EVALUATION OF SELECTED NUTRIENTS IN MULBERRY (*Morus alba L*) VARIETIES GROWN AT KARI STATION-THIKA, KIAMBU COUNTY

BY

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I56/CE/10377/2007

A Thesis Submitted in Partial Fulfillment of the Requirements for the Award of the Degree of Masters of Science (Applied Analytical Chemistry) in the School of Pure and Applied Sciences, Kenyatta University

Ngigi, Sabina
Evaluation of selected nutrients

March 2014
DECLARATION

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

Signature........................................Date.....................

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I56/CE/10377/2007

We confirm that the work reported in this thesis was carried out by the student under our supervision.

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Signature........................................Date.....................

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Department of Chemistry
Kenyatta University
DEDICATION

This work is dedicated to my dear parents who through God’s grace brought me up and nurtured me, my dear husband Raphael and our daughter Phoebe.
ACKNOWLEDGEMENTS

First and most important, I thank my Almighty God who granted me knowledge, wisdom and all other requirements that I needed to come this far. I sincerely thank my supervisors Prof. Hudson Nyambaka and Dr. Ruth Wanjau who tirelessly guided me with much patience and encouraged me throughout this research work. I also greatly thank the lecturers at the Department of Chemistry, Kenyatta University and more so the technical staffs for their assistance during the research work.

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God bless you all.
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<td>Association of Official Analytical Chemist</td>
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<tr>
<td>BSA</td>
<td>Bovine Serum Albumen</td>
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<td>EDXRF</td>
<td>Energy Dispersive X-Ray Fluorescence</td>
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ABSTRACT

Mulberry is a multipurpose plant whose use is well documented and it is perhaps one of the very rare tree species that can serve many functions of mankind namely fodder, fiber, fuel and food since its leaves and fruits are highly palatable and nutritious. Leaves and fruits are known to be rich in proteins, vitamins, carbohydrates and carotene, minerals such as calcium, iron, potassium and zinc. Different parts of this plant; leaves, fruits, roots, stem and shoot bark are known to have medicinal properties such as reducing blood sugar, blood pressure and cholesterol and the fruit juice prevents premature aging and impotence. In Kenya mulberry has been used for silkworm rearing, as part of diet for cattle, and in poultry rations and other ruminants. Recently mulberry has found its use as a vegetable and food supplement but levels of micro and macronutrients in leaves and fruits of different varieties are not well documented in Kenya. Different mulberry varieties are grown at KARI station-Thika for the purpose of feeding silkworms and the leaves are now being processed into powder form for human consumption. This study therefore determined the levels of nutrients in leaves and fruits of eight (8) different mulberry varieties (Ithanga, Thika, Embu, Limuru, S54, Kanva, Thailand and Ichinose). The leaves and fruit samples were collected and analyzed for potassium, calcium, zinc, iron, vitamins A (β-Carotene), C (Ascorbic acid) and E (α-tocopherol), carbohydrates and proteins. The Ca, Zn and Fe were analyzed using AAS, K using flame photometry, carbohydrates, β-Carotene, α-tocopherol and proteins content were analyzed using UV/Visible spectroscopy while ascorbic acid was analyzed by a titration method. The mean levels obtained in leaves and fruits ranged from 34.25±2.50 - 148.42±1.44 mg/100g for Ca, 141.81±2.50 - 435.93±1.40 mg/100g for K, 0.13±0.01 - 0.43±0.010 mg/100g for Zn, 0.55±0.01 - 5.62±0.00 mg/100g for Fe, 1.71±0.03 - 56.81±0.08 mg/100g for β-Carotene, 394.00±7.00 - 644.33±1.15 mg/100g for ascorbic acid, 11.06±0.94 - 16.845±0.30 mg/100g for α-tocopherol 87.12±6.92 - 426.36±9.1 mg/100g for carbohydrates and 1.24±0.34 - 49.55±0.29 mg/100g for proteins. Ichinose leaves recorded the highest levels of Ca and K, Thika leaves were significantly higher in zinc, Limuru leaves were higher in Fe, Kanva leaves were higher in carbohydrates, S54 leaves were significantly higher in vitamins A and E, Ithanga leaves were significantly higher in ascorbic acid and Thailand leaves were significantly higher in proteins. Embu fruits recorded significantly higher levels in Ca and K, Limuru fruits were highest in Zn, Thika fruits were higher in Fe and S54 fruits were higher in carbohydrates. The results showed that all varieties had substantial amount of the nutrients but in varying proportion and there were variations in levels of nutrients in the leaves and fruits of the same mulberry variety with levels being higher in leaves than in fruits of most varieties. Among all the nutrients studied, β-carotene and ascorbic acid levels in fresh mulberry leaves of all the varieties and also carbohydrate levels in Kanva variety were found to be higher than the RDA. However Kanva and Embu varieties were higher in most macronutrients while Thika and Limuru were higher in most micronutrients Therefore all the varieties can be used to provide cheaper and locally available source of nutrients. The results of the present study will be availed to relevant authorities and also to sensitize the public.
CHAPTER ONE: 1 INTRODUCTION

1.1 Background

People around the world have recognized the grim truth that ultimately the population growth will outstrip food suppliers with apocalyptic results. About 36 million people were reported to die every year as a result of hunger (FAO/SD, 2001). Approximately, 60% of the 10.9 million deaths each year among children under the age of five in the developing world are attributed to malnutrition (WHO, 2008). If the current average birth rate continues, the world's population will grow from the current 6.7 to 9.2 billion by the year 2050, most of which will be in the less developed countries, the countries least able to feed their children (WHO/UN, 2007). Feeding 9.2 billion people at the current dietary levels presents the staggering necessity of increasing the earth's food producing capacity to a rate never seen before. Malnutrition due to nutritionally inadequate diets is one of the major concerns in Kenya which has recently been rated poorly in food security due to high levels of poverty, inflation and famine, posing a great risk to malnutrition.

Nutrition is an input to and the foundation for health and development (WHO, 2007). Nutrients refers to mineral elements, vitamins or type of food providing what is needed for life and growth. Better nutrition means stronger immune systems, less illness and better growth and development. Healthy children learn better, healthy people are stronger, more productive and more able to create opportunities to gradually break the cycles of both poverty and hunger in a
sustainable way. Better nutrition is a prime entry part of ending poverty and a milestone to achieving better quality life (WHO, 2007). Food that is adequate in quantity and quality ensures an adequate consistent and dependable supply of energy and nutrients through sources that are affordable and social culturally acceptable at all times. Of special importance are vitamins A, C and E, certain B-group vitamins, proteins and minerals such as zinc iron, potassium and calcium (WHO, 2008).

Protein deficiency is a major nutritional problem in the developing world (FAO et al., 1997). The development of novel protein sources such as Fish Protein Concentrate (FPC) and Soybean Protein (SBP) (Mendez, 2002; Bhatia and Greer, 2008) has made significant contributions toward the alleviation of the world protein deficiency (Kuijer and Wielenga, 1999). However, there is still an estimated one billion people suffering from protein deficiency and malnutrition (WHO/FAO/UNU, 2007). New methods of feeding the underfed world population, especially in the less developed countries, have to be developed. Those methods that will guarantee a continuous protein supply require most serious attention since most malnutrition cases have been found to be a result of protein insufficiency. Protein malnutrition contributes to the high death rate among infants and children of the less developed countries and causes among the survivors debilitating weakness, higher susceptibility to disease and irreversible brain damage (WHO, 2002b; FAO, 2008). In most under developed countries, the majority of the people are vegetarian and even
where it is possible to obtain animal based foods the price is too high that most families afford them only once a week.

Iron deficiency affects a large number of children and women in developing countries, and is the only nutrient deficiency which is significantly prevalent in industrialized countries (WHO, 2008). The numbers are staggering: 2 billion people – over 30% of the world’s population are anemic, many due to iron deficiency, and in resource-poor areas, this is frequently exacerbated by infectious diseases (WHO, 2008). Iron deficiency affects more people than any other condition, constituting a public health condition of epidemic proportions. Iron deficiency and anaemia reduce the work capacity of individuals and entire populations, bringing serious economic consequences and obstacles to national development. Overall, it is the most vulnerable, the poorest and the least educated that are disproportionately affected by iron deficiency, and it is they who stand to gain the most by its reduction (WHO, 2002a).

Many people are known to die prematurely or suffer deliberating ill health conditions which to a large extent are preventable. Therefore a strategy needs to be adopted about securing a continuing improvement in the general health of the population. The health benefits of a diet rich in vegetables and fruits are many; lower blood pressure, reduced risk of heart diseases, stroke and probably some cancer, lower risk of eyes and digestive problems and a mellowing effect on blood sugar that can keep appetite in check (Decuyper, 2005). Therefore we should not underestimate the contribution of plant sources in total nutrition.
intake especially when most communities rely on plant source of nutrients and vitamins. Families must also be prevailed upon to have active vegetable gardens from which they can get vegetables for domestic consumption (Opundo, 2011). Protein sources of animal origin like milk, eggs, meat and fish are expensive because of their inadequate production and prohibitive cost and hence vegetable proteins of conventional source can meet most of the protein requirement of our population. Out of the non-conventional sources of proteins leaf protein can be best exploited because of its easy availability at low cost of production (FAO, 2008).

Sources of nutrients consumed by majority Kenyans come from plants especially green leafy vegetables; this can be made available during the dry season even when they are expected to be in limited supply (USAID, 2009). Vegetables such as cabbage, kale and spinach among others have been exploited for many years but increasing consumption of plant sources of nutrients, through introducing disease resistant and climate tolerant vegetable trees could play a role in improving future food security (USAID, 2009). This can be done through planting vegetable trees such as mulberry.

Utilization of mulberry in food industry to supplement human diet is well documented, it is appreciated for its fruit (consumed fresh, in juice or as preserves), as a delicious vegetable (young leaves and stems) and for its medicinal properties in infusions (mulberry leaf tea), fruits, roots, stem and shoot bark. The advantages of using mulberry as food source are high protein
and low fiber content, succulence, high extrability, low mucilage, low anti-
nutritional factors, easy cultivation and availability of the leaf throughout the
derg year under varied agro climatic conditions. If properly utilized it could provide a

Quite a number of mulberry varieties can grow in varied agro climatic
conditions in both temperate and tropical areas and in a variety of soils.
Mulberry also grows fast hence form a good component of forestry program due
to its amenability to vegetative propagation. The National Sericulture station has
domesticated varieties such as Thika, Embu, Limuru, Ithanga, Kanva, Ichionose,
Thailand and S-54 which are named according to the place from which they are
mostly dominant. There is need therefore to identify varieties that are rich in
most nutrients so that they can be exploited to improve health status and quality
of life of all Kenyans.

1.2 Problem statement and justification
Lack of nutrients presents major threat to health and development of population
the world over, particularly children and pregnant women in low income
countries. Although the body requires tiny amount of some nutrients,
consequences of their absence are severe. When the body is not given enough
of any one of the essential nutrients over a period of time, it becomes weak and
less able to fight infection (WHO, 2008).
Mulberry is an extremely versatile plant whose value is multifaceted and the potential for increasing and diversifying its uses is enormous. Being a multipurpose tree, mulberry could make a significant contribution to agriculture by providing a variety of useful products including food (nutrients) and forage. Studies have shown that mulberry leaves and fruits are rich in proteins, calcium, iron, phosphorus, potassium, carotene and vitamins and no ant nutritional factors or toxic compounds have been identified in this plant (Anshul and Vadomalai, 2011).

Due to the rich protein component, mulberry leaves can be utilized as protein supplement in mitigating problems of malnutrition, anaemia, and vitamin A deficiency and as a high protein and calcium enriched component in food substances. It is currently being used as a vegetable but the nutrient value of the different varieties grown in Kenya is not yet documented. Studies done in India on nutritional value do not specify the nutritional value in each variety. Therefore there is need to determine the levels of nutrients in different varieties grown in Kenya so as to establish the variety(s) with the most nutrients which can be best to use for human consumption.

1.3 Hypothesis

The levels of nutrients are significantly different in different mulberry varieties grown at Sericulture KARI Station, Thika
1.4 Objectives of the study

1.4.1 General objective

To evaluate nutrients in mulberry varieties grown at KARI station -Thika.

1.4.2 Specific objectives

i. To evaluate the levels of potassium, calcium, zinc and iron in leaves and fruits of the Thika, Embu, Limuru, Ithanga, Kanva, Ichinose, Thailand and S-54 Mulberry varieties.

ii. To evaluate levels of vitamins A(β-carotene), C(Ascorbic acid) and E(α-tocopherol) in leaves of the Thika, Embu, Limuru, Ithanga, Kanva, Ichinose, Thailand and S-54 Mulberry varieties.

iii. To evaluate levels of carbohydrates and proteins in leaves and carbohydrates in fruits of the Thika, Embu, Limuru, Ithanga, Kanva, Ichinose, Thailand and S-54 Mulberry varieties.

1.5 Significance of the study

The results of this study will be availed to the relevant authorities to encourage people to plant mulberry plant since it can be an alternative source of nutrients hence can play a major role in improving the economy through provision of necessary nutrients for a healthier population. The study will provide cheaper and locally available source of nutrients. Mulberry is a multipurpose plant which can be cultivated in a variety of soils and is a good component of forestry program due to its fast growth and amenability to vegetative propagation. It can be used to prepare different forms of food formulations that
may provide daily nutritional requirements for man and as a feed supplement to
enrich animal feeds.

1.6 Scope and limitations of the study

Leaves of eight varieties of mulberry and ripe fruits of six varieties from
Sericulture Station- Thika were analyzed for potassium, calcium, zinc, iron,
proteins and total carbohydrates. Fruits of Thailand and Ichinose varieties were
not analyzed since they were found to dry shortly after flowering. The fresh
leaves of all varieties were analyzed for proteins and vitamins A, C and E.
Fruits were analyzed for mineral elements and carbohydrates only. Factors
affecting the levels of nutrients including seasons, geographical regions, and
soil conditions were not taken into consideration.
CHAPTER TWO: LITERATURE REVIEW

2.1 The mulberry plant

The scientific name of Mulberry is *Morus alba* (L.) a genus belonging to the moraceae family of the Urticales Subclass. The domestication of mulberry must have started about 5000 of years ago as a requirement for silkworm rearing (Morus, 2009). Origins of most cultivated varieties are believed to be in the area of China, Japan and in the Himalaya foothills (FAO, 1990).

Mulberry is a genus of 10-16 species of deciduous trees native to warm temperate and subtropical regions of Asia, Africa, Europe, and the Americas, with the majority of the species native to Asia. It is a hardy deciduous tree/shrub growing in temperate, subtropical and tropical climates and is usually associated with sericulture, the production of silk with the silkworm (Morus, 2009). Mulberries are fast growing when young, but soon become slow growing and rarely exceed 10-15 meters tall. The leaves are alternately arranged, simple, often lobed, more often lobed on juvenile shoots than on mature trees, and serrated on the margin. The mature plant contains significant amounts of resveratrol, particularly in stem bark (Gong *et al*., 1995). The fruit is a multiple fruit, 2-3 centimeters (0.8 - 1.2 inches) long. The fruits when immature are white or green to pale yellow with pink edges. In most species the fruits are red when they are ripe. Plates 2.1, 2.2 and 2.3 show a young mulberry plant, a branch with fruits and harvested fruits respectively (Espinoza *et al*., 1990).
A fully ripened mulberry is dark purple to black, edible, and sweet with a good flavor in several species. The fruits of the white-fruited cultivar of the white mulberry on the other hand are green when unripe and white when ripe; the fruit in this cultivar is sweet, and has a very mild flavor compared with the dark fruits. Unripe fruit and green parts of the plant have a white sap that is intoxicating and mildly hallucinogenic (Cuixq et al., 2008). The fruits and leaves are sold in various forms as nutritional supplements. Mulberries can be grown from seed, and this is often encouraged as seedling-grown trees are generally of better shape and health. But they are most often planted from large cuttings which root readily. It is harvested by leaf picking or cutting whole branches or stems.
Plate 2.1: Young mulberry plant (Photo outside Sericulture Offices)

Plate 2.2: A mulberry plant with fruits

Source; Espinoza et al. (1999)
2.2 Uses of Mulberry

2.2.1 Sericulture

Sericulture or silk worm rearing is the rearing of silkworms for the production of raw silk. The most important use of mulberry globally is for the production of the silkworm that feeds exclusively on its leaves. The country with the largest area of mulberry is China with approximately 626,000 ha, then India with nearly 280,000 ha (Datta, 2000). Several other countries like Thailand and Brazil (35,000ha) still have some mulberry production but on a much smaller scale. The preferential food value of mulberry leaf for silkworm larvae is attributed to the presence of three stimulant factors in it, an attractant, a biting factor and a swallowing factor.

The substances that attract the larvae to the leaves have been identified as citral, linalyl acetate, linalol, terpinyl acetate and hexenol. Sitosterol (approximately 0.2 percent in leaves), together with some sterols and a water-soluble substance are the main factors that stimulates the biting action (Cuixq et al., 2008). Silk projects have been and are being started in various developing countries, particularly in Africa and Latin America. Regardless of how successful or sustainable they have been, these projects have been responsible for the introduction and dissemination of mulberry varieties to different soils and climatic conditions (FAO, 1990, Adolkar, 2007).
2.2.2 Fruit

The ripe fruit is edible and is widely used in pies, tarts, wines and cordials. Mulberry is highly appreciated for its delicious fruit, which is consumed fresh, or in the form of juice or conserves (Espinoza et al., 1999). Juice directly applied on head promotes healthy growth of hair and blackening. Direct use of the fruit keeps low cholesterol levels in the body, reduces blood sugar, eliminates abdominal distention, suppresses mutagenesis of carcinogens, treats fatigue, anemia, urinary incontinence, dizziness and constipation in the elderly, and is used as a laxative for sore throat, dyspepsia and melancholia (Vankatesh and Chauhan, 2011). It is also helpful in recuperating after long time sickness and consumption after child birth is good for women's health among other benefit. Without preservatives mulberry fruit juice can remain fresh for 3 months under cold storage while the bottled beverage remains fresh at room temperature for 12 months. Fruits are also used in preparation of jam, jelly, squash and other food products (Datta, 2000).

2.2.3 Wood

Mulberry wood is used for handicrafts, cabinetwork and for sporting woods like Grass-hockey sticks and tennis rackets especially in the Indian subcontinent. The thin tree branches pruned during the fall season (after the leaves have fallen) are cut and used to make very durable baskets which are used in a lot of village jobs related to agriculture and animal husbandry, they also provide very good fuel (Datta, 2000).
2.2.4 Landscaping

In Asia, Southern Europe and in Southern U.S.A mulberry trees are utilized for landscaping. Their resistance to pruning and their low water requirements make them very suitable plants for urban conditions, house gardens, street shade and city embellishment (Miyahara et al., 2001).

2.2.5 Medicine

A variety of medicinal properties have been attributed to the different parts of the mulberry plant (Datta, 2000). Leaves have been reported to possess diverse functional properties like reduction in blood glucose, blood pressure and cholesterol in addition to recovery from arteriosclerosis and also to increase the defense enzyme activity. A decoction of leaves is used as a gargle in inflammations of the throat. Leaves are also dried and used in infusions in Asia (Adolkar et al., 2007). Mulberry fruits contain pharmacologically important compounds, which reduce adverse oxidative reactions, enhance circulation, alleviate inflammatory processes that yield systematic consequences and improve the digestion and absorption of nutrients.

The root is reported to possess antihelminthic and astringent properties. Root juice reduces blood sugar in diabetic patients and has the capacity to agglutinate blood. Bark of the root help in killing round worms, tape worms and hook worms in the digestive system (Vankatesh and Chauhan, 2011). The shoot produces free flowing latex which has property of healing wounds, injuries and can be used as dermal ointment. The stem shoot bark is used as purgative to
reduce heat from lungs, promote urination and reduce edema (Datta, 2000). Mulberry has the function of nourishing blood. If the person who has anemia, pallor, dizziness, insomnia, and heart-palpitations regularly takes mulberry juice, they will experience good effects. Women who have the above symptoms after childbirth, or anyone after a long-time sickness or after a major operation, can take mulberry juice frequently as a restorative. Those who experience premature aging, such as graying hair and impotence, can take mulberry juice often. The effect will be better for blackening hair and beautifying when it is combined with other herbs. The mulberry juice can also be applied topically to the head to promote healthy hair growth (Shayo, 1997).

2.2.6 Forage

The leaf fodder of mulberry is reported to be of good quality and can be profitably utilized as a supplement to poor quality roughages. Silk producers have traditionally fed mulberry refusals, leftovers from silkworm feeding, to farm animals and to herbivorous carp in polyculture fish ponds (Gong et al., 1997). Some small farmers in East Africa, mainly in Tanzania and in Kenya, harvest foliage from mulberry trees and include it as part of the diet offered to ruminants in confinement (Devshmuck et al., 1993).

Scientists have found out that mulberry leaves could replace grain-based concentrates in lactating cows with excellent results since the leaves act as stimulant for lactation in the cattle (Miranda et al., 2002). Feeding experiments have shown that up to 6 kg of mulberry leaves per day can be fed to milk cows
without adversely affecting the health of animals or the yield and butter content of milk. It is estimated that 1 ha of mulberry garden can sustain 3-4 milking animals thereby adding about 8,000-10,000 Kshs to family income (Prasad and Reddy, 1991). Among goats on a diet of king grass, milk production increased along with the amount of mulberry they were fed and, dairy goats fed exclusively on mulberry and king grass produced an average of four litres of milk a day (Shayo, 1997).

In Guatemala, steers normally fed on sorghum silage grew more rapidly the more mulberry was added to their diet (Armad and Meuret, 1995). In trials with growing pigs, replacing 15% of a commercial concentrate with mulberry leaf increased daily weight gains from 680 g per day to almost 750 g per day. Angora rabbits reduced their intake of pellets by up to 40% when they were offered mulberry leaves, representing a considerable saving in feed costs (Anshul and Vadamalai, 2011). Other researchers have found that including dried mulberry leaf meal in the mash of laying hens leads to better egg yolk color and increased egg size and production. The long selection and improvement of mulberry has made it comparable to - and often better than - many other forage plants in terms of nutritional value and yield of digestible nutrients per unit of area, especially in tropical environments (Uzman et al., 2011).
2.2.7 Human food

Mulberry leaves contain all the essential nutrients required by mankind, they are considered as rich, nutritious and more palatable as compared to other leafy vegetables such as amaranth and spinach. The use of 50g Mulberry leaves as vegetables per person per day is recommended for good nutrition. The Chinese prepare mulberry parts in a great variety of ways. The leaves and root bark are traditionally processed with honey. Fresh leaves and fruit are sometimes juiced for internal consumption, but otherwise, all parts are dried for use in decoctions (internal use) or poultices (external use), to which many health benefits are attributed (Srivastava et al., 2009).

The leaves alone contain a wide variety of nutrients, including proteins, sugars, polyphenols, flavonoids, steroids, triterpenes, vitamins, and minerals (Langley, 1995). A group of nutritional scientists in India has suggested that the leaves of white mulberry might make a good food because they’re so rich in nutrients, especially protein. Since this predominantly vegetarian (mostly grain-based) diet is low in protein and too low in vegetables and fruits for good health, the highly nutritious, nontoxic, and inexpensive mulberry leaves are seen as a potential remedy (Langley, 1995). Mulberry leaves are also appreciated as a delicious vegetable especially young leaves and stems (Zepeda, 1991). Mulberry leaf is known to have high mineral content with ash value of up to 28% (Anshul and Vadamalai, 2011). It is low in saturated fat, cholesterol and sodium and is high in vitamin C, vitamin K, iron, dietary fiber, riboflavin, magnesium and potassium (Langley, 1995). The nutritional value and health
benefits of mulberries make them ideal for maintaining optimum health and weight loss (Srivastava et al., 2006). The nutrient value of the leaves is given in

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Range in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates (sugars, glucose and fructose)</td>
<td>7.8-9.2</td>
</tr>
<tr>
<td>Proteins (with essential amino acids)</td>
<td>15-28</td>
</tr>
<tr>
<td>Fatty acids (linoleic, stearic and oleic acids)</td>
<td>0.4-0.5</td>
</tr>
<tr>
<td>Malic acid producing sour taste</td>
<td>1.1-1.9</td>
</tr>
<tr>
<td>Fiber</td>
<td>0.9-1.3</td>
</tr>
<tr>
<td>Calcium</td>
<td>1.8-2.4</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.14-0.24</td>
</tr>
<tr>
<td>Potassium</td>
<td>1.90-2.87</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.47-0.63</td>
</tr>
</tbody>
</table>

Source: Espinoza et al. (1999)

The nutritional values above lacks exhaustive information on nutrients in leaves, hence the present study is necessary in order to find out the concentration of nutrients available in mulberry leaves. Leaf concentrate also contains a fairly good amount of nutritionally important pigments like carotenes and xanthophylls. It is therefore a source of food that contains beta-carotene and fat, the precursor of vitamin A forming an ideal supplement to prevent malnutrition (Espinoza et al., 1999). The constituents of the fruit have also been analyzed and data reported on nutrient available in fresh raw mulberry (*Morus nigra* L.) is shown on table 2.2
### Table 2.2: Constituents of Mulberry Fruits: Nutrition value per 100 g

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Nutrient Value</th>
<th>% of RDA</th>
<th>Nutrient</th>
<th>Nutrient Value</th>
<th>% of RDA</th>
<th>Mineral</th>
<th>Nutrient Value</th>
<th>% of RDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy</td>
<td>43 Kcal</td>
<td>2</td>
<td>vitamin A</td>
<td>25 IU</td>
<td>1</td>
<td>Ca</td>
<td>39 mg</td>
<td>4</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>9.80 g</td>
<td>7.50</td>
<td>vitamin C</td>
<td>36.4 mg</td>
<td>61</td>
<td>Cu</td>
<td>60 mcg</td>
<td>6.5</td>
</tr>
<tr>
<td>Protein</td>
<td>1.44 g</td>
<td>2.50</td>
<td>vitamin E</td>
<td>0.87 g</td>
<td>6</td>
<td>Fe</td>
<td>1.85 mg</td>
<td>23</td>
</tr>
<tr>
<td>Total Fat</td>
<td>0.39 g</td>
<td>2</td>
<td>vitamin K</td>
<td>7.8 mcg</td>
<td>6.5</td>
<td>Mg</td>
<td>18 mg</td>
<td>4.50</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0 mg</td>
<td>0</td>
<td>Carotene β</td>
<td>9 mcg</td>
<td>0</td>
<td>Se</td>
<td>0.6 mcg</td>
<td>1</td>
</tr>
<tr>
<td>Dietary Fiber</td>
<td>1.7 g</td>
<td>4.50</td>
<td>Folates</td>
<td>6 mcg</td>
<td>1.5</td>
<td>Zn</td>
<td>0.12 mg</td>
<td>1</td>
</tr>
</tbody>
</table>

Source: (USDA National Nutrient data base, 2009)

A similar study to the one whose results are presented in table 2.2 is necessary to evaluate nutritional composition of in mulberry varieties grown in Kenya.

### 2.3 Nutrients

Nutrients are food substances used in an organism’s metabolism or chemicals that an organism needs to live and grow. They may be consumed in large quantities (macronutrients) or in small quantities (micronutrients).

#### 2.3.1 Macronutrients

Macronutrients are class of nutrients needed in largest quantities. They include; potassium, calcium, magnesium, carbohydrates, proteins and fat.

#### 2.3.1.1 Potassium

The human body contains 95% potassium in the fluid within the body cells whose role is regulating the activity of muscles and nerves. The frequency and degree to which muscles contract, and the degree to which nerves become
excitable; both depend heavily on the presence of potassium in the right amount (He and Macgregor, 2001). Potassium plays an important role in muscle contraction and nerve transmission. Muscle and nerve cells have specialized channels for moving potassium in and out of the cell. When the movement of potassium is blocked, or potassium is deficient in the diet, activity of both muscles and nerves can become compromised.

The recommended dietary allowance for potassium is about 4700 mg per day (WHO, 2003). Potassium occurs naturally in a wide variety of foods such as parsley, cucumber, bell pepper, turmeric, apricots, ginger root, strawberries, avocado, banana, legumes, potato skin, tomatoes, tuna, halibut, cauliflower and cabbage. As a result, dietary deficiency of potassium is uncommon. However, potassium deficiency may be brought about by excessive fluid loss, through vomiting, diarrhea or sweating (Fedida and Hesketh, 2001).

The symptoms of potassium deficiency include; muscle weakness, confusion, irritability, fatigue, and heart disturbances. Athletes with low potassium stores may tire more easily during exercise, as potassium deficiency causes a decrease in glycogen storage (Subar, 1998). Elevated blood levels of potassium can be toxic, and may cause an irregular heartbeat or even heart attack. Under normal circumstances, the body maintains blood levels of potassium within a tight range, so it is not usually possible to produce symptoms of toxicity through intake of potassium-containing foods and/or supplements. However, high intakes of potassium salts (potassium chloride and potassium bicarbonate) may
cause nausea, vomiting, diarrhea, and/or ulcers. In addition, the kidneys play an important role in eliminating excess potassium from the body, so people suffering from kidney disease must severely limit their intake of potassium (Insel et al., 2010).

Research show that levels of potassium in some common vegetables such as cucumber, carrot, cauliflower, cabbage and spinach range from 124 to 400 mg/100mg while those of fruits like melon, strawberry, raspberry, blackberries, grapes, cherries and tomatoes range from 158 to 397 mg/100mg (Decuyper, 2005). Studies done on nutritional potential of some tropical vegetables leaf meal show that levels of potassium in three vegetables *Talinum tringulare*, *Amaranthus cruentus* and *Telfairia occidentalis* were 2.7, 4.8 and 3.7 g/kg respectively (Fasuyi, 2006). Levels of potassium in mulberry leaves from India were reported to be 194 mg/100g (Srivastava et al., 2006), which is equivalent to levels of fruits reported for fruits of *Moras nigra* from western Asia (USDA, 2009).

### 2.3.1.2 Calcium

Calcium is one of the alkaline earth metals, which is an essential constituent of leaves, bones, teeth and shells. It is required for normal clotting of blood, formation and development of skeleton, teeth, normal functioning of muscle and nerves. The major function of calcium is to act with phosphorous to build and maintain bones and teeth and for maintenance of healthy gums. It is also important in maintenance of regular heartbeat and in the transmission of nerve
Calcium lowers cholesterol levels, helps cardiovascular disease and it is also needed for muscle growth and contraction for the prevention of muscle cramps.

It may also increase the rate of bone growth and bone mineral density in children (Balch, 2006). It is essential for healthy blood, milk production, enzyme activator and help to regulate the heartbeat. Also calcium assists in the process of blood clotting and helps in regulation of accumulation of acid or alkali in the blood and regulates the passage of nutrients in and out of the cell wall. Sources of calcium include; milk and dairy products, bones, meats and eggs, although the main sources are dairy products and vegetables. Dietary calcium deficiency also has been associated with increased risk of hypertension and colon cancer. Excess calcium supplementation has been associated with some mineral imbalances such as zinc (Jackman et al., 1997).

Calcium has been shown to prevent colon cancer (MII, 2011). Supplemental calcium seems to suppress changes in the lining of colon associated with the onset of cancerous changes. Experiments have shown that human colon cancer cells replicate rapidly when deprived of calcium but slow their replication when calcium is restored (Kally, 2000). Recommended daily allowance of 1000 mg/day has been set (WHO, 2003). Deficiency is associated with lack of vitamin D than a low dietary intake of calcium. Recorded calcium levels in common vegetables such as cucumber, carrot, cauliflower, cabbage and spinach
vegetables range between 14 to 125 mg/100mg while those of fruits range from 10 to 32 mg/100mg (Decuyper, 2005).

Levels of calcium recorded in three vegetables *Talinum tringulare, Amaranthus cruentus Telfaria occidentalis* were 0.8, 2.0 and 1.8 g/kg respectively (Fasuyi, 2006). Common fruits like strawberry, tomatoes, raspberry, blackberries and grapes also contain calcium levels of 15, 32, 30 and 14 mg/100g respectively. A study on nutritional evaluation of fresh leaves of 10 mulberry genotypes reported calcium mean value of 372.97±32.403 mg/100g with a range of 236.89 to 730.11 mg/100g (Raj *et al.*, 2009). The calcium content of all genotypes was higher as compared to other common edible leaves like spinach (125 g/100g), cabbage (75 g/100g), amaranth (397 mg/100g), broccoli (100 mg/100g) and cauliflower (15 mg/100g) (Decuyper, 2005). The USDA (2009) reported calcium levels of 39 mg/100g for fruits of *Morus nigra* from Asia.

### 2.3.1.3 Carbohydrates

Carbohydrates are the most abundant organic compounds found in nature in which hydrogen and oxygen are combined with carbon. They are produced by green plants and by bacteria through photosynthesis process. The carbohydrate group consists principally of sugar, starch, dextrin, cellulose, and glycogen substances that constitute an important part of the human diet and that of many animals. Carbohydrates are the human body’s key source of energy, providing 4 calories of energy per gram (Blanco *et al.*, 2004).

The recommended dietary allowance for carbohydrates is 300g/day (Irz, 2003). When carbohydrates are broken down by the body, sugar glucose is produced;
glucose is critical to help maintain tissue protein, metabolize fat, and fuel the central nervous system. Glucose is absorbed into the bloodstream through the intestinal wall. Some of this glucose goes straight to work in our brain cells and red blood cells, while the rest makes its way to the liver and muscles, where it is stored as glycogen and to fat cells, where it is stored as fat (Gopalan, 1995). Carbohydrates may be present as isolated molecules or they may be physically associated or chemically bound to other molecules.

Molecules in which the carbohydrates are covalently attached to proteins are glycoproteins, whereas those in which the carbohydrates are covalently attached to lipids are glycolipids. Some carbohydrates are digestible by humans and therefore provide an important source of energy, whereas others are indigestible and therefore do not provide energy. Indigestible carbohydrates form part of a group of substances known as dietary fiber, which also include lignin. Consumption of significant quantities of dietary fiber has been shown to be beneficial to human nutrition, helping reduce the risk of certain types of cancer, coronary heart disease, diabetes and constipation. When the supply of carbohydrates is too low to adequately supply all the energy needs of the body, amino acids from proteins are converted to glucose. Sources of carbohydrates include; whole-grain breads and cereals, pasta, corn, beans, peas, and potatoes. Naturally occurring sugars are found in; fruits and many vegetables; milk products, honey, maple sugar, and sugar cane.

Levels of carbohydrates in sorrel, carrot and moringa from Nigeria were reported as 71.10, 87.0 and 110.0 mg/ml respectively (Mustapha and Babura,
2009) and levels of carbohydrates of 43.88% and 54.38% in *Moringa olifera* and *Ipomoea batatas* respectively (Ibok *et al.*, 2008). A study of carbohydrate levels in fresh leaves of 10 mulberry genotypes reported values ranging from 11.03 to 16.27% with mean value of $13.69 \pm 0.114 \%$ (Raj *et al.*, 2009) while those of seven mulberry fruit varieties from India were reported to range from 0.373 to 0.655 mg/g (Venkatesh and Chauhan, 2011). It is therefore necessary to evaluate the levels of nutrients in the varieties available in Kenya.

### 2.3.1.4 Proteins

Proteins are vital body nutrients, just as fats and carbohydrates, vitamins and minerals. However, proteins, together with dietary fats are required by the body in larger amounts than vitamins and minerals because they are the primarily body building block sources for new tissue (FAO, 2008). Proteins are essential to all bioprocesses and are converted into specialized body proteins in various parts of the body including the blood, muscles, enzymes, hormones, skin and hair. Hemoglobin in red blood cells, antibodies in blood plasma, insulin and hormones are all proteins or composed primarily of protein (Wardlaw *et al.*, 2002).

All enzymes, the biological catalysts which make possible the myriad of bioprocesses, are proteins such as (a) the digestive enzymes which are used to break down complex organic compounds into simpler ones for easy absorption in the body, (b) the enzymes of respiration which permit energy transfer from metabolic processes into muscle action, energy for synthesis of new matter,
heat energy and many other living functions and (c) the enzymes of the protoplasts in plant leaves which harness the energy from the sun in a complex process called photosynthesis (Badaloo et al., 2006). The main tissue protein assembling units are the essential amino acids which come only from the protein in foods. Thus, if protein supply is cut short, synthesis of body protein is impaired and may finally cease (Wardlaw et al., 2002).

Proteins are important class of biological macromolecules found in all organisms. They are made from elements such as carbon (C), oxygen (O), hydrogen (H), and nitrogen (N) and sulphur (S). They have a variety of uses in the body, including serving as a source of energy, as substrates (starter materials) for tissue growth and maintenance, and for certain biological functions, such as making structural proteins, transfer proteins, enzyme molecules, and hormone receptors (Sander et al., 2006). Proteins are also the major component in bone, muscle, and other tissues and fluids. The digestive enzymes are proteins, as are insulin and most other hormones. When used for energy, protein supplies an average of 4 kcal/g. Proteins are formed by the linking of different combinations of the twenty common amino acids found in food. Ten of these are essential for the human in the synthesis of body proteins (eight are essential throughout a human's life, whereas two become essential during periods of rapid growth, such as during infancy) (Wardlaw et al., 2002).

Protein may be found in a variety of food sources, those from animal sources (meat, poultry, milk, fish) are of high biological value because they contain all
of the essential amino acids while those from plant sources (wheat, corn, rice, and beans) are of low biological value because an individual plant source does not contain all of the essential amino acids. Therefore, combinations of plant sources must be used to provide these nutrients. Recommended dietary allowance for proteins has been set as 50 g per day (WHO/FAO/UNU, 2007). Excess intake of protein is not considered to be harmful for the average healthy individual. However, when protein intake is inadequate, but total caloric intake is sufficient, a condition known as kwashiorkor may occur (Khan et al., 2006).

Symptoms of kwashiorkor include an enlarged stomach, loss of hair and hair color, and an enlarged liver. Conversely, if protein and caloric intake are both inadequate, marasmus occurs. Marasmus presents with a stoppage of growth, extreme muscle loss, and weakness. Levels of crude proteins in two leafy vegetable of Moringa Oleifera and Ipomoea batatas have been reported as 19.35% and 23.39% respectively (Ibok et al., 2008). Other studies on nutritional potential of some tropical vegetables leaf meal have reported the levels of crude proteins of Talinum tringulare, Amaranthus cruentus and Telfairia occidentalis as 19.9, 23.0 and 35.1 g/kg respectively (Ukam, 2008). Levels of proteins in leaves of 10 mulberry genotypes in a range of 6.38 to 10.73% with mean value of 8.01±0.098% have been reported (Raj et al., 2009). When compared to green leafy vegetables like spinach (2.0 g/100g), fenugreek leaves (4.4 g/100g), amaranth (4.0 g/100g), bathua (3.7 g/100g), mustard leaves (4.0 g/100g) and bengal gram leaves (7.0 g/100g) Decuyper, 2005), mulberry leaves have higher protein value. Thus, leaf protein concentrate preparation
from mulberry leaves is an excellent supplement to protein deficient diets (Raj et al., 2009). Protein content of seven mulberry fruit varieties from India were reported to range from 0.128 to 0.281 mg/g (Venkatesh and Chauhan, 2011).

2.3.2 Micronutrients
These are class of nutrients needed throughout life in small quantities. They include; zinc, iron, cobalt, chromium, copper, iodine, manganese, selenium, molybdenum and vitamins.

2.3.2.1 Zinc
The amount of zinc in the body of an adult is between 1.5 and 3 g, with approximately 60 percent found in the muscles, 30 percent in the bones, and 6 percent in the skin (Emsley, 2001). The highest concentrations of zinc are located in the prostate gland and sperm in men, and in red and white blood cells. The retina of the eye, the liver, and the kidneys all have high concentrations of zinc, with small amounts existing in the hair. Though the body uses zinc in minute doses only, it is one of the most important trace elements needed. It is essential in protecting against oxidative damage from free radicals and in helping to repair DNA (Cerklewski, 1998). Zinc is a key building block for the production of hundreds of the body’s enzymes that are responsible for cell growth, reproduction and repair, regulating the body’s immune response through the production of T-cells, protein synthesis, insulin metabolism and wound healing, among others (Sander et al., 2006).
While zinc is naturally present in a number of food sources such as meat, poultry, dairy products, whole grains, nuts and beans, body is only able to absorb approximately 30 percent of what it takes in due to its low bioavailability in the body. It is only able to absorb approximately 30 percent of what it takes in hence so many people are zinc deficient. Even a minor zinc deficiency has been found to cause DNA damage, according to some studies (Cerklewski, 1998). Studies have shown that most common vegetables have trace levels with cucumber recording 4 mg/100mg of zinc (Decuyper, 2005) while fruits of strawberry, blackberry, raspberry cherries and grapes are also reported to have trace levels of zinc (Decuyper, 2005). Levels of 0.99 to 1.26 mg/100g of zinc were reported in fresh leaves of various mulberry genotypes (Raj et al., 2009) while levels reported for fruits were 0.12 mg/100g (USDA, 2009).

Zinc deficiency is one of the most common deficiencies in the world. It is estimated that up to two billion people have diets poor in zinc, a condition that can lead to a host of problems such as poor immune function, DNA damage, infertility, slow wound healing, cancer, and other diseases. Recommended dietary allowance for zinc ranges between 11 mg-15 mg/ day (WHO, 2008). Zinc in excess can cause nausea and vomiting, dry skin, dry mouth, and neuropathy, which can be characterized by numbness or tingling in the arms, legs, fingers and/or toes, unexplained pain in the extremities, a diminished ability to move the arms, hands, legs and/or feet, poor balance, and unsteady gait or stride (Subar et al., 1998).
2.3.2.2 Iron

Iron (Fe) is a component of red blood cells and the muscles that assist in the transportation of oxygen throughout the body. The amount of iron in the body of a healthy adult is about 3-4 g. It is distributed in the hemoglobin, 2.5 g red pigment of red blood cells in the myoglobin, 0.3 g in the muscle pigment and respiratory enzymes of tissues and 1 g in the liver, spleen bone marrow as an iron store (Davis, 1997). Iron is essential for the formation of hemoglobin and certain enzymes, many proteins and enzymes that maintain good health, transporting oxygen in the blood to all parts of the body, many metabolic reactions and the regulation of cell growth and differentiation, immune activity, proper functioning of the liver and protection against the actions of free radicals (Wardlaw, 2002).

Two thirds of iron in the diet is obtained from plant sources almost half being provided by cereal foods, meat and meal products contribute about 18% and vegetables 16% (Wardlaw, 2002). The Recommended dietary allowance for iron is about 18 mg/day (WHO, 2008). Iron content of leaves of 10 mulberry genotypes has been reported to range from 3.81 to 6.80 mg/100g with a mean value of 5.06±0.02 g/100g (Raj et al., 2009). Srivastava et al. (2006) have reported a range of 4.70 to 10.36 mg/100g of iron in leaves of six mulberry genotypes from India while USDA (2009) reported 1.85 g/100g of iron in mulberry fruits from Asia. Levels of iron in cabbage, spinach, broccoli and cucumber have been reported to have a range of 1-3 mg/100g while those of common fruits such as strawberry, blackberry, melon and grapes ranged from
1.00-3.26 mg/100g (Decuyper, 2005). Mulberry leaves found in Kenya thus need to be analyzed in order to know the levels of iron.

Iron deficiency is associated with impaired brain function, and iron deficiency in infants can result in impaired learning ability and behavioral problems (MII, 2011). Also associated with iron deficiencies are growth problems, some forms of deafness, breathlessness, and reduced bone density. Iron deficiency develops gradually and usually begins with a negative iron balance when iron intake does not meet the daily need for dietary iron. This negative balance initially depletes the storage form of iron while the blood hemoglobin level, a marker of iron status, remains normal. Iron deficiency leads to low levels of hemoglobin; it is stored as ferritin and if too much iron is stored ferritin become conglomerated forming haemosiderin leading to a condition known as Siderosis (Davis, 1997).

Iron deficiency anemia can be associated with low dietary intake of iron, inadequate absorption of iron, or excessive blood loss. Women of childbearing age, pregnant women, preterm and low birth weight infants, older infants and toddlers, and teenage girls are at greatest risk of developing iron deficiency anemia because they have the greatest need for iron. Women with heavy menstrual losses can lose a significant amount of iron and are at considerable risk for iron deficiency (Ayoola et al., 2010). Toxicity is rare but there is potential for iron toxicity because very little iron is excreted from the body. Thus, iron can accumulate in body tissues and organs when normal storage sites are full.
2.3.3 Vitamins

Vitamins are organic food substances found only in plant and animals. They are essential for our bodies to function properly, for growth, energy and for our general well-being. With very few exceptions the human body cannot manufacture or synthesize vitamin. They must be supplied in our diet or in man-made dietary supplements. Vitamins cannot be assimilated without ingestion of food hence it’s better to take them with a meal (Ayoola et al., 2010).

2.3.3.1 Vitamin A

Vitamin A is a pale yellow, fat-soluble substance. It is formed from an orange pigment in plants called carotene, which animals convert into vitamin A. β-carotene has the most significant provitamin A activity. Vitamin A plays an important role in cell growth, cell differentiation, reproduction, vision, and the immune system (Subar et al., 1998). It helps skin develop and stay healthy and promotes the growth of bones and teeth. It is present in the retina (the light-sensitive membrane at the back of the eye) and aids vision in low light. It also helps maintain the mucous membranes that trap microbes and fight infection (Holden et al., 1999).

Vitamin A is found in preformed form in foods such as liver and daily products or as provitamin A carotinoids in foods such as carrots, peas, pumpkins and green vegetables (Ihokeoronge, 1992). About 800-1000 μg per day has been set
as the recommended dietary allowance for vitamin A (FAO, 2001). The beta-carotene is one of the naturally occurring carotenoids, which is found in plants, have vitamin A activity and is regarded as the most important nutritionally (Cai et al., 2000). Deficiency of vitamin A may lead to liver disease, intestinal malabsorption and abnormal urinary losses during infections (Rock and Swendseid, 1992).

The beta carotene content of spinach, fenugreek leaves, amaranth, bathua and mustard leaves of 5.58, 2.34, 5.52, 1.74 and 2.62 mg/100g respectively have been reported (Gopalan, 1995), while that of fresh mulberry leaves has been reported to vary from 3.91 to 14.79 mg/100g with a mean value of 9.64 ± 0.09 mg/100g in 10 mulberry genotype (Raj et al., 2009). Carotenoid levels in fruit varieties from India are reported to range from 0.006 to 0.01 mg/g which were lower than those reported in leaves (Vankatesh and Chauhan, 2011). It is thus necessary to analyze for vitamin A in mulberry genotypes found in Kenya.

2.3.3.2 Vitamin E
This is a fat-soluble vitamin, which includes all tocopherol and tocotrical compounds with alpha-tocopherol being the most potent and of the greatest impact on the human body. This is the form found in most vitamin supplements. It acts as an anti-oxidant that protects against cellular damage by inhibiting peroxidation of polyunsaturated fatty acids in cell membranes (Ayoola et al., 2010). Vitamin E helps protect the body cells from oxidation which is a chemical reaction in the body that can lead to cell damage resulting
to chronic health problems such as heart disease. It is also a natural part of aging. Vitamin E supplementation, in addition to diet and lifestyle factors, can contribute to reducing the risk of heart disease and improving overall health. It is necessary for normal fertility in some animals for cell metabolism and it plays a role in forming red blood cells. Chronic intestinal malabsorption with steatorrhea may decrease absorption of tocopherol (FBN, 2010).

Vitamin E is found in vegetable oils, margarine, wheat germ, whole grains, nuts, and leafy green vegetables. The recommended dietary allowance for vitamin E is 15 mg/day (FAO, 2001). At doses above 720 mg, side effects can occur with prolonged use, and documented side effects include gastrointestinal complaints, creatinuria, and impaired blood coagulation, which subside rapidly with dose reduction (Kappus and Diplock, 1992). Although vitamin E deficiency is rare, people who eat extremely low-fat diets or who are unable to absorb dietary fats may be at risk and should consider supplements (FBN, 2010).

Research on the benefits of vitamin E for preventing cataracts, heart disease, and certain cancers has so far provided inconsistent and inconclusive results (Petrus et al., 2011). Although vitamin E is stored in the body, it has lower toxic effects than do overdoses of other fat-soluble vitamins. Because vitamin E can prevent blood clots, high doses—more than 1,000 mg per day—over a prolonged period may lead to bleeding problems.
Vitamin E deficiency compromises immunity because of damage to immune effectors cell by free radical reaction. A study of vitamin E levels in kales (*Brassica oleracea*) grown in Delta State, Nigeria gave values of 4.06 mg/100mg (Ukam, 2008). Vitamin E levels in cucumber of 0.03 μg/100g have been reported (Emebu and Anyik, 2011). Srivastava *et al.* (2006) reported Vitamin E in mulberry leaves of 36.4 mg/100g while USDA (2009) reported those in fruits of 0.87 mg/g, which indicates that fruits have lower levels of vitamin E than leaves. Levels of vitamin E in Mulberry grown in Kenya should also be documented.

### 2.3.3.3 Vitamin C

Vitamin C is a water-soluble vitamin also known as ascorbic acid. The chemical name for vitamin C is L-ascorbic acid. Its molecular formula is \( \text{C}_6\text{H}_8\text{O}_6 \); its molar mass is 176.12 g/mole. Ascorbic acid is found throughout the plant and animal kingdoms, occurring in citrus fruits, hip berries (such as rose hips), fresh tea leaves, tomatoes, broccoli, other fruits and vegetables, paprika, and the adrenal cortex of oxen. It can be obtained from any of these sources but was originally isolated from and identified in oxen (Ayoola *et al.*, 2010). It was the first vitamin to be prepared in pure form.

Ascorbic acid is a white solid that has a sharp, sour taste and dissolves in water. The pure compound is stable to air oxidation when dry, but when impure (as it is in many natural forms) it is readily oxidized when exposed to air and light. Vitamin C is a fairly strong reducing agent and decolorizes many dyes, its
aqueous solutions are rapidly oxidized by air; this reaction is accelerated in basic solution and in the presence of iron and copper ions. The vitamin C content of juices can decrease rapidly with time once the juice is exposed to air (Sies et al., 1995).

Ukam (2008) reported vitamin C levels in Kales (Brassica oleracea) grown in Delta State, Nigeria of 23.43 mg/100g. Ascorbic acid levels in fresh leaves of 10 mulberry genotypes that ranged from 142.99 to 370.08 mg/100g with a mean value of 272.86 ±7.56 mg/100g were reported by Srivastava et al. (2006). The value of ascorbic acid is comparable to the value (200-300 mg/100g) reported by Raj et al. (2009). Levels of ascorbic acid of spinach, fenugreek leaves, amaranth, bathua and mustard leaves of 28, 52, 99, 35, and 33 mg/100g, respectively have been reported by Gopalan (1995). Levels of vitamin C in fruits of Morus nigra, a species of mulberry of 36.4 mg/100g have been reported (USDA, 2009). There is therefore need to analyze vitamin C in mulberry genotypes grown in Kenya.

Vitamin C is essential to humans and it is involved in the synthesis of collagen, which constitutes about one-third of the total protein in the human body. It is important in the formation and repair of bones, teeth, and collagen—a substance in the skin, muscles, blood vessels, and other tissues. Vitamin C helps heal wounds and also helps the body absorb iron from plant foods. Vitamin C is widely distributed in both plants and animals occurring as both ascorbic acid and dehydroascorbic acid. It is found in foods such as green vegetables, citrus fruits, potatoes and organ meat (Combs, 1998). It plays a role
of metal iron metabolism, orate metabolism, maintains immune strength and inhibits viral growth (Weber et al., 1996). It can stimulate production of interferons, proteins that protects cells against viral attack. Being a water-soluble vitamin it is not stored in the body for long. Good sources of vitamin C need to be identified and consumed every day (Combs, 1998).

A deficiency of vitamin C results to scurvy which is characterized by weakness, swollen joints, bleeding gums, loose teeth, and delayed healing of wounds. Its symptoms are due to loss of the cementing action of collagen and include hemorrhages, loosening of teeth, and cellular changes in the long bones of children. Although unused vitamin C is quickly excreted in the urine, physicians recommend that people should not take more than 2,000 mg per day (IOM, 2000). A quantity of 60 mg vitamin C per day is enough to prevent the disease, and this is the recommended daily dietary allowance (WHO, 2003). Vitamin C is also involved in iron metabolism, and very large doses are effective in preventing or curing the common cold (Sies and Stahl, 1995). At large doses vitamin C causes problems such as diarrhea and the induction of kidney stones (Sies and Stahl, 1995).

2.4 Methods for analysis

Methods available for determination of mineral elements include; (INAA) Instrumental Neutron Activation Analysis (Matus et al., 2009), (ICP-AES) inductively coupled plasma atomic emission spectrometry (Tao and Aebersold, 2003) (EDXRF) Energy dispersive X-ray fluorescence spectroscopy (Beckhoff
et al., 2006), AAS inductively coupled plasma-mass spectrometry (ICP-MS) (Chen et al., 1999; Morton et al., 2002) and flame photometry (Christian, 2004). Some of the methods used for analyses of carbohydrates are (NAA) Neutron Activation Analysis, anthrone method (Matus et al., 2009), gravimetric titration and colorimetric (Christian, 2004). Proteins are analyzed by HPLC (Engel et al., 2010), (MALDI) Matrix Assisted Laser Desorption (Taira et al., 2012) mass spectrometry (Tao and Aebersold, 2003) Kjeidahl method (Doi et al., 2001), Enhanced Dumas method (Sapan and Lundblad, 2009), UV-visible spectroscopy (Taylor et al., 2006), Biuret assay, Lowery method of protein analysis and turbimetric method (Adeduntan et al., 2010). Methods for analysis of vitamins are capillary electrophoresis, HPLC and spectrophotometry (Engel et al., 2010) and titration (Engel et al., 2009).

The methods used in this study were AAS for analysis of mineral elements due to its high accuracy and precision, selectivity, sensitivity and speed at which the analysis done (Sawyer et al., 2007). Anthrone method was used for analysis of carbohydrates since it is simple, very sensitive, has a high specificity for carbohydrates and allows direct determination of reducing and non-reducing sugars (Kerenhap et al. 2007). The Ultra violet visible spectrometry was used to determine concentration of proteins, β-carotene, α-tocopherol. UV-visible techniques are fairly rapid, simple to carry and sensitive to low concentration of samples (Upstone, 2000). Ascorbic acid was determined by titration method since redox titration using triiodide and starch as an indicator is a reliable method to quantify amount of ascorbic acid in a solution (Okiei et al., 2009).
2.4.1 Atomic absorption spectrometry

The atomic absorption spectrometry is a physical method, which involves absorption of light by free atoms of an element at a wavelength specific to that element. This method is largely free from spectral interferences because each metal has its own characteristic absorption wavelength and the source lamp is composed of the element being determined (Pavel et al., 2005).

2.4.1.1 Principle of AAS

Basic principle is that when electromagnetic radiation is incident onto vapors of metallic atoms, the atoms will absorb radiation of their own specific resonance wavelengths (Taylor et al., 2006). Upon absorption of radiation, the atoms are transformed from a low energy state (ground state- $E_1$) to a higher energy state excited state- $E_2$. Transition $E_1$ to $E_2$ results from the absorption of radiation of frequency $\nu$ which is given by Equation 2.1

$$\nu = \frac{E_1 - E_2}{h} \quad \text{Equation 2.1}$$

Where $E_1$=grounds state energy and $E_2$=excited state energy (Skoog, 1996).

Atoms at the excited state revert back to the ground state by emitting radiation of the same frequency. The translations are always stimulated by the absorption of radiation from an external source. The measurement of the radiation absorbed, using Beer-Lambert’s law in such a transition forms the basis of AAS. According to Beer’s law absorbance is linearly related to the concentration $c$ of the absorbing species and to the path length $b$ of the radiation in the absorbing medium. This relationship is given by Equation 2.2.
\[ A = (\frac{\log P_0}{P}) = abc \]  

Equation 2.2

Where;

a- is proportionality constant called the absorptivity

b- is thickness in cm

c- is concentration in moles per liter

\( P_0 / P \) - refer to the power of radiation after it has passed through cells containing the solvent and the analyte respectively. When the concentration is expressed in moles per liter and \( b \) in cm, the proportionality constant is called the molar absorptivity and is given the special symbol \( \epsilon \). Thus, \( A = \epsilon bc \) where \( \epsilon \) has the units of \( \text{L cm}^{-1}\text{mol}^{-1} \)

2.4.1.2 Instrumentation of AAS

An atomic absorption spectrometer contains the following basic components; a light source that emits sharp line spectrum of elements to be detected, a nebulizer used for introducing sample into the flame and breaks liquid sample into the fine droplets (aerosols), a sample container to hold the sample, an atomizer, a monochromator for spectral dispersion of the source of radiation and wavelength selector for selecting wavelength of the analyte resonance line, a detector, amplifier and readout system. A Schematic diagram of an atomic absorption spectrometer is shown in figure 2.1.
Figure 2.1 Schematic diagram of atomic absorption spectrophotometer (Skoog, 1996)
Components of atomic absorption spectrometer are discussed below;

(a) Radiation source

There are two types of radiation sources namely;

i) Line source

The commonly used line source in an atomic absorption spectrometer is a hollow cathode lamp whose cathode is made up of metallic or alloy of element of interest. A hollow cathode lamp consists of a tungsten anode and cylindrical cathode (lining made of metal of interest) sealed in a glass tube that is filled with neon or argon gas at a pressure of 1-5 torr.

ii) Continuous source

The continuous source gives a wide range of radiation and includes deuterium lamp and mercury vapor lamp. It is less sensitive because only a small amount of radiation passed by monochromator is absorbed while a large portion falls on the detector. The continuous source is used for background correction.

(b) Atomizer

The two types of atomizers are electrothermal and flame atomizers. In electrothermal atomizer, few molecules of the sample are first evaporated at low temperature and ashed at higher temperatures in electrically heated graphite. After ashing, the temperature is increased to 2000-3000 °C to cause
atomization of the sample. In flame atomizer, the temperature is determined by flow rate and ratio of oxidant and fuel. In flame atomizer solvent is evaporated to produce solid molecular aerosol during dissolving process. Dissociation leads to atomic gas whereas some of the atoms ionize to give captions and electrons.

(c) Monochromators

These are analyzers that present monochromatic radiation to the detector. They are filters, prisms or gratings that disperse or separate radiation so that selected wavelength corresponding to particular energy of the analyte is transmitted. Diffraction grating is preferred to prisms as they offer accuracy over a wide range of wavelengths.

(d) Detectors

Detectors convert radiation energy into electrical signal. The various types used include phototube, photomultiplier tube and photodiode array detectors.

(e) Read out system

These are digital and interfaced with microprocessors that allow the programming of various aspects, bringing simplicity in operation. However, AAS is a single elemental method in which one element is determined in a series of samples and instrumental parameters optimized for the next element.

2.4.2 Flame photometry

In flame photometry, the radiation that is emitted is measured since the flame is used for both atomization and excitation. The sample solution is nebulized by a
flow of gaseous oxidant, mixed with gaseous fuel and carried into a flame where atomization and excitation occurs. The process may be summarized as shown in figure 2.2

![Diagram showing processes in flame](image)

**Figure 2.2 Schematic diagram of processes taking place in the flame**

The relationship between emission intensity and concentration of atoms in the sample provides basis for quantitative analysis. The schematic diagram of flame photometer is shown in figure 2.3

![Diagram showing flame photometer](image)

**Figure 2.3 Schematic diagram of flame photometer. (Skoog, 1996)**

### 2.4.3 UV/visible spectrophotometry

This is an analytical technique used in determination of molecular species and involves measurement of the amount of radiation absorbed by molecules or ions. It is known for its rapidity high sensitivity, a reasonably high detection
limit and affordable instrumentation (Skoog, 1996). Total energy in a molecule is a sum of contributions from electronic, rotational, transitional and vibrational energies molecular absorption depends on electronic structure of the molecule. The molecular absorption of the UV/visible region depends on the electronic structure of the molecule. The energy absorbed in the UV/visible region by molecules causes transition of valence electrons from ground state to excited state in the molecule. These transitions are $\sigma \rightarrow \sigma^*$, where an electron in a bonding $\sigma$ orbital of a molecule is excited to the corresponding antibonding orbital by the absorption of radiation, $n \rightarrow \sigma^*$ transition which takes place in saturated compounds containing atoms with unshared pair of electrons (nonbonding electrons) and $n \rightarrow \pi^*$ or $\pi \rightarrow \pi^*$ which require presence of an unsaturated group to provide the $\pi$ orbitals. The star (*) refers to excited state. The transition $n \rightarrow \sigma^*$ and $\sigma \rightarrow \sigma^*$ are in the vacuum UV, where atmospheric gases absorb and are not used in UV/visible spectroscopy.

Modern spectrophotometers operate in the range of 200 to 800 nm. Thus the transition $\pi \rightarrow \pi^*$ (ultraviolet) and $n \rightarrow \pi^*$ (near UV and visible) are the ones normally involved in UV/visible spectroscopy measurements (Solomon, 1990). Many nonabsorbing analytes can be determined by causing them to react with chromophoric reagents to produce products that absorb strongly in the ultraviolet and visible regions. The successful application of these color forming reagents usually requires that their reaction with the analyte be forced to near completion (Skoog, 1996). Absorption of radiation by molecules obeys beer-Lamberts law which states that $A=\varepsilon cl$ where $A$ is absorbance, $c$ is
concentration per liter $\epsilon$ is molar absorptivity and $l$ is path length in centimeters (Skoog, 1996). Absorbance depends on the concentration of the absorbing species, hence it is possible to quantitatively determine the amount of a given species present (Skoog, 1996).

2.4.3.1 Instrumentation of UV/visible spectrophotometry

The basic instrument is similar to that of AAS but an atomizer is not required. Most important components are; radiation source, monochromators, sample holder and detectors. Figure 2.4 summarizes the important parts.

![Schematic diagram of UV/Visible spectrophotometer](image)

Figure 2.4 Schematic diagram of UV/Visible spectrophotometer (Skoog, 1996).

2.4.4 Anthrone method

The anthrone method is a colorimetric method of determining the concentration of the total sugars in a sample. Sugars react with the anthrone reagent under acidic conditions to yield a blue-green color. The sample is mixed with sulfuric acid and the anthrone reagent and then boiled until the reaction is completed.
The solution is then allowed to cool and its absorbance is measured at 620 nm using the UV/visible spectrophotometer. There is a linear relationship between the absorbance and the amount of sugar present in the original sample. This method determines both reducing and non-reducing sugars because of the presence of the strongly oxidizing sulfuric acid. Like the other methods it is non-stoichiometric and therefore it is necessary to prepare a calibration curve using a series of standards of known carbohydrate concentration (Chang, 2003).

2.4.5 Direct measurement for determining proteins content

This method is based on UV/visible spectroscopy. It uses either the natural ability of proteins to absorb or scatter light in the UV/visible region of the electromagnetic spectrum, or they chemically or physically modify proteins to make them absorb or scatter light in this region. The tryptophan and tyrosine absorb ultraviolet light strongly at 280 nm, since the tryptophan and tyrosine content of many proteins remains fairly constant, the absorbance of proteins solutions at 280 nm can be used to determine their concentration. The advantages of this method are that the procedure is simple to carry out, it is nondestructive, and no special reagents are required (Skoog, 1996). First of all a calibration curve of absorbance versus protein concentration is prepared using a series of protein solution of known concentration. The absorbance of the unknown samples being analyzed is then measured at the same wavelength, and its protein concentration determined from the calibration curve (Chang, 2003).
2.4.6 Titration

Volumetric analysis of vitamin C uses a redox reaction titration to accurately determine the amount of vitamin C in a sample. Titrations can be completed on both acid-base reaction and oxidation-reduction reaction systems (Pomeranz and Meloan, 1994). Both types of reactions occur rapidly in aqueous solution, the balanced equations for such reactions can be determined, to know when the reactants have been mixed in stoichiometric ratios. Since vitamin C is a weak acid and also a good reducing agent, either type of reaction might be used (East and Nascimeentome, 2002). This analysis makes use of an oxidation-reduction reaction in which elemental iodine oxidizes ascorbic acid.

Iodine is chosen because it is a weak oxidizing agent so it does not oxidize substances other than the ascorbic acid in the sample. As a strong reducing agent, ascorbic acid reduces I₂ to I⁻ very easily. This reaction is used in conjunction with a starch indicator to determine the number of moles of vitamin C present. A number of reactions occur during a single titration (East and Nascimeentome, 2002). In this reaction, the ascorbic acid molecule gains oxygen (in the form of OH groups). Each iodine atom in the I₂ molecule accepts an electron and becomes a negatively charge iodide ion. Thus the ascorbic acid molecule is oxidized and the iodine molecule is reduced, this equation could be written as:

\[ C₆H₈O₆ + I₂ + 2H₂O \rightarrow C₆H₁₀O₈ + 2I⁻ + 2H^+ \]
From this equation it is apparent that one mole of iodine is required to react with one mole of ascorbic acid. As soon as more than an equivalent amount of iodine has been added, the extra iodine cannot be consumed by the ascorbic acid. Therefore, some iodine will remain unreacted in the flask (Pomeranz and Meloan, 1994). Excess iodine (I$_2$) reacts with iodide ions (I$^-$) to make a triiodide ion (I$_3^-$) which forms a very intense blue color when it comes into contact with starch. This color is due to incorporation of the ions within the molecular structure of the starch; we refer to this as formation of a starch-iodine complex.

To detect the end point starch is added to the solution in the flask at the beginning of the titration. As iodine is added from the burette the iodine will react with ascorbic acid and the blue color will not continue to disappear.

When all the ascorbic acid has been used up, the next drop of iodine solution has nothing to react with but the starch, and the blue color will remain in the solution. The end point of the titration is achieved when the blue color remains in the solution for at least 30 seconds because some blue color might form before ascorbic acid has a chance to react with the iodine; if so, it will take a little while for ascorbic acid to react with the iodine that was in the starch-iodine complex. Reaction scheme may be summarized as shown in table 2.3.
A) As I\textsubscript{2} is formed, it will react with ascorbic acid.

\[
\begin{align*}
\text{C}_6\text{H}_8\text{O}_6 + \text{I}_2 + 2\text{H}_2\text{O} \rightarrow \\
\text{C}_6\text{H}_{10}\text{O}_8 + 2\text{I}^- + 2\text{H}^+
\end{align*}
\]

B) As soon as all of the ascorbic acid is consumed the I\textsubscript{2} will react with I\textsuperscript{-} to form I\textsubscript{3}⁻.

I\textsubscript{2} + I\textsuperscript{-} \rightarrow I\textsubscript{3}⁻

C) This will react with the starch indicator to produce the blue-black starch-iodide complex.

I\textsubscript{3}⁻ + starch \rightarrow starch (I\textsubscript{3}⁻) complex (blue-black)

Source: (East and Nascimento, 2002).

The sudden appearance of the blue-black color will indicate that all of the ascorbic acid is consumed. Since the concentration of the standardized I\textsubscript{2} solution is known, the volume that was used, and the stoichiometry of the reaction, the amount of vitamin C present in the original sample can be calculated as follows:

i. Calculate the average volume of iodine solution used from the concordant titers.

ii. Determine how much titrant was required for your sample, for instance

If an average solution of 10 ML of iodine solution is needed to react with 0.25 g of vitamin C, then the amount of vitamin C in the sample that was neutralized by 6 ml of iodine can be determined as follows;

\[
\frac{10\text{ ML iodine solution}}{0.25\text{g Vitamin C}} = 6.0\text{ ML xAg Vitamin C}
\]

\[
\text{................. Equation 2.3}
\]

\[
40A = 6.0
\]

\[
A := 0.15\text{g Vitamin C in the sample}
\]

Other calculations such as g/l can be determined as explained by Ball (2005).
CHAPTER THREE: 3 MATERIALS AND METHODS

3.1 Research design

An experimental design was used in this study and it involved determination of concentration of selected macro and micronutrients in leaves and fruits of eight selected varieties of mulberry plant. Selected varieties were obtained from KARI Thika-Sericulture Station where different varieties are grown.

3.2 Sampling and sample pretreatment

Samples used for the analysis were harvested fresh from the mature plants whose fruits were fully ripened. Leaves and fruits were randomly picked from the upper, middle and lower parts of different branches (Doi et al., 2001). The collected leaves and fruits were then mixed thoroughly and placed in separate perforated self-sealing polythene bags to maintain their freshness. They were then labeled and taken to the laboratory where they were washed thoroughly under tap water and divided into three lots. Samples (both leaves and fruits) for metal elements analysis, carbohydrates and proteins were dried for two weeks, turning them frequently to avert fungal growth and ground into fine powder using a mechanical blender. Samples for vitamins analysis were blanched in hot water for 3 minutes. After cooling and draining the water these samples were packed in laminated plastic bags, immediately sealed and stored at 0 °C in a deep freezer awaiting analysis.
3.3 Cleaning of apparatus and glassware

All apparatus were initially cleaned with detergent by soaking in 2 M nitric acid for 48 hours and then rinsed severally with tap water followed by soaking overnight in 10% analytical grade nitric acid. Finally they were rinsed thoroughly with distilled water. The glassware was then dried in a hot oven at 120 °C and this cleaning procedure was repeated before each test.

3.4 Chemical reagents and solvents

All chemical reagents and solvents; potassium chloride, calcium carbonate, zinc nitrate, ferrous ammonium sulphate, potassium iodide, iodine, sulphuric acid, anthrone reagent, D-glucose, petroleum ether, dichloromethane, BSA and All-rac-α-tocopherol (chemically synthesized vitamin E) used throughout the study were of high quality analytical grade (Analar grade). They were sourced from Thomas Baker Chemicals Ltd, Mumbai India.

3.5 Instruments used for analysis

A Varian (model AA.10) instrument was used was for element analysis and its operating parameters were set according to the specification given by the manufacturer (Table 3.1). The flame photometer, (model CORNING 400) was used for analysis of potassium. The UV/visible spectrophotometer used was CECIL S/W Version ROO52 (model EE 2041, 2000 series) for analysis of carbohydrates and vitamins. Smart Spec Plus spectrophotometer from Bio-Rad Laboratories Inc USA was used for analysis of proteins.
Table 3.1 Operating conditions of AAS

<table>
<thead>
<tr>
<th>Operating Parameters/Elements</th>
<th>Zn</th>
<th>Ca</th>
<th>Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>776.5</td>
<td>422.7</td>
<td>248.3</td>
</tr>
<tr>
<td>Slit width (nm)</td>
<td>1.0</td>
<td>0.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Flame type</td>
<td>Air-acetylene</td>
<td>N₂O-acetylene</td>
<td>Air-acetylene</td>
</tr>
<tr>
<td>Oxidant flow rate (l/min)</td>
<td>1.5</td>
<td>4.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Sensitivity (ppm)</td>
<td>0.01</td>
<td>0.021</td>
<td>0.12</td>
</tr>
<tr>
<td>Detection limit (ppm)</td>
<td>0.001</td>
<td>0.0005</td>
<td>0.005</td>
</tr>
</tbody>
</table>


3.6 Preparation of stock and standards solutions

Appropriate weights of analar grade salts were used to make 1000 ppm stock solution. Standard solutions were made by serial dilution of stock solution with dilution factor not exceeding 10 in order to account for the background effects from the acids and to correct for changes resulting from all procedures, blank samples from deionised water were prepared following the same procedures as the samples and each of the blank samples were determined for the elements of interest. Calibration curves were constructed and used to validate the methods.

3.6.1 Potassium stock and standard solutions

Potassium stock solution was prepared by dissolving 1.916 g of potassium chloride in distilled water and diluting to 1L in a volumetric flask. A 100 ppm solution was prepared by transferring 10 ml to K stock solution 1000 ppm in a 100 ml volumetric flask and diluted to the mark with distilled water. Standard
solutions of concentrations 2, 3, 5, 8 and 10 ppm aliquots from 100 ppm K were made by serial dilution of stock solution.

3.6.2 Calcium stock and standard solutions
The stock solution for calcium (1000 ppm) was prepared by dissolving 2.497 g of calcium carbonate (dried at 100 °C for 2 hours) in 30 ml of 1M hydrochloric acid and adjusting volume to 1 litre with distilled water. Working standard solutions of concentrations 2, 3, 5, 8 and 10 ppm Ca were made by serial dilution of stock solution.

3.6.3 Zinc stock and standard solutions
The stock solution for zinc (1000 ppm) was prepared by dissolving 4.552 g of zinc nitrate in distilled water and adjusting volume to 1 litre. Working standard solutions of concentrations 1, 3, 5, 8, and 10 ppm of Zn were made by serial dilution of stock solution.

3.6.4 Iron stock and standard solutions
The stock solution for iron (1000 ppm) was prepared by dissolving 0.070 g of ferrous ammonium sulphate in distilled water and adjusting volume to 1 litre. Working standard solutions of concentrations 1, 3, 5, 8 and 10 ppm were made by serial dilution of stock solution.
3.6.5 Reagents and standards for carbohydrates analysis

3.6.5.1 Sulphuric acid and anthrone reagent

Sulphuric acid was prepared by dissolving 700 ml concentrated sulphuric acid and diluting it to 1 litre to make 12.5 M sulphuric acid. Anthrone reagent 0.1% W/V was prepared by dissolving 0.100 g of anthrone reagent in 12.5 ml sulphuric acid and the solution diluted to volume in a 100 ml volumetric flask. The solution was mixed thoroughly and kept in a refrigerator awaiting use every day.

3.6.5.2 Glucose stock and standard solutions

A 0.100 g mass of D-glucose analar grade was dissolved in distilled water and diluted to 100 ml mark in a volumetric flask. Standards were prepared by pipetting 1, 3, 6, 9, 12, and 15 ppm of stock solution into successive 100 ml volumetric flasks and their absorbance read to prepare the calibration curve.

3.6.6 Protein stock and standard solutions

Stock solution of 1 mg/ml of Bovine Serum Albumin (BSA) was prepared by dissolving 0.05 g of BSA in 50 ml distilled water. Portions of 0.1, 0.2, 0.3, 0.4 and 0.5 ppm of the stock solution were pipetted and separately diluted to 1 ml to obtain working standards whose absorbance were used to prepare the calibration curve.
3.6.7 Preparation of stock and standard solutions of β-carotene and α-tocopherol

The β-carotene stock solution was obtained from accurately weighed 0.020 g β-carotene dissolved in 20 ml petroleum ether: dichloromethane 9:1 in a 100ml volumetric flask and made up to the mark. Working standards of concentrations, 0.2, 0.4, 0.8, 1.6, 2.0 and 2.8 ppm β-carotene were made by serial dilution for calibration of the method. Alpha-tocopherol stock solution was obtained from accurately weighed 0.02 g All-rac-α-tocopherol dissolved in petroleum ether: dichloromethane 9:1 in a 100 ml volumetric flask. Working standards of concentrations 1, 2, 4, 8, and 10 ppm all-rac-α-tocopherol were made by serial dilution for calibration of the method.

3.6.8 Standards and reagents for vitamin C

Iodine solution was made by weighing 2.000 g of potassium iodide and adding it with 1.300 g of iodine into a 100 ml beaker. A volume of 20 ml of water was added, to dissolve the mixture and then made up to 1 litre in a volumetric flask. Starch indicator solution (1%) was prepared by dissolving accurately weighed 0.250 g of soluble starch in nearly boiling water. The solution was made up to a 100 ml in a volumetric flask and cooled before use.

3.7 Extraction and analysis of mineral elements

A mass of 1.000±0.001 g dried ground leaves was accurately weighed and put into a 250 ml kjedhal flask. A 12 ml aliquot of concentrated nitric acid was
added and the flask swirled to ensure that the entire sample was wetted. The mixture was heated on an electric hot plate set at medium heating for 10 minutes until brown fumes disappeared and allowed to cool to room temperature. Two milliliters of perchloric acid was added and the contents heated for continued digestion until the solution was clear and white fumes were observed. The digest was then cooled and filtered using whatman No. 42 filter paper into a 100 ml volumetric flask and diluted to the mark with distilled deionized water. Fruit samples were also extracted in the same way. Digested solution for all the varieties were transferred into clean plastic bottles, labeled and stored awaiting analysis.

The analysis of potassium was done by flame photometry where a 5 ml aliquot of wet digested sample solution was pipetted into 250 ml volumetric flask and then made to the mark with distilled water. A volume of 10 ml of the diluted sample was further diluted with distilled water in a ratio of 1:5. The samples were nebulized into a flame of flame photometer randomly and measurements taken. The same procedure was repeated using the working standards.

Calcium, zinc and iron were determined by AAS. Calcium was determined by pipetting a volume of 10 ml of wet digested sample solution into 250 ml volumetric flask followed by addition of 10 ml of 0.15% lanthanum chloride and adjusting the volume to the mark with distilled water. The samples were run in the AAS instrument and the absorbance readings taken. The working Ca standards were sequentially run using the same conditions and the absorbance
values recorded. A calibration graph of absorbance versus concentration was constructed from which the concentrations of Ca in samples were determined by interpolation.

Zinc and iron were determined by pipetting a volume of 10 ml of wet digested sample solution into 100 ml volumetric flask and diluted to the mark with distilled water. The standards and samples were nebulized into the flame of an atomic absorption spectrometer and readings taken. The calibration graphs were constructed from which the concentration of Zn and Fe were obtained by interpolation (Okalebo et al., 2002).

3.8 Extraction and analysis of available carbohydrates as glucose

A mass of 1±0.001 g oven dried ground leaves and fruits was accurately weighed with electronic analytical balance and put into a clean dry 100 ml conical flask. A 10 ml aliquot of distilled water was added and the flask swirled to ensure that the whole sample was wetted before stirring using a stirring rod. A 15 ml aliquot of perchloric acid was pipette and added to the mixture while stirring continued for about 10 minutes.

The mixture was allowed to stand for 1 hour to hydrolyze any starch to glucose and other sugars to reducing sugar. The extract was then filtered into a 100 ml volumetric flask, diluted to the mark with distilled water and then left to stand for 30 minutes. Deionized water was used as the blank and was treated in the same way as the samples. The green colored leaves and red colored fruits extracts were decolorized by adding a spatulaful of charcoal decolorizing
powder while stirring, left to stand for 30 minutes and then filtered back into clean dry plastic bottles ready for analysis.

To 1ml portions of the sample, duplicate blanks and series of diluted glucose standard solutions 5 ml aliquots of freshly prepared anthrone reagent were added in each test tube, stoppered with plastic stoppers and mixed thoroughly (Doi et al., 2001). The test tube contents were boiled in a water bath for 12 minutes to permit full color development. The solutions with glucose turned from yellow to blue green while blanks remained yellow. The test tubes were then cooled in a dessicator to room temperature. The blanks were transferred into 1 cm cuvettes and used to zero the spectrophotometer. Samples and series of standard glucose solution were transferred into 1 cm cuvettes and respective absorbance measured at 630 nm using a UV/visible spectrophotometer (Doi et al., 2001).

3.9 Extraction and analysis of proteins

A weight of 0.100 g of powdered leaves were accurately weighed in triplicate and put into eppendorf tubes and 1 ml extraction buffer prepared from 0.2 M NaCl, 0.001 M EDTA, 0.2% tritonX-100, 0.1 M Tris-Cl PH 7.8 and 4% mercaptetoethanol was added. The tubes were then vortexed for 3 minutes then put in a freezer at -20 °C for 30 minutes after which they were centrifuged at 10,000 RPM for 10 minutes and supernatant transferred to new tubes. Extracted samples were preserved in the fridge at 4 °C ready for analysis. A series of diluted BSA standards and diluted samples, ratio 1:50 were transferred into 1
cm glass cuvettes and their respective absorbance measured at 280 nm using distilled water as a blank to zero the spectrophotometer. Calibration curve of absorbance versus protein concentration was prepared using a series of protein standards of known concentration and concentration of samples determined from the calibration curve.

3.10 Extraction and analysis of β-carotene and α-tocopherol

A weight of 50 ±0.001 g blanched leaves was weighed cut into small pieces so as to facilitate easy grinding and ground into a fine pulp using a mortar with pestle, adding enough cold acetone to cover the sample. The extract was filtered with suction through a Butchner funnel. The mortar, funnel and residue were washed with small amounts of acetone, receiving the washings in the suction flask with the extract. The residue was returned again to the mortar, fresh acetone was added, and the sample was macerated again. Extraction and filtration was repeated until the residue was devoid of any color and washings were colorless. The extract was partitioned to petroleum ether by adding small portions of the acetone extract to about 100ml of the petroleum ether in a separating funnel and adding distilled water slowly, letting it flow along the walls of the funnel. Shaking was avoided to prevent formation of an emulsion which would lead to erroneous results (Seo et al., 2005).

The two phases were allowed to separate and the lower aqueous-acetone phase was discarded. Another portion of the acetone extract was added and the above operation repeated until all of the extract was transferred to the petroleum ether
while washing about 4-5 times with water to remove residual acetone. Petroleum ether phase was collected and excess water was dried over anhydrous sodium sulphate. Petroleum ether was evaporated off under reduced pressure to concentrate the carotenoid solution to about 10 ml in a rotary evaporator at a temperature not exceeding 40 °C.

The separation was carried out in a chromatographic column packed with silica gel and topped with 1-cm layer of anhydrous sodium sulphate to ensure that no residual water gets into the adsorbent. The concentrated sample solution was dissolved in 2 ml of petroleum ether then quantitatively spotted into the column, and eluted with petroleum ether. Separation of carotenoid was monitored visually in dim light to reduce the rate of carotene oxidation. The first yellow eluate was collected in a 25 ml flask and made to the mark with petroleum ether. Samples were kept in amber plastic bottles and analyzed on the same day (Ogubi, 2008).

Samples of β-carotene and series of β-carotene standard solution were transferred into 1 cm cuvettes and respective absorbance measured at 470 nm using a UV/visible spectrophotometer that was calibrated with standard solutions of pure β-carotene in petroleum ether before optical densities of β-carotene were read. Samples of α-tocopherol were also measured in 1cm cuvettes after calibrating the UV/visible spectrophotometer with standard solutions of pure all-rac-α-tocopherol (Ogubi, 2008).
3.11 Extraction and analysis of ascorbic acid

A mass of 50 ±0.001 g fresh leaves sample was weighed, cut into small pieces and ground in a mortar and pestle. Several portions of 10 ml distilled water were added while grinding the sample, each time decanting off the liquid extract into 100 ml volumetric flask. The extract was filtered with suction through a butchner funnel while rinsing the vegetable pulp with 10 ml portions of water and collecting all filtrate and washings in the volumetric flask. The extracted solution was made up to 100 ml with distilled water and stored in a plastic amber bottle awaiting analysis on the same day.

A volume of 25 ml vitamin C standard was pipetted into a 250 ml conical flask; about 1ml of 1% starch solution was added and titrated against iodine solution from a burette. Titration continued until the first sight of blue-black color was observed, same procedure was followed for with the extracts. The final volume of iodine solution was recorded and two more titrations were done to obtain concordant results. The average volume of iodine used was calculated which was used to determine the amount of vitamin C that had been neutralized from the sample extract (Engel et al., 2009).

3.12 Methods validation

Calibration was done by running standards and the absorbance readings obtained were used to calculate correlation coefficient (r) values. The calibration curves were established by a plot of absorbance readings against the corresponding concentration of standards with optimized instrumental conditions. Regression
analysis was used to evaluate the linearity of the established calibration curves. The method detection limit was calculated as the concentration that gives signals equal to three times the standard deviations blanks.

3.13 Data analysis

The data derived from various determinations of nutrients was arranged in tables and charts. Statistical analysis was done and t-test was used to compare the mean levels of the nutrients between leaves and fruits of different varieties. The mean values in the various mulberry varieties were compared by one-way ANOVA at 95% level (Sawyer and Beebe, 2007).
CHAPTER FOUR: 4 RESULTS AND DISCUSSION

4.1 Introduction

The concentration of macronutrients: potassium, calcium and carbohydrates in dried leaves and fruits and proteins in dried leaves are reported. Levels of trace elements (zinc and iron) also determined in dried leaves and fruits, and vitamins A, E and C determined in fresh leaves are reported. The levels of K, Ca, Zn Fe, carbohydrates, proteins and vitamins A (β-carotene), C (ascorbic acid) and E (α-tocopherol) were analyzed as indicated in chapter 3. Potassium was analyzed using flame photometry, while Ca, Zn Fe were analyzed using AAS. Carbohydrates, proteins, β-carotene and α-tocopherol were analyzed by UV-visible spectrophotometry and ascorbic acid by titration method. Mean levels were calculated based on dry weight for metal elements, carbohydrates and proteins while mean levels in vitamins were calculated based on fresh weight. The results are presented and discussed in this chapter.

4.2 Validation of method

Standards prepared from stock solutions were used to establish calibration curves where correlation coefficients and regression equation were determined. The sensitivity of the analysis method was taken as the slope of the calibration curve. The method detection limit was determined as the lowest concentration obtained by the instrumental signal equal to the blank signal plus three times the standard deviation of blank. The results are summarized in table 4.1
The correlation coefficients of all calibration curves were ≥ 0.986, which shows that they had high positive correlation (relationship) between concentration and absorbance. Correlation coefficient estimates how well the experimental points fit a straight line and it can take values in the range -1 ≤ r ≤ +1 (Miller and Miller, 1998). Limit of detection depends on the ratio of the magnitude of the analytical signal to the size of the statistical fluctuations in the blank signal (Okalebo et al., 2002). This study had detection limits that were ≤ 0.9 ppm which shows the method was applicable for the analysis. Sensitivity measures the ability of an instrument or method to discriminate between small differences in analytes concentration (Sawyer et al., 2006). The sensitivity of instrumental method was compared with the slope of the calibration graph which gave gradients higher than zero hence the method was sensitive.
4.3 Mean levels of mineral elements in leaves

Table 4.2 gives the mean values of mineral elements in leaves of the different varieties. Appendices 9 to 12 give graphical presentation of the mean levels of mineral elements in leaves and fruits of the different varieties.

Table 4.2: Mean levels of different elements in leaves

<table>
<thead>
<tr>
<th>Variety</th>
<th>Calcium (Mean ±SD)(n=3)</th>
<th>Potassium (Mean ±SD)(n=3)</th>
<th>Zinc (Mean ±SD)(n=3)</th>
<th>Iron (Mean ±SD)(n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ithanga</td>
<td>61.75±2.50</td>
<td>205.99±1.43</td>
<td>0.35±0.01</td>
<td>2.34±0.01</td>
</tr>
<tr>
<td>Thika</td>
<td>91.75±2.50</td>
<td>303.48±1.44</td>
<td>0.43±0.01</td>
<td>2.13±0.01</td>
</tr>
<tr>
<td>Embu</td>
<td>79.92±1.15</td>
<td>216.79±2.50</td>
<td>0.19±0.01</td>
<td>0.55±0.01</td>
</tr>
<tr>
<td>Limuru</td>
<td>84.08±2.75</td>
<td>319.31±1.17</td>
<td>0.30±0.01</td>
<td>3.34±0.01</td>
</tr>
<tr>
<td>S54</td>
<td>84.25±2.50</td>
<td>302.64±2.89</td>
<td>0.20±0.01</td>
<td>2.46±0.01</td>
</tr>
<tr>
<td>Kanza</td>
<td>94.25±2.50</td>
<td>302.64±2.89</td>
<td>0.27±0.01</td>
<td>0.86±0.01</td>
</tr>
<tr>
<td>Thailand</td>
<td>91.25±2.00</td>
<td>369.98±1.15</td>
<td>0.23±0.01</td>
<td>1.33±0.01</td>
</tr>
<tr>
<td>Ichinose</td>
<td>148.42±1.44</td>
<td>435.93±1.40</td>
<td>0.26±0.01</td>
<td>2.10±0.01</td>
</tr>
<tr>
<td>Overall mean</td>
<td>91.96±2.42</td>
<td>307.10±1.86</td>
<td>0.28±0.01</td>
<td>1.89±0.01</td>
</tr>
</tbody>
</table>

NB: Mean values with the same small letters within the same column are not significantly different.

The results presented in table 4.2 are individually discussed in the following subsections.

4.3.1 Mean levels of calcium

Mean levels of calcium in leaves ranged from 61.75±2.50 mg/100g in Ithanga variety to 148.42±1.44 mg/100g in Ichinose variety. Ichinose variety recorded
the highest calcium levels while Ithanga recorded the lowest calcium levels. Levels of calcium in Thika, Kanva and Thailand varieties did not differ significantly, also calcium levels in Embu, Limuru and S54 were not significantly different. Calcium levels of all the varieties analyzed in this study were lower than those reported by Raj et al. (2009) on fresh leaves of 10 mulberry genotypes from India whose mean was reported to be 372.97 mg/100g. However they were comparable to those of common vegetables such as cabbage, spinach and cucumber which ranged from 14 to 125 mg/100g (Decuyper, 2005; Rumez et al., 2006). In addition calcium levels in this study were higher than 0.8 g/kg reported for *Talinum tringulare*, but lower than *Amaranthus cruentus* *Telfairia occidentalis* whose levels were reported as 2.0 and 1.8 g/kg respectively (Fasuyi, 2006). Mulberry leaves can therefore be considered as source of calcium just like commonly consumed green vegetables such as cabbage, spinach and cucumber. To meet the required daily allowance of 1000 mg/day of calcium (WHO, 2003), one would be required to take approximately 1087.43 g/day of dried mulberry leaves if considered as the only source of calcium.

4.3.2 Mean levels of potassium

Mean levels of potassium in leaves ranged from 205.99±1.43 mg/100g in Ithanga variety to 435.93±1.40 mg/100g in Ichinose variety. Ichinose recorded potassium levels that were significantly higher than the other varieties while Ithanga recorded levels that were significantly lower than all other varieties. Ithanga and Embu recorded potassium levels that were not significantly
different although they differed from those of other varieties. Limuru, Thika, Kanva and S54 recorded levels that were also not significantly different, this means that these four varieties would provide equivalent amount of potassium. Potassium levels in all the varieties were higher than those tabulated by Srivastava et al. (2006) of 194 mg/100g on Indian mulberry leaves and also higher than most common vegetables such as cabbage spinach and kale which ranged from 14 to 125 mg/100g. They were also lower than tropical vegetables such as Talimum tringulare, Amaranthus cruentus and Telfairia occidentalis whose levels were reported as 2.7, 4.8 and 3.7 g/kg respectively (Fasuyi, 2006). In addition, mean levels in the present study were lower than the RDA for calcium of 4700 mg/day (WHO, 2003), therefore about 1530.45 g/day of mulberry leaves would be required to meet the recommended daily allowance if consumed as the only source of potassium.

4.3.3 Mean levels of zinc

Mean levels of zinc in leaves ranged from 0.19±0.010 in Embu variety to 0.43±0.01 mg/100g in Thika variety. Zinc levels in Thika variety were significantly higher while those of Embu were significantly lower than all other varieties. This means that consuming Thika leaves would provide the highest amount of zinc while Embu would provide the least amount. Kanva and Ichinose varieties were not significantly different and the same case applied to Embu and S54 but all the other varieties differed significantly. This study established that the levels of zinc in the eight varieties were slightly lower than the range of 0.99-1.26 mg/100g reported by Raj et al. (2009) but high than most
common vegetables like spinach, cabbage, leek and cauliflower which were recorded to have trace amounts by Decuyper (2005). Mulberry leaves could therefore be considered as a better source of zinc compared with commonly consumed vegetables although these levels were lower than the RDA levels of zinc of 11-15 mg/day (WHO, 2008). An amount equivalent to 5357.14 g/day would have to be consumed to meet the RDA for zinc.

4.3.4 Mean levels of iron

The range of iron levels in leaves was from 0.55±0.01 mg/100g in Embu variety to 3.34±0.01 mg/100g in Limuru variety. Limuru variety had the highest level while Embu variety had the lowest level. All the varieties recorded iron levels that were significantly different meaning that leaves of different mulberry varieties contain different levels of iron. This study recorded levels of iron that were lower than a range of 4.70 to 6.80 mg/100g reported for 10 mulberry genotypes from India that by Raj et al. (2009), although they did not meet the RDA of 18 mg/day for iron (WHO, 2008). An amount equivalent to 953.38 g/day of mulberry leaves would be required to meet the RDA for iron. The levels in the present study were comparable to those of most vegetables like cabbage, spinach and kale (Decuyper, 2005; Rumeza et al., 2006) which ranged from 1 to 3 mg/100g. Therefore from the results of this study, mulberry leaves should be included in the human diet as a source of iron.
4.4 Mean levels of mineral elements in fruits

The levels of Ca, K, Zn and Fe were detected in the six varieties of mulberry fruits. Fruits of Ichinose and Thailand were not available for analysis because they dried after flowering. Table 4.3 gives the mean levels of different nutrients in fruits of the different varieties.

Table 4.3: Mean levels of mineral elements concentration in fruits

<table>
<thead>
<tr>
<th>Variety</th>
<th>Calcium</th>
<th>Potassium</th>
<th>Zinc</th>
<th>Iron</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ithanga</td>
<td>52.58±1.44d</td>
<td>192.48±1.61c</td>
<td>0.13±0.01a</td>
<td>3.08±0.01e</td>
</tr>
<tr>
<td>Thika</td>
<td>45.92±1.44c</td>
<td>192.64±1.44c</td>
<td>0.23±0.01e</td>
<td>5.62±0.00f</td>
</tr>
<tr>
<td>Embu</td>
<td>53.42±1.44d</td>
<td>245.98±1.44d</td>
<td>0.26±0.01d</td>
<td>1.15±0.01a</td>
</tr>
<tr>
<td>Limuru</td>
<td>40.92±1.44b</td>
<td>176.81±2.50b</td>
<td>0.28±0.01e</td>
<td>1.56±0.02c</td>
</tr>
<tr>
<td>S54</td>
<td>34.25±2.50a</td>
<td>141.81±2.50a</td>
<td>0.23±0.01c</td>
<td>1.46±0.01b</td>
</tr>
<tr>
<td>Kanza</td>
<td>52.25±0.87d</td>
<td>193.48±1.44c</td>
<td>0.15±0.01b</td>
<td>1.82±0.01d</td>
</tr>
<tr>
<td>Overall</td>
<td>46.56±1.52</td>
<td>190.53±1.82</td>
<td>0.21±0.01</td>
<td>2.45±0.01</td>
</tr>
</tbody>
</table>

NB: Mean values with the same small letters within the same column are not significantly different

The results presented in table 4.3 are individually discussed in the following subsections.

4.4.1 Mean levels of calcium

Levels of calcium in fruits ranged from 34.25±2.50 mg/100g in S54 to 53.42±1.4 mg/100g in Embu variety. Embu variety had the highest level of calcium while S54 had the lowest. Levels of calcium in fruits of Ithanga, Embu and Kanva had no significance difference while the other varieties recorded
levels that were significantly different. All varieties in this study recorded calcium levels in fruits that were higher than those reported for Asian mulberry fruits of 39 mg/100g (USDA, 2009). Calcium levels in strawberry (15 mg/100g), raspberry (15 mg/100g), grapes (14 mg/100g), blackberries (30 mg/100g) and melon (10 mg/100g) reported by Decuyper (2005) were lower compared to the levels recorded in the present study. Therefore, mulberry fruits can be considered as a good source of calcium than most commonly used fruits, although the levels reported in the present study were lower than the RDA levels for calcium of 1000 mg/day (WHO, 2003). This implies that an amount of about 2147.77 g/day of dried mulberry fruits would have to be consumed in order meet the RDA for calcium.

4.4.2 Mean levels of potassium

Mean levels of potassium in fruits ranged from 141.81±2.50 in S54 to 245.98±1.44 mg/100g in Embu variety. Embu variety had significantly higher levels while S54 had significantly lower levels of potassium. Mean levels of potassium in Ithanga, Thika and Kanva were not significantly different which implies that they provide same level of potassium, but the potassium levels recorded for Embu Limuru and S54 were significantly different. Levels of potassium in this study were comparable to a range of 158-194 mg/100g reported by Decuyper (2005) in common fruits such as melon, strawberry, raspberry, blackberries, grapes, cherries and tomatoes. On the other hand these levels were comparable to 194 mg/100g reported by USDA (2009) on Morus nigra fruits from western Asia. However these levels did not meet the RDA
of 2466.80 g/day of mulberry fruits would have to be consumed to meet the RDA if taken as the only source of potassium. Figure 4.1 shows the graph representing the mean levels of potassium in different fruit varieties.

![Graph showing mean levels of potassium in different fruit varieties](image)

**Figure 4.1: Concentration of potassium in fruits mg/100g**

**NB:** Mean values with the same small letters are not significantly different

From figure 4.1 it can be seen that the levels of potassium in fruits follow the order S54 < Limuru < Thika < Kanza < Ithanga < Embu

### 4.4.3 Mean levels of zinc

Mean levels of zinc in fruits ranged from 0.13±0.01 mg/100g in Ithanga to 0.28±0.01 mg/100g in Limuru variety. Limuru variety recorded the highest
4.4.3 Mean levels of zinc

Mean levels of zinc in fruits ranged from 0.13±0.01 mg/100g in Ithanga to 0.28±0.01 mg/100g in Limuru variety. Limuru variety recorded the highest while Ithanga had lowest levels of zinc. Therefore Limuru variety is a better source of zinc compared to the other varieties studied. The mean levels of zinc in Thika and S54 varieties did not differ significantly while those of Kanva, Embu, Ithanga and Limuru differed significantly. This study recorded zinc levels that were higher than 0.12 mg/100g reported by USDA (2009) on fruits of Morus nigra. Decuyper (2005) reported trace levels of zinc in most common fruits like strawberry, blackberry, raspberry cherries and grapes unlike those recorded in the present study implying that mulberry fruits are a better source of zinc than most fruits. The levels in the present study were lower than the RDA for zinc of 11-15 mg/day (WHO, 2008), therefore to meet the required RDA a larger amount of about 7142.69 g/day of dried mulberry fruits would have to be consumed. Figure 4.2 shows the graph representing the mean levels of zinc in different fruit varieties.
Figure 4.2: Concentration of zinc in fruits mg/100g

NB; Mean values with the same small letters are not significantly different

From figure 4.2 it can be seen that the levels of zinc in fruits follow the order Ithanga<Kanva< Thika< S54 < Embu<Limuru.

4.4.4 Mean levels of iron

Mean levels of iron in fruits ranged from 0.15±0.01 mg/100g in Embu variety to 5.62±0.00 mg/100g in Thika variety. Thika variety had the highest mean levels of iron while Embu had the lowest. Thika variety had mean levels of iron that were significantly higher while Embu had levels that were significantly
lower. All the six varieties recorded mean levels of iron that were significantly different. Iron levels recorded in fruits of these varieties were found to be higher than those of 1.85 mg/100g of iron in mulberry fruits reported by USDA (2009) on mulberry fruits from Asia and comparable to fruits such as strawberry, blackberry, melon and grapes (Decuyper, 2005; Rumez et al., 2006) which ranged from 1.00-3.26 mg/100g. Therefore mulberry fruits can be considered as a better source of iron compared to most common fruits, but a higher amount of about 734.69 g/day of dried mulberry fruits would have to be consumed in order to meet an RDA of 18 mg/day (WHO, 2008). Figure 4.3 shows the graph representing the mean levels of iron in different fruit varieties.
From figure 4.3 it can be seen that the levels of iron in fruits follow the order Embu < S54 < Limuru < Kanva < Ithanga < Thika.
4.5 Comparison of mineral elements in leaves and fruits

Levels of mineral elements in leaves and fruits of the varieties whose both leaves and fruits were studied were compared. The mean values of K, Ca, Zn and Fe between leaves and fruits of six mulberry variety are given on table 4.2. The levels of all analytes were not significantly different between leaves and fruits of all the six varieties since all p-values were < (0.05) except levels of zinc in Limuru variety since p (0.0668) > p (0.05).

<table>
<thead>
<tr>
<th>Variety</th>
<th>Nutrient</th>
<th>Concentration mg/100g (Mean ±SD)n=3</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Leaves</td>
<td>Fruit</td>
</tr>
<tr>
<td>Embu</td>
<td>Calcium</td>
<td>79.92±1.15</td>
<td>53.42±1.4</td>
</tr>
<tr>
<td></td>
<td>Iron</td>
<td>0.55±0.01</td>
<td>1.15±0.01</td>
</tr>
<tr>
<td></td>
<td>Potassium</td>
<td>216.79±2.50</td>
<td>245.98±1.44</td>
</tr>
<tr>
<td></td>
<td>Zinc</td>
<td>0.19±0.01</td>
<td>0.26±0.01</td>
</tr>
<tr>
<td>Ithanga</td>
<td>Calcium</td>
<td>61.75±2.50</td>
<td>52.58±1.4</td>
</tr>
<tr>
<td></td>
<td>Iron</td>
<td>2.34±0.01</td>
<td>3.08±0.01</td>
</tr>
<tr>
<td></td>
<td>Potassium</td>
<td>205.99±1.43</td>
<td>192.48±1.61</td>
</tr>
<tr>
<td></td>
<td>Zinc</td>
<td>0.35±0.01</td>
<td>0.13±0.01</td>
</tr>
<tr>
<td>Kanva</td>
<td>Calcium</td>
<td>94.25±2.50</td>
<td>52.25±0.87</td>
</tr>
<tr>
<td></td>
<td>Iron</td>
<td>0.86±0.01</td>
<td>1.82±0.01</td>
</tr>
<tr>
<td></td>
<td>Potassium</td>
<td>302.64±2.89</td>
<td>193.48±1.44</td>
</tr>
<tr>
<td></td>
<td>Zinc</td>
<td>0.27±0.01</td>
<td>0.15±0.01</td>
</tr>
<tr>
<td>Limuru</td>
<td>Calcium</td>
<td>84.08±2.75</td>
<td>40.92±1.44</td>
</tr>
<tr>
<td></td>
<td>Iron</td>
<td>3.34±0.01</td>
<td>1.56±0.02</td>
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<tr>
<td></td>
<td>Potassium</td>
<td>319.31±57.17</td>
<td>176.81±2.50</td>
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<td>Zinc</td>
<td>0.30±0.01</td>
<td>0.28±0.01</td>
</tr>
<tr>
<td>S54</td>
<td>Calcium</td>
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<td>34.25±2.50</td>
</tr>
<tr>
<td></td>
<td>Iron</td>
<td>2.46±0.01</td>
<td>1.46±0.01</td>
</tr>
<tr>
<td></td>
<td>Potassium</td>
<td>300.14±1.44</td>
<td>141.81±2.50</td>
</tr>
<tr>
<td></td>
<td>Zinc</td>
<td>0.20±0.01</td>
<td>0.23±0.01</td>
</tr>
<tr>
<td>Thika</td>
<td>Calcium</td>
<td>91.75±2.50</td>
<td>45.92±1.44</td>
</tr>
<tr>
<td></td>
<td>Iron</td>
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<td>5.62±0.00</td>
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<tr>
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<td>Potassium</td>
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<td>192.64±1.44</td>
</tr>
<tr>
<td></td>
<td>Zinc</td>
<td>0.43±0.01</td>
<td>0.23±0.01</td>
</tr>
</tbody>
</table>
From Table 4.4, Embu variety recorded levels that were not significantly different between leaves and fruits. Fruits were found to have higher levels of potassium, zinc and iron compared to the leaves of the same variety, but leaves had higher levels of calcium (79.92±1.15 mg/100g) compared to (53.42±1.4 mg/100g) in fruits. This shows that leaves of Embu variety can be considered as a good source of calcium while fruits could provide K, Zn and Fe. Levels of all nutrients in leaves of this variety are lower than those recorded by Raj et al (2009) in leaves of 10 mulberry genotypes which ranged from 236-730 mg/100g while those of fruits were found to be higher than those recorded by Vankatesh and Chauhan (2011) on 7 Indian mulberry varieties. All levels of both leaves and fruits of Embu variety reported in this study were higher than those in common vegetables and fruits and therefore should be encouraged for human consumption (Emsley, 2001).

Ithanga leaves were found to have higher levels of calcium, potassium and zinc compared to the fruits of the same variety, but fruits recorded higher levels of iron (3.08±0.01 mg/100g) compared to (2.34±0.01 mg/100g) of iron in leaves. Ithanga leaves recorded iron and potassium levels that are comparable to those reported by Raj et al (2009) on 10 mulberry genotypes which had a mean of 5.06±0.02 mg/100g while those of zinc and calcium were lower which could be due to the different soils from which they were grown. Levels of calcium, iron and potassium in leaves of Ithanga were also comparable to those of common vegetables like cabbage and spinach which ranged from 1 to 3 mg/100g (Decuyper, 2005). However zinc levels were lower than levels of 10 mulberry
genotypes Raj et al. (2009), which ranged from 0.9-1.26 mg/g. Levels of potassium in leaves were higher than those reported by Srivastava et al. (2006) of 194 mg/100g while those of fruits were comparable to 194 mg/100g reported by USDA (2009).

Kanva leaves were found to have higher levels of calcium, potassium and zinc compared to the fruits of the same variety, but fruits had higher levels of iron (1.82±0.01 mg/100g) compared to (0.86±0.01 mg/100g) of iron in leaves. Levels of all minerals in leaves of Kanva were lower than a range of 0.9-1.26 mg/100g reported for different mulberry genotypes reported by Raj et al. (2009) except potassium levels which were found to be higher than 194 mg/100g potassium reported for Indian leaves. Levels of iron and zinc in fruits of Kanva variety were lower than 1.26 mg/100g of fruits in Morus nigra from Asia (USDA, 2009) while calcium levels in fruits were higher than 39 mg/100g reported by USDA (2009). Levels in this study are in agreement with those recorded in common vegetables and fruits (Decuyper, 2005), therefore Kanva leaves and fruits in Kenya should be considered as sources of nutrients.

Leaves of Limuru variety were found to have higher levels of calcium, potassium, zinc and iron compared to the fruits of the same variety. Limuru leaves are therefore better source of Ca, K, Zn and Fe than fruits. Levels of calcium in this variety were found to be lower than a mean value of 372.97 mg/100g reported by Raj et al. (2009) on leaves of 10 mulberry genotypes although, they were comparable to those of common vegetables. Fruits had
higher levels of calcium than 1.85 mg/100g reported on the Asian *Morus nigra* fruits by USDA (2009) which were also higher than those of common fruits reported by Decuyper (2005). Potassium levels in leaves of this study were higher than 194 mg/100g obtained by Srivastava *et al.* (2006) and also higher than a range of 124-400 mg/100g reported for vegetables such as cucumber, carrot, cauliflower, cabbage and spinach.

Levels of iron in leaves were lower than a mean of 5.06±0.02 1.85 mg/100g reported by Raj *et al.* (2009) on mulberry genotypes while fruits had levels that were in agreement with 1.85 mg/100g reported by USDA (2009) on Asian fruits. Levels of zinc in leaves were lower than those reported by Raj *et al.* (2009) which ranged from 0.99-1.26 mg/100g but higher than those of common vegetables since most common vegetables were reported to have trace levels of zinc (Decuyper, 2005). Fruits of Limuru variety had higher levels of zinc 0.12 mg/100g reported for Asian mulberry fruits (USDA, 2009). Leaves and fruits of Limuru variety in Kenya should therefore be considered as a source of food.

S54 leaves were found to have higher levels of calcium, potassium and iron compared to the fruits of the same variety, but fruits had higher levels of zinc (0.23±0.00 mg/100g) compared to (0.20±0.01 mg/100g) in leaves. Calcium levels in leaves were lower than 236 mg/100g reported by Raj *et al.* (2009) and also lower in fruits than those reported by USDA (2009) of 39 mg /100g. Levels of Fe reported in this study were lower than a mean of 5.06±0.02 mg/100g reported by Raj *et al.* (2009), but comparable to those reported for
common vegetables like cabbage, spinach and kale (Decuyper, 2005; Rumeza et al., 2006) which ranged from 1 to 3 mg/100g, however all fruit levels of S54 variety were lower than 1.85 mg/100g recorded by USDA (2009). The leaves of S54 variety recorded zinc levels that were higher than 0.99-1.26 mg/100g reported by Raj et al. (2009) and Srivastava et al. (2006) and also higher than trace levels reported in common fruits and vegetables reported by Decuyper (2005). We can therefore conclude that leaves of S54 variety can be considered as a good source of Ca, K and Fe while fruits are a better source of Zn compared to commonly consumed fruits.

Thika leaves were found to have higher levels of Ca, K and Zn compared to the fruits of the same variety, but fruits had higher levels of iron (5.62±0.00 mg/100g) compared to (2.13±0.01 mg/100g) of iron in leaves. The Ca, Fe and Zn levels in leaves were lower than a mean value of 372.97±32.403 mg/100g, 5.06±0.02 mg/100g and a range of 0.99-1.26 mg/100g respectively reported by Raj et al. (2009) and Srivastava (2006) on mulberry varieties. The leaves of Thika variety recorded higher levels of Ca than cabbage, cauliflower and cucumber (Decuyper, 2005) while fruits recorded Ca levels that were lower than a range of 10 -32 mg/100g of Ca reported for fruits such as strawberry, blackberry, raspberry, cherries and grapes by Decuyper (2005). The K levels were higher in leaves than 194 mg/100g reported on mulberry genotypes by Srivastava (2006) while fruits had levels that were comparable to those reported by USDA (2009). The leaves of Thika variety are therefore better source of Ca,
K and iron compared to the fruits while the fruits are a good source of iron compared to the leaves.

### 4.6 Mean levels of vitamins in different mulberry varieties

Table 4.5 gives the mean levels of vitamins in 8 different varieties. Appendix 13, 14 and 15 show graphically the levels of \( \beta \) carotene, ascorbic acid and \( \alpha \)-tocopherol leaves respectively.

#### Table 4.5: Mean values of vitamins concentration in leaves

<table>
<thead>
<tr>
<th>Variety</th>
<th>( \beta ) Carotene (mg/l00g) Mean ±SD</th>
<th>Ascorbic acid (mg/l00g) Mean ±SD</th>
<th>( \alpha )-Tocopherol (mg/l00g) Mean ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ithanga</td>
<td>12.44±0.76( ^e )</td>
<td>644.33±1.15( ^h )</td>
<td>13.48±0.25( ^c )</td>
</tr>
<tr>
<td>Thika</td>
<td>19.27±0.03( ^f )</td>
<td>555.00±1.73( ^l )</td>
<td>11.08±3.70( ^a )</td>
</tr>
<tr>
<td>Embu</td>
<td>9.40±0.03( ^d )</td>
<td>584.00±3.61( ^g )</td>
<td>11.99±0.30( ^b )</td>
</tr>
<tr>
<td>Limuru</td>
<td>6.90±0.13( ^c )</td>
<td>394.00±7.00( ^a )</td>
<td>12.24±0.31( ^b )</td>
</tr>
<tr>
<td>S54</td>
<td>56.81±0.08( ^g )</td>
<td>464.33±4.04( ^c )</td>
<td>16.85±0.30( ^d )</td>
</tr>
<tr>
<td>Kanza</td>
<td>56.58±0.14( ^g )</td>
<td>543.33±2.89( ^g )</td>
<td>16.83±0.18( ^d )</td>
</tr>
<tr>
<td>Thailand</td>
<td>1.17±0.03( ^a )</td>
<td>530.33±5.69( ^d )</td>
<td>11.25±0.94( ^a )</td>
</tr>
<tr>
<td>Ichinose</td>
<td>2.25±0.04( ^b )</td>
<td>451.67±5.51( ^b )</td>
<td>11.06±0.94( ^a )</td>
</tr>
<tr>
<td>Overall mean</td>
<td>20.60±0.16</td>
<td>520.87±5.27</td>
<td>13.10±0.87</td>
</tr>
</tbody>
</table>

NB: Mean values with the same small letters within the same column are not significantly different.

The results presented in table 4.5 are individually discussed in the following subsections.

#### 4.6.1 Mean levels of \( \beta \)-carotene

The levels of \( \beta \)-carotene ranged from 1.17±0.03 in Thailand leaves to 56.81±0.08 mg/100g in S54 leaves. S54 had the highest while Thailand variety
had the lowest. The levels of β-carotene in S54 and Kanva were significantly higher and not significantly different while the other varieties differed significantly. S54 and Kanva can be recommended as good sources of β-carotene compared to the other varieties. The β-carotene levels reported in this study were higher compared to those of fresh mulberry genotypes reported by Raj et al. (2009) which were reported to range from 3.91-14.79 mg/100g with a mean value of 9.46±0.09. Levels in this study were also higher than levels reported on common vegetables such as spinach amaranth that ranged between 1.74 and 5.58 mg/100g as reported by Gopalan (1995). Mulberry leaves were found to have higher levels of β-carotene than the RDA (800-1000 μg/day) therefore a range of about 3.88 - 4.84 g/day of fresh leaves would be recommended for human consumption.

4.6.2 Mean levels of ascorbic
Ascorbic levels in leaves of the eight varieties ranged from 394.00±7.00 in Limuru variety to 644.00±1.15 mg/100g in Ithanga variety. Ithanga leaves had significantly higher levels while Limuru leaves had significantly lower levels. Ithanga leaves can therefore be considered as the best source of ascorbic acid compared to the other varieties. All the varieties recorded ascorbic acid levels that were significantly different implying that they would provide different amount of ascorbic acid. Levels of ascorbic acid recorded in this study were higher than a range of 142.99-370.08 mg/100g reported by Raj et al. (2009) on leaves of 10 mulberry genotypes. The value of ascorbic acid of spinach, fenugreek leaves, amaranth, bathua and mustard leaves was reported as 28, 52,
99, 35, and 33 mg/100g, respectively by Gopalan et al. (1995), which were lower compared to those of the present study. Other levels reported on kale from Nigeria by Ukam (2008), as 23.43 mg/100g showed that mulberry leaves had higher levels of ascorbic acid. Levels of ascorbic acid in mulberry varieties recorded in this study were beyond the RDA of 60 mg/day (WHO, 2008); therefore only about 1.15 g/day of fresh leaves would be required to meet the RDA.

4.6.3 Mean levels of α-tocopherol
The levels of α-tocopherol as shown in table 4.5 ranged from 11.06±0.94 in Ichinose leaves to 16.85±0.30mg/100g in S54 leaves. The levels in S54 variety were the highest while levels in Ichinose variety were the lowest. Levels in S54 and Kanva varieties recorded significantly higher levels that did not differ significantly which imply that they can be considered as good sources of α-tocopherol than the other varieties. Thika, Ichinose Thailand, recorded lower levels that were not significantly different meaning that they would also provide equivalent amount of α-tocopherol. Embu and Limuru varieties were also not significantly different. Levels of α-tocopherol in this study were lower than 36.4mg/100g reported for mulberry leaves from India by Srivastava et al. (2006). Ukam (2008) reported 4.06 mg/100g in kale (Brassica oleracea) while those of m.maderaspanata tropical leaves were reported to be 0.194 mg/100g by Petrus et al. (2010) which were lower compared to levels recorded in this study. The levels of α-tocopherol in the present study did not meet the RDA of
15 mg/day for α-tocopherol which implies that 114.5 g/day of fresh mulberry fruits would have to be consumed in order to meet the RDA.

4.7 Mean levels of carbohydrates and proteins

The results of the mean levels of carbohydrates in leaves and fruits of eight varieties and proteins in leaves of six varieties are tabulated in table 4.6.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Carbohydrates (n=3) mg/100g (Mean ±SD)</th>
<th>Proteins in Leaves mg/100g (Mean ±SD) n=3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaves</td>
<td>Fruits</td>
</tr>
<tr>
<td>Ithanga</td>
<td>87.12±6.92^a</td>
<td>184.09±4.55^b</td>
</tr>
<tr>
<td>Thika</td>
<td>166.66±9.34^g</td>
<td>163.49±6.94^a</td>
</tr>
<tr>
<td>Embu</td>
<td>116.97±6.94^c</td>
<td>234.55±4.60^d</td>
</tr>
<tr>
<td>Limuru</td>
<td>153.49±2.62^f</td>
<td>211.97±2.63^c</td>
</tr>
<tr>
<td>S54</td>
<td>135.30±6.9^e</td>
<td>271.35±7.72^f</td>
</tr>
<tr>
<td>Kanva</td>
<td>426.36±9.10^h</td>
<td>241.52±6.96^g</td>
</tr>
<tr>
<td>Thailand</td>
<td>122.73±9.10^d</td>
<td>ND</td>
</tr>
<tr>
<td>Ichinose</td>
<td>90.76±1.5^b</td>
<td>ND</td>
</tr>
<tr>
<td>Overall</td>
<td>162.42±6.56</td>
<td>217.83±5.57</td>
</tr>
<tr>
<td>Mean</td>
<td>ND: Not Determined</td>
<td>18.74±0.60</td>
</tr>
</tbody>
</table>

NB; Mean values with the same small letters within the same column are not significantly different.

The results presented in Table 4.6 are individually discussed in the following subsections. The levels of carbohydrates in leaves ranged from 87.12±6.92 mg/100g in Ithanga leaves to 426.36±9.10 mg/100g in Kanva variety. Kanva variety recorded the significantly higher levels while Ithanga recorded significantly lower levels. On the other hand mean levels of carbohydrates in
fruits ranged from 163.495±6.94 in Thika variety to 271.35±7.72 mg/100g in S54 variety. Fruits of S54 variety had the highest levels while Thika variety had the lowest. The levels of carbohydrates recorded for both leaves and fruits were significantly different. From the results it is seen that S54 fruits are the best sources of carbohydrates compared to the other five varieties of fruits that were studied. The mean values of carbohydrates in leaves and fruits are shown in Figure 4.5.
> Ithanga while the order of carbohydrates concentration in fruits is S54 > Kanva > Embu > Limuru > Ithanga > Thika.

Leaves of Thika, and Kanva had higher levels of carbohydrates than fruits of the same varieties while Ithanga, Embu, Limuru and S54 fruits had higher levels of carbohydrates than leaves of the same variety. Carbohydrate levels reported by Raj et al. (2009) had a range of 11.03-16.26 % on mulberry leaves genotypes while Vankatesh and Chauhan (2011) reported a range of 0.373-0.655 mg/g on 7 mulberry fruits from India which were lower compared to levels reported in this study.

This study recorded carbohydrate levels that were higher compared to those of vegetables reported by Mustapha (2009) which recorded 71.1, 87.0 and 110.0 mg/100g in sorrel, carrot and moringa leaves. Levels of carbohydrates reported by Rumez et al. (2006) in commonly used vegetables such as cabbage (4.8 mg/100g) spinach (4.0 mg/100g) and tomato (3.9 mg/100g) are very low compared to mulberry leaves. Levels of carbohydrates in this study were lower than the RDA of 300 mg/day except Kanva leaves. An amount of about 184.7 g of both leaves and vegetables would be required to meet the RDA for all the other varieties. Mulberry can therefore be used to provide carbohydrates and more so by diabetic patients.
4.7.2 Mean levels of proteins in leaves

The levels of proteins in leaves ranged from 1.24±0.31 in Ichinose leaves to 49.55±0.29 mg/100g in Thailand leaves. Thailand variety had the highest while Ichinose variety had the lowest. The levels of proteins in S54 and Ichinose varieties were not significantly different while all the other varieties recorded significantly different levels. Thailand leaves are therefore the best source of proteins compared with all the other varieties while S54 and Ichinose would provide equivalent amount of proteins. Levels of proteins in the present study were lower compared to levels reported for leaves of 10 mulberry genotypes which ranged from 6.38 to 10.73% with mean value of 8.01±0.098 % (Raj et al., 2009).

The levels reported for tropical vegetables ranged from 19.9 to 35.1 g/kg (Ukam, 2008) were higher than the levels recorded in the present study. Proteins of common vegetables have been recorded in cabbage (1.6 mg/100g), carrot (1.5 mg/100g), Lettuce (1.2 mg/100g), cauliflower (1.8 mg/100g) and spinach (2.1 mg/100g) by Rumeza et al. (2006). All the levels in the above vegetables are very low compared to those of mulberry leaves recorded in this study, but these levels do not meet the RDA of 50 g /day of proteins (WHO/FAO/UNU, 2001). An amount equivalent to 2668.09 g/day of dried mulberry leaves would be required to meet the RDA.
CHAPTER FIVE: 5 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions.

The following conclusions can be made from the present study;

i) All mulberry varieties analyzed in this study were found to contain substantial amounts of K, Ca, Zn, Fe, β carotene, α-tocopherol, carbohydrates and proteins, with none recording high levels in all the nutrients analyzed.

ii) There were variations in levels of K, Ca, Zn, Fe, in the leaves and fruits of the same mulberry variety with levels being higher in leaves of most varieties.

iii) Variations were noted in the levels of vitamins in leaves of different mulberry varieties with higher levels of ascorbic acid followed by β-carotene then α-tocopherol.

iv) Carbohydrates levels in the different mulberry varieties analyzed varied in leaves and fruits with leaves of Ithanga, Embu, Limuru and S54 recording higher levels of carbohydrates than fruits of the same varieties.

v) Levels of proteins differed in all the mulberry varieties studied except in S54 and Ichinose varieties and the levels recorded in all varieties were found to be higher compared to other studied mulberry varieties and to most common vegetables.

vi) The nutritional quality of fresh mulberry leaves was found to be better compared to those of other commonly consumed green vegetables.
5.2 Recommendations

5.2.1 Recommendations from this study

The study established that both leaves and fruits of the varieties of mulberry considered had substantial levels of the nutrients with relation to levels reported for commonly consumed vegetables hence the need to sensitize the public on the nutritional value of leaves and fruits. Mulberry should be cultivated not only for silkworm rearing but also as a sources of nutrients to boost food security, foster development and support sustainable land care. Nutrients available in leaves and fruits may also be used by people with ill health conditions in reducing blood sugar, blood pressure and cholesterol levels by formulating supplements and enriching food for people with these conditions.

Leaves of Ichinose, Thika and Thailand varieties had higher levels of most nutrients while fruits of Embu variety had higher levels of most mineral elements that were analyzed, therefore these varieties should be considered by planting them mostly in areas that they grow well as the name of the variety suggests.

5.2.2 Recommendations for further study

The following may be recommended for further study:
i) The present study analyzed nutrients in leaves and fruits of 8 mulberry varieties hence analysis of other metal elements and vitamins and other mulberry varieties need to be considered for further study.

ii) This study considered leaves and fruits only therefore analysis of other parts such as roots and the bark of the mulberry varieties are can be considered.

iii) Factors affecting levels of nutrient such as geographical regions seasonal variation and soil types were not considered in this study hence the need to assess the effect of these factors on levels of nutrients for mulberry varieties grown in different regions and at different seasons.

iv) The present study analyzed total proteins present in mulberry leaves. A study to find out the types of amino acids present in leaves and fruits is necessary.

v) Mulberry leaves and fruits are known to have medicinal properties (Cuixq et al., 2008) a study should be carried out to assess the medicinal properties of the different parts and varieties of mulberry found in Kenyan.

vi) Mulberry leaves are known to be highly palatable to livestock, therefore a study should be carried out too find out the suitability of leaves as a component of animal feed.
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APPENDICES

APPENDIX 1

Calibration curve for potassium

\[
y = 0.073x + 0.01 \\
R^2 = 0.996
\]
APPENDIX 2

Calibration curve for calcium

\[ y = 0.027x + 0.000 \]
\[ R^2 = 0.997 \]
APPENDIX 3

Calibration curve for zinc

Absorbance

Concentration mg/ml

\[ y = 0.085x + 0.025 \]

\[ R^2 = 0.994 \]
PPENDIX 4

Calibration curve for iron

\[ y = 0.004x + 0.000 \]

\[ R^2 = 0.997 \]
PPENDIX 5

Calibration curve for total carbohydrates

\[ y = 0.029x + 0.001 \]

\[ R^2 = 0.993 \]
APPENDIX 6

Calibration curve for total proteins

\[ y = 0.966x + 0.005 \]

\[ R^2 = 0.988 \]
APPENDIX 7

Calibration curve for β-carotene

\[ y = 0.794x + 0.068 \]

\[ R^2 = 0.996 \]
APPENDIX 8

Calibration curve for α-tocopherol

\[ y = 0.294x + 0.121 \]

\[ R^2 = 0.991 \]
APPENDIX 9

Concentration of potassium in leaves and fruits mg/100g

<table>
<thead>
<tr>
<th>Variety</th>
<th>Leaves</th>
<th>Fruit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ithanga</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thika</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Embu</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limuru</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kanva</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thailand</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ichinose</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX 10

Concentration of zinc in leaves and fruits mg/100g

Key

- Leaves
- Fruit
APPENDIX 11

Concentration of iron in leaves and fruits mg/100g

![Graph showing concentration of iron in leaves and fruits for different varieties.](image)

**Key**
- Leaves
- Fruit
APPENDIX 12

Concentration of calcium in fruits mg/100g

<table>
<thead>
<tr>
<th>Variety</th>
<th>Concentration of Ca in fruits mg/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ithanga</td>
<td>d</td>
</tr>
<tr>
<td>Thika</td>
<td>c</td>
</tr>
<tr>
<td>Embu</td>
<td>d</td>
</tr>
<tr>
<td>Limuru</td>
<td>b</td>
</tr>
<tr>
<td>SS-4</td>
<td>a</td>
</tr>
<tr>
<td>Kanva</td>
<td>d</td>
</tr>
</tbody>
</table>
APPENDIX 13

Concentration of β-carotene in leaves mg/100g

Variety

- Ithanga
- Thika
- Embu
- Limuru
- SS4
- Kanza
- Thailand
- Ichinose
APPENDIX 14

Concentration of ascorbic in leaves mg/100g

Variety

Ithangs
Thika
Embu
Limuru
SS4
Kanva
Thailand
Ichinose

Concentration of Ascorbic acid in leaves mg/100g
APPENDIX 15

Concentration of α-tocopherol in leaves mg/100g

Variety

Concentration of α-tocopherol mg/100g
# APPENDIX 16

Data for the analyzed levels of nutrients (mg/100g)

<table>
<thead>
<tr>
<th>Leaves</th>
<th>Variety</th>
<th>Calcium</th>
<th>Potassium</th>
<th>Zinc</th>
<th>Iron</th>
<th>Carbohydrates</th>
<th>β-Carotene</th>
<th>Ascorbic Acid</th>
<th>α-tocopherol</th>
<th>Proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ithanga</td>
<td>61.75±2.50^a</td>
<td>205.99±1.43^a</td>
<td>0.35±0.01^e</td>
<td>2.34±0.01^f</td>
<td>87.12±6.92^a</td>
<td>12.44±0.76^e</td>
<td>644.33±1.15^g</td>
<td>13.49±0.25^d</td>
<td>21.87±1.10^e</td>
<td></td>
</tr>
<tr>
<td>Thika</td>
<td>91.75±2.50^c</td>
<td>303.48±1.44^b</td>
<td>0.43±0.01^f</td>
<td>2.13±0.01^e</td>
<td>166.66±9.34^g</td>
<td>19.27±0.03^f</td>
<td>555.00±1.73^e</td>
<td>11.08±3.70^a</td>
<td>18.16±0.31^d</td>
<td></td>
</tr>
<tr>
<td>Embu</td>
<td>79.92±1.15^b</td>
<td>216.79±2.50^a</td>
<td>0.19±0.01^a</td>
<td>0.55±0.01^a</td>
<td>116.97±6.94^c</td>
<td>9.40±0.03^d</td>
<td>584.00±3.61^f</td>
<td>11.99±0.30^b</td>
<td>9.70±0.81^e</td>
<td></td>
</tr>
<tr>
<td>Limuru</td>
<td>84.08±2.75^b</td>
<td>319.31±1.17^b</td>
<td>0.30±0.01^d</td>
<td>3.34±0.01^b</td>
<td>153.48±2.62^f</td>
<td>6.90±0.13^c</td>
<td>394.00±7.00^a</td>
<td>12.24±0.31^h</td>
<td>40.56±0.61^f</td>
<td></td>
</tr>
<tr>
<td>S54</td>
<td>84.25±2.50^b</td>
<td>300.14±1.44^b</td>
<td>0.20±0.01^a</td>
<td>2.46±0.01^g</td>
<td>135.30±6.9^e</td>
<td>56.81±0.08^g</td>
<td>464.33±4.04^c</td>
<td>16.85±0.30^d</td>
<td>1.59±0.53^a</td>
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<td>0.86±0.01^b</td>
<td>426.36±9.10^f</td>
<td>56.58±0.14^g</td>
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<td>122.73±9.10^d</td>
<td>1.17±0.03^a</td>
<td>530.33±5.69^d</td>
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One-way anova showed a significant difference all the p-values <0.0001 at 95% confidence level

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<thead>
<tr>
<th>Fruits</th>
<th>Variety</th>
<th>Calcium</th>
<th>Potassium</th>
<th>Zinc</th>
<th>Iron</th>
<th>carbohydrates</th>
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<td>192.48±1.61^c</td>
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One-way anova showed a significant difference all the p-values were <0.0001 at 95% confidence level