NUTRITIONAL COMPOSITION AND BIOACTIVE COMPONENTS IN SLENDERLEAF (*CROTALARIA OCHROLEUCA* AND *CROTALARIA BREVIDENS*) VEGETABLE AT VARIOUS GROWTH STAGES

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Nutritional Composition and Bioactive Components in Slenderleaf (*crotalaria ochroleuca* and *crotalaria brevidens*) Vegetable at Various Growth Stages

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university:

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DEDICATION

This thesis is dedicated to my dear parents: **Jean Jacob SAHOU**, **Rachel Yeyinou KEKEH** for all your sacrifices for the sake of my education and training;

May this work, fruit of your efforts, meet your expectations and be a testimony of my gratitude towards you.

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LIST OF ABBREVIATION

AOAC	Association of Official Analytical Chemists
ANOVA	Analysis of Variance
ARN	Acid RiboNucleic
BWB	Boiling Water Bath
DNA	DeoxyriboNucleic Acid
DPPH	DiPhenyl Picryl Hydrazyl
FAO	Food and Agricultural Organization
GENSTAT	General Statistical Package
HPLC	High Performance Liquid Chromatography
IPGRI	International Plant Genetic Resources Institute
IUPAC	International Union of Pure and Applied Chemistry
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KDHS	Kenya Demographic and Health Survey
LDLC	Low Density Lipoprotein Cholesterol
LSD	Least Significant Difference
mRNA	messenger RiboNucleic Acid
PAHO	Pan American Health Organization
RDA	Recommended Dietary Allowance
RDI	Recommended Daily Intake
TLC	Thin Layer Chromatography
TCA	Trichloroacetic Acid
UNICEF	United Nations Children's Fund
USDA	United State Department of Agriculture
USA	United State of America
WHO	World Health Organization

ABSTRACT

In Africa, one child out of five suffers from chronic malnutrition. In Kenya, 50% of the children have calcium, iron and zinc deficiency and 30% of children suffer chronic malnutrition. African traditional leafy vegetables have a role in alleviating this malnutrition. Such vegetables include slenderleaf. Though some work has been done on this vegetable, the relationship between growth stages on one side and nutritional, non nutritional, bioactives and antinutrients composition on the other side is not well researched.

The objectives of this study were to determine the nutrition composition, the bioactive components and the antinutrient content of 10 accessions of slenderleaf at three different growth stages. Slenderleaf were grown in JKUAT farm between January 2011, and February 2012.

Fresh leaves were harvested at 8 weeks, 13 weeks and 16 weeks after planting. The leaves were destalked, weighed, and ground. The proximate analysis (Moisture, protein, and ash) were determined and results were reported on dry matter basis. Vitamin C, β-Carotene and Total carotenoids were determined and results were reported on fresh matter basis. Atomic absorption spectrophotometer was used to determine the minerals (Iron, Zinc and Calcium). Phytates were determined using HPLC. The diPhenyl picryl hydrazyl method was used for the antioxidant determination and for the tannins vanillin-hydrochloric acid was used.

For nutrients contents (moisture, protein, ash and mineral), it was noticed significant ($p \leq 0.05$) differences in the slenderleaf accessions due to maturity stage. The nutrient content was significantly ($p \leq 0.05$) higher during flowering stage than the other two stages (moisture 73.98 %, ash 7.16 %, protein 33.07 %, iron 14.45 mg/100g, zinc 3.88 mg/100g, calcium 9.32 mg/100g). There were significant ($p \leq 0.05$) lower values of nutrient content at the after flowering stage. There were significant ($p \leq 0.05$) differences in bioactive (vitamin C, β-carotene, total carotenoids, flavonoid and vitamin E) content among the different accessions of slenderleaf. The significant ($p \leq 0.05$) highest values were observed during flowering stage compare to the two other stages (vitamin C 25.17 mg/100g, β-carotene 10.08 mg/100g, flavonoid 31.51 mg/100g and vitamin E 3.83 %). There were significant ($p \leq 0.05$) differences in the antinutrients (tannins, phytate and oxalate) content of slenderleaf at different maturity stages. For the antinutrients, there was significant ($p \leq 0.05$) higher values at the after flowering stage (tannins 70.39 mg/100g, phytate 63.14 mg/100g and oxalate124.73 mg/100g) while the significant ($p \leq 0.05$) lowest values were observed at the during flowering stage.

For all the samples and the different variables analyzed (nutrients, bioactives and antinutrients) were significantly ($p \leq 0.05$) different from each other at the three growth stages.

For each of the three growth stages we also noticed some significant ($p \leq 0.05$) difference between some accessions which were having significant ($p \leq 0.05$) higher level compared to others. Before the flowering growth stage, the nutrients, the bioactives and the antinutrient content of the plants were all at their highest level. During flowering, the nutrients and the bioactive content of the plant were at their highest level while the antinutrient content was at their lowest level. Finally after the flowering growth stage, the anti-nutrients content increased as the nutrients and the bioactive contents decreased to their lowest level.

It could then be concluded that the growth stage affected the nutritional value of the plant and also the accession. Therefore for an optimal nutritional value, the plant needs to be harvested before end of the flowering stage.

CHAPTER ONE
INTRODUCTION

1.1 Background

Plants play a central role in food, nutrition and health of the households in the world (Johns, 2001; IPGRI, 2004). Leafy vegetables and fruit form a significant part of the traditional diets of agricultural communities (Bhattacharjee, 2006; Maundu *et al.*, 2007). About 200 indigenous plant species are used as leafy vegetables in Kenya, but only a few have been fully domesticated, while the majority are wild (Mkiwa, 2008). African leafy vegetables (ALVs) have recently been attracting research attention for both their nutrition quality and alleged health benefits (Maundu *et al.*, 2007). They are often easier to grow, resistant to pests and diseases and are quite acceptable to local tastes. It is commonly argued that vegetable consumption reflects cultural backgrounds (Olaimer-Anyara *et al.*, 2007; Akpavi, 2008; Oniang'o *et al.*, 2007).

Unfortunately, due to the perpetual environmental, economic, and sociocultural changes, a good number of ALVs formerly very appreciated for consumption are neglected, under-exploited and threatened with extinction (Padulosi, 2004). That partly explains the paradox according to which Africa, one of the zones most endowed in biodiversity (Myers*et al.*, 2000; White, 2001; Wieringa *et al.*, 2004), remains the continent on which the populations suffer the most malnutrition and food deficiency diseases (FAO, 2002).

One of the neglected but important ALVs (among the top ten in Kenya) is slenderleaf (Abukutsa-Onyango *et al.*, 2007). The plant is one of the ALVs that has been grown and consumed in Africa, especially in Kenya for a long time (Kigen, 2008)[1]. It is reported that its young leaves are a good source of several vitamins and minerals (Abukutsa-Onyango *et al.*, 2007). The plant has also been known to be beneficial in treating stomach-related ailments and malaria (Woomer, 2000). The plant is also regarded as a multi-purpose crop in agricultural production and has a high germination rate (83% after five days). It is mainly cultivated by local communities in Western and Coast counties of Kenya for the purpose of food, restoring fertility to soils and to combat weeds in areas where cultivable land is a problem (Polhill, 1982). It can be intercropped with regular food or cash crops to improve soil

fertility and raise productivity. The green leaves can be harvested two to three times a week for human food (Beverly, 1991).In general, it is thought that bioactive food components are predominantly found in plant foods such as whole grains, fruit, and vegetables (Leung *et al*., 1968). The plant as any other ALVs is high in antioxidant activity factors (Czepa *et al*., 2004). Those factors are the ones expressed as bioactives components. β-carotene in the plant is also a most effective vitamin A precursor, and has been reported to protect humans against certain types of cancer (Edward *et al*., 2008) and cardiovascular diseases (Florence *et al*., 2009). Latter it can increase macular pigment concentration in eyes and may improve visual function (Hill *et al*., 1970). Recent research has demonstrated that β-carotene can act as powerful antioxidants and to help guard against aging, cancers and diseases (IPGRI, 2001).

1.2 Statement of the problem

According to the KDHS (2009), the nutritional status of children under five had improved only slightly in the past few years. At the national level, 35% of children under five were stunted (low height-for-age). Overall, 7% of children were wasted (low weight-for-height) and 16% were underweight (low weight-for-age). These data shows that malnutrition is still a serious public health problem in Kenya and requires urgent attention. Though the most pressing form of malnutrition in Kenya is protein-energy malnutrition, which affects infants, preschool, and school children (Ngare *et al.*, 2006), there is also concern about micronutrient deficiencies.

Slenderleaf is a popular traditional vegetable in some parts of East Africa, with good nutritional properties. Research priorities have previously focused mostly on survey and agronomy. By that fact, there is very little reported on its key compositional aspects (including its nutrients and antinutrients) in relation with the stage of growth.

1.3 Justification

Hunger and malnutrition in Africa have been on the increase since independence of most African countries in the 1960's. During the 1970s, it is estimated that 30 million people were directly affected by famine and malnutrition and nearly 12 million children under the age of five die annually (Imbumi, 2007). By the 1980's, over 150 million people were affected by one form or another of malnutrition (Frehiwot *et al.*, 1998). About 5 million children died in 1984 alone in Africa. Currently, hundreds of millions of people suffer from disease, blindness and other problems associated with malnutrition (Meso, 2005). Malnutrition is an underlying

cause in 55% of these deaths. Iron deficiency anemia alone is a contributing factor in over 20% of post-birth maternal deaths in Africa (Bonham *et al.*, 2002). In 2002, about 43 million people worldwide suffered from varying degrees of brain damage due to iodide deficiency (Bekele *et al.*, 2002).

Recent studies on slenderleaf show that its health promoting and protecting attributes are clearly linked to its nutritional (vitamin and mineral) and non-nutrients (bioactive phytochemicals) contents (Smith, 2007). Adequate micronutrient intake by women has important benefits for both women and their children. Breastfeeding children benefit from micronutrient supplies that mothers receive, especially iron (KDHS, 2009). Micronutrient deficiency is a serious problem in Kenya according to KDHS (2009). Though it is evident that the proportion of women receiving micronutrient supplementation has increased considerably over the last five years, there is need to sustain this trend in order to further reduce deficiencies of these crucial micronutrients among women and children (PAHO/WHO, 2003; WHO, 2005).

Few studies had been done on slenderleaf but they are not conclusive and the alleged plant's health benefits have not been established by studies (Vorster *et al.*, 2007). There is serious need to document scientifically the nutritional composition in order to adequately fill that gap. This will provide information on slenderleaf to various stakeholders[2].

The study will help to increase knowledge on the plant, promote indigenous vegetables, and therefore increase its production/consumption, as a useful tool to decrease food insecurity and malnutrition.

1.4 Objectives

1.4.1 General 0bjective

To determine the nutrient, the antioxidant activity of the bioactive components and the antinutrient content of slenderleaf different accessions at various growth stages.

1.4.2 Specific objectives

1. To determine the nutrient composition of different accessions of slenderleaf at various growth stages.
2. To quantify antioxidant active of the bioactive components in different accessions of slenderleaf at various growth stages.
3. To profile the antinutrient (phytate, oxalate and tannins) in different accessions of slenderleaf at various growth stages.

1.5 Hypothesis

- There is significant difference in the nutrients content of slenderleaf at different growth stages of different accessions.
- There is significant difference in the antioxidant active of the bioactive components of slenderleaf at different growth stages of different accessions.
- There is significant difference in the antinutrients content of slenderleaf at different growth stages of different accessions.

CHAPTER TWO
LITERATURE REVIEW

2.1 African Leafy Vegetables

African Leafy Vegetables (ALVs) foods have an exceptional place in African cuisine (Owuor *et al.*, 2002; Akpavi, 2008). For anyone interested in rural women's welfare, ALVs offer an important entry point and provide an important economic pillar upon which women's rural livelihood is supported (Myers *et al*., 2000). On the view of Johns, one woman producing ALVs provides employment to two others, a wholesaler and a retailer. They contain high level of minerals (Calcium, Iron, and Phosphorus) and significant amounts of vitamins and proteins (Johns, 2001).

2.2 Slenderleaf

2.2.1 Overview

Slenderleaf is found almost throughout tropical Africa except the northeastern and most southern parts and most islands of the Indian Ocean. Outside Africa, it has become naturalized in Brazil, the United States (Florida), Australia, New Guinea and China. In Kenya, slenderleaf grows mainly in Nyanza, Coast and Western Provinces. It can grow at elevations up to 2000 metres but does best in warm areas. According to Meso (2007) reveals that the plant is known under different names depending on the community: kamusuusuu by **Kamba** (Eastern province), kipkururiet by **Kipsigis**, kimiro by **Luhya**, mito by **Luo**.

Slenderleaf is one of the African Leafy Vegetables (ALVs) packed with nutritional value and grown for its young leaves and shoot, and is used as a cooked vegetable (Alan *et al.*, 2002). Despite this there has been a relatively restricted number of works on Slenderleaf in recent years; knowledge that is nowadays available on the plant offers a chink of understanding on different aspects of Slenderleaf including its botany characteristics and its agronomics conditions. Work from Alton (1994) enabled better understanding of its consumption patterns and its various potentials. Indeed, the plant is so rich in nutrients and low in fat and calories that it is even considered a super food. In many communities, the plant is eaten for its bitterness (just as pepper is taken for its 'hot' taste). However, some people find this bitter taste unpleasant and reduce it by blending with other, blander vegetables such as cabbage. Although *C. brevidens* is poor leaf yielder, it has strong taste and is used to spice food (Meso, 2005). It can be stored for a couple of days in the refrigerator and can be cooked into dishes

that require high temperatures (Bast *et al.*, 2008). HoeschleZeledon *et al.,* (2004) reported that a number of medicinal applications have been reported where the leaves are used to cure stomach-ache, swelling and malaria (Nekesa *et al.*, 2008). The roots are used to treat sore throat and mouth thrush. Shoot are used for livestock fodder and seeds are feed to poultry (Padulosi, 2000).

2.2.2 Botanical description

Slenderleaf as an African Leafy Vegetables is a plant from the family of *Fabaceae* (Pea family), the Genus of *Crotalaria L*. (Rattlebox) and presents two different species differing in taste and pod size (Ray-Yu *et al.*, 2009):

- *Crotalaria ochroleuca G. Don:* mild taste and big pod's cross section. *Crotalaria ochroleuca* is an erect much branched annual or short-lived perennial herb that grows to a height of up to 2.5m tall with branches with short hairs. Leaves are bright green, alternately arranged, tri-foliate, with petioles 1-6.5 cm long. Leaflets are linear to lanceolate or elliptical lanceolate. Inflorescence is a terminal raceme which is up to 50 cm long, with many flowers. Flowers are bisexual with a creamish or pale yellow corolla, ovary is superior and one-celled. Fruit is a broadly cylindrical pod that turns black when dry and is many seeded. Seeds are oblique-cordiform about 3.5 mm long, smooth, pale yellow to orange (Schippers, 2004)

- *Crotalaria brevidens Benth:* bitter taste (due to the presence of alkaloids and phenolic compounds), and small pod's cross section. *Crotalaria brevidens* is an erect much branched annual or short-lived perennial herb that grows to a height of up to 2m tall, with branches with short slightly spreading hairs. It is very similar to *Crotalaria ochroleuca* but the main distinguishing features are the bluish green leaves and the narrowly cylindrical pod, often slightly curved at the ends with seeds that range in colour from pale yellow, turning orange to dark red (Abukutsa-Onyango, 2004).

2.2.3 Agronomic conditions, crop husbandry and Ecology

White (2001) found that the genus to which slenderleaf belongs is the largest in tropical Africa and is commonly encountered in open places from mountains to semi-deserts. Most of the species are annual, perennial, herbs or shrubs (Martin*et al.*, 1970). It is an erect much-branched herb (usually 0.5–1.2m high) with green stems ascending branches (Myers*et al.*, 2000).

The leaves (wider and longer for *C. brevidens)* are either simple or divided into 3 long narrow leaflets of 13cm long by 3cm wide (Meso, 2007). Leaves are eaten as a cooked green vegetable, usually in combination with other greens (because of its bitter taste). Most of the species have numerous yellow flowers with very conspicuous reddish purple veins which later on bear tough-skinned seed pods that are inflated (Fig 1). The plant is seldom found in the wild and is grown from seeds and the maturity period takes around 6 months. Early growth is slow and it may take about 8 weeks until the first harvest. The plant dies after about 6 months.

Plate 1: Slenderleaf (*Crotalaria ochroleuca*) in pots at the glasshouse Photo: Abukutsa-Onyango, 2009

Seeds are small kidney-shaped, yellow and turn from orange to dark red. They are cultivated in rows or fertilized, raised beds (Fig 2). Fedders *et al*., (1998) showed that they are shortly stalked, longitudinally compressed on one side slightly, and blunt end with persistent style. The number of seed contained in a single pod depends on species but ranges from 5 to 50 seeds. The seed germinates in 3–4 days.

Plate 2: Seeds of slenderleaf . Photo: Abukutsa-Onyango, 2006

The pods are slightly longitudinally compressed on one side, black when dry with a very short stalked. According to Grace (1999), the pods are usually of varying sizes from long narrow and wide (7cm long by 2cm wide) to short and thinner for *C. brevidens* (Fig 3).

Plate 3: Pods of Crotalaria ochroleuca

According to Angeles *et al.*, (1993), slenderleaf grows in open localities with adequate sunshine at 300–2000 m altitude. It is favored by warm conditions, and after the crop is well established and has formed long taproots and long lateral roots, it can tolerate rather dry conditions. It does not suffer much from diseases and even less from pests. However under wet conditions the whole crop may be destroyed by blight just before it starts flowering. Aphids and thrips can be observed, but are rarely a serious menace. During fruit development, pod borers may enter and interfere with seed development. The holes in the pods will allow water to enter and destroy the seeds further through rot.

Slenderleaf thrives in open localities with adequate sunshine at elevations of between 300 and 2500 m above sea level and require warm growing seasons. It is favoured by warm temperatures. The optimal temperature range for slenderleaf grow and produce optimally is 20-30 ^{o}C. It can be cultivated in a wide range of soils as long as they have high organic matter content, fertile, well drained and with a pH 5.5-6.8. Slenderleaf can give good yields in soils with low nitrogen content. After the crop is well established and has formed long tap roots and long lateral roots, it can tolerate rather dry conditions but cannot tolerate water-logging. It is drought tolerant and can do well in rainfall of less than 500mm. (Schippers, 2004)

2.2.4 Consumption pattern

Ngugi's (1999) work revealed that the leaves are grown and widely consumed (young leaves and shoots cooked) as boiled or fried in oil vegetable in western Kenya (Fig 4) and slightly bitter, cooked normally with milk or other vegetables to counteract this. It is also used as potherb in stews or soup

Plate 4: View of one typical Slenderleaf dish Photo: Abukutsa-Onyango, 2006

2.2.5 Nutritional constituents

Elias *et al.*, (2011) says that slenderleaf like many vegetables is a potential supplier of supplementary proteins, minerals and vitamins. In the body, proteins are important for synthesis of new proteins or tissues (replacement for adults and for growth of children), to provide energy, to convert fat and glucose. Vitamins are essential organics substances needed in small amount in the diet for the normal function, growth and maintenance of body tissues. According to UNICEF (2008), minerals are known for many functions. Millogo *et al.*, (2001) reported that they helps to maintain the osmotic balance between intra and extra cellular fluid, important in muscle contraction and transmission of nervous impulses, synthesis and maintenance of bones tissues, essential in the synthesis of DNA and RNA.

The amounts of vitamins and proteins contents in the plant are significant (FAO, 2002). 100g of fresh slenderleaf contain level of calcium, iron and vitamins that would provide 100% of the daily requirement and 40% for the proteins (Abukutsa-Onyango, 2003). The plant is therefore a valuable source of nutrition in rural areas where they contribute substantially to

protein, mineral and vitamin intake (Mnzava, 1997). But we still have so many varieties whose compositions are not known and that is the purpose of our study.

2.2.6 Health benefits

Work from Olembo *et al*., (1995) showed that slenderleaf have medicinal properties and have been known to heal stomach-related ailments. Slenderleaf is occasionally used for its fibres and also known to suppress nematode populations. The bark from fresh roots is chewed, and the juice swallowed as a treatment for boils (Woomer, 2002).

The plant has been reported to contain anti-nutrients factors causing stomach-ache if eaten in excess or over a long period. Orech (2005) on his side revealed that this can emanate from toxicity problems due to its phytochemicals. In recent years, researchers have discovered that Slenderleaf is rich in calcium, iron, beta-carotene, and vitamins A, C, and K which can act as a preventative against lung diseases (Bhattacharjee, 2006). Research in Australia found that a diet high in beta-carotene foods like Slenderleaf can decrease the chances of skin cancer (FAO, 2002).

These factors need to be deeply investigated as some of the phenols can be anti-oxidants. About 20 species of *Crotalaria* in tropical Africa according to Polhill (1982) are known to cause poisoning. The more lethal forms of poisoning affect the nervous systems; lungs and liver (Bilabina *et al.,* 1991). Poisoning seems to be serious at flowering and seed stages, probably due to accumulation of toxic alkaloid substance (*Crotaline*) found in the bitter type. However, the leaves seem to be non-toxic before flowering (Rupper *et al.*, 1984).

2.2.7 Production, management and availability

Slenderleaf, introduced a century ago, has become part of the food culture in the sub-continent. Maziya-Dixon et *al*., (2005) reported that in Kenya, slenderleaf is relatively available and affordable particularly during the rainy seasons but were found to be among the least consumed foods. Despite the abundance of slenderleaf, it remains underutilized due to production, agronomic, processing, distribution and marketing limits, and lack of nutritional characterization information (Smith, 2007).

Slenderleaf is a vegetable of small-scale production since no individual data are available (grouped together with statistics on other green vegetables). Shackleton *et al.,* (2009) reported

that leaves are sold in Nairobi and most major towns of western Kenya and Nairobi. Field observation showed that trading is generally through networks and along well-established patterns. Production, handling and marketing are mostly done by women who harvest, packs and transports the fresh vegetables to the nearest buying point, usually by a roadside. Women vendors from urban centers buy and transport vegetables to strategic wholesale urban markets. Their counterparts in the retail sector purchase and transfer the vegetables to strategic retail points. At each vegetable exchange point, a profit of well over 75% is made (Nekesa *et al.*, 2007).

2.2.8 Challenges and opportunities in the Kenyan context

Oniang'o *et al.,* (2007) reported that African Leafy Vegetables (ALVs) conservation and sustainable utilization of traditional plants are keys contributing to national development, food security and the alleviation of poverty in the next 30 years. Some of the major challenges are particularly hygiene, heavy metal contamination, poor quality seeds and lack of technical packages for optimal production. In Kenya, like the rest of agricultural activities, slenderleaf production relies mostly on rain-fed agriculture (Maundu, 1993). Producers believe that irrigation and crop husbandry will lead to the improvement in production and reduction of seasonality in the plant's availability.

The poor state of roads worsens during the rainy season in most of the rural areas increasing wastage. Each community tends to have a typical species leading to the fact some are only consumed in particular part of the country (Oniang'o *et al.*, 2007).

2.2.9 Processing techniques

Processing techniques may vary with the community but the following are some of the common examples:

Boiling: used to soften the leaves and may be salted and mixed with butter added or fried to add flavor. The vegetable is eaten along with ugali.

Mashing with maize mixture: The vegetable may be cooked with maize, a mixture of maize, a pulse, pumpkins or a starchy tuber like English potato. These are mashed together. Butter may be added or the food fried.

Cooking with cereals or dried tubers flour: we add flour to the vegetable boiled in water (mixture cooked together). The mixture may be solid (ngunzakutu - Kamba) or

semi-solid. In times of famine (depends on rain), vegetables may be boiled and eaten with nothing else (Dell'Orco, 2003).

2.3 Micronutrients deficiencies

Micronutrient deficiencies result from inadequate dietary intake, impaired absorption, limited bioavailability, excessive losses, increased requirement or combination of these factors (KDHS, 2008). They also occur due to use of plant based staple diets, which have their micronutrients differing according to type of food, soil type and condition, agronomic practices and methods of food preparation. Consumption of animal protein, poultry and fish, which are rich in these minerals, is often low because of economic, cultural and religious constraints. Beverly (1991) found that micronutrient interactions are also very important because they occur at the metabolic level as well within the diet and affect the bioavailability of nutrients.

2.3.1 Minerals deficiency

The mineral composition of different foods differs. Among the main mineral deficiency condition of public health concern, we include iron, zinc and calcium (Armento, 1991).

Iron: According to KDHS (2009) survey, low iron content in diet is common, as is a low bioavailability of the iron in the diets consumed which are mainly plants based. This is compounded by rapid growth and tissue synthesis in infants and children, menstrual losses in fertile women and fetal demands for iron in expectant mothers (Sahou, 2008). Iron deficiency during pregnancy is associated with low birth weight babies, premature delivery and even prenatal and fetal death (Hedger *et al.*, 1994). During childhood, iron deficiency leads to impaired cognitive performance and motor development (Lozoff *et al*., 1991), decrease linear growth rate (Angeles *et al*. 1993). In adults iron deficient leads reduced work capacity (Scholz *et al*., 1997). It also negatively influences the normal defense systems against infection (Srikania *et al*., 1976; Joyson *et al*., 1972). Iron is however combines with protein to form hemoglobin, it is important for assuming gaseous exchange and helps prevent malfunctioning of the body which may cause anemia (Twamasi *et al.,* 2001).

Zinc: Research conducted by Aukeket *et al*., (1986) revealed that infants, children and women of reproductive age have high requirements for zinc, and the factors inhibiting iron absorption often is zinc absorption. Meat is the best dietary source of zinc (but the usual meat

intake of many populations is very low). King *et al.*, (1990) and Arbonnier (2002) found that a lack of data on zinc nutrition and the difficulties in assessing zinc status, particularly marginal zinc status, have led to zinc deficiency not being recognized as a major nutritional problem in less developed countries. However, when the attempts are made to assess both iron and zinc status, the prevalence of zinc deficiency appears to be similar to that of iron deficiency. Zinc deficiency does not appear to have any pronounced effect on iron absorption or metabolism but some reports suggest that copper absorption may increase when zinc status is impaired, even if the deficiency is only marginal (Polberger *et al*., 1996). Zinc deficiency has been reported to be responsible for growth retardation, hypogonadism, and delay in sexual maturation in selected malnourished children (Rimbach *et al.*, 2001; Burton *et al.*, 1986). According to Lawson-Body *et al*., (2007), in cases observed it is found that the factors which make zinc unavailable (cereal with high phytate content, staple diet, clay eating or soils) or cause abnormal losses of zinc in the body (chronic blood loss from parasitism) or a combination of these appear to be the underlying causes in addition to generally adequate diet deficient in animal protein (Millogo-Rasolidimby *et al.*, 2001).

Calcium: Basta *et al*., (1979) revealed in his work that calcium serves as one of the main structural skeletal elements (Sahou, 2008). The skeletal calcium is in dynamic equilibrium with the constituents of the body fluids and other tissues and the rate of exchange is greater than the rate of original deposition of new bone. The mostly readily mobilized calcium is found in the trabecular portion of bones. It is used when the calcium requirement of an individual increases as during pregnancy and lactation. The dentine and enamel of teeth are metabolically more stable and do not yield calcium with similar ease. Research from Assimos *et al.,* (2000) showed that the blood calcium serves several functions, it is essential for the normal functioning of the nerves tissues. A reduce blood calcium level increase the irritability of nervous tissues; very low calcium level may cause characteristic tetany with convulsions. Concentration of the calcium above the normal range is also essential for a normal pulse and calcium deficiency contributes to rickets. The calcium requirement is obviously increased in pregnancy to satisfy the demand created by growth and development of the fetal skeletal system. Good prenatal calcium nutrition and accumulation of calcium reserves is essential for optimal fetal development. The requirement of the mother consists of her own as well as that of the maternal skeleton to supply the fetus. Lactating mothers should have a markedly increased calcium supply to meet her needs and the calcium requirement for milk production (Holmes *et al.,* 2000).

2.3.2 Mineral interactions

Sienera (2006) stipulated that micronutrient interactions are important because they affect the bioavailability (absorption and use) of micronutrients despite their presence in diet. They are two fundamentally different types of micronutrients interactions that can occur in biological system:

- In the first type two (or more) trace elements shear the same absorptive pathways. This means that high concentration of one element may interfere with absorption of another element. Hill and Maundu (1994), who postulated that elements that have a similar coordination number and a similar configuration in water solution could compete for absorptive pathways, provided the conceptual framework for this type of interaction. Through experiments in animals they demonstrated that essential elements such as zinc and copper can interact and that an excess of one element can induce the deficiency of another. They also demonstrated that a toxic element like cadmium could inhibit the absorption of an essential element like zinc. This type of interaction is well recognized and even used in some clinical applications: treatment of Wilson's diseases patients with high doses of zinc (Simopoulos, 1994).
- The second type of interactions, stipulated by Cohen *et al*., (1985), occurs when a deficiency of one element affects the metabolism of another element as shown by the example of copper deficiency that causes iron deficiency anemia. Because copper is an essential component of celluloplasmin, or as it more accurately should be called, ferroxidase I, copper deficiency causes a dramatic decreases in ferroxidase activity, which in turn prevents the mobilization of iron from stores (by being oxidized from +2 to +3) and its incorporation into hemoglobin. Another example is the homeostatic up regulation of iron deficiency. This up-regulation also dramatically increases the absorption of essential elements, such as manganese, and toxic element such as cadmium n (Gutteridge *et al.*, 2000).
- **Iron and zinc:** It shows two clear possibilities of an interaction: intestinal co-adaptation and direct competition for uptake pathways. For experiments carried out by Hambidge *et al*., (1983), high amounts of iron tend to interfere with zinc absorption (Breskin *et al*., 1983) it appeared that iron uptake is not affected by zinc but excess iron can affect zinc uptake when given together (Rimbach *et al.*, 2001; Sandstrom *et al*., 1985).

2.4 Bioactive compounds

2.4.1 Phytochemicals

Phytochemicals are not something produced by a big chemical company, but plant chemical of health benefit that we should eat every day (Kushad *et al.*, 2003). They are natural compounds that are found in fruits and vegetables, grains and legumes. Phytochemicals are chemicals found in plants that protect plants against bacteria, viruses, and fungi (Zidorn *et al.*, 2005). For Hofmann, eating large amounts of brightly colored fruits and vegetables (yellow, orange, red, green, white, blue, purple), whole grains/cereals, and beans containing phytochemicals may decrease the risk of developing certain cancers as well as diabetes, hypertension, and heart disease, help prevent cell damage, prevent cancer cell replication, and decrease cholesterol levels (Hofmann *et al.*, 2004).

Research has also shown slenderleaf's phytochemicals effectively help in lowering cholesterol levels and in the body's detoxification (enzymes clear potentially carcinogenic substances out of your system). The action of phytochemicals varies by color and type of the food. They may act as antioxidants or nutrient protectors, or prevent carcinogens (cancer causing agents) from forming (Ganzera *et al.*, 2005). For Stuppner *et al.*, (2005), each type of fruit or vegetable may contain hundreds of different phytonutrients. Phytochemicals, such as carotenoids and flavonoids, are found in plants. That helps protect the plant against bacteria, viruses and fungi (Chweya *et al.*, 1997). These phytochemicals may have cancer fighting properties and generally cannot be taken in supplement form, according to the Yale Medical Group. There is no recommended dietary allowance for phytochemicals.

Specific sources of phytochemicals

The list below is a partial list of phytochemicals found in foods (Rosselot *et al.*, 2005; Mnzawa, 1997):

- **Allicin**, according to Tian *et al.*, (2005), is found in onions and garlic. Allicin blocks or eliminates certain toxins from bacteria and viruses.
- **Anthocyanins** are found in red and blue fruits (such as raspberries and blueberries) and vegetables. Work from Halkier *et al.*, (2006) showed that they help to slow the aging process, protect against heart disease and tumors, prevent blood clots, and fight inflammation and allergies.

- **Biflavonoids** are found in citrus fruits. Gershenzon *et al.,* (2005) revealed that they improve skin tone, increase energy, endurance, aging, and fatigue. It enhances immune function, and is natural alternatives, to use for dermatitis, cold and flu.
- **Carotenoids** are found in yellow, red, deep green fruits and vegetables (tomatoes, parsley, oranges, pink grapefruit, and spinach). They help to reduce and minimize the oxidative damage caused by free radicals (Schieberle *et al.*, 2004) and can protect against some cancers (lung, skin, uterine, cervix, gastrointestinal tract), macular eye disease, cardiovascular problems, macular degeneration, cataracts, and other sicknesses linked to oxidative or free radical damage. They also help to rejuvenate the body by promoting the growth of healthy. The beneficial actions are optimal when a variety of carotenoids are consumed together, rather than alone.
- **Flavonoids** are found in fruits, vegetables, wine, green tea, onions, apples, kale, and beans and have extensive biological properties that promote human health and help reduce the risk of disease. Grosch *et al.*, (2004) revealed that they extend the activity of vitamin C, act as antioxidants, protect LDL cholesterol to oxide into HDL, inhibit platelet aggregation, have anti-inflammatory and anti-tumor action.
- **Indoles** are found in broccoli, cabbage, kale, Brussel sprouts, and turnips (also known as "cruciferous" vegetables). Work from Belitz *et al.*, (2004) found that they contain sulfur and activate agents that destroy cancer-causing chemicals.
- **Isoflavones** are found in soybeans and products which consumption has many health benefits: protection against breast and prostate cancer, menopausal symptoms, heart disease and osteoporosis. Many of the health benefits of soy are derived from its isoflavones. However work from Wrolstad (2005) showed that some critics claim that isoflavones can increase the incidence of epithelial hyperplasia and cause goiter and hyperthyroidism.
- **Lignans** are found in flaxseed and whole-grain products. Their perceived health beneficial properties extend beyond hormone-dependent breast, prostate cancer and osteoporosis to include brain function, cardiovascular disease, immune function, inflammation, and reproduction. Women who eat healthy amounts of plant foods rich in lignans reduce their risk of developing breast cancer (American Journal of Clinical Nutrition, 2010).
- **Lutein** is found in leafy green vegetables. As for Boots *et al.*, (2008), it may prevent macular degeneration and cataracts as well as reduce the risk of heart disease and breast cancer.

- **Lycopene** is found primarily in tomato products. When cooked, it appears to reduce the risk for cancer and heart attacks (Haenen *et al.*, 2008).
- **Phenolics** are found in citrus fruits, fruit juices, dried and fresh plums, raisins, eggplant, cereals, legumes, and oilseeds. According to Bast *et al.*, (2008), they are studied for: slowing the aging process, protecting against heart disease and tumors, and fighting inflammation, allergies, and blood clots.

Table 1: Phytochemicals found in some foods and their benefits

Phytochemicals	Food	Benefits
Flavonoids	Apples	Protect against cancer, cholesterol lowering, fight heart disease, reduce inflammation
Flavonoids (saponins)	Beans	Protect against cancer, cholesterol lowering
Ellagic Acid	Berries	Prevent cellular changes that can lead to cancer, protect aging brain
Indoles, isothiocyanates	Broccoli	Protect against cancer, stroke and heart disease
Beta-carotene	Carrots	Antioxidant
Flavonoids (limonene)	Citrus fruits	Antioxidant, inhibit tumor formation, decrease inflammation
Isoflavones	Flaxseed	Protect against cancer, lower cholesterol
Allium (allyl sulfides)	Garlic	Protect against cancer and heart disease, boost immune system
Isoflavones	Grains	Protect against cancer, lower cholesterol
Flavonoids (quercitin)	Red grapes	Protect against cancer and heart disease
Allium (allyl sulfides)	Onions	Protect against cancer and heart disease, boost immune system
Isoflavones	Soy	Protect against cancer and heart disease, improve bone density, reduce menopausal symptoms
Flavonoids	Tea, Tomatoes	Protect against cancer and heart disease, fight infection

Source: Mnzawa, 1997: United States Department of Agriculture. 1980. USDA/CDCP. 1982

2.4.2 Antioxidants

Antioxidants are substances that inhibit the oxidation process and act as protective agents. They protect the body from the damaging effects of free radicals (by-products of the body's normal chemical processes). Free radicals attack healthy cells, which changes their DNA, allowing tumors to grow. Research from Padayatty *et al.*, (2003) is underway to investigate the role of antioxidants in decreasing the risk of developing cancer.

Antioxidants include:

- **Vitamin C (ascorbic acid)**

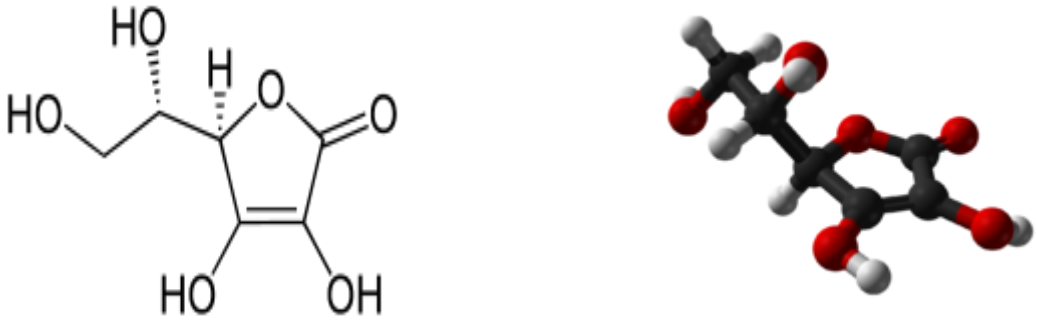


Figure 1: Structure of Vitamin C

IUPAC name: 2-oxo-L-threo-hexono-1,4- lactone-2,3-enediol

Synonyms: L-ascorbic acid
Formula: $C_6H_8O_6$
Routes: Oral
Source: Sebastian *et al.* (2003).

Excretion: renal
Bioavailability: rapid & complete
Protein binding: negligible

Fedders found that Vitamin C is an essential nutrient for humans and certain other animal species. In living organisms ascorbate acts as an antioxidant by protecting the body against oxidative stress (Fedders *et al.*, 1998). It is also a cofactor in at least eight enzymatic reactions including several collagen synthesis reactions that cause the most severe symptoms of scurvy when they are dysfunctional (these reactions are especially important in wound-healing and in preventing bleeding from capillaries).

Ascorbate (an Ion of ascorbic acid) is required for a range of essential metabolic reactions in all animals and plants (Yaohui *et al.*, 2003). It is made internally by almost all organisms although notable mammalian group (excepting bats, guinea pigs, monkeys and human beings). Ascorbic acid is also not synthesized by some species of birds and fish. All species

that do not synthesize ascorbate require it in the diet. Deficiency in this vitamin causes the disease scurvy in humans. It is also widely used as a food additive (Christopher *et al.*, 2003). Scurvy has been known since ancient times. People in many parts of the world assumed it was caused by a lack of fresh plant foods. Ascorbic acid was finally isolated in 1932 and commercially "synthesized" (this included a fermentation step in bacteria) in 1934. The uses and recommended daily intake of vitamin C are matters of ongoing debate, with RDI ranging from 45 to 95 mg/day.

According to the USDA Nutrient Database for Standard Reference, the following foods are rich in vitamin C including citrus fruits, pawpaw, broccoli, and pepper. The recommended dietary allowance (RDA) for vitamin C is 75 mg/day for women and 90 mg/day for men (Alton, 1994).

- **Beta carotene**

  Beta carotene, also known as provitamin A, may help decrease the risk of developing cancer. According to the American Cancer Society, this nutrient may help prevent normal cells from becoming cancerous, although it's not yet clear if it can actually help prevent cancer in people (IPGRI, 1997).

  Good sources of beta carotene are dark green leafy and yellow-orange fruits and vegetables. In the body, beta carotene is converted to vitamin A. Eating foods rich in beta carotene is recommended to possibly decrease the risk of developing stomach, lung, prostate, breast, and head and neck cancer. However, according to Omenn *et al.*, (1996), more research is needed before a definite recommendation on beta carotene consumption can be made (Ichikawa *et al.*, 1980). Overdosing on beta carotene is not recommended. Large doses can cause the skin to turn a yellow-orange color, a condition called carotenosis. High intake of beta carotene in supplement form may actually cause lung cancer in people at risk, such as smokers.

  While there is a recommended dietary allowance for vitamin A (900 micrograms a day for men and 700 micrograms a day for women), there is not one for beta carotene. Examples of some foods high in beta carotene include carrots, spinach, squash, orange, sweet potatoes.

- **Vitamin E**

  Vitamin E is essential for our bodies to work properly. Vitamin E helps to build normal and red blood cells, as well as working as an antioxidant. Research is finding

evidence that vitamin E may protect against prostate and colorectal cancer. The recommended dietary allowance for vitamin E is 15 milligrams per day (Maundu, 1999). The adult upper limit for vitamin E is 1,000 milligrams per day. Good sources of vitamin E include sunflower oil, olive oil, and groundnut.

Some sources of vitamin E are high in fat. A synthetic form of a vitamin E is available as a supplement. According to Gissi *et al.,* (1999), vitamin E supplementation is probably not needed for most individuals because vitamin E is a fat-soluble vitamin and is stored in our bodies. Very high doses of vitamin E can also interfere with the way other fat-soluble vitamins work. Also, large doses of vitamin E from supplements are not recommended for people taking blood thinners and some other medications, as the vitamin can interfere with the action of the medication (Kabuye *et al*., 1999).

2.5 Antinutrients and toxic components

2.5.1 Oxalates

Oxalates are naturally-occurring substances found in plants, animals, and in humans. Work from Assimos *et al.,* (2000) revealed that in chemical terms, oxalates belong to a group of molecules called organic acids, and are routinely made by plants, animals, and humans. Our bodies always contain oxalates, and our cells routinely convert other substances into oxalates. For example, vitamin C is one of the substances that our cells routinely convert into oxalates. In addition to the oxalates that are made inside of our body, Holmes *et al*., (2000) revealed that oxalates can arrive at our body from the outside, from certain foods that contain those.

Foods that contain oxalates

The following are some examples of the most common sources of oxalates, arranged by food group (Endourol *et al.,* 1999). It is important to note that the leaves of a plant almost always contain higher oxalate levels than the roots, stems, and stalks.

- **Fruits:** blackberries, blueberries, raspberries, strawberries, currants, kiwifruit, concord (purple) grapes, figs, tangerines, and plums
- **Vegetables:** spinach, Swiss chard, beet greens, collards, okra, parsley, leeks and quinoa (are among the most oxalate-dense vegetables), celery, green beans, rutabagas, and summer squash would be considered moderately dense in oxalates (Stoller *et al.*, 1996).
- **Nuts and seeds:** almonds, cashews, and peanuts
- **Legumes:** soybeans, tofu and other soy products

- **Grains**: wheat bran, wheat germ, quinoa
- **Other:** cocoa, chocolate, and black tea (Parivar *et al.*, 1996)

Table 2: Oxalate levels in some foods

	LOW (Less than 5mg per serving)	**MEDIUM** (10 - 25mg per serving)	**HIGH** (26 - 99mg per serving)
Beverages	Water; Fruit juices (Apple, Lemon); Milk; Wine	Beer; Coffee; Fruit juice (Grape, Orange, Tomato)	Beer (lager draft); Chocolate milk; Cocoa; Tea (black)
Fruits	Avocado, Grapes; Mangoes; Watermelon	Apples; Apricots; black berries, Cherries; Pineapple	Blackberries, dried Figs, Kiwi; Peeled Lemon, Orange
Grains	Cornflakes (Kellog's); Egg noodles; white rice	Cooked barley, white bread, cooked macaroni, brown rice	Bread; Graham (crackers, flour); Wheat (bran, germ)
Condiments	Salt; Mustard (Dijon); Vinegar; Nutmeg (dry)	Cinnamon; Ginger; Pepper;Basil (fresh); Malt (powder)	Pepper (in excess of 1 tsp/day); Soy; Ginger (1 tsp);
Legumes,Nuts, and Seeds	Coconut ; Lentils	Sunflower seeds; Cashews ; Split peas	Green beans, Peanuts & butter; Sunflower seeds
Vegetables	White cabbage, Cauliflower; red pepper, Lettuce	Onions; Broccoli; Carrots; Cucumber;fresh tomato	Celery; Eggplant; Kale; Okra; Parsley; Pumpkin ; Spinach;
Meats	Beef; Chicken; Fish; Ham; Lamb ; Pork	Kidney (beef) ; Liver ; Sardines	

Source: Yount, 2005. Low Oxalate Cookbook, Pg 224. Graham, NC 27253.

Oxalates and health

Conditions that require strict oxalate restriction

Hanson *et al.*, (1989) revealed that there are a few, relatively rare health conditions that require strict oxalate restriction. These conditions include absorptive hypercalciuria type II, enteric hyperoxaluria, and primary hyperoxaluria. According to Low *et al.*, (1996), dietary oxalates are usually restricted to 50 milligrams per day under these circumstances (these

relatively rare health conditions are different than a more common condition called nephrolithiasis in which kidney stones are formed, 80% from calcium and oxalate). What does 50 milligrams of oxalate look like in terms of food? One cup of raw spinach in leaf form (not chopped) weighs about one ounce, and contains about 200 milligrams of oxalate, so 50 milligrams for the day would permit a person to consume only 1/4 cup of raw spinach (and no other oxalate sources could be eaten during the day).

Oxalates and kidney stones

Restriction of dietary oxalate helps prevent formation of calcium oxalate stones in individuals who have previously formed such stones. Since intake of dietary oxalate accounts for only 10-15% of the oxalate that is found in the urine of individuals who form calcium oxalate stones, many researchers believe that dietary restriction cannot significantly reduce risk of stone formation (Curhan, 1999; Frankos *et al*., 1989).

In addition to the above observation, recent research studies have shown that intake of protein, calcium, and water influences calcium oxalate affect stone formation as much as, or more than intake of oxalate. Finally, some foods that have traditionally been assumed to increase stone formation because of their oxalate content (like black tea) actually appear in more recent research to have a preventive effect (Mallin *et al*., 2003; Kelsay *et al*., 1988). For all of the above reasons, when healthcare providers recommend restriction of dietary oxalates to prevent calcium oxalate stone formation in individuals who have previously formed stones, they often suggest "limiting" or "reducing" oxalate intake rather than setting a specific milligram amount that should not be exceeded. "Reduce as much as can be tolerated" is another way that recommendations are often stated (Prather *et al*., 1983).

The effect of cooking on oxalates

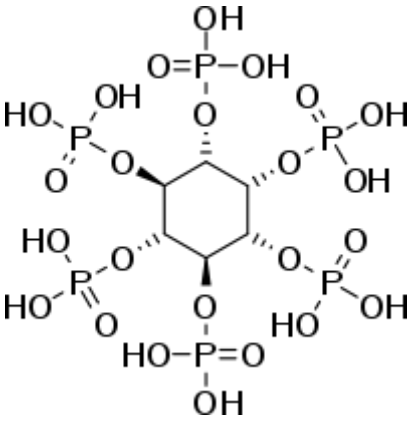
Cooking has a relatively small impact on the oxalate content of foods. According to Kikunaga *et al*., (1988), repeated food chemistry studies have shown no statistically significant lowering of oxalate content following the blanching or boiling of green leafy vegetables (Thompson *et al*., 1989). A lowering of oxalate content by about 5-15% is the most you should expect when cooking a high-oxalate food. It does not make sense to overcook oxalate-containing foods in order to reduce their oxalate content. Because many vitamins and minerals are lost from overcooking more quickly than are oxalates, the overcooking of foods (particularly

vegetables) will simply result in a far less nutritious diet that is minimally lower in oxalates (Takahashi *et al*., 1988).

According to researcher Susan *et al*., (1998), low oxalate vegetable options include kale, mustard greens and collard greens. Susan has found that boiling vegetables significantly reduces their oxalate content. For example, kale that has been steamed has an oxalate content of 8.8 mg, whereas the same amount of boiled kale contains 4.9 mg. That's a 40% reduction (Shiundu *et al.*, 2007).

2.5.2 Phytates

Stoller *et al*., (1996) observed that phytic acid is the principal storage form of phosphorus in many plant tissues, especially bran and seeds. Phytate is not digestible to humans or no ruminant animals. It makes unabsorbable certain important minor minerals (zinc and iron), and also macro minerals (calcium and magnesium).



Molecular Formula: $C_6H_{18}O_{24}P_6$

Molar mass: 660.04 g mol^{-1}

Figure 2: Structure of Phytate

Source: Guttieri *et al*., M. (2006).

Phosphorus in phytate form is, in general, not bioavailable to nonruminant animals because they lack the digestive enzyme phytase, which is required to separate phosphorus from the phytate molecule. On the other hand, ruminants readily use phytate because of the phytase produced by rumen microorganisms (Prakash *et al*., 1993).

The bioavailability of phytate phosphorus can be increased by supplementation of the diet with the enzyme phytase. Also, viable low-phytic acid mutant lines have been developed in several crop species in which the seeds have drastically reduced levels of phytic acid and concomitant increases in inorganic phosphorus. However, reported germination problems have hindered the use of these cultivars thus far (Guttieri *et al*., 2006).

The use of sprouted grains will reduce the quantity of phytic acids in feed, with no significant reduction of nutritional value. Work from Guttieri *et al*., (2006) revealed that phytates also have the potential to be used in soil remediation, to immobilize uranium, nickel and other inorganic contaminants.

Chemistry

Zahoor *et al*., (2010) revealed that phytic acid is found within the hulls of nuts, seeds, and grains. In-home food preparation techniques can reduce the phytic acid in all of these foods. Simply cooking the food will reduce the phytic acid to some degree. More effective methods are soaking in an acid medium, lactic acid fermentation, and sprouting (Pal *et al*., 1993).

Phytic acid has a strong binding affinity to important minerals, such as calcium (depending on pH), magnesium, iron (effect reduced by ascorbic acid), and zinc. When a mineral binds to phytic acid, it becomes insoluble, precipitates and will be no absorbable in the intestines. This process can therefore contribute to mineral deficiencies in people whose diets rely on these foods for their mineral intake. Contrary to that, Sienera (2006) revealed that one study correlated decreased osteoporosis risk with phytic acid consumption. It also acts as an acid, chelating the vitamin niacin, the deficiency of which is known as pellagra.

Therapeutic uses

Phytic acid may be considered a phytonutrient, providing an antioxidant effect. Phytic acid's mineral binding properties may also prevent colon cancer by reducing oxidative stress in the lumen of the intestinal tract. Researchers now believe phytic acid, found in the fiber of legumes and grains, is the major ingredient responsible for preventing colon cancer and other cancers (Alan *et al*., 2002).

In vitro studies using a cell culture model have suggested phytic acid may have a neuroprotective effect by chelating iron. Similar types of cell-culture studies have found

phytic acid significantly decreased apoptotic cell death induced by 1-methyl-4-phenylpyridinium. Phytic acid, at least in rodents, is known to cross the blood-brain barrier, and so, there is a strong possibility that neuroprotection occurs *in vivo* as well (Galen *et al*., 2002).

Phytic acid's chelating effect may serve to prevent, inhibit, or even cure some cancers by depriving those cells of the minerals (especially iron) they need to reproduce. The deprivation of essential minerals like iron would, much like other systemic treatments for cancers, also have negative effects on noncancerous cells.
A randomized, controlled trial in breast cancer patients showed no effect on chemotherapy-induced anemia or tumor markers, but the patients reported subjectively feeling better. Research from Ganzera *et al*., (2005) revealed that phytic acid is one of few chelating therapies used for uranium removal. As a food additive, phytic acid is used as a preservative, as E391.

Biological and physiological roles

In seeds and grains, phytic acid and its metabolites have several important roles. Most notably, phytic acid functions as a phosphorus store, as an energy store, as a source of cations and as a source of myoinositol (a cell wall precursor). Phytic acid is the principal storage forms of phosphorus in plant seeds (Larry *et al*., 2003).

In animal cells, myoinositol polyphosphates are ubiquitous, and phytic acid (myoinositol hexakisphosphate) is the most abundant, with its concentration ranging from 10 to 100 μM in mammalian cells, depending on cell type and developmental stage. The interaction of phytic acid with specific intracellular proteins has been investigated *in vitro*, and these interactions have been found to result in the inhibition or potentiation of the physiological activities of those proteins. The best evidence from these studies suggests an intracellular role for phytic acid as a cofactor in DNA repair by non homologous end-joining (Michael *et al*., 2003). Other studies using yeast mutants have also suggested intracellular phytic acid may be involved in mRNA export from the nucleus to the cytosol. There are still major gaps in the understanding of this molecule, and the exact pathways of phytic acid and lower inositol posphate metabolism are still unknown. As such, the exact physiological roles of intracellular phytic acid are still a matter of debate (Reddy *et al*., 2001).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study site

Slenderleaf accessions were collected from different geographical locations within Western Kenya. The study was carried out in Jomo Kenyatta University of Agriculture and Technology (JKUAT) between January 2011 and February 2012. JKUAT is in Juja, Central Kenya under the geographical coordinates 1^0 11' 0" S, 37^0 7' 0" E. The area is majorly covered by cambisols and vertisols, the average annual rainfall is 856 mm, and the mean annual temperature is 18.9^0C (Muchena *et al.,* 1978). The crops were grown in JKUAT farm (CRBD design with three replications and ten treatments - Accessions) and the analysis done in the Food Science laboratory in JKUAT.

3.2 Planting and Agronomic pratices

The plant was grown in 5 x 5 meter plots in an open field and poultry manure mixed with soil at a rate of 20 ton per hectare was applied to the plots. Seeds of each accession were selected, mixed with soil at the rate of 1:10 and drilled in the respective plots at a space of 30 cm between crops. After two weeks, thinning was done to leave an inter-row spacing of 15 cm to ensure optimum growing conditions. The seed germinated in 3–4 days. Harvesting was done by removing the leaves at three different growth stages: before (8 weeks after seedling germination), during (11 weeks after seedling germination) and after flowering (15 weeks after seedling germination).

3.3 Study design

This was a laboratory (CRD design with three replications and ten treatments) based study where samples of slenderleaf leaves at three growth stages, were harvested from the farm, and analyzed to determine the relevant nutritional component (Moisture, Ash and Protein, Vitamin C, vitamin E and β-carotene content), bioactive components (Flavonoids, Vitamin C, Vitamin E and β-carotene) and antinutrients (tannin, phytates and oxalates). The seeds were planted on thirty three plots with different accessions (Plate 5). Analyses were carried out in triplicate and results were presented in term of percentage (%) and/or mg/100g. The means were analyzed using GenStat, and were considered significantly different at Significance level of 5 %. The differences in means were analyzed using T-test.

Plate 5: Accessions of Slenderleaf in JKUAT farm

Initially thirty three (33) different accession seeds were collected from various places of the country. The 33 accession seeds were planted in the farm and they were harvested at the three various growth stages for the preliminary analysis. All the laboratory analysis was done on the 33 accessions. The means were compared and based on the growth characteristics; some accessions were presenting the same characteristics without significant difference. Those accessions presenting the same values were grouped in 10 major groups and in each of the 10 groups; the accession presenting the significantly higher value was picked. More research was carried out on the 10 selected accessions (Table 3).

Table 3: Accessions characteristics and classification

	Accessions	**Description**
1	1	*Crotalaria Ochroleuca* (thick and big pods, mild species)
2	2	*Crotalaria Ochroleuca* (thick and big pods, mild species)
3	8	*Crotalaria Ochroleuca* (thick and big pods, mild species)
4	11	*Crotalaria Ochroleuca* (thick and big pods, mild species)
5	14	*Crotalaria Ochroleuca* (thick and big pods, mild species)
6	18	*Crotalaria Ochroleuca* (thick and big pods, mild species)
7	19	*Crotalaria Brevidens* (slim and thin pods, bitter species)
8	20	*Crotalaria Ochroleuca* (thick and big pods, mild species)
9	25	*Crotalaria Ochroleuca* (thick and big pods, mild species)
10	33	*Crotalaria Ochroleuca* (thick and big pods, mild species)

3.4 Sample handling and preparation

Fresh leaves were harvested in the farm (Plate 6), brought to the laboratory, and destalked to separate leaves from the stems. The leaves were then weighed. Some of the leaves were dried at 105°C for an hour then ground into a fine powder using a mortar and pestle, to await analysis for ash, minerals and protein. The other fresh leaves were used within 24 hours (particularly for Vitamin C and beta-carotene).

.

Plate 6: Researcher collecting samples from JKUAT farm

3.5 Nutrition Composition

3.5.1 Proximate composition

Proximate analysis including moisture, protein and ash were determined according to AOAC methods specification 950.46 (AOAC, 1995).

Moisture determination

The moisture content was determined by oven drying method as per the AOAC method (AOAC, 1995). About 2g of fresh leaves of the sample was accurately weighed into a moisture dish and transferred to a hot-air-oven previously heated to temperatures of 105°C and drying done until constant weight was attained. The final weight of the sample was taken after cooling in a desiccator.

The model of hot-air-oven used to perform this analysis was the Adventec - Electric muffle furnaces (KL 420). For general purpose like drying the crucibles, the glassware, the moisture

dishes, a general oven was used. The model was MRK- convection type (Mitamura Riken Kogyo).

Protein determination

The protein was determined using the Kjedahl method. About 1g of the pre dried (at 105°C for an hour) sample was weighed into a digestion flask together with a catalyst composed of 5g of K_2SO_4 and 0.5g of $CUSO_4$ and 15 ml of concentrated H_2SO_4. The mixture was heated in a fume hood till the digest color turned blue. The digest was cooled, transferred to a volumetric flask and topped up with distilled water. 10 ml of diluted digest was transferred into the distilling flask and washed with about 2 ml distilled water. 15 ml of 40% NaOH was added and washed with about 2 ml distilled water. Distillation was done to a volume of about 60 ml and titrated using 0.02N-HCl. Appearance of an orange color signified the end point of the titration.

Ash determination

Ash was determined using the dry ashing method. A sample weight of between 2-5 g was measured in pre-conditioned crucibles. The samples were first charred by flame to eliminate smoking before being incinerated at 550°C in a muffle furnace to the point of white ash (about 18 hours).

The residues were cooled in desiccators and the weights taken. Analysis was performed using an Advantec – Electric muffle Furnaces (KL 420).

Determination of mineral composition

The total ash obtained was digested by concentrated nitric acid and perchloric acid (1:1v/v). Calcium was determined using emission flame photometer, while iron and zinc were determined using atomic absorption spectrophotometer using standard methods (AOAC, 1995, Method 950.46-32.1.30).

Analysis was performed using a Shimadzu AA 6200 atomic absorption flame (AAS) – emission spectrophotometer. Shimadzu AA 630-12 atomic absorption flame (AAS).

3.6 Bioactive components

3.6.1 Vitamin determination

Vitamin C

The ascorbic acid content was determined by 2,6-dichlorophenolindophenol (DCPI) method (AOAC method). A 5 g of fresh slenderleaf leaves was ground using a mortar and pestle with acid washed sand and 10 ml of TCA reagent. The content was transferred into a flask and topped up with TCA. The extract was mixed well and filtered immediately. Then 10 ml was titrated with indophenol solution until pink color appeared. For the blank, 10 ml of water was used and titrated with indophenols until rose pink color appeared.

β-carotene and total carotenoids

β-carotene and total carotenoids were determined as described by Rodriguez *et al.*, (2002). A sample of 5 g of fresh leaves was mixed with 3 g of hyflosupercel and 50 ml of acetone, ground and filtered. Then 25 ml of petroleum ether was added in a separatory funnel and a small portion of acetone extract was then added. Distilled water was slowly added, and the lower acetone phase discarded. Butylated hydroxytoluene and an equal volume of potassium hydroxide in methanol were added to the carotenoids solution in petroleum ether in the dark at room temperature. The mixture was washed with distilled water to remove the potassium hydroxide using a separatory funnel. The carotenoid phase was collected and dried with sodium sulphate.

The total carotenoid content of the extract was determined by measuring the absorbance in a spectrophotometer using the extinction coefficient. β-carotene was read at 440 ŋm while total carotenoids was read at 450 ŋm (Bhaskarachary *et al.*, 1995).

The equipment used for the analysis was Shimadzu UV–160 1 PC UV–visible spectrophotometer.

3.6.2 Flavonoid determination

Qualitative analysis was carried out to ascertain the presence of the different phytochemicals before quantitative analysis was done. Phytochemicals were also identified on thin layer chromatography (TLC) using reference standards and the concentrations were determined with the help of a spectrophotometer. Consequently, a high-performance liquid chromatography (HPLC) was used for quantitative determination of bioactive compounds in extracts.

Plant tissues were lyophilized after harvest and ground to a homogeneous powder. Samples (0.5 g) of lyophilized whole leaf were extracted in 125 ml round bottom flasks by steeping in 25 ml of chloroform for 30 min. The extract was filtered and the filtrate set aside. The

extraction flask and filtered solids were rinsed with an additional 15 ml of fresh chloroform. The filtrate from the rinse was then combined with the original filtrate and the resulting solution evaporated to dryness with a rotary evaporator. The dry solids were redisolved in a mixture of 20.0 ml methanol and 5.0 ml acetone using sonication to assist in dissolving of all solid material.

Five milliliters (5 ml) of dilute ammonia solution was added to a portion of the aqueous filtrate of the extract followed by addition of concentrated H_2S0_4. A yellow coloration observed indicated the presence of flavonoids. The yellow coloration disappeared on standing. Quantity was determined according to the method of Harborne (1998) where 5 g of the sample was boiled in 50 ml of 2 M HCl solution for 30min under reflux. It was then allowed to cool and filtered through Whatman No 42 filter paper. A measured volume of the extract was treated with equal volume of ethyl acetate starting with drop. The flavonoid precipitated was recovered by filtration using weighed filter paper. The resulting weight difference gave the weight of flavonoid in the sample.

The model of equipment used for the analysis was HPLC Shimadzu LC 10AS, the liquid chromatograph LC 10AS, the auto injector SIL 10A, the refractive index detector RID 6A, the system controller SCL 10A, the UV-vis detector SPD 10AV, the colum oven CTO 10A and the chromatopac C-R7A plus.

3.6.3 Vitamin E determination

Vitamin E was used as a measure of antioxidant activity. About 20 g of dried vegetable pieces was homogenized with 100 ml of distilled water using a Moulinex blender, boiled for 3 min and allowed to stand at room temperature for 24 h before filtration. Filtrates were diluted to produce a 200 mgl^{-1} of extract needed for the antioxidant assays**.**

The antioxidant activity was assayed in linoleic acid model system using the ferric thiocyanate method as described by Kikuzaki *et al.,* (1993) and modified by Odukoya *et al*., (2005a-c). The α-Tocopherol (Sigma) was used as standard antioxidant while a blank of distilled water was run with each assay. The radical-scavenging activity was determined using diphenyl picryl hydrazyl radical (DPPH) according to Ayoola *et al.,* (2006).

For the standard curve, the following concentrations of the extracts were prepared, 0.05, 0.1, 0.5, 1.0, 2.0 and 5 mg/ml in methanol in cuvettes placed in a spectrophotometer (Analar grade). Vitamin C was used as the antioxidant standard at concentrations of 0.02, 0.05, 0.1, 0.2, 0.5 and 0.75 mg/ml. Linear regression analysis was used to predict concentrations of the unknown.

One ml of the extract was placed in a test tube, and 3 ml of methanol added followed by 0.5 ml of 1 mM DPPH in methanol. The mixture was shaken vigorously and left to stand for 5 min. A blank solution was prepared containing the same amount of methanol and DPPH. The absorbance of the resulting solution was measured at 517 nm with a spectrophotometer. The radical scavenging activity was calculated and the activity of each extract was calculated as %inhibition of lipid peroxidation.
The equipment used was Shimadzu UV – 160 1 PC UV – visible spectrophotometer.

3.7 Antinutrients

3.7.1 Tannins determination

About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were then added. A brownish green or a blue-black coloration indicated presence of tannins. This tannin qualitative test was determined by the Folis-Denis colorimetric method described by Kirk *et al.,* (1998).

The quantitative determination of the tannins was done according to the Vanillin-hydrochloric acid method (Burns, 1963; Price *et al*., 1978). To 0.25 g of sample, 10 ml HCl in methanol was added. 5 ml of HCl in methanol was added to the residue from the first extraction and the extraction process was repeated. The aliquots of the first and second extracts were combined and made up to 25 ml volume. To 1 ml of standard and sample extract, 5 ml of Vanillin-HCl reagent was added.

Five grams of the sample was dispersed in 50 ml of distilled water and shaken. The mixture was allowed to stand for 30 min at 28^{o}C before it was filtered through whatman No. 42 filter paper. Two milliliters of the extract was dispersed into a 50 ml volumetric flask. Similarly 2 ml standard tannin solution (tannic acid) and 2 ml of distilled water were put in separate volumetric flasks to serve as standard and reagent was added to each of the flask and the 2.5 ml of saturated Na_2C0_3 solution was added. The content of each flask was made up to 50 ml

with distilled water and allowed to incubate at 28^{o}C for 90 min. Their respective absorbance (standard solutions, sample extracts and blanks) was measured in a UV–VIS spectrophotometer at 260 nm using the reagent blank to calibrate the instrument at zero.

A standard curve of absorbance against catechin concentration was prepared from the readings of the catechin standard solutions. The blank absorbances were subtracted from the samples absorbances and the corrected absorbance used into the regression equation to determine the concentration of the sample extracts.
The Shimadzu UV – 160 1 PC UV – visible spectrophotometer was used for that analysis.

3.7.2 Phytate determination

HPLC analysis of phytic acid according to Camire *et al.,* (1982) was used. Fifty mg of sample was weighed and 10 ml of 3% H_2SO_4 added. The mixture was shacken and filtered. The filtrate was transferred to a boiling water bath (BWB) and 3 ml of $FeCl_3$ solution added. Centrifugation was then done and the supernatant discarded. The precipitate was washed with distilled water, centrifuged and the supernatant discarded. A 3 ml portion of 1.5N NaOH was added to the residues and the volume brought to 30 ml with distilled water. Cooled samples were centrifuged and the supernatant transferred. The precipitate was rinsed with distilled water, centrifuged and the supernatant taken. Samples of 20 nµl of the supernatant were injected into a HPLC.

The analysis was done using a HPLC model Shimadzu LC 10AS, the liquid chromatograph LC 10AS, the auto injector SIL 10A, the refractive index detector RID 6A, the system controller SCL 10A, the UV-vis detector SPD 10AV, the colum oven CTO 10A and the chromatopac C-R7A plus.

3.7.3 Oxalate determination

Atomic absorption spectrophotometer using a calcium hollow cathode lamp was used (AOAC, 974.24). After sample preparation, 25 ml filtrate was pipetted, 5 ml tungstophosphoric acid reagent added, mixed, left to stand for more than five hours, then filtered. A portion of 20 ml filtrate was pipetted, ammonia solution added drop wise to pH 4.0-4.5, and 5 ml acetate buffer solution added. The solution was stirred, left to stand

overnight and centrifuged to compact the precipitate and the supernatant decanted. The precipitate was broken into fine suspension with jet of cold calcium oxalate wash liquid, the centrifugation repeated and the precipitate drained completely.

The precipitate and 5 ml of sulphuric acid was diluted and labeled solution 1. Solution 1 (2 ml) was pipetted into 10 ml of lanthanum solution, diluted with water and labeled solution 2. 15 ml of solution 2 was transferred into 2 ml lanthanum solution and diluted with water.

The absorption of the sample was determined in an atomic absorption spectrophotometer using a calcium hollow cathode lamp. The model of equipment used was Shimadzu AA 6200 atomic absorption flame (AAS) – emission spectrophotometer. Shimadzu AA 630-12 atomic absorption flame (AAS).

All tests were run in triplicate, and analyses of all samples run in duplicate and averaged.

3.8 Data quality, Data Analysis

Each sample analysis was carried out in triplicate. Relevant standards were used in the determination of the various parameters (minerals, vitamin C, β-carotene and total carotenoids).

Descriptive statistics were used to describe the results of the nutrient composition. Data obtained was subjected to analysis of variance (ANOVA) and treatment means that differed significantly ($p \leq 0.05$) were separated by the Least Significant Difference (LSD).

CHAPTER FOUR
RESULTS & DISCUSSIONS

4.1 Nutrients Composition

4.1.1 Proximate composition

4.1.1.1 – Moisture content

Moisture content for all the accessions before, during and after flowering varied significantly ($p \leq 0.05$) as shown in Table 4. The moisture content varied from 82.68 to 90.17 % before flowering; from 69.42 to 77.32 % during flowering and between 55.40 to 65.52 % after flowering. The mean of all the moisture values before flowering was of 86.52 %, during flowering it was of 73.98 % and after flowering it was of 59.47 %. The means were significantly ($p \leq 0.05$) different. We noticed that the significantly ($p \leq 0.05$) highest value of moisture content was observed before flowering and the significantly ($p \leq 0.05$) lowest value observed after flowering. This was due to the growth stage and we can then say that there was a decrease of moisture as the vegetable matured.

For the accessions, the means were between 70.68 % and 76.12%. According to the Table 4, accessions 1, 2, 11, 14, 19, 20 and 33 were not significantly ($p \leq 0.05$) different from each other, but they were from accessions 8 and 18. There was also significant difference ($p \leq 0.05$) in moisture among the various accessions. Accession 18 had significantly ($p \leq 0.05$) highest moisture while accession 1 was the one that showed the significantly ($p \leq 0.05$) lowest value.
There was no significant ($p \leq 0.05$) interaction between stage of growth and the accessions. The moisture content in the accessions was not linked with stage of growth. The Moisture content was not positively correlated with stage of growth of the samples.

The body cannot function properly without enough water (60% of the body weight). Water helps transport oxygen, fat and glucose to your working muscles, regulate your body temperature, digest food and eliminate waste products (Dr William, 2005). Fruits and vegetables contain large quantities of water in proportion to their weight, which are absorbed by the body when these foods are eaten (Sandra Bastin, 1994).

Table 4: Effect of stage of harvesting on Moisture Content of Slenderleaf accessions.

Accession	Moisture (%) Stage of growth			Mean (accession)
	BF	**DF**	**AF**	
1	86.08 ± 1.1	69.42 ± 0.6	56.54 ± 0.4	**70.68**[a]
2	85.30 ± 1.3	73.53 ± 1.5	56.91 ± 1.1	**71.91**[ab]
8	89.40 ± 0.3	77.10 ± 0.1	58.32 ± 0.3	**74.94**[c]
11	88.10 ± 0.7	72.68 ± 0.2	55.40 ± 0.4	**72.06**[ab]
14	84.60 ± 4.0	77.32 ± 0.2	58.86 ± 0.4	**73.59**[b]
18	90.17 ± 0.6	75.38 ± 0.2	62.81 ± 0.5	**76.12**[d]
19	87.93 ± 1.1	72.50 ± 0.5	56.91 ± 0.3	**72.45**[b]
20	88.17 ± 0.4	74.46 ± 0.5	57.96 ± ≤0.1	**73.53**[b]
25	82.77 ± 0.1	73.90 ± 0.5	65.52 ± 0.5	**74.06**[c]
33	82.68 ± 2.0	73.55 ± 1.1	65.46 ± 0.4	**73.89**[bc]
Mean	**86.52**[c]	**73.98**[b]	**59.47**[a]	
LSD (5%)	0.51	0.49	0.52	

Value= Mean ± S.D on fresh weight basis. Each value is a mean of 3 replicates. Means followed by the same letter are not significantly different (p≤5%).

BF= Before Flowering; DF= During Flowering; AF= After Flowering

There was a significant ($p \leq 0.05$) reduction on the concentration of all the moisture during maturity; which is likely to affect the nutritional value of the vegetable. However, moisture content was within the range and similar to those reported by Abukutsa-Onyango (2004) in *Crotalaria brevidens* in respect to the reducing moisture content with maturity. The loss of Moisture content with maturity was quite high, which may be due to the effect of the quality of the soil and the light.

As each crop grows toward harvest, it is important to allow the crop to attain its optimal moisture content. This will make for the best form, color, and taste. Low moisture can leave vegetables bitter while high moisure content can leave vegetables tasteless (Aman, 2005).

4.1.1.2 – Ash content

Ash content for all the accessions before, during and after flowering varied significantly ($p \leq 0.05$) as shown in Table 5. The ash content varied from 4.98 to 10.21 % before flowering; from 2.78 to 8.76 % during flowering and between 2.43 to 18.52 % after flowering. The mean of all the ash values before flowering was of 7.59 %, during flowering it was of 7.16 % and after flowering it was of 9.66 %. The means were significantly ($p \leq 0.05$) different. We noticed that the significantly ($p \leq 0.05$) highest value of ash content was observed after flowering and the significantly ($p \leq 0.05$) lowest value observed during flowering. The mean Ash Content was significantly higher ($p \leq 0.05$) in the samples after flowering than before flowering.

For the accessions, the means were between 6.18 % and 10.99%. According to the Table 5, accession 14 had significantly higher ($p \leq 0.05$) ash content than the other accessions (10.99%) while Accession 11 had significantly lower ($p \leq 0.05$) ash content than the other accessions. All the accessions were all significantly ($p \leq 0.05$) different from each other. There was also significant difference ($p \leq 0.05$) in ash among the various accessions.

There was no significant ($p \leq 0.05$) interaction between stage of growth and the accession. The ash content in the accessions was not linked with stage of growth.

The amount of ash represents the amount of mineral matter in the vegetable. It is a measure of the total amount of minerals present within a food, whereas the mineral content is a measure of the amount of specific inorganic components present within a food (Bonnie, 1999). The quality of many foods depends on the concentration and type of minerals they contain, including their taste, appearance, texture and stability.

It is often important to know the mineral content of foods during processing because this affects the physicochemical properties of foods (Shrimpton, 1993). Although minerals represent a small proportion of dry matter (often less than 7%), they play an important role from a physicochemical, technological and nutritional point of view. It is part of nutritional evaluation and the first step in preparing a food sample for determination of specific elemental analysis (Mark, 1993).

The results of mean ash content appear to be higher than the 1.6 g per 100 g reported by Maundu (1999). However, these results were within the normal range for ash content in leafy vegetables (Andzouana, 2012). The variation in the ash content may be attributed to external non controlled factors (quality of the soil, water, period of harvest and place) that can affect the plant (Ayaz, 2006).

Moisture content has been established as an important indicator to a food's nutrition and longevity (Gupta, 2011). It is one of the most important characteristics in consumer sensory perception of food. Change in moisture content will dramatically affect flavor and texture as well as physical and chemical properties because of water activity. The presence of free moisture is directly related to water activity (Emebu, 2011).

Table 5: Effect of stage of harvesting on Ash Content of Slenderleaf accessions.

Ash (%)				
Accession	**Stage of growth**			**Mean (accession)**
	BF	**DF**	**AF**	
1	10.21 ± 0.3	8.57 ± 0.1	5.38 ± 0.4	**8.05^{c}**
2	8.58 ± 0.6	6.33 ± 1.2	9.73 ± 0.2	**8.21^{c}**
8	8.62 ± 0.1	8.53 ± 0.5	14.9 ± 0.1	**10.68^{d}**
11	7.48 ± 0.1	8.62 ± 0.4	2.43 ± 0.6	**6.18^{a}**
14	9.26 ± 0.2	8.76 ± 0.9	14.95 ± 0.1	**10.99^{d}**
18	4.98 ± 0.4	8.06 ± 0.8	10.16 ± 0.4	**7.73^{b}**
19	5.83 ± 0.8	3.16 ± 0.7	18.52 ± 1.3	**9.17^{c}**
20	7.86 ± 0.3	8.68 ± 0.3	13.17 ± 1.7	**9.90^{c}**
25	7.63 ± 0.4	8.15 ± 0.9	2.49 ± 0.1	**6.09^{b}**
33	5.54 ± 0.5	2.78 ± 0.1	4.91 ± 1.1	**4.41^{a}**
Mean	**7.59^{b}**	**7.16^{a}**	**9.66^{c}**	
LSD (5%)	0.16	0.34	0.42	

Value= Mean ± S.D on fresh weight basis. Each value is a mean of 3 replicates. Means followed by the same letter are not significantly different (p≤5%).

BF= Before Flowering; DF= During Flowering; AF= After Flowering

.4.1.1.3 – Protein content

Proteins content for all the accessions before, during and after flowering varied significantly ($p \leq 0.05$) as shown in Table 6. The protein content varied from 2.54 to 3.38 % before flowering; from 3.01 to 3.69 % during flowering and between 1.23 to 1.97 % after flowering. The mean of all the proteins values before flowering was of 3.02 %, during flowering it was of 3.31 % and after flowering it was of 1.61 %. The means were significantly ($p \leq 0.05$) different. We noticed that the significantly ($p \leq 0.05$) highest value of protein content was observed during flowering and the significantly ($p \leq 0.05$) lowest value observed after flowering. There was a decrease of protein as the vegetable matured. It was during flowering stage that the mean protein content (3.31%) was significantly higher ($p \leq 0.05$) than before (3.02%) or after flowering (1.61%).

For the accessions, the means were between 2.31 % and 3.01 %. According to the Table 6, accession 20 had significantly higher ($p \leq 0.05$) protein content than the other accessions (3.01 %) while accession 1 had significantly lower ($p \leq 0.05$) protein content than the other accessions. All the accessions were all significantly ($p \leq 0.05$) different from each other. There was also significant difference ($p \leq 0.05$) in proteins among the various accessions.

There was no significant ($p \leq 0.05$) interaction between stage of growth and the accession. The ash content in the accessions was not linked with stage of growth.

Although protein is certainly an essential nutrient which plays many key roles in the way our bodies function, we do not need huge quantities of it (Rodriguez, 2009). Only about one calorie out of every 10 we take in needs to come from protein. Protein is made up of amino acids (building blocks) and we need all nine of these amino acids for our body to make protein (Mariga, 2012).

It is crucial to make sure your body gets enough protein to stay strong. Protein functions to build and maintain your body, fight off disease, keep energy levels high to help stay alert all day (Nnamani, 2009). Since vegetables are a great source of nutrients with high levels of vitamins and minerals, it's important to include them in your diet, just not as your main protein source.

Table 6: Effect of stage of harvesting on Protein Content of Slenderleaf accessions.

	Protein (%)			
Accession	**Stage of growth**			**Mean (accession)**
	BF	**DF**	**AF**	
1	2.54 ± 0.1	3.01 ± 0.1	1.37 ± 6.8	**2.31[a]**
2	2.89 ± 0.9	3.20 ± 2.1	1.67 ± 1.9	**2.58[b]**
8	2.92 ± 0.1	3.21 ± 1.8	1.73 ± 3.3	**2.62[b]**
11	3.11 ± 0.7	3.32 ± 0.8	1.60 ± 5.0	**2.67[b]**
14	2.96 ± 0.2	3.21 ± 2.1	1.57 ± 3.3	**2.58[b]**
18	2.92 ± 0.5	3.24 ± 1.3	1.73 ± 0.7	**2.63[b]**
19	3.33 ± 0.3	3.63 ± 1.8	1.67 ± 0.7	**2.87[c]**
20	3.38 ± 0.3	3.69 ± ≤0.1	1.97 ± 3.3	**3.01[d]**
25	3.12 ± ≤0.1	3.26 ± 0.1	1.53 ± 0.3	**2.64[b]**
33	3.02 ± 0.4	3.31 ± 0.7	1.23 ± 1.3	**2.52[b]**
Mean	**3.02[b]**	**3.31[c]**	**1.61[a]**	
LSD (5%)	0.35	0.26	0.30	

Value= Mean ± S.D on fresh weight basis. Each value is a mean of 3 replicates. Means followed by the same letter are not significantly different (p≤5%).

BF= Before Flowering; DF= During Flowering; AF= After Flowering

The results indicate that as the slenderleaf matures, protein content increases till the flowering stage, after which it then starts reducing. These protein levels were much lower than those reported by Makobo *et al.*, (2010) of 43 – 50 %; and 38.5 % reported by Oliverian and De Carvalho (1975). These results of protein content were, however, consistent with the reported data of Mibei (2011) in ALV. This could account for 6 – 10 % of the daily protein requierement. Slenderleaf have been reported to be amoung the ALVs an excellent protein source. The increase of protein content during flowering stage can be due to the increase in dry matter content.

Most vegetables protein are considered to be incomplete, It is therefore important to add high protein foods (meat, dairy, or beans) in your daily diet (Acikgoz, 2011).

4.1.2 Mineral content of slenderleaf accessions

4.1.2.1 Iron content

Iron content for all the accessions before, during and after flowering varied significantly ($p \leq 0.05$) as shown in Table 7. The iron content varied from 8.07 to 13.93 mg/100g before flowering; from 12.82 to 16.10 mg/100g during flowering and between 9.42 to 13.62 mg/100g after flowering. The mean of all the iron values before flowering was of 10.80 mg/100g, during flowering it was of 14.45 mg/100g and after flowering it was of 11.32 mg/100g. The means were significantly ($p \leq 0.05$) different. We noticed that the significantly ($p \leq 0.05$) highest value of iron content was observed during flowering and the significantly ($p \leq 0.05$) lowest value observed before flowering. There was a decrease of iron as the vegetable matured. The mean Iron Content was significantly ($p \leq 0.05$) higher in the samples during flowering than after and before flowering.

For the accessions, the means were between 10.88 mg/100g and 14.03 mg/100g. According to the Table 7, accessions 1, 2, 14 and 33 were not significantly ($p \leq 0.05$) different from each other, but they were from accessions 11, 18 and 19. There was also significant difference ($p \leq 0.05$) in iron among the various accessions. Accession 19 had significantly ($p \leq 0.05$) highest moisture while accession 2 was the one that showed the significantly ($p \leq 0.05$) lowest value.

There was no significant ($p \leq 0.05$) interaction between stage of growth and the accession. The iron content in the accessions was not linked with stage of growth. The iron intake of infants is of particular interest because iron deficiency anaemia has been associated with a number of adverse effects in infants and young children, including impaired motor and mental development (Andraca *et al*., 1997).

Iron is needed by infants for the proper growth and formation of healthy blood cells and prevention of iron-deficiency anemia. This mineral is a vital component of hemoglobin, the part of red blood cells that carries oxygen throughout the body; myoglobin, the part of muscle cells that stores oxygen; and many enzymes in the body. A child's growth and development depends on iron, and studies show that inadequate iron intake can have long-term consequences on learning, attention span and behavior (Krebs *et al*., 2006). Most babies are born with enough iron stores for the first 6 months of life; after 6 months, infants need a diet rich in iron to meet their needs.

The results for iron content in the slenderleaf were similar to the results obtained by Shackleton (2009) for iron content in African indigeneous leafy vegetables, who also observed a peak of iron content in the vegetables at flowering stage, after which the iron content decreased. These results were, however, generally higher than those reported by Habwe (2009), (13.6 ± 0.52 mg/100g) in African leafy vegetables.

Iron, one of the most abundant metals on Earth, is essential to most life forms and to normal human physiology (Byaruhanga, 2001). Iron is an integral part of many proteins and enzymes that maintain good health. In humans, iron is an essential component of proteins involved in oxygen transport (Ferguson, 1997. It is also essential for the regulation of cell growth and differentiation. The body daily iron requirement (18 mg/day) intake for adult could be provided by an adequate intake of vegetable (Tietz *et al.,* 1994).

Table 7: Effect of stage of harvesting on Iron Content of Slenderleaf accessions.

	Iron (mg/100g)			
Accession	**Stage of growth**			**Mean (accession)**
	BF	**DF**	**AF**	
1	9.90 ± 0.3	13.91 ± 0.3	9.66 ± 0.6	**11.16^{a}**
2	10.40 ± 1.4	12.82 ± 0.2	9.42 ± 0.4	**10.88^{a}**
8	13.93 ± 0.7	15.97 ± 1.1	10.13 ± 0.2	**13.34^{bc}**
11	12.35 ± 0.4	13.22 ± 0.1	11.39 ± 0.5	**12.32^{b}**
14	10.70 ± 2.0	14.60 ± 0.5	10.37 ± 0.6	**11.89^{a}**
18	8.96 ± 0.6	16.04 ± 2.7	13.62 ± 0.9	**12.87^{b}**
19	12.79 ± 0.2	16.10 ± 1.4	13.20 ± 0.5	**14.03^{c}**
20	10.67 ± 1.4	12.93 ± 1.1	12.71 ± 1.0	**12.10^{ab}**
25	8.07 ± 0.8	15.73 ± 0.7	12.47 ± 0.2	**12.09^{ab}**
33	10.21 ± 0.5	13.18 ± 0.3	10.23 ± 0.8	**11.21^{a}**
Mean	10.80^{a}	14.45^{c}	11.32^{b}	
LSD (5%)	**2.5**	**1.26**	**2.30**	

Value= Mean ± S.D on fresh weight basis. Each value is a mean of 3 replicates. Means followed by the same letter are not significantly different (p≤5%).

BF= Before Flowering; DF= During Flowering; AF= After Flowering

4.1.2.2 Zinc content

Zinc content for all the accessions before, during and after flowering varied significantly ($p \leq 0.05$) as shown in Table 8. The zinc content varied from 2.98 mg/100g to 3.9 mg/100g before flowering; from 3.51 to 4.18 mg/100g during flowering and between 1.86 to 7.4 mg/100g after flowering. The mean of all the zinc values before flowering was of 3.50 mg/100g, during flowering it was of 3.88 mg/100g and after flowering it was of 2.84 mg/100g. The means were significantly ($p \leq 0.05$) different. We noticed that the significantly ($p \leq 0.05$) highest value of zinc content was observed during flowering and the significantly ($p \leq 0.05$) lowest value observed before flowering. There was a decrease of zinc as the vegetable matured. The mean zinc content was significantly ($p \leq 0.05$) higher in the samples during flowering than after and before flowering.

For the accessions, the means were between 3.01 mg/100g and 5.03 mg/100g. According to the Table 8, accession 25 had significantly higher ($p \leq 0.05$) zinc content than the other accessions (5.03 mg/100g) while accession 11 had significantly lower ($p \leq 0.05$) zinc content than the other accessions. All the accessions were all significantly ($p \leq 0.05$) different from each other. There was also significant difference ($p \leq 0.05$) in zinc among the various accessions.

There was no significant ($p \leq 0.05$) interaction between stage of growth and the accession. The zinc content in the accessions was not linked with stage of growth. Zinc is an essential mineral that is involved in numerous aspects of cellular metabolism, growth, development and is a component of many enzymes in the body (Kelsay, 1983).. It is required for the catalytic activity of approximately 100 enzymes and it plays a role in immune function, protein synthesis, DNA synthesis, growth, appetite, sperm production, cell division, supports normal growth and development during pregnancy, childhood, and adolescence and is required for proper sense of taste and smell. It helps the body break down carbohydrates, fats and proteins so they can be used for energy (Prakash, 1993). Zinc boosts immunity and also helps the body heal wounds and maintain normal blood glucose levels. Research suggests that it also has a role in improving recall skills, reasoning and attention (Krebs *et al.*, 2006). The zinc content of breast milk gradually decreases over time, so it's important to introduce foods rich in zinc when infants progress to solid foods.

The general trend of the different accessions showed that the zinc content significantly ($p \leq 0.05$) increased from before flowering to during flowering and then significantly ($p \leq 0.05$) dropped after flowering. These results are similar to those reported by Shackleton (2009) in African Indigenous Vegetables.

Zinc is normally required in the body in trace amount and is involved in the normal functioning of the immune system (Ferguson, 1997). One thing that can assist therefore to prevent harmful effects of zinc deficiency (growth retardation) could be a regular consumption of Slenderleaf. A daily intake of zinc is required to maintain a steady state because the body has no specialized zinc storage system (Ferguson, 1997).

Table 8: Effect of stage of harvesting on Zinc Content of of Slenderleaf accessions.

Zinc (mg/100g)				
Accession	**Stage of growth**			**Mean (accession)**
	BF	**DF**	**AF**	
1	3.27 ± 1.6	3.73 ± 0.4	2.35 ± 3.1	**3.12^{b}**
2	39 ± 4.9	3.78 ± 0.2	2.56 ± 1.9	**3.41^{c}**
8	39 ± 2.0	3.79 ± 2.8	2.24 ± 0.5	**3.31^{c}**
11	3.67 ± 3.1	3.51 ± 2.5	1.86 ± 0.2	**3.01^{b}**
14	2.99 ± 0.2	3.91 ± 1.4	2.36 ± 1.1	**3.08^{b}**
18	3.85 ± 3.2	3.87 ± 1.7	2.39 ± 3.8	**3.37^{c}**
19	3.65 ± 3.9	4.18 ± 0.6	2.45 ± 2.6	**3.43^{c}**
20	2.98 ± 1.1	3.87 ± 0.3	2.84 ± 0.9	**3.23^{b}**
25	3.36 ± 0.6	4.33 ± 1.7	7.40 ± 0.5	**5.03^{a}**
33	3.47 ± 0.2	3.78 ± 0.4	1.98 ± ≤0.1	**3.07^{b}**
Mean	**3.50^{b}**	**3.88^{c}**	**284^{a}**	
LSD (5%)	0.35	0.26	0.30	

Value= Mean ± S.D on fresh weight basis. Each value is a mean of 3 replicates. Means followed by the same letter are not significantly different (p≤5%).

BF= Before Flowering; DF= During Flowering; AF= After Flowering

4.1.2.3 – Calcium content

Calcium content for all the accessions before, during and after flowering varied significantly ($p \leq 0.05$) as shown in Table 9. The calcium content varied from 2.14 mg/100g to 18.43 mg/100g before flowering; from 8.93 to 9.73 mg/100g during flowering and between 3.34 to 3.77 mg/100g after flowering. The mean of all the calcium values before flowering was of 9.57 mg/100g, during flowering it was of 9.32 mg/100g and after flowering it was of 3.56 mg/100g. The means were significantly ($p \leq 0.05$) different. We noticed that the significantly ($p \leq 0.05$) highest value of calcium content was observed before flowering and the significantly ($p \leq 0.05$) lowest value observed after flowering. There was a decrease of calcium as the vegetable matured.

For the accessions, the means were between 5.11 mg/100g and 10.46 mg/100g. According to the Table 9, accessions 1, 2, 18 and 25 were not significantly ($p \leq 0.05$) different from each other, but they were from accessions 33, 20, 19, 14 and 8. There was also significant difference ($p \leq 0.05$) in calcium among the various accessions. Accession 14 had significantly ($p \leq 0.05$) highest calcium while accession 8 and 19 were the ones that showed the significantly ($p \leq 0.05$) lowest value.

There was no significant ($p \leq 0.05$) interaction between stage of growth and the accession. The zinc content in the accessions was not linked with stage of growth. Calcium plays an important role in bone and tooth development, blood clotting and maintenance of healthy nerves and muscles.

Calcium is necessary for the growth and maintenance of strong teeth and bones, nerve signaling, muscle contraction, and secretion of certain hormones, prevents blood from clotting and enzymes (Yamaguchi, 2003). A deficiency in calcium can lead to numbness in fingers and toes, muscle cramps, convulsions, lethargy, loss of appetite, and abnormal heart rhythm. Finding calcium in vegetables is a concern. While there is some evidence that oxalates in vegetables can hinder calcium absorption, they are still a good source of calcium, and the calculated daily value (DV) already takes into account absorption and bio-availability (McDowell, 1989).

The general trend is that the calcium content increased as the slenderleaf matured, till the flowering stage where it peaked, and then it started decreasing.

These results of calcium content in slenderleaf are in agreement to the observations of Leung (1986) about the calcium content in kales. The big difference between the stages of maturity might be attributed to the increase in the dry matter.

The calcium recommendation for adults age 19-50 years and men 51-70 years is 1000 mg per day. An intake of 1200 mg of calcium is recommended for women over 51 years and for men over 70 (Shills and Young, 1998; Ishida *et al.*, 2000). Protein's effect on calcium needs and bones remains uncertain (Yamaguchi, 2003).

Table 9: Effect of stage of harvesting on Calcium Content of Slenderleaf accessions.

Calcium (mg/100g)				
Accession	**Stage of growth**			**Mean (accession)**
	BF	**DF**	**AF**	
1	10.23 ±0.2	9.24 ± 0.3	3.41 ± 0.1	**7.63**[a]
2	12.50 ± 0.1	8.97 ± 0.7	3.34 ± ≤0.1	**8.27**[a]
8	2.58 ± 0.1	8.99 ± 0.1	3.75 ± 0.1	**5.11**[bc]
11	2.45 ± 0.3	9.73 ± 0.9	3.77 ± 0.1	**5.31**[c]
14	17.74 ± 0.2	9.42 ± 5.7	3.63 ± ≤0.1	**10.26**[bc]
18	13.61 ± 0.2	9.35 ± 0.2	3.43 ± 0.1	**8.79**[a]
19	2.14 ± ≤0.1	9.45 ± 0.3	3.75 ± 0.5	**5.11**[bc]
20	2.68 ± 0.3	9.62 ± ≤0.1	3.41 ± 0.2	**5.24**[bc]
25	13.31 ± 0.5	8.93 ± 0.1	3.56 ± 0.6	**8.6**[a]
33	18.43 ± 0.4	9.42 ± 0.5	3.54 ± 0.1	**10.46**[b]
Mean	**9.57**[a]	**9.32**[c]	**3.56**[b]	
LSD (5%)	10.35	8.26	10.30	

Value= Mean ± S.D on fresh weight basis. Each value is a mean of 3 replicates. Means followed by the same letter are not significantly different (p≤5%).

BF= Before Flowering; DF= During Flowering; AF= After Flowering

4.1.3 Vitamin content of slenderleaf accessions

Vitamin C content

Vitamin C content for all the accessions before, during and after flowering varied significantly ($p \leq 0.05$) as shown in Table 10. The vitamin C content varied from 10.56 mg/100g to 18.95 mg/100g before flowering; from 21.91 to 28.83 mg/100g during flowering and between 10.37 to 15.81 mg/100g after flowering. The mean of all the vitamin c values before flowering was of 13.24 mg/100g, during flowering it was of 25.17 mg/100g and after flowering it was of 12.59 mg/100g. The means were significantly ($p \leq 0.05$) different. We noticed that the significantly ($p \leq 0.05$) highest value of vitamin c content was observed during flowering and the significantly ($p \leq 0.05$) lowest value observed after flowering. There was a decrease of vitamin c as the vegetable matured.

For the accessions, the means were between 15.48 mg/100g and 19.23 mg/100g. According to the Table 10, accessions 11, 14, and 33 were not significantly ($p \leq 0.05$) different from each other, but they were significantly ($p \leq 0.05$) different from accessions 1, 2, 20 and 25. There was also significant difference ($p \leq 0.05$) in vitamin C among the various accessions. Accession 1 had significantly ($p \leq 0.05$) higher vitamin C than accession 12, 13 and 33 which had the significantly ($p \leq 0.05$) lowest vitamin C values.

There was a significant ($p \leq 0.05$) interaction between stage of growth and the accessions. The vitamin C content in the accessions was linked with stage of growth.

Vitamins C builds collagen, healthy gums, teeth and blood vessels and is essential for connective tissue formation and maintenance, fights infections, keeping skin and joints firm and strong, immune system stimulation, works as anti-oxidant, and enhances iron utilization among other roles (Bramley, 2000). It is essential nutrients mostly available from vegetables. Vitamin C is an essential nutrient required by the body for the development and maintenance of scar tissue, blood vessels, and cartilage. Vitamin C is also necessary for creating ATP, dopamine, peptide hormones, and tyrosine (Sies, 1991). As a powerful antioxidant, vitamin C helps lessen oxidative stress to the body and is thought to lower cancer risk (Bramley, 2000). Vitamin C can increase the absorption of iron (especially the iron found in plant foods) and may help lower the risk of dietary iron deficiency (Sies, 1991).

The results of Vitamin C content are similar to those results reported by Abukutsa-Onyango (2004) on the same vegetable in Kenya.

Consumption of 100 g of raw Slenderleaf can provide up to 29.23 mg/100g of vitamin C daily requirement. Vitamin C is however highly sensitive to air, light, temperatures and is also soluble in water. Hence most of it (up to 78%), has been reported to be lost during cooking of vegetables (Bramley, 2000). Shorter cooking time is therefore recommended to avoid greater loses of this vitamin. Human beings cannot synthesize vitamin C, which is a very important antioxidant. Vitamin C is an important co-factor in protein chemistry, protecting against cancer and heart disease and has many other uses (Sies, 1991).

Table 10: Effect of stage of harvesting on Vitamin C Content of of Slenderleaf accessions.

Vitamins C (mg/100g)				
Accession	**Stage of growth**			**Mean (accession)**
	BF	**DF**	**AF**	
1	18.95 ± 3.8	22.93 ± 7.3	15.81 ± 0.8	**29.23^{e}**
2	12.35 ± 2.6	24.09 ± 5.6	14.06 ± 0.6	**16.83^{b}**
8	18.45 ± 1.0	24.66 ± 6.3	12.21 ± 2.1	**18.44^{cd}**
11	10.56 ± 0.8	21.91 ± 0.5	14.02 ± 0.3	**15.49^{a}**
14	11.52 ± 0.5	24.25 ± 7.7	11.31 ± 1.1	**15.69^{a}**
18	11.48 ± 4.1	28.57 ± 12.5	11.69 ± 0.4	**17.25^{bc}**
19	12.17 ± 1.8	26.05 ± 4.0	12.43 ± 0.3	**16.88^{ba}**
20	11.58 ± 1.7	28.78 ± 12.4	12.20 ± 0.5	**17.52^{c}**
25	11.21 ± 0.7	28.83 ± 8.1	11.81 ± ≤0.1	**17.28^{c}**
33	14.17 ± 0.9	21.65 ± 1.3	10.37 ± 6.6	**15.48^{a}**
Mean	**13.24^{b}**	**25.17^{a}**	**12.59^{c}**	
LSD (5%)	4.87	4.22	4.12	

Value= Mean ± S.D on fresh weight basis. Each value is a mean of 3 replicates. Means followed by the same letter are not significantly different (p≤5%). BF= Before Flowering; DF= During Flowering; AF= After Flowering

β- Carotene content

β-Carotene content for all the accessions before, during and after flowering varied significantly ($p \leq 0.05$) as shown in Table 11. The β-Carotene content varied from 7.32 to 8.67 mg/100g before flowering; from 8.03 to 14.22 mg/100g during flowering and between 2.48 to 8.26 mg/100g after flowering. The mean of all the β-Carotene values before flowering was of 8.06 mg/100g, during flowering it was of 10.08 mg/100g and after flowering it was of 5.12 mg/100g. The means were significantly ($p \leq 0.05$) different. It was observed that the significantly ($p \leq 0.05$) higher values of β-Carotene content were observed during flowering in comparison to after flowering. There was a decrease of β-Carotene as the vegetable matured. The mean β- Carotene content increased and reached the peak at the flowering stage (10.08 mg/100g) after which it started reducing as the slenderleaf matured further.

For the accessions, the means were between 6.65 mg/100g and 9.83 mg/100g. According to the Table 11, accessions 8 and 33 were not significantly ($p \leq 0.05$) different from each other, but they were from accessions 2, 20, 19 and 11. There was also significant difference ($p \leq 0.05$) in β-Carotene among the various accessions. Accession 11 had significantly ($p \leq 0.05$) highest β-Carotene while accession 33 was the one that showed the significantly ($p \leq 0.05$) lowest value.

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There was a significant ($p \leq 0.05$) interaction between stage of growth and the accessions. The β-Carotene content in the accessions was linked with stage of growth.

β-Carotene is one of the most important food additives and is widely used as a colorant in foods and beverages (Faure, 1999). β-Carotene is also a most effective vitamin A precursor, and has been reported to protect humans against certain types of cancer (Steinmetz, 1996), bone calcification, eye degeneration, neuronal damages and cardiovascular diseases (Gaziano, 1992). β-carotene is an important biological compound that is widely distributed in fruits and vegetables. It is converted in the human body to retinol (vitamin A) that is essential for proper function of the retina, skin and mucous membrane (Rao, 2002). β-carotene protects human cells (most mucous membranes and skin) from the destructive effects of UV radiation

(Richelle et al., 2002). As an antioxidant deactivates harmful free radicals, thus slows down the ageing process.

There was a significant ($p \leq 0.05$) interaction between Accession 2 and the during flowering stage of growth. These results are in agreement with those reported by Mibei (2011) who observed β-carotene content in the same range in ALVs.

The results of β-Carotene in this study are higher compare to those reported by Akubugwo *et al.*, (2008) where he found out that the values are of 3.29 mg/100g. Since β-carotene in plant is normally converted to vitamin A (retinol) with the help of enzymes from the liver, then taking 100 g of raw Slenderleaf can therefore provide some of the vitamin A required in the body. This is needed for healthy skin, protects against infections, antioxidant and immune booster, essential for night vision (Kitade, 2002).

Table 11: Effect of stage of harvesting on β- Carotene Content of Slenderleaf accessions.

	β-carotene (mg/100g)			
Accession	**Stage of growth**			**Mean (accession)**
	BF	**DF**	**AF**	
1	8.59 ± 0.3	9.87 ± 0.1	4.45 ± 11.3	**7.64^{b}**
2	8.56 ± 0.8	10.00 ± 1.5	4.03 ± 0.7	**7.53^{b}**
8	8.55 ± 0.6	8.97 ± 0.6	2.48 ± 0.2	**6.67^{a}**
11	8.67 ± 1.4	14.22 ± 15.8	6.61 ± 0.3	**9/83^{e}**
14	7.32 ± 1.3	11.85 ± 0.2	3.92 ± 0.5	**7.69^{b}**
18	7.53 ± 0.3	8.03 ± 0.4	6.70 ± 3.9	**7.42^{b}**
19	7.56 ± 0.6	10.36 ± 1.6	8.26 ± 0.3	**8.73^{d}**
20	7.73 ± 0.4	10.31 ± 1.8	5.67 ± 0.2	**7.90^{c}**
25	8.15 ± 3.3	8.58 ± 0.5	5.77 ± 1.9	**7.5^{b}**
33	7.98 ± 0.5	8.65 ± 0.3	3.33 ± 2.4	**6.65^{a}**
Mean	**8.06^{b}**	**10.08^{c}**	**5.12^{a}**	
LSD (5%)	0.20	0.11	0.15	

Value= Mean ± S.D on fresh weight basis. Each value is a mean of 3 replicates. Means followed by the same letter are not significantly different (p≤5%).

BF= Before Flowering; DF= During Flowering; AF= After Flowering

4.2 Bioactive components

4.2.1 Flavonoid content

Flavonoids content for all the accessions before, during and after flowering varied significantly ($p \leq 0.05$) as shown in Table 12. The flavonoids content varied from 20.49 mg/100g to 34.52 mg/100g before flowering; from 26.58 to 36.33 mg/100g during flowering and between 19.23 to 22.57 mg/100g after flowering. The mean of all the flavonoids values before flowering was of 26.26 mg/100g, during flowering it was of 31.51 mg/100g and after flowering it was of 20.79 mg/100g. The means were significantly ($p \leq 0.05$) different. We noticed that the significantly ($p \leq 0.05$) highest value of flavonoids content was observed during flowering and the significantly ($p \leq 0.05$) lowest value observed after flowering.

For the accessions, the means were between 23.48 mg/100g and 28.41 mg/100g. According to the Table 12, accessions 25, 19 and 11 were not significantly ($p \leq 0.05$) different from each other, but they were from accessions 8 and 18. There was also significant difference ($p \leq 0.05$) in flavonoids among the various accessions. Accession 18 had significantly ($p \leq 0.05$) highest flavonoids while accession 33 was the one that showed the significantly ($p \leq 0.05$) lowest value.

There was a significant ($p \leq 0.05$) interaction between stage of growth and the accessions. The flavonoids content in the accessions was linked with stage of growth.

Bioactive have become synonymous with good health and been shown to have the potential to reduce the risk of cancer, cardiovascular disease, osteoporosis, inflammation, type 2 diabetes, and other chronic degenerative diseases (Singleton, 1999). It is most famous for its antioxidant and anti-inflammatory health benefits, as well as its contribution of vibrant color to the foods we eat (Warda, 2009). They are a class of compounds thought to prevent certain types of chemical damage caused by an excess of free radicals, charged molecules that are generated by a variety of sources including pesticides, smoking and exhaust fumes. Destroying free radicals may help fight cancer, heart disease, stroke and other immune compromising diseases (Stulzer, 2006). As an especially delicate group of nutrients with respect to cooking heats, flavonoids are often front and center in development of best cooking

methods able to preserve nutrients. Many of the natural antioxidants, especially flavonoids, seem to be very important in the prevention of these diseases (Tosun, 2009).

Similar results, expressed in dry matter basis, were found by Grace Ngugi (1999) in her research on the nutritional composition of edible part of *Slenderleaves*. For all the different stages of growth, values are within the normal daily requirement allowed. Reports show that these phytochemicals reduce LDL (Anderson, 2004), prevent blood clotting which can reduce the risk for a heart attack or a stroke (Zhou, 2004).

Flavonoids are class of secondary plant metabolites with significant antioxidant and chelating properties. Antioxidant activity of flavonoids depends on the structure and substitution pattern of hydroxyl groups (Sharififar et al., 2008).

Table 12: Effect of stage of harvesting on Flavonoids Content of of Slenderleaf accessions.

Flavonoids (mg/100g)				
Accession	**Stage of growth**			**Mean (accession)**
	BF	**DF**	**AF**	
1	23.23 ± 0.2	35.35 ± 0.3	20.67 ± 0.7	**26.42^{ab}**
2	27.82 ± 0.8	29.66 ± 0.1	22.57 ± 2.7	**26.68^{ab}**
8	34.52 ± 0.1	28.05 ± 1.8	22.26 ± 0.2	**28.28^{b}**
11	26.55 ± 0.2	26.58 ± 1.1	20.57 ± 0.1	**24.57^{a}**
14	26.50 ± 0.4	30.49 ± 0.1	21.73 ± 1.2	**26.24^{ab}**
18	30.03 ± 0.9	33.38 ± 1.3	21.81 ± 0.1	**28.41^{b}**
19	23.17 ± 2.7	33.20 ± 5.2	20.31 ± 0.3	**25.56^{a}**
20	29.73 ± 2.5	31.38 ± ≤0.1	19.37 ± 0.1	**26.83^{ab}**
25	20.50 ± 0.1	36.33 ± 0.2	19.23 ± 0.2	**25.35^{a}**
33	20.49 ± 0.2	30.63 ± 0.3	19.33 ± 0.3	**23.48^{a}**
Mean	**26.26^{b}**	**31.51^{c}**	**20.79^{a}**	
LSD (5%)	0.35	0.26	0.30	

Value= Mean ± S.D on fresh weight basis. Each value is a mean of 3 replicates. Means followed by the same letter are not significantly different (p≤5%).

BF= Before Flowering; DF= During Flowering; AF= After Flowering

4.2.2 – Vitamin E Composition

Vitamin E content for all the accessions before, during and after flowering varied significantly ($p \leq 0.05$) as shown in Table 13. The vitamin E content varied from 2.54 % to 4.07 % before flowering; from 2.52 to 5.33 % during flowering and between 1.26 to 11.55 % after flowering. The mean of all the vitamin E values before flowering was of 2.95 %, during flowering it was of 3.83 % and after flowering it was of 1.4 %. The means were not the same showed that the means were significantly ($p \leq 0.05$) different. We noticed that the significantly ($p \leq 0.05$) highest value of vitamin E content was observed during flowering and the significantly ($p \leq 0.05$) lowest value observed after flowering. There was a decrease of vitamin E as the vegetable matured.

For the accessions, the means were between 2.13 % and 3.13 %. Some accessions were not significantly ($p \leq 0.05$) different. According to the Table 13, accessions 14 and accession 8 were significantly ($p \leq 0.05$) different from each other and also they were from accessions 11 and 20. There was also significant difference ($p \leq 0.05$) in vitamin E among the various accessions. Accession 11 had significantly ($p \leq 0.05$) highest vitamin E while accession 14 was the one that showed the significantly ($p \leq 0.05$) lowest value.

There was a significant ($p \leq 0.05$) interaction between stage of growth and the accessions. The vitamin E content in the accessions was linked with stage of growth.

Vitamin E is a group of 8 fat-soluble vitamins characterized by its antioxidant properties (Malleshi, 1986). it protects the body's cell membranes by trapping free radicals and preventing them from spreading. Vitamin E works together with the body's other antioxidant defense systems and in addition to its antioxidant action, it contributes to platelet aggregation and inflammatory responses (Hurrell, 2003). Vitamine E has been shown to stimulate the phagocytic action of macrophages and synthesis of several immune cell types, which increases the protection against infection (Anderson, 2005).

Adequate amounts of vitamin E can help protect against heart disease, cancer, and age related damage (macular degeneration). While too much vitamin E from supplements can lead to excessive bleeding, or hemorrhaging (Clinton, 1998). Vitamin E is not notably sensitive to

heat, but it is sensitive to light and oxygen. Foods that are rich in Vitamin E should be stored in a dark place and in firmly sealed containers (Cantuti-Castelvetri, 2000).

These results were similar to the vitamin E levels reported by Ray-Yu Yang (2009) in African Indigenous Vegetables.

Fruits and vegatables are the second largest source of Vitamin E. According to Velioglu (1998), they do not contain high levels of Vitamin E (between 1 and 1.8 mg per 100 g for the richest sources), but the size of the portions consumed (we eat 100 and 200 g of vegetables) makes them a significant source of Vitamin E (12 % and 18 % of our Vitamin E intake comes from fruits and vegetables). Genuine vitamin E deficiency is unusual in adult men ususally characterized by nervous system problems. In most cases, a deficiency is the result of serious and prolonged difficulty with lipid absorption and metabolism (Hurrell, 2003).

Table 13: Effect of Stage of harvesting on Vitamin E Content of Slenderleaf accessions.

Vitamin E (%)				
Accession	**Stage of growth**			**Mean (accession)**
	BF	**DF**	**AF**	
1	2.55 ± 0.2	3.86 ± 0.4	1.30 ± ≤0.1	**2.57^{b}**
2	2.62 ± 0.1	3.83 ± 0.1	1.26 ± 0.3	**2.57^{b}**
8	2.80 ± ≤0.1	3.60 ± ≤0.1	1.33 ± 0.7	**2.58^{b}**
11	2.61 ± ≤0.1	5.33 ± 0.2	1.44 ± 0.5	**3.13^{c}**
14	2.54 ± 0.1	2.52 ± ≤0.1	1.34 ± 0.5	**2.13^{a}**
18	3.17 ± 0.1	3.86 ± 0.2	1.38 ± 0.1	**2.80^{bc}**
19	3.15 ± 0.4	3.5 ± 0.3	1.55 ± 0.5	**2.72^{bc}**
20	4.07 ± ≤0.1	3.73 ± 0.1	1.44 ± 0.1	**3.08^{c}**
25	2.98 ± 0.7	3.83 ± 0.1	1.52 ± 0.2	**2.78^{bc}**
33	3.03 ± 0.1	4.35 ± 0.5	1.42 ± 0.2	**2.93^{c}**
Mean	**2.95^{b}**	**3.83^{c}**	**1.40^{a}**	
LSD (5%)	0.35	0.26	0.30	

Value= Mean ± S.D on fresh weight basis. Each value is a mean of 3 replicates. Means followed by the same letter are not significantly different (p≤5%).

BF= Before Flowering; DF= During Flowering; AF= After Flowering

4.3 Anti-nutrient content of slenderleaf

4.3.1 Tannin content

Tannins content for all the accessions before, during and after flowering varied significantly ($p \leq 0.05$) as shown in Table 14. The tannins content varied from 22.20 to 53.69 mg/100g before flowering; from 26.23 to 56.48 mg/100g during flowering and between 60.02 to 87.40 mg/100g after flowering. The mean of all the tannins values before flowering was of 35.87 mg/100g, during flowering it was of 38.65 mg/100g and after flowering it was of 70.39 mg/100g. The means were significantly ($p \leq 0.05$) different. We noticed that the significantly ($p \leq 0.05$) highest value of tannins content was observed after flowering and the significantly ($p \leq 0.05$) lowest value observed before flowering. There was an increase of tannins as the vegetable matured.

For the accessions, the means were between 41.03 mg/100g and 58.82 mg/100g. Some accessions were not significantly ($p \leq 0.05$) different. According to the Table 14, accessions 19, 20 and 25 had the same superscripts letters so they were not significantly ($p \leq 0.05$) different from each other, but they were from accessions 33, 14, 8 and 1. There was also significant difference ($p \leq 0.05$) in tannins among the various accessions. Accession 1 had significantly ($p \leq 0.05$) highest tannins while accession 20 was the one that showed the significantly ($p \leq 0.05$) lowest value.

There was a significant ($p \leq 0.05$) interaction between stage of growth and the accessions. The tannins content in the accessions was linked with stage of growth.

Also known as natural organic tannins, tannin is an astringent, bitter plant polyphenolic compound that binds to and precipitates proteins and various other organic compounds including amino acids and alkaloids (McGee, 2004). However, the term "tannin" by extension is widely applied to any large polyphenolic compound containing sufficient hydroxyls and other suitable groups. Tannin compounds are widely distributed in many species of plants, where they play a role in protection from predation, and perhaps also as pesticides, and in plant growth regulation (Boralle, 1993). Likewise, the destruction or modification of tannins with time plays an important role in the ripening of fruit and the aging of wine. Tannins are found in leaf, bud, seed, root, and stem tissues (Katie, 2006)..

Tannins determination was important because of the alleged interference with mineral absorption (Rose 1982). The results of the tannin content are similar to those observed by Mibei (2011) in his report on antinutrient components of some ALV.

Tannins have the ability to precipitate proteins from aqueous solutions. They are polyphenols that bind proteins and hence with their bioavailability. In the body, others studies on tannins have been as well shown it as phytochemical having some antioxidant activity (Drabble, 1907). Tannins have traditionally been considered antinutritional, but it is now known that their beneficial or antinutritional properties depend upon their chemical structure and dosage.

A review published found insufficient consensus for the hypothesis that the specific intake of food and drink containing tannins may play a meaningful role in reducing the risk of cardiovascular disease (Van der Linden, 2012)

Table 14: Effect of Stage of harvesting on Tannin Content of Slenderleaf accessions.

	Tannins (mg/100g)			
Accession	**Stage of growth**			**Mean (accession)**
	BF	**DF**	**AF**	
1	53.69 ± 1.4	44.43 ± 0.4	78.33 ± 0.3	**58.82^{e}**
2	33.46 ± ≤0.1	43.06 ± 0.3	75.17 ± 0.1	**50.56^{c}**
8	30.58 ± ≤0.1	48.55 ± 0.1	87.40 ± 0.4	**55.51^{d}**
11	42.45 ± 0.1	56.48 ± 0.5	60.26 ± 0.2	**53.06^{cd}**
14	50.55 ± 0.2	46.25 ± 0.2	60.02 ± ≤0.1	**52.27^{c}**
18	30.29 ± 0.2	33.51 ± 0.1	63.85 ± 1.2	**42.55^{ab}**
19	22.20 ± 0.1	28.33 ± 0.2	74.74 ± 1.0	**41.76^{a}**
20	25.77 ± 0.1	33.24 ± 0.1	64.08 ± ≤0.1	**41.03^{a}**
25	29.34 ± 0.1	26.39 ± 0.4	72.48 ± 3.5	**42.74^{a}**
33	40.36 ± 0.4	26.23 ± 0.2	67.60 ± 0.1	**44.73^{b}**
Mean	**35.87^{a}**	**38.65^{b}**	**70.39^{c}**	
LSD (5%)	2.35	2.26	2.30	

Value= Mean ± S.D on fresh weight basis. Each value is a mean of 3 replicates. Means followed by the same letter are not significantly different (p≤5%).

BF= Before Flowering; DF= During Flowering; AF= After Flowering

4.3.2 Phytate content

Phytate content for all the accessions before, during and after flowering varied significantly ($p \leq 0.05$) as shown in Table 15. The phytate content varied from 29.69 to 41.48 mg/100g before flowering; from 19.29 to 34.05 mg/100g during flowering and between 43.15 to 76.46 mg/100g after flowering. The mean of all the phytate values before flowering was of 37.55 mg/100g, during flowering it was of 27.00 mg/100g and after flowering it was of 63.14 mg/100g. The means were significantly ($p \leq 0.05$) different. We noticed that the significantly ($p \leq 0.05$) highest value of phytate content was observed after flowering and the significantly ($p \leq 0.05$) lowest value observed during flowering. There was an increase of phytate as the vegetable matured.

For the accessions, the means were between 37.33 mg/100g and 48.17 mg/100g. Some accessions were not significantly ($p \leq 0.05$) different. According to the Table 15, accessions 8 did not have the same superscripts letters with the other accessions so they were significantly ($p \leq 0.05$) different from each other and also from accessions 20, 19, and 33. There was also significant difference ($p \leq 0.05$) in phytates among the various accessions. Accession 33 had significantly ($p \leq 0.05$) highest phytate while accession 8 was the one that showed the significantly ($p \leq 0.05$) lowest value.

There was a significant ($p \leq 0.05$) interaction between stage of growth and the accessions. The phytate content in the accessions was linked with stage of growth.

Simple cooking can reduce the phytic acid to some degree. According to Seaman (2003), Phytic acid has a strong binding affinity to important minerals (calcium, iron, and zinc) which becomes insoluble, precipitates and will be nonabsorbable in the intestines leading to mineral deficiencies (pellagra).

It may be considered a phytonutrient, providing an antioxidant effect. Phytic acid's mineral binding properties may also prevent colon cancer. Researchers now believe phytic acid, found in the fiber of legumes and grains, is the major ingredient responsible for preventing colon cancer and other cancers (López-González, 2008). Phytic acid's chelating effect may serve to prevent, inhibit, or even cure some cancers. The deprivation of essential minerals like iron

would, much like other systemic treatments for cancers, also have negative effects on noncancerous cells. One study correlated decreased osteoporosis risk with phytic acid consumption (Dendougui, 2004).

Phytates determination was important because of the alleged interference with mineral absorption (Rose 1982). Phytates have been long considered as antinutrients because of their ability to interact with dietary protein, starch and minerals. It is associated with nutritional diseases such as rickets in children and osteomalacia in adults

Phytate is also a beneficial phytochemical and in the body has antioxidant activity (Watzl *et al.,* (1999). .it has been suggested that health benefits associated with dietary fiber such as delayed nutrient absorption, decreased cancer risk, increase fecal bulk and lowering of blood lipid, also may be attributed to phytates (Thompson, 1995).

These results are similar to those reported by Mibei (2011) found in some ALV.

Table 15: Stage of harvesting effect on Phytates Content of Slenderleaf accessions.

	Phytates (mg/100g)			
Accession	**Stage of growth**			**Mean (accession)**
	BF	**DF**	**AF**	
1	36.54 ± 2.5	25.21 ± 1.5	63.34 ± 5.8	**41.69^{b}**
2	35.86 ± 2.2	27.13 ± 1.0	61.95 ± 1.9	**41.65^{b}**
8	34.42 ± 0.3	31.04 ± 0.1	46.54 ± 6.8	**37.33^{a}**
11	40.70 ± ≤0.1	34.05 ± 0.3	43.15 ± 3.5	**39.3^{ab}**
14	29.69 ± ≤0.1	20.55 ± ≤0.1	70.33 ± 0.1	**40.19^{b}**
18	36.04 ± ≤0.1	30.70 ± ≤0.1	65.85 ± 0.1	**44.19^{c}**
19	41.05 ± ≤0.1	28.55 ± ≤0.1	60.47 ± 0.1	**43.36^{c}**
20	38.38 ± 0.2	19.29 ± 0.2	67.67 ± 4.6	**41.78^{b}**
25	41.29 ± 0.3	26.04 ± 0.1	76.46 ± 0.2	**47.93^{d}**
33	41.48 ± 0.1	27.44 ± 0.1	75.60 ± 0.6	**48.17^{d}**
Mean	**37.55^{b}**	**27.00^{a}**	**63.14^{c}**	
LSD (5%)	1.35	1.26	1.30	

Value= Mean ± S.D on fresh weight basis. Each value is a mean of 3 replicates. Means followed by the same letter are not significantly different (p≤5%). BF= Before Flowering; DF= During Flowering; AF= After Flowering

4.3.3 Oxalate content

Oxalate content for all the accessions before, during and after flowering varied significantly ($p \leq 0.05$) as shown in Table 16. The oxalate content varied from 85.94 to 100.36 mg/100g before flowering; from 67.38 to 75.38 mg/100g during flowering and between 85.2 to 160.2 mg/100g after flowering. The mean of all the oxalate values before flowering was of 92.79 mg/100g, during flowering it was of 72.16 mg/100 g and after flowering it was of 124.73 mg/100 g. The means were significantly ($p \leq 0.05$) different. We noticed that the significantly ($p \leq 0.05$) highest value of oxalate content was observed after flowering and the significantly ($p \leq 0.05$) lowest value observed during flowering. There was an increase of vitamin c as the vegetable matured.

For the accessions, the means were between 84.58 mg/100g and 107.99 mg/100g. Some accessions were not significantly ($p \leq 0.05$) different. According to the Table 16, accessions 18, 19 and 20 had the same superscripts letters so they were not significantly ($p \leq 0.05$) different from each other, but they were from accessions 11, 8, 14 and 25. There was also significant difference ($p \leq 0.05$) in oxalate among the various accessions. Accession 25 had significantly ($p \leq 0.05$) highest oxalate while accession 20 was the one that showed the significantly ($p \leq 0.05$) lowest value.

There was a significant ($p \leq 0.05$) interaction between stage of growth and the accessions. The oxalate content in the accessions was linked with stage of growth.

Oxalates are naturally-occurring substances found in plants, animals, and in humans. In chemical terms, oxalates belong to a group of molecules called organic acids, and are routinely made by plants. Our bodies always contain oxalates, and our cells routinely convert other substances into oxalates (Hanson, 1989).

The formation of kidney stones containing oxalate is an area of controversy in clinical nutrition with respect to dietary restriction of oxalate. About 80% of kidney stones formed by adults in the U.S. are calcium oxalate stones (Assimos, 2000)

It is important to note that the leaves of a plant almost always contain higher oxalate levels than the roots, stems, and stalks (Freidig, 2011).

For all the different stages of growth, values are within the normal daily requirement allowed.

Cooking has a relatively small impact on the oxalate content of foods. Repeated food chemistry studies have shown no statistically significant lowering of oxalate content following the blanching or boiling of green leafy vegetables (Simpson, 2009). A lowering of oxalate content by about 5-15% is the most you should expect when cooking a high-oxalate food. It does not make sense to overcook oxalate-containing foods in order to reduce their oxalate content. Because many vitamins and minerals are lost from overcooking more quickly than are oxalates, the overcooking of foods (particularly vegetables) will simply result in a far less nutritious diet that is minimally lower in oxalates (Kikunaga, 1988).

Table 16: Effect of Stage of harvesting on Oxalate Content of Slenderleaf accessions.

Oxalate (mg/100g)				
Accession	**Stage of growth**			**Mean (accession)**
	BF	**DF**	**AF**	
1	85.94 ± 2.1	74.35 ± 1.3	127.6 ± 0.1	**95.96**[b]
2	90.70 ± 0.7	74.03 ± 3.9	129.1 ± 5.9	**97.94**[b]
8	100.36 ± 0.3	72.42 ± 2.8	127.6 ± 0.1	**100.13**[c]
11	90.48 ± 0.3	75.38 ± 0.3	127.4 ± 0.8	**97.75**[b]
14	98.83 ± 4.2	70.40 ± 0.4	145.9 ± 0.1	**105.04**[d]
18	98.36 ± 4.8	67.38 ± 0.1	94.3 ± 1.6	**86.68**[a]
19	98.31 ± 0.6	70.22 ± 0.3	85.2 ± 1.6	**84.58**[a]
20	88.30 ± 0.5	68.51 ± 0.3	93.5 ± 0.1	**83.44**[a]
25	87.40 ± 2.1	74.38 ± 0.4	156.5 ± 0.5	**106.09**[e]
33	89.25 ± 0.2	74.52 ± 0.5	160.2 ± 18.6	**107.99**[e]
Mean	**92.79**[b]	**72.16**[a]	**124.73**[c]	
LSD (5%)	26.35	30.26	25.30	

Value= Mean ± S.D on fresh weight basis. Each value is a mean of 3 replicates. Means of the same parameter followed by the same letter are not significantly different ($p \leq 5\%$).

BF= Before Flowering; DF= During Flowering; AF= After Flowering

CHAPTER FIVE
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

- There were significant ($p \leq 0.05$) differences in nutrients and phytochemical contents in the slenderleaf accessions due to maturity stage. Generally, the nutrient and phytochemical content in the slenderleaf leaves was significantly higher during and before flowering than before flowering.
- There were significant ($p \leq 0.05$) differences in bioactive and phytochemicals content in the slenderleaf accessions due to maturity stage.
- There were significant ($p \leq 0.05$) differences in the antinutrients content of slenderleaf at different stages of maturity. Generally as the slenderleaf accessions matured, the tannin, phytate and oxalate content increased.

5.2 Recommendations and Suggestion

Some of the key recommendations are:

- For optimal nutrient and phytochemical value, the slenderleaf leaves should be harvested before the end of the flowering stage. Generally after the flowering stage, the nutrient value will decrease as the antinutrient content increases.

5.3 Suggestion for further research

- Further research needs also to be done on the plant in order to identify the different mechanisms for the transformation processes that affect the nutritional quality of the plant and reduce its toxic components.

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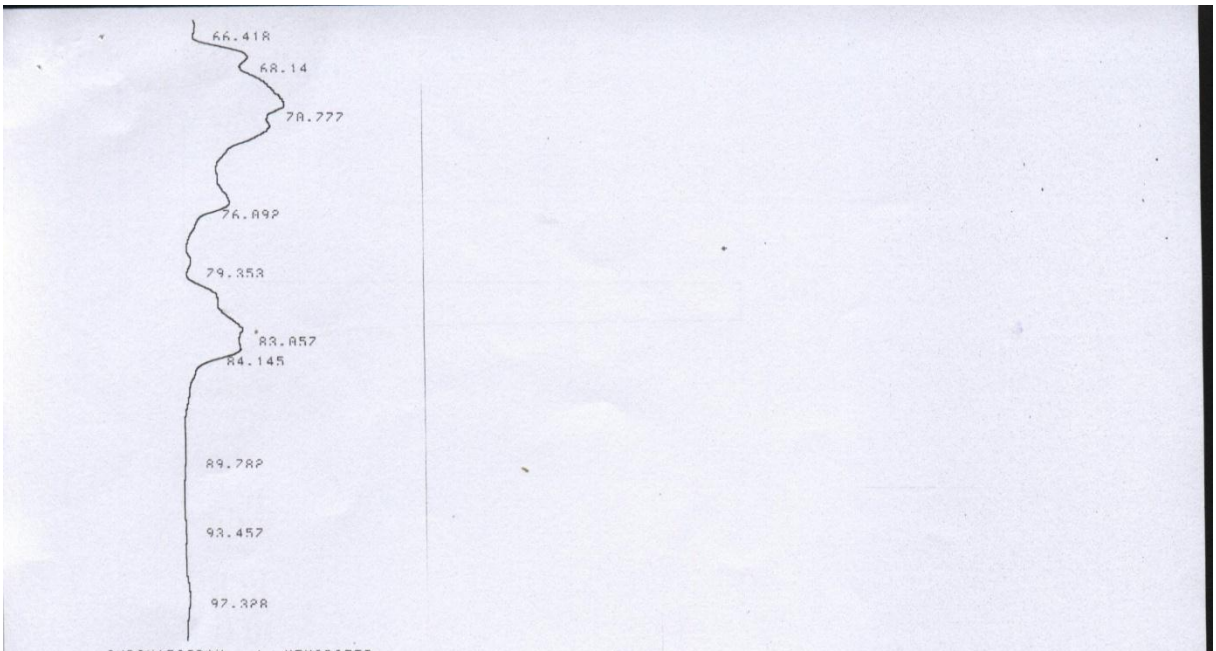
APPENDICES

Appendice I: Scanned copy of part of the Chromatography result for Moisture content

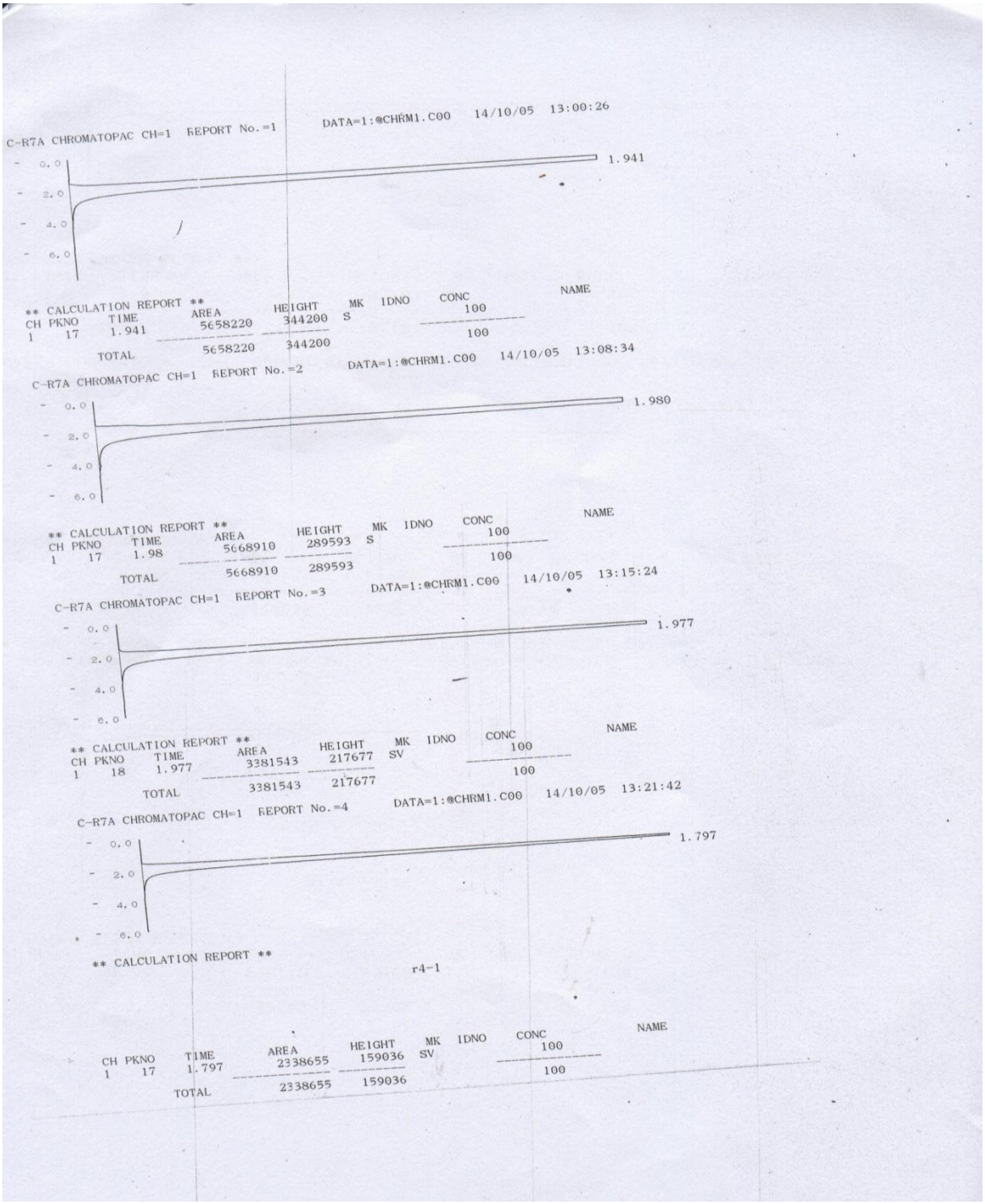


CHROMATOGRAM 1 MEMORIZED

CHROMATOPAC C-R6A FILE 5
SAMPLE NO A METHOD .41
REPORT NO 11325

PKNO	TIME	AREA	MK	TYPE	CONC	NAME
1	6.155	818			0.0022	
2	7.612	1923764	F		51.3762	
3	8.512	1284982	SV		34.3169	
4	11.867	3655	T		0.0098	
5	12.927	1018	T		0.0027	
6	13.28	1098	TV		0.0029	
7	13.848	673	TV		0.0018	
8	14.433	1101	T		0.0029	
9	14.623	4047	TV		0.0108	
10	15.465	1288	TV		0.0034	
11	16.5	220	T		0.0006	
12	16.625	141	TV		0.0004	
13	16.865	150	TV		0.0004	
14	17.498	142	T		0.0004	
15	18.298	300	T		0.0008	
16	19.133	241			0.0006	
17	20.697	615			0.0016	
18	21.183	2087	V		0.0056	
19	21.745	883	V		0.0024	
20	22.15	670	V		0.0018	
21	22.693	444	V		0.0012	
22	23.817	1042	V		0.0028	
23	26.615	10504	V		0.0281	
24	27.315	3840	V		0.0103	
25	28.075	5036	V		0.0134	
26	30.883	4279837	SV		11.4298	
27	35.705	8342	T		0.0223	
28	41.5	14718	T		0.0393	
29	43.303	8131	TV		0.0217	
30	45.703	6453	TV		0.0172	
31	48.303	2534004	TV		6.7672	
32	56.875	4217	TV		0.0113	
33	61.9	6592	TV		0.0176	
34	64.077	3751	TV		0.01	
35	66.418	11328	TV		0.0302	
36	68.14	67437	TV		0.1801	
37	78.777	346514	TV		0.9254	
38	76.892	1012109	TV		2.7033	
39	79.353	7941	TV		0.0193	
40	83.857	1110823	TV		2.9665	

Appendice 2: Scanned copy of part of the HPLC result for Vitamin E (After Flowering)



C-R7A CHROMATOPAC CH=1 REPORT No.=1 DATA=1:@CHRM1.C00 14/10/05 13:00:26

1.941

** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC 100	NAME
1	17	1.941	5658220	344200	S		100	
	TOTAL		5658220	344200			100	

C-R7A CHROMATOPAC CH=1 REPORT No.=2 DATA=1:@CHRM1.C00 14/10/05 13:08:34

1.980

** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC 100	NAME
1	17	1.98	5668910	289593	S		100	
	TOTAL		5668910	289593			100	

C-R7A CHROMATOPAC CH=1 REPORT No.=3 DATA=1:@CHRM1.C00 14/10/05 13:15:24

1.977

** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC 100	NAME
1	18	1.977	3381543	217677	SV		100	
	TOTAL		3381543	217677			100	

C-R7A CHROMATOPAC CH=1 REPORT No.=4 DATA=1:@CHRM1.C00 14/10/05 13:21:42

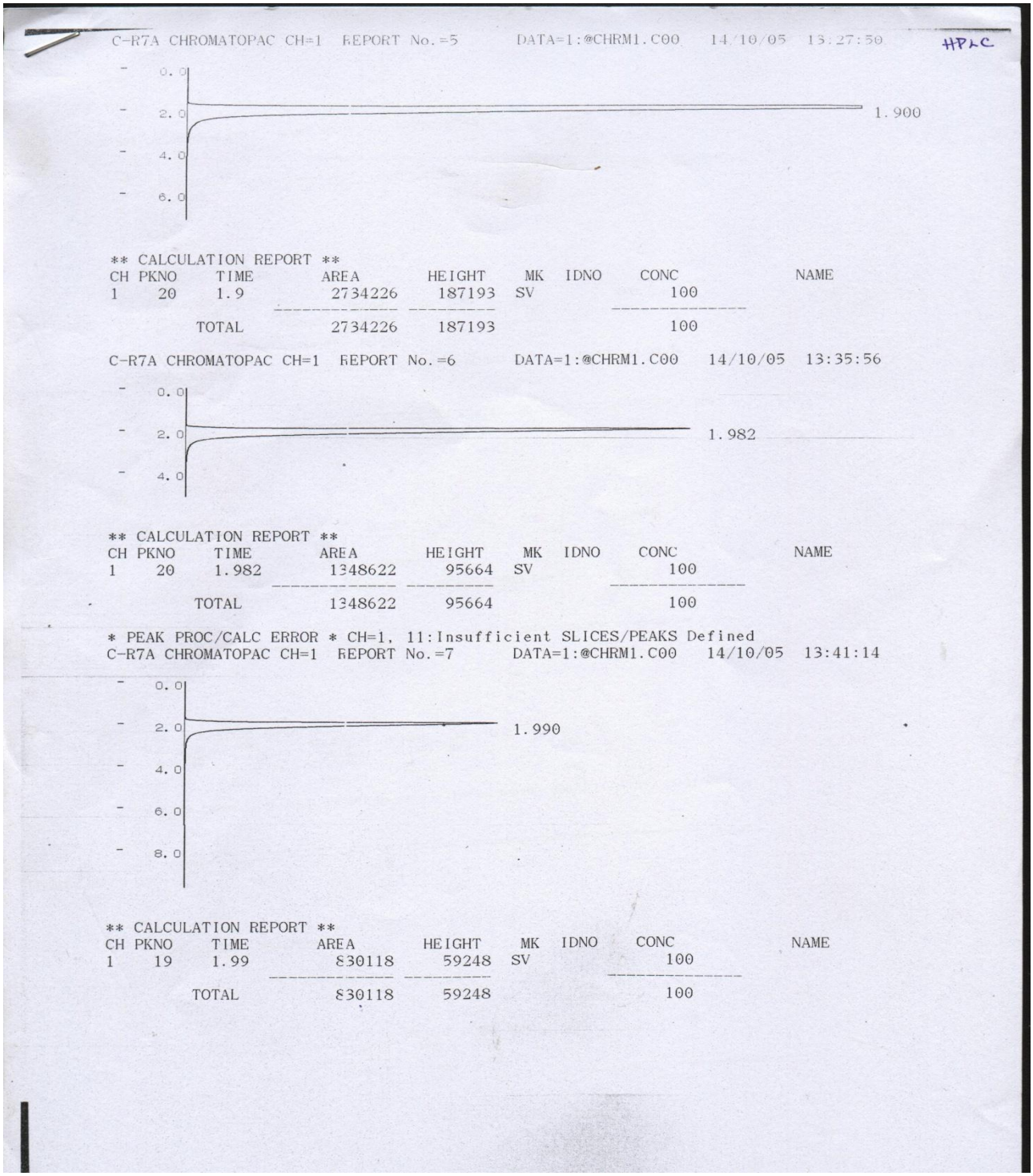
1.797

** CALCULATION REPORT **

r4-1

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC 100	NAME
1	17	1.797	2338655	159036	SV		100	
	TOTAL		2338655	159036			100	

Appendice 3: Scanned copy of part of the HPLC result for Phytates Content



C-R7A CHROMATOPAC CH=1 REPORT No.=5 DATA=1:@CHRM1.C00 14/10/05 13:27:50 HPLC

0.0
2.0
4.0
6.0
1.900

** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	20	1.9	2734226	187193	SV		100	
		TOTAL	2734226	187193			100	

C-R7A CHROMATOPAC CH=1 REPORT No.=6 DATA=1:@CHRM1.C00 14/10/05 13:35:56

0.0
2.0
4.0
1.982

** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	20	1.982	1348622	95664	SV		100	
		TOTAL	1348622	95664			100	

* PEAK PROC/CALC ERROR * CH=1, 11:Insufficient SLICES/PEAKS Defined

C-R7A CHROMATOPAC CH=1 REPORT No.=7 DATA=1:@CHRM1.C00 14/10/05 13:41:14

0.0
2.0
4.0
6.0
8.0
1.990

** CALCULATION REPORT **

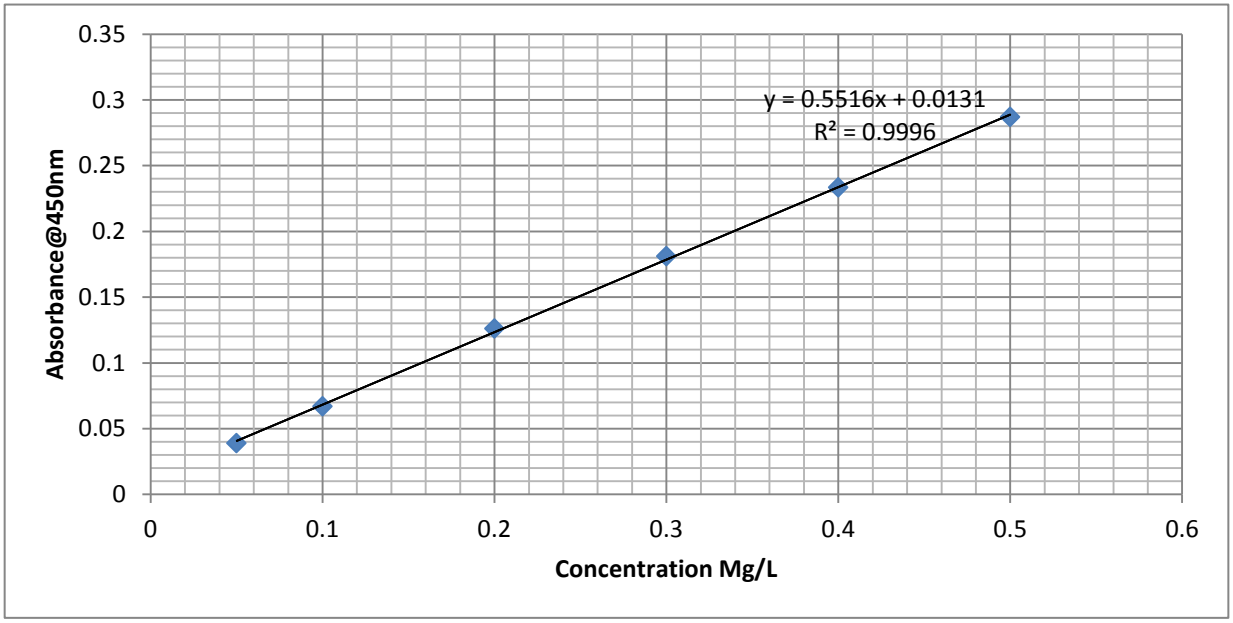
CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	19	1.99	830118	59248	SV		100	
		TOTAL	830118	59248			100	

Appendice 4 : Samples standard tables and graphs

Zinc standard

Concentration	Absorbance
0.05	0.039
0.1	0.067
0.2	0.126
0.3	0.181
0.4	0.2334
0.5	0.287

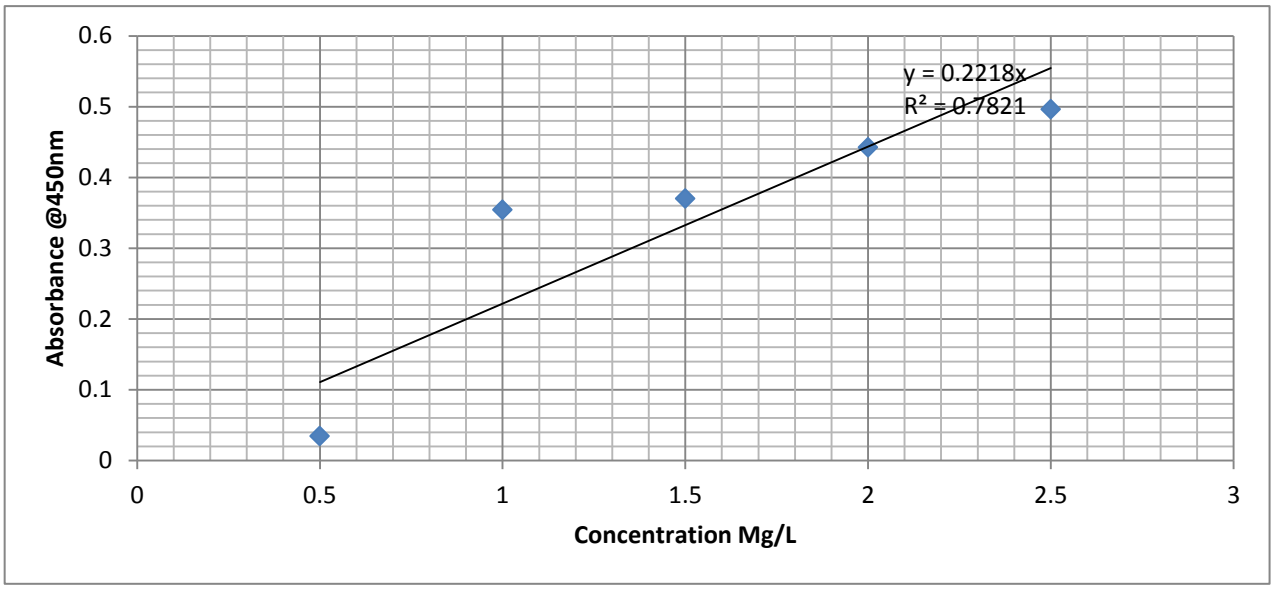
A graph of absorbance against concentration of Zinc standard



Iron standard

Concentration	Absorbance
0.5	0.034
1.0	0.354
1.5	0.370
2.0	0.442
2.5	0.496

A graph of absorbance against concentration of Iron standard



The standard β- carotene

Concentration (Mg/L)	**Absorbance@450nm**
0.00	0.00
1.0	0.013
2.0	0.065
4.0	0.073
8.0	0.126
10.0	0.167