Determination of Selected Heavy Metals in Leaves and Petiole of Parts of *Croton Macrostachyus* Collected from Hidabu Abote, North Shoa, Oromia Region, Ethiopia

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Abstract: *In this study, the level of Ca, Mg, Fe, Zn, Ni, Cr, Pb and Cd in leaves and petiole parts of Croton macrostachyus were determined using flame atomic absorption spectroscopy. A wet digestion method was used to digest a 0.5 g of both samples with 2 mL of $HClO_4$ (70%) and 2 mL of HNO_3 (69%) for a digestion time of 2.25 h at a digestion temperature of 270 ^oC. A series of solution were prepared from stock solution to construct calibration curves for each metal investigated. The recovery analysis was made by spiking 0.5 g of leave and petiole parts of Croton macrostachyus samples at concentration levels of 1565 μg/g of Ca, 992 μg/g of Mg, 116 μg/g of Fe, 12 μg/g of Zn, 1.75 μg/g of Pb, 1.08 μg/g of Cd, 1.68 μg/g of Ni and 1.84 μg/g of Cr. The method offers a good regression coefficients (0.9998-0.9999), limit of detection (0.03-4.23 μg/g), and limit of quantification (0.1-14.1μg/g). The percentage recovery of target analytes were in the ranges of 95.5%-104% which is in agreement with accepted ranges. The concentration of Ca, Mg, Fe, Zn, Pb, Cd, Ni and Cr in leaves of Croton macrostachyus, were 12,137, 5,038, 592, 65.3, 2.86, 1.26, 2.73 and 2.38 μg/g, respectively. Similarly, the concentration of Ca, Mg, Fe, Zn, Pb, Cd, Ni and Cr in petiole of Croton macrostachyus 10,815, 4,251, 482, 57.2, 2.64, 1.12, 2.74 and 2.21 μg/g, correspondingly. The amount of Fe, Zn, Cd, Ni and Cr were above the tolerable limit of WHO for herbal medicine.*

Keywords: *Croton macrostachyus, Heavy metals, Leaves, Medicinal plant and Petiole.*

INTRODUCTION

Herbal medicinal plants are commonly cultivated in garden or wild growing (Hailemariam and Bibiso, 2019). Medicinal plants are natural resources of biologically active ingredients and used as therapeutic healings and in variable pharmaceutical preparations (Adie and Adekunle, 2017; Bardarov and Mihaylova, 2014). The treatment of diseases using medicinal plants and plant based is as old as history of mankind (Bachheti *et al.*, 2011). Medicinal plants have important attributes including medication, economic, ecological and cultural services. Medicinal plants are the world most important means to treat diseases and infections (Adie and Adekunle, 2017; Hailemariam and Bibiso, 2019). About 80% of the world population is dependent on medicine originated from plant source for their healthcare requirements largely due to the high cost of modern pharmaceuticals, but also because the traditional medicines are generally more acceptable from a cultural and spiritual perspective (Aregahegn *et al.*, 2015, Kassaye *et al.*, 2009). The healing capacity of medicinal plant extracts had long been discovered and widely used before pathogenic microbes recognized as a causative agent of infectious diseases (Assefa *et al.*, 2020).

Medicinal plants are also representing a major reservoir of novel compounds for new drug identification. Several medicinal plant-derived bioactive compounds are used for the management of a variety of chronic disorders and infectious diseases (Baquar, 1995). Medicinal plants are rich with their secondary metabolites and a number of phytochemicals have been developed into effective modern drugs (Demma, 2009). The best examples of these plant-derived therapeutic agents are vinblastine and vincristine, two alkaloid drugs commonly used in chemotherapy, which are derived originally from *Catharanthus roseus* (Madagascar periwinkle). An additional example is etoposide, a non-alkaloid anticancer drug derived from the North American *Podophyllum* species (Mayapple) (Bhanot *et al.*, 2011).

Ethiopia has a long history of using traditional medicines from plants and has developed methods to fight diseases using it (Admasu and Yohannes, 2018). Ethiopia is a centre of diversity for a number flora and fauna. *Croton macrostachyus* is one of the common medicinal plants in Ethiopia. *Croton macrostachyus* belongs to the Euphorbiaceae, a very large family with 300 genera and 8,000 to 10,000 species and the most abundant plant in the tropics. Its common names in different parts of Ethiopia are Bisana (Amharic and Shinasha), Makanissa and Bakkanissa (Afan Oromo), Wusha, Masincho (Sidama) and Islami, Tambuk and Tambush (Tigrigna) (Aregahegn *et al.*, 2015). *Croton macrostachyus* is used in Ethiopia to treat malaria, snakebite, internal worms, headache, rabies, ascarasis, gonorrhea, tinea diseases, fungal disease, and sexually transmitted disease. The plant is also used for fever and wounds of domestic animals (Chandravanshi, 2017, Liu *et al.*, 2009). In Hidabu Abote Woreda, the plant is commonly in use to preserve and remove hair from skin of sheep and goat during traditional skin processing for different purposes including carpet, belt, knapsack and buckskin sack. Furthermore, they are using this plant to treat dandruff and other fungal disease.

Plants are an important agent to transfer trace metals from soil to human being through food chain (Liu at al., 2009). The amount of metals in medicinal plants depends on the nature of soil, the capacity of plants to accumulate metals, rainfall, fertilizers, pesticides and atmospheric dusts (Kitata and Chandravanshi, 2012; Olajire and Ayodele, 2003). The deficiency of trace metals causes diseases and overdose may result in toxicity to human leading to malfunctioning of organs and central nervous system (Aregahegn *et al.*, 2015). Intake of heavy metals during medication and foods may results in bio-accumulation in organisms, leading to health hazards like symptoms of chronic toxicity, injury to the kidneys, familiarity of renal and damage to liver (Abou-Arab *et al.*, 1999, Malede, 2019). The World Health Organization (WHO) has established standards for the quality control of medicinal plants including the botanical identification, classification, identification of contaminants and determination of active principles. The WHO recommends qualitative and quantitative assays of heavy metals in phytotherapeutics, especially in raw materials of doubtful origin and plants produced by intensive agricultural means (Bachheti, 2011; Mamani *et al.*, 2005).

Heavy metals have both a curative and a preventive role in combating diseases. They can be dangerous and may result in toxicity to human life disturbing normal functioning of organs and central nervous system (Aregahegn *et al.*, 2015). The medicinal plants are one of an important link to the transfer of trace elements from soil to human. Thus, it is crucial to determine the trace level of metals in *Croton macrostachyus* parts used for medication. This plant is widely in use as a curing medicine by many residents in North Shoa, Oromia Regional State, Ethiopia. Thus, the study intended to determine Ca, Mg, Fe, Zn Ni, Cr, Pb and Cd in the leaves and petiole of *Croton macrostachyus* collected from Hidabu Abote by flame atomic absorption spectroscopy.

MATERIALS AND METHODS

Description of the Study Area

The samples were collected from Hidabu Abote woreda of North Shoa Zone, Oromia Region, Ethiopia. Hidabu Abote is bordered on the south by Kuyu woreda, on the west by Wara Jarso woreda, on the north by the Jamma River and on the east by Degem woreda. The capital of the woreda is Ejere town and its distance from Addis Ababa (Capital city of Ethiopia) is 161.9 km. The latitude and longitude of the woreda is 9° 54' 52" N and 38° 31' 08" E, respectively with elevation of 2347 m above sea level.

Sample Collection and Pre-treatment

The sample of leaves and petioles of the plant part, estimated to have similar age, were collected randomly from ten *Croton macrostachyus* trees. The samples were put in separate clean plastic bags and labeled accordingly. Samples were treated with tap water to avoid unwanted materials, rinsed with distilled and de-ionized water and finally, air dried. About 500 g of each sample were put on acid-washed porcelain dish, dried in drying oven set at 105 ^oC for 2 h. All samples were cooled to room temperature, ground in mortar with pestle and sieved using 1000 μm sieve. All sub-samples were mixed to have representative samples for leaves and petioles samples. Then, the resulting samples (leave and petiole samples) were stored in clean plastic sample holder (bags).

Reagents and Chemicals

Analytical reagents used for this research work were 69% HNO_3 and 70% $HClO_4$ (both were from UNI-CHEM® Chemical reagents, India). The reagents were used during digestion of the samples. A 99.9% lanthanum nitrate hydrate (Blulux laboratories, Haryana, India) was also used to eliminate the interference from Mg and Ca in the sample solution during analysis. Stock standard solution of Ca, Zn, Fe, Mg, Pb, Cd, Ni and Cr at concentration level of 1000 mg/L, were prepared using their respective nitrate salts (Sigma-Aldrich Corp., St. Louis, USA). 10 mg/L standard solutions of each metal were also prepared for the preparation of series of concentration for the calibration curves. Deionized water was used for dilution, sample preparation and others during analysis.

Apparatus and Instruments

Teflon (PTFE, China) knives were used to cut leaves and petioles of the *Croton macrostachyus* into pieces. Drying of samples was carried out using an oven (Digitheat, J.P. Selecta, Spain). Ceramic pestle and mortar were used for grinding samples. The samples were digested on Kjeldahl heating apparatus (Gallenkamp, England) using round bottom flasks (100 mL) fitted with reflux condenser. Volumetric flasks (25, 50 and 100 mL), pipettes (Pyrex, USA), measuring cylinders (Duran, Germany) and micropipettes (Dragonmed, 1-10 µL, 100-1000 µL, Shanghai, China) were used during analysis. Whatmanfilter paper no. 42 was used to filter samples. 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 was used to analyse the amount of target metals in each samples.

Optimization of Digestion Procedure

The digestion parameters including reagent volume for HNO_3:$HClO_4$ ratio (1:2, 2:1, 2:2, 1:3 and 3:1), temperature (220, 270 and 300 ^oC), digestion time (2.0, 2.25, 2.5, 2.75 and 3.0 h) and mass of the samples (0.25, 0.5 and 0.75 g) were optimized by varying one parameter at a time for the digestion of leave and petiole parts of *Croton macrostachyus* samples. Thus, for a complete digestion of 0.5 g of the dry leaves and petiole parts of *Croton macrostachyus* samples, 2 mL of $HClO_4$ (70%) and 2 mL of HNO_3 (69%) for digestion time 2.25 h using digestion temperature of 270 ^oC was chosen and used for further experiments.

The optimum points were chosen based on digest clarity, minimal digestion and minimal fusion time, reagent volumes consumption, simplicity, and proper use of sample amounts. On the other hand, alternative therapies offer certain disadvantages. They call for more reagents, a longer or shorter digestion period, and a higher or lower temperature leading to the formation of turbid solution and colored sample solutions. Moreover, the blank solution was prepared in seven replicate and digested following the same procedure.

Method Validation

The calibration curve for each target metal was prepared from five calibration points. The detection limits (LODs) and quantification limits (LOQ) were obtained by multiplying the pooled standard deviation of the reagent blank (n=7) by three and ten, respectively (Tolcha *et al.*, 2021). The recovery analysis was done by spiking 0.5 g of leave and petiole samples (Aregahegn *et al.*, 2015) at concentration level of 1565 µg/g of Ca, 992 µg/g of Mg, 116 µg/g of Fe, 12 µg/g of Zn, 1.75 µg/g of Pb, 1.08 µg/g of Cd, 1.68 µg/g of Ni and 1.84 µg/g of Cr. Each sample was analyzed for their respective spiked metals by atomic absorption spectrophotometer. Each recovery test for both samples was performed in triplicate. The spiked samples were treated in under the same experimental conditions used to digest real sample and reported in percent recovery (%R).

RESULTS AND DISCUSSION

Method Validation

Method validation is the process of providing that analytical method is acceptable for its intended purpose. The analytical method was validated by evaluating its R^2, LODs, LOQs and %R. The detection wavelengths, R^2, LODs and LOQs of each metal are given in Table 1. The regression coefficient of the method was between 0.9998 and 0.9999. The LODs value ranged between 0.03-4.23 μg/g. LOQ is also in the range of 0.1-14.1μg/g. The percentage recoveries of target analytes were in the ranges of 95.5% - 104% which is in agreement with accepted ranges (Tolcha *et al.*, 2020). The obtained analytical results clearly indicate as the method is sensitive, robust and reliable.

Table 1: Method detection limits for *Croton macrostachyus* leaves and petiole samples

Metal	Wavelength (nm)	R^2	LODs (μg/g)		LOQs (μg/g)		%R	
			Leaves	Petiole	Leaves	Petiole	Leaves	Petiole
Ca	**422.7**	**0.9998**	**4.23**	**3.21**	**14.1**	**10.7**	**98.6 ± 6**	**97.3± 4**
Mg	**285.2**	**0.9998**	**2.9**	**2.38**	**9.67**	**7.93**	**99.5 ± 3**	**98.4± 2.5**
Fe	**248.3**	**0.9999**	**0.1**	**0.12**	**0.33**	**0.4**	**99.3 ± 1.5**	**98.5± 1.6**
Zn	**324.8**	**0.9999**	**0.12**	**0.87**	**0.40**	**2.9**	**102 ± 3.4**	**101± 2.8**
Pb	**217.0**	**0.9996**	**0.16**	**0.13**	**0.53**	**0.43**	**98.1 ± 2.2**	**99.5± 3.7**
Cd	**228.9**	**0.9998**	**0.14**	**0.11**	**0.47**	**0.37**	**95.5 ± 5**	**96.8± 2.6**
Ni	**357.9**	**0.9999**	**0.08**	**0.03**	**0.27**	**0.1**	**104 ± 1.6**	**102.3± 2**
Cr	**341.5**	**0.9999**	**0.12**	**0.11**	**0.4**	**0.37**	**103 ± 1.8**	**101 ± 1.6**

A = Absorbance, x = Concentration

Concentration of Essential and Nonessential Metals in Croton Macrostachyus Samples

Concentrations of both essential (Ca, Mg, Fe, Zn, Ni and Cr) and nonessential (Pb and Cd) elements in leaves and petiole parts of *Croton macrostachyus* were determined using flame atomic absorption spectroscopy and the outcome were presented in Tables 2. The data obtained depicted the samples have varying concentration of target heavy metals. This variation may be due to the geochemical characteristics of a soil and the ability of plants to accumulate these elements (Weldegebrie *et al.*, 2012). All the analyzed metals were detected in the two samples. Calcium is the most abundant metal detected in the two samples while cadmium is the least among the metals studied.

From the Table 2, the average amount of target metals (µg/g) in leave and petiole parts of *Croton macrostachyus* is significantly high. The average amount of Ca metal is significantly more than other target metals on both samples. Similarly, higher levels of other analyzed metals were also detected with following the descending order given as follows:
Ca > Mg > Fe > Zn > Pb > Ni > Cr > Cd in both samples.

Table 2: Mean concentration (X ± SD, n = 3) of essential and toxic metals in *Croton macrostachyus* leaves and petiole samples

Metal	**X ± SD**		**WHO safe limit (µg/g)**
	Leave sample (µg/g)	Petiole sample (µg/g)	
Ca	12,137 ± 650	10,815 ± 476	-
Mg	5,038 ± 84	4,251 ± 53	-
Fe	592 ±69	482 ±46	15
Zn	65.3 ± 6	57.2 ± 3.7	50
Pb	2.86 ± 0.07	2.64 ± 0.06	10
Cd	1.26 ± 0.04	1.12 ± 0.03	0.3
Ni	2.73 ± 0.08	2.74 ± 0.05	1.5
Cr	2.38 ± 0.09	2.21 ± 0.08	2

X = mean SD = standard deviation

Leaves and petiole parts of *Croton macrostachyus* is commonly in use in Hidabu Abote Woreda for medical propose and treatment of skin of sheep and goat during traditional skin processing for different proposes. The skin of sheep and goats is wrapped up with leaf of *Croton macrostachyus* for about three to five days to remove hair and preserve the skin not to spoil. The skin removing capacity may be due to chromium metal in leaves and the preserving capacity may be due to photochemical contained in leaves of *Croton macrostachyus*.

The mean concentration of Ca metal determined in leaf and petiole parts of *Croton macrostachyus* samples were found to be 12,137 and 10,815 µg/g, respectively. The maximum residue limit for Ca in medicinal plant is not set by FAO/WHO. Calcium is an essential mineral useful for the skeletal, endocrine, cardiovascular and neurological systems including contraction of muscle, to strengthen bones and teeth, activation of oocyte, nerve impulse, clotting of the blood, transmission, to regulate heart beat and to balance fluid within a cells. Exposure to overdose of calcium from supplements and bioaccumulation can affect bones, reduce absorption of nutrient, produce kidney stones and affect health of heart (Pravina *et al.*, 2013). Therefore, the users of leaf and petiole parts of *Croton macrostachyus* may be affected by high amount of Ca metal.

The results depicted in Table 2 indicates the concentration of Mg metal in leaf and petiole parts of *Croton macrostachyus* are 5038 and 4251 µg/g, respectively. There is no maximum residue limit for Ca in medicinal plant set by FAO/WHO. Magnesium has an important role to play in nearly all physiological functions, including the regulation of many bodily functions through its action as a cofactor for numerous enzyme systems. Magnesium is necessary for the structural function of nucleic acids and the mitochondria, proteins and is required for both anaerobic and aerobic energy production. It also plays a critical role in Ca^{2+} and potassium transportation across cell membranes (Laires *et al.*, 2004). The calcium deficiency in female and male may results in reproductive failure, migraine, increasing of blood pressure, heart disease, osteoporosis and diabetes (James *et al.*, 2018).

From the Table 2 iron was found to be 592 and 482 µg/g in leaf and petiole parts of *Croton macrostachyus* samples, respectively. The Fe concentration values obtained in both samples was beyond the permissible limit of WHO/FAO (15 µg/g). Iron is part of fundamental metabolic reactions including production of energy and replication of DNA. In mammals, iron transports oxygen in the blood as hemoglobin. On the other hand, overdose of iron may cause inflammation of gut, increase production of free radical and peroxidation reactions in the mucosa of the gut (Stoffel *et al.*, 2020).

The concentration of zinc determined in leaf and petiole parts of *Croton macrostachyus* samples was found to be 65.3 and 57.2 μg/g, respectively. The Zn concentration values obtained from the two samples was above the permissible limit of WHO/FAO (50 μg/g). Zn is an important element accountable for a number of enzymatic processes and takes part in functioning of genetic materials, immune reactions, proteins, foetus development, wound healing and production of sperm cells. It is can also prevent diarrhea at normal levels (Bhowmik *et al.*, 2010). The excessive Zn intake resulted in sideroblastic anemialeukopenia, hypochromic microcytic anemia, lymphadenopathy, hypocupremia, neutropenia and hypoferremia (Bedassa *et al*., 2017).

The trace level of lead in leaves and petiole parts of *Croton macrostachyus* samples was determined to be 2.86 and 2.64 μg/g, respectively. The detected amount of lead in the studied samples was less than the allowed limit for medicinal plant, 10 μg/g, reported by WHO. However, on repeated exposure and as result of bioaccumulation, the users may be affected by diseases like fatigue, headache, abdominal cramps, joint pain and nausea (Fikadu and Mekassa, 2022).

The Cd contents found in the leaves and petiole parts of *Croton macrostachyus* samples were 1.26 and 1.12 μg/g, respectively. Its amount in both samples is more than the maximum permissible level set by WHO (0.3 μg/g) for medical plants. Cadmium can cause irritation of stomach, vomiting and severe diarrhea. It is also known as a carcinogenic agent. The result shows there should be a continuous awareness creation for people using this medicinal plant (Fikadu and Mekassa, 2022; Lokhande *et al.*, 2009).

The amount of nickel in both parts of *Croton macrostachyus* samples was also determined and its concentration level was found to be 2.73 and 2.74 μg/g, respectively. The amount of Ni in both samples is above recommended residue levels in medicinal plants set by WHO (1.5 μg/g). Even though, Ni toxicity in human is not a very common occurrence because of its absorption by the body is very low, it may cause health effects including irritation to the skin. Exposure can also harm the lungs, stomach, and kidneys (Lokhande *et al.*, 2009). The main concern in handling nickel is its ability to produce allergic dermatitis. Ni is also a known carcinogen (Fikadu and Mekassa, 2022).

The average concentration of chromium determined in the analyzed leaves and petiole parts of *Croton macrostachyus* samples was found to be 2.38 and 2.21 µg/g (Table 2), respectively. The value obtained is higher than the allowed level (2 µg/g) set by WHO. Therefore, people who are using this medicinal plant can be affected by disease like dermatitis, skin ulcerations, allergic skin reactions, mucous membrane ulcerations, reaction of asthmatic allergy, nasal septum perforation, gastro-enteritis, deficiency of hepatocellular and renal oligo anuric, and bronchial carcinomas (Baruthio, 1992).

Comparison of Metal Composition of Leaves and Petiole Parts of *Croton Macrostachyus*

The data obtained from this research work (Table 3) indicated the metal composition of the two samples showed variation. The concentration of all metals in leave of *Croton macrostachyus* is higher than metal concentration in petiole of *Croton macrostachyus* samples. This may in fact that leaves of *Croton macrostachyus* is rich in phytochemicals including alkaloid, flavonoids, saponins, tannins, steroids, phenols, terpenoids and anthraquinones (Ezeabara and Okonkwo, 2016) which may have tendency to bind the target metals.

Table 3: Comparison of concentration of metals in leaves and petiole parts of Croton macrostachyus

Metal	**X ± SD**	
	Leave sample (µg/g)	Petiole sample (µg/g)
Ca	12,137 ± 650	10,815 ± 476
Mg	5,038 ± 84	4,251 ± 53
Fe	592 ±69	482 ±46
Zn	65.3 ± 6	57.2 ± 3.7
Pb	2.86 ± 0.07	2.64 ± 0.06
Cd	1.26 ± 0.04	1.12 ± 0.03
Ni	2.73 ± 0.08	2.74 ± 0.05
Cr	2.38 ± 0.09	2.21 ± 0.08

Statistical Analysis

Statistical analysis is used to know the presence or absence of significant difference in mean concentration of each metal between the analyzed leave and petiole parts of *Croton macrostachyus* samples. The statistical analysis for t-test at 95% confidence limit ($p < 0.05$) indicated that the difference among means in some metals from the two samples were significant. The amount of Cd, Pb and Cr in studied samples shows significant difference when tested at confidence level of 95% ($p < 0.05$). Insignificant mean concentration variation was observed for Ca, Mg, Fe, Zn and Ni in both samples at confidence limit of 95% ($P > 0.05$). The variation in distribution of Cd, Pb and Cr in leave and petiole of the *Croton macrostachyus* leaves might due to the chemical composition of the leaves and petiole (Aregahegn *et al.*, 2015)

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CONCLUSIONS

Croton macrostachyus is very important traditional medicine that occurred in different part of Africa including Ethiopia. In this study *Croton macrostachyus* leaves and petiole samples were analysed for their selected metal composition. *Croton macrostachyus* accumulated appreciable amounts of essential and non-essential metals in its leaves and petioles. Ca and Mg were the most abundant nutrients among the analyzed metals followed by, Fe and Zn. There is no permissible level set for Ca by FAO/WHO. The concentration values of Mg, Fe, Zn, Ni, Cr and Cd were more than allowed level set by WHO. The finding of this study showed that *Croton macrostachyus* leaves and petiole can be good source of mineral nutrients if further research is conducted to predict appropriate dose to be taken. The *Croton macrostachyus* leaves is traditionally used to remove hair during local skin processing for different purposes including carpet, belt, knapsack and buckskin sack. The hair removal capacity of the leaves of *Croton macrostachyus* may be due to high concentration of Cr in it.

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Conflicts of Interest

The author declares there is no conflict of interest.

Ethical standards: Not applicable.

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