

**ANALYSIS OF ESSENTIAL TRACE ELEMENTS IN SELECTED
MEDICINAL PLANTS USED IN KENYA**

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the Degree of Master of Science in Applied Analytical Chemistry in the School of
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DECLARATION

I hereby declare that this is my original work and has not been presented for the award of a degree in any University.

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DEDICATION

This thesis is dedicated to my dear wife Harriet Kagendo for being there during the easy and difficult moments and our sons, Steve and Manuel for providing me with the inspiration to complete this work. Also to my friend and colleague, Jack Wasike, for his invaluable moral support as I laboured to produce this work. *“The race is not for the swift or the battle to the strong, nor does food come to the wise or wealth to the brilliant or favour to the learned; but time and chance happen to them all”*(Ecclesiastes 9:11).

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ABBREVIATIONS AND ACRONYMS

DW	Dry weight
EDXRF	Energy Dispersive X-ray Fluorescence
IAEA	International Atomic Energy Agency
MCA	Multichannel Analyzer
RDA	Recommended Dietary Allowance
RDI	Recommended Dietary Intake

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ABSTRACT

Kenya is endowed with nature where hundreds of medicinal plants are available. During photosynthesis and respiration process in plants, animals and other organisms, ions of metal elements play a major role with a few of the elements being essential to the body as nutrients. Trace elements Zn, Cr, V and Se with known immunological response and healing properties were analysed from selected medicinal plants available in Kenya. These plants were; *Prunus africana*, *Urtica massaica*, *Maytenus obscura*, *Maytenus putterlickiodes*, *Azadiracta indica* (Neem), *Mondia whytei*, *Zanthoxylum usambarensense*, *Maerua edulis*, *Trigonella foenum-graecum* (fenugreek) and *Glycyrrhiza glabra*. The concentrations of elements were determined using Energy Dispersive X-ray Fluorescence Spectrometer (EDXRF). The levels of zinc varied from 25.94±1.89 to 70.58±4.70 mg/kg (mean 45.94± 12.42 mg/kg). Vanadium from 1.69±0.18 to 9.99±0.86 mg/kg with an average level of 5.89± 2.09 mg/kg. Chromium from 1.44±0.30 to 6.94±0.59 mg/kg with a mean of 3.49±1.32 mg/kg. For selenium the levels varied from 53.21±5.45 to 124.01±4.41 µg/kg with a mean of 90 ±19.17 µg/kg. The levels of the trace elements were compared with recommended dietary intake (RDI) and were found to provide these essential elements as part of therapeutic utility. The levels in different plant parts were found not to be statistically significantly different (P>0.05) except for vanadium levels in *urtica massaica* (P=0.05). The results of this study will be used to sensitize the public on the presence of essential trace elements in the studied medicinal plants and to contribute to the advancement of knowledge.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

The world population is experiencing an upsurge of diseases some of which have no cure while others are becoming resistant to the available conventional drugs. Some of these diseases are HIV/AIDS, tuberculosis, malaria, diabetes, cardiovascular diseases and cancer. There has been great effort to search for a cure of these diseases in herbal plants. Almost all diseases have a direct link to a decline or compromise on the immune system (Roitt, 1975; Hamilton *et al.*, 1988; Walsh, 1989).

World health organization (WHO) estimates that 80 % of the current world population currently uses the medicinal plants for some aspect of their primary healthcare (WHO, 1992; Obianjunwa *et al.*, 2004). Resurgent use of medicinal plants coincides with the “greening” of society and an enhanced interest in natural systems and questioning of an over-dependence on synthetic drugs to maintain health (Eisenberg *et al.*, 1993). Many conventional drugs and their precursors are derived from plants but there is a fundamental difference between administering a pure chemical and the same chemical in plant matrix. “There exist advantages of chemical complexity in herbal medicines that form a synergy with the human body” (Ron *et al.*, 2000). Trace elements are part of the chemical complexity of the herbal plants. Chemistry and bioavailability of trace elements in different plants and sources is diverse (Nicholas and Andrian, 1975). There is need to analyse the levels of trace elements in medicinal plants for their quality control (Arceusz *et al.*, 2010). Analysis of trace elements with known immunological effect and/or curing properties in herbal plants is therefore of great importance.

Many metal complexes have powerful anti-microbial activities and are already in common day-to-day use in medicine for example silver bandages for treatment of burns, zinc antiseptic creams, zinc lozenges for the common cold (Macknin, 1999), magnesium drugs for the treatment of ulcers, and metal clusters as anti-HIV drugs (Mocchegiani and Muzzioli, 2000; Kupka and Fawai, 2002). The potential for development of metal-based drugs is enormous and is of great importance with the evolution of drug-resistant bacteria and threats from a range of viral infections. Analysis of trace elements in herbal medicines is thus valuable in development of metal-based drugs. Plant based preparations containing these beneficial elements in the form of natural metallo-organic compounds are thought to be superior as they are more effective and have minimal side effects than administering these metals in the form of drugs prepared from highly refined chemical compounds (Ron *et al.*, 2000).

Selenium has been shown to cure arthritis, induce cytotoxicity to *Plasmodium falciparum*, malaria parasite, and to be active in chemoprevention of cancer (White, 1987; Tarp, 1995; Taguchi *et al.*, 2004; Klein, 2009). Its supplement restores age-related decline in immune cell functions and improves immune function and poliovirus handling in adults with marginal selenium status (Roy *et al.*, 1995; Broome *et al.*, 2004).

Chromium is known to be active in glucose metabolism thus controlling the availability of energy to the body (Murray *et al.*, 2000). Chromium supplementation enhances insulin action and has also been shown to have an effect on humoral and cell-mediated immune responses and host resistance to disease (Khangarot *et al.*, 2002).

Vanadium stimulates immunological response (Qureshi *et al.*, 1999). Zinc supplements increases the humoral response after vaccination, demonstrating antiviral activity and it activates serum thymulin, which stimulates thymocyte proliferation, producing T-cells and enhancing the antibody response (Girodon, 1999).

The medicinal plants analyzed in this study have been reported to have remedial effect on various conditions. *Urtica masaica* plant is used in management of gout since it lowers the amount of uric acid (Mitchell and Breyer, 1962). *Maytenus obscura* is used for treatment of tumors (Kokwaro, 1976). There is general lack of vigor resulting in general lethargy among those recuperating from disease and the elderly. Herbal plants such as *Trigonella foenum-graecum*, *Mondia whytei* and *Maytenus putterlickiodes* are used to boost vigour and are reputed as aphrodisiac. *Trigonella foenum-graecum* is reputed to “make an old man into a young man” (Kokwaro, 1976). *Azadirachta indica* has been used in treatment of malaria among other disease (Badam *et al.*, 1987). The plants studied are a source of biologically important elements, which may play part in therapeutic properties.

Majority of Kenyans live below the poverty line and are unable to access the conventional medical care. For such population, medicinal plants can provide a cheaper, easily available and sustainable source of medicines. Plants that can boost the immune system would be of great benefit to these people since their use would result in less infections and speedy recovery from diseases (WHO, 1992).

1.2 Statement of the Problem

The emergence of drug resistant diseases has made people look for alternative medicines. There is a rekindled interest in the use of natural products for improved health among the general population in Kenya. There is a marked proliferation of packaged herbal medicines and many manufacturing companies are exploiting this interest. Many non governmental organizations working among those infected and affected by the HIV/AIDS pandemic have created great awareness of the role of nutrition in the form of herbal medicine and foods in boosting the immune system and thus managing and controlling the progress of the infections. Herbal plants are increasingly being viewed as an extension of nutrition since they are also natural products with minimal side effects. The search for active ingredients in plant materials by phytochemists, pharmacists and chemists has been going on for a long time. There has been no major successes in the recent past of extracting these active ingredients from plants and hence no major development of medicines using this approach. The reason is that the final plant extracts have ended up not being the useful disease remedies sought. Search for new drugs in plants is focused mainly on organic compounds. There is need to get trace elements profiles for medicinal plants in order to obtain a complete picture of the plants constituents.

1.3 Purpose of Study

This study sought to analyze some known herbal medicinal plants for the presence and levels of Cr, Zn, Se and V, which have an immunological effect and are thus useful in preventing and curing of diseases. Energy dispersive x- ray florescence spectrometer was used to determine the mean levels of trace elements in various medicinal plants.

1.4 Scope and Limitations of the Study

- i. The soils on which the plants were obtained were not analyzed for the trace elements.
- ii. Many trace elements are known to have an immunological response but in this study only four were considered and from a few selected medicinal plants.
- iii. Seasonal variation may have an effect on element concentration but our study did not consider it.
- iv. Only those parts of a given plant with known medicinal application was analyzed

1.5 Hypothesis

The mean values of trace elements within different plant parts are equal.

1.6 Objectives of the Study

1.6.1 General Objective

To determine levels of some trace elements with known immunological response and curative properties in selected medicinal plants used in Kenya.

1.6.2 Specific Objective

To determine the levels of Zn, Cr, V and Se in *Prunus africana*, *Urtica doica*, *Maytenus obscura*, *Maytenus putterlickiodes*, *Azadiracta indica*, *Mondia whytei*, *Zanthoxylum usambarense*, *Maerua edulis*, *Trigonella foenum-graecum*, and *Glycyrrhiza glabra* medicinal plants using EDXRF.

CHAPTER TWO

LITERATURE REVIEW

2.1 Elemental Chemical Composition of Plants

The extent to which elemental chemical composition in plants exists, is highly variable and is governed by different factors. The common concentrations of elements in plants growing on various, but non-polluted soils show quite a large variation for each element. Adsorption by roots is the main pathway of trace elements to plants but other tissues have been observed to absorb trace elements (Kabata and Pendias, 2004). Transport of ions within plant tissue and organs involves movement in phloem and xylem, storage, accumulation and immobilization. The distribution and accumulation patterns of trace elements vary considerably for each element, kind of plant, and growth season. Elements such as copper and zinc seem to be distributed more uniformly throughout the plant (Pendias, 2004). Other elements are accumulated in roots, especially when their metal supply is sufficient (Grusak and Eduardo, 1999; Kabata and Pendias, 2004).

Trace elements are defined as mineral elements that occur in living systems in micrograms per gram or less and are considered essential for good health. These are cobalt, copper, iodine, iron, manganese, molybdenum, chromium, zinc, vanadium and selenium. Persuasive evidence has recently appeared that indicate other trace elements; boron, aluminum, arsenic, fluorine, lithium, nickel and silicon. However, conclusive evidence for essentiality such as a defined biochemical function is lacking for some of these elements (Underwood, 1977; Bowman *et al.*, 2001; Food and Nutrition Board, 2001). Metallic ions or elements form part of structural components of tissues, cofactors and co-enzymes involved in transport processes and maintenance of homeostatic balance. The essential elements have incomplete electronic configuration which contribute to the formation of chemical

bonds, attractions between positive and negative ionic charges, polarity of molecules or the electronegativity of atoms. These have a bearing on the biological functions such as the maintenance of homeostatic balance, transport processes in the cell, bio-availability and immunity (Underwood, 1977; Chandra, 1985; Balch and Balch, 2000). Trace elements of interest in this study and their levels in herbal plants are discussed in the following sections.

2.1.1 Zinc

Zinc in the earth's crust is about 65 $\mu\text{g/g}$ while in the human body it is approximately 33 $\mu\text{g/g}$ (David, 1990). It is widely distributed throughout all cells in the body but is particularly concentrated in the nucleus, and in specialized areas of the brain in the production of neurotransmitters, pancreas, adrenal gland, bones, liver, prostate and testes (Sandstead and Lofgen, 2000). In plants, it averages at 20 $\mu\text{g/g}$ (Bob *et al.*, 2000). Man's age and disease status affect dietary uptake and resorption of zinc, which is an essential component of more than 270 enzymes including RNA and DNA. It therefore contributes, via enzymes, a catalytic role, a regulatory role including controlling and co-ordinating cell growth, and a structural role. It influences various organ functions having a secondary effect on the immune system, maturation and functioning of leucocytes and affects the effects of immunostimulants (Lepage *et al.*, 1999; Rink and Gabriel, 2000; Zinpro, 2000; Serfor *et al.*, 2002). Zinc is used in the management of HIV/AIDS to prevent opportunistic infections by boosting the immune system (Mocchegiani and Muzzioli, 2000). It promotes the normal function of cells and membranes and helps in the development and repair of tissues (Hamilton *et al.*, 1988).

Deficiency of zinc results in neuromotor deficit and severe derangement (Sandstead and Lofgen, 2000). Studies have demonstrated the success of Zn supplementation in reducing infertility and

sterility through its involvement in prostate gland function and the growth and maturity of reproductive organs (Tom, 1998). The RDA for infants and children between ages 0-6 years is 3-10 mg while adolescents and adults is between 10-15 mg (NRC/NAS, 1980).

Giridhar *et al.* (2013) found the concentration of zinc to be high in *Centella asiatica*, *Vitex negundo*, *Datura metel* and *Acorus calamus* with values of 93 ± 2 mg/kg, 48 ± 1 mg/kg, 47 ± 0.6 mg/kg and 46 ± 0.3 mg/kg respectively. Djama *et al.* (2011) also found high levels of zinc in leaves of *Phyllanthus amarus*, *Isolona cooperi* and *Ocimum americanum* with values of 37.8 mg/kg, 12.1 mg/kg and 40.2 mg/kg respectively. Sakina *et al.* (2013) reported levels in ten Sudanese medicinal plants to be between 1.43 and 18.11mg/kg. Konieczynski and Wesolowski (2007) found levels in *Urtica massaica* to be 52.23 ± 1.5 mg/kg whereas Subramanian *et al.* (2012) found levels in *Trigonella faenum-graecum* to be 9.85 ± 2.54 mg/kg.

2.1.2 Selenium

Selenium in the earth's crust is about 0.05 μ g but its concentration in the human body is unknown (David, 1990). In land plants, it averages between 0.01 and 1 mg per kilogram (Horst, 1990). Selenium is required as an essential cofactor of antioxidant enzymes such as glutathione peroxidase that targets harmful hydrogen peroxide in the body protecting cells from membrane damage and oxidative stress which slows the ageing process (Sun *et al.*, 1995; Ito *et al.*, 1998; Balch and Balch, 2000) and decreases plasma and LDL lipid peroxidation, producing antiatherogenic effect, thus reducing incidences of hyperlipidaemia, hypertension and other cardio vascular diseases (Hussein, 1997; Coppinger and Diamond 2001). Selenium is important guardian of blood cells, liver and lungs. Its deficiency is associated with cancer, cardiovascular disease, diabetes and arthritis. Selenium has

been shown to cure arthritis, induce cytotoxicity to *Plasmodium falciparum* that causes drug resistant malaria and to be active in chemoprevention of cancer (White, 1987; Tarp, 1995; Fleet and Mayer, 1997; Bjelakovic *et al.*, 2004; Taguchi *et al.*, 2004; Stranges *et al.*, 2006). Oral selenium supplements have been shown to increase the plasma selenium concentration, resulting in heightened sperm motility and a decreased risk of miscarriages (Scott, 1998; Hawks and Turek, 2001). It has also been shown to increase the humoral immunity therefore reducing vulnerability to respiratory tract infections and reducing associated morbidity and mortality (Graspo *et al.*, 1989; Roy *et al.*, 1995; Girodon, 1999; Broome *et al.*, 2004).

Selenium deficiency results in a decrease in activity of Se-dependent enzymes, especially if the vitamin E status is also compromised. Lack of antioxidants in the heart, liver and muscles eventually leads to tissue death and organ failure. Low selenium status has also been a suggested cause of Keshan's disease (juvenile cardiomyopathy) and Kashin-Beck Disease-chondrodystrophy (Coppinger and Diamond, 2001; Broome *et al.*, 2004). The RDA for infants and children between 0-6 years is in the range of 0.01-0.12 mg while for adolescents and adults is 0.05-0.2 mg (NRC/NAS, 1980).

Muchemi *et al.* (2015) found selenium levels to range from 30 to 153.8 $\mu\text{g}/\text{kg}$ among spices by AAS. Giridhar *et al.* (2015) reported the levels of selenium to be slightly high in *Anisomeles malabarica* and *Anacyclus yrethrum* with values of $2.4 \pm 0.6 \text{ mg}/\text{kg}$ and $1.06 \pm 0.7 \text{ mg}/\text{kg}$ respectively. In a study of in Cote d'ivoire on medicinal plants using EDXRF, found selenium in only one plant at levels of $0.6 \pm 0.4 \text{ mg}/\text{kg}$ (Djama *et al.*, 2011).

2.1.3 Chromium

Chromium in the earth crust is ≈ 100 $\mu\text{g}/\text{kg}$ and in the human body is 0.09 $\mu\text{g}/\text{kg}$ (David, 1990). Chromium is a mineral that becomes part of a naturally occurring co-ordination complex between chromium, nicotinic acid and the amino acids glycine, glutamate, cysteine or glutathione and aids in insulin utilization, blood sugar control and it's also important in building muscle and reducing obesity (Devlin, 1997; Murray *et al.*, 2000). By controlling blood sugar, chromium helps maintain normal cholesterol levels and improves high-density lipoprotein levels. It increases humoral and cell mediated immunity lymphocytes (Chang *et al.*, 1996; Castro, 1998; Khangarot *et al.*, 2002). Insulin becomes less effective in chromium deficiency with the result of impaired glucose tolerance and the symptoms of deficiency. These include glucose intolerance, symptoms typical of diabetes and arteriosclerosis (Marks *et al.*, 1996; Murray *et al.*, 2000).

The RDA for infants and children from 0-6 years is between 0.01-0.12 mg while for adolescents and adults is between 0.05-0.2 mg (NRC/NAS, 1980).

Analysis of ten Sudanese medicinal plants recorded chromium levels range of 1.98 ± 0.3 to 13.3 ± 0.6 mg/kg (Sakina *et al.*, 2013) while a study of antiparasitic medicinal plants gave levels of Cr of 0.0 to 6.2 mg/kg (Djama *et al.*, 2011). Grankina *et al.* (2013) using the method of ICP-AES reported levels of chromium in medicinal plants to vary between 0.26 and 3.12 mg/kg. Using AAS, Odongo (2013) found the levels of chromium to vary from 0.49 ± 0.02 to 1.60 ± 0.01 $\mu\text{g}/\text{Kg}$ in herbal plants found in Tharaka Nithi County with levels in *Prunus africana* bark at 1.34 ± 0.02 and *Carissa edulis* bark at 1.38 ± 0.01 mg/kg. Cherop (2009) found the levels of chromium in stem bark of *Prunus africana* from Koibatek to be 12.31 ± 5.47 mg/kg.

2.1.4 Vanadium

In the earth crust, the concentration of vanadium is ≈ 150 mg/kg while in the human body is ≈ 0.3 mg/kg (David, 1990). Higher plants on average accumulate 1 mg/kg (Hugh, 1973).

Vanadium is present in the heart and blood vessels, kidney, spleen, liver, bone, testes, and has a role in normal iodine metabolism and/or thyroid function and inhibits cholesterol formation in blood vessels thus useful in heart attack prevention, hypertension, arteriosclerosis, improves insulin action, and mimics the function of insulin thus useful for the control of diabetes (Hamel and Duckworth, 1995; Sakurai, 2002). Vanadium stimulates immunological response. There is a greater risk of birth defects, including bone deformities, and reduced production of milk in vanadium deficient animals (Sharma *et al.*, 1981; Qureshi *et al.*, 1999). The RDA for vanadium is less than 1 mg/kg (David, 1990).

In a study of anti parasitic medicinal plants using EDXRF, levels of vanadium were found to be 13.2 ± 0.5 mg/kg and 5.5 ± 1.0 mg/kg in *Isolona cooperi* and *Mareya micrantha* leaves respectively (Djama, 2011).

A study of a perennial herbaceous plant *Sesuvium portulacastrum* using AAS found the levels of vanadium to be 4.39 ± 0.31 mg/kg in leaves and 9.76 ± 0.47 mg/kg in stems (Gathii, 2011). Diana, *et al.* (2004) found levels in flowering aerial parts of herbal plants to have a highest value of 5.02 mg/kg while in *Adhatoda vasica* leaves were 19 ± 1.11 mg/kg (Manoj *et al.*, 2014).

Table 2.1: RDA for trace elements of interest

Element	Adults and adolescents	Infants and children	Toxicity
Zn	10 – 15 mg National Academy of Sciences,1998)	3 – 10 mg	Doses larger than 25 mg may cause anaemia and copper deficiency
Se	55-70 µg (Whanger,2003)	0.01	Doses larger than 200 µg can be toxic
Cr	50-200µg (Kumplainen in <i>et al</i> ,1979)	–	Doses larger than 200 µg are toxic and may cause concentration problems and fainting
V	10µg (Whanger,2003)	–	1.8 mg toxic

2.2 Medicinal Plants

These are plants with known medicinal properties and are alternative medicine. Medicinal plants differ from the other plants in that they have a noted pharmacological effect on the health of a human being and animals when administered. The plants contain healing substances called medicaments or chemotherapeutic agents. The medicaments form medicinal preparations such as solutions, ointments, tablets and capsules and are used as prescribed. Some methods used for plant medicaments preparation are maceration, infusion and decoction. In this study, ten herbal plants were studied. These plants are; *Urtica masaica* L. (stinging nettle), *Trigonella foenum-graecum* L (fenugreek), *Glycyrrhiza glabra* L. (licorice), *Prunus africana* Kalkman (Hook F), *Azadirachta indica* A. Juss, *Zanthoxylum usambarensis* (Engl) Kokwaro, *Maytenus obscura* (A. Rich) Cuf., *Maytenus putterlickiodes* (Loes) Exell and Mendoca *Mondia whitei* (Hook F.), *Maerua edulis* (Gilg and Gilg-Ben). A brief description of each of the plant is given in the following sections.

2.2.1 *Trigonella foenum-graecum* L (Fenugreek)

The plant (Figure 1) is originally from south-eastern Europe and western Asia. The plant grows in many parts of the world including India, Sub-sahara Africa and the united States (Acharya, 2008). It is a member of the bean family and grows as an erect annual with long slender stems reaching 30 to 60 cm in height. The plant bears grey-green, tripartite, toothed leaves. White or pale yellow flowers appear in sunny seasons and develops into long, slender, sword-shaped seed pods with a curved, beak-like tip. Each pod contains about 10 to 20 small, yellowish-brown, angular seeds as shown in figure 2. These are dried to form the commercial spice. The plant thrives in full sun on rich, well-drained soils and has a spicy odor that remains on the hands after contact (Acharya, 2008).



Figure 2.1: *Trigonella foenum-graecum* plant



Figure 2.2: *Trigonella foenum-graecum* seeds

The seeds of fenugreek contain the most potent medicinal effects of the plant. A poultice made from pulverized seeds is applied locally to alleviate the pain of gout, neuralgia, boils, inflamed haemorrhoids, tumorous growth, heals cracked dry skin on hands, lips, throat and mouth wounds, abscesses, arthritis, bronchitis, tuberculosis, swollen glands and digestive problems (Gomez, 2003). Fenugreek has a reputation as a skin softener and has both an ancient and modern reputation as an aphrodisiac transforming "an old man into a young man" (Gomez, 2003). Seeds stabilizes blood

sugar in patients with diabetes and do not lower high-density lipoprotein cholesterol levels (Sharma *et al.*, 1990; 1996).

The leaves contain at least seven saponins, known as graecunins. These compounds are glycosides of diosgenin. Seeds contain 0.1% to 0.9% diosgenin and are extracted on a commercial basis (Sauvaire and Baccou, 1978; Madar and Stark, 2002). Plant tissue cultures from seeds grown under optimal conditions produces as much as 2% diosgenin with smaller amounts of gitongenin and trigogenin. The seeds also contain the saponin fenugrin B (Gangrade *et al.*, 1979; Elujoba, 1987) Several coumarin compounds have been identified in fenugreek seeds as well as a number of alkaloids (for example trigonelline, gentianine, carpaine). A large proportion of the trigonelline is degraded to nicotinic acid and related pyridines during roasting (Gupta *et al.*, 1986). The C-glycoside flavones vitexin, vitexin glycoside, and the arabinoside isoorientin have been isolated from the plant (Adamska and Lutomski, 1971). Three minor steroidal saponins also have been found in the seeds: smilagenin, sarsapogenin, and yuccagenin (Gupta *et al.*, 1986). The mucilages of the seeds of several plants, including fenugreek, have been determined and their hydrolysates analysed (Karawya *et al.*, 1980). Fenugreek gel consists chiefly of galactomannans characterized by their high water-holding capacity. These galactomannans have a unique structure and may be responsible for some of the characteristic therapeutic properties attributed to fenugreek (Madar, 2002).

2.2.2 *Glycyrrhiza glabra* L. (Licorice)

The plant (Figure 3) is commonly known as licorice and is native to Southern Europe. India and parts of Asia. It is an herbaceous perennial, growing to 1 m in height, with pinnate leaves about 7–15 cm (3-6 inch) long, with 9–17 leaflets. The flowers are 0.8–1.2 cm (1/3 to 1/2 inch) long, purple to

pale blue, produced in a loose inflorescence. The fruit is an oblong pod, 2–3 cm (1 inch) long, containing several seeds. The roots are stoloniferous as shown in figure 4 (Balakrishna, 2006).



Figure 2.3: *Glycyrrhiza glabra* plant



Figure 2.4: *Glycyrrhiza glabra* roots

Boiled liquid of the root alleviate problems of hypoglycemia, loosens bronchus secretions, treat ulcers, digestive ailments such as epigastric bloating and flatulence and helps skin in the process of elimination of toxins and promotes transpiration (Ross, 2001; Ben *et al.*, 2002; Gomez, 2003). A sweet substance obtained from the root is used in brewing, for confectionery, and for flavouring to disguise the taste of nauseous preparation (Albert, 1980; Somjen *et al.*, 2004).

The compound glycyrrhizin (or glycyrrhizic acid), found in the plant roots has been proposed as being useful for liver protection in tuberculosis therapy (Liu, 2008). Glycyrrhizin has also demonstrated antiviral, antimicrobial, anti-inflammatory, hepatoprotective and blood pressure-increasing effects *in vitro* and *in vivo*, as is supported by the finding that intravenous glycyrrhizin slows the progression of viral and autoimmune hepatitis (Liu, 2008; Chien, 2011). The plant has also demonstrated promising activity in one clinical trial, when applied topically against atopic dermatitis (Reuter, 2010). Additionally, it has also proven itself effective in treating hyperlipidaemia (a condition of high amount of fats in the blood) (Hasani, 2010), treating inflammation-induced skin

hyperpigmentation (Callender, 2011; leyden, 2011) and prevents neurodegenerative disorders and dental caries (Kannappan, 2011; Gazzani, 2012; Messier, 2012). The antiulcer, laxative, antidiabetic, anti-inflammatory, antitumour immunomodulatory and expectorant properties of the plant have been investigated (Shibata, 2000). Isoliquiritigenin (2,4,4- trihydroxychalcone) compound isolated from the plant roots has shown anti-cancer properties (Mariel *et al.*, 2010).

The scent of plant roots comes from a complex and variable combination of compounds, of which anethole is up to 3% of total volatiles. Much of the sweetness comes from glycyrrhizin, which has a sweet taste, 30–50 times the sweetness of sugar. The sweetness is very different from sugar, being less instant, tart, and lasting longer. The isoflavene glabrene and the isoflavane glabridin, found in the roots are phytoestrogens (Tamir *et al.*, 2001; Somjen *et al.*, 2004).

In a study of various medicinal plants using ICP-AES, chromium levels in licorice were found to be 2.80 ± 0.12 mg/kg (Sann, 2013) and in another study done by Grankina (2013) using Synchrotron Radiation X-ray Fluorescence Analysis (SRXFA) reported the levels of chromium to be 22 mg/kg, zinc as 30 mg/kg and Selenium as 0.09 mg/kg.

2.2.3 *Prunus africana* (Hook F) Kalkman

The plant is commonly known as the red stinkwood and is an evergreen tree native of sub-Saharan Africa, Madagascar, Sao Tome and Grand Comore. A mature tree can grow to 10-25 meters long and is open branched. The bark is black to brown with a fissured, scaly characteristic as seen in figure 5 and its fruits are bitter (Stewart, 2003). This plant is known as “muiiri” among the Kikuyu community of Kenya (Balch and Balch, 1997).



Figure 2.5: *Prunus africana* trunk

The bark extract is useful for benign prostatic hyperplasia and has been taken for various urinary difficulties including frequent urination, stangury or painful urination, urine retention, and nocturnal urination. The herb is also beneficial to prostatitis and adenomatous fibrosclerosis (Murray and Pizzorno, 1998; Kurt, 2000).

Some of the major phytochemicals are docosanol, b-sitosterol and docosyl (E)-ferulate, tetracosanol, trans-ferulic acid esters of docosanol and tetracosanol, fatty acids comprising myristic, palmitic, linoleic, oleic, stearic, arachidic, behenic and lignoceric acids; sterols (sitosterone and daucosterol) and triterpenes (ursolic acid, friedelin , 2- α -hydroxyursolic acid , epimaslinic acid and maslinic acid (Uberti *et al.*, 1990).

In a study of medicinal plants found in Tharaka Nithi County using AAS, levels of chromium in this plant bark was reported to be 1.34 ± 0.02 mg/kg and for zinc to be 1.05 mg/kg (Odongo, 2013) while Cherop (2009) reported levels of chromium in the bark to be 12.31 ± 5.47 mg/kg.

2.2.4 *Azadirachta indica* A.Juss

The tree is also known as Neem, and Indian Lilac. It is native to Indian subcontinent and is cultivated in India and the tropical regions of the world such as Indonesia, Australia, West Africa (Isman, 2006; Ahmed, 2002) and here in Kenya. The plant typically grows in tropical and semi-tropical regions. It is commonly known as “murubaine” here in kenya as a reference to its ability to cure forty diseases.

Neem is a fast-growing tree (figure 6) that can reach a height of 15–20 metres. It is evergreen with the opposite, pinnate leaves been 20–40 centimetres (7.9–15.7 inch) long, with 20 to 31 medium to dark green leaflets about 3–8 centimetres (1.2–3.1 inch) long. The terminal leaflet is often missing and the petioles are short. It has white and fragrant flowers with bisexual flowers existing on the same individual tree. The fruits are smooth (glabrous) olive-like drupe (figure 7), which varies in shape from elongate oval to nearly roundish, and when ripe are 1.4–2.8 centimetres (0.55–1.10 inch) by 1.0–1.5 centimetres (0.39–0.59 inch). The fruit skin (exocarp) is thin and the bitter-sweet pulp (mesocarp) is pale yellow and very fibrous. The mesocarp is thick with dimentions of 0.3–0.5 centimetres (0.12–0.20 inch). The white, hard inner shell (endocarp) of the fruit encloses one, rarely two or three, elongated seeds (kernels) having a brown seed coat (Chatterjee, 2003; Singh, 2009).



Figure 2.6: *Azadirachta indica* plant



Figure 2.7: *Azadirachta indica* fruits

Some of the diseases cured by the plant are arthritis, diabetes, fever, malaria, syphilis, worm infestations and also purifies and detoxifies blood (Badam *et al.*, 1987; Koul *et al.*, 1990; Kraus, 2009; NRC, 1992). It is useful as a contraceptive and in teeth cleaning (Elvin and Lewis, 1980). Its fruits and seeds are the source of neem oil, which is important for healthy hair, improves liver function, detoxify the blood, and balance blood sugar levels. Neem leaves treats skin diseases like eczema and psoriasis. Extract from its leaves is a key ingredient in non-pesticidal management, providing a natural alternative to synthetic pesticides (Isman, 2006). The seeds are ground into a powder that is soaked overnight in water and sprayed onto the crop. It acts as an anti-feedant, repellent, and egg-laying deterrent, protecting the crop from damage. The insects starve and die within a few days (Isman, 2006). Neem also suppresses the hatching of pest insects from their eggs and has been shown to avert termite attack as ecofriendly and economical agent. Neem cake is often sold as a fertilizer (Chatterjee, 2003).

In an elemental analysis of anti-diabetic medicinal plants using XRF technique, levels of zinc and Chromium were reported as 58.1 and 3.88 mg/kg in *Azadirachta indica* leaves (Ray *et al.*, 2004). In

another study using AAS, the levels of zinc and chromium were reported to be 6.08 ± 1.23 mg/kg and 0.17 ± 0.01 mg/kg respectively while Sahito *et al.* (2003), reported mean levels of 45.6 and 1.98 mg/kg for zinc and chromium in their leaves respectively.

2.2.5 *Urtica massaica* L.

The plant (Figure 8) is often called common nettle or stinging nettle. It is an herbaceous perennial flowering plant native to Europe, Asia, North America and northern Africa. It has widely spreading rhizomes and has soft green serrated leaves having stinging hairs, which act as hypodermic needles. Human and animal wastes may be responsible for elevated levels of phosphates and nitrogen in the soil, providing an ideal environment for nettles to grow (Michael *et al.*, 2003). The Kikuyu people of Kenya call it “Thabai”. It grows well in the highlands regions of Kenya (Beentje, 1994).



Figure 2.8: *Urtica massaica* plant

The leaves are used as a purgative, as diuretic and remedy for oedema, haemorrhoids, *Urticaria*, jaundice, dysentery, bronchial catarrh, eczema, diabetes, gout, prostatitis, rheumatism, high blood pressure, allergic rhinitis, natural remedy to treat or prevent baldness. The roots are recommended as a diuretic, for relief of benign prostatic hyperplasia (BPH) and other prostate problems, and in excessive menstrual bleeding, diarrhea and in intestinal worms infestation (Mitchell and Breyer, 1962; Riehemann *et al.*, 1999; Westfall, 2003; Safarinejad, 2005; Kianbakhat, 2013). Some of the

phytochemicals isolated in *Urtica doica* are histamine, acetylcholine, formic acid, serotonin and leukotrienes (Michael *et al.*, 2003).

Konieczynski and Wesolowski (2007) found zinc levels in *Urtica massaica* leaves to be 52.23 ± 1.5 , mg/kg while Iwona *et al.* (2009) found the levels of zinc in leaves to range between 19 to 25 mg/kg. Vanadium levels were found to be 10 mg/kg in a study of medicinal plants in Romania (Diana *et al.*, 2014).

2.2.6 *Zanthoxylum usambarensis* (Engl.) Kokwaro

The plant is found in Ethiopia, Kenya, Tanzania and Rwanda (Lulekal, 2008). It is a much-branched tree which can grow up to 15 m tall with conical woody protuberances 2–3 cm long ending in sharp straight thorns 5–9 mm long. The bark is rough with longitudinal ridges and furrows as deep as 5 cm, greyish brown, peeling yellow underneath. The branches have sharp straight to slightly upcurved dark red prickles 6–12 mm long. Leaves alternate imparipinnately compound with 2–8 pairs of leaflets (figure 9). Fruits (figure 10) are usually a pair of almost globose follicles (Beentje, 1994; Lulekal, 2008). The plant is known as “mugucuwa” among the Kikuyu people of Kenya. It is found in dry forests or its remnants, such as clump thickets or secondary bushlands and has white or yellow flowers (Kokwaro, 1976; Beentje, 1994).



Figure 2.9: *Zanthoxylum usambarensis* plant



Figure 2.10: *Zanthoxylum usambarensis*
Seed pods

Bark decoction serves as an emetic to treat malaria and rheumatism. The bark and roots are used as a cough mixture (Kokwaro, 1976; Beentje, 1994). Dried stem infusion treats kidney infections; fresh stem bark is crushed and used to poultice swollen joints. In Kenya and Tanzania *Zanthoxylum usambarensis* is an important medicinal plant. A stem bark and root bark decoction is commonly taken for the treatment of malaria, backache, painful joints and rheumatism, emetic and purgative, a cough mixture and to treat pneumonia (Kokwaro, 1976). The fruits and the leaves are chewed to treat mouth infections, intestinal worms, diarrhoea, dysentery, cough, vomiting and stomachache. An infusion of the fruit is mixed with milk to treat fever, sore throat, tonsillitis and chest pains. A hot decoction of the seeds treat malignant catarrhal fever and respiratory tract infections. The young twigs are used as chew sticks for dental hygiene (Lulekal, 2008).

A variety of compounds, especially alkaloids, has been isolated from the stem bark and root bark. The main alkaloids isolated from both the stem bark and root bark are the aporphine alkaloids magnoflorine, chelerythrin and the berberine alkaloid N-methyl canadine. A minor alkaloid isolated from the stem bark and root bark is the canthinone alkaloid canthin-6-one. Minor alkaloids from the stem bark are the tetrahydroprotoberberine alkaloid usambarine, the benzophenanthridine alkaloids nitidine and oxychelerythrine, the quinoline alkaloid N-methylplatydesmine, the aporphine alkaloids

tembetarine and norchelerythrine, the isoquinoline alkaloid usambanoline and the benzyloisoquinoline alkaloid oblongine. Other compounds isolated from the stem bark and root bark are sesamine and piperitol-3,3-dimethylallyl ether as well as the aliphatic amide pellitorine. From the roots the coumarins toddaculin, phellopterin, pimpinellin, toddalolactone and *o*-methylcedrelopsin were isolated. Canthin-6-one possesses a broad spectrum of fungicidal, trypanocidal and leishmannicidal activities, besides its low toxicity. Pellitorine has significant insecticidal properties (Flaas, *et al.*, 2002).

2.2.7 *Maytenus obscura* (A.Rich) Cuf.

The plant (Figure 11) is found in subtropical and tropical regions, Africa, China, Brazil, Paraguay, Uruguay and Argentina and in southern regions of Saudi Arabia (Rojo *et al.*, 2010). It is found in riverine forest, drier forest margins and evergreen bush land in Kenya (Beentje, 1994).



Figure 2.11: *Maytenus obscura* plant

A decoction of leaves is mixed with soup and drunk hot for treatment of internal injuries and cancer or tumors while bark extract has antibacterial activity (Kokwaro, 1976; Rao, 1996). It is widely used in folk medicine as antiseptic, antiasthmatic, fertility-regulating agents, antitumor, antiulcer (Rao, 1996; Mohammed and Perwez, 2014) and antidiabetic (Karau, 2014) activities.

Among the phytochemicals isolated from the plant are proteins, lipids, carbohydrates, phenols, flavonoids, saponins, triterpenoids, alkaloids and anthraquinones, sesquiterpenes, spermidines, and agarofurans (Mohamed and Perwez, 2014).

The levels of various trace elements reported by Karau (2014) in leaves of some herbal plants were Se (1.0 ± 0.00 mg/kg), Cr (59.1 ± 0.35 mg/kg), V (6.3 ± 0.04 mg/kg) and Zn (13 ± 0.03 mg/kg) and in stem were Se (1.7 ± 0.03 mg/kg), Cr (30.49 ± 0.19), V (1.2 ± 0.02 mg/kg) and Zn (17 ± 0.07 mg/kg).

2.2.8 *Maytenus putterlickiodes* (Loes.) Exell & Mendonça

The plant (figure 12) is found in Angola (Benguela, Huila); Congo (Katanga); Ethiopia; Kenya; Tanganyika; Transvaal (Kruger National Park) and dry deciduous woodland, thickets and termite mounds 200–1600 m. Shrub or small tree 1–3 m high, often bushy or straggling, with spines of up to 1.8 cm in length, terminal or axillary on short branches without latex; branches flattened, angular, reddish-brown. *M. putterlickiodes* is easily distinguishable from all other species of *Maytenus* by its pubescent leaves and large flowers with a prominent disk (Beentje, 1994).



Figure 2.12: *Maytenus putterlickiodes* plant

Roots are good for enhancement of appetite, regulating internal body injuries, treating irregular menstruation and stimulating sexual desires while leaves are used for hookworm infestation (Kokwaro, 1976; Hedberg *et al.*, 1983; Hyde, 2015).

2.2.9 *Mondia whytei* (Hook F.)

The plant (Figure 13) is a perennial, woody, rather robust and vigorous climber that grows from a large tuberous rootstock. The roots are aromatic and apparently taste like ginger or liquorice and have an aroma similar to vanilla. The leaves are attractive, large (100–300 x 50–150 mm), opposite, with a deeply notched heart-shaped base and 30–55 mm long stalks. The stipules are well developed and consist of frilly teeth. The flowers are borne in branched inflorescences. They are large and relatively short-lived (die after 3–4 days). The reddish-purple corolla lobes have a green margin. Plants flower from October to March in the Southern, and from May to August in the Northern distribution area of the species. The large fruits (75–100 x 44 mm) are almost woody and contain many seeds (Venter *et al.*, 2009). The plant is commonly known as white's ginger and as “kumukombelo” among the luhya people where the plant is commonly found in Kenya. The roots (Figure 14) are sold in towns in Kenya and other African countries. The plant has disappeared in central Kenya due to over exploitation and increase in demand for agricultural land (Mukonyi, 2002).



Figure 2.13: *Mondia whytei* plant



Figure 2.14: *Mondia whytei* roots

The roots are eaten as flavouring agent, appetizing agent, aphrodisiac and anti-depressant. The food flavoring agent has been identified as 2-hydroxy-4-methoxy benzaldehyde (Oketch, 2012). The root extract treats gonorrhoea, sexual stimulates and is given to women after childbirth (Beentje, 1994). Aqueous extract of *Mondia whytei* roots have shown androgenic effects, testosterone production and fertility of male rats (Watcho *et al.*, 2004). The roots have been formulated into a high value powder as a source of nutrients and minerals by Kenya Forest Research Institute. Zinc levels in the roots have been reported to be in the range of 30 and 70mg/kg (Mukonyi, 2001).

2.2.10 *Maerua edulis* (Gilg & Gilg-Ben)

The plant (Figure 15) is found in Eastern DR Congo, Uganda, Kenya, Tanzania, Zambia, Malawi, Mozambique, Zimbabwe, Swaziland and northern S. Africa (Beentje, 1994; Samie *at al.*, 2010). It is a much-branched, evergreen, perennial plant, with a woody rootstock that is often large and swollen, growing to a height of 1-3 metres. It varies in habit from a herbaceous plant with more or less woody stems, to a true shrub with stiff branches and it regenerates quickly from its thick woody root. The fruit is a yellow-orange, globose or ovoid capsule 15 - 30mm long, containing one to four seeds with

a sweet flavour and they are mainly eaten by children herding animals and during periods of famine (Beentje, 1994; Samie *et al.*, 2010).



Figure 2.15: *Maerua edulis* plant

Its tuber treats fungal infections, wounds and venereal diseases (Samie *et al.*, 2010; Hyde *et al.*, 2015). The roots purify water, chewed against thirst, boiled fruits are eaten as food while leaves decoction are used on sore joints (Beentje, 1994).

2.3 Analytical Methods for Determination of Trace Elements

There are a number of methods used in the determination of trace elements which include; voltammetric and potentiometric methods (Liptrot *et al.*, 1986; Mondham *et al.*, 2000), Inductively coupled plasma mass spectroscopy (ICP-MS) (Diana *et al.*, 2004; Kgabi, 2010), inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Popescu *et al.*, 2009), atomic absorption spectroscopy (AAS) (Chuang *et al.*, 2000; Khan *et al.*, 2011), flame emission spectroscopy, proton induced X-ray emission (Lockman *et al.*, 2014) and Energy dispersive X-ray fluorescence spectroscopy (EDXRF) (Sakina *et al.*, 2013). The method used in this study was EDXRF as it was easy to use, readily available, required no reagents and is a multi element analyzer.

2.3.1 Energy Dispersive X-ray Fluorescence Theory

In EDXRF analysis, the atoms in the sample are bombarded with energetic primary x-rays, causing inner shell ionization. The ionized atoms return to the ground state by transition of electrons from an outer shell to the inner shell. As a result, there is emission of fluorescence X-rays equal in energy to the difference in electron energy levels. The energy of this fluorescence X-ray emission is a function of the atomic number of the element and therefore a characteristic of that element. This relationship serves as the basis for the quantitative analysis by EDXRF. X-rays interact with the atom of the material through processes such as photoelectric absorption, incoherent scattering and coherent scattering effects (Bertin, 2006). Each interaction has a certain probability of occurring, referred to as a cross-section. The fractions of the photons that pass through the material after the interaction is given by equation

$I = I_0^{-\mu\rho d}$ Where:

I = Intensity of the photon beam after traversing an absorbing material

I_0 = Initial intensity of the photon beam

ρ = Density of the absorbing material (g cm^{-3})

d = Thickness of material

μ = Total mass absorption coefficient (sum of the scattering coefficients and the photoelectric absorption coefficient expressed in $\text{cm}^3 \text{g}^{-1}$).

For photoelectric absorption effect, the incident photon energy is completely absorbed by the electron leading to an ejection of the electron from the sub-shell. The ejected electron is called a photoelectron. For ejection to occur, the incident photon must have enough energy to overcome its binding energy in a given shell. In the energy level from which the electron has been ejected, a vacancy, “a hole” is created. To restore stability, the atom must rearrange its electrons in the various electron shells. When an electron is transferred from a higher level to a lower energy level, the atom

loses a certain amount of energy emitted from the atom in the form of electromagnetic radiation called characteristic X-ray radiation (Bertin, 2006; Beckhoff *et al.*, 2006).

Incoherent scattering effect involves a photon of primary energy colliding with a weakly bound electron assumed to be at rest. Thus, the part of the incident photon energy is converted into the kinetic energy of the struck electron with the original direction of the photon changing. Coherent scattering occurs when the low energy photons are scattered by elements with high atomic numbers, as more tightly bound atomic electrons are involved. The mass of the atomic nucleus is much higher than that of the incident photon; hence, photons do not undergo change in energy (Bertin, 2006).

The EDXRF system consists of excitation sources of which there are various excitation sources that are used. These include ^{55}Fe and ^{109}Cd . Quantitative analysis is performed by exciting the samples using a ^{109}Cd radioisotope source. The preamplifier converts the burst of electrons, resulting from the absorption of X-rays, into a voltage signal, which may be conveniently transmitted to the measurement system. It also minimizes any source of noise, which may degrade the resolution of the spectrum. The voltage amplifier converts voltage signals into 0 - 10 V range so that pulse height analysis can be performed. It also filters off low and high frequencies in order to improve signal to noise ratio and energy resolution. The detector used was a 30 mm x 30 mm x 10 mm thick Canberra Si (Li) detector located inside a crystal and having a 25 mm beryllium window entrance. The resolution of the detector was 184 eV at 5.9 KeV Mn K_{α} line.

The detector and the preamplifier assembly are mounted in the cooling system at liquid nitrogen temperature of 77 K to minimize electronic noises. Multichannel analyzer (MCA) measures heights

of amplified output pulses and converts these amplitudes into an integer number in analog-digital converter. The number of times a pulse height has been detected is accumulated in the memory (channels) address to give a distribution of pulse heights. The pulse height distribution is converted to the X-ray energy spectrum by appropriate MCA calibration. The MCA also includes a microprocessor, which is pre-programmed to perform simple data analysis operations like energy calibration, integration and subtraction of background for various qualitative and quantitative information. The software package used in this study was S100 Canberra. It acquires stores and retrieves spectral data for display. Other facilities include energy calibration, which enables peak identification and identity determination. The qualitative peak identification is facilitated by a generated chart, which has corresponding energy values of K and L lines for each element. The AXIL (Analysis of X-Ray spectra by Iterative Least square fitting) program that fits a polynomial function to the actual spectra calculates the background. Net areas of the elements of interest present in the sample are calculated after spectrum deconvolution. Excitation source holder is used as a sample holder, shielding and a collimator at the same time. Figure 2.16 is a schematic diagram of the EDXRF system (Grieken and Markowicz, 2002).

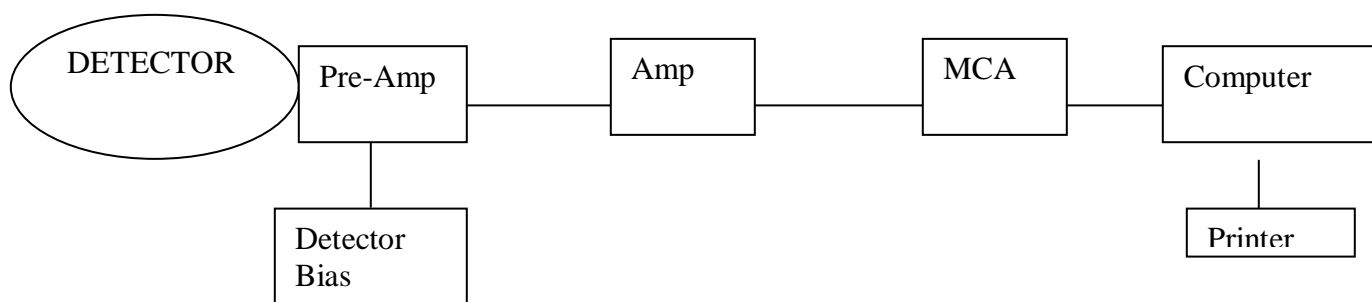


Figure 2.16: Electronic set up of the EDXRF system.

The basis of quantitative X-ray fluorescence analysis relates to the measured intensity of characteristic X-rays from the analyte and their concentration. The analyte intensity and its concentration are related by an expression, which assumes a monochromatic excitation radiation on a homogenous sample. The intensity of the radiation is given by the equation;

$$I_i = G_o \cdot K_i \cdot \varepsilon(E)_i \cdot (\rho d)_i \cdot \left[\frac{1 - \exp(-a \rho d)}{a \rho d} \right]$$

Where, the geometrical constant , $G_o = I_o \cdot \Omega_1 \cdot \Omega_2$. and the relative detection efficiency

$K_i = \sigma_i^{ph}(E) \cdot (1 - 1/J_k)_i \cdot \omega_k^i \cdot f_k^i$. The last term in the equation on intensity of radiation above represents the absorption correction factor, which depends on sample composition (IAEA, 2005).

I_o = The intensity of primary radiation which is dependent on the activity of the excitation source.

Ω_1 and Ω_2 = Solid angles of the source and the detector as seen from the sample in conformity with the geometry respectively

K_i = The relative detection efficiency.

$\sigma_i^{ph}(E)$ = The photoelectric mass absorption coefficient of element i at energy (E).

“K” = The relative probability for photoelectric effect in shell.

ω_k^i = The fluorescence yield for element i in shell “K”.

f_k^i = The ratio of the intensity of a given K line to the intensity of the whole series.

$\varepsilon(E)_i$ = the relative efficiency of the detector for photons of energy E_i .

$(\rho d)_i$ = The mass per unit area of element i in the sample.

Bertin (2006), reported the advantages of EDXRF to other systems as;

- i. Elemental analysis by use of characteristic X-ray methods is relatively easy due to specificity of the X-ray spectra.
- ii. The requirement for sample preparation is frequently minimal and reduces the cost per test.
- iii. The technique allows for analysis of samples of a variety of sizes; ie thick, moderate and thin.
- iv. The technique is relatively sensitive.

- v. There is minimal dependence on the use of prepared standards in evaluation of elemental concentration.
- vi. The system can allow for a wide range of the analyte to be determined i.e. ~1ppm to percent levels.
- vii. Systems allows for the simultaneous analysis of a wide range of elements.
- viii. The analyte may be in various forms, for example solid, liquids, powder or slurry filter.
- ix. X-ray analysis is sometimes non-destructive.
- x. For liquid samples, the uniform distribution of the precipitate on the filter paper is an added advantage.
- xi. Spectral or chemical matrix interference can be eliminated through calibration and spectral deconvolution.

Bertin (2006) also reported some limitations of EDXRF which include;

- i. Fluorescent x-rays can be easily absorbed by the sample itself (self-absorption), this requires close match of the sample matrix to that of the calibration standards.
- ii. Sample fusion's enhances the XRF measurements technique by minimizing particle size effects but sometimes refractory minerals dissolve slowly and do not give satisfactory fusion's

The detection limits (LDL) are determined using equation below;

$$\text{Det. Lim.} = \{ 3.C\sqrt{N_B} \} / N_P$$

Where:

C is the concentration of the element in parts per million (ppm).

N_p is the net peak area for the element, and

N_B is the net background area under the element peak.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample Collection and Pretreatment

Fenugreek seeds, Licorice roots and *Mondia whytei* roots were acquired from Ngara, Kawangware, Kangemi and River-road markets respectively in Nairobi county. *Prunus africana*, *Z. usambarensis*, and *M. obscura* were obtained from Thogoto forest in Kiambu County, and Ngong forest in Nairobi County at different sites. *Urtica massaica* was sourced from Limuru in Kiambu county and Narok county, *Azadirachta. Indica* was from Voi in Kwale county and Ngong forest in Nairobi County. *Maerua edulis* plant was acquired from Embu County while *Maytenus putterlickiodes* was sourced from Kangundo in Machakos County. The samples were collected from mature plants. The leaves for analyses were evenly mixed between young and old leaves to provide a reliable average. Where a given species was sourced from different places, the samples were evenly mixed. The samples were collected in clear plastic paper bags, washed with distilled water, chopped or cut into small pieces and open dried. The leaves were ground into a fine powder using blender model CT/404. The stem barks and roots were ground in a Wiley mill separately.

3.2 EDXRF Standards

Certified reference material (Bowen Kale) supplied by International Atomic Energy Agency (IAEA) were analysed. The results of the analysis needed to be within the IAEA and were compared with the IAEA certified range to ensure reliability of the analytical method.

3.3 EDXRF Instrumentation

The EDXRF spectrometer used consisted of Cd-109 radioisotopes source; Si (Li) detector, an ORTEC spectroscopy shaping amplifier model 571, ORTEC high voltage supply bias model 459, ORTEC liquid nitrogen monitor, a Canberra multichannel analyzer (S-100) interfaced with a 486 personal computer. The system was calibrated and validated (using reference material “Bowen kale” and was optimized by adjusting the amplifier’s shaping and peaking times desirably in order to obtain an optimum energy resolution and count rate performance.

The Canberra S-100 multi-channel analyzer interfaced with a 486 personal computer was used for spectral data storage and quantitative analysis done using AXIL and QAES software (IAEA, 2005). The energy resolution of the Si (Li) detector used was 195 eV for manganese (Mn) $K\alpha$ line at 5.9 Kev. For each pellet sample, the intensity measurements were taken on sample alone and sample with multi-element target accordingly for correction of absorption matrix effects. Corrections were applied using Emission Transmission Technique (IAEA, 2005). Subsequently, elemental concentration values were calculated using the intensity equation developed for intermediate samples based on fundamental parameters. The prepared pellet was irradiated with Cd-109 radioisotope source for 5000 seconds and 100 seconds for corrections of absorption with a molybdenum target (Bertin, 2000; Jenkins, 1999; Markowicz, and Van-Grieken, 2002).

3.4 Sample Preparation and Analysis

The sample, in powdered form, was passed through sieves of different sizes to obtain particle sizes of approximately less than 50 μm . For each sample, fine pellets 2.5 cm in diameter weighing 200

mg/cm² were prepared for EDXRF analysis. This was done by adding approximately 0.5 g of sample in a steel die and applying 10-15 tons of pressure (Jenkins, 1999).

Three pellets were prepared for analysis. Each pellet sample was irradiated using a ¹⁰⁹Cd radioactive source for a period of 5000 seconds. For purposes of correcting matrix effects the following steps were taken; the intensity measurements were taken on sample alone, sample with multielement target and multi target alone. Corrections were applied using emission transmission technique (Van-Grieken and Markowicz, 2002). Subsequently, elemental concentration values were calculated using the intensity equation developed for intermediate samples based on fundamental parameters (IAEA, 2005). In this study, the concentrations of the following elements were determined; zinc, chromium, vanadium and selenium.

3.5 Statistical Analysis

Analysis of variance was done by SPSS statistical package. Standard error was used to give differences between the treatment means. Spectral data analysis for elemental concentration was done using MCA S-100, AXIL and QAES computer software.

CHAPTER FOUR

RESULTS, DISCUSSION, CONCLUSION AND RECOMMENDATIONS

4.1 Results and discussion

The results of the evaluation of analytical procedure are presented in table 4.1 and are used for the validation of the analytical method. The results of the concentration levels of trace elements observed in the various plants are presented in tables 4.2 to table 4.5, discussed and analysis of variance done

4.1.1 Element Levels in Reference Material

The element levels obtained for Bowen Kale standard reference material are shown in Table 4.1.

Table 4.1: Elemental analysis of Bowen kale compared to the certified values (mg/kg)

Element	Experimental	Certified	% error
Ti	3.33±0.30	3.19±1.40	4.20
Mn	15.1±2.80	14.2±1.60	6.34
Fe	111.5±3.50	119.3±4.80	6.54
Zn	35.49±1.70	32.29±2.70	9.91
Cu	4.45±0.60	4.89±0.66	9.00
Se	0.12±0.18	0.13±0.10	7.69
Pb	2.25±0.90	2.49±0.50	9.64
Br	22.35±0.60	24.9±2.40	10.24
Sr	78.0±4.80	75.7±2.93	3.04
Ni	0.85±0.02	0.90±0.01	5.56

The average accuracy of the determinations was found to be within the recommended range of ±10%. This was an indication that calibration was done well and the results of the analysis would be reliable.

4.1.2 Zinc Levels

The levels of zinc are presented in table 4.2.

Table 4.2: Zinc levels (mg/kg)

<i>Sample</i>	<i>Part(s)used</i>	<i>Mean± SD n = 3</i>	<i>P-Value</i>
<i>Urtica massaica</i>	Leaves	70.58±4.70	0.325
	Roots	65.61±4.13	
<i>Maytenus obscura</i>	Leaves	59.97±4.68	0.609
	Bark	43.47±3.90	
<i>Maytenus Putterlikioides</i>	Leaves	43.59±2.12	0.461
	Roots	40.48±1.52	
<i>Azadirachta indica</i>	Leaves	45.18±5.10	0.845
	Bark	37.51±3.90	
	Fruits	33.27±2.18	
<i>Prunus Africana</i>	Leaves	57.47±3.14	0.795
	Bark	43.16±2.61	
<i>Zanthoxylum usambarensis</i>	Leaves	37.82±4.98	0.568
	Roots	46.03±4.8	
	Bark	29.46±2.07	
<i>Mondia whytei</i>	Roots	55.91±4.78	
<i>Maerua edulis</i>	Leaves	57.33±3.03	0.526
	Roots	42.54±1.54	
	Fruits	25.94±1.89	
<i>Glycyrrhiza glabra</i>	Roots	54.22±4.75	
<i>Trigonella Foenum-graecum</i>	seeds	29.27±2.82	

The analysis of variance at $\alpha=0.05$ show $P > 0.05$ so we fail to reject the null hypothesis. There is no statistical significant difference in the mean of zinc found in the different parts of a given plant i.e all the mean values are not different for a given plant.

The levels were highest in *Urtica massaica* leaves and roots which were found to have mean values of 70.58±4.70 mg/kg and 65.61±4.13 mg/kg respectively. There was no significant difference between levels in leaves and roots ($p > 0.05$). Konieczynski and Wesolowski (2007) found zinc

levels in *Urtica massaica* leaves to be 52.23 ± 1.5 mg/kg comparable to 65.61–70.58 mg/kg in this study while Iwona *et al.* (2009) found the levels to be in the range of 19 to 25 mg/kg. This shows that even for similar species, the habitat affected the trace element levels an observation made in other studies (Underwood, 1997). Zinc levels in *Urtica massaica* grown in different soils were 113 mg/kg (Tack and Verloo, 1996). The very high levels in this study were due to differences in soil characteristics.

The levels of zinc in *Maytenus obscura* were high in leaves and stem bark with mean values of 59.97 ± 4.68 and 43.47 ± 3.90 mg/kg respectively. The mean levels in both parts were not significantly different ($p > 0.05$). The leaves extracts of *Maytenus obscura* are used for treatment of internal injuries, and as fertility regulating agent while the bark extract have antibacterial activity conditions that are correlated with zinc therapy (Rink and Gabriel, 2000; Zinpro, 2000). Karau (2014) found levels of Zn in leaves to be 13mg/kg and in stem bark to be 17 mg/kg using EDXRF, which are far lower than those in this study. *Maytenus putterlikoioides* has reasonably high values of 43.59 ± 2.12 mg/kg in leaves and 40.48 ± 1.52 mg/kg in roots. These levels are not significantly different ($p > 0.05$) to each other. Root extracts are used for internal body injuries (Kokwaro, 1976). The levels in *Azadirachta indica* leaves, stem bark and fruits were also relatively high with mean values of 45.18 ± 5.10 mg/kg, 37.51 ± 3.90 mg/kg and 33.27 ± 2.18 mg/kg respectively. Levels between leaves, bark, and fruits were not significantly different ($p > 0.05$).

Prunus africana had levels in leaves and bark of 57.47 ± 3.14 mg/kg and 43.16 ± 2.61 mg/kg respectively which were not significantly different ($p > 0.05$), with the levels in leaves showing a higher concentration. For zinc requirements, leaves would provide higher levels than the bark. The

bark extract is used for prostate conditions (Tom, 1998). Ondongo (2013) reported levels of zinc in the bark to be 1.05 mg/kg using EDXRF, a level that is lower than the one reported in this study.

The zinc levels in *Zanthoxylum usambarensis* leaves, roots and stem bark were 37.82 ± 4.98 mg/kg, 46.03 ± 4.8 mg/kg and 29.46 ± 2.07 mg/kg respectively. The levels between leaves, roots and bark were not significantly different ($p > 0.05$). *Mondia whytei* and Licorice roots had high levels of zinc of 55.91 ± 4.78 mg/kg and 54.22 mg/kg respectively. Licorice has anti-microbial and anti-inflammation activities as well as healing of atopic dermatitis (Callender, 2011) while *Mondia whytei* is a sexual stimulant which has also androgenic effects (Beentje, 1994; Watcho, *et al.*, 2004), conditions well correlated with zinc functions. Mukonyi (2001) reported levels of between 30 and 70mg/kg in *Mondia whytei* while Grankina *et al.* (2013), reported values of 30mg/kg in licorice. These values were close to those obtained in this study. Fenugreek seeds had the lowest levels of zinc of 29.27 ± 2.82 mg/kg. A poultice of the seeds alleviates pain in gout, inflamed haemorrhoids and heals cracked skin (Gomez, 2003). The seeds are used as spices in cooking and are therefore a good source of dietary zinc. The levels of zinc in *Maerua edulis* leaves, roots and fruits were 57.33 ± 3.03 mg/kg, 42.54 ± 1.54 mg/kg and 25.94 ± 1.89 mg/kg respectively. These levels are not significantly different ($p > 0.05$) from each other. The fruits had the lowest while the leaves had the highest levels of zinc. The tuber is used in the treatment of fungal infections, wounds and sore joints conditions, which are correlated with zinc (Beentje 1994 and Hyde *et al.*, 2015).

Glycyrrhiza glabra roots had mean zinc levels of 54.22 ± 4.75 while in *Trigonella Foeniculum-graecum* The levels were 29.27 ± 2.82 .

The levels of zinc were compared with the RDI value of 12.5 mg/kg (National Academy of Science, 1998) and were found to be significantly high ($P < 0.5$). That leaves had generally high levels of zinc followed by the roots and bark while the fruits and seeds had the lowest. The mean levels were found not to be statistically significantly different in the various plant parts. *Urtica massaica* leaves were identified as the best source for zinc.

4.1.3 Vanadium levels

The levels of vanadium are presented in table 4.3

Table 4.3: Vanadium levels (mg/kg)

<i>Sample</i>	<i>Part(s)used</i>	<i>Mean± SD</i> <i>n = 3</i>	<i>P-Value</i>
<i>Urtica massaica</i>	Leaves	5.31±1.36	0.05
	Roots	4.26±0.48	
<i>Maytenus obscura</i>	Leaves	7.83±0.59	0.783
	Bark	5.05±0.23	
<i>Maytenus Putterlikoioides</i>	Leaves	5.38±0.53	0.384
	Roots	5.29±0.44	
<i>Azadirachta indica</i>	Leaves	6.74±0.49	0.783
	Bark	3.81±0.29	
	Fruits	5.19±0.42	
<i>Prunus africana</i>	Leaves	7.60±0.42	0.935
	Bark	5.48±0.52	
<i>Zanthoxylum usambarensis</i>	Leaves	3.96±0.30	0.803
	Roots	1.69±0.18	
	Bark	3.48±0.29	
<i>Mondia whytei</i>	Roots	6.63±0.55	
<i>Maerua edulis</i>	Leaves	5.67±0.48	0.998
	Roots	9.00±0.44	
	Fruits	6.65±0.45	
<i>Glycyrrhiza glabra</i>	Roots	9.99±0.86	
<i>Trigonella foenum-graecum</i>	seeds	9.19±0.65	

The analysis of variance at $\alpha=0.05$ show $P > 0.05$ so we fail to reject the null hypothesis. There is no statistical significant difference in the mean of vanadium found in the different parts of a given plant

except in the case of *urtica massaica* where $p=0.05$ in which case we reject the null hypothesis. There is a statistical significant difference in the mean of vanadium found in *urtica massaica* leaves and its roots i.e all the means are different.

Vanadium levels were high in licorice roots, fenugreek seeds and *Maerua edulis* roots with mean values of 9.99 ± 0.86 mg/kg, 9.19 ± 0.65 mg/kg and 9.00 ± 0.44 mg/kg respectively. Fenugreek seeds stabilize blood sugar in patients with diabetes and do not lower high-density lipoprotein cholesterol (Sharma *et al.*, 1990; 1996). Licorice has antidiabetic properties, treat hyperlipidaemia and improves immune function (Hasani *et al.*, 2010; Shibata, 2000; Chien, 2011). All these conditions are well correlated with vanadium functions. which among others; control diabetes mellitus (Sakurai, 2002), prevent heart attack and stimulates immune response (Sharma *et al* 1981; Qureshi *et al.*, 1999). At RDA levels of 0.01mg, these two plants can readily provide such requirements at about 1g of sample. Fenugreek is used as a spice and licorice as a flavouring agent and the amounts involved in such processes would provide the RDI (0.01mg/kg) very easily without reaching the toxicity level of 1.8mg.

Levels of vanadium in *Urtica massaica* leaves and roots are 5.31 ± 1.36 mg/kg and 4.26 ± 0.48 mg/kg respectively. The mean levels are significantly different ($p=0.05$) from each other. The leaves are a better source of vanadium as they are consumed as a vegetable. Diana *et al.* (2004) found a mean level of 10 mg/kg in the leaves a level higher than that found in this study. Vanadium levels in *Maytenus obscura* leaves and stem bark were found to be 7.83 ± 0.59 mg/kg and 5.05 ± 0.23 mg/kg respectively. The two levels are not statistically significantly different ($p>0.05$). The levels in *Maytenus putterlikioides* leaves was 5.38 ± 0.53 mg/kg and its roots was 5.29 ± 0.44 mg/kg which were not significantly different ($p>0.05$) from each other. *Azadirachta indica* plant had vanadium levels of

6.74±0.49 mg/kg, 3.81±0.29 mg/kg and 5.19±0.42 mg/kg for the leaves, bark, and fruits respectively. The levels between the various parts are not statistically significantly different ($p>0.05$) from each other.

In *Prunus africana* plant, vanadium levels in leaves and bark were 7.60±0.42mg/kg and 5.48±0.52mg/kg respectively. The levels are not significantly different ($p>0.05$). In *Zanthoxylum usambarensis* plant, the levels for the leaves, roots and bark were 3.96±0.3 mg/kg, 1.69±0.18 mg/kg and (3.48±0.29 mg/kg) respectively. Levels are not significantly different ($P>0.05$) from one another. Vanadium levels in *Maerua eduli* leaves, roots and fruits were 5.67±0.48 mg/kg, 9.00±0.44 mg/kg and 6.65±0.45 mg/kg respectively. The levels are not significantly different ($p>0.05$). *Mondia whytei* has levels of 6.63±0.55 mg/kg in its roots. The roots have been used as flavouring and appetizing agent and it can provide a good source of vanadium. *Glycyrrhiza glabra* roots had mean vanadium levels of 9.99±0.86 while in *Trigonella Foenum-graecum* the levels where 9.19±0.65.

4.1.4 Chromium levels

The levels of chromium are presented in table 4.4.

Table 4.4: Chromium levels (mg/kg)

Sample	Part(s)used	Mean± SD n = 3	P-Value
<i>Urtica massaica</i>	Leaves	3.61±0.29	0.947
	Roots	3.36±0.34	
<i>Maytenus obscura</i>	Leaves	2.47±0.22	0.235
	Bark	2.16±0.21	
<i>Maytenus Putterlikioides</i>	Leaves	4.00±0.29	0.878
	Roots	4.39±0.41	
<i>Azadirachta indica</i>	Leaves	5.09±0.46	0.825
	Bark	2.72±0.19	
	Fruits	3.17±0.29	
<i>Prunus africana</i>	Leaves	4.16±0.17	0.592
	Bark	3.73±0.36	
<i>Zanthoxylum usambarensis</i>	Leaves	1.90±0.13	0.992
	Roots	2.90±0.22	
	Bark	1.44±0.12	
<i>Mondia whytei</i>	Roots	3.03±0.28	
<i>Maerua edulis</i>	Leaves	4.10±0.20	0.896
	Roots	5.13±0.22	
	Fruits	2.66±0.19	
<i>Glycyrrhiza glabra</i>	Roots	3.85±0.31	
<i>Trigonella foenum-graecum</i>	Seeds	6.94±0.59	

The analysis of variance at $\alpha=0.05$ show $P > 0.05$ so we fail to reject the null hypothesis. There is no statistical significant difference in the mean of chromium found in the different parts of a given plant i.e all the mean values are not different for a given plant.

Trigonella foenum graecum (Fenugreek) seeds had the highest level of Chromium of 6.94 ± 0.59 mg/kg. The seeds extract has been known to stabilize blood sugar in diabetic and a positive effect on high blood pressure (Sharma *et al.*, 1990; 1991.; Gomez, 2003). At RDA values of between 0.05 and 0.2 mg, just about 30 g of the seeds would be enough to provide the RDI levels in the diet.

Chromium analysis in different spice and herbal plant species from western Anatolia, Turkey found levels varied from 0.1–9.7 mg/kg (Umit *et al.*, 2005). Values comparable to those obtained in this study. The levels were also high in *Maerua edulis* roots/tubers at 5.13 ± 0.22 mg/kg with its leaves having levels of 4.10 ± 0.20 mg/kg and fruits a low value of 2.66 ± 0.19 mg/kg. The levels in the plant parts were not significantly different ($P > 0.05$).

Levels in *Urtica massaica* were low, leaves (3.61 ± 0.29 mg/kg) and roots (3.36 ± 0.34 mg/kg). The levels between the various parts were not significantly different ($P > 0.05$). The leaves are consumed as a vegetable and a consumption of about 70g would provide an RDA of 0.2mg. The leaves extract are used against diabetes and high blood pressure conditions correlated with vanadium (Kianbakhat, 2013). *Maytenus obscura* leaves (2.47 ± 0.22 mg/kg) and bark (2.16 ± 0.21 mg/kg) levels are low and are significantly different among the parts. Karau (2014) found the levels to be 5.91 ± 0.35 mg/kg. *Maytenus putterlickioides* levels are slightly higher with leaves 4.00 ± 0.29 mg/kg and roots 4.39 ± 0.41 mg/kg. The levels between the different parts are not significantly different. *Azadirachta indica* leaves have high levels 5.09 ± 0.46 mg/kg with the bark (2.72 ± 0.19 mg/kg), and fruits (3.17 ± 0.29 mg/kg) having low values. The levels are not significantly different for the different plant parts ($P > 0.05$). The leaves have been reported as a remedy for the treatment of diabetes (Murray *et al.*, 2000). Other studies have found levels in leaves to be 3.88 mg/kg (Ray *et al.*, 2004) and 1.98 mg/kg (Sahito *et al.*, 2003). These values were slightly lower than those found in this study. *Prunus africana* levels in leaves (4.16 ± 0.17 mg/kg) and bark (3.73 ± 0.36 mg/kg) were relatively high and were significantly different. Odongo (2013) found the levels to be 1.34 ± 0.02 mg/kg while Cherop (2009) found values of 12.31 ± 5.47 mg/kg. *Zanthoxylum usambarensis* had low levels that were not significantly different among the plant parts. The levels were; leaves (1.90 ± 0.13 mg/kg), bark

(2.90 ± 0.22 mg/kg) and bark (1.44 ± 0.12 mg/kg). Licorice roots had levels of 3.85 ± 0.31 mg/kg) which are relatively high. Sann, (2013) found levels of 2.8 ± 0.12 mg/kg while Grankina *et al* (2013) reported high values of 22 mg/kg. *Mondia whytei* levels in the roots were 3.03 ± 0.28 mg/kg. The level of chromium in all the plant samples was compared with RDI value of 0.2 mg/kg and was found to be significantly high ($P > 0.5$).

4.1.5 Selenium levels

The levels of selenium in the different herbal plants are presented in table 4.5.

Table 4.5: Selenium levels ($\mu\text{g}/\text{kg}$)

Plant Species	Part(s)used	Mean \pm SD	P- value
<i>Urtica massaica</i>	Leaves	86.75 \pm 7.94	0.343
	Roots	124.01 \pm 4.41	
<i>Maytenus obscura</i>	Leaves	115.55 \pm 7.72	0.195
	Bark	104.57 \pm 2.78	
<i>Maytenus Putterlikoioides</i>	Leaves	99.56 \pm 5.03	0.743
	Roots	83.86 \pm 2.05	
<i>Azadirachta indica</i>	Leaves	97.05 \pm 5.55	0.806
	Bark	90.66 \pm 4.86	
	Fruits	78.88 \pm 4.41	
<i>Prunus africana</i>	Leaves	74.78 \pm 1.91	0.767
	Bark	72.47 \pm 2.66	
<i>Zanthoxylum usambarensis</i>	Leaves	86.09 \pm 4.45	0.266
	Roots	122.33 \pm 11.20	
	Bark	79.72 \pm 5.27	
<i>Mondia whytei</i>	Roots	53.21 \pm 5.45	
<i>Maerua edulis</i>	Leaves	94.76 \pm 3.57	0.911
	Roots	111.43 \pm 2.04	
	Fruits	103.16 \pm 6.60	
<i>Glycyrrhiza glabra</i>	Roots	83.27 \pm 7.10	
<i>Trigonella foenum-graecum</i>	Seeds	66.19 \pm 4.37	

The analysis of variance at $\alpha=0.05$ show $P > 0.05$ so we fail to reject the null hypothesis. There is no statistical significant difference in the mean of selenium found in the different parts of a given plant i.e all the mean values are not different for a given plant.

Selenium levels ranged from 53.21 ± 5.45 $\mu\text{g/kg}$ for *Mondia whytei* to 124.01 ± 4.41 $\mu\text{g/kg}$ for *Urtica massaica* roots with an average of 90 ± 19.17 $\mu\text{g/kg}$ and a range of 70.80 $\mu\text{g/kg}$. In *Urtica massaica*, the levels in roots 124.01 ± 4.41 $\mu\text{g/kg}$ were not statistically significantly different from those in leaves 86.75 ± 7.94 $\mu\text{g/kg}$ ($p > 0.05$). *Maytenus obscura* has equally high levels in leaves (115.55 ± 7.72 $\mu\text{g/kg}$) and bark (104.57 ± 2.78 $\mu\text{g/kg}$) with the levels not significantly different ($P > 0.05$). The levels of selenium in *Maytenus putterlikoioides* leaves and roots were 99.56 ± 5.03 $\mu\text{g/kg}$ and 83.86 ± 2.05 $\mu\text{g/kg}$ respectively. The levels in the two parts are not statistically significantly different ($P > 0.05$). Selenium levels in *Azadirachta indica* leaves, bark and fruits were 97.05 ± 5.55 $\mu\text{g/kg}$, 90.66 ± 4.86 $\mu\text{g/kg}$ and 78.88 ± 4.41 $\mu\text{g/kg}$ respectively. There was no statistical significant difference between leaves, bark and fruits ($P > 0.05$). Selenium level in *Prunus africana* leaves and bark were 74.78 ± 1.91 $\mu\text{g/kg}$ and 72.47 ± 2.66 $\mu\text{g/kg}$ respectively. The levels were not significantly different ($P > 0.05$). In *Zanthoxylum usambarenses*, selenium levels in leaves, roots and bark were 86.09 ± 4.45 $\mu\text{g/kg}$, 122.33 ± 11.20 $\mu\text{g/kg}$ and 79.72 ± 5.27 $\mu\text{g/kg}$ respectively. The levels in leaves, roots and bark are not significantly different ($P > 0.05$). In *Maerua edulis*, selenium levels in leaves (94.76 ± 3.57 $\mu\text{g/kg}$), roots (111.43 ± 2.04 $\mu\text{g/kg}$) and fruits (103.16 ± 6.60) are not significantly different as shown in Table 6. The levels in *Glycyrrhiza glabra* and *Trigonella foenum-graecum* were 83.27 ± 7.10 $\mu\text{g/kg}$ and 66.19 ± 4.37 $\mu\text{g/kg}$ respectively (Table 6).

In a study of other plants with medicinal properties selenium levels were found to range from 30 to 153.8 µg/kg among spices, which are close to those obtained in this study (Muchemi *et al.* 2015) The levels of selenium in herbal plants were compared with the RDI requirement of 55-70 µg/kg and found to be significantly high ($p>0.05$).

4.2 Conclusion

This study analysed the presence and levels of Zn, Cr, Se and V in some selected herbal plants used for treatment of various diseases. All the four elements were present in the plants at varying concentration. The mean levels in different plant parts of a given plant were not statistically significantly different for almost all cases ($p>0.05$) except for vanadium levels in *urtica massaica* leaves and roots ($p<0.05$). Therefore we fail to reject the null hypothesis in the former cases but we reject the null hypothesis in the later.

These plants contain appreciable concentration levels of the trace elements which have shown healing and immunological functions. The leaves had generally higher levels of the trace elements for a given species than the roots or fruits. *Urtica doica* leaves had the highest levels of Zn and Se, *Glycyrrhiza glabra* roots the highest levels of V while *Trigonella foenum-graecum* (imported from India) had highest levels of Cr. The data obtained in this study enhances the understanding of the pharmacological effects of the medicinal plants and provide useful information for formulation of dosages, quality assurance and safety information.

4.3 Recommendations

4.3.1 Recommendations From This Study

- i. *Urtica massaiica* showed high values of the studied trace elements and the fact that it is eaten as a vegetable, it`s use should be encouraged.
- ii. Fenugreek seeds have also shown high levels of trace elements and is also consumed as a spice and as a legume It`s cultivation is thus recommended.

4.3.2 Recommendation for Further Work

- i. In the course of the study, it was observed that important species were facing pressure of over exploitation and we recommend that those plants be protected to conserve the species.
- ii. There is need to determine the percentage levels of extractable trace elements from the herbal plants by the various methods of preparation.

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APPENDIX

Publications

Njue, W., Wanjau, R. and Njenga, I. (2016). Analysis of Essential Trace Elements by EDXRF in Selected Medicinal Plants Used in Kenya. *The International Journal of Science & Technoledge*, 4:1

ABSTRACT

Kenya is endowed with nature where hundreds of medicinal plants are available. During photosynthesis in plants and respiration in animals and other organisms, metal elements play a major role with a few of the elements being essential to the body as nutrients. Trace elements for example Zn, Cr, V and Se with known immunological response and healing properties were analysed in selected medicinal plants available in Kenya. These plants were; *Prunus africana*, *Urtica massaica* (stinging nettle), *Maytenus obscura*, *Maytenus putterlickiodes*, *Azadiracta indica* (Neem), *Mondia whytei*, *Zanthoxylum usambarensense*, *Maerua edulis*, *Trigonella foenum-graecum* (fenugreek) and *Glycyrrhiza glabra* (licorice). The concentrations of elements were determined using Energy Dispersive X-ray Fluorescence Spectrometer (EDXRF). The levels of zinc varied from 25.94±1.89 to 70.58±4.70 mg/kg (mean 45.94± 12.42 mg/kg). Vanadium from 1.69±0.18 to 9.99±0.86 mg/kg with an average level of 5.89± 2.09 mg/kg. Chromium from 1.44±0.30 to 6.94±0.59 mg/kg with a mean of 3.49±1.32 mg/kg. Selenium the levels varied from 53.21±5.45 to 124.01±4.41 µg/kg with a mean of 90 ±19.17 µg/kg. The levels of the trace elements were compared with recommended dietary intake (RDI) and were found to provide these essential elements as part of therapeutic utility. The levels in different plant parts were found to vary significantly (P<0.05) and in some cases not significantly (P>0.05) for a given species. The plants can provide the elements as per required daily intake (RDI)

Key words: Trace elements, EDXRF, Medicinal plants