British Journal of Applied Science & Technology
13(5): 1-6, 2016, Article no.BJAST.17726
ISSN: 2231-0843, NLM ID: 101664541

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Atomic Absorption Spectrometric Determination of Some Heavy Metals from the Leaves of *Moringa stenopetala* Grown in Gamo Gofa Zone, Ethiopia

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJAST/2016/17726
Editor(s):
(1) Jon S. Gold, East Stroudsburg University, East Stroudsburg, PA, USA.
(2) Singiresu S. Rao, Prof. at Department of Mechanical and Aerospace Engineering, University of Miami, Coral Gables, USA.
Reviewers:
(1) Gregorio Guadalupe Carbajal Arizaga, Universidad de Guadalajara, Mexico.
(2) Anonymous, National Research Centre, Egypt.
(3) Anonymous, Federal University of Sao Joao Del Rei, Brazil.
(4) Anonymous, Ecole Normale Superieure, France.
(5) Anonymous, Landmark University, Nigeria.
Complete Peer review History: http://sciencedomain.org/review-history/12626

Original Research Article

Received 25th March 2015
Accepted 23rd October 2015
Published 10th December 2015

ABSTRACT

The plant *Moringa* (*Moringa stenopetala*), eaten as a vegetable, is a deciduous plant cultivated in the southern part of Ethiopia. It is indigenous to Ethiopia, distributed in the lowland ecology of the country. The present study was conducted with the objective of analyzing some trace metallic contents (Fe, Cr, Pb and Cu) in the leaves of *Moringa* plant and to identify their levels. The work was investigated based on samples collected from Arba Minch area, Gamo Gofa administrative zone. Atomic Absorption Spectrometry (AAS) was used to analyze the mentioned trace metals in this plant. The results showed: Fe, 1.18 mg/kg; Cr, 0.44 mg/kg; Cu, 0.67 mg/kg; Pb, 0.63 mg/kg. Thus, out of the four analyzed trace metals, Fe was found in larger amount compared to others and Cr was the smallest.

Keywords: Atomic absorption spectrometry; Moringa stenopetala; microkjeldhal; trace metals.

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1. INTRODUCTION

Nearly all plants have been and will stay very important to mankind, animals as well as to environments. They turn out primary and secondary metabolites which include a broad range of functions, many of which have been subsequently subjugated by humans for their beneficial job in an array of applications. The most significant of these bioactive ingredients of plants are the secondary metabolites which include phenolic compounds, alkaloids, tannins, phytosterols and terpenoids [1].

Moringa is one such group whose various species have not been investigated fully notwith-standing the enormous reports regarding the various parts of a few species' potentials such as cardiac and circulatory stimulants [1]. Among different types of *Moringa* tree, *Moringa stenopetala* is a traditional medicinal and nutritional plant.

Moringa stenopetala is native to the horn of Africa, particularly in southern Ethiopia, north Kenya and Easten Somalia. In Ethiopia, it is found in many arid zones of the southern Ethiopia most extensively between Arba Minch, Negelle, and Welayta Sodo [2-4]. It is commonly called '*Shifarw*' in Amharic [5]. It is widely distributed at an altitude range of about 1100 to 1600 m. The cabbage tree is small (up to 12 m), with a much branched crown and some time with multi-trunks. The leaves are bi-pinnate or tri-pinnate with about five pairs of pinnate and three to nine elliptic or ovate leaves lets on each pinna. The fruits are long reddish pods with a grayish bloom [6]. It is the major vegetable crop in the region and can be used as forage as well as for purification of water. The plant has several medicinal uses in areas where it is native. Local people use the plant parts to treat malaria, leish maniasis and hypertension [3,6]. The plant is used as traditional medicine [7]. In general, *Moringa stenopetala* is used for clarification and purification of water to make it potable. A powder made by grinding the seeds is found to be more effective at coagulating substances in suspension [8]. It has blood pressure lowering effect too.

The edible part of the *Moringa* leaves are exceptionally nutritive. The leaves, which are undertaken as a food source in the tropics because the tree is covered with green leaves during dry time of the year (when other foods are limited), are used as kale or cabbage for human consumtion and animal feed. Over five million people depend on this plant as vegetable source. Immature leaves of *Moringa stenopetala*, which are part of the staple diet of the population in the Gamo Gofa zone [9], contain different trace inorganic substances. However, the presence of small amount of cynogeniglucosides in *Moringa stenopetala* leaves may have health peril in areas of high incidence of endemic goitre as an exacerbating factor if consumed for a long period of time [10].

This study is done for the objectives of providing base line data and specifying the levels of four elements namely iron, copper, lead and chromium as they can disturb levels in the body. The varieties of concentration of these four metals in *Moringa Stenopetala* leaves are compared with permissible limit of metals in plants as recommended by World Health Organization [11]. Generally, this study is focused to solve the problem related with consuming *Moringa stenopetala leaves* by determining the concentration of these heavy metals employing the most widely used technique of Atomic Absorption Spectrometry (AAS) [12]. From inorganic biochemistry point of view, the type of study is important as the deficiency or excess of the above elements is known to cause a number of serious metabolic / growth / physiological as well as toxic problems.

2. EXPERIMENTAL

2.1 Sampling

From Gamo Gofa zone (about 500 km south of Addis Ababa, Ethiopia) the consumable part of the *Moringa stenopetala* (as judged by consumers) were collected in pre-cleaned plastic bags. The collected samples were washed thoroughly with distilled water before breakdown [13]. The leaves were cut to separate the stems using knife, dried (in an oven at a temprature between 60-70℃) to constant weight, crushed in mortar and sieved through a 2 mm sieve. The sieved sample was stored in clean plastic bottle until used for digestion. The dried *Moringa* leaves sample was protected from sunlight and weighed.

2.2 Digestion of Samples

0.5 g of dehydrated and homogenized M*oringa stenopetala* samples were transferred in to a 150 ml round bottom flask. 4.5 ml of a mixture of HNO_3 (70%), $HClO_4$ (70%) and H_2O_2 (30%) were

added to the flask with a volume ratio of 2.5:1:1 and the mixture was digested on a microkjeldhal digesting apparatus by setting the temperature first at 60℃ for 30 min and then increased to 210℃ for the next 2:30 h. Then, the digested solution was allowed for 5min without dismantling the condenser from the flask to cool, and for 10 min after removing the condenser. To the cooled solution and precipitate formed, 10 ml of deionized water was added to be cooled and minimize dissolution of filter paper by the digest residue. Then the solution was filtered with Whatman filter paper [13].

The round bottom flask was wetted with 10 ml deionized water and the solution was filled with deionized water to the mark (50 ml). Three separate digestions of the samples were carried out. The digested samples were kept in refrigerator until the levels of each metal in the sample solution were determined by AAS. The blank solutions were prepared followed by the same digestion procedure as the sample.

2.3 Determination of the Concentration of the Metals in *Moringa stenopetala* Sample Solutions

Standard solutions containing 100 mg**/**L were prepared from standard stock solutions that contained 1000 mg**/**L. These secondry standards were diluted with deionized water to obtain four working standards for each metal of interest. Cu, Fe, Cr and Pb were measured with Atomic Absorption Spectrometery (AAS).Three replicate determinations were carried out on each sample. The four elements were determined by in the Abs mode.

3. RESULTS AND DISCUSSION

The quality of results obtained for Fe, Cr, Pb and Cu metals analysis using AAS are affected by the calibration and standard solution preparation procedure. The instrument was calibrated using five series of standards.

The calibration graphs of the four metal standard solutions were drawn using the standard solution data and the unknown concentrations of each metal was determined using the slope equation from the calibration graph. Graphs of the four heavy metal standards are shown in the following Figs. 1, 2, 3 and 4.

The unknown concentration of the metals were determined from the graphs and the metal Fe has the highest concentration and Cr metal has the lowest concentration in the sample. The concentration of the metals are Fe 1.18 mg/kg, Cr 0.44 mg/kg, Pb 0.63 mg/kg and Cu 0.67 mg/kg calculated from the graph using the equation of the line.

Table 1. The instrumental working conditions for analysing metals using AAS

S.N.	Metals	Wavelength (nm)	Slit width	Lamp current	Energy
1.	Fe	248.3	0.2	5.0	3.331
2.	Cr	357.9	0.7	2.0	3.623
3.	Cu	324.9	0.7	1.5	3.775
4.	Pb	283.2	0.2	4.5	3.338

Table 2. Concentration of standards, samples, wavelength and absorbance of each metal

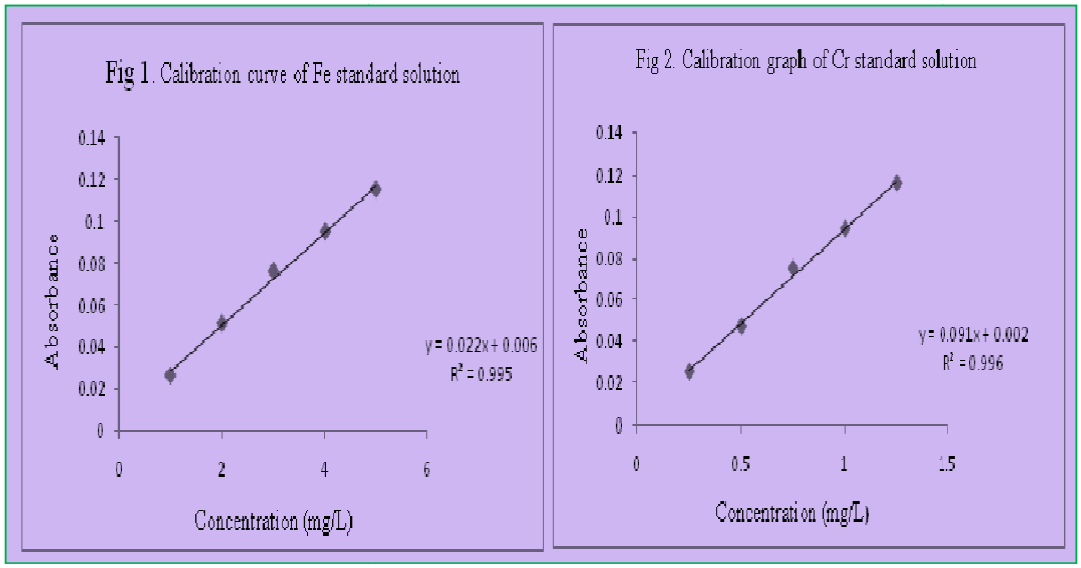
S.N.	Metals	Conc of standards (mg/L)	Absorbance
1	Fe	1, 2, 3, 4, 5, sample	0.026, 0.051, 0.076, 0.095, 0.115, 0.032, respectively.
2	Cr	0.25, 0.5, 0.75,1, 1.25, sample	0.025, 0.047, 0.075, 0.094, 0.116, 0.042, respectively.
3	Cu	0.5, 1, 1.5, 2, 2.5, sample	0.015, 0.023, 0.032, 0.041, 0.051, 0.017, respectively.
4	Pb	0.25, 0.5, 0.75, 1, 1.25, sample	0.061, 0.092, 0.124, 0.162, 0.110, respectively.

Table 3. Correlation coefficients of the calibration curves for determinations of metals by AAS

S.N.	Metals	Conc. of intermediate standards (mg/L)	Correlation coefficient of calibration curve
1.	Fe	100	0.995
2.	Cr	100	0.996
3.	Cu	100	0.998
4.	Pb	100	0.998

3.1 Optimization of Digestion Methods of *Moringa stenopetala* Samples

One of the basic necessities for sample preparation for analysis is to get an optimum condition for digestion. The optimum condition is the one which leads to: Minimum reagent volume consumption, minimum digestion time, minimum residue (clear solution) and ease of simplicity. Optimizing of the digestion method involved some changes of parameters such as reagent volume, digestion temperature and digestion time. Accordingly, three procedures were tested for digestion of *Moringa* (Table 4). Based on the above listed criteria, the optimal digestion procedure chosen was the one that fulfilled the selected criteria for complete digestion of 0.5 g of the dry sample powders, with 2.5 mL of HNO_3 (72%), 1 mL $HClO_4$ (70 %) and 1 mL H_2O_2 (30%) for a total of 3 h. The mixture was digested smoothly by setting the temperature first to 60℃ for 30 min and then increased to 210℃ for the next 2 h and 30 min. Then the digested solution was allowed to cool for 5 min without dismantling the condenser from the flask and for 10 min after removing the condenser. The procedures that required higher reagent volume longer, digestion time and colored digested solution were rejected [12].



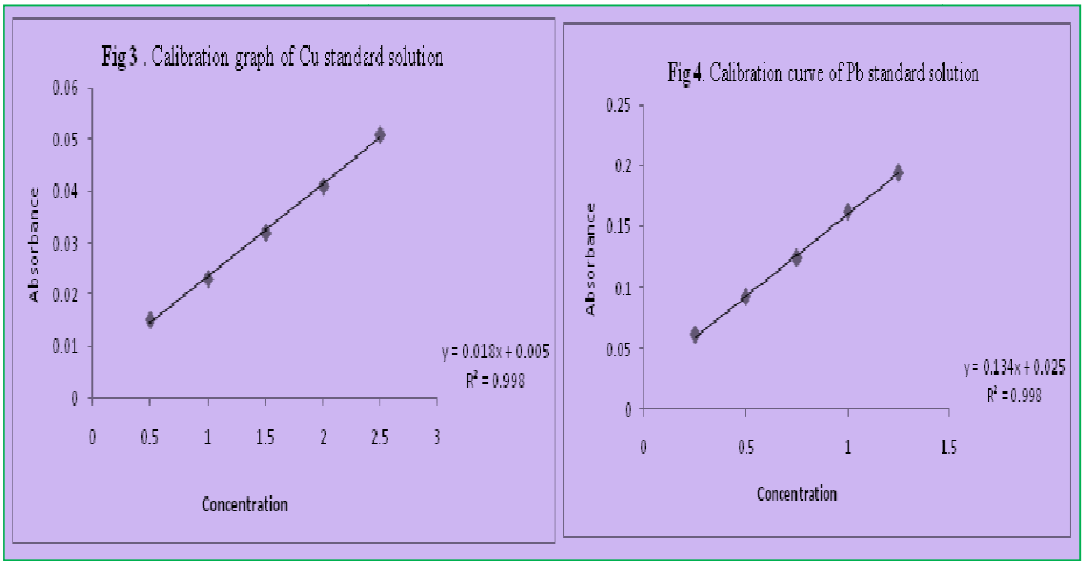


Table 4. Procedures tested during optimization of method for digestion of samples

S.N.	Sample trial	Reagents	*T_i (℃)	*T_f (℃)	Digestion time (h)	Colour of digested sample after filtration
1	0.5 g	3 ml HNO_3 (72%) 1 ml $HClO_4$ (70) 1 ml H_2O_2 (30%)	60	210	3	light brown
2	0.5 g	3 ml HNO_3 (72%) 1 ml $HClO_4$ (70) 1 ml H_2O_2 (30%)	60	210	3	light brown
3	0.5 g	3 ml HNO_3 (72%) 1 ml $HClO_4$ (70) 1 ml H_2O_2 (30%)	60	210	3	light brown

** T_i and T_f = Initia and final temperatures, repectively*

The concentrations of the four trace elements (Fe, Cr, Pb and Cu) in the digested and diluted solutions of *Moringa* were identified with AAS. The levels of total metal contents in *Moringa* samples show that *Moringa* is a source of nutrients. The concentration of the metals determined in the analysis were Fe 1.18 mg/kg, Cr 0.44 mg/kg, Pb 0.63 mg/kg and Cu 0.67 mg/kg. So that the result shows out of the four analyzed trace metals, Fe was found in the large amount compared to other with the concentration of 1.18 mg/kg and Cr was the smallest one (0.44 mg/kg). The amounts of the analyzed metals in the *Moringa* samples were arranged in an increasing order of their concentration Cr<Pb<Cu<Fe and the concentration of these metals is less than the permissible limit of metals for plants recommended by WHO.

4. CONCLUSION

The analysis of heavy metals from the leaves of *Moringa stenopetala* using Atomic Absortion Spectroscopy can be determined in wet digestion method. The optimized wet digestion routine for analysis was found effective for all of the trace heavy metals. This investigation helps to know different minerals that are found in the *Moringa* plant leaves and to identify which are indispensable or nonindispensable elements to consider the consumption of the *Moringa*. Fe has higher concentration compared to the other four metals. Based on the current standing, eating *Moringa* contributes appreciable amount of trace metals for the individuals. However; according to this study, the concentration of these four metals become less than the permissible limit of metals in plants as suggested by WHO. The permissible limit of Cu, Cr, Pb for plants is 10 mg/kg, 1.3 mg/kg and Pb 2 mg/kg, respectively, as suggested by WHO [11]. Therefore, according to this study, consuming of *Moringa stenopetala* leaves is recommended because of the less concentration of these trace heavy metals that cannot lead health problem to the consumer.

It is suggested that a better understanding of the health related effect and chemical profile of *Moringa* be developed through a multidisciplinary approach with the full involvement of *Moringa* growers. Because most of Ethiopian farmers use fertilizer and pesticides to get good and attractive crop without considering their side effects on consumers since *Moringa* is consumed directly after harvesting. The findings derived from such an approach should then be counter balanced with a realistic understanding of the negative health effect of *Moringa* use. In general, in south western part of Ethiopia, Arba Minch, Gamo Gofa Zone the number of consumers of *Moringa* is increasing through time to time due to its economic importance and the available hearsay simply on off-putting and positive aspect of *Moringa* on economic, social, cultural and agricultural values of the country.

In relation to the ever increasing human consumption of *Moringa* and the benefit from this yield an overall investigation of its chemical profile and disadvantage remains an instantaneous issue because of the presence of small amount of cynogeniglucosides in *Moringa stenopetala* leaves may have health peril in areas of high incidence of common goitre as an exacerbating factor if consumed for a long period of time.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
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