the mean concentrations of various metals, in the same *C. macrostachyus* species plant in different part of the country. From the study, it was revealed that all the metals were accumulated to greater or lesser extents by plant leave studied.

Table 5. Mean concentration ($X \pm SD$, $n = 9$, $\mu\text{g/g}$ dry weight) of essential and toxic metals in *C. macrostachyus* leaves samples.

Metal	Concentration of metal ($\mu\text{g/g}$) dry weight basis			
	Akaki leaves	Abomsa leaves	Bonga leaves	Dilla leaves
Ca	12,040 \pm 840	7,976 \pm 370	5,823 \pm 81	8,202 \pm 430
Mg	4,961 \pm 32	2,361 \pm 54	2,384 \pm 29	1,971 \pm 20
Fe	581 \pm 40	329 \pm 7.1	269 \pm 6	192 \pm 4
Mn	283 \pm 5	157 \pm 7.5	1,770 \pm 70	421 \pm 9
Zn	60.5 \pm 2.3	34.5 \pm 2.6	33.6 \pm 1.1	19.7 \pm 0.6
Cu	18.6 \pm 0.7	8.20 \pm 0.70	7.75 \pm 0.59	6.31 \pm 0.48
Co	2.18 \pm 0.07	3.45 \pm 0.26	3.36 \pm 0.11	1.97 \pm 0.06
Cr	8.75 \pm 0.66	8.15 \pm 0.22	2.13 \pm 0.09	6.02 \pm 0.26
Ni	2.62 \pm 0.11	2.15 \pm 0.16	3.16 \pm 0.26	3.8 \pm 0.10
Cd	1.08 \pm 0.09	0.75 \pm 0.02	ND	ND
Pb	2.19 \pm 0.09	1.15 \pm 0.02	2.07 \pm 0.12	1.05 \pm 0.1

From Table 5, it is clear that Ca was present in all the samples collected from four different regions with concentration ranging from 5,823 to 12,040 $\mu\text{g/g}$. Among all these regions the highest concentration of Ca was found in Akaki *C. macrostachyus* leaf powder and the lowest in Bonga *C. macrostachyus* leaf powder. The concentration of Ca found in Abomsa and Dilla *C. macrostachyus* leaves powder were 7,976 and 8,202 $\mu\text{g/g}$. The higher concentrations of Ca are very significant because Ca is known to enhance the qualities of bones and teeth and also of neuromuscular systemic and cardiac functions.

The Mg concentration varied from 1,971 to 4,961 $\mu\text{g/g}$. Dilla *C. macrostachyus* leaves had the lowest Mg concentration (1,971 $\mu\text{g/g}$) while Akaki *C. macrostachyus* leaves had the highest (4,961 $\mu\text{g/g}$). The Mg concentration of Abomsa and Bonga *C. macrostachyus* leaves were 2,361 and 2,384 $\mu\text{g/g}$, respectively. Like Ca, Mg is critical to many cell functions.

The abundance of Ca and Mg in the *C. macrostachyus* leaves is also in agreement with the previous studies on other plants, which indicated that these two elements were the most abundant elements in many medicinal plants and tea leaves [32, 33].

The Fe concentration varied in the range from 192 $\mu\text{g/g}$ in Dilla *C. macrostachyus* leaves to 581 $\mu\text{g/g}$ in Akaki *C. macrostachyus* leaves. The concentration of Fe found in Abomsa and Bonga *C. macrostachyus* leaves powder were 329 and 269 $\mu\text{g/g}$, respectively. The plant in all the four regions accumulates Fe above the limits proposed by FAO/WHO in edible plants (20 $\mu\text{g/g}$). However, for medicinal plants the WHO limits not yet been established for Fe. Sheded *et al.* [34] reported that the range of Fe in their study was between 261 to 1,239 $\mu\text{g/g}$ in selective medicinal plants of Egypt, thus the present result is in good agreement with this range.

Coming to the case of Mn, an appreciable amount of Mn was present in all the areas of the study, its concentration is maximum in Bonga *C. macrostachyus* leaves 1,770 $\mu\text{g/g}$ and

minimum in Abomsa *C. macrostachyus* leaves powder (157 µg/g). The concentration of Mn found in Akaki and Dilla *C. macrostachyus* leaves powder were 283 and 421 µg/g, respectively. Similar to Fe, the plants in all the four regions accumulated Mn above the limits proposed by FAO/WHO in edible plants (2 µg/g). However, for medicinal plants the WHO limits not yet been established for Mn, the range of Mn in five medicinal plants studied in Spain had concentration ranging from 46 to 2134 mg/kg [35].

From Table 5, it is clear that Zn was present in all the medicinal plants with concentration ranging from 19.7 to 60.5 µg/g. Among all these study areas the highest concentration of Zn is found in Akaki *C. macrostachyus* leaves powder (60.5 µg/g) and the lowest concentration is found in Dilla *C. macrostachyus* leaves powder (19.7 µg/g). The concentration of Zn found in Abomsa and Bonga *C. macrostachyus* leaves powder are comparable, 34.5 and 33.6 µg/g, respectively. The permissible zinc limit set by FAO/WHO in edible plants was 27.4 µg/g. After comparison, metal limit in the studied medicinal plant with those proposed by FAO/WHO it is found that only Dilla *C. macrostachyus* leave is within this limit while all others study area accumulate Zn above this limit. However, for medicinal plants the WHO limits not yet been established for Zn. According to Bowen and Allaway, the range of Zn in agricultural products was between 15 to 200 µg/g [36].

Copper was detected in all samples with varying concentrations. Leaves powder of *C. macrostachyus* from Akaki contain the highest amount (18.6 µg/g) of Cu while least concentration was exhibited in the leaves powder of *C. macrostachyus* from Dilla (6.31 µg/g). Abomsa and Bonga *C. macrostachyus* leaves powder contains comparable amount of Cu, 8.20 and 7.75 µg/g, respectively. Although the results are not in the permissible limit of Cu set by FAO/WHO in edible plants 3.0 µg/g, the results obtained from our medicinal plant are in the permissible limit of Cu set by China and Singapore in medicinal plants, which were 20 and 150 µg/g, respectively. Reddy and Reddy [37] also reported that the range of Cu contents in the 50 medicinally important leafy material growing in India were 17.6 to 57.3 µg/g.

The Co content was observed to be the highest, 3.45 µg/g in Abomsa *C. macrostachyus* leaves powder and relatively comparable in Bonga *C. macrostachyus* leaves powder (3.36 µg/g). The concentration of Co found in Akaki and Dilla *C. macrostachyus* leaves powder are 2.18 µg/g and 1.97 µg/g, respectively. There are no established criteria for Co in medicinal plants. Basgel and Erdemoglu [38] determined Co concentration ranged between 0.14 to 0.48 µg/g in seven herbs in Turkey.

Another essential element, Cr, acts as a co-factor in the insulin synthesis and in the cholesterol and blood triglycerides control. The Cr content was observed to be the highest, 8.75 µg/g in Akaki *C. macrostachyus* leaves powder and relatively comparable in Abomsa *C. macrostachyus* leaves powder (8.15 µg/g). The concentration of Cr found in Bonga and Dilla *C. macrostachyus* leaves powder are 2.13 and 6.2 µg/g, respectively.

Abomsa *C. macrostachyus* leave accumulated lowest Ni that is, 2.15 µg/g and Dilla *C. macrostachyus* leave accumulate maximum that is 3.80 µg/g. The concentration of Ni found in Bonga and Akaki *C. macrostachyus* leaves powder were 3.16 and 2.62 µg/g, respectively. The permissible limit set by FAO/WHO in edible plants was 1.63 µg/g. However, for medicinal plants the WHO limits not yet been established for Ni. Ni toxicity in human is not a very common occurrence because its absorption by the body is very low [39].

It is well known that Pb and Cd are toxic metals because of their effect in disordering the biological activity once taken into the body. In this study, Cd was not detected in Dilla and Bonga *C. macrostachyus* leaves powder. Elevated Cd concentrations were determined in Abomsa *C. macrostachyus* leave 0.75 µg/g next to Akaki *C. macrostachyus* leave 1.08 µg/g. The permissible limit for Cd set by FAO/WHO in edible plants was 0.2 µg/g. However, for medicinal plants the permissible limit for Cd set by WHO, China and Thailand was 0.3 µg/g. The relatively high concentrations of Cd found in the *C. macrostachyus* samples are certainly

due to industrial pollution as well as the addition of some fertilizers and herbicides on the farm and farm sides.

The content of Pb in the *C. macrostachyus* leaves analyzed varied between 1.05 and 2.19 $\mu\text{g/g}$ which is lower than the permissible limit for medicinal plant set by China, Malaysia, Thailand and WHO (10 mg kg^{-1}). The maximum concentrations of Pb were determined in Bonga *C. macrostachyus* leaves ($2.07 \mu\text{g/g}$) next to Akaki *C. macrostachyus* leaves ($2.19 \mu\text{g/g}$). The concentration of Pb found in Abomsa and Dilla *C. macrostachyus* leaves were 1.15 and 1.05 $\mu\text{g/g}$, respectively.

Most of the studied sites showed more or less comparable pattern in mineral accumulation in the plant. Thus the following pattern in decreasing order was notice: $\text{Ca} > \text{Mg} > \text{Fe} > \text{Mn} > \text{Zn} > \text{Cu} > \text{Cr} > \text{Ni} > \text{Pb} > \text{Co} > \text{Cd}$.

Concentration of metals in a 3, 6, 12 and 24 hours infusion

As shown in Table 6, the trend of average concentration of metals in the 24 h infusion was $\text{Ca} > \text{Mg} > \text{Zn} > \text{Mn} > \text{Fe} > \text{Cu} > \text{Cr} > \text{Ni} > \text{Co} > \text{Pb} > \text{Cd}$. Similarly, the pattern of the average concentration of metals in the 12 h infusion was $\text{Ca} > \text{Mg} > \text{Fe} > \text{Zn} > \text{Mn} > \text{Cu} > \text{Cr} > \text{Ni} > \text{Co} > \text{Pb} > \text{Cd}$. Ca was appreciably extracted with the amount, 1,776 $\mu\text{g/g}$ followed by Mg, 80.9 $\mu\text{g/g}$ in the 24 hour infusion sample. Among the trace metals, Zn (7.88 $\mu\text{g/g}$) was leached in larger amount followed by Mn, Fe and Cu with concentrations (3.40, 3.20 and 2.31 $\mu\text{g/g}$), respectively. Other trace metals, Cr, Ni and Co were also extracted in lower concentration in the 24 h and 12 h of extraction time. The lower concentrations of these metals are most likely due to the small intake of these metals by the plant and also due to their low extraction efficiency in the water as these transition metals are known to form stable complexes with organic molecules present in the plant leaves. The toxic heavy metals Cd and Pb were also detected in the 24 hour infusion sample. The amount of Cd and Pb detected in this infusion sample were 0.098 $\mu\text{g/g}$ and 0.185 $\mu\text{g/g}$, respectively.

Table 6. Mean concentration ($X \pm \text{SD}$, $n = 9$, $\mu\text{g/g}$ wet weight) of essential and toxic metals in infusion samples.

Metal	Concentration of metal ($\mu\text{g/g}$) wet weight basis in infusions extracted for			
	3 hour	6 hour	12 hour	24 hour
Ca	716 \pm 19	904 \pm 29	1,091 \pm 40	1,776 \pm 41
Mg	16.7 \pm 0.9	17.9 \pm 1.2	40.5 \pm 0.5	80.9 \pm 2.2
Fe	1.39 \pm 0.02	1.53 \pm 0.02	3.34 \pm 0.04	3.20 \pm 0.24
Mn	2.17 \pm 0.03	2.65 \pm 0.20	2.99 \pm 0.07	3.40 \pm 0.04
Zn	0.674 \pm 0.1	1.68 \pm 0.02	3.02 \pm 0.10	7.88 \pm 0.39
Cu	1.94 \pm 0.07	2.10 \pm 0.14	2.23 \pm 0.004	2.31 \pm 0.11
Co	0.157 \pm 0.01	0.165 \pm 0.01	0.184 \pm 0.013	0.224 \pm 0.008
Cr	0.144 \pm 0.02	0.454 \pm 0.012	0.539 \pm 0.18	1.23 \pm 0.09
Ni	0.203 \pm 0.004	0.237 \pm 0.008	0.238 \pm 0.01	0.267 \pm 0.013
Cd	ND	ND	0.050 \pm 0.003	0.098 \pm 0.007
Pb	0.0148 \pm 0.001	0.082 \pm 0.007	0.136 \pm 0.004	0.185 \pm 0.004

The percentage of leaching of the metals was also calculated and results are shown in Figure 1. As can be seen from the figure, each of the metal was extracted from the *C. macrostachyus* leaves to the water at different rate. For all the metals studied, maximum percentage extraction was observed at 24 h of infusion time followed by the subsequent lower extraction times. This highest leaching rate is appreciable for the essential metals. Whereas, for some of the trace metals and toxic elements, rate of extraction was more pronounced with infusion time. For instance, Cd was not detected in the infusion time of 3 and 6 h, while 2.80% and 15.5% was

leached for Pb for the extraction time of 3 and 6 h, respectively. The extraction efficiencies for these two metals proportionally increased with time of infusion. Thus we recommend to the traditional practitioners to infuse their samples at lower period of time as possible so as to minimize the risk of these two heavy metal toxicity.

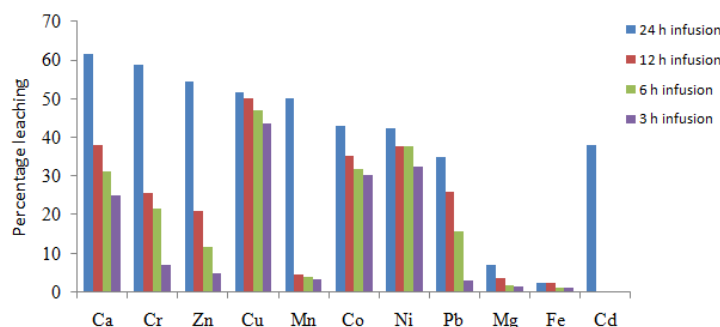


Figure 1. Percentage leaching of metals in 24 h, 12 h, 6 h and 3 h *C. macrostachyus* leaves infusions.

Table 7. Metal concentrations ($\mu\text{g/g}$) of *C. macrostachyus* leaves powder in wet weight basis and range of concentration of metals ($\mu\text{g/g}$) in different infusion time.

Metal	<i>C. macrostachyus</i> leaves powder in ($\mu\text{g/g}$) fresh weight basis ^a	<i>C. macrostachyus</i> leaves infusion in ($\mu\text{g/g}$) fresh weight basis ^b
Ca	2,887	716 – 1,776
Mg	1,190	16.7 – 80.9
Fe	139	1.39 – 3.34
Mn	67.9	2.17 – 3.40
Zn	14.5	0.674 – 7.88
Cu	4.46	1.94 – 2.31
Co	0.52	0.157 – 0.224
Cr	2.10	0.144 – 1.23
Ni	0.63	0.203 – 0.267
Cd	0.26	0.05 – 0.098
Pb	0.53	0.0148 – 0.185

^aConversion were made for Akaki leaves sample using a conversion facto of 4.17. ^bInfusion time between 3 and 24 h.

Generally, for most of the metals investigated their concentration in the extract increase with prolonged period of infusion time, i.e. highest concentration were noticed in the 24 h followed by 12, 6 and 3 h. This might be due to the fact that the solvent might get the opportunity to penetrate the matrix and solvate the ions and hence easily leached in to the filtrate. Furthermore, wide variations in extent of extraction were also noticed among metals (Table 6, Figure 1). Range of metals extracted with time for Akaki leaves sample is given in Table 7.

Comparison of metals in the *C. macrostachyus* leaves powder samples

The concentrations of the elements in the analyzed samples were quite varied. The order of the metal concentration from Akaki *C. macrostachyus* leaves powder and Abomsa *C. macrostachyus* leaves powder is almost similar except that the Akaki *C. macrostachyus* leaves powder with lower mean concentration of Co than Ni and Pb. The trend in metal concentration for that of the Bonga and Dilla *C. macrostachyus* leaves powder were the same for Ca, Mg and

Fe concentration. However, the concentration of Fe was greater than that of Zn and the concentration Cr were lower than the concentration of Ni which were not true for Dilla *C. macrostachyus* leave powder. Ca was found at highest concentration than other metals in all the four sample sites, with the mean concentration ranging from 5,823 $\mu\text{g/g}$ (Bonga *C. macrostachyus* leaves powder) to 12,040 $\mu\text{g/g}$ (Akaki *C. macrostachyus* leaves powder). Similarly, Mg was the next highest than other metals all the sites. The concentration of Fe was the third next to Mg but Mn was the next highest than the other metals for Bonga (1,770 $\mu\text{g/g}$) and Dilla (421 $\mu\text{g/g}$) sites.

Zn was found to be the third highest amount out of the microelements analyzed with mean concentration ranging from 19.7 $\mu\text{g/g}$ (Dilla *C. macrostachyus* leaves powder) to 60.5 $\mu\text{g/g}$ (Akaki *C. macrostachyus* leaves powder) followed by Cu with mean concentration range 6.31 $\mu\text{g/g}$ (Dilla *C. macrostachyus* leaves powder) to 18.6 $\mu\text{g/g}$ (Akaki *C. macrostachyus* leaves powder). The lowest concentration observed for all the four sites was that of Cd, ranging from 0.75 $\mu\text{g/g}$ (Abomsa *C. macrostachyus* leaves powder) to 1.08 $\mu\text{g/g}$ (Akaki *C. macrostachyus* leaves powder). Overall, the concentrations of each metal in the four sites are comparable.

Generally, Akaki *C. macrostachyus* leaves powder contained higher concentration of all the metals than other *C. macrostachyus* leaves samples but its Mn, Co and Ni contents were least. Abomsa *C. macrostachyus* leaves powder contained higher concentration of Fe, Zn, Cu, Cr and Cd next to Akaki *C. macrostachyus* leaves powder while Dilla *C. macrostachyus* leaves powder contained least amount of Mg, Fe, Zn, Cu, Co, Cd and Pb as can be seen from Table 5. This trend of concentration is also similar for the other *C. macrostachyus* leaves samples.

Comparison of the metal content in C. macrostachyus leaves and infusion samples with other medicinal plants

Results of the present study were compared with reported data on other medicinal plants from other parts of the world. Results of the comparison are given in Table 8. Looking at the table diversified concentration of metals in different species was noticed. However, the range of metals in the present study falls within the range of the reported data. For some of the species, higher concentrations for some of the metals were noticed compared to the present study while lower concentrations were found for some of the others. These variations might be attributed due to variation in species and family of the plants analyzed. Furthermore, environmental and geographical variations might have significant effect on the nutrients accumulation in the plants.

The metals concentrations in the infusion samples were also compared with other medicinal plants leaves from other parts of the world (Table 9). Looking at the table, extent of extractions from the leaves in to the infusions was comparable with the present study for most of the metals investigated. Higher concentrations in the infusion were noticed for those metals in higher concentration in the leaves for almost all of the plants compared. While small concentrations were observed for those whose concentrations were smaller in the leaves. Generally speaking, the range of mineral concentrations in the infusion of the present study was within the range of the plants compared.

Table 8. Comparison of metal concentration ($\mu\text{g/g}$) in *C. macrostachyus* leaves with other medicinal plant leaves.

A

Medicinal Plant	Origin	Concentration ($\mu\text{g/g}$) of metals in medicinal plants					Ref.
		Ca	Mg	Mn	Fe	Zn	
<i>Taraxacum officinale</i> Weber	Spain	29247	4461	101	853	68	[36]
<i>Eucalyptus globulus</i> Labill	Spain	18621	1616	2134	89	23	[36]
<i>Plantago major</i> L.	Bulgaria	48022	6405	46	373	56	[36]
<i>Mentha piperita</i> L.	Bulgaria	21131	5483	116	376	45	[36]
<i>Matricaria chamomilla</i> L.	Egypt	926	2642	76	701	49	[36]
<i>Calotropis procera</i> Ait.	Nigeria	18900	-	232	1872	71.7	[36]
<i>Acalypha wilkensiana</i>	Nigeria	28400	-	44.4	760	75.3	[40]
<i>Euphorbia hirta</i> Linn.	Nigeria	8120	-	52	536	191	[40]
<i>Pelargonium graveolens</i> L.	Egypt	-	-	26.4	516	12.1	[39]
<i>Marjorana hortensis</i> L.	Egypt	-	-	28	671	10.6	[39]
<i>Gynostemma pentaphyllum</i>	Thailand	5583–34070	1756–7739	43.4–259	126–2321	25.4–61.9	[33]
<i>Camellia sinensis</i>	Thailand	1384–6550	784–2549	229–1512	20.9–318	10.1–55.4	[33]
<i>Morus alba</i>	Thailand	15286–25182	3078–5188	75.3–353	89.5–408	19.2–34.4	[33]
<i>Croton macrostachyus</i>	Ethiopia	5,823–12,040	1971–4961	157–1,770	192–581	19.7–60.5	This study

B

Medicinal Plant	Origin	Concentration ($\mu\text{g/g}$) of metals in medicinal plants					Ref.
		Cu	Ni	Cr	Cd	Pb	
<i>Taraxacum officinale</i> Weber	Spain	27	-	-	-	7	[36]
<i>Eucalyptus globulus</i> Labill	Spain	10	-	-	-	6	[36]
<i>Plantago major</i> L.	Bulgaria	13	-	-	-	6	[36]
<i>Mentha piperita</i> L.	Bulgaria	19	-	-	-	8	[36]
<i>Matricaria chamomilla</i> L.	Egypt	20	-	-	-	3	[36]
<i>Calotropis procera</i> Ait.	Nigeria	138	235	199	-	-	[40]
<i>Acalypha wilkensiana</i>	Nigeria	146	204	34.6	-	-	[40]
<i>Euphorbia hirta</i> Linn.	Nigeria	235	241	-	-	-	[40]
<i>Marjorana hortensis</i> L.	Egypt	3.95	25.3	2.15	2.05	14.4	[39]
<i>Gynostemma pentaphyllum</i>	Thailand	5.14–15.5	0.53–6.60	0.43–12.4	0.021–4.77	0.36–64.4	[33]
<i>Camellia sinensis</i>	Thailand	3.08–22.4	2.28–9.19	0.21–10.5	0.002–0.10	0.060–53.9	[33]
<i>Morus alba</i>	Thailand	5.72–11.1	0.37–2.17	0.25–1.42	0.001–0.022	0.12–1.18	[33]
<i>Croton macrostachyus</i>	Ethiopia	6.31–18.6	2.15–3.80	2.13–8.75	0.75–1.08	1.05–2.19	This study

Table 9. Comparison of metal contents of *C. macrostachyus* leaves infusion with other medicinal plant leaves infusion.

Medicinal plant	Concentration ($\mu\text{g/g}$) of metals in infusion of medicinal plants				Ref.
	Cu	Cr	Cd	Pb	
<i>Taraxacum officinale</i> Weber	0.18	-	-	0.011	[36]
<i>Eucalyptus globulus</i> Labill	0.068	-	-	0.049	[36]
<i>Plantago major</i> L.	0.11	-	-	0.007	[36]
<i>Mentha piperita</i> L.	0.11	-	-	0.025	[36]
<i>Matricaria chamomilla</i> L.	0.21	-	-	0.036	[36]
<i>Gynostemma pentaphyllum</i>	1.62–7.99	0.00–1.41	0.103–2.31	0.113–8.02	[33]
<i>Camellia sinensis</i>	1.20–8.43	0.00–0.69	0.004–0.024	0.0004–3.16	[33]
<i>Morus alba</i>	1.48–7.39	0.00–0.40	0.00–0.028	0.0075–0.15	[33]
<i>Croton macrostachyus</i>	1.94–2.31	0.14–1.2	0.05–0.098	0.015–0.18	This study

Statistical analysis

Analysis of variance (ANOVA) [41] was used to evaluate the difference in concentration of metals among the sampling sites were significant or not. Except between Abomsa/Dilla *C. macrostachyus* leaves powder samples, there was a significant difference ($p < 0.05$) in mean concentrations of Ca at 95% confidence interval between all the four samples of *C. macrostachyus* leave powder when pair wise comparison was made. For Mg, Fe, Mn and Cd significant difference ($p < 0.05$) at 95 % confidence interval was observed in their mean concentrations between all the four *C. macrostachyus* leave powder samples. The same was true for Cu and Ni except between Abomsa and Dilla whilst, no significant difference ($p > 0.05$) in Zn and Cu concentration were observed for all the pair wise comparison except between Akaki and Abomsa *C. macrostachyus* leaf samples. Similarly, significant variation was in Cr and Pb concentration for all the pair wise comparison tests except between Akaki and Abomsa for Cr and Akaki and Bonga for Pb.

Absence of significant difference in some mineral nutrient among samples from Akaki, Abomsa, Bonga and Dilla may indicate that these areas are under the same geographical location and share common climatic conditions. Similarly presence of significant difference in concentration for some minerals of *C. macrostachyus* leaves powder might aroused from the chemical composition of the leaves itself, the specific environmental conditions in which the plant was grown or the studied area might contains higher concentration of mineral nutrient in the soil [41].

CONCLUSION

Generally, *C. macrostachyus* (an Ethiopian traditional medicinal plant) accumulated appreciable amounts of major and trace metals in its leaves. Ca and Mg were the most abundant nutrients among the analyzed metals followed by, Fe, Mn and Zn. All the metals enriched in the extract proportionally with extraction time but extent of leaching in the extract was significantly pronounced for the trace metals and the toxic metals (Cd and Pb) for prolonged period of infusion. Thus, beside its medicinal value, *C. macrostachyus* leaves can be good source of mineral nutrients if further research is conducted to predict appropriate dose to be taken.

Statistical analysis revealed that significant variation in metal concentrations among the sampling sites was observed for some of the metals when pair wise test was made while insignificant variation were seen for the remaining metals. Existence of significant variation in the level of metals for the samples studied might be difference in environmental and/or climatic conditions. Difference in soil parameters might also have significant effect for the variation. Absence of significant variation might be due to the fact that similarity in the aforementioned parameters among the stated areas.

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REFERENCES

1. Lanfranco, G. E. *J. Biotechnol.* **1999**, 2, 1.
2. Demma, J.; Engidawork, E.; Hellman, B. *J. Ethnopharmacol.* **2009**, 122, 136.
3. Kassaye, K.D.; Amberbir, A.; Getachew, B.; Mussema, Y. *Ethiop. J. Health Develop.* **2006**, 20, 127.

4. Baquar, S.R. *The Role of Traditional Medicine in Rural Environment in Traditional Medicine in Africa*, Issaq, S. (Ed.), East Africa Educational Publishers Ltd.: Nairobi; **1995**; pp 141–142.
5. World Health Organization (WHO) *General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine*, WHO: Geneva; **2001**.
6. Derbie, A.; Chandravanshi, B.S. *Biol. Trace Elem. Res.* **2011**, 141, 317.
7. Gebre, A.; Chandravanshi, B.S. *Bull. Chem. Soc. Ethiop.* **2012**, 26, 329.
8. Abebe, D. *The role of herbal remedies and the approaches towards their development in Proceedings of the Workshop on Development and Utilization of Herbal Remedies in Ethiopia*, Nazareth, **1996**; p 29.
9. Tadeg, H.; Mohammed, E.; Asres, K.; Gebre-Mariam, T. *J. Ethnopharmacol.* **2005**, 100, 168.
10. Gidey M. *An Ethanobotanical Study of Medicinal Plants Used by the Zay People in Ethiopia*, CBM: Skriftserie 3, Uppsala; **2001**, pp 81–89.
11. Desissa, D.; Binggeli, P. *Uses and conservation status of medicinal plants used by the Shinasha people*; **2000**; Available at <http://members.multimania.co.uk/ethiopianplants/shinasha.html>. Accessed on 20 June 2012.
12. Breitenbach, F.V. *The Indigenous Trees of Ethiopia*, 2nd ed., Ethiopian Forestry Association: Addis Ababa; **1963**; p 306.
13. Gidey, M.; Teklehaymanot, T.; Animut, A.; Mekonnen, Y. *J. Ethnopharmacol.* **2007**, 110, 516.
14. Shukla, P.; Misra, S.P. *An Introduction to Taxonomy of Angiosperms*, Vikas Publishing House Pvt. Ltd.: Delhi; **1979**; p 556.
15. Heywood, V.H. *Flowering Plants of the World*, Andromeda Oxford Ltd.: Oxford; **1993**; p 335.
16. Tane, P.; Tatsimo, S.; Connolly, J.D. *Tetrahedron Lett.* **2004**, 45, 6997.
17. Wakjira, K. *Seed germination physiology and nursery establishment of Croton macrostachyus Hochst. Ex Del.* M.Sc. Dissertation, Addis Ababa University, Addis Ababa, Ethiopia, **2007**.
18. Yibralign, Z. *Phytochemical investigation on the stem bark of Croton macrostachyus (Bisana)*. M.Sc. Dissertation, Addis Ababa University, Addis Ababa, Ethiopia, **2007**.
19. Olajire, A.A.; Ayodele, E.T. *Bull. Chem. Soc. Ethiop.* **2003**, 17, 11.
20. Cundeva, K.; Pavlovovska, G.; Stafilov, T. *J. Braz. Chem. Soc.* **2007**, 18, 1207.
21. Yemane, M.; Chandravanshi, B.S.; Wondimu, T. *Food Chem.* **2008**, 107, 1236.
22. Atlabachew, M.; Chandravanshi, B.S. *J. Food Comp. Anal.* **2008**, 21, 545.
23. Atlabachew, M.; Chandravanshi, B.S.; Redi, M. *Biol. Trace Elem. Res.* **2010**, 138, 316.
24. Weldegebriel, Y.; Chandravanshi, B.S.; Wondimu, T. *Ecotoxicol. Environ. Saf.* **2012**, 77, 57.
25. Kitata, R.B.; Chandravanshi, B.S. *Bull. Chem. Soc. Ethiop.* **2012**, 26, 27.
26. Aregahegn, A.; Chandravanshi, B.S.; Atlabachew, M. *Afr. J. Food Agric. Nutr. Dev.* **2013**, 13, 7870.
27. Schumacher, M.; Bosque, M.A.; Domingo, J.L.; Corbella, J. *Bull. Environ. Contam. Toxicol.* **1991**, 46, 320.
28. Somers, E. *J. Food Sci.* **1983**, 39, 215.
29. Deckelbaum, R.J.; Palm, C.; Mutno, P.; Declerck, F. *Food Nutr. Bull.* **2006**, 27, 335.
30. Liu, D.; Kottke, I. *J. Biosci.* **2004**, 29, 329.
31. Raskin, I.; Kumar, P.B.A.; Dushenkov, S.; Salt, D.E. *Curr. Opin. Biotechnol.* **1994**, 5, 285.
32. Piotr, K.; Zbigniew, F.; Anna, D.; Peter, O. *Sci. Total Environ.* **2007**, 381, 99.
33. Al Moaruf, O.; Muibat, O.; Asiata, O.; Isiaka, A.; Nureni, O. *Food Chem.* **2004**, 85, 67.
34. Sheded, G.M.; Pulford, I.D.; Hamed, I.A. *J. Arid Environ.* **2006**, 66, 210.

35. Sumontha, N.; Nuchanart, R.; Jutamaad, S. *J. Agric. Food Chem.* **2006**, *54*, 6939.
36. Rashed, M. *J. Arid Environ.* **1995**, *29*, 185.
37. Reddy, P.R.; Reddy, S.J. *Chemosphere* **1997**, *34*, 2193.
38. Basgel, S.; Erdemoğlu, S.B. *Sci. Total Environ.* **2006**, *359*, 82.
39. Lokhande, R.; Singare, P.; Andhele, M.; Acharya, R. *Radiochem.* **2009**, *51*, 321.
40. Obiajunwa, E.; Adeleke, A.; Olanrewaju, O. *J. Radioanal. Nucl. Chem.* **2002**, *252*, 473.
41. Miller, J.N.; Miller, J.C. *Statistics and Chemometrics for Analytical Chemistry*, 5th ed., Pearson, Harlow: England; **2005**.