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## TRACE ELEMENTS CONTENT OF SELECTED KENYAN ANTIDIABETIC MEDICINAL PLANTS

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## ABSTRACT

Diabetes mellitus has experimentally been shown to be managed by medicinal plant extracts. Among the factors attributable to the hypoglycemic potential of the medicinal plants, are the trace elements present in them. This study was designed to determine the content of trace elements in five Kenyan antidiabetic medicinal plants traditionally used to manage diabetes mellitus using Energy Dispersive X-ray Fluorescence (EDXRF) and Atomic Absorption Spectroscopy (AAS) techniques. The elements Fe, Zn, Pb, Mg, Cr, Cu, Ni, Mn, Mo and Sr were identified and their contents estimated. The results of the present study provide justification for the usage of these medicinal plants in the treatment of diabetes mellitus since they are found to contain the elements Mg, Cr, Zn, Cu, Ni, Mn and, which play vital roles in blood glucose reduction, thereby aiding in management of diabetes mellitus. Our results show that the analyzed medicinal plants can be considered as potential sources for providing a reasonable amount of the required elements other than diet to the patients of diabetes mellitus. Moreover, these results can be used to set new standards for prescribing the dosage of the herbal drugs prepared from these plant materials.

**Keywords:** EDXRF, AAS, Diabetes mellitus, Trace elements, Medicinal plants.

## INTRODUCTION

Diabetes mellitus has been shown to be associated with abnormalities in the metabolism of some trace elements such as zinc, chromium, copper, magnesium and manganese<sup>1,2</sup>. Macro and micronutrients have been investigated as potential preventive and therapeutic agents for diabetes mellitus and common diabetic complications of diabetes<sup>3,4</sup>.

Literature regarding the active components of such types of plant extracts, which may be responsible for lowering blood glucose levels, is still unavailable. Believable, some trace elements, such as zinc, copper, manganese, and selenium, play a major role in protecting the insulin secreting pancreatic  $\beta$ -cells, which are sensitive to free radical damage<sup>3</sup>.

It is against this background that this study was designed in order to determine the presence, identity and levels of antidiabetic trace elements in five medicinal plants traditionally used in management of diabetes mellitus in Kenya. The five plants have previously been bioscreened for their antidiabetic potential and found to lower blood glucose levels appreciably in alloxan-induced diabetic mice<sup>5,6</sup>. It is justifiably postulated that their hypoglycemic potential is due to, among others, antidiabetic trace elements contained in them.

## MATERIALS AND METHODS

## Collection of medicinal plants

The plants used in this study were collected from their native habitats on the basis of ethnobotanical information. They were collected with bioconservation aspects in mind from Siakago and Gachoka divisions of Mbeere North and South districts of Kenya. A traditional medical practitioner named *Njuki wa Maringa* provided the information on the identity of the plant to collect, what part to collect, the precise locality where it grows, when curative potency is maximal and the mode of preparation. The plants collected and studied were *Bidens pilosa*, *Erythrina abyssinica*, *Aspilia pluriseta*, *Strychnos henningsii* and *Catha edulis*. An acknowledged authority in taxonomy authenticated the botanical identity of the plants and a voucher specimen was deposited at the National Museums of Kenya Herbarium, Nairobi. The plants collected and studied were *Bidens pilosa*, *Erythrina abyssinica*, *Aspilia pluriseta*, *Strychnos henningsii* and *Catha edulis*. The voucher specimen numbers for the collected and studied plants are *Hypogly 001/2004 (Bidens pilosa)*, *Hypogly*

*021/2004 (Erythrina abyssinica)*, *Hypogly 003/2004 (Aspilia pluriseta)*, *Hypogly 004/2004 (Strychnos henningsii)* and *Hypogly 005/2004 (Catha edulis)*.

## Initial preparation of plant materials

The parts of the plants collected were root barks, stem barks and leaves. The stems and roots were harvested and their barks peeled off while still fresh and cut into small pieces and then dried at room temperature for different periods of time depending on the succulence of the plant materials. Leaves were collected while green and dried in the same way. The root barks, stem barks and the leaves were separately ground when completely dry using an electric mill. The powdered plant materials were kept at room temperature away from direct sunlight in closed dry plastic bags.

## Trace element analysis

The different trace elements present in the extracts were investigated by use of Energy Dispersive X-ray Fluorescence (EDXRF) and Atomic Absorption Spectrophotometry (AAS).

## Energy Dispersive X-Ray Fluorescence (EDXRF)

0.3g of the freeze-dried material was weighed and made into pellets of 2.5 cm in diameter and weighing 100-200 mg/cm<sup>2</sup>. The pellets were made using a pellet press machine. The pellets were weighed and their weights recorded. The EDXRF system consists of an X-Ray spectrometer and a radioisotope excitation source. The radiation from the radioactive source, Cd109 ( $T_{1/2} = 453$  days and activity = 10 mCi) are incident on the sample, which emits the characteristic X-Rays. These X-Rays are detected by Si (Li) detector (EG&G Ortec, 30mm2x10mm sensitive volume, 25 $\mu$ m Be window) with energy resolution of 200eV at 5.9keV Mn K $\alpha$  - line. The spectral data analysis was collected using personal computer based Cariberra S-100 multichannel analyser (MCA). The acquisition time applied in the EDXRF measurements was 1000seconds. The X-Ray spectrum analysis and quantification was done using IAEA QXAS software<sup>7</sup>, which is based on the Fundamental Parameters Method (FPM)<sup>8,9</sup>. A print out of the results in the personal computer was taken.

## Atomic Absorption Spectrophotometry (AAS)

This technique was used for analysis of Magnesium, Vanadium and Chromium.

### Preparation of standard solutions

Standard stock solutions of 1000ppm for AAS were used as supplied by the manufacturers (Aldrich Chemical Co., Inc.).

### Preparation of working standards

Suitable aliquots of standard stock solutions of each element were taken in a series of 100ml volumetric flasks. The solutions were diluted to volume using distilled-deionised water, mixed thoroughly and transferred into plastic beakers. This was done for each element when its analysis was due. For each element, working standard solutions were prepared within a given range (1ppm, 5ppm, 10ppm, 15ppm, 20ppm, and 25ppm). The relationship between concentration and absorbance was linear. In case of Magnesium, 2ml of 5% Lanthanum solution was added to each series of the working standard solution before diluting them to volume. Standard blank reagents for each element were prepared by adding all the reagents, except the target element being determined.

### 20% Hydrochloric acid (w/w)

The solution was prepared by transferring 548 ml of 36% hydrochloric acid into 300 ml of distilled deionised water contained in 1 litre volumetric flask. The solution was then made to 1L volume with distilled deionised water, mixed thoroughly and kept in plastic bottles ready for use during analysis.

### Lanthanum solution

Lanthanum solution (50 mg/ml) was prepared by dissolving 12.6g of Lanthanum chloride in 100 ml distilled-deionised water. The solution was then made to volume using distilled deionised water in 250ml volumetric flask. After mixing thoroughly, the solution was kept in clean plastic bottle and used during the determination of Magnesium in the plant materials.

### Digestion of plant materials

Each plant material that was collected was brought to solution by wet oxidation/digestion. The procedure was repeated two times. Wet oxidation for determination of Magnesium (Mg), Vanadium (V), and Chromium (Cr) was done as follows:

The dried samples of known weights were transferred into 100ml Pyrex beakers and to each beaker, 10 ml of concentrated Nitric acid (HNO<sub>3</sub>) was added, and then allowed to soak thoroughly. 3 ml of 60% Perchloric acid (HClO<sub>4</sub>) was added to each beaker, warmed on hot plate slowly at first, until frothing ceased. Heating was then intensified until all HNO<sub>3</sub> evaporated. When charring occurred, the mixture was cooled, 10 ml of concentration HNO<sub>3</sub> was added and heating continued until white fumes of HClO<sub>4</sub> were observed. The final solution was cooled and 25 ml of 20% Hydrochloric acid (HCl) was added. The solution was then transferred into 100 ml

volumetric flask by filtering through Whatman filter paper No.1. The solutions were then made to volume and shaken well to allow proper mixing before the contents were transferred to plastic

sample bottles. The samples were kept in a freezer awaiting analysis.

### Determination of the total elemental content

Sample solutions for analysis of Magnesium were prepared by withdrawing 1 ml of the digested

sample solution into 100 ml volumetric flasks. 5% Lanthanum solution was added in each flask and the mixture diluted to volume using distilled-deionised water. However, for analysis of Vanadium and Chromium, the digested sample solutions were analysed without dilution.

After setting the AAS instrument to the right conditions for each element, the respective standard and sample solutions were aspirated into the flame in turns to determine their respective absorbance. At least four standard solutions were aspirated between 6-10 samples to monitor the stability of the working conditions. Distilled-deionised water was always flushed into the flame to re-establish the zero absorbance. For each sample and element, the above procedure was repeated two times. The mean absorbance for each sample solution and standard solutions were calculated and recorded. To prepare a calibration curve for each element, a graph of mean absorbance against corresponding concentrations of the standard solutions was plotted. In all cases, the graphs were linear and the best fitting straight line was obtained by using Microsoft Excel computer software, which also helped to convert absorbance readings to concentrations of elements in each sample analysed with better accuracy than manual graphical method. The programmes gave concentrations of the diluted and undiluted samples directly. Concentration values obtained for the diluted samples were corrected by multiplying with the respective dilution factors. The final values expressed as mg/100g dry matter, were recorded. These values were obtained by using the expression:

$$\text{Elemental content} = a/w \times 100$$

where, a is the amount of element (mg) in 100 ml sample analysed

w is the dry weight (g) of the material analysed

### RESULTS

Upon analysis of trace elemental composition of the five antidiabetic medicinal plants, it was found that iron, zinc, magnesium, chromium and lead were present in detectable quantities in all the five plants at varying levels; copper was present in detectable quantities in two plants *Bidens pilosa* and *Aspilia plurisetata*; manganese in *Catha edulis*, nickel in *Aspilia plurisetata* and molybdenum in *Bidens pilosa* (Table 1 and 2).

Table 1: Trace elements present in the antidiabetic plants as determined by EDXRF

Elements	Plants and elemental quantity (µg/g)				
	<i>B. pilosa</i>	<i>A. plurisetata</i>	<i>C. edulis</i>	<i>E. abyssinica</i>	<i>S. henningsii</i>
Mn	<5	<5	8.9 ± 0.5	<5	<5
Fe	281.0 ± 2.6	75.2 ± 6.2	46.0 ± 5.2	66.6 ± 12.3	32.9 ± 7.6
Ni	<5	8.2 ± 0.5	<5	<5	<5
Cu	14.9 ± 2.2	6.7 ± 1.0	<5	<5	<5
Zn	53.0 ± 4.8	35.1 ± 4.5	44.7 ± 3.7	50.3 ± 7.4	7.0 ± 1.8
Sr	<1	<1	<1	<1	<1
Mo	7.6 ± 3.5	<1	<1	<1	<1
Pb	40.8 ± 3.5	6.8 ± 0.8	9.4 ± 0.2	5.7 ± 0.6	5.7 ± 0.2

Table 2: Trace elements present in the antidiabetic plants as determined by AAS

Plants	Elemental quantity (mg/g)		
	Magnesium (Mg)	Chromium (Cr)	Vanadium (V)
<i>B. pilosa</i>	879.7 ± 3.1	110.1 ± 0.0	<1
<i>A. plurisetata</i>	823.6 ± 2.3	118.9 ± 10.8	<1
<i>C. edulis</i>	791.7 ± 1.2	97.9 ± 1.5	<1
<i>E. abyssinica</i>	1021.4 ± 6.3	103.4 ± 1.7	<1
<i>S. henningsii</i>	865.5 ± 2.8	182.1 ± 46.2	<1

## DISCUSSION

The hypoglycemic activity of the five studied medicinal plants can justifiably be attributed to, among others, the trace elements in them. These metal ions play vital roles in hypoglycemic antidiabetic activity. Literature is rich with information regarding the mode of antidiabetic activity of these elements.

As shown in table 2, Magnesium was appreciably detected in all the five studied antidiabetic plants. Involvement of Magnesium in glucose homeostasis is multifactorial. It is a cofactor in the glucose transport system of hepatocyte plasma membranes and it regulates hepatocyte mitochondrial functions. It catalyzes the various enzymes involved in the phosphorylation of glucose in its anaerobic metabolism, as well as, in its oxidative decarboxylation in the citric acid cycle and can modulate the mechanisms of energy transfer from high energy phosphate bonds<sup>10</sup>. Magnesium also plays a role in the release of insulin and the maintenance of the pancreatic  $\beta$ -cells<sup>11</sup>. It can presumably increase the affinity and the number of insulin receptors.

It has been shown that the main action of insulin on target tissues involves an ionophore effect on Magnesium and calcium. The translocation of Magnesium seems particularly correlated to the peptide mediator and to transphosphorylation reactions. Insulin deficiency induces a drop in 1,25-dihydroxycholecalciferol, and modifies secretion of parathyroid, calcitonin and gastrointestinal tract peptide hormones; which in turn favour the occurrence of Magnesium depletion<sup>12</sup>.

It was established in this study that Zinc and Chromium were present in all the studied plants in significant quantities (Table 1 and 2). Abnormalities in the metabolism of chromium and zinc have been associated with diabetes. Impairment of chromium and zinc status has been reported as aggravating factors in the progression of diabetes. Chromium potentiates the action of insulin, acting as a cofactor<sup>13-16</sup>. Thus, it may improve blood glucose levels in individuals experiencing blood glucose fluctuations associated with diabetes. It, therefore, may not be surprising to find an inverse relationship between serum chromium levels and blood glucose control<sup>17</sup>.

Zinc and insulin concentrations in the pancreas change in the same direction in a variety of situations in humans<sup>3</sup>. Zinc plays vital roles in insulin biosynthesis, storage, and secretion<sup>18</sup>. It no wonder Zinc deficiency is associated with a number of metabolic disturbances including impaired glucose tolerance, insulin degradation, and reduced pancreatic insulin content<sup>19</sup>. It is also postulated to improve glycaemia, and a restored zinc status in type 2 diabetics may counteract the deleterious effects of oxidative stress, thereby helping to prevent complications associated with diabetes mellitus<sup>20</sup>.

Copper was detected in appreciable quantities in *B. pilosa* and *A. plurisetata* (table 1). Owing to its redox chemistry, copper is both a powerful enzyme catalyst and a dangerous reactant that generates hydroxyl radical. Although virtually all cells must acquire copper to drive important biochemical reactions, the potential toxicity of copper demands an exquisite level of vectorial transport and homeostatic control<sup>21</sup>. Abnormal copper metabolism can lead to several chronic pathogenesis, such as diabetes or diabetic complications<sup>22</sup>.

As table 1 shows, Manganese was found in detectable quantities in *C. edulis*. It acts as a cofactor for the antioxidant enzyme, Manganese Superoxide Dismutase (MnSOD). The levels of this enzyme are reported to be lower in the white blood cells of diabetics than in those of non diabetic controls<sup>2</sup>. By still obscure mechanisms, Manganese is essential for glucose metabolism and deficiency may result in glucose intolerance similar to diabetes mellitus in some animal species<sup>23</sup>. However, studies examining the Manganese status of diabetic humans have generated contradictory results, where diabetics showed lower level of Manganese than that of normal subjects but the difference did not reach to the level of statistically significant difference<sup>24</sup>.

Nickel was found in detectable levels in *A. plurisetata* (Table 1). Recent studies in rats and humans indicate that Nickel deprivation depresses growth, reproductive performance, and plasma glucose

and that it alters the distribution of other elements in the body, including calcium, iron, and zinc<sup>25,26</sup> (Yarat et al., 1992; Nielson, 2000).

## CONCLUSION

Interest in the biochemical and clinical consequence of trace element metabolism has been steadily increasing. Trace elements have important physiological effects when present at concentrations other than those associated with classical toxicity or with extreme deficiency. This study has established that the studied antidiabetic plants have appreciable quantities of some of the trace elements associated with glucose lowering effects. It is, therefore, hereby underscored that the antidiabetic potential of these plants can be attributed to, among others, some of the trace elements present in them.

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