DETERMINATION OF LEVELS OF SELECTED ESSENTIAL ELEMENTS IN THE MEDICINAL PLANTS USED BY CHUKA COMMUNITY, MERU-KENYA USING AAS

S. O. Adongo*

J. Murungi*

R. Wanjau*

Abstract:

In this paper, we determine the levels of Zn, Mg and Fe in eight medicinal plants using flame atomic absorption spectroscopy (FAAS) is established. The results show that all the mean concentrations of Fe, Mg and Zn considered as essential elements are significantly different from one sample to another ($p \leq 0.05$ at 95% confidence interval). The levels of iron are significantly higher in the leaves and stem of *Vernonia lasiopus* ranging between 4.77-4.81 ppm as compared to others. The levels of zinc ranged from 0.35-0.37 ppm in the leaves of *Eucalyptus saligna* to 4.18-4.29 ppm in the leaves of *Aloe secundiflora* which had the highest concentration. The levels of magnesium were significantly higher in *Aloe secundiflora* (163.00±1.00 ppm) as compared to others.

Key words: Essential elements, medicinal plants, Chuka community.

* Department of Chemistry, School of Pure and Applied Chemistry, Kenyatta University, P.O. BOX 43844 – 00100, Nairobi, Kenya.

1. Introduction:

Traditional medicine has been in use for different pharmacological activities since time immemorial (Okemo, 1996) and has remained a pillar component in health care systems of resource poor communities in developing countries who are faced with worsening economic situation of the Sub-Sahara African countries and diseases such as HIV/AIDS, malaria, cancer, diabetes and tuberculosis (TB) (Balick and Cox, 1996; Mworia, 2000). The World Health Organization estimated that 80% of the current population in the world uses medicinal plants for some aspect of primary healthcare (Choudhry *et al.*, 2004).

In Kenya, 90% of the population has used medicinal plants at least once for various health conditions (Chirchir *et al.*, 2006). The Chuka community Kenya uses some of the traditional herbs for curative purposes. It is thought that some medicinal plants contain elements of vital importance for human metabolism, disease prevention and healing (Obianjunwa *et al.*, 2004).

It is thought that some medicinal plants contain elements of vital importance for human metabolism, disease prevention and healing (Obianjunwa *et al.*, 2004; Rajurkar and Damame, 1997). The elemental contents of the medicinal plants are very important and need to be screened for their quality control (Arceusz *et al.*, 2010). High levels may be toxic or interfere with absorption of other essential elements. Low levels may lead to deficiency of the elements in the body. Iron deficiency is the most common nutrient deficiency; affecting more than 1 billion people worldwide (Grodner *et al.*, 2000). More than 40 elements have been considered essential to life systems for the survival of both mammals and plants. Elements such as Zn, Fe and Mg are important for the normal functioning of the body. For example, zinc is a trace element required as a co-factor by more than 200 enzymes (Grodner *et al.*, 2000). Zinc is essential for proteins and synthesis of cells' genetic material DNA and RNA (Sizer and Whitney, 2003). It helps the pancrease with its digestive functions, helps metabolize carbohydrate, protein and fat, liberate vitamin A from storage in the liver and dispose of damaging free radicals (Sizer and Whitney, 2003). Magnesium is involved in more than 300 essential metabolic reactions (Spencer *et al.*, 1994). It is needed for the use of energy from energy-yielding nutrients and directly affects metabolism of potassium, calcium and vitamin D (Sizer and Whitney, 2003). On the other hand, iron is an essential component of hundreds of proteins and enzymes (Wood *et al.*, 2006; Beard and Dawson, 1997). Heme is an iron-containing compound found in a number of biologically important molecules. Hemoglobin and myoglobin are heme-containing proteins that are involved in the transport and storage of oxygen (Yip and Dallman, 1996; Brody, 1999).

Levels of these essential elements (Zn, Fe and Mg), higher than the permissible levels, over-dose or prolonged ingestion of medicinal plants with higher levels of these essential elements may lead to the chronic accumulation of different elements which causes various health problems. The study therefore sought to determine the levels of Zn, Fe and Mg in 8 common medicinal plants in this region. It is hoped that the research findings will be used to formulate policies and initiate action to improve the use of traditional medicine for better health and even their conservation. The results obtained will be available to the relevant authorities and to the public.

Although the efficacy of medicinal plants for curative purposes is often accounted for in terms of their organic constituents like essential oils, vitamins, glycolsides, etc. now, it has been established that metal content has also a significant role to play in biological activity either with organic molecules such as metallo-enzymes or independently like redox or catalytic reaction. Higher dose or prolonged ingestion of medicinal plants can lead to the chronic accumulation of different elements which causes various health problems (Sharma *et al.*, 2009).

2. Material and Methods:

The traditional medicinal plants were transversely collected randomly in triplicate from three selected sampling sites namely; Upper Chuka (forest), Middle Chuka (Chuka town) and lower Chuka (extending to Kaanwa). Specimen of plants mentioned for medicinal uses were collected and identified The leaves and stem barks of the medicinal plants were obtained by cutting them using a panga while the roots were dug using a jembe. They were mixed thoroughly and carried in polythene bags to the laboratory. They were then washed carefully and thoroughly using tap water to remove foreign matter and rinsed in de-ionized water (Sidney, 1984). They were spread to dry in the air at room temperature. The leaves of the plants were ground into a fine powder using a blender model number CT/ 404, put in transparent polythene bags and kept in lockable cupboards under lock and key. The stem barks and roots were ground in a Wiley mill. All glassware and plasticware were washed in detergent then soaked with 10% analytical grade nitric acid for 24 hours. They were then rinsed in tap water followed by distilled de-ionized water. The glassware were dried in the oven at 105 °C while the plasticware were dried on an open rack. They were all stored in clean dry drawers. Stock solutions were prepared from analar grade granulated metals of zinc, iron and magnesium of high purity (99.9%). Each of them was first dried at 105 °C, cooled in desiccators prior to weighing. Analytical grade nitric acid and hydrogen peroxide solutions were obtained from Thomas Baker Chemicals Ltd. Mumbai India whereas zinc, iron and magnesium were purchased from Fluka Chemie GmbH Aldrich chemical company, inc. USA.

Each ground sample was weighed accurately using an electronic analytical balance and put in clean dry conical flasks. A known volume of concentrated analytical grade nitric acid was added to each flask. The mixture was then subjected to wet digestion using an electric digester with occasional addition of a few drops of analytical grade hydrogen peroxide. This was done in the fume chamber. After complete digestion, the sample solution was left to cool, and then filtered using Whatman filter papers (125mm Ø) into 100 ml volumetric flasks. The conical flasks were rinsed with deionised water and content poured into the filtering mixture. Deionised water was then added to top up to the mark. The filtrate were transferred into dry plastic val tubes, closed, labelled and kept in the lockable cupboard. The same procedure was repeated for all the other samples. A reagent blank was prepared in a similar manner. After cooling, the remaining content was filtered into a 100 ml volumetric flask. Deionised water was used to top up to the mark. The aqueous solution was transferred into a plastic val tube, labeled and then kept in the cupboard.

Flame absorption spectroscopy (FAAS) was used to determine the levels of Zn, Fe and Mg. Atomic absorption spectrophotometer (AAS) with model specification; Spectr AA. 10 was used for analysis. The analysis was done in replicates under the same conditions as standards and blanks. For better precision, standards were measured before and after the sample solutions. The blank solution was measured between standards and samples to ensure stability of the base line.

3. **Results:**

To evaluate the linearity of the established calibration curves, regression analysis was used. The absorbance readings and concentrations of ideal standards were used to calculate correlation coefficient (r) values for Zn, Fe and Mg. Table 1 shows the results obtained.

Table 1: Correlation coefficient (r) values of AAS calibration curves

Element	Correlation values (r)
Mg	0.99950
Zn	1.00000
Fe	0.99499

The linearity of the established calibration curves was good and the performance of the AAS instrument had the ability to provide accurate results. The linear ranges of the obtained calibration curves were determined by considering concentration ranges of the linear portions. Provided in Table 2 herein is the range of the concentration of the standards (ppm) along the linear portions of calibration curves for the selected essential elements in the medicinal plants obtained from AAS.

Table 2: Concentration of standards and linear ranges of AAS calibration curves

Element	Linear range (ppm)	Concentration range of standards (ppm)
Mg	0.00 – 5.00	0.40 – 5.00
Zn	0.00 – 5.00	1.00 – 5.00
Fe	0.02 – 10.00	2.00 – 10.00

The results in Table 2 indicate the established calibration curves are linear over a wide range of concentrations making it enough to bracket the elemental concentrations of the medicinal plants analysed in this study and also bring elements that are naturally abundant in medicinal plants into the working range through the dilution. Determination of accuracy of the AAS involved ‘’spiking” the samples with 5 ppm of standards of the respective elements under study and re-analyzing the “spiked” sample to determine the percentage recovery. Table 3 below shows the percentage recovery after “spiking”.

Table 3: Percentage recovery

Element	Sample conc. (ppm)	New conc. (ppm)	% recovery
Fe	4.65	9.64	99.8
Mg	0.74	5.73	99.8
Zn	0.67	5.66	99.8

From the results in the Table 3 above, it is evident that the % recovery is above 99.5% showing that the results obtained using AAS are accurate and that the samples were prepared effectively, handled well without contamination and the AAS instrument used for analysis was accurate.

Table 4 below shows that the Chuka community uses different medicinal plants to treat various ailments. The parts of the plants used for each plant is also indicated.

Table 4: The 8 common plants in traditional medicine of the Chuka community, parts used and the ailments they treat

LOCAL NAME	SCIENTIFIC NAME	PART(S) USED	AILMENTS
mukuura	*Bauhinia tomentosa*	stem bark	Cough and malaria
Kithunju	*Aloe secundiflora*	Leaves	malaria, pneumonia, wounds , chest pains, chicken diseases, arthritis, rheumatism
Muriguriguj	*Anthocleista glandiflora*	stem bark	chest pains, cold, malaria,amoeba, worms
Mukawa	*Carissa edulis*	Roots	malaria, pneumonia, chest pains, colds, cough,

			stomachache, paining joints, worms, asthma
Mwiria	*Prunus africana*	stembark	amoeba, typhoid,chestpains, colds, malaria, general body weakness
Mwatha	*Vernonia lasiopus*	leaves	amoeba, antirabies ,worms
Mubau	*Eucalyptus saligna*	leaves, stem bark	chicken pox, cold, fever, toothache, measles
Mutuntu	*Croton microstachyus*	juice, roots, stem bark	wounds, cough, cold, cleansing digestive and blood circulation system, bleeding (blood clotting)

Levels of Fe, Mg and Zn in 8 medicinal plants were determined using flame atomic absorption spectroscopy (FAAS). Tables 6, 7 and 8 below show a summary of mean levels of these essential elements obtained from 8 medicinal plants analysed in this study.

Table 5: Iron levels (ppm) in the common medicinal plants

Botanical name	Part (s) used	Mean±SD	Range
Croton macrostachyus	Leaves	2.57±0.03	2.54-2.60
Anthocleista grandiflora	Stembark	2.31±0.01	2.30-2.31
Aloe secundiflora	Leaves	4.65±0.06	4.59-4.70
Eucalyptus saligna	Leaves	1.82±0.03	1.79-1.84
Bauhinia tomentosa	Leaves	0.76±0.02	0.74-0.78
Bauhinia tomentosa	Stem bark	2.44±0.05	2.39-2.48
Prunus africana	Leaves	1.68±0.02	1.67-1.71

Prunus africana	Stem bark	3.04±0.01	3.04-3.05
Caesalpinia volkensii	Leaves	1.47±0.02	1.45-1.49
Vernonia lasiopus	Leaves & stems	4.78±0.02	4.77-4.81

Table 6: Zinc levels (ppm) in selected medicinal plants

Botanical name	Part (s) used	Mean±SD	Range
Croton macrostachyus	Leaves	0.91±0.01	0.90-0.91
Anthocleista grandiflora	Stembark	0.44±0.00	0.44-0.44
Aloe secundiflora	Leaves	4.24±0.06	4.18-4.29
Eucalyptus saligna	Leaves	0.36±0.01	0.35-0.37
Bauhinia tomentosa	Leaves	0.45±0.01	0.45-0.46
Bauhinia tomentosa	Stem bark	1.15±0.00	1.15-1.15
Prunus africana	Leaves	1.05±0.01	1.05-1.06
Prunus africana	Stem bark	0.86±0.00	0.86-0.86
Caesalpinia volkensii	Leaves	1.27±0.12	1.14-1.35
Vernonia lasiopus	Leaves & stems	3.37±0.04	3.32-3.40

Table 7: Magnesium levels (ppm) in selected medicinal plants

Botanical name	Part (s) used	Mean±SD	Range
Croton	Leaves	77.17±1.04	76.00-78.00

macrostachyus			
Anthocleista grandiflora	Stembark	8.83±0.29	8.50-9.00
Aloe secundiflora	Leaves	163.00±1.00	162.00-164.00
Eucalyptus saligna	Leaves	11.50±0.00	11.50-11.50
Bauhinia tomentosa	Leaves	14.50±1.32	13.00-15.50
Bauhinia tomentosa	Stem bark	20.33±0.29	20.00-20.50
Prunus africana	Leaves	37.17±0.29	37.00-37.50
Prunus africana	Stem bark	10.50±0.00	10.50-10.50
Caesalpinia volkensii	Leaves	12.17±0.29	12.00-12.50
Vernonia lasiopus	Leaves & stems	26.17±0.29	26.00-26.50

4. Discussions:

The study reveals that all the mean concentrations of seleted essential elements were significantly different from one sample to another ($p \leq 0.05$ at 95% confidence interval). The levels of iron in the present study were significantly higher in the leaves and stem of *Vernonia lasiopus* ranging between 4.77-4.81 ppm as compared to others while the leaves of *Bauhinia tomentosa* showed the lowest level of iron (0.76±0.02 ppm). Stem bark of *Bauhinia tomentosa* had higher levels of iron ranging between 2.39-2.48 ppm than leaves of the same plant. The stem bark of *Prunus africana* also had higher levels of Fe (3.04±0.01 ppm) than the leaves of the same plant. The permissible limit set by FAO/WHO in edible plants was 20 ppm (FAO/WHO, 1984). Comparing metal limit in the studied medicinal plants with those proposed by FAO/WHO (FAO/WHO, 1984) it is evident that all the medicinal plants did not accumulate Fe above this limit. However, for medicinal plants the limits for Fe have not been established (WHO, 2005). Fe forms an integral part of cytochromes, haemoglobin, myoglobin, metalloflavoproteins and certain enzymes such as catalase and peroxidases. Thus, Iron is absolutely essential for transport of oxygen to the tissue and for operation of oxidation systems within the tissue cells, without which life would cease within a few seconds. In view of the positive role of iron on immune system the use of this plant in the treatment of cold may be attributed to considerable amounts of iron present in them. Given the level of Fe in the analysed samples, it is evident that the herbal plants can serve as a source of Iron especially in anaemic patients. The dietary limit of Fe in the food is 10-60 mg/day (Kaplan *et al.,* 1993).

The levels of zinc in the medicinal plants range from 0.35-0.37 ppm in the leaves of *Eucalyptus sali*gna to 4.18-4.29 ppm in the leaves of *Aloe secundiflora* which had the highest concentration.

Zinc plays an important role in various cell processes including normal growth, brain development, behavioural response, bone formation and wound healing. Since zinc could reduce respiratory infections such as pneumonia by up to 45 % and malaria cases by 35 % (Fox, 1998), the use of *Vernonia lasiopus* in treating malaria could be attributed to the significant level of zinc in the herb. Moreover, the significant level of zinc in *Aloe secundiflora* may explain its wide use against lung diseases like expectorants, cough, asthma, bronchitis and pneumonia (Fortin *et al.*, 1997*). Eucalyptus saligna* has antituberculosis properties (Goldstein *et al.,* 1990; Ngugen *et al.*, 1994) and this this may be attributed to the presence of zinc in the medicinal plant. Patients with sickle cell disease have been observed with zinc deficiency syndromes and some measure of success has been recorded in the use of natural products such as zinc in the management of sickle cell anemia (Zemel *et al*, 2002; Prasad, 2002).

Leaves of *Aloe secundiflora* may be effective in the management of sickle cell disease. A study showed that alcohol and aqueous extracts of *Aloe vera* exhibit anti–sickling activities in patients with sickle cell disease (Ejele and Njoku, 2008). This might be attributed to the presence of zinc in the leaf extract of the plants. The permissible limit set by FAO/WHO (FAO/WHO, 1984) in edible plants was 27.4 ppm. After comparison, metal limit in the studied medicinal plants with those proposed by FAO/WHO (1984) it was found that all the medicinal plants were within this limit. However, for medicinal plants, the limits have not yet been established for Zn (WHO, 2005). The dietary limit of Zn was put at 100 ppm according to a study (Jones, 1987).

The levels of magnesium were significantly higher in *Aloe secundiflora* (163.00±1.00 ppm) as compared to others while stem bark of *Anthocleista grandiflora* showed the lowest level of magnesium (8.83±0.29 ppm). Stem bark of *Bauhinia tomentosa* had higher levels of magnesium (0.41±0.01 ppm) than leaves of the same plant (0.29±0.03 ppm) while the leaves of *Prunus africana* had higher levels of magnesium (0.74±0.01 ppm) than the stem bark of the same plant.

Magnesium levels in the fluid surrounding cells affect the migration of a number of different cell types which may be important in wound healing (Rude and Shils, 2006). The significant levels of magnesium in *Aloe secundiflora* and *Croton macrostachyus* might explain their use in treatment of wounds (Rude and Shils, 2006). High-dose oral Mg has been found to be useful in migraine sufferers, reducing frequency and/or number of days with migraine headache. This might explain the use of *Aloe secundiflora* in treating bone related ailments as well as headache since it has a high level of magnesium. Magnesium is thought to cause dilatation of cerebral blood vessels thus reducing cerebral ischemia. It is also thought that the magnesium blocks calcium receptors by inhibiting *N*-methyl-D-aspartate receptors in the brain (Shealy *et al.*, 1992) Magnesium also produces a peripheral (predominantly arteriolar) vasodilatation (Mark *et al.*, 2001) thus reducing the blood pressure.

5. Conclusion:

The medicinal plants from Chuka community have minerals that are within permissible levels and they are generally safe for use as far as concentration of inorganic elements is concerned. All the plant samples analysed by AAS show the existence of the selected essential elements in varied levels. *Aloe secundiflora* had significantly the highest levels of Zn and Mg compared to the other plant samples analysed in this study. Some of the medicinal plants analysed could be suitable as suppliments for certain metal deficiencies for example *Aloe secundiflora* could be a good source of magnesium.

REFERENCES:

- Arceusz, A., Radecka, I. and Wesolowski, M. Identification of diversity in elements content in medicinal plants belonging to different plant families. Food Chemistry. 2010; 120: 52-58.

- Balick, M.J. and Cox, P.A.R. Plants people and culture. The Science of ethnobotany, Scientific American Library, New York, USA. 1996; pp. 219.
- Beard, J.L. and Dawson, H.D. Iron. In: O'Dell, B.L.; Sunde, R.A. (eds). Handbook of nutritionally essential minerals, New York, Marcel Dekker Inc. 1997; pp. 275-334.
- Brody, T. Nutritional Biochemistry, 2nd Ed. San Diego, Academic Press. 1999.
- Choudhry, R.P., Acharyu, R., Nair, A.G.C., Reddy, A.V.R.; Garg, A.N. Availability of essential trace elements in medicinal herbs used for diabetes mellitus and their possible correlations. Journal of Radioanalytical and Nuclear Chemistry. 2004; 120 (2): 85-93.
- Chirchir, J., Mungai, G. and Kariuki, P. Indigenous knowledge and conservation of natural resources: Resource medicinal plants utilisation in Eastern Africa. Proceedings of national museums of Kenya first scientific conference, 15th-17th Nov 2006; pp. 106-111.
- Ejele, A.E. and Njoku, P.C. Anti sickling potential of *Aloe vera* extract. Journal of Science, Food and Agriculture. 2008; 88: 1482-1485.
- FAO/WHO. Contaminants. In Codex Alimentarius, vol. XVII, Edition 1. FAO/WHO, Codex Alimentarius Commision, Rome. 1984.
- Fortin, D., Modou, Lô, Maynard, G. Plantes, médicinales du sahel. Enda Edition, Dakar. 1997; pp. 280.
- Fox, B.A. Food Science-A Chemical approach. University of London Press Limited. 1998; pp. 57.
- Goldstein, M., Simonetti, G. and Watschinger, M. (). Complete guide to trees and their identification. Macdonald and Co., Ltd., London. 1990; pp. 219.
- Grodner, M., Anderson, S., Deyoung, S. Foundation and clinical applications of nutrition, 2nd Ed. Mosby Inc., St. Louis, Missouri. 2000; pp. 45-107.
- Jones, J.W. Determination of trace elements in food by inductively coupled plasma atomic emission spectrometry; Elements in Health and Disease. 1987.
- Kaplan, L.A., Pesce, A.J., Kazmierczak, S.C. Theory, analysis, correlation, In: Clinical Chemistry, 4th Ed. Mosby. 1993; pp. 707.

- Mark, L.P., Prost, R.W., Ulmer, J.L., Smith, M.M., Daniels, D.L. and Strottmann, J.M. Pictorial review of glutamate excitotoxicity: fundamental concepts for neuroimaging. American Journal of Neuroradiology. 2001; 22: 1813–1824.
- Mworia, A.G. Status, value and management of indigenous plants of Upper Imenti Forest Reserve. Meru District. Kenya. Unpublished Msc Thesis. Kenyatta University. 2000; pp. 1-20.
- Ngugen, T., Lai, T. and Neguyen, T. Study of the cough suppression activity of E. camaldulensis and E. exserta. Tap Chi Duoc Hoc. 1994; 1: 18-19.
- Obianjunwa, E.I., Adeleke, C., Adebajo, O. and Omubuwajo, O. Essential and trace element contents of some Nigerian medicinal plants. Journal of Radioanalytical and Nuclear Chemistry. 2004; 252 (3): 473-476.
- Okemo, P.O. Antimicrobial efficacy of selected medicinal plants used by Kenyan herbal doctors. Unpublished Ph.D thesis. Kenyatta University, Nairobi. 1996; pp. 1-22.
- Prasad, A.S. Zinc deficiency in patients with sickle cell disease. American Journal of Clinical Nutrition. 2002; 75: 181 – 182.
- Rajurkar, N.S. and Damame, M.M. Elemental analysis of some herbal plants used in the treatment of cardiovascular diseases by NAA and AAS. Journal of Radioanalytical and Nuclear Chemistry. 1997; 219 (1): 77-80.
- Rude, R.K. and Shils, M.E. Magnesium. In: Shils, M. E.; Shike, M.; Ross, A. C.; Caballero, B.; Cousins, R. J. eds. Modern Nutrition in Health and Disease 10th Ed. Baltimore, Lippincott Williams & Wilkins. 2006; pp. 223-247.
- Sharma, K.R., Agrawal, M. and Marshall, M.F. Heavy metals in vegetables collected from production and market sites of a tropical urban area of India. Food and Chemical Toxicology. 2009; 47: 583-591.
- Shealy, N.C., Cady, R.K., Veehoff, D., Houston, R., Burnette, M. and Cox, R.H. The neurochemistry of depression. American Journal of Physchiatry in Medicine. 1992; 2: 13–16.
- Sidney, W. Official methods of analysis, 14th Ed. A.O.A.C. Inc., Washington DC. 1984; pp. 38.

- Sizer, F. and Whitney, E. Nutrition concepts and controversies, 9th Ed, Thompson Wadsworth. Canada. 2003; pp. 284.
- Spencer, H., Norris, C. and Williams, D. Inhibitory effects of zinc on magnesium balance and magnesium absorption in man. Journal of the American College of Nutrition. 1994; 13 (5): 479-484.
- Wood, R.J. and Ronnenberg, A.G. (2006). Iron. In: Shils, M.E., Shike, M., Ross, A.C., Caballero, B. and Cousins, R.J. (eds). Modern nutrition in health and disease, 10th Ed. Philadelphia, Lippincott Williams & Wilkins; pp. 248-270.
- WHO. Prevalence and severity of anaemia and cancer in developing countries. Geneva. 2005; 5: 217-223.
- WHO. Quality Control Methods for Medicinal Plant Materials, Revised, Geneva. 2005
- Yip, R. and Dallman, P.R. Iron. In: Ziegler, E. E. and Filer, L. J. (eds). Present knowledge in nutrition, 7th Ed. Washington, DC, ILSI Press. 1996; pp. 277-292.
- Zemel, B. S., Kawchak, D. A., Fung, E.B. Effect of zinc supplementation on growth and body composition in children with sickle cell disease. American Journal of Clinical Nutrition. 2002; 75: 300 – 303.