PROXIMATE AND ESSENTIAL NUTRIENTS EVALUATION OF TWO INDIGENOUS VEGETABLES: *COMMELINA AFRICANA AND AMARANTHUS THUNBERGII* FROM AINAMOI DIVISION, KERICHO COUNTY

BY

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DECLARATION

Declaration by the Student

I declare that this thesis is my original work and has not been presented for examination in any academic institution. No part of this work may be reproduced without the prior permission of the author and/or University of Eldoret.

Signature…… Date……

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Declaration by the Supervisors

This thesis has been submitted for examination with our approval as University supervisors.

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DEDICATION

This thesis is dedicated to my family and my parents for their inspiration, moral and financial support throughout the time i was carrying out this study.

ABSTRACT

In Kenya there are many indigenous leafy vegetables available. Despite this there is inadequate information on their nutritional diversity and how they can best be used in reducing malnutrition. This study was carried out to investigate the nutritional and mineral diversity of *Amaranthus thunbergii* and C*ommelina africana.* The vegetables were harvested 3-4 weeks after germination during the rainy season in various farms in Ainamoi, Kericho County. Soil samples where the vegetables grow were also collected. A total of one kilogram of each vegetable sample was collected. The samples were hand washed with tap water to remove soil particles and oven dried at 60 ^{0}C for 24 hours. They were then reduced to fine powder using a mechanical blender. The vegetables were analyzed for their proximate contents using Association Official of Analytical Chemists (AOAC) methods. Mineral concentration was determined using AAS and colorimetry. The soil samples were analyzed for the minerals in order to compare it's correlation with that in the vegetables. The results showed that moisture content was lower in *Commelina africana* (4.13%) than in *Amaranthus thunbergii* (4.25%) *Commelina africana* had a higher protein content (16.05%).The fat content of *Amaranthus thunbergii* (4.69%) was significantly higher than that of *Commelina africana* (1.72%). The crude fiber content 9.02% and 10.87% for *Amaranthus thunbergii* and *Commelina africana* respectively. These were comparable with that of other vegetables. The minerals detected in the vegetables and in the soil samples were phosphorous, iron, calcium sodium, potassium, magnesium, manganese zinc and cobalt in decreasing order of concentrations. The transfer factors of minerals from the soil to the vegetables ranged from 0.59 (Mg) to 0.90 (Ca) for *Commelina africana* while for *Amaranthus thunbergii* it ranged from 0.20 (Co) to 0.92 (Ca). The results obtained in this study indicate that the two vegetables are a good source of key nutrients, which can be used in mitigation of malnutrition and providing alternative cheaper food sources. It also shows that the mineral in the soil were transferred to the vegetables.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ACRONYMS

AAS	Atomic Absorption Spectroscopy
AIV	African Indigenous Vegetables
ATP	Adenosine Triphosphate
DPG	Diphosphoglycerate
DW	Dry Weight
EDL	Electrodeless Discharge Lamp
FAO	Food and Agriculture Organization
FP	Flame Photometer
LOD	Limit of Detection
RDA	Recommended Daily Allowance
RDI	Recommended Daily Intake
SOD	Superoxide Dismutase
TALV	Traditional African Leafy Vegetables
TAV	Traditional African Vegetables
TLV	Traditional Leafy Vegetables
UV-Vis	Ultra Violet Visible
WHO	World Health Organization

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CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

The health of an individual depends on the qualities and quantities of food stuff he/she consumes. The food requirements are simply needed to supply the minimum requirements of the six groups of nutrients: carbohydrates, fats, proteins, mineral elements, vitamins and water (Balch, 2006). According to Whitney & Rolfes (2007), vegetables, contain low calories and negligible quantities of utilizable energy. Hence they are ideal for obese people who can satisfy their appetite without consuming much carbohydrate. Although there are low levels of proteins in vegetables, there is increasing awareness of the importance of vegetables in maintaining health, particularly in areas where animal proteins are scarce (Lichtenstein *et al.*, 2012). Vegetables contribute to the mineral, vitamin and fiber contents of diets (Liu, 2007).

Minerals are naturally occurring inorganic substances with a definite chemical composition and an ordered atomic arrangement. Among the plants, vegetables are excellent sources of minerals and contribute to RDA of these essential nutrients (Sparks, 2003). Minerals are very important ingredients for normal metabolic activities of body tissues. Out of 92 naturally occurring minerals, 25 are present in living organisms. They are constituents of bones, teeth, blood, muscles, hair and nerve cells. Vitamins cannot be properly assimilated without the correct balance of minerals (Sonni, 2002). Most vegetable plants consumed in tropical Africa are cultivated by farmers or consumers. Wild tropical plants used as leafy vegetables are increasingly being abandoned by the rural people. Vegetables are the fresh

and edible portions of herbaceous plants .They are an important class of food substances and are highly beneficial for the maintenance of health and prevention of diseases. They contain valuable food ingredients, which can be successfully utilized to build up and repair the body. Vegetables are valued mainly for their high carbohydrate, vitamins and mineral contents. There are three different types of vegetables: they may be edible roots, stems, leaves, fruits or seeds. Each group contributes to diet in its own way (Hanif *et al.*, 2006)

Indigenous leafy vegetables play an important role in the tradition and food culture of African households and some are also used for medicinal purposes (Van Rensburg *et al.*, 2004). Traditional vegetables offer variety in family diets and help to ensure household food security (Cooke, 2007). Indigenous leafy vegetables are known as sources of many nutrients, vitamins, antioxidants, minerals and important proteins (Akula and Odhav, 2008).

In Kenya and many African countries vegetables are very abundant after the rainy season but become scarce in the dry season. Most of these vegetables are consumed by rural people. In the rural areas, especially in areas where they grow, wild vegetables are under-utilized when compared to introduced varieties like kales and cabbage, due to flavor and unfamiliar taste impacted on the food (Flyman & Afolayan, 2007; Ogoye-Ndegwa, 2003). Leafy vegetables collected from the wild play an important role in traditional diets in rural areas. In some cultures such as the Luhya, Kisii, Luo and Mijikenda, traditional indigenous vegetables are a common food in the diet. While some may be collected from the wild, a sizeable number have now been cultivated, including *Cleome gynandra*, and *Crotalaria*,

Solarium, and *Amaranthus* species (Maundu *et al.*, 2009). Table 1.1 below shows some of the common vegetables in Kenya

Table 1.1: Common types of indigenous vegetables in Kenya

Type/species	Common name	Areas commonly found
Adansonia digitata	baobab	Kitui, Coast
Amaranthus hybridus	amaranth	Countrywide
Amaranthus dubius	amaranth	Countrywide
Amaranthus lividus	amaranth	Kisii, Kericho, Nyanza, Western, central Rift Valley
Amaranthus spinosus	spiny amaranth	Nyanza, countrywide
Asystasia mysorensis		Nairobi, West Pokot, Western, Nyanza
Basella alba	vine spinach	Nairobi, Coast, Western, Nyanza, central Rift Valley
Brassica carinata	kandhira	Nyanza, Western
Gynandropsis gynandra	spider herb	Kisii, Nyanza, Western, Coast, Central and northern Rift Valley, Nairobi
Corchorus trilocularis		Nairobi, Coast, Western, Nyanza, central Rift Valley
Corchorus olitorius	jute	Nairobi, Coast, Western, central Rift Valley, Nyanza
Crotalaria ochroleuca		Nairobi, Western, Nyanza, central Rift Valley
Crotalaria brevidens		Nairobi, Western, Nyanza, central Rift Valley
Digera muricata		Northern Rift Valley, Coast
Ipomoea aquatica		Coast, Malindi
Kedrostis pseudogijef		Voi
Launaea cornuta		Western, Nyanza, Coast
Sesamum calycinum	onyulo	Nyanza, Western

Solanum nigrum	black nightshade	Nairobi, Nyanza, Western, Coast, Central Rift Valley, countrywide
Vigna unguiculata	cowpea	Countrywide

(Maundu *et al.*,1999).

Surveys indicate that there are over 7000 plant species across the world that are cultivated or harvested from the wild for food (Bharucha & Pretty, 2010). Ecologically, there is a great deal of variation within Kenya which has extremes of environments. Land rises from the coastal zone and the lowlands of the north and north-east-where day temperatures exceed 40°C - to the cool highlands and mountain tops in the center of the country, including Mt Kenya with a summit at 5,199 m, which is permanently snow covered (Githeko & Ndegwa, 2001). This great altitudinal range significantly influences rainfall and temperatures in various areas of the country, which in turn dictate the dominant vegetation types. Precipitation ranges from 150 mm annually in the dry low-lying deserts of the north and north-east to over 2,500 mm on the slopes of Mt Kenya (Githeko & Ndegwa, 2001). Likewise, vegetation ranges from almost bare rock and sand dunes in the deserts through *Acacia-Commiphora* bushland to grassland with scattered trees, dry highland forests, tropical rain forests and to alpine vegetation (Wickens, 2013). This wide ecological range has resulted in a rich flora of about 7,100 distinct plant species and several thousand subspecies and varieties. Some of these species have a wide, almost world-wide distribution (e.g. some weedy species such as *Amaranthus* species), while others, or their subspecies or varieties may have a more limited distribution (Given & Maxted, 2005). Some, for example *Bridelia taitensis*, are only found within the country and others occur in even more restricted areas; for example the yet-to-be-described *Salacia* species

(*ndendela)* has only been reported from a single hill (Thui hill) that covers an area of less than 4 hectares.

These neglected and underutilized species play a crucial role in food security, income generation and food culture of the rural poor. Lack of attention in the past has meant that their potential value is mostly under-estimated and under-exploited. Many neglected and underutilized species are nutritionally rich and are adapted to low input agriculture (Toledo & Burlingame, 2006).

1.2 Statement of the problem

The last century has brought more change for the people of Kenya than perhaps any other before. Western culture and modern science and technology are encroaching on traditional practices and eroding local knowledge. Modern times have brought new food habits and even several new crops. The plants from which traditional foods were obtained are now suffering a double tragedy: genetic erosion and loss of traditional knowledge on how to grow and use them. In Ainamoi division within Kericho County and many areas around it, diets are based on fewer and fewer plant species: one in particular-maize-is becoming an increasingly dominant and widespread staple food to the detriment of the health of families and national food security. This, coupled with low incomes and a misguided preference for expensive exotic foods, has contributed significantly to malnutrition.

Traditional farming systems, which are associated with specific traditional crops, varieties and technologies, are being abandoned, also resulting in increasingly monotonous diets and the loss of food-plant resources and indigenous knowledge about them. Specialized

habitats such as indigenous forests and wetlands are being destroyed, similarly endangering specific forms and varieties of plants and sometimes resulting in the loss of entire species.

1.3 Justification

Vegetables are considered as natural sources of nutrients gifted by God to human beings. In developing nations numerous types of wild edible plants are exploited as food sources and provide adequate levels of nutrients to the people. Studies which have been carried out indicate that these plant resources play a significant role in nutrition, food security and income generation

Many traditional plant foods are characteristically energy rich and play a crucial nutritional role during hunger periods. They may be equally important during periods when people have less time for food preparation, such as during peak agricultural seasons, or in arid regions where seasonal food-supply fluctuations are particularly acute. *Commelina* species for example, is strategically available at the beginning of the rainy season before other species can be harvested.

Food insecurity can be reduced by motivating communities to increase their consumption of indigenous and traditional dark green leafy vegetables. However, to recommend these foodstuffs as contribution to an improved diet, knowledge about the nutrient content of the traditional vegetables is required. It should also be considered that soil and climatic conditions of different regions results in a significant difference in food composition of foods produced and therefore data cannot simply be borrowed between countries or regions.

In addition to this mineral analysis on the soils where the plants grow should be done to determine the correlation with that in the vegetables. This information would be useful to farmers on how they can improve the soil composition in their farms and in turn the quality of the vegetables.

1.4 Objectives

1.4.1 General Objective

The aim of this study was to investigate the mineral composition and proximate constituents of the leaves of C*ommelina africana* and *Amaranthus thunbergii* from Ainamoi , Kericho county.

1.4.2. Specific Objectives

i. To determine the proximate nutrient composition (carbohydrate, proteins, ash content, moisture, fat, fiber and energy content) of *Commelina africana* and *Amaranthus thunbergii* from Ainamoi , Kericho county.
ii. To determine the overall mineral composition (phosphorous, iron, calcium sodium, potassium, magnesium, manganese zinc and cobalt) of *Commelina africana* and *Amaranthus thunbergii*.
iii. To compare the concentration of the minerals in the soil and in the leaves of the two indigenous vegetables.
iv. To compare concentration levels of the different minerals in the two selected indigenous vegetables with the recommended daily allowances of the minerals by .World Health Organization

CHAPTER TWO

LITERATURE REVIEW

2.1 Traditional Leafy Vegetables

Leafy vegetables are plant species of which the leafy parts, which may include young, succulent stems, leaves and very young fruit, are used as vegetable (Mensah *et al.,* 2008). According to Ambrose-Oji (2009) there are several terms used in describing traditional African vegetables (TAV): indigenous African vegetables (IAV); African indigenous vegetables (AIV) traditional leafy vegetables (TLV); African leafy vegetables (ALV); traditional African leafy vegetables (TALV or TLV) , and all are subject to contested meanings. In the context of these thesis traditional leafy vegetables follows the definition by United Nations Food And Agricultural Organization FAO (1998) which states that traditional leafy vegetables are categories of plants whose leaves, fruits or roots are acceptable and used as vegetables by urban and rural communities through custom, habit and tradition. In essence, traditional leafy vegetables refer to leafy vegetable which grow naturally and have been harvested and used for culinary purposes for a long time in history to date (Gockowski *et al.,* 2003).

Quite a large number of African indigenous leafy vegetables have long been known and reported to have health protecting properties. Several of these indigenous leafy vegetables continue to be used for not only culinary purposes but also prophylactic and therapeutic purposes by rural communities (Smith & Eyzaguirre, 2007; Gockowski *et al.,* 2003; van Rensburg *et al.,* 2007). The folklore knowledge of the health promoting and protecting attributes of African indigenous leafy vegetables is clearly linked to their nutritional and non-nutrient phytochemical properties. The two main traditional leafy vegetables widely

domesticated in Africa, include amaranth (*Amaranthus* species) and African eggplant (*Solanum aethiopicum*) (Maundu *et al.,* 2009).

2.2 Nutritional importance of African leafy vegetables

Traditional leafy vegetables play a very important role in the livelihoods of rural communities as they form an integral part of the subsistence strategy of people in many developing countries. Locally available leafy vegetables serve as alternatives to staple food during periods of food deficit (Legwaila *et al.,* 2011). This is mainly because most have been found to contain a variety of important nutrients and minerals.

Traditional leafy vegetables have been found to be a great source of minerals such as calcium, copper, magnesium, zinc, iron and potassium (Orech *et al.,* 2007). Turan *et al.* (2003) for instance reported that the potassium, calcium, magnesium and protein contents of wild vegetables in Turkey were all higher than cultivated species such as spinach, pepper, lettuce, and cabbage. Other studies have reported that these indigenous leafy vegetables contain several vitamins including vitamin E, carotenoids and vitamin C. For instance, studies conducted by Flyman & Afolayan (2006) in South Africa confirmed the importance of wild vegetables as sources of Vitamin C and Vitamin A. In addition, studies conducted on wild East African vegetables by Vainio-Mattila, 2000 in Tanzania underscored the wild plants' significant contribution as sources of micronutrients including vitamin C and E. Noteworthy, each different green leafy wild vegetable contains a different percentage of each mineral, so it is best to rotate their consumption.

Traditional leafy vegetables have also been found to contain non-nutrient bioactive phytochemicals that have been linked to protection against cardiovascular and other

degenerative diseases (Kumar *et al.*, 2009; Uusiku *et al.*, 2010). Furthermore, they have been reported to possess antibiotic, probiotic and prebiotic properties (Erasto & Mbwambo, 2009; Mahasneh & Abbas, 2010), and contain antioxidants and phytochemicals that help protect people against non-communicable diseases (Lako *et al.*, 2007; Gupta & Prakash, 2009). Nevertheless, Orech *et al.* (2005) observed that some of these phytochemicals found in some leafy vegetables may pose toxicity problems when consumed in large quantities or over a long period of time.

2.3 Botany of the two TLVs in this Study

2.3.1 *Commelina africana*

Commelina africana is a perennial herb with tuberous fleshy roots; stem creeping or straggling. Leaves arranged spirally, with purple tinge, ciliate along the free margins; blade generally lanceolate. Flowers are bisexual, zygomorphic, yellow, rarely protruding from the spathe; lower petal. Seeds are variable in size, cylindrical-rectangular in outline, *Commelina africana* is a variable species, in which many varieties are distinguished. The typical variety, var. *africana*, is a cultivated plant grown as a vegetable. *Commelina africana* is easily distinguished from the other *Commelina* species by its yellow instead of blue, purplish or pink flowers. The flowers open from 7–10 a.m. Figure 2.1 shows a matured *Commelina africana plant* (Mudau, 2007).

Figure 2.1: *Commelina africana* Plant

(Source: Author, 2015)

Some common and vernacular names for *Commelina africana* include Yellow commelina, Wandering Jew, Dayflower (England). Comméline, africaine (France), Kongwa (Sweden) (Van de burg, 2004) and loblobitiet (Kipsigis), Mukengesya (Kamba).

Commelina africana is indigenous and widespread in Africa, occurring from Senegal to Ethiopia, and south to South Africa. It occurs also in Saudi Arabia, Yemen and Australia. In Kenya, Uganda and Tanzania the leaves are cooked and eaten as a vegetable. They are chopped and boiled in water or in fresh or sour milk. Sesame seeds and groundnut paste are added for flavor and consistency. This vegetable is eaten with the staple food as a substitute for more preferred vegetables. Many other uses are reported for *Commelina africana*. In Kenya and Tanzania the leaves are fed to livestock, especially pigs and rabbits. The flowers provide bee forage. In Kenya an infusion of the plant is used as a wash to reduce fever, and pounded stalks are used to treat colds and coughs in children. Fluid from the stem is applied locally to cure eye diseases (Hyde *et al.*, 2015).

The Zulu of South Africa bathe the body, especially of a child, with a cold infusion in cases of restless sleeping. Similarly, an infusion of the leaves is sprinkled over the resting place of a restless child in Zimbabwe. The Sotho in southern Africa take a decoction of the plant with *Tephrosia capensis* for treatment of a 'weak heart' and nervousness. In DR Congo the root is used for the same purpose. The plant cooked with *Haplocarpha scaposa*, *Helichrysum pilosellum* or the root of *Cotyledon decussata* is given by Sotho as medicine to young women to cure infertility. Also, an infusion of the plant is drunk and its ash is rubbed over the loins as a fertility charm. In Zimbabwe and South Africa a concoction of the root is used as treatment for venereal diseases and to treat women with menstrual cramps. This preparation is also used for pelvic pains and bladder complaints (Van Wyk and Gericke, 2000).

Commelina africana occurs in secondary growth and disturbed localities, and as a weed on farms. In Senegal it grows in marshes. After the onset of the rains, the plant sprouts earlier than other plants and it is therefore useful as a fodder plant after prolonged drought. The same applies for its use as a vegetable; it is available earlier than commonly cultivated species. (Van de burg, 2004)

In Kenya, Uganda and Tanzania *Commelina africana* is not cultivated or protected by local people. It is common as a weed and therefore easily accessible. The leaves are collected in the rainy season for immediate use, they are not stored. There are also no germplasm collections or breeding programmes known to exist for *Commelina africana*. This is because of its abundance in Africa (Van de burg, 2004) and (Mudau, 2007)

2.3.2 *Amaranthus thunbergii*

Amaranthus thunbergii is a leafy vegetable growing to a height of 0.5 m (1ft 8in). It is frost tender. The flowers are either male or female, but both sexes can be found on the same plant and are pollinated by Wind. Its leaves are glabrous with green flowers, in dense axillary clusters normally extending well down towards the base of the plant, the clusters approximate above, the superior leaves scarcely reducing, or sometimes so rapidly so that a few upper clusters are leafless; male and female flowers are intermixed, the males more numerous in the upper clusters(Hyde *et al.*,2015)

Figure 2.2 shows the *Amaranthus thunbergii* plant in its natural habitat.

Figure 2.2: *Amaranthus thunbergii*

(Source: Author, 2015)

2.4 Importance of some Micronutrients and Macronutrients Analyzed

2.4.1 Iron

Iron has the longest and best described history among all the micronutrients. It is a key element in the metabolism of almost all living organisms. In humans, iron is an essential component of hundreds of proteins and enzymes (Qureshi *et al.,* 2005; Bendich & Zilberboim, 2010). Haeme is an iron-containing compound found in a number of biologically important molecules. Haemoglobin and myoglobin are haeme-containing proteins that are involved in the transport and storage of oxygen. Haemoglobin is the primary protein found in red blood cells and represents about two thirds of the body's iron. The vital role of hemoglobin in transporting oxygen from the lungs to the rest of the body is derived from its unique ability to acquire oxygen rapidly during the short time it spends in contact with the lungs and to release oxygen when needed during its circulation through the tissues. Myoglobin functions in the transport and short-term storage of oxygen in muscle cells, helping to match the supply of oxygen to the demand of working muscles (Zaater, 2012).

The Recommended Dietary Allowance (RDA) for iron shown in Table 2.1 was revised in 2001 and is based on the prevention of iron deficiency and maintenance of adequate iron stores in individuals eating a mixed diet (Food and Nutrition Board, 2001).

Table 2.1: Recommended Dietary Allowance (RDA) for Iron

Life Stage	Age Group	Males (mg/day)	Females (mg/day)
Infants	0-6 months	0.27	0.27
Infants	7-12 months	11	11

Children	1-3 years	7	7
Children	4-8 years	10	10
Children	9-13 years	8	8
Adolescents	14-18	11	15
Adults	19-50	8	18
Adults	51 years and older	8	8
Pregnancy	All ages		27
Breast-feeding	18 years and younger		10
Breast-feeding	19 years and older		9

(IOM, 2001)

2.4.2 Zinc

Zinc is a metal. It is called an "essential trace element" because very small amounts of zinc are necessary for human health. Numerous aspects of cellular metabolism are zinc-dependent. Zinc plays important roles in growth and development, the immune response, neurological function, and reproduction. On the cellular level, the function of zinc can be divided into three categories namely, catalytic, structural and regulatory (Eide, 2006). Nearly 100 different enzymes depend on zinc for their ability to catalyze vital chemical reactions. Zinc-dependent enzymes can be found in all known classes of enzymes (Beyersmann & Haase, 2001).

It plays an important role in the structure of proteins and cell membranes. A fingerlike structure, known as a zinc finger motif, stabilizes the structure of a number of proteins. For example, copper provides the catalytic activity for the antioxidant enzyme copper-zinc superoxide dismutase (CuZnSOD), while zinc plays a critical structural role (Laity *et al.*, 2001; Eide, 2006). The structure and function of cell membranes are also affected by zinc. Loss of zinc from biological membranes increases their susceptibility to oxidative damage and impairs their function (Matthews & Sunde, 2002).

Zinc finger proteins have also been found to regulate gene expression by acting as transcription factors.It also plays a role in cell signaling and has been found to influence hormone release and nerve impulse transmission (Nieto, 2002).It is also important in apoptosis, a critical cellular regulatory process with implications for growth and development, as well as a number of chronic diseases (Chinnusamy *et al.,* 2007).

The RDA for zinc is listed by gender and age group in Table 2.2. Since a sensitive indicator of zinc nutritional status is not readily available, the RDA for zinc is based on a number of different indicators of zinc nutritional status and represents the daily intake likely to prevent deficiency in nearly all individuals in a specific age and gender group (Food and Nutrition Board, 2001)

Table 2.2: Recommended Dietary Allowance (RDA) for Zinc

Life Stage	**Age**	**Males (mg/day)**	**Females (mg/day)**
Infants	0-6 months	2	2
Infants	7-12 months	3	3
Children	1-3 years	3	3
Children	4-8 years	5	5
Children	9-13 years	8	8
Adolescents	14-18 years	11	9
Adults	19 years and older	11	8
Pregnancy	18 years and younger		12
Pregnancy	19 years and older		11
Breast-feeding	18 years and younger		13
Breast-feeding	19 years and older		12

(IOM, 2001)

2.4.3 Sodium

Although Na is often maligned as a cause of high blood pressure, it also plays several essential roles in the body. Sodium helps control blood pressure and regulates the function of muscles and nerves, which is why sodium concentrations are carefully controlled by the body. However, most people consume far more sodium than their bodies need (Sfgate,2012).

Sodium is dissolved in the blood and plays a key role in maintaining blood pressure. It attracts and holds water, so the sodium in the blood helps maintain the liquid portion of the blood. On the other hand, if one consumes too much sodium, ones body may hold onto extra water, increasing the volume of ones blood. Since one blood vessels cannot expand to accommodate this increased blood volume, ones blood pressure will rise. High blood pressure is a risk factor for many diseases, including heart problems and stroke (Vollmer *et al.,* 2001).

Both muscles and nerves require electrical currents to function properly. Muscle and nerve cells generate these electrical currents by controlling the flow of electrically charged molecules, including sodium. For muscle cells, these electrical currents stimulate contraction of the muscle. Nerves, on the other hand, need electrical activity to communicate with other nerves. Cells use molecular pumps to keep sodium levels outside the cell high. When an electrical current is needed, cells can allow the positively charged sodium ions into the cell, generating a positive electrical current (Hille, 2001).

Since Na is an integral part of nerve and muscle function, it is not surprising that too little or too much sodium in the body can affect both of these organ systems. Low levels of sodium, called hyponatremia, can cause muscle spasms, cramps, headache, irritability, restlessness, nausea and fatigue. More serious signs of hyponatremia include confusion, hallucinations, decreased consciousness and coma. Too much sodium, also known as hypernatremia, can make one lethargic or restless. Hypernatremia may also cause increased deep tendon reflexes, muscle spasticity and seizures (Kimura, 2013).

The RDA for sodium is currently no more than 2.3 g, or 2,300 mg, of sodium daily for healthy adults. That is equivalent to about 1 tsp. of salt a day. According to the Institute of Medicine, the maximum daily intakes for sodium are the following: ages 1 to 3, 1,500 mg; ages 4 to 8, 1,900 mg; ages 9 to 13, 2,200 mg, and ages 14 to 18, 2,300 mg (Garriguet, 2007). People who are sensitive to sodium should only consume 1,500 mg of sodium per day. Sensitive populations include adults over 50, blacks or those diagnosed with hypertension, diabetes or chronic kidney disease (Karppanen & Mervaala, 2006).

2.4.4 Phosphorus

Phosphorus is an essential mineral that is required by every cell in the body for normal function (Soetan *et al.,* 2010). The majority of the phosphorus in the body is found as phosphate (PO_4^{3-}). Approximately 85% of the body's phosphorus is found in bone (Food and Nutrition Board, Institute of Medicine, 2001).

Phosphorus is a major structural component of bone in the form of a calcium phosphate salt called hydroxyapatite. Phospholipids are major structural components of cell

membranes. All energy production and storage are dependent on phosphorylated compounds, such as ATP and creatine phosphate (Rey *et al.,* 2009).

Nucleic acids, which are responsible for the storage and transmission of genetic information, are long chains of phosphate-containing molecules. A number of enzymes, hormones, and cell-signaling molecules depend on phosphorylation for their activation. Phosphorus also helps to maintain normal acid-base balance by acting as one of the body's most important buffers. Additionally, the phosphorus-containing molecule 2,3-diphosphoglycerate (2,3-DPG) binds to haemoglobin in red blood cells and affects oxygen delivery to the tissues of the body (Kalantar-Zadeh *et al.,* 2010).

The recommended dietary allowance (RDA) as shown in Table 2.3 for phosphorus was based on the maintenance of normal serum phosphate levels in adults, which was believed to represent adequate phosphorus intake to meet cellular and bone formation needs (Food and Nutrition Board, 2001).

Table 2.3: Recommended Dietary Allowance (RDA) for Phosphorus

Life Stage	Age	Males (mg/day)	Females (mg/day)
Infants	0-6 months	100	100
Infants	7-12 months	275	275
Children	1-3 years	460	460
Children	4-8 years	500	500
Children	9-13 years	1 250	1 250
Adolescents	14-18 years	1250	1250
Adults	19 years and older	700	700

Pregnancy	18 years and younger	-	1 250
Pregnancy	19 years and older	-	700
Breast-feeding	18 years and younger	-	1 250
Breast-feeding	19 years and older	-	700

(IOM, 2001)

2.4.5 Potassium

Potassium is an essential dietary mineral and electrolyte. The term electrolyte refers to a substance that dissociates into ions in solution, making it capable of conducting electricity. Normal body function depends on tight regulation of potassium concentrations both inside and outside of cells (He & MacGregor, 2001).

It is the principal positively charged ion in the fluid inside of cells, while sodium is the principal cation in the fluid outside of cells. Potassium concentrations are about 30 times higher inside than outside cells, while sodium concentrations are more than ten times lower inside than outside cells. The concentration differences between potassium and sodium across cell membranes create an electrochemical gradient known as the membrane potential. A cell's membrane potential is maintained by ion pumps in the cell membrane, especially the sodium, potassium-ATPase pumps. These pumps use ATP to pump sodium out of the cell in exchange for potassium. Their activity has been estimated to account for 20% to 40% of the resting energy expenditure in a typical adult (Hille, 2001).

The large proportion of energy dedicated to maintaining sodium/potassium concentration gradients emphasizes the importance of this function in sustaining life. Tight control of cell

membrane potential is critical for nerve impulse transmission, muscle contraction, and heart function (Shinoda *et al.,* 2009).

In 2004, the Food and Nutrition Board of the Institute of Medicine established an adequate intake level (AI) as shown in Table 2.4 for potassium based on intake levels that have been found to lower blood pressure, reduce salt sensitivity, and minimize the risk of kidney stones (Food and Nutrition Board, 2001).

Table 2.4: Adequate Intake (AI) for Potassium

Life Stage	Age	Males (mg/day)	Females (mg/day)
Infants	0-6 months	400	400
Infants	7-12 months	700	700
Children	1-3 years	3 000	3 000
Children	4-8 years	3 800	3 800
Children	9-13 years	4 500	4 500
Adolescents	14-18 years	4 700	4 700
Adults	19 years and older	4 700	4 700
Pregnancy	14-50 years	-	4 700
Breast-feeding	14-50 years	-	5 100

(IOM, 2001)

2.4.6 Cobalt

Cobalt is one of the vital trace mineral that is known as a constituent of vitamin B12. The body requires a small amount of this mineral in order to conduct its daily growth and maintenance. The amount of cobalt that is in foods is based on the amount of mineral that is in the soil where the food sources are grown or consumed.

It is essential to humans as well as to animals. It is known as the main constituent of cobalamin, also known as vitamin B12, that is basically cobalt's biological reservoir as an "ultra-trace" element. In ruminant animals, the bacteria found in them convert cobalt salts into a compound that can only be produced by the bacteria (Czarneck *et al*,.2015).

The cobalt atom in vitamin B12 is attached and surrounded to a deoxyadenosyl group, methyl group, and a cyano group or hydroxyl group. The human body has a need for cobalt that is not in the ionic form of the metal but rather, for a performed metallovitamin which cannot be synthesized from a simple dietary meal. Thus, the content of vitamin B12 of foods is essential is the overall human nutrition.

The activity and function of cobalt is essentially the same as vitamin B12, hence, meaning that cobalt plays a major role in the process of erythropoiesis, the process wherein erythrocytes or red blood cells are produced. When in the form of CoC_{12}, cobalt assists in regulating casein and phosvitin phosphatases and other certain phosphoprotein phosphatases. Along with Nickel (Ni) and Manganese (Mn), cobalt can be a good alternative for Zinc (Zn) in the carboxypeptidase, carbonic anhydrase, angiotensin-converting enzyme, and metalloenzymes.

Health experts suggest that adults need around 1.5 µg of vitamin B12 daily; the daily B12 RDA is 2.4 µg for adults and adolescences. To date no Cobalt RDA has been established. However, excessive deficiency of cobalt is known to lead to certain health disorders.

2.4.7 Manganese

Manganese is a trace mineral that is present in tiny amounts in the body. It is found mostly in bones, the liver, kidneys, and pancreas. Manganese helps the body form connective tissue, bones, blood clotting factors, and sex hormones. It also plays a role in fat and carbohydrate metabolism, calcium absorption, and blood sugar regulation. Manganese is also necessary for normal brain and nerve function (Hambidge, 2003).

Manganese is a component of the antioxidant enzyme superoxide dismutase (SOD), which helps fight free radicals. Free radicals occur naturally in the body but can damage cell membranes and DNA. They may play a role in aging, as well as the development of a number of health conditions, including heart disease and cancer. Antioxidants, such as SOD, can help neutralize free radicals and reduce or even help prevent some of the damage they cause (Evans & Halliwell, 2001).

Low levels of manganese in the body can contribute to infertility, bone malformation, weakness, and seizures. It is fairly easy to get enough manganese in your diet -- this nutrient is found in whole grains, nuts, and seeds -- but some experts estimate that as many as 37% of Americans do not get the recommended dietary intake (RDI) of manganese in their diet. The American diet tends to contain more refined grains than whole grains, and refined grains only provide half the amount of manganese as whole grains (Keen *et al*., 1996).

However, too much manganese in the diet could lead to high levels of manganese in the body tissues. Abnormal concentrations of manganese in the brain, especially in the basal ganglia, are associated with neurological disorders similar to Parkinson's disease. Early life

manganese exposure at high levels, or low levels, may impact neurodevelopment. Elevated manganese is also associated with poor cognitive performance in school children (IOM, 2001).

2.4.8 Moisture Content

The moisture content of food material is important because it affects the physical and chemical aspects of the food which relates with freshness and stability for the storage of the food for a long period of time. It also determines the actual quality of the food before consumption and to the subsequent processing in the food sector by food producers (isengard, 2001).

Importance of moisture analysis in food includes: Legal and labelling requirements whereby legal limitations regarding the amount of water present in the food is necessary for producing some of the specific products. It also determines the shelf life of the food which is dependent on the moisture content of the food. This is because microbial activity of the food materials favour with moisture availability in the food. Moisture rich foods are easily susceptible to the microbial attack.

The quality of the food is determined in terms of the food texture, taste, and appearance, but the moisture content of the food is a determining factor of the quality and the stability of the processed food products. Food processing operations are involved with the amount of moisture content present in the food item which is going to be processed for a specific purpose.

The moisture determination methods include: dry methods, direct distillation methods, electrical methods and chemical methods. The method chosen is influenced by the following: amount of water in food, kind of apparatus used, type of food handled, how quick results are needed, reason for moisture content determination and accuracy of the results needed(Mulvaney,1995).

2.4.9 Ashing

The ash content is a measure of the total amount of minerals present within a food, whereas the mineral content is a measure of the amount of specific organic components present within a food, such as Ca, Na, K and Cl (Nanda *et al.,* 2003).

Determination of ash and mineral content of foods is important, most of all being nutritional labeling in which the concentration and type of minerals present must often be stipulated on the label of a food. Quality of many foods depends on the concentration and type of minerals they contain, including their taste, appearance, texture and microbiological stability, in which, high mineral contents are used to retard the growth of certain microorganisms. Nutrition of some minerals is essential to a healthy diet (for example, calcium, phosphorus, potassium and sodium) whereas others can be toxic (for instance, lead, mercury, cadmium and aluminum). It is often important to know the mineral content of foods during processing because this affects the physicochemical properties of foods. There are three main types of analytical procedure used to determine ash contents of foods. These are dry ashing, wet ashing and low temperature plasma dry ashing. The method chosen for a particular analysis depends on the reason for carrying out the analysis, the type of food analyzed and the equipment available.

2.4.10 Carbohydrates

Carbohydrates are one of the main types of nutrients and the one needed in the largest amounts by the body (Mann & Truswell, 2012). Between 45 and 65 percent of calories should come from carbs, according to the Dietary Reference Intakes set by the Institute of Medicine (Trumbo *et al.,* 2002). Carbohydrates are often maligned for contributing to weight gain, but they are needed for your body to function well and should be part of every person's diet (Mann & Truswell, 2012).

The role of carbohydrates is to provide energy, as they are the body's main source of fuel, needed for physical activity, brain function and operation of the organs. All the cells and tissues in the body need carbohydrates, and they are also important for intestinal health and waste elimination. Once in the body, carbohydrates are easily converted to fuel (Burke *et al.,* 2012).

The two types of carbohydrates are simple and complex. Simple carbohydrates, also called simple sugars, include sugars founds in fruits, vegetables and milk, as well as sugars added during food processing (Pigman, 2012). Complex carbohydrates, also called starches, include whole-grain breads and cereals, starchy vegetables and legumes. Most complex carbohydrates contain fiber, which helps digestive health and increases satiety, reducing overeating and weight gain. Additionally, high-fiber foods help lower cholesterol and decrease the risk of heart disease (Mann & Truswell, 2012).

The Institute of Medicine report (IOM, 2002) established a Recommended Dietary Allowance (RDA) for carbohydrate of 130 g per day for adults and children. This value is

based upon the amount of carbohydrate (sugars and starches) required to provide the brain with an adequate supply of glucose.

2.4.11 Proteins

Proteins can consist of a single chain of less than 100 amino acids up to a complex structure of several chains with hundreds of folds and a three-dimensional shape (Roberts *et al.*, 2012). These larger proteins, called structural proteins, provide structure and shape to cells, organs and connective tissue (Schulz & Schirmer, 2013).

Actin and myosin are two specialized types of filament protein present in human muscles. When stimulated by a signal from the central nervous system, these two proteins act in unison to shorten in length, causing the muscle to contract (de Lanerolle & Serebryannyy, 2011).

Antibodies, another protein, help the body fight infection. For each antigen that enters the body, there is a separate and distinct antibody to fight it. Antibodies immobilize and sequester antigens until white blood cells can destroy them (Harrison *et al.*, 2011).

The body performs thousands of biochemical reactions a day to function properly. These reactions require energy, and many have significantly high energy thresholds that can delay essential reactions (UniProt Consortium, 2010). Proteins called enzymes assist in lowering the activation energy of hundreds of reactions, helping them to proceed thousands of times faster than they would in a normal environment. A well-known example of an enzyme is lactase, which facilitates the metabolism of lactose, or milk sugar, in the small intestine to aid in digestion (Bonetta, 2010).

Hormones are proteins that send signals and coordinate activities throughout the body. Examples include insulin, which facilitates glucose metabolism and controls blood glucose levels; thyroid hormones, which regulate metabolism, body temperature and the synthesis of other proteins; and gonadotropins, which stimulate the production of sperm and ova (Chang, 2010).

The body can use protein for its energy needs when carbohydrates are depleted. When needed, proteins degrade into their component amino acids, which are then oxidized in the same process as glucose to create energy. However, prolonged use of protein for energy can cause problems if not enough protein remains to perform its essential functions, according to "Human Physiology" (Schulz & Schirmer, 2013).

2.5 Determination of Nutrient Contents

Mineral analysis is performed using various methods such as colorimetric method, gravimetric method, atomic absorption spectrometry, and flame photometry among others. Vitamin contents are analyzed by spectrophotometry, high performance liquid chromatography, titrimetry, bioassay, just to name a few (Prapasri *et al.,* 2011).

The three main types of analytical procedures used to determine ash contents of foods are dry ashing, wet ashing and low temperature plasma dry ashing. Depending on the form of the water present in a food, the method used for determining moisture may measure more or less of the water present. Moisture determination methods include: dry methods, direct distillation methods, electrical methods and chemical methods (James, 2013).

The methods for determining nutritional contents of foods differ according to the type of reaction being investigated. Instrumental methods of analysis are mostly based on measurement of an intensive chemical property which is proportional to the concentration of the substance. It is important to have a selective analytical method by which the concentration of single species can be determined in the presence of others, independent of the solution (Lusweti, 2000).

2.5.1 Flame Photometry

The underlying principle of flame photometry may be explained when a liquid sample containing a metallic salt solution under investigation is introduced into a flame. The following steps normally take place in quick succession; the solvent gets evaporated leaving back the corresponding solid salt which undergoes vaporization and gets converted into its respective gaseous state. This is followed by the progressive dissociation of either a portion or all of the gaseous molecules to give rise to free neutral atoms. The resulting neutral atoms are excited by the thermal energy of the flame in which they are fairly unstable, and hence instantly emit photons and eventually return to the ground state. The resulting emission spectrum caused by the emitted photons and its subsequent measurement forms the fundamental basis of flame emission spectroscopy (James, 2013).

Upon their return to a lower or ground electronic state, the excited atoms and molecules emit radiations that are characteristic for each. The emitted radiation passes through a monochromator that isolates the desired spectral feature which is then registered by a photodetector whose output is amplified and read on a meter or recorder (Pomeranz, 2013).

2.5.2 Atomic Absorption Spectroscopy

Atomic absorption spectroscopy (AAS) is an analytical method, having been described by Walsh in 1955.its a procedure for the quantitative determination of chemical elements using the absorption of optical radiation by free atoms in the gaseous state (McCarthy, 2012).The Principle of Atomic absorption spectrophotometry is based upon the absorption of radiation by free atoms, usually in the ground state. By selecting a wavelength for a given element that corresponds to an optical transition between atoms in the ground state and atoms in an excited level, the absorption of the radiation leads to a depopulation of the ground state. The value of the absorption is related to the concentration of the atoms in the ground state, and therefore, to the concentration of the element (James, 2013).

By measuring the amount of radiation absorbed, a quantitative determination of the amount of analyte can be made. An atomic absorption spectrometer will therefore consist of a primary radiation source which produces the radiation to be absorbed, a source of free atoms with an associated sample introduction system, an optical dispersive system, a detector, and electronics for data acquisition, processing, and editing. The presence of free atoms must be obtained in the path between the primary radiation source and the detector (James, 2013).

The absorption obeys Beer's law. The most commonly-used primary radiation sources are the hollow-cathode lamp (HCL) and the electrodeless discharge lamp (EDL). They both belong to low pressure discharges. The hollow-cathode lamp consists of a hollow cathode made of a highly pure metal whose spectrum is to be produced with an inner diameter in the 2-5 mm range. A high voltage and a current of up to 30 mA are to produce a discharge

which takes place entirely in the hollow cathode. The fill gas is either argon or neon. Neon is preferred for elements with high ionization potential. A transparent silica window is used for light transmission. The success of the AAS method is clearly related to the availability of HCLs (James, 2013).

2.5.3 Colorimetric analysis

It's a technique used to determine the concentration of colored compounds in solution. A colorimeter is used to test the concentration of a solution by measuring its absorbance at a specific wavelength of light (Greenan *et al*,. 1995).

2.6 Related Studies

Studies, which have been carried out on other vegetables, to determine their proximate and mineral composition of other vegetables include; evaluation of the nutritional composition of three traditional vegetables *Cnidoscolus chayamansa* (iyana ipaja), *Solaniumnodiflorum* (Ogumo*)*, and *Senecio biafrae* (worowo)) in Iree, Osun State, Nigeria. The findings indicated that *Cnidoscolus chayamansa* had higher protein content (5.91%) and carbohydrate content (8.88%) but there was no significant difference in the crude fibre value and that of *Seneci obiafrae*. *Senecio biafrae* had higher moisture content (89%) while *Solanium nodiflorum* had higher ash and fat content which were significantly different from the other vegetables. *Cnidoscolus chayamansa* had higher values in all the mineral contents determined and these were significantly different from other vegetables. There were no significant difference in potassium, calcium and iron contents of *Solanium nodiflorum* and *Senecio biafrae.* From the findings of this study the three vegetables are

good sources of nutrients which could be consumed for normal growth (Adaleke and Abiodun, 2010)

The proximate and mineral analysis of the some vegetables: *Telfaria occidentalis*, *Ocimum gratissimum*, *Hyptis suaveolens*, *Talinum triangulare*, *Amaranthus hybridus*, and *Corchorus olitorius*, were carried out to evaluate their nutritional value. Results showed high mean moisture contents ranging from 45.47% to 84.00%, low ash content ranging from 1.10% to 10.10%, and protein content ranging from 3.20% to 21.95%. Elemental analysis in mg/100 g indicated that the leaves and stem of the vegetable crops showed that they are rich sources of sodium, calcium, and potassium. There was complete absence of iron and magnesium in the vegetable crops. This result revealed that these vegetables are rich sources of nutrients and minerals essential for human growth and development.

Proximate composition, Amino acids profile, mineral content and anti-nutritional factors of tender leaves of *Amaranthus viridis* were evaluated using standard methods of analyses. The leaves had the following proximate compositions on dry weight (DW) basis: ash (21.05%), crude protein (35.11 ± 0.33%), crude lipid (5.26 ± 0.30%), crude fibre (14.04 ± 0.35%), available carbohydrate (24.54 ± 0.71%) and calorific value (530.34 ± 0.01 kcal/100g). The amino acids profile indicates that the leaves are good source of essential amino acids for adults. The leaves are rich in K, Mg, Fe, Mn and Cu when compared to their respective RDA values. Tannins (7,530.21 ± 5.21 mg/100gDW) and phytate (1,326.92 ± 16.57 mg/100gDW) were the plant's predominant anti-nutrients while total oxalate (202.50 ± 6.50 mg/100gDW) and soluble oxalate (97.50 ± 3.75 mg/100gDW) were in appreciable concentration. The amounts of hydrogen cyanide (13.07 ± 2.38 mg/100gDW)

and nitrate (25.35 ± 2.74 mg/100gDW) were below the critical values. The results are an indication that the *Amaranthus viridis* leaves had the potential to be used as source of nutrients in alleviating macro- and micro- nutrient deficiencies.

The leaf cabbage (*Brassica oleracea var. acephala*), is a traditional local vegetable widely grown in rural and urban areas and consumed mostly by the poor in southern and eastern Africa. It is easy to propagate and is highly productive throughout the year. Three lines of leaf cabbage were evaluated for their nutritional value against a commonly grown exotic vegetable, Swiss chard (*Beta vulgaris* var.*cicla*). Swiss chard was superior to the leaf cabbage lines in protein, total ash, vitamin A, sodium andiron content. On the other hand, the leaf cabbage lines had significantly higher quantities of fiber, carbohydrate, fat, calcium, vitamin C and energy than Swiss chard. The leaf cabbage types tested have many good nutritional attributes that justify their genetic improvement through breeding in aspects such as protein, vitamin A and iron content.

Four different green leafy vegetables commonly consumed in southern parts of Nigeria were analyzed with a view to determine the nutrient composition of these vegetables. The vegetables are jute leaf (*Corchorus olitorius*), mint leaf (*Ocimum gratissimum*), water leaf (*Talinum triangulare*) and fluted pumpkin leaf (*Telfaria occidentalis*). The results revealed that *Talinum triangulare* had the highest amount of moisture (89.47 %) while *Corchorus olitorius* had highest ash, crude fiber, protein, lipid and carbohydrate content (0.83%), (0.33%), (6.21%), (5.08%) and (6.25%) respectively. The vitamin analysis also revealed that *Telfaria occidentalis* contained the highest ascorbic acid, niacin and thiamin content (356.11 mg/100 g), (0.74 mg/100 g) and (0.08 mg/100 g) respectively. *Talinum triangulare* had the highest amount of riboflavin (0.18 mg/100 g). The results obtained in

this work clearly indicate that the four leafy vegetables are cheap and readily available source of the nutrients analyzed.

Eight vegetable species viz., *Solanum melongena, Trianthema portulacastrum, Abelmoschus*

esculentus, Spinacia oleracea, Praecitrullus fistulosus, Luffa acutangula, Cucurbita moschata and *Cucumis sativus* were evaluated for their nutritional values using standard techniques for proximate, macro and micronutrient analysis. In proximate analysis, ash, carbohydrate, proteins, fiber, fats and moisture (both dry and wet) were assayed while Cu, Ni, Zn, Pb, Co, Cd, Fe, Cr, Ca and Na were evaluated in micronutrients analysis using AOAC methods and atomic absorption spectrometric techniques. The species showed variable results in proximate analysis, however, *Cucurbita moschata* have revealed higher percentage of carbohydrates, fibers, and energy values. The results showed that *Trianthema portulacastrum* (a wild vegetable) had the highest concentrations of the micronutrients like Cu, Zn, and Fe compared to the other seven species while it had highest concentration of Ca. Proximate and nutrient analysis of such wild and cultivated vegetables can help us to determine the health benefits achieved from their use in marginal communities.

Folliage of Hairy indigo *(Indigofera astragalina)* obtained from Sokoto state, Nigeria were studied for their proximate analysis and mineral composition. The proximate composition revealed the presence of moisture (51.00±0.50% fresh weight), ash (8.17±0.58% dry weight, DW), crude lipid (5.0±0.5% DW), crude fiber (2.67±0.29% DW), crude protein (8.23±0.11% DW) and carbohydrate (75.94±0.64%). The energy value was found to be 578.87 kcal/100 g. The minerals composition revealed the presence of potassium

(14.55±0.17 mg/100 g), sodium (0.33±0.16 mg/100 g), calcium (11.49±0.34 mg/100 g), magnesium (10.89±0.32 mg/100 g),

phosphorus (0.39±0.01 mg/100 g), copper (0.02±0.00 mg/100 g), zinc (0.11±0.00 mg/100 g), iron (20.95±3.84 mg/100 g) and manganese (0.43±0.01 mg/100 g). These results revealed that the leaves of Hairy indigo *(Indigofera astragalina)* contained essential nutrients which compare favorably well with those of wild edible leaves in literatures.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Reagents

All the chemicals and solvents used were of analytical reagent grade and were used without further purification. They include: selenium powder, lithium sulphate, Hydrogen peroxide (30%), Sulphuric acid (concentrated), Sodium hydroxide and Nitric acid. Other reagents used were sodium citrate, sodium hypochlorite, sodium nitroprusside, sodium salicylate and sodium tartrate obtained from Unilab and Berk and Spencer Acids Limited.

3.2 Sampling

The leaves of the vegetables were hand-picked in farms in six zones in Ainamoi within Kericho Central district. 600g of each type of vegetable were collected and taken to the university of Eldoret botany laboratory for identification. The plant material was identified and authenticated. Six soil samples were also collected in the same farms where the vegetable samples were collected and put in plastic bags. The soil samples were taken using a soil auger and the sample depth incorporated was 4-6 inches.

3.3 Sample Preparation

The vegetable samples were hand washed in running tap water to remove soil and oven dried at 60 ^{0}C for 24 hours then reduced into fine powder using a mechanical blender. The ground samples were then kept in air tight containers. About 600 g of the leaves yielded 112 g and 148 g for C*ommelina africana* and *Amaranthus thunbergii* respectively. Figure 3.1 below shows the ground leaf samples

Figure 3.1: Ground samples (Source: Author, 2015)

The soil samples were dried in the oven at 100 ^{0}C for 24 hours, stored in desiccators until cooled. It was then mixed and homogenized by spreading it on a cloth and pulling each corner in succession over its diagonal partner, this was repeated three times. It was weighed and stored in a sample bottle and stored for later analysis

3.4 Plant sample digestion

3.4.1 Digestion mixture

The digestion mixture was prepared by adding 0.42 g selenium powder and 14 g lithium sulfate to 350 mL 30 % hydrogen peroxide and mixing it well. 420 mL concentrated H_2SO_4 was added slowly while cooling in an ice bath and stored at 2 ^{0}C.

3.4.2 Digestion procedure

0.3g of the vegetables and soil samples was put into dry and cleaned labeled digestion tubes.4.4 mL of the digestion mixture was added to each tube and also to reagent blanks of each sample .It was then digested at 360 ^{0}C for 2 hours (The digestion was complete when the solution turned colourless).The tubes were removed from the digester and cooled. About 25 mL of distilled water was then added, mixed well and made up to 50 mL distilled

water and allowed to settle (Okalebo *et al.*, 1998).The digested solution of each sample was kept in well stoppered bottles for further analysis.

3.5 Proximate analysis

This was carried out on the blended and homogenized leaf samples in triplicate. The determinations included moisture content, crude fiber, and ash content and crude protein, crude fat and total carbohydrates by (difference).

3.5.1 Determination of moisture content on dried weight basis

An empty evaporating dish was washed and dried in the oven, allowed to cool in the dessicator and weighed (w_1). About 3g of the sample was weighed and put into the dish. The sample plus evaporating dish was weighed and recorded as (w_2) and transferred into the oven, maintained at 105°C and kept there for three hours. The sample was removed, allowed to cool in the dessicator and then weighed. This process was continued until a constant weight was obtained and recorded as (w_3)

$$\% \text{ moisture content} = \frac{\textbf{weight loss due to drying}}{\textbf{weight of sample}} \times 100$$

$$\% \text{ moisture content} = \frac{W2-W3}{W2-W1} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{ (1)}$$

3.5.2 Determination of Ash

A crucible with its lid was washed, rinsed, dried in the oven and allowed to cool in a dessicator. The weight of empty crucible plus its lid, (w_1) was measured using an analytical balance. About 3g of the sample was put into the crucible and covered with its lid. The new weight was taken and recorded as (w_2). The crucible with its content was then transferred

into the muffle furnace maintained at 550^{o}C and kept there for about 6 hours for complete ashing. The ash obtained plus crucible was allowed to cool in the desiccators and weighed (w_3). Figure 3.2 shows the ashed samples

Figure 3.2: Ashed samples (Source: Author, 2015)

$$\% \text{ Ash Content} = \frac{\boldsymbol{weight\ of\ ash}}{\boldsymbol{weight\ of\ sample}} \times 100$$

$$\% \text{ Ash Content} = \frac{\boldsymbol{w3-w1}}{\boldsymbol{w2-w1}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

3.5.3 Determination of Crude Fat

About 50g of the sample was weighed (w_1) into a clean piece of cotton cloth. The sample was then wrapped securely in the cloth by tightening a thread around it. The wrapped sample was then immersed in the thimble of the Soxhlet extractor, using n-hexane and maintained at a temperature of 60^{o}C. This set-up was left in this condition for up to 5 hours

so that all the fat in the sample was extracted. The oil was concentrated from the oil-solvent mixture by removing the defatted sample from the thimble and distilling off some, but not all, of the solvent from the mixture in the flask. This extract was then exposed to air overnight for complete evaporation of the residual solvent. In the morning, the flask with its content was weighed continuously until a constant weight (w_2) was obtained. The flask was then emptied, cleaned thoroughly, oven-dried at 100^{o}C and weighed (w_3)

$$\% \text{ Crude Fat Content} = \frac{\boldsymbol{weight\ of\ extracted\ fat}}{\boldsymbol{weight\ of\ sample}} \text{ x } 100$$

$$\% \text{ Crude Fat Content} = \frac{\boldsymbol{w2-w3}}{\boldsymbol{w1}} \times 100 \quad \text{..... (3)}$$

3.5.4 Determination of total nitrogen

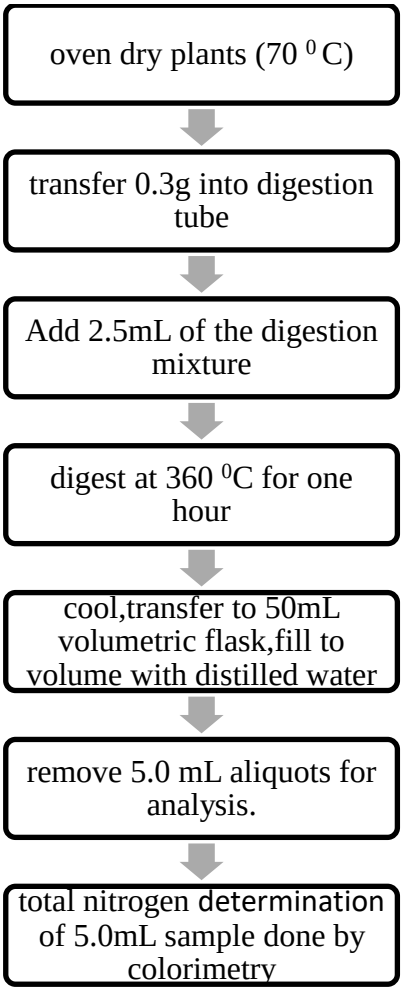
The total nitrogen was determined colorimetrically.

3.5.4.1 Reagents preparation

The reagents were made one day before being used and kept in the dark. Reagent N1was prepared by dissolving 17g sodium salicylate,12.5 g sodium citrate and 12.5 g sodium tartrate together in about 375 mL water. 0.06g nitroprusside was added and made up to 0.5 L of distilled water. Reagent N2: 15 g of sodium hydroxide was dissolved in about 375 mL distilled water and allowed to cool. 5 mL of sodium hypochlorite mixed well and made up to 0.5 L.

The oven dried plant samples (0.3g) were transferred into digestion tubes 2.5ml of the digestion mixture was added and digested for two hours. The mixture was then cooled and transferred into 50 mL volumetric flask and filled with distilled water to volume. Two 0.2

mL aliquots of each sample and the blanks were taken into clearly labeled test tubes. 5.0 mL of reagent N1 was added and vortexed, 5.0 ml of reagent N2 was also added and vortexed and allowed to stand for two hours .The absorbance was measured at 650 nm. A calibration curve was plotted and the concentration of nitrogen in each sample read off. The scheme below shows a summary of the procedure for determination of total nitrogen in the samples.



% Nitrogen Content = $\frac{c \times v \times f}{w}$ ..... (4)

Where:-

c = the corrected concentration of N in the sample

v = volume of digest

f = dilution factor

w = weight of sample

The final step was to estimate the % Nitrogen in the sample and hence the Crude Protein by multiplying that value by the general factor; 6.25

% Crude protein = % Nitrogen x 6.25

6.25 is the general factor of plant-derived food sample.

3.5.5 Estimation of crude fiber

Two grams of moisture free and fat free material of each sample was treated with 200ml of 1.25% H_2SO_4 and boiled for 30 minutes. After filtration and washing, the residue was treated with 1.25% NaOH and boiled for 30 minutes. It was filtered and washed with hot water. The washed residue was dried in the oven at 130 ^{0}C to a constant weight and cooled in the dessicator. The residue was scrapped into a pre-weighed crucible, weighed, ashed at 550 ^{0}Cfor two hours, cooled in a dessicator and reweighed. Crude fiber content was expressed as percentage loss in weight on ignition.

3.5.6 Total Carbohydrates Estimation

The Total Carbohydrates content was estimated by difference. The percentage Total Carbohydrates content is equal to the sum of the percentage Moisture, Crude Protein, Ash, Crude fiber and Crude Fat contents, subtracted from100.

% Carbohydrates = *100 - (%Mo + % Ash + % f + % p)*..... (5)

Where; *% Mo* = Percentage moisture content

% As = Percentage ash content

% f = Percentage fat

% p = Percentage protein

3.5.7 Energy content

This is calculated with the formula below:

Energy (Kcal/g) contents = *(%Fat x 9) + (%Protein x 4) + (% Carbohydrate x 4)*……

(6)

3.5.8 Mineral Analysis

The digested solution was used for determination of nutritionally valuable minerals such as: Na, K, Ca, Mg, Zn, Fe, Co and Mn. Sodium and potassium were determined by flame photometry (U.K, model 405). Ca and Mg were determined by Atomic Absorption spectrophotometry (Varian, USA) while Zn, Fe, Co and Mn were determined by AAS (Shimadzu AA-6300 GFA-EX7i). Phosphorous was determined by colorimetry

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Introduction

The two indigenous vegetables (*Amaranthus thunbergii* and *Commelina africana*) were analyzed for their nutritional and mineral composition. The nutritional composition was examined in terms of % moisture, total fat, total ash, crude protein, fiber, carbohydrate content and energy content. On the other hand mineral composition was ascertained in terms of the net content of sodium potassium, calcium, magnesium, iron, manganese, phosphorous, cobalt and zinc.

The soils were also analyzed for the same mineral contents which were also analyzed in the vegetables. This was done to determine the correlation between soil nutrient level and the nutritional composition of the vegetables.

4.2 Method validation

Performance of instruments used was tested using regression analysis and reproducibility.

4.2.1 Regression

Linear response of the instrument to the standard mineral concentration for the different minerals studied was determined by plotting respective calibration graphs.

The Limit of Detection (LOD) was determined by multiplying the Standard Deviation (SD) of the response by 3 then dividing by the slope of the calibration curve. SD was obtained from a linear regression function (LINEST) in the Ms Excel. Table 4.1 gives a summary of the results.

Table 4.1: Correlation Coefficients, Limit of Detection and Regression Equation

Parameter	R	LOD (ppm)	Regression equation
Cobalt	0.9952	0.2210	y = 0.903x + 0.408
Phosphorus	0.9997	0.1230	y = 0.823x + 0.648
Sodium	0.9939	0.6010	y = 0.902x + 0.381
Iron	0.9991	0.2340	y = 0.972x + 0.101
Manganese	0.9999	0.0336	y = 0.953x + 0.171
Potassium	0.9968	0.8746	y = 1.700x - 2.400
Zinc	0.9971	0.4025	y = 0.722x + 0.549
Calcium	0.9940	0.6011	y = 0.902x + 0.383
Magnesium	0.9092	2.5080	y = 0.615x + 1.410

Key: R; Correlation Coefficients, LOD; Limit of Detection

4.2.2 Reproducibility

This was carried out by a standard recovery in which three replicate blanks were spiked with standard solution to make a 3 ppm solution for each parameter and three readings from the instrument recorded. The mean, standard deviation, % recovery, repeatability relative standard deviation (RSD_r), reproducibility relative standard deviation (RSD_R) and Horwitz Ratio (HorRat) were determined and tabulated in table 4.2.

Table 4.2: Reproducibility test

Parameter	Mean conc (ppm)	SD	% Recovery	RSD_r	RSD_R	HorRat Value
Phosphorus	3.16	0.1312	105	5.5854	13.45	0.33
Cobalt	3.12	0.1305	104	4.3164	13.48	0.357
Sodium	3.03	0.1381	101	4.5881	13.51	0.31
Iron	3.10	0.1478	103	4.7728	13.50	0.35
Manganese	3.17	0.1577	105	4.9685	13.44	0.36
Potassium	3.09	0.1588	103	5.1386	13.50	0.38
Zinc	3.09	0.1540	104	4.5517	13.47	0.37
Calcium	3.03	0.1392	101	4.5887	13.54	0.33
Magnesium	3.12	0.6343	104	20.3164	13.48	1.507

Key: SD; Stand Deviation, RSD_r; Repeatability Relative Standard Deviation, RSD_R; Reproducibility Relative Standard Deviation

4.3 Nutritional Analysis

The nutritional content of the two indigenous vegetables (*Amaranthus thunbergii* and *Commelina africana)* was gauged in terms of percentage moisture content, total ash, crude fat content, crude protein content, crude fiber content and total carbohydrates content.

4.3.1 Percentage Moisture Content (On Dry Weight Basis)

The results of the experimental measurement of the percentage moisture content (dry weight basis) of the two vegetables are shown in Table 4.3. The percentage moisture

content of each vegetable represents the mean value of three replications. The standard deviation of each experimental point is also given.

Table 4.3: Proximate composition of the two vegetables (*Amaranthus thunbergii* and *Commelina africana*)

Parameter	**Concentration (% DW)** ***Amaranthus thunbergii***	**Concentration (% DW)** ***Commelina africana***
Moisture	4.20±0.05	4.13±0.01
Ash content	29.61±0.04	18.98±0.04
Crude protein	4.64±0.01	16.05±0.04
Crude fat	4.69±0.03	1.72±0.03
Carbohydrates	30.34±0.04	37.62±0.06
Crude fiber	9.02±0.03	10.87±0.04
Energy content (kcal/100 g)	182.13±0.03	230.16±0.02

Key: DW; Dry Weight

The moisture contents of the two vegetable species (*Amaranthus thunbergii* and *Commelina africana*) were 4.20±0.05% and 4.13±0.01% respectively. These values were more or less the same within a variation of 0.1%. The percentage moisture content of the vegetables was very low because the leaves were oven dried before analysis. Since this percentage of moisture was found in the dry milled (DM) samples, it indicates that freshly harvested leaves will contain higher amount of moisture. The low moisture content suggests that the leaves can be kept for a relatively long time. The moisture content of any

food can be used as a measure of its keeping quality. The values obtained in this study were lower than those reported by Nnamani *et al.* (2009) who did an assessment of moisture content of three underutilized indigenous leafy vegetables of Ebonyi State, Nigeria. Their findings showed that the moisture contents were 9.6, 10.2 and 10.8 percent in *Z. zanthoxyloides, V. doinana* and *A. cissampeloides,* respectively. The values were also lower than those reported by Seidu *et al.* (2012) who determined the moisture content of three common vegetable namely *Xanthosoma sagittifolia, Moringa oleifera* and *Talinum trangulare*. Their results showed that the moisture content of dried samples ranges from 10.28% for *Xanthosoma sagittifolia* to 12.48% for *Talinum trangulare.*

4.3.2 Total Ash Content

Ashing is the process of mineralization for preconcentration of trace substances prior to chemical analysis. Ash is one of the components in the proximate analysis of biological materials, consisting mainly of salty, inorganic constituents. The results of the experimental measurement of the total ash of the two vegetables are shown in Table 4.3. There was minimum variation of ash contents between the two sampled vegetables. The mean ash contents on dry matter were 29.61±0.04% and 18.98±0.04% for *Amaranthus thunbergii* and *Commelina africana,* respectively. These values are comparable to those reported in many related studies. Odhav *et al.* (2007) who did a preliminary assessment of nutritional value of three traditional leafy vegetables (*Chenopodium album, Sonchus asper, Solanumnigrum, Urtica urens*) in KwaZulu-Natal in South Africa reported that the ash contents were 16.08%, 23.08% and 27.75% respectively. Gupta *et al.* (2005) reported even lower values in their study of nutrient and antinutrient content in two underutilized green

leafy vegetables, namely, *Polygala erioptera, Delonix elata, Digera arvensis* and C*occulus hirsutus.* The findings indicated that the total ash content ranged from 0.77% to 3.54%.

4.3.3 Crude Fat Content

The results of the experimental measurement of the crude fat content of the two vegetables are shown in Table 4.3. The crude fat content was highest in *Amaranthus thunbergii* (4.69±0.03%) compared to *Commelina africana* (1.72±0.03%). The fat content range observed in this study compared favourably with results of Kwenin *et al.* (2011) who assessed the nutritional values of the leaves of *Xanthosoma sagittifolia, Talinum triangulare, Amaranth cruentus* and *Moringa oleifera*. The fat content ranged from 1.33% to 3.19%. The values of this study also compared favourably with the fat content values reported by Oduro *et al.*(2008) who assessed the nutritional potential of two leafy vegetables: *Moringa oleifera* and *Ipomoea batatas.* Their findings indicate that the crude fat content was 0.38% for *Moringa oleifera* and 1.91% for *Ipomoea batatas.* However, the results of this study were far much lower than those reported by Ndlovu and Afolayan (2008) who performed nutritional analysis of the South African wild vegetable *Corchorus olitorius L.* The findings showed that the crude fat content ranged from 17.20% to 18.30%. The difference in values can be attributed to genetic factors as noted by Kwenin *et al* . (2011).

4.3.4 Crude Protein Content

The results of the experimental measurement of the crude protein content of the two vegetables are shown in Table 4.3. The protein content of the two indigenous vegetables ranged between 4.64±0.01% and 16.05±0.04% with *Commelina africana* showing very

high value (16.05%) compared to *Amaranthus thunbergii* (4.64%). The results observed for *Commelina africana* were in range with that reported by Agunbiade *et al.* (2012) who assessed the hypoglycaemic activity of *Commelina africana* and *Ageratum conyzoides* in relation to their mineral composition. The findings showed that the protein content for *Commelina africana* and *Ageratum conyzoides* were 17.86% and 15.67%, respectively. The crude protein value of *Amaranthus thunbergii* compared favorably with those by Kwenin *et al.* (2011) who reported crude protein content of 4.46% for *Amaranth cruentus.*

According to Kris-Etherton *et al.* (2002), a diet providing 10 - 15% of its crude protein is said to be sufficient to human beings, as excess calories consumption leads to certain cardiovascular disorders such as atherosclerosis, cancer and aging thus these two vegetables have the capability to provide the required protein content.

4.3.5 Crude Fiber Content

The results of the experimental assessment of the crude fiber content of the two vegetables are shown in Table 4.3. Crude fiber content was expressed as percentage loss in weight on ignition. According to table 4.3, Crude fibre ranged from 9.02±0.03% in *Amaranthus thunbergii* to 10.87±0.04% in *Commelina africana*. The substantial amount of fiber in these two vegetables shows that they can help in keeping the digestive system healthy and functioning properly. Fibre aids and speeds up the excretion of waste and toxins from the body, preventing them from sitting in the intestine or bowel for too long, which could cause a build-up and lead to several diseases (Adeniyi *et al.,* 2012).

The values reported in this study were in range with those of a study by Seidu *et al.* (2012) who reported that for *Talinum tringulare, Moringa oleifera* and *Vernonia amygbalina* the

crude fiber content values were 8.88%, 8.19% and 9.50%, respectively. However, the values for this study were much higher than those of a study by Adeniyi *et al.* (2012) who reported crude fibre ranges from 0.33% in *Corchorus olitorius* to 0.21% in *Talinum triangulare.*

4.3.6 Total Carbohydrate Content

The results of the experimental assessment of the total carbohydrates content of the two vegetables are shown in Table 4.3. Based on the results obtained the mean total carbohydrates content ranged from 37.62±0.04% for *Commelina africana* to 30.34±0.06% for *Amaranthus thunbergii*. These values were much higher than those reported by Mensah *et al.* (2008) who did a systematic survey of a green leafy vegetable (*Basella rubra*) from Edo State of Nigeria. The results showed that the carbohydrate content was 2.9% which was very low. However, the carbohydrate results of this study are lower than those reported by Seidu *et al.* (2012) who reported the carbohydrate levels for *Xanthosoma sagittifolia, Talinum triangulare, Moringa oleifera, Vernonia amydalina* as 77.22%, 65.77%, 50.27% and 73.74% respectively.

The National Academies Institute of Medicine recommends that in order to meet the body's daily nutritional needs while minimizing risk for chronic disease, adults should get 45% to 65% of their calories from carbohydrates daily (Sox & Greenfield, 2009). This implies that the two vegetables in this study can not provide enough daily body carbohydrate nutritional needs unless supplemented with other carbohydrate meals.

4.3.7 Energy content

The calorific value of *A. thunbergii* and *C. africana* from table 4.3 were estimated to be 182.13±0.03 kcal/100g and 230.16±0.02 kcal/100g, respectively, which is low compared to 248.8 kcal/100g reported in some Nigerian leafy vegetables (Isong *et al*.,1999). Asibey-Berko and Tayie (1999) also reported high energy content in some Ghanaian green leafy vegetables such as C*orchurus tridens* (283.1 kcal/100g).This shows that the plant leaves has low calorific value which is in agreement with general observation that vegetables have low energy values (Lintas,1992).

4.4 Mineral Analysis

The concentration of phosphorus, sodium, potassium, cobalt, zinc, manganese, magnesium, calcium and iron in the two indigenous vegetable species (*Commelina africana* and *Amaranthus thunbergii*) were determined using two methods; atomic absorption spectrometry (AAS) and flame photometry. The results of the concentration of the different studied nutrients in the two vegetables are presented in table 4.4 below.

Table 4.4: Concentration of the different studied nutrients in the two vegetables (mg/100g)

Parameter	***Amaranthus thunbergii***	***Commelina africana***
P	78.80 ±0.03	80.10 ±0.01
Na	8.80 ±0.04	11.40 ±0.03
K	8.20 ±0.03	8.90 ±0.05
Co	0.01 ±0.00	0.03 ±0.06

Zn	4.18 ±.30	5.04 ±0.03
Mn	8.30 ±0.04	6.60 ±0.02
Fe	26.20 ±0.35	24.40 ±0.3
Mg	48.60 ±0.06	8.87 ±0.03
Ca	18.30 ± 0.05	13.60 ±0.02

4.4.1 Phosphorus

The concentration of phosphorus in the two vegetables was 80.10±0.01 mg/100g for *Commelina africana* and 78.80± 0.03 mg/100g for *Amaranthus thunbergii.* This implies that the two vegetables have nearly the same content of phosphorus. The main function of phosphorus is in the formation of bones and teeth. According to Mason (2007), the daily phosphorus requirement ranges from 275 mg for kids to 700 mg for adults. This implies that the two vegetables cannot offer sufficient daily phosphorus supply unless taken voluminously.

These findings are in line with those of Kwenin *et al.* (2011) who did an assessment of the nutritional value of three African indigenous green leafy vegetables in Ghana. The findings showed that the phosphorus content was 80.10 mg/100g, 78.90 mg/100g, 81.90 mg/100g and 74.00 mg/100g for *Xanthosoma sagittifolia, Amaranth cruentus, Talinum triangulare* and *Moringa oleifera,* respectively.

However, the values of this study are much higher than those of a study by Ndlovu and Afolayan (2008) who did a nutritional analysis of the South African wild vegetable *Corchorus olitorius* and found out that the phosphorus content was 25.80 mg/100g. The

findings of this study were also much higher than those of Okwu (2008) who assessed the nutritional content of two Nigerian vegetables and reported phosphorus values of 0.33 mg/100g for *Garcnia kola* and 1.60 mg/100g for *Aframomum melegueta.*

4.4.2 Sodium

The concentration of Na in the two vegetables was 11.40± 0.03 mg/100g for *Commelina africana* and 8.80±0.04 mg/100g for *Amaranthus thunbergii.* This implies that the concentration of Na in *Commelina africana* was higher than those of *Amaranthus thunbergii.* These values were higher than those of *Telferia occidentalis* (1.17 mg/100g), *Vernonia amygdalina* (3.75 mg/100g), *Gnetum africana* (1.50 mg/100g), *Piper guineense* (0.07 mg/100g), *Cochorus olitorius* (0.33 mg/100g) and *Talinum triangulareisimum* (0.28 mg/100g) as reported by Mensah *et al*.(2008).

The sodium content of the two vegetables in this study were, however, comparatively lower than that of *Gnetum africanum, Xanthosom asagittifolium, Lasianthera africana and Heinsia crinite* which were 92.00 mg/100g, 26.00 mg/100g, 63.80 mg/100g and 179.00 mg/100g, respectively, as reported by Ukam (2008).

Sodium is used in the body to control blood pressure and blood volume. Additionally, the body needs sodium for the muscles and nerves to work properly (Vollmer *et al.,* 2001; Pimenta *et al.,* 2009). The U.S. Food and Drug Administration recommend that individuals consume no more than 2,300 milligrams of sodium per day (Havas *et al.,* 2007). This implies that these two vegetables supply low amounts of dietary sodium but this is easily supplemented by addition of common table salt to foods.

4.4.3 Potassium

The concentration of K in the two vegetables was 8.90±0.05 mg/100g for *Commelina africana* and 8.20±0.03 mg/100g for *Amaranthus thunbergii*. The potassium content of these two vegetables was higher than that of *Amaranthus cruentus* (4.82 mg/100 g), *Telferia occidentalis* (2.45 mg/100g), *Vernonia amygdalina* (3.75 mg/100g), *Gnetum africana* (0.08 mg/100g), *Piper guineense* (3.92 mg/100g), *Basella rubra* (2.32 mg/100g) and *Ocimum grattisimum* (2.34 mg/100g) as reported by Mensah *et al*. (2008). However, the potassium content of the two vegetables in this research were lower than those of *Amaranthus hybridus* (54.20 mg/100g) reported by Akubugwo *et al.* (2007).

World Health Organization (2013) recommends a daily potassium intake ranging from 3g for children to 5g for adults. This implies that these two vegetables can supply sufficient potassium to the body.

4.4.4 Cobalt

The concentration of Co in the two vegetables was 0.03±0.06 mg/100g for *Commelina africana* and 0.01±0.00 mg/100g for *Amaranthus thunbergii*. This implies that cobalt levels in the vegetables were very low which was attributed to the low concentrations of cobalt in the soil.

The cobalt content of these two vegetables was comparable with that of *Myrciaria dubia* (0.02 mg/100g) as reported by Justi *et al.* (2000). However, the values were lower than those of *Garcnia kola* and *Aframomum melegueta* which had cobalt values of 0.55 mg/100g and 0.60 mg/100g as reported by Okwu *et al.* (2005).

4.4.5 Zinc

The concentration of Zn in the two vegetables was 5.04±0.03 mg/100g for *Commelina africana* and 4.18±0.03 mg/100g for *Amaranthus thunbergii.* The Zn values of this research were higher than those of *Amaranthus cruentus* (0.7 mg/100g) and *Amaranthus dubius* (1.5 mg/100g) as reported by Yang and Keding (2009). Kinabo *et al.* (2004) reported contents of Zn in African nightshade to be 0.57 mg/100g which is lower than those observed in the current study. Additionally, the current values were far much lower than those reported by Mutune *et al.* (2013) for *Solanum villosum, Betavulgaris,Brassica carinata, Amaranthus, Curcubita moschata, Vignaunguiculata, Cochorus clitorius, Crotalaria* and *Brassica oleracea* which had zinc concentrations ranging from 1.56 to 4.91 mg/100g. However, the values of this study were in range with those of *amaranth* (4.08 mg/100g) collected in various markets in Dar el Salaam as reported by Raja *et al.* (1997).

Zinc is an important trace mineral that people need to stay healthy. This element is second only to iron in its concentration in the body (Swaine, 2000).Zinc is found in cells throughout the body. It is needed for the body's defensive (immune) system to properly work. It plays a role in cell division, cell growth, wound healing, and the breakdown of carbohydrates (Thompson& Manore, 2012).Zinc is also needed for the senses of smell and taste (Haas, 2006). During pregnancy, infancy and childhood, the body needs zinc to grow and develop properly (Balch, 2006).

The Recommended Dietary Allowance (RDA) for Zinc ranges from 3mg/day for kids to 11 mg/day for adults (Fenech, 2002). This implies that these two vegetables can supply sufficient Zn content to human body if taken daily in bulk.

4.4.6 Manganese

The concentration of Mn in the two vegetables was 6.60±0.02 mg/100g for *Commelina africana* and 8.30±0.04 mg/100g for *Amaranthus thunbergii*. The Mn values for the two vegetables were comparable to those of five *Amaranthus* accessions reported by Keni *et al.* (2007) to be ranging from 6.64 to 9.18 mg/100g. The values were higher compared with those of *Colocassia esculenta* (5.0 mg/100g) and *Ocimum gratissimum* (5.3 mg/100g) as reported by Thomas and Oyediran (2008).

4.4.7 Iron (Fe)

The concentration of Fe in the two vegetables was 24.40±0.03 mg/100g for *Commelina africana* and 26.20±0.35 mg/100g for *Amaranthus thunbergii.* These levels were in range with those of *Talinum triangulare* (28.21 mg/100g) reported by Kwenin *et al.* (2011) and *Amaranthus cruentus* (20.57 mg/100g), *Solanum scabrum* (26.60 mg/100g) reported by Kamga *et al.* (2013).

However, these levels were higher than those of *Solan0um aethiopicum* (3.34 mg/100g), *Abelmoschus callei* (4.22 mg/100g) as reported by Kamga *et al.* (2013) and also other five *Amaranthus* accessions reported by Mnkeni *et al.* (2005) to have Fe levels ranging from 6.50 to 7.50 mg/100g.

The Recommended Dietary Allowance (RDA) for Fe ranges from 5 mg/day for kids to 30 mg/day for adults (Trumbo *et al.*, 2001). This implies that these two vegetables can't supply sufficient Fe content to human body unless taken in bulk.

4.4.8 Magnesium

The concentration of Mg in the two vegetables was 8.87±0.03 mg/100g for *Commelina africana* and 48.60±0.06 mg/100g for *Amaranthus thunbergii.* The magnesium content of these two vegetables was higher than that of *Amaranthus cruentus* (2.53 mg/100 g), *Telferia occidentalis* (0.65 mg/100g), *Vernonia amygdalina* (0.45 mg/100g), *Gnetum africana* (0.30 mg/100g), *Piper guineense* (1.01 mg/100g), *Basella rubra* (0.06 mg/100g) and *Ocimum grattisimum* (0.43 mg/100g) as reported by Mensah *et al*. (2008). However, the magnesium content of the two vegetables in this research were lower than those of *Amaranthus hybridus* (231.20 mg/100g) reported bw3y Akubugwo *et al.* (2007).

World Health Organization (2013) recommends a daily magnesium intake ranging from 30 g for children to 420 g for adults. This implies that these two vegetables can supply sufficient magnesium to the body if taken voluminously.

4.4.9 Calcium

The concentration of Ca in the two vegetables was 13.60±0.02 mg/100g for *Commelina africana* and 18.30±0.05 mg/100g for *Amaranthus thunbergii.* These levels were lower than those of *Amaranthus hybridus L.*(44.15 mg/100g) reported by Akubugwo *et al.* (2007) and *Colocassia esculenta* (240 mg/100g), *gratissimum* (175 mg/100g) reported by Thomas & Oyediran (2008). However, these levels were higher than those of *Amaranthus cruentus* (2.05 mg/100g), *Vernonia amygdalina* (2.25 mg/100g), *Piper umbellatum* (2.36 mg/100g) as reported by Mensah *et al.* (2008).

The DV for calcium is 1,000 mg for adults and children aged 4 years and older. Foods providing 20% or more of the DV are considered to be high sources of a nutrient. This

implies that these two vegetables can't supply sufficient Ca content to human body unless taken in bulk.

4.5 Concentration of the Different Minerals in Soil

The concentration of phosphorus, sodium, potassium, cobalt, zinc, manganese, magnesium, calcium and iron in the soils where the two indigenous vegetable species (*Commelina africana* and *Amaranthus thunbergii*) were growing were determined using atomic absorption spectrometry (AAS), colorimetry and flame photometry. The results of the concentration of the different studied nutrients in the soil samples are presented in Table 4.5 below.

Table 4.5: Concentration of the different studied nutrients in the soil samples

Soil samples (mg/100g)	
P	89.40±0.035
Na	14.99±0.04
K	10.08±0.16
Co	0.05±0.03
Zn	6.25±0.27
Mn	10.18±0.35
Fe	39.94±0.07
Mg	32.91±17.91
Ca	19.77±0.25

The nine minerals analyzed in this study were available in soil with mean concentration ranging from0.05±0.03 mg/ 100g to 89.40±0.035 mg/ 100g. Levels of all the minerals in

soil were found to be higher than the corresponding concentrations in the two vegetables. To determine whether there was a significant difference between the concentration of the different minerals in the soil and in the vegetables, a paired t-test was conducted. The results are shown in Table 4.6 below:

Table 4.6: Paired t-test results

	Paired Differences							
				95% Confidence Interval of the Difference				
	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t value	DF	2-tailed test
Soils - Vegs	5.05857	5.33236	2.01544	0.12696	9.99018	2.510	6	0.031

The results in Table 4.6 show that the p value was 0.031 implying that there existed a significant difference at $p<0.05$ (95% confidence level) between the concentration of the different minerals in the soils and in the vegetables. This means that most of the nutrients in the soils were transferred to the vegetables. This can be attributed to the high transfer factors attested for the different nutrients as shown in Table 4.7 below.

Table 4.7: Transfer factors of the nutrients from soil to vegetables (mg/100g)

	Transfer Factor	
Nutrient	***Commelina Africana***	***Amaranthus thunbergii***
P	0.90±0.10	0.88±0.03
Na	0.76±0.01	0.59±0.04
K	0.88±0.02	0.81±0.02
Co	0.60±0.30	0.20±0.00

Zn	0.81±0.25	0.67±0.01
Mn	0.65±0.10	0.82±0.02
Fe	0.61±0.01	0.66±0.01
Mg	0.59±0.02	0.67±0.01
Ca	0.68±0.03	0.92±0.02

From the results provided in Table 4.7 above, the transfer factors for *Commelina africana* ranged from 0.59 (Mg) to 0.90 (P) while for *Amaranthus thunbergii*, the transfer factors ranged from 0.20 (Co) to 0.92 (Ca). Considering the two vegetables the order for soil to plant transfer factors was Mg<Co<Fe<Mn<Ca<Na<Zn<K<P for *Commelina africana* and Co<Na<Fe<Zn<Mg<K < Mn<P<Ca for *Amaranthus thunbergii*. In general cobalt had the lowest transfer factor in both vegetables while phosphorus had the highest.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The research sought to determine the nutritional composition of two main indigenous vegetables; *Commelina africana* and *Amaranthus thunbergii* from Ainamoi within Kericho County. The nutritional values were assessed based on the contents of carbohydrates, proteins, fats, moisture and essential minerals including cobalt, zinc, manganese, iron, phosphorus, sodium and potassium. Based on the findings, it can be concluded that *Amaranthus thunbergii* is more nutritious than *Commelina africana.* This is because *Amaranthus thunbergii had* higher values of moisture (4.2 %), total ash (29.61%), crude fat (4.69%), fiber (9.02%), total Fe (26.20 mg/100g), total Mn (8.30 mg/100g), total Mg (48.60 mg/100g) and total Ca (18.30 mg/100g). Compared to *Amaranthus thunbergii, Commelina africana* had high values of fiber (10.87%) and crude proteins (16.05%). It also had higher values of P, Na, K, Co and Zn which were 80.10, 11.40, 8.90, 0.03 and 5.04 mg/100g, respectively.

There was a positive correlation between the concentration of minerals in the soil and the vegetables. This means that most of the minerals in the soils were transferred to the vegetables. The concentrations of most of the minerals in the vegetables for example, Mn, Co, K and Zn met the daily RDA of minerals. The others can meet the RDA if taken voluminously or taken with supplements.

From researches throughout the world including this research it is evident that indigenous vegetables both domesticated and non-domesticated have high levels of essential nutrients and minerals.

5.2 Recommendations

Based on the findings of this study, the following recommendations were offered in an attempt to raise the knowledge and usability of indigenous vegetables:

1. Instead of concentrating on commercial food crops, extension efforts should now be aimed at maintaining, popularizing and improving the accessibility of a wide range of the two species, as this can do much to improve nutrition and food security.
2. Efforts should be directed at maintaining the maximum possible diversity in our food plants and use them for everyone's well-being. To achieve this, it is necessary to:
    i. Make sure that families eat more traditional vegetables;
    ii. Discard the false and unwarranted notion that traditional foods are inferior;
    iii. Take the initiative to grow those species that can be grown and to manage others in the wild while preserving their habitats and ecosystems, even in people’s back yards;
    iv. Promote and keep alive indigenous knowledge about edible plants, methods of preparation, local names, among others, pass this knowledge to the next generation and, where possible, document it;

v. Identify rare and endangered varieties and liaise with the National Gene Bank at the Kenya Agricultural Research Institute (KARI) for long-term conservation.

vi. Studies should be conducted to ascertain the possibility of domestication of different indigenous vegetables to avoid their extinction from the flora species.

Future studies should focus on the possibility of improving the nutrition values of different indigenous vegetables.

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APPENDICES

Appendix I: Moisture Content Determination (Raw Data)

		Amaranthus thunbergii	*Commelina Africana*
Sample 1	W1	27.900	27.543
	W2	27.897	27.440
Sample 2	W1	26.960	27.730
	W2	26.843	27.625

Appendix II: Ash Content Determination (Raw Data)

		Amaranthus thunbergii	*Commelina africana*
Sample 1	W1	27.970	27.543
	W2	25.848	25.468
Sample 2	W1	27.960	27.730
	W2	25.908	25.720

Appendix III: Crude Fat Determination (Raw Data)

		Amaranthus thunbergii	*Commelina africana*
SAMPLE 1	Thimble + sample W1	13.667	11.787
	Thimble w2	3.568	3.458
Fat obtained	Flask + fat	99.434	81.626
	Flask	98.960	81.483

Appendix IV: Fibre Content Determination (Raw Data)

		Amaranthus thunbergii	*Commelina africana*

SAMPLE 1	Empty crucible W1	26.36	15.12
	Fibre + crucibleW2	26.64	15.201
	Crucible + ash	26.437	14.892

Appendix V: Crude Protein Determination (Raw Data)

Nitrogen Standards	**Absorbance**
0ppm	0.000
10ppm	0.165
20	0.327
30	0.535
40	0.909
50	0.958
60	0.996
70	1.076
80	1.355
90	1.385
Samples	**Absorbance**
L1	2.340
L1	2.325
S1	0.287
S1	0.272
S2	0.270
S2	0.268
A1	2.100
A2	2.150

NB: Samples were analyzed in duplicate

Appendix VI: Phosphorus Composition Determination (Raw Data)

Phosphorous Standards	**Absorbance**
0 ppm	0.000
0.2	0.222
0.4	0.312
0.6	0.453
0.8	0.617
1.0	0.803
1.2	0.950
1.4	1.095
Samples	**Absorbance**
L1	1.640
L1	1.720
S1	0.906
S1	0.902
S2	1.002
S2	0.988
A1	1.620
A1	1.560

NB: Samples were analyzed in duplicate

Appendix VII: Sodium Composition Determination (Raw Data)

Sodium standards	**Absorbance**
0 ppm	0
2	26
4	50
6	70
8	89
10	100
Samples	**Absorbance**
A1	2
A1	2 × 100
L1	1
L1	1 × 100
S1	3
S1	1 × 25
S2	7
S2	6 × 10

NB *100=Dilution Factor

Appendix VIII: Potassium Composition Determination (Raw Data)

Potassium standards	**Absorbance**
0 ppm	0
2	14
4	35
6	56
8	75
10	100
Samples	**Absorbance**
A1	12
A1	13 × 100
L1	27
L1	27 × 100
S1	1
S1	1 × 25
S2	24
S2	21 × 10

Appendix IX: A Summary of the Raw Data of AAS Results for Co, Zn, Mn and Fe

	Co(ppm)	**Abs**	**Zn (ppm)**	**Abs**	**Mn (ppm)**	**Abs**	**Fe (ppm)**	**Abs**
A1	0.02	0.000	0.7866	0.0561	1.026	0.0249	1.781	.0106
A1	0.04	0.00	0.8572	0.0613	1.090	0.0264	1.888	.0112
L1	0.00	0.00	0.0359	0.0023	1.217	0.0295	5.167x10	.0310
L1	0.01	0.00	0.0883	0.0058	1.204	0.0292	4.509x10	.027
S1	0.03	0.00	0.4576	0.0318	0.976	0.0237	1.300	.0077
S1	0.04	0.00	0.5531	0.0389	1.253	0.0304	1.061	.0063
S2	0.01	0.00	0.0899	0.0059	1.511	0.0366	7.700x10	0.046
S2	0.00	0.00	0.000	0.000	1.079	0.0262	5.257	0.0315

NB: x10 is number of dilutions for samples whose concentrations were outside the graph.

The original results are on the hard copy

Appendix X: A Summary of the Raw Data of AAS Results for Mg

Contr AA 700 5/14/2015 10:42

Operator: Government Chemist

Laboratory Results file

Instrument: ContrAA 700#160Ca 0775 Technique: Flame Comment

Ca						
Action	Sample	True	Conc	Abs	Actual Value	Actual
BLK				0.005		
STD		1	1.0000	0.32588		
STD		2	2.0000	0.67912		
STD		3	3.0000	0.95093		
STD		4	4.0000	1.2000		
STD		5	5.0000	1.64414		
UNK 1	A1		0.4549	1.19845	0.4549	ppm
UNK 2	A1(Dupl)		0.4557	1.18407	0.4557	ppm
UNK 3	L1		0.4863	1.16441	0.4863	ppm
UNK 4	L1(Dupl)		0.4844	1.15052	0.4844	ppm
UNK 5	S1		0.5303	1.34142	0.5303	ppm
UNK 6	S1(Dupli)		0.5232	1.32846	0.5232	ppm
UNK 7	S2		0.5658	1.53648	0.5658	ppm
UNK 8	S2(Dupli)		0.5767	1.54417	0.5767	ppm

Appendix XI: A Summary of the Raw Data of AAS Results for Mg

Contr AA 700 5/14/2015 11:28

Operator: Government Chemist

Laboratory Results file:

Instrument: ContrAA 700 #160Mg 0775 Technique: FlameComment

Mg						
Action	Sample	True Value	Conc	Abs	Actu	Actual
BLK				0.00123		
STD		1	1.0000	0.19804		
STD		2	2.0000	0.37758		
STD		3	3.0000	0.54958		
STD		4	4.0000	0.69525		
STD		5	5.0000	0.83147		
UNK 1	A1		0.2833	0.16585	0.2833	ppm
UNK 2	A1(Dupli)		0.2732	0.15233	0.2732	ppm
UNK 3	L1		0.2869	0.40972	0.2869	ppm
UNK 4	L1(Dupli)		0.2835	0.39651	0.2835	ppm
UNK 5	S1		0.2494	0.57595	0.2494	ppm
UNK 6	S1(Dupli)		0.2528	0.58825	0.2528	ppm
UNK 7	S2		0.2552	0.80772	0.2552	ppm
UNK 8	S2(Dupli)		0.2416	0.78548	0.2416	ppm