DETERMINATION OF ESSENTIAL TRACE ELEMENTS IN SELECTED MEDICINAL PLANTS AND THEIR AQUEOUS EXTRACTS

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THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN THE SCHOOL OF PURE AND APPLIED SCIENCES, KENYATTA UNIVERSITY

APRIL 2011
DECLARATION

This thesis is my own work and has not been presented for a degree in any other university or any other award.

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DEDICATION

This thesis is dedicated to my loving parents Mama Naomi and Baba Ndubi Wo’koki for their struggle to give me education among other values of life. They are the reason I strive for excellence.
ACKNOWLEDGEMENTS

My most sincere gratitude goes to God for giving me health, strength and daily bread. I also wish to sincerely acknowledge and appreciate my supervisors, Prof. J. Murungi, Prof. H. Nyambaka and Prof. P. K. Mbugua for their scholarly advice, wise counsel and the invaluable time they put in to make this work a success. I cannot forget Dr. E. Dindi of International Atomic Energy Agency, Austria for his financial assistance and moral support. I am greatly indebted to the entire Chemistry Department of Kenyatta University and Department of Geology and Mines for their ready assistance at all times.

My sincere thanks also go to my wife Lucy for her support, love and encouragement. My entire family cannot be forgotten. Their prayers, moral support and encouragement steered me to this end. My colleagues were also of great help. They gave me insightful academic suggestions, provided moral support and made my years of study a worthwhile experience. Last but not least, my sincere thanks go to friends and well wishers since I cannot mention each one of them. Thank you all.
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ABBREVIATIONS AND ACRONYMS

AAS      Atomic absorption spectrophotometry
ADDI     Average daily dietary intake
DPASV    Differential pulse anodic stripping voltammetry
GTF      Glucose tolerance factor
MPMPs    Multi-purpose medicinal plants
NAA      Neutron activation analysis
PPM      Parts per million
SES      School equipment supplies
WHO      World health organization
ABSTRACT

It is becoming increasingly evident that trace elements such as chromium (Cr), manganese (Mn), zinc (Zn), vanadium (V) and iron (Fe) are therapeutically useful as they play vital role in immune boosting. It is therefore possible that effectiveness of certain medicinal plants may be due to either interaction of the metals with organic molecules or these trace elements alone are effective in improving or boosting immunity. There has been an increased use of medicinal plants due to high cost of drugs, drug resistance and increased awareness that medicinal plants contain valuable compounds. Great emphasis has been on organic compounds in these plants yet trace elements also play a vital role in the effectiveness of drugs. Hence there is need to determine the levels of these elements in medicinal plants and their aqueous extract. This work reports the determination of the levels of chromium, manganese, zinc, vanadium and iron in some selected medicinal plants and their aqueous extract used in Kenya. The medicinal plants were identified by a plant taxonomist. Sample of *Markhamia platycalyx* were collected randomly from Kakamega forest while *Erythrina abyssinica, Prunus africana, Croton macrostachyus* and *Euphorbia candelabrum* were collected from Nyeri. For each plant, samples were collected from three different regions separated by at least 10 km. A total of 105 samples were collected for each plant. Digestion was done using a mixture of acids and the level of these trace elements in digested sample and aqueous extract determined using atomic absorption spectrometry (AAS) and differential pulse anodic stripping voltammetry (DPASV). Dixon’s Q-test was used for the elimination of outlying data. Correlation analysis was done for levels of trace elements in plants and their aqueous extract. *Erythrina abyssinica* was found to be especially rich in trace elements with vanadium concentration being lowest (0.79 μg/g) while iron concentration being highest (452 μg/g). *Markhamia platycalyx* contained the lowest levels of trace elements ranging from 2.5 μg/g chromium to 87.4 μg/g iron. This could probably explain why *Markhamia platycalyx* with low levels of trace elements is used to treat the least number of diseases compared to other herbs analysed in this study. There was high level of iron (65 μg/g to 452 μg/g) in the five medicinal plants analysed while vanadium showed the lowest level (≤ 0.79 μg/g). Chromium was in the range of 1.9 μg/g to 5.6 μg/g, zinc 10.5 μg/g to 158.7 μg/g while manganese levels ranged from 13.2 μg/g to 219.2 μg/g. Vanadium was not detected in any of the aqueous extract. Chromium in the aqueous extract was in the range of 1.8 μg/g to 4.3 μg/g though not detected in *Prunus africana* and *Croton macrostachyus*. Manganese concentration in the aqueous extract ranged from 4.3 μg/g to 45.6 μg/g while that of iron 17.7μg/g to 224.5 μg/g. High levels of trace elements were detected in aqueous extract obtained from *Erythrina abyssinica* and *Croton macrostachyus*. The extract from these two plants are used to treat diseases which are as a result of deficiency or malnutrition. Extracts from *Erythrina abyssinica, Croton macrostachyus* and *Euphorbia candelabrum* can act as supplement for people with deficiency in these trace elements. The results obtained by atomic absorption spectrometry (AAS) and Differential pulse anodic stripping voltammetry (DPASV) showed no significance difference.
CHAPTER 1
INTRODUCTION

1.1 Background

Many human diseases are as old as mankind and therefore their understanding and treatment or management is important. Diseases have negative impact not only to the individual and family involved but also to the entire nation. The stigma caused to the family when one of its members has terminal illness is great (WHO, 2003). Diseases are known to have a negative impact on economic growth of a nation (WHO, 2003). The manpower hours wasted by sick workers seeking treatment at the expense of working affects the output of a nation.

There are various approaches to treatment of common diseases, one of which is by use of medicinal herbs (Kokwaro, 1993). Most of the common diseases have cure and for those which do not have, scientists are in constant search for their cure. However some diseases which have existed for a long time such as malaria and whose cure has been known for a long time are increasingly becoming resistant to the known drugs (WHO, 2003). Malaria is a worsening problem world-wide with more than 110 million people suffering from its infection every year and up to 2 million die (National Research Council, 1986). A growing number of countries are now affected by drug resistant strains of the parasites. Other diseases such as diabetes and cancer have no known appropriate cure; the drugs available commercially are mainly used for the management of these diseases. There are cases where herbal medicine is known to cure certain disease, but when pure active organic compound are isolated, their activity disappears, or is reduced. It is therefore
possible that their activity is enhanced by complexing with metal ions (Akbarov and Aripkhodzhaeva, 2000).

Drugs used for treating various diseases use organic molecules as the active ingredients (Abrams and Murrer, 1993). In some countries, especially third world countries, most people do not use conventional medicine because they cannot afford them (Black, 2003). Such people resort to the use of medicinal herbs because they are readily available within their locality. Furthermore, people consider medicinal herbs to have minimal side effects since they are natural (Food and Nutrition Board, 1980). A study on the bio-economic value of multi-purpose medicinal herbs for the rehabilitation of dry lands in Sub-Saharan Africa, suggests that the 2004 global market for herbal medicines, including herbal products and raw materials was estimated to be 65 billion US dollars (WHO, 2003). Recent market studies in South Africa’s Kwazulu Natal province and Burkina Faso showed that the estimated annual value of medicinal herb products is 13 million US dollar and 7.5 million US dollars respectively (WHO, 2003). The same report indicates that Kenya’s budget for medicine in 2002 was 16 million US dollars and the national healthcare system provides for conventional drug needs of only 30 per cent of the population. The 70 per cent of those who cannot afford them rely on traditional forms of medication. Some of these medicinal herbs are known to treat not only one disease but a number of diseases (WHO, 2003).
It is becoming increasingly evident that some of these medicinal herbs contain active compounds of trace elements such as chromium, manganese, zinc, iron and vanadium which may be involved in treatment (Abrams and Murrer, 1993). The importance of adequate trace elements for protection of animals and humans against infections has been known since last quarter of 20th century (Spears, 2000). Investigations have shown that changes in the intracellular environment induced by alterations in micronutrient status can directly influence viral virulence and in addition can cause immune system dysfunction (Beck et al., 2003).

Zinc is essential for growth and reproduction in animals. In adolescents, delayed sexual maturation and stunned growth has been reported to be a result of low zinc levels (Black, 2003). Zinc also aids wound healing and is believed to be required for mobilizing vitamin A from storage sites in the liver (Carl, 1975). Chromium on the other hand is essential for the formation of glucose tolerance factor (Offenbacher and Pi-Sunyer, 1988). Its deficiency also leads to inadequate metabolism of amino acids and an increased risk of arteriosclerosis (Lukaski, 1999). Vanadium is suggested to play a role in building healthy bones and teeth (Mohammad et al., 2001) and in treatment of cancer (Narla et al., 2000). Iron plays an important role in the chemistry of living organisms being found at the active centre of many biological molecules. Iron deficiency causes anemia while manganese is known to cure certain allergic syndromes (Abrams and Murrer, 1993). The importance of trace elements in maintenance of good health requires continued monitoring of their levels in food and plants used by man and animals. Medicinal herbs such as Markhamia platycalyx, Prunus africana, Erythrina abyssinica, Croton
Macrostachyus, and Euphorbia candelabrum have been used over years in treatment of diseases (Kokwaro, 1993). Aqueous extracts of these medicinal herbs obtained from either leaves or stem-bark is used in treatment.

1.2 Statement of the problem and justification

Studies have shown that severe malnutrition produces striking diseases, which indicate a close relationship between nutrition and defense system of the host (Gershwin et al., 1985). There exists interrelation between malnutrition and infectious disease, the two largest health problems (Beisel, 1977). Proper nutrition and nutrient therapy have helped many schizophrenics, some of whom had given up as hopeless after both drug and psychotherapy treatments failed (Carl, 1975). Primary deficiencies of certain essential elements have been recognised as a major contributor to a number of common chronic illnesses (Hendler and Rorvik, 2001). Research during the last quarter of the 20th century established the importance of adequate trace element nutrition for protection of animals and humans against infections (Spears, 2000). In this research the standard paradigm was to catalog the impact of induced and genetic trace element deficiencies on the numbers and effectors activities of the various classes of leukocytes in blood, marrow and lymphoid organs (Shankar and Prasad, 1998). The data overwhelmingly showed that the impairment in the immune system due to trace element deficiency can be sufficient to increase the risk of morbidity and mortality due to viral, microbial and parasitic infections (Fraker and King, 2001).
For many years patients have used conventional medicine to treat different kinds of diseases. However due to high cost of drugs and disease resistance to drugs, most people have resorted to use of medicinal herbs. Medicinal herbs are known to contain active organic molecules as well as immune boosting essential trace elements (Food and Nutrition Board, 2000). Much of the study on medicinal herbs has been directed towards the active organic molecules present; leaving out trace elements some of which are known to enhance the activity of organic compounds by complexing with them (Abrams and Murrer, 1993). Furthermore, traditional methods of making aqueous extract only extracts small percentages of organic molecules (Kokwaro, 1993). This could mean that the aqueous extract contains mainly immune boosting trace elements which may be responsible for their therapeutic property. People have developed a store of empirical information concerning the therapeutic values of local plants (Kokwaro, 1993). However information on chemical composition has been insufficient making it difficult to prescribe appropriate dosages. Some of the plants used for direct or specific treatment of particular diseases are worth further investigation in order to determine their chemical composition. Some side effects from the use of medicinal plants may be due to over-dosage from lack of knowledge on its chemical composition since a high concentration of trace elements are toxic (Kokwaro, 1993).

There are several methods by which the drug plants or their parts are prepared before they are given to a patient. One of the common methods is by boiling the plant parts (Kokwaro, 1993). In some cases the parts are soaked in cold or warm water and the
infusion used either internally or externally. The third method is by burning the plant part and the resultant ash applied to the affected body part.

The knowledge of the levels of trace elements in medicinal herbs and their aqueous extract will enhance effective use of these herbs in treatment of various diseases. Also the traditional methods of making the aqueous extract may be extracting only a small fraction of these elements leading to so much wastage. There has never been accurately defined dosage for patients using such medicinal herbs (Kokwaro, 1993). If the healing power of these medicinal herbs lies in the presence of trace elements in them, and if the concentration of such elements is well known, then it will be possible to prescribe appropriate dosage. The results obtained from this research will therefore be useful in effective use of medicinal herbs to cure various ailments. The parts of the plant used to treat various diseases as obtained from literature were also the parts studied for each plant in this study.

1.3 Hypothesis

(i) Trace elements are present in substantial amounts in medicinal herbs

(ii) The traditional method of preparing aqueous extract only extracts a small percentage of these elements.
1.4 Objectives of the study

1.4.1 General objective

The aim of this project was to determine the levels of zinc, manganese, iron, vanadium and chromium in selected medicinal plants and their aqueous extract used in Kenya.

1.4.2 Specific objectives

(i) To determine the levels of chromium, manganese, vanadium, iron and zinc in leaves of *Markhamia platycalyx*, leaves and stem bark of *Prunus africana*, stem bark of *Erythrina abyssinica*, leaves of *Croton macrostachyus* and stem bark of *Euphorbia Candelabrum* growing in selected parts of Kenya using atomic absorption spectrophotometry (AAS).

(ii) To determine the amount of these metals in aqueous extract of *Markhamia platycalyx*, *Prunus africana*, *Erythrina abyssinica*, *Croton macrostachyus* and *Euphorbia candelabrum* using AAS.

1.5 Significance of the research

The information obtained from this research can be used to encourage people to grow plants identified as having significant levels of these trace elements which will result in improved forest cover and reduced poverty. The information can also be useful in identifying the medicinal herbs that can supplement for these elements in human in case of deficiency. The results of this research can also create awareness on the need to improve on the methods of preparing the extracts.
1.6 Scope and limitations

There are many medicinal plants but due to time and financial limitations only a few were selected. Further more, there are many species of the same plant but this was not taken into consideration. Also the study did not determine bio-available elements in patients using the herbs or the levels in the soil where these herbs grow. Soil condition, climatic conditions and age of the plant is known to affect levels of metals in plants, and this was not taken into consideration. Some medicinal herbs may contain heavy metals which may cause adverse side effects. This study however did not determine the levels of heavy metals in these herbs. The study determined the levels of trace elements in aqueous extracts. It did not however consider the use of other solvents to prepare the extracts.
2.1 Trace elements and human health

For a number of years now the role of trace elements in various biochemical processes has been underlined. It has been shown that the normal growth of plants, animals and man depend on the continuous and sufficient supply of certain trace elements (Vivek and Garg, 1997). The biological effect of an element depends on its concentration in the organism. Trace metal overload, like deficiency, suppresses immune cell function and increases the risks of morbidity due to infectious diseases (Kumar et al., 2003). Primary deficiencies of certain essential elements such as chromium, zinc, iron, manganese and vanadium have been recognized as a major contributor to a number of common chronic illnesses (Hendler and Rorvik, 2001). The role played by each of these elements in human health is outlined in the following subsections.

2.1.1 Iron

From antiquity, man has recognized the special role of iron in health and disease (Paul and Garg, 2007). It plays an important role in the chemistry of living organisms being found at the active centre of many biological molecules. Most of the iron in animals exists in complex forms bound to protein, either as porphyrin or heme compounds. It is also a component of many enzymes. An adult human body contains around 4 g of iron, about three-quarters of which is in the form of haemoglobin (Carson, 1986).
Iron deficiency is one of the most common deficiency diseases in humans. Its deficiency leads to low haemoglobin levels and thus anaemia. Women, because of menstruation, and children, because of an enhanced requirement for growth, are particularly susceptible to anaemia (Carson, 1986). During reproductive years adult women lose an average of 20 mg iron monthly as a result of menstruation and approximately 800 mg for each pregnancy. These losses are in addition to an approximately 1 mg daily from extoliation of intestinal epithelial cells (Bratter and Schramel, 1980).

A large proportion of the world’s population is iron deficient and its sufficiency can be achieved relatively easily through dietary intake. Daily iron requirements are 10 mg for adult males, 15 mg for female, 30 mg for pregnant females and 6-12 mg for children (Clarkson and Rowson, 1999). Iron dietary sources include liver, fish, spinach and whole grains (Clarkson and Rowson, 1999). The iron content of most feed ingredients is highly variable, reflecting differences in soil and climatic conditions. Its level in herbage plants is basically determined by the species and type of soil on which the plants grow (Reddy et al., 1987).

A wide range of iron chelators inhibit ribonucleotide reductase and this is the reason for cytotoxic properties of such molecules as tropolone (Abrams and Murrer, 1993). Some iron chelators also function as free radical scavengers, for instance, hydroxyurea which inhibits the enzyme activity. The ability of iron chelators to inhibit ribonucleotide reductase has led to several proposals for therapeutic application (Ferguson, 1990). Anti-malarial activity of desferrioxamine has been demonstrated in a range of plasmodium
species in rats and man (Ferguson, 1990). Malaria is a worsening problem world wide and the introduction of an iron chelator to control such infection is a novel approach, and as chelation is particularly critical at the late trophozoite stage, it may prove possible to limit treatment to relatively short time periods (Carson, 1986).

Conditions of iron overload have been observed in humans and include those in idiopathic hemochromatosis (genetic disorder in which excessive iron is absorbed), transfusion hemosiderosis and in humans who consume diets containing as much as 200 mg iron per day. Kumar et al. (2003) reported iron concentration in most medicinal herbs above 500 μg/g except arjuna, red sandal and tulsi which all contain iron in a smaller range of 160 μg/g to 324 μg/g. Similar studies done by Basgel and Erdemoglu (2006) in medicinal herbs used in Turkey reported iron concentration in the range of 224 μg/g to 502.7 μg/g.

2.1.2 Manganese
Manganese is recognized as an essential nutrient for humans. However, in excessive amounts it is also a toxic substance. Manganese occurs in relatively constant amounts in tissues and organs of both plants and animals, and is especially concentrated in the reproductive organs (Grossman and Wendel, 1985). It was first recognized as an essential mineral element for growth and reproduction in mice and rats in 1931. Interest in manganese nutrition was greatly stimulated a few years later by the discovery that a deficiency of this element was largely responsible for the crippling disease of chickens known as perosis (Pennington and Young, 1991).
Soil manganese concentrations range from less than 1 ppm to as much as 7000 ppm with an average of 500 to 600 ppm (Pennington and Young, 1991). Its two most important valence states in biological systems are +2 and +3. Liver, bones, pancreas and kidney have relatively high levels while muscles have very little. The skeleton accounts for about 25 per cent of total body manganese (Berman, 1980). This reserve, however, is not readily used when dietary intake is low. The oral absorption of manganese into the body is naturally slow and is transported in blood serum by β-globulin (Berman, 1980). Excess intake is generally reflected in excess concentrations in hair, but deficient intakes are not consistently reflected in lower levels (Grimble, 1994). Its absorption apparently occurs equally well throughout the length of the small intestine (Berman, 1980). It competes directly with cobalt and iron for binding sites, thus excess iron or cobalt could induce its deficiency. Absorbed manganese may either remain free or rapidly become bound to α2-macro-globulin before traversing the liver to enter the systematic circulation where it becomes oxidized to the manganic state or become bound to transferrin (Grimble, 1994). Basgel and Erdemoglu (2006) observed an increase in liver manganese when dietary manganese was elevated.

Like other essential trace elements, manganese can function both as an enzyme activator and as a constituent of metalloenzymes (Pond and Maner, 1984). It activates many enzymatic reactions associated with the metabolism of organic acids, carbohydrates, nitrogen and phosphorus. It is part of several enzymes such as arginase, pyruvate and carboxylase which are required for protein and energy metabolism in mucopolysaccharide formation (Herber and Stoeppler, 1994). While the number of
manganese metalloenzymes is limited, the enzymes that can be activated by manganese are numerous. They include hydrolases, kinases, decarboxylases and transferases (Sappey, 1994). Manganese is essential for development of the organic matrix of the bone, which is composed largely of mucopolysaccharide and also cures certain allergic syndromes (Beck et al., 2001). Glucose utilization is impaired by manganese deficiency.

Necropsy has revealed gross abnormalities in the pancreas such as aplasia or marked hypoplasia of all cellular components having deficiency in manganese and it may in some way be involved in insulin formation or activity (Ajit et al., 1999). Abnormalities in cell function and ultrastructure, particularly involving the mitochondria, occur due to manganese deficiency (Ajit et al., 1999). The element also plays a role in immunological function (Ajit et al., 1999). Its interaction with neutrophils and microphages has been demonstrated, possibly through interaction with the plasma membrane of cells employed in the immune response (Abrams and Murrer, 1993). Its deficiency or toxicity can affect brain function (Bratter and Schramel, 1980). Humans with convulsive disorders, including epileptics, showed whole blood manganese concentrations significantly below normal (Bratter and Schramel, 1980).

The human requirement for manganese is not well defined, but the estimated safe and adequate intake is 2.0 to 5.0 mg/day for adults and 0.3 to 3.0 mg daily for infants and children (Bratter and Schramel, 1980). Manganese is highly concentrated in the outer layers of grains so that the inclusion of mill products increases its intake. Meat and fish are generally low in manganese, approximately 5-15 ppm while milk is 20-40 μg/l. Its
deficiency in human reduces uptake of vitamin K (Bratter and Schramel, 1980). Clinical signs of deficiency include inability to elevate depressed clotting proteins in response to vitamin K, hypocholesterolemia, slowed growth of hair and nails, weight loss and reddening of hair and beard. Toxicity in humans is characterized by a severe psychiatric disorder (Iocura manganica) resembling schizophrenia, followed by a permanently crippling neurological disorder clinically similar to Parkinson’s disease (Berman, 1980). A number of studies have been done on medicinal herbs to determine the level of manganese in them. Lozak et al. (2002) reported manganese concentration of 188 mg/kg in mint leaves while Basgel and Erdemoglu (2006) reported manganese concentration in the range of 23 mg/kg to 244 mg/kg in various medicinal herbs.

2.1.3 Zinc

Zinc is an essential element to humans and under normal conditions about 1-2 mg of zinc are required daily (Berman, 1980). Zinc bioavailability from food ranges from 10 to 40 per cent (National Academy of Science, 1976). About 5 to 10 per cent of the dietary zinc intake is absorbed and this depends on the age, content of zinc in the body and the dietary composition. Phytates and fibers decrease zinc absorption while zinc deficient organisms show a tremendous increase in zinc absorption (National Academy of Science, 1976). High concentrations are found in the male reproductive system, muscles, bone, liver, kidney, pancreas, thyroid and endocrine glands. The cause of zinc deficiency in man include excessive intake of cereal food with high phytate and fiber contents, which renders the zinc ingested unavailable for absorption. Zinc deficiency in animals and
humans is manifested as growth retardation, impaired development functions, malabsorption syndrome, renal and liver diseases (Neve, 1996).

Zinc functions mainly as an essential co-factor (an activator of enzymes) or structural component for over 70 enzymes in body cells (Eleanor and Mary, 1984). A number of enzymes including alkaline phosphate, alcohol dehydrogenase, carboxyl peptidate, glutamic dehydrogenase, leucine amino peptidase, RNA polymerase and DNA polymerase are zinc dependent (Neve, 1996). Humans and birds exhibit gross bone disorders, poor wound healing and susceptibility to infections due to zinc deficiency (Berman, 1980). Research has revealed that increased plasma glicocorticoid level and decreased cellular zinc content in zinc deficient animals contribute to a reduction in the B- and T-cells in bone marrow (Fraker and King, 2001). Zinc deficiency during pregnancy results in congenital malformations of the embryo particularly by affecting growth of proliferating tissues. The consequences of more subtle metabolic interactions, as in cirrhosis, and the basis of genetic or teratological defects have not been examined widely and offer rich investigative opportunities (Berman, 1980). Zinc also plays an important role as antioxidant that helps limit the oxidation of low-density lipoprotein cholesterol and thereby helps to prevent coronary artery disease (Neve, 1996).

There have been encouraging findings about the apparent benefits of zinc supplementation for select target populations. For example, individuals with HIV are at high risk of developing zinc and other micronutrient deficiencies as a result of decreased appetite, malabsorption and increased losses of endogenous zinc due to diarrhea and
recurrent infections (Kumar et al., 2003). Progression to stage IV of the disease is associated with decreased plasma zinc, inactivation of thymulin due to dissociation of bound zinc and reduced numbers of CD4 T-cells (Kumar et al., 2003). In addition to increased plasma zinc levels, thymulin activity and absolute numbers of CD4 cells, zinc-supplemented individuals have been found to maintain weight and experience fewer opportunistic infections (Kumar et al., 2003).

A major success regarding the beneficial impact of improving trace element nutritional status and the immune system during the past several years concerns the demonstration that zinc supplementation of children between the age of 1-5 years significantly decreases the incidence and duration of diarrhea and the incidence of pneumonia (Basgel and Erdemoglu, 2006). Animal sources of zinc include oysters and other seafoods, liver, meat and eggs. Whole grains also supply a significant amount of zinc (Eleanor and Mary, 1984).

With regard to excess zinc, supplementation of healthy adult men with 300 mg of zinc/day for 6 weeks impairs chemotaxis and phagocytic activity of neutrophils against bacteria and lymphocytic response to mitogenic stimulation (Ajit et al., 1999). Symptoms of acute toxicity include decreased blood concentration of high-density lipoprotein (HDL), the form of lipoprotein thought to be protective against heart disease (Berman, 1980). In addition to this, vomiting, dehydration, electrolyte imbalance, abdominal pain, lethargy, dizziness and muscular in coordination (Shankar and Prasad, 1998). Kumar et al. (2003) analysed levels of essential elements in Pragya-peya, a herbal drink and its 12
herbal constituents. Zinc content was found to be in the range of 26.5 μg/g to 56.9 μg/g with the lowest and highest contents being in *arjuna* and *aagya-ghas* respectively.

### 2.1.4 Chromium

Chromium occurs in oxidation states of +2, +3 and +6. However its most stable oxidation state is +3 (Anderson, 1989). It is one of the least toxic metals at low concentration and is in fact essential for fat and carbohydrate metabolism in mammals, forming part of the glucose tolerance factor (Lukaski, 1999). Impaired glucose tolerance and type two diabetes are associated with adverse changes in lipid profiles and increased risk of cardiovascular disease (Ajit *et al.*, 1999). In 1957 Schwarz and Mertz extracted from pork kidney a compound they called GTF, which restored impaired glucose tolerance in rats; with chromium identified as the active component (Cohen *et al.*, 1995). It appears to be an essential trace element because it potentiates insulin action (Cohen *et al.*, 1995).

Offenbacher and Pi-Sunyer (1988) hypothesized that chromium forms a complex between insulin and insulin receptors that facilitates the insulin-tissue interaction. They reported that suboptimal chromium intake in humans led to detrimental changes in glucose, insulin and glucagons of subjects with slightly impaired glucose tolerance. Deficiencies may be as a result of inadequate dietary intake and bioavailability as in overly refined junk foods, increased body loses and intravenous feeding. Deficiency of chromium may lead to glucose intolerance in the body, anxiety, fatigue, inadequate metabolism of amino acids and an increased risk of arteriosclerosis in addition to impaired glucose tolerance factor (Lukaski, 1999). There is evidence that tissue level of
chromium decline with increasing age, pregnancy and in malnourished children (Offenbacher and Pi-Sunyer, 1988).

Since chromium is poorly absorbed, very high oral intakes are necessary to attain toxic level. Symptoms of acute toxicity include dermatitis, gastrointestinal ulcers as well as liver and kidney damage if taken in large dosages over prolonged periods (Lukaski, 1999). Food sources of chromium include eggs, beef, brewers yeast, green pepper, apples, bananas, spinach and molasses (Hathcock, 1997). It is suggested that human requirements for chromium can be met by typical plant food concentrations (Hathcock, 1997). Analysis done on various medicinal herbs has shown low levels of chromium. Naidu et al. (1998) reported chromium concentration in the range of 0.3 μg/g to 2 μg/g, which can not be sufficient to compensate for chromium deficiency.

### 2.1.5 Vanadium

The chemistry of vanadium is complex because the element can exist in oxidation states from -1 to +5 and can form polymers. Oxidation states +4 and +5 are the most important in biological systems (Srivastava, 2000). It is widely distributed in nature, occurring in many plants and animals. Vanadium deficiency is known to reduce wing and tail feather growth in chicks consuming a diet containing less than 10 ppb Vanadium (National Research Council, 1994). Vanadium is also known to play a role in building healthy bones and teeth (Mohammad et al., 2001). It also has significance in the treatment of cardiovascular disease (Cohen et al., 1995) as well as treatment of cancer (Narla et al., 2000).
There has been discussion about evidence behind the suggestions that vanadium might have a role in the regulation of $\text{Na}^+$, $\text{K}^+$, phosphoryl transfer enzymes, adenylate cyclase and protein kinase (Srivastava, 2000). It may also act as an enzyme cofactor in the form of vanadyl in metabolism of the hormones, glucose, lipids and teeth but no specific biochemical function has been identified (Cohen et al., 1995). Recommended daily intake for vanadium is 1 mg/day to 4 mg/day (Srivastava, 2000). The main sources of vanadium in the diet include oat straw, peas, liver, fish, mushrooms and corn (Clarkson and Rowson, 1999). Fats and oils and fresh fruits and vegetables contain the least vanadium, ranging from 1 to 5 ppb (Srivastava, 2000). It is relatively toxic, with a threshold level near 10-20 mg/day or 10-20 ppm of diet (Clarkson and Rowson, 1999). Kumar et al. (2003) reported vanadium concentration of 0.5 μg/g to 5.2 μg/g in various medicinal herbs while Basgel and Erdemoglu, (2006) reported 0.9 μg/g to 7.6 μg/g.

2.2 Medicinal plants and their use in treatment
The therapeutic or medicinal properties of plants are normally dependent upon the presence of certain active components. Plants contain a structural framework based on cellulose and lignin, and the important cells contain protoplasm which in turn contains chlorophyll, starches, sugars and inulins (Kokwaro, 1993). Plants also contain trace elements which vary from plant to plant, and their influence in an animal body is also variable. The other basic compounds associated with plants which have medicinal values are oils and fats. Many essential oils have the power to hinder bacterial growth hence used for treating wound infections (Kokwaro, 1993). The Acacia species are known to contain tannins and are mainly used to treat diarrhoea. Croton and Euphorbia species on
the other hand contain Toxalbumins and are known in treatment of ulcers and eye infections (Kokwaro, 1993).

There is ample evidence of herbs being used in the treatment of diseases and for revitalizing body system in almost all ancient civilizations (Steiner, 1986). Plants are the main stay of many medicines having mystical and almost supernatural healing power. There are many references to the curative properties of several herbs in the ancient Indian literature, *Rigveda* (Kanias *et al.*, 1993). Herbs play a significant role in modern times, when the damaging effects of food processing and over medication have assumed alarming proportions. These are now being increasingly used in cosmetics, foods as well as alternative medicine (Bakhru, 1998).

**2.2.1 Euphorbia candelabrum**

*Euphorbia candelabrum* has a number of medicinal values. The extract from the stem-bark is given to women after birth to clear out the afterbirth (Kokwaro, 1993). The extract is also used to treat malaria. Most *Euphorbia* species described as medicinal contain resin as the active components (Kokwaro, 1993). Appendix 14 shows a photograph of *Euphorbia candelabrum* plant. *Euphorbia candelabrum* is also used in traditional Ethiopian medicine. Mixed with clarified honey, its sap is used as a purgative to cure syphilis, and when mixed with other medicinal plants as a salve to treat the symptoms of leprosy (Pankhurst, 1990).
2.2.2 *Prunus africana*

*Prunus africana* is applied in treatment of stomach-ache (Kokwaro, 1993). The stem-bark is boiled and the resultant red extract drunk as a remedy for stomach-ache. The aqueous extract from the leaves is also used to reduce fever. Appendix 15 shows a photograph of *Prunus africana* whose stem-bark has been peeled off for medicinal use. Historically, the powdered *Prunus africana* bark was used as a tea for relief of bladder and urinary disorders. European scientists began laboratory investigations into the active constituents in the bark, and this led to the development of the modern lipophilic (fat soluble) extracts used today (Neuwinger, 2000). Successfully researched in Europe, studies show Pygeum Bark to be exceptional for helping to maintain a healthy prostate (Neuwinger, 2000). The phytosterols, particularly beta-sitosterol, are found in numerous plants and are said to be anti-inflammatory, inhibiting the synthesis of prostaglandins (Neuwinger, 2000).

2.2.3 *Croton macrostachyus*

*Croton macrostachyus* is used in treatment of coughs, malaria and venereal diseases (Kokwaro, 1993). The fame of this medicinal herb is based on superstition. A patient suffering from mumps is supposed to go round the tree singing and the mumps is supposed to gradually disappear after that. Appendix 16 shows a photograph of *Croton macrostachyus* plant. In West Africa different plant parts in decoction are taken to treat constipation, stomach-ache and female infertility, and are used externally to treat stitch-like pain in the side and Guinea worm sores (Maundu, 2005). In Ethiopia *Croton macrostachyus* has many uses. A leaf extract is applied against itchy scalp. A decoction of the leafy twigs mixed with *Justicia schimperiana* is taken to treat jaundice and
smallpox. The preparation is taken with pepper, butter and milk. An infusion of the leafy branches is used as a mouthwash to treat toothache. The leaves or young shoots are eaten to treat fever and oedema and mashed leaves are applied to haemorrhoids. Crushed leaves in water are taken to treat tapeworm infection. Bark maceration is drunk as an abortifacient and uterotonic, to expel a retained placenta (Pankhurst, 1990). In Western Kenya people lick the ash of burnt leaves as a cough remedy. A leaf decoction is also taken to treat cough and stomach problems. A root decoction is taken to treat indigestion (Maundu, 2005). In Central Kenya a root decoction is taken to treat malaria; leaf juice is put on wounds to improve blood clotting, and also to treat sores, warts and ringworm (Maundu, 2005). Despite the many medicinal uses, not much research has been done concerning the chemical composition and pharmacology of the different plant parts and more research is warranted.

### 2.2.4 Erythrina abyssinica

_Erythrina abyssinica_ is used to treat a wide range of diseases. It is used for treating eyes when there is an inflammation of the eyelids (Kokwaro, 1993). The stem-bark is boiled with goat’s meat and the extract used for treating malaria, gonorrhoea and syphilis. Appendix 17 shows a photograph of _Erythrina abyssinica_ plant.

### 2.2.5 Markhamia platycalyx

_Markhamia platycalyx_ is used for treatment of throat as well as eye diseases (Kokwaro, 1993). Leaves obtained from the herb are boiled and the extract swallowed. This plant is
mainly found in western Kenya. The parts of the medicinal herb used and the disease treated by each of the medicinal herb is listed in table 2.1

**Table 2.1**  
**Diseases and plants used in their treatment**

<table>
<thead>
<tr>
<th>Disease treated</th>
<th>Plant used in treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Throat infection</td>
<td><em>Markhamia platycalyx</em> (leaves)</td>
</tr>
<tr>
<td>Eye disease</td>
<td><em>Markhamia platycalyx</em> (leaves), <em>Erythrina abyssinica</em> (stem bark)</td>
</tr>
<tr>
<td>Coughs</td>
<td><em>Croton macrostachyus</em> (leaves)</td>
</tr>
<tr>
<td>Fever</td>
<td><em>Prunus africana</em> (leaves / stem bark)</td>
</tr>
<tr>
<td>Skin rushes</td>
<td><em>Croton macrostachyus</em> (leaves)</td>
</tr>
<tr>
<td>Stomach-ache</td>
<td><em>Prunus africana</em> (leaves / stem bark)</td>
</tr>
<tr>
<td>Venereal disease</td>
<td><em>Croton macrostachyus</em> (leaves), <em>Erythrina abyssinica</em> (stem bark)</td>
</tr>
<tr>
<td>Wounds</td>
<td><em>Euphorbia candelabrum</em> (stem bark)</td>
</tr>
<tr>
<td>Malaria</td>
<td><em>Croton macrostachyus</em> (leaves) <em>Euphorbia candelabrum</em> (stem bark) <em>Erythrina abyssinica</em> (stem bark)</td>
</tr>
</tbody>
</table>


Besides lipids, proteins and carbohydrates required for human growth, supply of optimum quantities of inorganic micronutrients is also essential (Prasad, 1993). Several micronutrients such as Cr, Mn, Fe and Zn constitute a small fraction of our diet and play an important role in metabolic processes. Their excess or deficiency may disturb normal biochemical functions of the body (Iyengar, 1989). Two main criteria considered for essentiality of elements are; first, its absence from diet results in departure from normal growth and metabolism; and second, the replacement of an element may suppress pathological symptoms (Dean, 2003).

Various workers have reported the analysis of medicinal herbs of their respective countries. Obiajunwa *et al.* (2001) determined essential and trace elements contents of
some Nigerian medicinal plants. Vega-carrillo et al. (1997) reported elemental distribution in medicinal plants used in folklore medicines in Mexico. Research done on various medicinal herbs in Serbia and Montenegro showed that these plants contain relatively high concentration of trace elements which could be useful in their therapeutic application (Abrams and Murrer, 1993).

Basgel and Erdemoglu (2006) observed that medicinal herbs contain significant levels of trace elements and the levels in herbs and their infusions presented a wide variability. Lozak et al. (2002) reported 239 mg/Kg and 107 mg/Kg iron in mint and nettle preparations respectively while that of manganese was 188 mg/Kg in mint leaves. Castro (1998) reported 2.20 ± 0.88 µg/g chromium in different aromatic plants used as herbal medicine. Lozak et al. (2002) reported 51.0 mg/Kg zinc in mint leaves while Kumar et al. (2003) reported vanadium in a range of 0.5 µg/g to 5.2 µg/g in some medicinal herbs. In recent years, much attention has been paid to the analysis of traditional Chinese medicines by using a variety of analytical techniques (Prasad, 1993). However, very little has been done on elemental analysis in medicinal herbs used in Kenya. In the present study, some medicinal plants and their aqueous extract used in Kenya were analysed. Local and botanical names of all the analysed herbs, the part used for treatment and their uses are listed in Table 2.2
### Table 2.2
#### Plant part used for treatment and disease treated

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Local name</th>
<th>Plant part used</th>
<th>Disease treated</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Markhamia platycalyx</em></td>
<td>Lusiola (Luhya)</td>
<td>leaves</td>
<td>Eye infection</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Throat diseases</td>
</tr>
<tr>
<td><em>Prunus africana</em></td>
<td>Muiri (Kikuyu)</td>
<td>Leaves/stem bark</td>
<td>Fever and flu</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Stomach-ache</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Prostrate cancer</td>
</tr>
<tr>
<td><em>Erythrina abyssinica</em></td>
<td>Muuti (Meru)</td>
<td>Stem bark</td>
<td>Eye inflammation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Gonorrhoea</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Syphilis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Malaria</td>
</tr>
<tr>
<td><em>Croton macrostachyus</em></td>
<td>Mutuntu (Meru)</td>
<td>Leaves</td>
<td>Skin rushes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Coughs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Malaria</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Venerial disease</td>
</tr>
<tr>
<td><em>Euphorbia candelabrum</em></td>
<td>Ol-pongoni (Maasai)</td>
<td>Stem bark</td>
<td>Malaria</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>wounds</td>
</tr>
</tbody>
</table>

Source: Kokwaro, (1993)

Phytotherapy, which is the healing with plant is old and has become as increasing popular as the history of humanity and botanical medications (Basgel and Erdemoglu, 2006). However the vast majority of the medicinal herbal products are unlicensed and are not required to demonstrate efficacy, safety or quality (Ernst, 1998). In addition, the interest in chemical composition of medicinal herb products is growing because of ongoing developments in nutrition and in biochemical surveying and mineral prospecting (Rodushkin et al., 1999). Even though herbal products are often promoted as natural and therefore harmless, herbal remedies are, by no means free from adverse effects (Basgel and Erdemoglu, 2006). The debate on whether phytomedicines should be submitted to proper licensing procedures incorporating standards for quality, safety and efficacy is therefore continuing (Basgel and Erdemoglu, 2006). Unknown effects of some of the medicinal herbs have been observed. This is mainly due to drug interactions, drug contamination and mistaken plant identities (Ernst, 1998). Little is known about the
relative safety of medicinal herbs compared to conventional medicine although, for some medicinal herbs the risk may be less than for conventional drugs (Fuh et al., 2003). Many medicinal herbs can present a health risk depending on the concentration of trace elements in them (Garcia et al., 2000). An important link in the transfer of trace elements from soil to man is plants. The level of essential elements in plants is conditional, the content being affected by the geochemical characteristic of the soil and by the ability of plants to selectively accumulate some of these elements (Bin et al., 2001). Bioavailability of the elements depends on the form of their bond with the constituents of the soil (Basgel and Erdemoglu, 2006). Plants readily assimilate through the roots such elements which dissolve in water and occur in ionic forms.

**2.3 Methods of analysis**

Due to the importance of the mineral and trace elements present in medicinal herbs, several studies have been carried out to determine their levels by using atomic absorption spectrometry (AAS), electrochemical methods and neutron activation analysis (Krachler et al., 2002). Spectroscopic methods of analysis are effective and sufficient for the analysis of trace metals. This is because they are highly specific, sensitive and rapid. These techniques include X-ray fluorescence (XRF), atomic absorption spectroscopy (AAS) and neutron activation analysis (NAA). Other methods that can be used include electrochemical techniques such as ion-selective electrode and polarography. This study used atomic absorption spectrophotometry and anodic stripping voltammetry for the analysis of metals due to their sensitivity, accuracy, low interferences and availability.
2.3.1 Atomic Absorption Spectrophotometry (AAS)

Atomic absorption spectrometry is a powerful instrumental technique for the determination of heavy metals in liquid samples. It provides a total metal content of the sample. The growth of analytical atomic absorption spectrophotometry has been phenomenal (Dulsik, 1999).

When electromagnetic radiation is incident onto a vapour of metallic atoms, the atoms interacts with light radiation of their own specific resonance wavelengths resulting in absorption of radiation. Upon absorption of radiation, the atoms are transformed from a low energy state (ground state) characterized by $E_1$ to a higher energy state (excited state) characterized by $E_2$. The transition $E_1$ to $E_2$ results from the absorption of radiation of frequency, $\gamma$ given by the expression given in equation 1.

$$\gamma = \frac{E_1 - E_2}{h} \quad \text{.......................... (1)}$$

where $h$ is Plank’s Constant (Skoog and Leary, 1992).

The excited atoms may revert back to ground state by emitting radiation of the same frequency. The transitions are always stimulated by the absorption of radiation from an external source. The measurement of the radiation absorbed in such a transition forms the basis of AAS (Skoog and Leary, 1992). Radiation source should emit stable, intense radiation of the resonance wavelength of the element to be determined and should guarantee high signal to noise ratios. The radiation sources commonly used in AAS are the hollow cathode lamps consisting of the test element and the gaseous discharge lamps (arc lamps).
The atomizers convert the metal ions in the liquid sample to atomic vapour. Air-acetylene and nitrous oxide-acetylene flames are commonly used, although electrically heated graphite atomizers are also used for special analytical work. Nebulizers convert the liquid samples into small droplets before the sample enters the atomizer. This is achieved by use of a gas moving at high velocity. Monochromator on the other hand selects a given absorption line from spectral lines emitted from the light source or background emission from the flame. The most common monochromators in AAS are prisms and gratings. Gratings have the advantage of constant performance throughout the electromagnetic spectrum and are often used. However, prisms have higher performance in the ultraviolet region (Skoog and Leary, 1992).

The photomultiplier tube detector is used in most modern instruments. In the photomultiplier tube, there is an evacuated envelope, which contains a photocathode, a series of dynodes, which amplify the optical signals at the anode. A photon from the monochromator strikes the photocathode and dislodges an electron which is then accelerated to dynode one. The accelerated electron in turn ejects two or more electrons from dynode one. These electrons are accelerated to dynode two, three and so on resulting in the ejection of more and more electrons which eventually reach the anode as an amplified electron current. The amplified electron current from the photomultiplier tube is then fed to an electrical amplifier, which is then read out on an analogue or digital display. Most modern instruments are interfaced to a computer processor (Skoog and Leary, 1992).
This method is generally free from spectral interference or radiation interference since each metal has its own characteristic wavelength (APHA-AWWA, 1975) and the source lamp is composed of the element being determined. Detection limits vary with the metal and the specific instrument but it is generally less than 0.1 µg/ml (Chatwal and Anand, 1991). The incorporation of appropriate computer software in modern atomic absorption spectrophotometers has made AAS one of the most rapid analytical techniques.

**2.3.2 Voltammetric analysis techniques**

Voltammetry is an electrochemical technique in which the current-potential behaviour at an electrode surface is measured. The potential is varied in some systematic manner to cause electro-active chemical species to be reduced or oxidized at the electrode. The resultant current is proportional to the concentration of the chemical species. Electrochemical principles are well discussed in details in the literature (Heyrovsky and Zuman, 1965; Kolthoof and Lingane, 1952). There are various voltammetric techniques which include polarography and stripping voltammetry.

**2.3.2.1 Polarography**

The polarographic method of analysis is based on the current against voltage curves arising at a microelectrode when diffusion is the rate determining step in the electrochemical reaction. Polarography allows selectivity through control of electrode potential. It has utility for finger print purposes as well as analytical ones. Polarography provides sensitivity to the parts-per-billion level for many electro-active substances. Polarography was introduced as an electroanalytical technique by Heyrovsky (Heyrovsky
and Zuman, 1965). Applications of polarographic techniques range from the simple classical (DC) polarography to the more complex differential pulse anodic stripping voltammetry (DPASV) (Willard et al., 1986).

Polarographic analysis is based on the measurement of diffusion current versus potential. The half-wave potentials (E_{1/2}) correspond to the inflection point of the polarographic waves, where the current is half the limiting current. The value of E_{1/2} is characteristic of the substance under analysis and this can be compared to literature values hence enabling qualitative analysis to be carried out. This value may be a function of the conditions of the solution under analysis that is supporting electrolyte, pH, solvent system and reference electrode. Quantitative analysis is carried out by comparing the wave heights corresponding to the diffusion limiting currents of redox with those of standards or comparing them to those of spiked standards.

Reduction reaction follows the process shown in equation 2.

\[ \text{M}^{n+} (aq) + n e^- \rightarrow \text{M} (Hg) \]

where, \( n \) = number of electrons involved in the reduction process

The equilibrium potential, E, of the amalgam electrode is dependent on the ratio of the concentration of the metal ions \( m^{n+} \) in solution to the concentration of the metal in the amalgam and is represented by the Nernst Equation, given in equation 3.

\[ E = E^o + \frac{RT}{nF} \ln \frac{C_{m(aq)}^{n+}}{C_{m(Hg)}} \]
where,
\[ E^0 = \text{Standard Electrode Potential} \]
\[ R = \text{Gas Constant} \]
\[ \ln = \text{Natural log} \]
\[ F = \text{Faraday Constant} \]
\[ C_{m^{n+}(aq)} = \text{Concentration of the metal ion in solution} \]
\[ C_{m(Hg)} = \text{Concentration of the metal in amalgam} \]

Since an amalgam is formed when the metal ions discharge at the surface of the electrode, it follows that the concentration in the amalgam must be proportional to the current so produced as shown in equation 4.
\[ C_{m(Hg)} = K'I \quad ............4 \]

where, \( K' = \text{proportionality constant} \)
\[ I = \text{current} \]

The concentration of substances is determined in a solution containing an excess of an indifferent electrolyte referred to as the supporting electrolyte. The supporting electrolyte suppresses the migration of the ions to be determined, thus ions are transported to the surface of the mercury electrode exclusively by diffusion. Under these conditions, limiting current, \( I_d \) (maximum diffusion current) is proportional to the concentration of the reducible species in the test solution, as shown in equation 5.
\[ I_d = K'' C_{M^{n+}(aq)} \quad ............5 \]

where, \( K'' = \text{proportionality constant, and} \)
\[ C_{M^{n+}} = \text{Concentration of reducible species in the test solution.} \]
The limiting current corresponds to the complete depletion of ions in the solution to be determined near the drop surface. At any given time after the reaction has started, the current is I lower than \( I_d \), so that the concentration of the metal ions is different from zero and corresponds to a value \( C^{\infty}_{M^{n+}} \), which determines the electrode potential according to equation (3). Under these conditions, the diffusion current is equal to what is given in equation 6.

\[
I = k'' \left( C^{\infty}_{M^{n+} (aq)} - C_{m^{n+} (aq)} \right) \quad \ldots \ldots \text{6}
\]

Three modes of transport are responsible for the migration of ions in the solutions under analysis. First, migration due to the existence of an electric field. Its effect is eliminated by the presence, in the supporting electrolyte, ions in concentrations of about 50 to 80 times the ions under investigation. The second, convection, caused by mechanical agitation of the solution is reduced to negligible proportions by keeping the solution still. The third, diffusion, caused by a concentration gradient between the electrode surface and bulk of the solution, is the mode that is of interest in polarographic analysis.

### 2.3.2.2 Stripping voltammetry

Stripping voltammetry is a two-step technique in which the first step consists of electrolytic deposition of a chemical species into an inert electrode surface at a constant potential. This pre-concentration step can either involve an anodic or cathodic process. The second step consists of the application of a voltage scan to the electrode which causes the electrolytic dissolution, or stripping, of the various species in the amalgam back into solution at characteristic potential. The remarkable sensitivity of stripping
voltammetry is attributable to the pre-concentration that takes place during deposition. For pre-concentration to take place, the deposited material must as a necessity adhere to the electrode surface. Although there are exceptions, mercury is generally the electrode of choice. Stripping voltammetry can be used to determine those chemical species that will be retained by the mercury, by formation of either an amalgam or an insoluble mercurous salt.

The demand for the detection and quantitation of trace components in complex samples has always been on the increase (Batley and Florence, 1978). The need to establish the levels of trace elements in food, drinking water and waste-water effluents has led to stringent public legislation and industry-wide quality assurance programs which have been directed towards monitoring components of a sample at sub-ppm levels. Trace technique of stripping voltammetry has been used in trace analysis with relative ease and success in a variety of analytical applications. With minimal sample preparations, this electrochemical technique is routinely capable of identifying and quantifying trace components from $10^{-5}$ mol/dm$^3$ to $10^{-9}$ mol/dm$^3$ with excellent sensitivity (Batley and Florence, 1978).

The ideal working electrode must be stationary, have a reproducible surface area and low residual current. Solid electrodes such as gold, platinum, glassy carbon, wax impregnated graphite and carbon paste demonstrate such qualities and have been used successfully. Although solid electrodes give a sensitive response, they generally can be used for the analysis of only one species. When a solid electrode is employed in the analysis of
several species, it is nearly impossible to obtain the required homogeneity of the deposited materials prior to the stripping step (Dulski, 1999). The most practical electrode for stripping voltammetry employs mercury as the electrode surface. Because of their general versatility and convenience, the hanging mercury drop electrode (HMDE) and the thin film mercury electrode (TFME) are used (Dulski, 1999).

The hanging mercury drop electrode is the best working electrode for stripping voltammetry because of its extremely reproducible surface. All the characteristics of the dropping mercury electrode (Heyrovsky and Zuman, 1965), which makes it the most suitable electrode for routine analytical determinations, also apply to the hanging mercury drop electrode. The entire stripping voltammetry experiment is performed on one mercury drop. The drop is then dislodged and a new drop is dispensed for next experiment. Since the electrode is replaced for each experiment the condition of the electrode is not a variable in the analysis. This is not true for solid electrode (Vandecasteele and Block, 1993).

It is imperative that the hanging mercury drop electrode used in stripping voltammetry should be able to dispense a drop with an area that is reproducible to within one per cent. The measured current in an electrochemical experiment is proportional to the electrode area. Since the current from a standard is compared to the current from the sample, an error in the surface area of the drop will lead directly to an error in the calculated sample concentration. Stripping voltammetry with a hanging mercury drop electrode is a much
more convenient technique to implement since hanging mercury drop electrodes are automatically dispensed with the push of a button (Vandecasteele and Block, 1993).

The perennial problem of the hanging mercury drop electrodes is in maintaining the drop on the end of the capillary. The mercury drop can fall off in which case the experiment must be aborted. The ability to hold is a function of the mechanical construction of the electrode. The performance characteristics of the hanging mercury drop electrodes can often be improved by siliconizing the interior bore of the capillary (Vandecasteele and Block, 1993). Siliconizing is performed by coating the bore with a material such as dimethyldichlorosilane. Siliconizing enhances the hydrophobic nature of the capillary and minimizes the deleterious effects of minor imperfections in the surface of the glass.

In Thin film mercury electrode, a thin film mercury electrode is prepared by depositing a film of mercury onto a glassy carbon electrode. Although other electrodes may be used, glassy carbon usually gives excellent results. The thin film mercury electrode is generally used only for anodic stripping voltammetry. Such electrodes are most useful when maximum sensitivity is required (Vandecasteele and Block, 1993). The thin film mercury electrode exhibits high sensitivity because only an extremely small amount of mercury is incorporated into the film, resulting in the formation of a very concentrated amalgam during the deposition step. The stripping peaks that are obtained with a thin film mercury electrode tend to be sharper than those observed with hanging mercury drop electrode (Vandecasteele and Block, 1993). The thin film mercury electrode can be prepared by placing the glassy carbon electrode in a well-stirred solution of 2.5 mg/l reagent grade
mercuric nitrate made slightly acidic with acid at -0.4 V vs. SCE for 5 minutes. Once the thin film mercury electrode is generated it must be protected from oxygen to prevent oxidation of the film. Also, because the layer of the deposited mercury is extremely thin, the use of thin film mercury electrode should be limited to analyte concentration of less than $10^{-7}$ mol/dm$^3$.

The thin film mercury electrode can also be prepared in situ by adding 2.5 mg/l Hg$^{2+}$ directly to the sample solution and depositing mercury and the analyte simultaneously (Florence, 1986). The experiment is begun with a completely clean electrode, usually glassy carbon. The mercury and the deposited analyte are removed from the surface either mechanically or electrolytically following the completion of experiment. Because the same electrode surface is used for repetitive analysis, the condition of the surface is a major consideration. Steps must be taken to ensure that the surface of the thin film mercury electrode is as reproducible as possible prior to each analysis (Florence, 1970). Failure to guarantee a consistent surface may give rise to irreproducible results, since the current due to a particular analyte concentration is dependent upon a reproducible electrode surface. This problem however, is not a consideration with a hanging mercury drop electrode since a new mercury drop is used for each determination.

The thin film mercury electrode is recommended only when maximum sensitivity is required. Because of the care required to obtain consistent results, the thin film mercury electrode cannot be considered appropriate for routine analytical purposes. It can be used
to analyse metals in water at concentration in the order of 1 part per trillion (Nurnberg et al., 1976).

The sensitivity of voltammetric methods can be greatly enhanced by the use of a pre-concentration, or accumulation step in which the element is accumulated at the electrode by a faradaic process (anodic or cathodic). Anodic stripping voltammetry consists of a deposition potential that is more negative than the half-wave potentials of the metals to be determined and an anodic (positive going) scan to oxidize the reduced metal back into solution. During deposition, the elemental metal and the mercury on the electrode form an amalgam. Anodic stripping voltammetry can only be used to determine those metals that exhibit appreciable solubility in mercury.

For deposition the applied potential is more negative than $E_{1/2}$ of $M^{n+}$, and equation 7 shows the reduction of the ion.

$$M^{n+}_{(aq)} + ne^- \rightarrow M_{(Hg)} \ldots \ldots \ldots \ldots 7$$

Stripping: Scan in the positive direction, peak current is proportional to the concentration of $M$, as shown in equation 8.

$$M_{(Hg)} \rightarrow M^{n+}_{(aq)} + ne^- \ldots \ldots \ldots \ldots 8$$

It is worth noting that concentrations of ions exceeding 10 mg/l can cause inter-metallic formation in a hanging mercury drop electrode and in such cases differential pulse polarography is applied instead of differential pulse anodic stripping voltammetry (Dean, 2003). The hanging mercury drop electrode used with the differential pulse waveform is the most versatile electrode technique combination. Not only is the sensitivity high for
trace determinations, but also the tendency for inter-metallic formation is minimized in all but the most concentrated solutions. Stripping voltammetry is an indispensable technique in trace analysis.

In the anodic form of stripping voltammetry, the metal concerned is reduced at a controlled potential for a definite time under fixed conditions of geometry and stirring. The final anodic dissolution, or stripping process, involves a linear anodic scan in which the metal is oxidized. The resulting stripping voltammograms shows peaks, their heights or areas, of which are generally proportional to the concentrations of the electro-active metal ion, and the potentials of which have the same qualitative interpretations as their half-wave potentials in polarography.

Aqueous solutions exposed to air contain concentrations of dissolved gaseous oxygen as high as $10^{-3}$ mol/dm$^3$ at room temperature and pressure. Dissolved oxygen interferes in stripping analysis as it does in classical polarography. Depending upon the pH, oxygen undergoes reduction in three steps.

$$\text{O}_2 (g) + 2\text{H}^+ (aq) + 2e^- \rightarrow \text{H}_2\text{O}_2 (g) \quad \text{..........9}$$
$$\text{O}_2 (g) + 2\text{H}_2\text{O} (l) + 2e^- \rightarrow \text{H}_2\text{O}_2 (g) + 2\text{OH}^- (aq) \quad \text{..........10}$$
$$\text{O}_2 (aq) + 4\text{H}^+ (aq) + 4e^- \rightarrow 2\text{H}_2\text{O} (l) \quad \text{..........11}$$

Equations 9 and 10 occur at an $E_{1/2}$ of between $-0.5 \pm 0.05V$ and $1.3 \pm 0.005V$ vs. saturated calomel electrode (SCE). This is also the same range of potential that most reductions of metals occur and hence oxygen may bring about the overlapping of peaks in addition to metal peaks. The formation of hydrogen peroxide in 9 and 10 is an invidious
reaction to other electro-active species since it can function as both an oxidizing or reducing agent. The presence of the peroxide ion may also cause pH changes within the vicinity of the hanging mercury drop electrode increasing the chances of precipitation of heavy metals in turn diminishing the diffusion currents. As a result of the foregoing reactions, it is necessary to ensure that all traces of oxygen are removed. This can be done using the vanadous chloride scrubbing system. Nitrogen used in de-aeration needs to be of high purity. It is necessary to clean it to remove any traces of oxygen.

Conditioning is a term that denotes electrolytic cleaning of the electrode surface. A specified potential is applied to the electrode for a controlled time in order to remove contaminants or materials not removed during the stripping step from the electrode surface. Conditioning is not required with a hanging mercury drop electrode because a new drop is used for each determination. On the other hand, conditioning is a necessity with a thin film mercury electrode because the same electrode surface will be used in subsequent determinations. When the thin film mercury electrode is used to determine metals, the conditioning potential should be positive with respect to the half-wave potentials of the analyte to ensure the oxidation of the metals back into solution. If the thin film mercury is being formed in situ, the conditioning potential may be set positive of the oxidation potential of mercury to provide a clean electrode surface for the deposition step. The solution is stirred during conditioning. A typical conditioning time is 60-120 seconds.
The deposition potential is applied to the working electrode to cause the material of interest to be deposited onto the surface of the working electrode. The solution is generally stirred during deposition to maximize analyte-electrode contact. The selection of the deposition step depends upon whether the material to be determined is to be oxidized or reduced. For a reducible metal, the deposition potential should be negative with respect to the half-wave potential of the metal but not so negative such that the decomposition of the electrolyte is encountered. For oxidation materials the deposition potential should be selected so that it is positive with respect to the half-wave potential. The deposition time must be carefully controlled. It is an important experimental parameter that is unique to stripping voltammetry. If more sensitivity is required, the analyst simply increases the deposition time. This increases the degree of pre-concentration, making a greater amount of deposited analyte available at the electrode during the stripping step.

During equilibration, the deposition potential is applied to the working electrode but stirring is halted. This allows convection current from the stirring to decrease to a negligible level and also allows time for the amalgam to stabilize. An excitation waveform is applied from the voltammetric analyzer which electrolyses the deposited material back into solution. The current is measured vs. the applied potential. The deposited material will strip at potentials very close to their half-wave potentials. The measured current at these potentials is proportional to the concentration of the analyte in the original sample. Either a dc or differential pulse waveform may be used during the
stripping step. For this study, AAS and DPASV were used because of their availability and sensitivity.

**Figure 22-17** A modern dropping mercury electrode with mechanical control of drop size and time. (Courtesy of EG&G Princeton Applied Research, Princeton, NJ.)
CHAPTER 3

MATERIALS AND METHODS

3.1 Sampling procedure

The selection of plants to be analysed in this study was based on information gathered from literature. The medicinal herbs were classified into nine groups based on the diseases they treat which include throat infection, eye disease, coughs, fever, skin rushes, stomach-ache, venereal disease, wounds and malaria. Determination of levels of trace elements was done in two stages; total concentration of trace elements in part of the plant used for treatment and the concentration in the aqueous extract.

3.2 Samples and sample size

The choice of areas where samples were collected was based on regions where a particular medicinal herb was readily available and therefore widely used as obtained from literature. The medicinal herbs were identified with the help of a plant taxonomist. Leaves of *Markhamia platycalyx*, Baker spragne (Beentje, 1994) which is widely used in western Kenya was collected from Kakamega forest. Leaves and stem bark of *Prunus africana* Hoof f. kalkm, stem bark of *Erythrina abyssinica* Lam. Ex Dc, leaves of *Croton macrostachyus* Hochst. Ex Delile and stem bark of *Euphorbia candelabrum* Kotschy (Beentje, 1994) which are widely used in Mount Kenya region were obtained from Nyeri. For each medicinal herb, samples were collected from three different places between Tumutumu, Gatitu and Kamakwa in a region separated by at least 10 km. From each of the three regions 35 samples of all the five plants were collected. A total of 105 samples
were therefore collected for each herb. The sample size was determined using equation 12 and 13 which yielded samples that were representative.

\[ n_o = \frac{z^2 pq}{e^2} \] ..........................12

Where, \( n_o \) = the sample size
\( Z^2 \) = the abscissa of the normal curve that cuts off an area at the tails at a given confidence level
\( e \) = the desired level of precision
\( p \) = is the estimated proportion of an attribute that is present in the population
\( q = 1-p \)

The value for \( Z \) is found in statistical tables which contain the area under normal curve.

In this study, a maximum variability of \( P=0.5 \) at 95\% confidence level and ±5\% precision was used. The resulting sample size was thus determined.

\[ n_o = \frac{z^2 pq}{e^2} = \frac{(1.26)^2 (0.5)(0.5)}{(0.05)^2} = 159 \]

Since the population of medicinal plants was small, the sample size was reduced slightly. This is because a sample size provides proportionately more information for a small population than for a large population. The sample size \( (n_o) \) was thus adjusted using equation 13.

\[ n = \frac{n_o}{1 + \frac{n_o - 1}{N}} \] .................................13

where \( n \) is the sample size and \( N \) is the population size. Since the sampling affected 300 medicinal plants, the final sample size was hence determined.
$$n = \frac{159}{1 + \frac{159 - 1}{300}} = 105$$

Specific parts used for treatment including leaves and barks were obtained from the plants. On average about 50 g of either stem bark or leaves sample was collected from each medicinal herb. The samples were put in plastic bags, sealed and transported to the laboratory for analysis.

### 3.3 Sample treatment

The fresh samples were washed with tap water, distilled water, and then rinsed with de-ionized water. The samples were then air dried in the laboratory for two weeks before being ground to a homogenous fine powder in a mortar. The samples were then stored in appropriate vials or glass bottles awaiting analysis.

### 3.4 Chemicals and reagents

The chemicals used were of high quality analytical grades (Analar grade). These included standards, buffers, and acids. The chemicals were purchased from School Equipment Supplies and Kobian Ltd, Nairobi.

### 3.5 Cleaning glassware and plastics

All glassware were thoroughly washed with a detergent, chromic acid and tap water. They were rinsed with distilled water followed by 1:1 nitric acid solution and finally with distilled de-ionised water before drying overnight in the oven at 80°C. Plastic wares were
thoroughly cleaned with detergents and then rinsed with nitric acid followed by distilled de-ionised water and dried in the open. Voltammetric cells were soaked in 50% v/v analytical grade nitric acid overnight prior to analysis. They were then rinsed several times with distilled de-ionised water.

3.6 Experimental procedure

3.6.1 Preparation of stock solutions

3.6.1.1 Zinc

A weight of 0.501 g of pure zinc metal was weighed and put in a 500 ml volumetric flask containing about 100 ml of de-ionised water and 5 ml of concentrated hydrochloric acid and allowed to react. After all the zinc had dissolved the content was adjusted to the mark using concentrated hydrochloric acid. The resultant solution contained 1000 µg/ml of Zn$^{2+}$ ions.

3.6.1.2 Iron

A weight of 3.52 g of ammonium ferrous sulphate hexahydrate (98.8% BDH) was weighed and transferred into a 500 ml volumetric flask and dissolved in about 100 ml of de-ionised water. 5 ml of a 2M sulphuric acid solution was added to oxidize the Fe$^{2+}$ ions. The contents were finally made to the mark with sulphuric acid. The final solution contained 1000 µg/ml Fe$^{3+}$ ions.
3.6.1.3 Manganese

A weight of 1.44 g of dry potassium permanganate crystals (99.2% BDH) was weighed and put into a pyrex conical flask, dissolved in about 100 ml of de-ionised water and 10 ml of concentrated sulphuric acid added slowly. The permanganate ions were reduced to Mn$^{2+}$ by dropwise addition of a 10 per cent sodium metasulphite solution until the solution became colourless. 2 ml of concentrated nitric acid were added to oxidize the excess sulphuric acid. This solution was then cooled and transferred into a 500 ml volumetric flask and finally made up to the mark with de-ionised water. The resulting solution contained 1000 µg/ml Mn$^{2+}$ ions.

3.6.1.4 Vanadium and chromium

Vanadium and chromium standards were obtained from Buck Scientific Company, USA. They were prepared in 1% nitric acid to keep the metal in free ionic state. Their concentration was 1000 µ/ml.

3.7 Sample preparation

3.7.1 Preparation of aqueous extracts

The aqueous extracts were prepared using 1.0 g of plant material and 45 cm$^3$ of distilled de-ionised water. The dried samples were boiled in water to obtain the extracts. In order to obtain appropriate time for preparing the extracts, trials at different times were done. Boiling for 30 minutes was found adequate to prepare the extracts. It was observed that element concentrations in the extract were not significantly changed after 30 minutes of boiling. The sample solution was cooled and filtered (Whatman No.40 filter paper) into
50 ml volumetric flask. The filter papers were washed and the washings added to the filtrate. The filtrate was made to the mark using distilled de-ionised water. Samples of the extract were preserved in a deep freezer without any further treatment for analysis. Each analysis was done in triplicate.

3.7.2 Sample digestion

The samples were digested according to the procedure described by van loon (1980). One gram of plant material was weighed into the digestion tube. 10 ml of concentrated nitric acid was added and the solution digested by heating for 10 minutes and then allowed to cool. 5 ml perchloric acid was then added and further heating carried out until white fumes of perchloric acid was liberated. The sample solution was then cooled and filtered into 50 ml volumetric flask using a whatman No. 40 filter paper and made up to the mark with distilled de-ionised water for analysis by AAS and polarography (Chakrabarti, 1983).

The blank solution was prepared by heating 10 ml concentrated nitric acid for 10 minutes and then allowing it to cool. After cooling 5 ml perchloric acid was added and further heating carried out until white fumes of perchloric acid was liberated. The solution was then cooled and diluted to 50 ml mark using distilled de-ionised water. The samples and the standards were treated with potassium chloride solution at the rate of 2 ml per 100 ml of sample or standard. The potassium chloride acted as ionization suppressant. The sample and standard were aspirated into the nitrous oxide–acetylene flame and the absorbance measured under appropriate conditions. The absorbance of the standards were
used to establish the calibration curve and the content of the various metals in the samples determined by direct comparison. The calibration curves for all the elements are shown in the Appendices.

3.7.3 Preparation of ultra pure hydrochloric acid and ammonia

Ultra pure hydrochloric acid and ammonia were required for adjusting the pH of sample solutions to prevent introducing contaminants into the solution under analysis. Purification by isothermal distillation at room temperature was used in preparation of pure solutions. 500 ml of concentrated hydrochloric acid and concentrated ammonia were separately placed in a dessicator in which there was 200 ml of distilled de-ionised water. They were then left to equilibrate for a period of 72 hours. The solutions so prepared had approximately 1 mol dm$^{-3}$ of the respective solutions (Chakrabarti, 1983).

3.7.4 Preparation of ultra pure ammonium citrate buffer (supporting electrolyte)

A weight of 42.5 g of citric acid (monohydrate) was dissolved in 750 ml distilled de-ionized water. The pH was adjusted to 3.0 with hydrochloric acid. The solution was diluted to one litre with distilled de-ionised water. Such solution may have contained traces of some trace elements hence it was necessary to remove them for it to work well as a supporting electrolyte. This was achieved by controlled potential electrolysis in a cell made of platinum anode and mercury pool as cathode. 100 ml of supporting electrolyte was put in the cell and electrolysed for 24 hours at a current of 0.2 mA. The solution was stirred continuously during the process by bubbling nitrogen gas through it. Trace metal dissolved in the solution were deposited in the mercury pool. The electrolysed solution
was then extracted from the cell using a pipette and stored. The extraction was done with the cell still on to avoid re-dissolving of the metals which occur if the current is switched off with the solution still in contact with the mercury.

### 3.7.5 Preparation of potassium chloride suppressant

Potassium chloride used as ionization suppressant was prepared as described by (Skoog and Leary, 1992). A weight of 95 g of analytical grade potassium chloride was weighed and transferred to a 1 litre volumetric flask and dissolved in distilled de-ionised water. It was then made to the mark with distilled de-ionised water.

### 3.7.6 Preparation of vanadous chloride scrubbing agent

In this project vanadous chloride was used as the scrubbing agent. It was prepared by boiling 2 g of ammonium metavanadate with 25 ml of concentrated hydrochloric acid and diluting with distilled deionised water to 250 ml. The solution so produced was pale-green and contained vanadium in low oxidation states. It was then transferred into the glass washing tower and amalgamated zinc added to reduce it to the +2 oxidation state. The amalgamated zinc was prepared by placing 13 g of powdered zinc in a beaker, covering it with distilled deionised water and adding three drops of concentrated hydrochloric acid. Amalgamation was achieved by the addition of mercury. In addition to this basic scrubbing system, a washtower containing 0.2 mol dm$^{-3}$ ammonium citrate at pH 3 was attached. Its purpose was to ensure that the moisture content of the nitrogen gas bubbling through the analyte was of the same concentration as the supporting electrolyte and to remove any traces of vanadous chloride solution picked up by the gas; Nitrogen to
be scrubbed was first bubbled through the vanadous chloride solution, then through the ammonium citrate solution. Exhaustion of the scrubbing solution was detected when the characteristic bluish-green colour changed to violet colour. Regeneration was achieved by either, adding a few millilitres of concentrated hydrochloric acid, more amalgamated zinc or both. With the scrubbing tower ready white spot nitrogen gas supplied by BOC Kenya limited was connected to the tower input, while the output of this tower was connected to the input of a second tower containing the same electrolyte as that in the analysis cell which was 0.2 mol dm\(^{-3}\) ammonium citrate solution buffer. The output of the second tower was connected to the nitrogen input part of the model 303A. Saturation of the gas with supporting electrolyte prevents sample concentration changes because of evaporation. Traces of vanadous chloride solution in the gas may also contaminate the sample.

3.8 Sample analysis

3.8.1 Stripping voltammetry

The samples were analysed under the instrumental conditions as shown in Table 3.1

<table>
<thead>
<tr>
<th>Working electrode conditions</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Drop size:</td>
<td>Medium</td>
</tr>
<tr>
<td>Mode of measurement:</td>
<td>Differential Pulse</td>
</tr>
<tr>
<td>Modulation Amplitude</td>
<td>25 mV</td>
</tr>
<tr>
<td>Purge time</td>
<td>5 minutes</td>
</tr>
<tr>
<td>Initial / Deposition potential</td>
<td>-1.20V Vs Ag/AgCl</td>
</tr>
<tr>
<td>Measurement / Drop time</td>
<td>0.5 sec</td>
</tr>
<tr>
<td>Current range</td>
<td>5 (\mu)A to 200 (\mu)A as appropriate</td>
</tr>
<tr>
<td>Deposition time</td>
<td>120 seconds</td>
</tr>
<tr>
<td>Equilibration time</td>
<td>30 seconds</td>
</tr>
<tr>
<td>Scan direction</td>
<td>‘ + ‘</td>
</tr>
<tr>
<td>Scan rate</td>
<td>20 mV / sec</td>
</tr>
</tbody>
</table>

(Courtesy of VG Instruments, Inc.)
The polarographic instrument (Model 303 A) was connected to the polarographic analyzer/stripping voltameter (Model 264 A) which is an electric polarographic instrument capable of performing dc, sample dc, normal pulse, cyclic voltammetry and differential pulse polarographic analysis, as well as dc and differential pulse stripping analysis.

The voltameter was interfaced with an X-Y plotter (Phyne LY 16100-11) and a personal computer (a Tatung Model No. 145 Bs) for recording. The software program, POLR4 was used to carry out voltammetric recordings. Sufficient standards additions were made to each sample so as to increase the sample stripping peaks by 50-500%.

3.8.2 Analysis of metals by differential pulse anodic stripping voltammetry (DPASV)

The physical and chemical components for analysis were prepared in order to get reliable results. The capillary tube was discharged of mercury into a reservoir and then 1 mol dm$^{-3}$ nitric acid sucked through it by plunging the one end of the capillary into a rubber sucker. This was then followed by distilled de-ionised water. The capillary was siliconised in order to ensure that the test solution does not penetrate the capillary and to make the interior bone of the capillary hydrophobic. The capillary was therefore able to hold the drop and prevent it from falling off. The capillary was rinsed with methanol and air dried at 65 °C for two hours. The tip of opposite ferrule was then placed in a fresh vial of siliconizing fluid. Excess fluid was removed from the opposite end of the ferrule. The capillary was filled as recommended in the manufacture’s manual (EG & G Princeton Applied Research Corporation, 1980). It was ascertained that no air was trapped in the
capillary by a considerable retraction of the mercury thread when a drop was dislodged. Before the analysis it was ensured that the capillary was well filled by ensuring that the suck buck did not exceed 5 mm.

The reference electrode was a simple silver/silver chloride electrode that made contact with the analyte via porous Vycor frit. The frit was replaced periodically due to contamination after prolonged use. The glass sleeve of the reference electrode was filled with the filling solution (saturated with silver chloride) making sure that it was free of bubbles. This was done from time to time since when the electrodes are stored in water for days, shifting of peak potentials occur due to dilution of the filling solution by water diffusing into the reference electrode. The counter electrode was a Teflon sheathed platinum wire installed at the factory and required no maintenance. It was washed frequently with analytical grade nitric acid followed by rinsing with large amounts of distilled de-ionised water.

The best pH for the analysis of samples was first determined by analyzing specific samples at different pH’s and observing at which pH the instrument was most sensitive to the sample, giving the highest peak. 9 ml of the digested sample and 1 ml of ultra pure ammonium citrate solution were put in the analysis cell. The pH was then adjusted to 5.8 after which the solution was blanketed with nitrogen gas and deposition step of ions carried out for 2 minutes. The stirrer was then switched off to allow the convection currents to cease after which stripping was carried out. The procedure was repeated with
cumulative addition of 10µl of a mixed standard consisting of 10 mg/l of each of the ions Zn$^{2+}$, Cr$^{3+}$ and Mn$^{2+}$ until a total of 100 µl had been added.

### 3.8.3 Atomic absorption spectroscopy

Make 210 Vap atomic adsorption spectrophotometer was used in the analysis of metals. Table 3.2 is a guide to the instrumental conditions using flame techniques.

<table>
<thead>
<tr>
<th>Operating parameter</th>
<th>Zn</th>
<th>Mn</th>
<th>Fe</th>
<th>V</th>
<th>Cr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wave length (nm)</td>
<td>213.9</td>
<td>279.5</td>
<td>248.4</td>
<td>437.9</td>
<td>425.4</td>
</tr>
<tr>
<td>Slit width (nm)</td>
<td>0.2</td>
<td>0.2</td>
<td>0.3</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Lamp current (mA.)</td>
<td>10</td>
<td>10</td>
<td>15</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>Sensitivity (µg / g)</td>
<td>0.01</td>
<td>0.04</td>
<td>0.06</td>
<td>0.05</td>
<td>0.08</td>
</tr>
<tr>
<td>Detection limit (µg / g)</td>
<td>0.003</td>
<td>0.005</td>
<td>0.005</td>
<td>0.008</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Detection limit is the lowest concentrations that can give an absorbance detectable above the noise range while sensitivity is a measure of the instrument’s response to the analyte and by convention, shows the concentration of each element required to absorb 1% of the incident light energy. This corresponds to an absorbance value of 0.0044 (Richard *et al.*, 2005). Elements with greater sensitivity will have the lowest concentration values in that category. The values of sensitivity in Table 3.2 are the amounts in ppm required to give the absorbance reading of 0.200.
3.9 Data analysis

The results obtained were arranged in tables. Dixon’s Q-test was used for the elimination of outlying data. Student t-test was used to determine if there was a significant difference between the results obtained by the two methods of analysis, AAS and DPASV. Correlation coefficient, r, was used to estimate how well the experimental results were distributed to fit a straight line. Correlation analysis was done for levels of trace elements in plants and the aqueous extracts.
CHAPTER 4
RESULTS AND DISCUSSION

4.1 Introduction

The levels of five trace elements in selected medicinal plants and their aqueous extracts were analysed using Atomic Absorption Spectrophotometry (AAS). Differential Pulse Anodic Stripping Voltametry (DPASV) was used for method validation. The results are presented in the following sections.

4.2 Calibration curves

The calibration curves obtained by the respective metal ion standards were used for quantitative determination of elements in samples and blank and are presented in Appendix 9-13. The standards were prepared such that they approximated the overall composition of the actual samples and covered a reasonable concentration range of the analyte. Table 4.1 gives calibration equation and the Spearson Correlation Factor, $r$ for the elements using AAS and DPASV. The detection limit values defined as the concentration of element corresponding to three times the Standard Deviation from the digestion blanks where $n = 15$ was also calculated and is shown in Table 4.1

<table>
<thead>
<tr>
<th>Technique</th>
<th>Element</th>
<th>Regression line</th>
<th>r-value</th>
<th>Detection limit (μg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAS</td>
<td>Zinc</td>
<td>$Y=0.0777X+0.0007$</td>
<td>0.9942</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Manganese</td>
<td>$Y=0.0869X+0.0800$</td>
<td>0.9970</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>Iron</td>
<td>$Y=0.0829X-0.0033$</td>
<td>0.9894</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>Vanadium</td>
<td>$Y=0.085X-0.0018$</td>
<td>0.9980</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>Chromium</td>
<td>$Y=0.085X-0.0040$</td>
<td>0.9983</td>
<td>0.020</td>
</tr>
<tr>
<td>DPASV</td>
<td>Zinc</td>
<td>$Y=0.0778X+0.0008$</td>
<td>0.9939</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>Manganese</td>
<td>$Y=0.0867X+0.0831$</td>
<td>0.9892</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>Chromium</td>
<td>$Y=0.0861X+0.0047$</td>
<td>0.9958</td>
<td>0.025</td>
</tr>
</tbody>
</table>
From table 4.1, the correlation coefficients are above 0.98 reflecting good correlation since they are linear over a wide range of analyte concentration. When the regression line was drawn so as to come as close to the point as possible, it gave best fitting straight line, \( y = ax \pm b \) where, \( a \) is the slope and \( b \) is the \( y \)-intercept. The \( y \)-intercept is close to the origin and \( r \)-values close to 1 hence giving a linear relationship. Therefore the elemental concentration data for samples analyzed in this study is reliable.

**4.3 Method validation**

The levels of zinc, manganese and chromium in medicinal herbs were analysed using Differential Pulse Anodic Stripping Voltammetry. The concentrations of trace metals in the medicinal plants were determined using equation 14.

\[
C_\mu = \frac{I_1 v C_s}{I_2 v + (I_2 - I_1) V} \tag{14}
\]

where \( I_1 \) - sample peak height

\( I_2 \) - standard addition peak height

\( v \) - Volume of standard solution added

\( V \) - volume of original sample

\( C_s \) - concentration of standard solution

\( C_\mu \) - concentration of original sample

Since the volume of the spiking solution added was very small the equation above was simplified to equation 15.
\[ C_\mu = \frac{I_1\nu C_S}{(I_2 - I_1)} \] 

This equation is valid for 10 ml of sample and 10-100 µl standard additions. The concentration of zinc, manganese and chromium determined in each of the five medicinal herbs using DPASV are listed in Table 4.2

<table>
<thead>
<tr>
<th>Medicinal plant</th>
<th>Zn (X ± SD)</th>
<th>Mn (X ± SD)</th>
<th>Cr (X ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Markhamia platycalyx (leaves)</td>
<td>20.9±1.1</td>
<td>14.5±1.5</td>
<td>2.0±0.2</td>
</tr>
<tr>
<td>Prunus africana (leaves)</td>
<td>11.7±0.8</td>
<td>80.1±1.2</td>
<td>ND</td>
</tr>
<tr>
<td>Euphorbia candelabrum (stem-bark)</td>
<td>39.9±0.8</td>
<td>75.8±2.1</td>
<td>3.6±0.2</td>
</tr>
<tr>
<td>Croton macrostachyus (leaves)</td>
<td>33.2±0.7</td>
<td>36.2±1.7</td>
<td>2.4±0.1</td>
</tr>
<tr>
<td>Erythrina abyssinica (stem-bark)</td>
<td>151.9±1.3</td>
<td>208.1±2.1</td>
<td>4.9±0.6</td>
</tr>
</tbody>
</table>

ND, not detected.

From the results in Table 4.2 zinc was abundant in *Erythrina abyssinica* (151.9±1.3 µg/g) and lowest in *Prunus Africana* (11.7±0.8 µg/g). *Erythrina abyssinica* had high levels of manganese (208.1±2.1 µg/g) and chromium (4.9±0.6 µg/g) while *Markhamia platycalyx* had the lowest levels (14.5±1.5 µg/g) and (2.0±0.2 µg/g) respectively. No chromium was detected in *Prunus africana*. Among the three trace elements, chromium had the least concentration in all the medicinal herbs analysed.

To validate the results obtained by AAS, the concentrations obtained by both DPASV and AAS were compared as in the table 4.3
Table 4.3
Comparison of concentration of Zn, Mn, and Cr (µg/g) in medicinal plants by AAS and DPASV (n=15)

<table>
<thead>
<tr>
<th>Medicinal plant</th>
<th>Zn (X±SD)</th>
<th>Mn (X±SD)</th>
<th>Cr (X±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AAS</td>
<td>DPASV</td>
<td>AAS</td>
</tr>
<tr>
<td><em>Markhamia platyclayx</em> (leaves)</td>
<td>22.7±1.4</td>
<td>20.9±1.1</td>
<td>13.2±0.5</td>
</tr>
<tr>
<td><em>Prunus africana</em> (leaves)</td>
<td>10.5±2.5</td>
<td>11.7±0.8</td>
<td>84.2±1.6</td>
</tr>
<tr>
<td><em>Euphorbia candelabrum</em> (stem-bark)</td>
<td>44.2±1.8</td>
<td>39.9±0.8</td>
<td>82.9±2.5</td>
</tr>
<tr>
<td><em>Croton macrostachyus</em> (leaves)</td>
<td>32.4±3.3</td>
<td>33.2±0.7</td>
<td>35.6±1.7</td>
</tr>
<tr>
<td><em>Erythrina abyssinica</em> (stem-bark)</td>
<td>158.7±0.9</td>
<td>151.9±1.3</td>
<td>219.2±1.1</td>
</tr>
</tbody>
</table>

ND, not detected

A two tailed student t-test carried out on the two sets of results at a 95% confidence limit showed no significant difference between the results of the two methods. The calculated student t-test values were lower than the expected values (Tables 4.4). It can be seen that the agreement between the results obtained with both independent determination methods is very satisfactory.
Table 4.4  
Values of student T-test of trace elements in medicinal herbs by AAS/DPASV

<table>
<thead>
<tr>
<th>Medicinal plant</th>
<th>Element</th>
<th>T-calculated</th>
<th>T-expected</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Markhamia platycalyx</em> (leaves)</td>
<td>Zinc</td>
<td>1.980</td>
<td>2.362</td>
</tr>
<tr>
<td></td>
<td>Manganese</td>
<td>0.206</td>
<td>2.362</td>
</tr>
<tr>
<td></td>
<td>Chromium</td>
<td>0.050</td>
<td>2.362</td>
</tr>
<tr>
<td><em>Prunus africana</em> (leaves)</td>
<td>Zinc</td>
<td>0.871</td>
<td>2.365</td>
</tr>
<tr>
<td></td>
<td>Manganese</td>
<td>1.202</td>
<td>2.365</td>
</tr>
<tr>
<td></td>
<td>Chromium</td>
<td>0.905</td>
<td>2.365</td>
</tr>
<tr>
<td><em>Euphorbia candelabrum</em> (stem-bark)</td>
<td>Zinc</td>
<td>0.702</td>
<td>2.045</td>
</tr>
<tr>
<td></td>
<td>Manganese</td>
<td>0.662</td>
<td>2.045</td>
</tr>
<tr>
<td></td>
<td>Chromium</td>
<td>0.044</td>
<td>2.045</td>
</tr>
<tr>
<td><em>Croton macrostachyus</em> (leaves)</td>
<td>Zinc</td>
<td>0.721</td>
<td>2.451</td>
</tr>
<tr>
<td></td>
<td>Manganese</td>
<td>0.826</td>
<td>2.451</td>
</tr>
<tr>
<td></td>
<td>Chromium</td>
<td>0.066</td>
<td>2.451</td>
</tr>
<tr>
<td><em>Erythrina abyssinica</em> (stem-bark)</td>
<td>Zinc</td>
<td>0.058</td>
<td>2.306</td>
</tr>
<tr>
<td></td>
<td>Manganese</td>
<td>0.083</td>
<td>2.306</td>
</tr>
<tr>
<td></td>
<td>Chromium</td>
<td>0.111</td>
<td>2.306</td>
</tr>
</tbody>
</table>

The extended range of the metal levels in the analysed samples was also utilized to check the accuracy of DPASV over AAS by means of correlation graphs. The resulting graphs (Appendix 6-8) gave results in Table 4.5

Table 4.5  
Correlation coefficient of trace elements by AAS and DPASV

<table>
<thead>
<tr>
<th>Metal</th>
<th>Correlation coefficient, $r^2$</th>
<th>Regression line</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc</td>
<td>0.9990</td>
<td>$Y = 0.9533X + 0.2712$</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.9993</td>
<td>$Y = 0.942X + 0.8034$</td>
</tr>
<tr>
<td>Chromium</td>
<td>0.999</td>
<td>$Y = 0.8873X - 0.1023$</td>
</tr>
</tbody>
</table>

Correlation analysis is used to check if the values of two variables are associated. The correlation coefficient is a number between -1 and 1. In general, the correlation expresses the degree that on an average, two variables change correspondingly (Valenta et al.,
A regression line is a line drawn through a scatter plot of two variables. The line is chosen so that it comes as close to the points as possible. The line in the plot is the best fitting straight line. A straight line depicts a linear trend in the data. In addition an equation of the regression line can be calculated. How well this equation describes the data is expressed as a correlation coefficient, $R^2$. The closer $R^2$ is to 1.00, the better the fit (Valenta et al., 1981).

Research done by Prerna et al. (2004) to compare DPASV and AAS a good linearity was observed with a linear regression coefficient of 0.998 while Nikola and Maja (1988) obtained correlation coefficient of 0.9466. In this study the correlation coefficient of 0.999 was obtained. This is close to 1.00 which shows agreement between Differential Pulse Anodic Stripping Voltammetry and Atomic Absorption Spectrophotometry. The results presented in this study using AAS are therefore highly satisfactory.

### 4.4 Concentration of elements in plants using AAS

Five elements were determined in five medicinal herbs commonly used in Kenya using AAS. The results obtained are listed in Table 4.6. From the results in Table 4.6 the levels of elements analysed varied from one plant to another. It was also observed that within a give plant, the concentration of different elements presented a wide variability.
Table 4.6
Mean concentrations (µg/g) of elements in some medicinal plants using AAS technique  
(n = 15)

<table>
<thead>
<tr>
<th>Medicinal plant</th>
<th>Zn ((\bar{X} \pm SD))</th>
<th>Mn ((\bar{X} \pm SD))</th>
<th>Fe ((\bar{X} \pm SD))</th>
<th>V ((\bar{X} \pm SD))</th>
<th>Cr ((\bar{X} \pm SD))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Markhamia platycalyx(leaves)</td>
<td>22.7±1.4</td>
<td>13.2±0.5</td>
<td>87.4±1.7</td>
<td>ND</td>
<td>2.5±0.1</td>
</tr>
<tr>
<td>Prunus africana(leaves)</td>
<td>10.5±2.5</td>
<td>84.2±1.6</td>
<td>65.4±0.4</td>
<td>0.13±0.01</td>
<td>ND</td>
</tr>
<tr>
<td>Euphorbia candelabium(stem bark)</td>
<td>44.2±1.8</td>
<td>82.9±2.5</td>
<td>154.2±3.5</td>
<td>0.79±0.02</td>
<td>4.1±0.3</td>
</tr>
<tr>
<td>Croton macrostachyus(leaves)</td>
<td>32.4±3.3</td>
<td>35.6±1.7</td>
<td>281.3±1.8</td>
<td>0.09±0.01</td>
<td>1.9±0.1</td>
</tr>
<tr>
<td>Erythrina abyssimica(stem bark)</td>
<td>158.7±0.9</td>
<td>219.2±1.1</td>
<td>452.8±2.6</td>
<td>0.79±0.02</td>
<td>5.6±0.4</td>
</tr>
</tbody>
</table>

ND, not detected

4.4.1 Concentration of elements in leaves of *Markhamia platycalyx*

*Markhamia platycalyx* had low levels of manganese (13.3±0.5 µg/g) while zinc concentration (22.7±1.4 µg/g) and iron concentration (87.4±1.7 µg/g) was second lowest. Chromium concentration was (2.5±0.1 µg/g). No vanadium was detected in *Markhamia platycalyx* at detection limit of 0.008 µg/g. In general *Markhamia platycalyx* had low elemental content as compared to other herbs analysed. This could be attributed to varying geo-environmental conditions and local soil characteristics since the samples were obtained from different regions (Western Kenya) as compared to other herbs which were obtained from Nyeri region. The herb is used to treat minor ailments like eye and throat infections. Low levels of trace elements could explain its limited application in treatment. Vanadium was detected in all the herbs in the range of 0.09-0.79 µg/g except in *Markhamia platycalyx*. This level of vanadium is lower than reported by Kumar *et al.*
(2003) where a range of 0.5-5.2 μg/g was detected in a number of herbal plants. Many vanadium compounds have been described to pose special therapeutic properties being used for the treatment of diabetes (Marzban et al., 2002).

4.4.2 Concentration of elements in leaves of *Prunus africana*

*Prunus africana* had the lowest levels of zinc (10.5±2.5 μg/g) and iron (65.4±0.4 μg/g). Its vanadium content was 0.13±0.01 μg/g. Vanadium compounds have been found to be antidiabetic and cancer protective (Mukherjeem et al., 2004). This may explain the use of *Prunus africana* in treating of prostrate cancer. Manganese concentration was (84.2±1.6 μg/g). Chromium was however not detected in *Prunus africana* at detection limit of 0.02 μg/g. Except *Markhamia platycalyx* and *Prunus africana*, iron concentration found in all the other herbs was higher than those reported by Lozak et al. (2002) who obtained 107 mg/kg in a herbal plant, *Urtica dioica*.

4.4.3 Concentration of elements in stem bark of *Euphorbia candelabrum*

*Euphorbia candelabrum* is recommended for treating wounds (Kokwaro, 1993). Its zinc content (44.2±1.8 μg/g) is of special interest because of wound healing ability of this element (Kokwaro, 1993). This herb had the highest levels of vanadium at 0.79±0.02 μg/g while its chromium concentration (4.1±0.3 μg/g) and zinc concentration (44.2±1.8 μg/g) was second highest after *Erythrina abyssinica*. The level of iron in the herb was 154.2±3.5 μg/g.
4.4.4 Concentration of elements in leaves of *Croton macrostachyus*

Among the five elements analysed, iron levels were highest (281.3±1.8 µg/g) in *Croton macrostachyus*. Zinc concentration was 32.4±3.3 µg/g while manganese was 35.6±1.7 µg/g. Chromium concentration was 1.9±0.1 µg/g while vanadium was least at 0.09±0.01 µg/g. *Croton macrostachyus* has been found to be highly effective for curing cold and coughs as well as enhancing body resistance against many diseases where trace elements such as iron, manganese and zinc may be responsible. Analysis done on *Pragya peya* by Kumar *et al.* (2003), which is used as a nervine tonic, for curing cold and cough in India revealed that the herb is rich in iron, manganese, zinc and vanadium with iron being the most enriched. This shows a close link between *Croton macrostachyus* and *Pragya peya* in terms of medicinal application and elemental content. Equally important is that the two herbs are also used to treat skin diseases. It is likely that trace elements in these herbs play a vital role.

4.4.5 Concentration of elements in stem bark of *Erythrina abyssinica*

The high elemental content in *Erythrina abyssinica* is a likely indicator of its ability to cure a wide range of diseases. It is rich in iron (452.8 ± 2.6 µg/g), an important mineral that enters into the vital activity of blood and glands. It also had the highest levels of zinc (158.7±0.9 µg/g), manganese (219.2±1.1 µg/g) and chromium (5.6±0.4 µg/g). It is likely that the herb could have wide applicability. The fact that it is rich in immune boosting trace elements suggests that it can be used to boost the immunity of patients suffering from various ailments. For example it can be used for managing diabetes since it has been shown that zinc level in blood of diabetic patients is low (Kumar *et al.*, 2003).
Iron deficiency is the most prevalent nutritional deficiency in humans (Reddy et al., 1987) and is commonly caused by insufficient dietary intake, excessive menstrual flow or multiple births. In this case, it results especially in anaemia. Iron is important because it eliminates phlegm and strengthens the function of the stomach. The stem bark of *Erythrina abyssinica* contains higher amounts of iron and may be advised to compensate for iron deficiency and also suitable for stomach disorders.

Except for *Erythrina abyssinica*, zinc content in the studied herbal drugs was lower than what Lozak et al. (2002) reported at 51.0 mg·kg in *Salvia officinalis* leaves. Manganese content ranged from 13.2-219.2 μg/g. Lozak et al. (2002) reported a concentration of manganese of 188 mg/kg in *Cassia anqustifolia* leaves used as herbal drug. Except for *Erythrina abyssinica* manganese content in the studied medicinal herbs was lower than 219.2 μg/g. Iron content of the herbs varied with a wide range of 65.4-452.8 μg/g among all the five herbs.

Chromium concentration of the herbs was in the range of 1.9–5.6 μg/g with the highest value in *Erythrina abyssinica*. Garcia et al. (2000) reported that presence of Cr in spices and aromatic herbs is higher than other foods and beverages. Castro (1998) reported chromium values of 2.20 ± 0.88 μg/g in different aromatic plants. Chromium is an essential element since it is a cofactor for insulin and a component of the glucose tolerance factor (Anderson, 1989). The recommended dietary intake extends from 50 to 250 μg/g. The risk of chromium deficiency increases under all situations in which highly purified foods are fed at the exclusion of a varied diet. Chromium deficiency can cause an
insulin resistance, impair glucose tolerance and may be a risk factor in artherosclerotic
disease (Mertz, 1982). The chromium content in the plants analysed is relatively low and
cannot be sufficient to compensate a chromium deficiency. The variation in elemental
contents as observed in this study could be due to preferential uptake of the elements by a
particular plant species from the soil. Soil characteristics together with environmental
conditions play an important role in the nutrients content (Manahan, 1984).

Choudhury et al. (2008) reported chromium and vanadium concentration range of 1-2
µg/g while zinc concentration was 25-60 µg/g in antidiabetic herbs. These elements play
an important role in Diabetes mellitus (Choudhury et al., 2008). The same study showed
a wide range of concentration of manganese (26.7-250 µg/g). Lokhande et al. (2010)
alysed 12 Indian plants used in Indian Ayurvedic system, where specific parts of
different medicinal plants like bulb, tuber, flower and rhizomes were used for the study,
and reported iron concentrations in the range of 70-523 µg/g while zinc was 45-98 µg/g.
In a similar study done on plant species, especially those used in the treatment of diseases
such as hypertension, diabetes and asthma reported iron levels in Lippia multiflora, O.
canum and R. vomitoria in the range of 20-753 µg/g, Kofi (2010). Zinc ranged from 43.5
µg/g in B. diffusa to 495.0 µg/g in C. anisata. Manganese concentration was
disproportionately very high in only a few species, including R. vomitoria (1455 µg/g)
followed by G. sylvesre(1190 µg/g) and V. africana (556 µg/g).
4.5 Concentration of elements in aqueous extracts

The aqueous extract samples were prepared by boiling one gram of the plant sample for a period of 30 minutes in 45 cm$^3$ of distilled water and levels of elements determined in order to gauge the uptake. After boiling and filtering, the extracts were analyzed directly without any further treatment.

4.5.1 Optimization of aqueous extraction time

It was necessary to optimize the boiling time. One gram of sample was boiled in 45 cm$^3$ of de-ionized water for 25, 30 and 35 minutes. The results for optimization of boiling time is presented in Table 4.7

<table>
<thead>
<tr>
<th>Element</th>
<th>Boiling time(min)</th>
<th>Markhamia platycalyx (leaves)</th>
<th>Prunus africana (leaves)</th>
<th>Euphorbia candelabnum (stem-bark)</th>
<th>Croton macrostachyus (leaves)</th>
<th>Erythrina abyssinnica (stem-bark)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>25</td>
<td>10.3</td>
<td>3.9</td>
<td>10.2</td>
<td>18.9</td>
<td>33.9</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>13.0</td>
<td>4.2</td>
<td>10.7</td>
<td>19.2</td>
<td>35.0</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>13.1</td>
<td>4.3</td>
<td>10.7</td>
<td>19.3</td>
<td>35.3</td>
</tr>
<tr>
<td>Mn</td>
<td>25</td>
<td>4.0</td>
<td>31.3</td>
<td>15.8</td>
<td>6.3</td>
<td>45.2</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>4.4</td>
<td>32.6</td>
<td>16.5</td>
<td>6.7</td>
<td>45.7</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>4.4</td>
<td>32.6</td>
<td>16.6</td>
<td>6.7</td>
<td>45.7</td>
</tr>
<tr>
<td>Fe</td>
<td>25</td>
<td>21.5</td>
<td>16.0</td>
<td>46.5</td>
<td>45.5</td>
<td>220.1</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>22.2</td>
<td>17.8</td>
<td>47.4</td>
<td>48.3</td>
<td>224.1</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>22.3</td>
<td>17.9</td>
<td>47.5</td>
<td>48.3</td>
<td>224.6</td>
</tr>
<tr>
<td>V</td>
<td>25</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Cr</td>
<td>25</td>
<td>1.8</td>
<td>ND</td>
<td>2.9</td>
<td>ND</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1.9</td>
<td>ND</td>
<td>3.1</td>
<td>ND</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>1.9</td>
<td>ND</td>
<td>3.2</td>
<td>ND</td>
<td>4.4</td>
</tr>
</tbody>
</table>

ND, not detected
It was observed that element concentration in the aqueous extracts were not significantly changed after 30 minutes of boiling. Therefore boiling for 30 minutes was found sufficient for preparing the aqueous extracts from the medicinal herbs.

Since these herbs are consumed as aqueous extract in Kenya, no further treatment was applied on the extracts. The results of the concentration of elements after boiling one gram of each sample in 45 cm$^3$ of distilled water is presented in Table 4.8

<table>
<thead>
<tr>
<th>Medicinal plant</th>
<th>Zn ($\bar{X} \pm SD$)</th>
<th>Mn ($\bar{X} \pm SD$)</th>
<th>Fe ($\bar{X} \pm SD$)</th>
<th>V ($\bar{X} \pm SD$)</th>
<th>Cr ($\bar{X} \pm SD$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Markhamia platycalyx</em> (leaves)</td>
<td>13.2±1.7</td>
<td>4.3±0.9</td>
<td>22.3±1.5</td>
<td>ND</td>
<td>1.8±0.2</td>
</tr>
<tr>
<td><em>Prunus Africana</em> (leaves)</td>
<td>4.3±1.2</td>
<td>32.7±1.6</td>
<td>17.7±1.3</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td><em>Euphorbia candelabrum</em> (stem bark)</td>
<td>10.7±1.7</td>
<td>16.4±2.3</td>
<td>47.3±2.1</td>
<td>ND</td>
<td>3.2±0.4</td>
</tr>
<tr>
<td><em>Croton macrostachyus</em> (leaves)</td>
<td>19.1±1.3</td>
<td>6.7±1.1</td>
<td>48.5±2.9</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td><em>Erythrina abyssinica</em> (stem-bark)</td>
<td>35.2±2.3</td>
<td>45.6±2.7</td>
<td>224.5±3.5</td>
<td>ND</td>
<td>4.3±0.5</td>
</tr>
</tbody>
</table>

ND, not detected

4.5.2 Concentration of zinc in aqueous extract

From table 4.8 the zinc content in the extracts was in the range of 4.3-35.2 µg/g. The highest level was determined in *Erythrina abyssinica* (35.2±2.3 µg/g) while the lowest
was in *Prunus africana* 4.3±1.2 µg/g). Zinc is an important element responsible for many enzymatic processes and is involved in the working of genetic materials, proteins, immune reactions, wound healing, development of foetus and sperm production (Abdulla *et al.*, 1993). *Euphorbia candelabrum* has been found to be highly effective for wound healing where zinc may be responsible.

### 4.5.3 Concentration of manganese in aqueous extract

Manganese content in the extracts was in the range of 4.3-45.6 µg/g. The highest level was determined in *Erythrina abyssinica* (45.6±2.7 µg/g) while the lowest was in *Markhamia platycalyx* (4.3±0.9 µg/g). This is comparable to the range of 4.30-49.1 mg/kg reported by Basgel and Erdemoglu (2006) in *Foeniculum vulgare* infusion and *Rosa caninae* infusion. *Erythrina abyssinica* is known to treat the highest number of diseases among the herbs analysed (Kokwaro, 1993). In this work the aqueous extract obtained from this herb had the highest levels of iron, manganese and zinc which enhance body resistance against many diseases.

### 4.5.4 Concentration of iron in aqueous extract

The highest level of iron was determined in extracts from *Erythrina abyssinica* (224.5±3.5 µg/g) while the lowest level was in *Prunus africana* (17.7±1.3 µg/g). Extracts from *Croton macrostachyus* contained 48.5±2.9 µg/g, *Euphorbia candelabrum* contained 47.3±2.1 µg/g while *Markhamia platycalyx* had 22.3±1.5 µg/g. Kumar *et al.* (2003) reported iron content of 476 µg/g in *Pragya-peya* infusion.
4.5.5 Concentration of vanadium in aqueous extract

Vanadium was not detected in any of the aqueous extracts at the detection limit of 0.008 μg/g. Kumar et al. (2003) reported levels of vanadium below 0.97 μg/g in aqueous extract obtained from Arjuna bark used as herbal drug.

4.5.6 Concentration of chromium in aqueous extract

The level of chromium in aqueous extracts was the lowest among the elements analysed. It was highest in Erythrina abyssinnica (4.3±0.5 μg/g) and lowest in Markhamia platycalyx (1.8±0.2 μg/g). It was however not detected in Prunus africana and Croton macrostachyus at detection limit of 0.02 μg/g. Basgel and Erdemoglu (2006) did not detect chromium in infusions obtained from various herbs except in those from Rosa caninae and Urtica dioical. Chromium compounds are considered insulin-like (Paul et al., 2007). Chromium, required for maintenance of normal glucose metabolism is directly related to the function of insulin by way of the glucose tolerance factor (GTF) and Cr (III) complexes play a key role in carbohydrate and lipid metabolism (Yang et al., 2006). The Cr-(phenylalanine)$_3$ complex has been shown to be insulin sensitive (Yang et al., 2006). In literature, Prunus africana is recommended for stomach-ache, fever and flu.

4.6 Comparison of the levels of elements in the plants with daily requirements

Comparison was made to determine how well these herbs can supplement for essential trace elements. Average daily dietary intake (ADDI) for the studied elements are given in Table 4.9
Table 4.9
Daily average up-take of elements by a person weighing 70 kg

<table>
<thead>
<tr>
<th>Element</th>
<th>ADDIs μg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc</td>
<td>15,000</td>
</tr>
<tr>
<td>Manganese</td>
<td>2,800 (2000-5000)</td>
</tr>
<tr>
<td>Iron</td>
<td>15,000 (10,000-28,000)</td>
</tr>
<tr>
<td>Vanadium</td>
<td>40</td>
</tr>
<tr>
<td>Chromium</td>
<td>50-200</td>
</tr>
</tbody>
</table>

(Mahan and Scott, 1996)

It must be realized that, in Table 4.9, the element concentrations in the aqueous extract were given for 1 g of the corresponding herb. It is therefore necessary to have a clear guideline on the use of medicinal herbs so that the amount of these trace elements consumed can be correctly established.

When the intake values listed in Table 4.9 were compared with those given in Table 4.8 and depending on the metal levels in the aqueous extracts, some of the extracts may be good supplements of essential elements. *Erythrina abyssinica* can be a good supplement for zinc, manganese, iron and chromium. To meet average daily dietary intake of zinc, about 426 g of the herb should be used to prepare 50 cm$^3$ of aqueous extract while for manganese 61 g is needed to make 50 cm$^3$ of aqueous extract. In the case of iron, 67 g of the herb is needed whereas 29 g is needed incase of chromium. It is likely that the use of this herb can meet the average daily dietary intake of manganese, iron and chromium but not zinc. This is because an average of between 50 g to 100 g is used to prepare the aqueous extracts. These extracts are mainly consumed twice a day, morning and evening (Kokwaro, 1993). The levels of elements in aqueous extracts obtained from other herbs may not meet the average daily dietary intake but can still supplement for the essential
elements. However, the consumption rate of the aqueous extracts should be under strict control.

4.7 Comparison of concentration of elements in the herbs and aqueous extracts.

The percentage of each element extracted is indicated in Table 4.10. The percentages were based on the mean concentration of the elements. It was observed that the proportion of trace elements extracted showed a wide variability.

Table 4.10
Percentage of trace elements extracted into aqueous extracts

<table>
<thead>
<tr>
<th>Medicinal plant</th>
<th>Percentage of Zn extracted</th>
<th>Percentage of Mn extracted</th>
<th>Percentage of Fe extracted</th>
<th>Percentage of Cr extracted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Markhamia platycalyx (leaves)</td>
<td>58.1</td>
<td>32.6</td>
<td>25.5</td>
<td>72</td>
</tr>
<tr>
<td>Prunus africana (leaves)</td>
<td>40.9</td>
<td>38.8</td>
<td>27.1</td>
<td>NIL</td>
</tr>
<tr>
<td>Euphorbia candelabrum (stem-bark)</td>
<td>24.2</td>
<td>19.8</td>
<td>30.6</td>
<td>78</td>
</tr>
<tr>
<td>Croton macrostachyus (leaves)</td>
<td>58.9</td>
<td>18.8</td>
<td>17.2</td>
<td>NIL</td>
</tr>
<tr>
<td>Erythrina abyssinica (stem-bark)</td>
<td>22.2</td>
<td>20.8</td>
<td>49.6</td>
<td>76.8</td>
</tr>
</tbody>
</table>

*Erythrina abyssinica* had the highest level of zinc extracted into the aqueous extract at (35.2 µg/g) which comprises of 22.2 per cent. Despite the fact that the smallest percentage of zinc was extracted from *Erythrina abyssinica* (22.2%) as compared to the other herbs, its extract is still the richest source of zinc. In *Euphorbia candelabrum* 24.2 per cent of zinc was found in the aqueous extract whereas in *Prunus africana* 40.9 per cent was found. The highest percentage of zinc in the extracts was reported in *Markhamia platycalyx* and *Croton macrostachyus* as 58.1 and 58.9 per cent respectively.
A relatively low percentage of manganese was determined in the extracts. The highest percentage of extraction was obtained from *Prunus africana* (38.8%). *Markhamia platycalyx* had 32.6 per cent of manganese in the extracts whereas *Euphorbia candelabrum*, *Croton macrostachyus* and *Erythrina abyssinica* had very close percentages extracted in the range of 19.8%, 18.8% and 20.8% respectively. Manganese is especially important for several enzymatic and biochemical processes (Abdulla et al., 1993).

*Erythrina abyssinica* had 49.6 per cent of iron being extracted. *Euphorbia candelabrum* had 30.6 per cent of iron being extracted. Among all the elements analysed, this was the highest percentage of element obtained from *Euphorbia candelabrum*. In *Markhamia platycalyx* and *Prunus africana* 25.5% and 27.1% of iron respectively was determined. The lowest level of iron determined in the extract was in *Croton macrostachyus* (17.2%).

An interesting observation is that high percentage of chromium was extracted from *Euphorbia candelabrum* (78%), *Erythrina abyssinica* (76.8%) and *Markhamia platycalyx* (72%) although its levels in aqueous extracts is low compared to levels of zinc, manganese and iron.

The aqueous extract obtained from *Erythrina abyssinica* was the richest source of trace elements even if the percentage of element extracted is high. Previous studies have shown that all the five elements are necessary to human health (Hac et al., 1997; Kuo et al., 2000). However they may be toxic in high concentrations. It must be noted that all of the
herbs considered in this study are not consumed directly but are prepared in form of aqueous extracts. In spite of the fact that the concentrations of the elements such as zinc, manganese and iron in the herbs seems high, they do not completely get extracted. Therefore, rather than the concentrations in the herbs, those in the extracts are more significant when considering the daily uptake. For example the concentration of zinc in *Croton macrostachyus* is 32.4 μg/g while that of manganese is 35.6 μg/g. However 58.9% of zinc and 18.8% of manganese leached into the extracts. Hence the extracts contain 19.1 μg/g of zinc and 6.7 μg/g of manganese. The level of element concentration in the aqueous extracts may be affected by a number of parameters such as organic matrix of corresponding herb, original mineral content and natural pH of water used to prepare the extract (Manhan, 1984). In addition, since no further acidic or basic reagent treatment was applied to the extracts solubility characteristics of mineral and organic matrix of the herb in water may be the most important one.

Correlation analysis was done for levels of these elements in plants and aqueous extracts. The correlation curves (Appendix 2-5) resulted that give the regression line. The regression lines visually depict a linear relationship between concentration of chromium in herbs and aqueous extracts as compared to that of zinc, iron and manganese. In addition to visually depicting the trend in the concentration in herbs and extracts with a regression line, the equation of the regression line was calculated to determine correlation coefficient, $R^2$. How well this equation describes the data is expressed as a correlation coefficient $R^2$. The closer $R^2$ is to 1.00, the better the fit (Valenta *et al.*, 1981). Table 4.11 shows the regression line and correlation coefficient.
Table 4.11
Regression line and correlation coefficient for levels in plants and aqueous extracts

<table>
<thead>
<tr>
<th>Element</th>
<th>Regression line</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc</td>
<td>Y = 0.1983X + 4.877</td>
<td>0.8503</td>
</tr>
<tr>
<td>Manganese</td>
<td>Y = 0.212X + 2.2981</td>
<td>0.8793</td>
</tr>
<tr>
<td>Iron</td>
<td>Y = 0.4539X - 18.703</td>
<td>0.8393</td>
</tr>
<tr>
<td>Chromium</td>
<td>Y = 0.7788X - 0.0383</td>
<td>0.9985</td>
</tr>
</tbody>
</table>

From the results in Table 4.11, correlation coefficient value for chromium is closer to 1.00 indicating that the concentration of chromium in medicinal herbs and aqueous extracts are well correlated. This implies that most of the chromium in the medicinal herbs is extracted during the preparation of the extract.

The correlation coefficient values for zinc, manganese and iron indicate deviation from 1.00. This shows a somewhat poor relationship. The implication of this is that during preparation of aqueous extracts from the medicinal herbs analysed, less zinc, manganese and iron infuses into the extract as compared to chromium.
5.1 Conclusion

• The concentration of zinc, manganese, iron, vanadium and chromium were determined in *Markhamia platycalyx*, *Prunus africana*, *Erythrina abyssinica*, *Croton macrostachyus*, and *Euphorbia candelabrum* by AAS. Concentration of zinc, manganese and chromium was also determined using DPASV. The results indicated that the mean content vary over a wide range from one medicinal herb to another. *Erythrina abyssinica* had the highest levels of all elements while *Markhamia platycalyx* leaves had the lowest levels of manganese and vanadium. *Prunus africana* leaves had the lowest levels of zinc, iron and chromium.

• Among the elements analyzed, level of iron was the highest in all plants except *Prunus africana* in which manganese levels were highest. Vanadium levels were lowest in *Markhamia platycalyx*, *Erythrina abyssinica* and *Euphorbia candelabrum* while chromium was lowest in *Prunus africana* and *Croton macrostachyus*.

• This research also showed that there is great variation in levels of trace elements in medicinal herbs and their aqueous extracts. The highest percentage extracted was that of chromium in *Euphorbia candelabrum* at 78 per cent while none was extracted from *Croton macrostachyus*. Therefore the preparation of extracts by boiling extracts small percentage of trace elements hence need to be improved.
In spite of the fact that concentration of elements such as iron, manganese and zinc in medicinal herbs analyzed was high, low levels were extracted. The highest level of zinc extracted was in *Croton macrostachyus* whereas the lowest was in *Erythrina abyssinica*. Iron which was in high concentrations in most plants had low level of it being extracted, highest being in *Erythrina abyssinica* and lowest in *Croton macrostachyus*. Though chromium concentration was low, high proportion of it was extracted, highest levels being in *Euphorbia candelabrum*. High level of manganese was extracted from *Prunus africana* whereas *Croton macrostachyus* gave the lowest. No vanadium was detected in the aqueous extracts.

### 5.2 Recommendations from this work

- Due to mineral deficiency in most people as a result of consuming over refined food this study recommends that extracts from *Erythrina abyssinica*, *Croton macrostachyus* and *Euphorbia candelabrum* be used as a supplement for those with deficiencies in these trace elements.

- With regard to the curative effect of the herbal plants and their elemental levels the study recommends further analyses of these medicinal herbs from different parts of Kenya for a cross-checking of their elemental contents and for better understanding of the therapeutic actions of medicinal herbs with regard to their elemental levels.

- Since levels of elements studied vary depending on plants, the amount consumed should be measured carefully to avoid excessive intake of one of the elements especially since
iron and manganese were much higher in most of the plants studied as compared to other elements.

5.3 Recommendations for further research

• The vast majority of the medicinal herbs products are unlicenced and are not required to demonstrate efficacy or safety. Even though they are often promoted as natural and therefore harmless, herbal remedies are, by no means, free from adverse effects due to heavy metal poisoning such as lead and mercury. The herbs may be easily contaminated during growth and processing. This study therefore recommends that further research be done to determine if these medicinal herbs contain heavy metals that can result in adverse effects to patients using them.

• The study recommends that the levels of trace elements absorbed by patients using these herbs be determined by analyzing their blood samples as well as the levels of elements in the soil where these medicinal herbs grow.

• Various solvents such as organic solvents should be used to prepare extracts in order to determine those that extract highest amounts of trace elements. Such solvents could replace water as a solvent in preparing extracts. This is especially so if the solvents can be consumed by human beings with no adverse effects.
• The nature of complexes of active compounds of herbs with elements needs to be determined by doing speciation study. It is also necessary to determine other nutrients in the herbs.

• The study also recommends that the levels of these elements in other medicinal plants be determined in order to compare with the results obtained in this study.
REFERENCES


APPENDICES

APPENDIX 1

Statistical treatment of data

1. Mean

The mean concentrations of each of the samples were calculated for triplicate determination using equation;

\[ X = \frac{1}{n} \sum_{i=1}^{n} x_i \]

Where \(x_i\) is the \(i^{th}\) term of the determination or the set of data and \(n\) is the number of determinations

2. Student t-Test

Used to determine whether two sets of data from two methods of analysis differ significantly. The t value obtained is compared to the critical value

\[ t = \frac{r / \sqrt{n-2}}{\sqrt{1-r^2}} \]

where \(r\) is the correlation coefficient given by

\[ r = \frac{\sum_i (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_i (x_i - \bar{x})^2 \sum_i (y_i - \bar{y})^2}} \]

3. Standard deviation, \(s\)

This was used to determine dispersion values about the mean. The equation below was used to calculate standard deviation.
\[ S = \sqrt{\frac{\sum_{i=1}^{n}(x_i - x)^2}{n - 1}} \]
APPENDIX 2

Fig. 4a

Correlation between levels of Zn in herbs and concoction

\[ y = 0.1983x + 4.877 \]

\[ R^2 = 0.8503 \]
Correlation between levels of Mn in herbs and concoction

\[ y = 0.2112x + 2.2981 \]

\[ R^2 = 0.8793 \]
Correlation between levels of Fe in herbs and concoction

$y = 0.4539x - 18.703$

$R^2 = 0.8393$
Correlation between levels of Cr in herbs and concoction

\[ y = 0.7748x - 0.0383 \]

\[ R^2 = 0.9985 \]
Correlation between DPASV and AAS

determination of Zn

\[ y = 0.9533x + 0.2712 \]

\[ R^2 = 0.9991 \]
Fig. 4f

Correlation between DPASV and AAS
determination of Mn

\[ y = 0.942x + 0.8034 \]
\[ R^2 = 0.9994 \]
APPENDIX 8

Fig. 4g

Correlation between DPASV and AAS
determination of Cr

\[ y = 0.8873x - 0.1023 \]

\[ R^2 = 0.9975 \]
APPENDIX 9

Fig. h

Calibration curve for Zn ion standard

\[ y = 0.0779x + 0.0004 \]

\[ R^2 = 0.9927 \]
APPENDIX 10

Fig. i

Calibration curve for Mn ion

y = 0.0886x + 0.0043
R² = 0.9959
Calibration curve for Fe ion standard

\[ y = 0.0821x - 0.0018 \]

\[ R^2 = 0.9864 \]
Fig. k

Calibration curve for Cr ion standard

\[ y = 0.085x - 0.0004 \]

\[ R^2 = 0.9967 \]
Fig. 1

Calibration curve for V ion standard

\[ y = 0.085x - 0.0018 \]

\[ R^2 = 0.9961 \]
APPENDIX 14

Photograph 1, *Euphorbia candelabrum*
APPENDIX 15

Photograph 2, *Prunus africana*
APPENDIX 16

Photograph 3, *Croton macrostachyus*
APPENDIX 17

Photograph 4, *Erythrina abyssinica*