

**INFLUENCE OF NITROGEN APPLICATION ON
PHYTOCHEMICALS, NUTRITIONAL AND SENSORY
CHARACTERISTICS OF ACCESSIONS OF *Cleome
gynandra* HARVESTED AT DIFFERENT
DEVELOPMENTAL STAGES**

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**Influence of Nitrogen application on Phytochemicals, Nutritional
and Sensory characteristics of accessions of *Cleome gynandra*
harvested at different developmental stages**

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**A thesis submitted in partial fulfilment for the degree of Master of
Science in Food Science and Technology in the Jomo Kenyatta
University of Agriculture and Technology**

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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 family who has always been the source of love, strength, support and encouragement.

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ABBREVIATIONS AND ACRONYMS

AA	Antioxidant activity
AAS	Atomic absorption spectroscopy
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
Conc.	Concentration
DMRT	Duncan Multiple Range Test
DMB	Dry matter basis
DWB	Dry weight basis
DPPH	Diphenyl Picryl Hydrazyl Radical
EC₅₀	Efficient Coefficient
FAO	Food and Agricultural Organization
GAE	Gallic Acid Equivalent
HPLC	High Performance Liquid Chromatography
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KEBS	Kenya bureau of standards
QE	Quercetin equivalent
SEM	Standard Error of the Mean
TAE	Tannic acid equivalent
UV-Vis	Ultra violet visible spectrophotometer
WHO	World Health Organization

ABSTRACT

Spider plant is one of indigenous vegetable whose consumption is fast rising both in rural and urban areas. This study was designed to determine the effect of Calcium Ammonium Nitrate (CAN) fertilizer and cattle manure on the accumulation of total phenolics, total flavonoids, tannins and alkaloids as the secondary metabolites of interest during growth and storage of vegetable spider plant (var. *Cleome gynandra* L). Field trials were set up at the JKUAT Horticultural Farm and Ruiru in 2011 and 2012. This was followed up by two seasons of green house trials set out in the greenhouse in JKUAT between March and June, and June and September 2012. Five accessions of spider plant; Control, MLSF17, UGSF14, UGSF36 and UGSF9 were grown at three fertilizer levels in a split-plot design in field trials. Vegetables were harvested at 4, 5, 6, 7, and 8 weeks after planting. Nitrogen fertilization improved the nutritional quality of spider plants in terms of proximate and mineral compositions. Use of CAN fertilizer led to significant increases in accumulation of nitrates and alkaloids, and significant reductions in the amount of total phenolics, total flavonoids and tannins. Antioxidant activity positively correlated with both the total polyphenols and total flavonoids recorded. Nitrogen did not however, show any significant difference with sensory attributes. MLSF 17 and UGSF 14 exhibited superior characteristics in nutritional and phytochemical compositions. The 6th week after planting proved the most optimum harvesting period to derive the maximum nutrition. Spider plants have therefore a high potential to contribute to the reduction in malnutrition, especially among people in rural areas where it constitutes an important part of the diet. In addition, they grow quickly, require little input and can

be harvested within a short period of time (4-9 weeks after planting). This is especially so when produced using CAN rates of not more than 2.6g N/plant or 40Kg N/ha. With proper fertilizer management, it is possible to obtain boost high yields and at the same time ensure high quality *C.gynandra*. In addition, maturity at harvest influences the vitamin and phytochemical contents. The phenolic content including that of quercetin, are also enhanced by use of manure, low levels of N as CAN and with advancing maturity of the vegetables at harvest. Application of manure/fertilizer and the stage of maturity of *Cleome gynandra* are critical in determining the phytochemical, nutritional and sensory characteristics of the vegetable.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Spider plant (*Cleome gynandra*), a member of the family *Capparaceae*, grows widely in the tropics. They are a group of fast growing C₄ herbs that yield a harvestable product within 4-6 weeks after planting, which makes them attractive to most farmers. In some countries such as Kenya, they are largely grown by women thus providing them with a degree of financial independence and healthy nutrition (IPGRI, 2003).

Spider plant is reported to deliver health benefits in addition to fulfilling physiological nutritional needs (Dillard and German, 2000; Surh, 2003; Scalbert *et al.*, 2005). Their consumption is associated with protection against major diseases including cancer and cardiovascular diseases (Wargovich 2000; Kaur and Kapoor 2001; Scalbert *et al.*, 2005). The protective action of the vegetables has been attributed to the presence of antioxidants (Chu *et al.*, 2000; Prior and Cao, 2000). Research has shown that the majority of the antioxidant and antimicrobial activities may arise from secondary metabolites rather than just from vitamins C and E, and β -carotene alone (Kähkönen *et al.*, 1999). Moreover, a positive correlation between total phenolics and antioxidant activity in this vegetables and fruits has been reported (Gil *et al.*, 2000; Pyo *et al.*, 2004; Khandaker *et al.*, 2008). Hollman (1997) says that quercetin is one of the strongest antioxidants among flavonoids. It is reported they chelate metals, scavenge oxygen free radical and prevent oxidation of low density

lipoprotein in *in vitro* studies (de Whalley *et al.*, 1990; Kandaswami and Middleton, 1994).

Earlier studies have established the abundance of antioxidants in spider plant leaves (Sokkanha and Tiratanakul, 2006; Khandaker *et al.*, 2008) and that there was a general trend towards increased antioxidant activity with increased total phenolics content. These beneficial effects of phenolics make it necessary to understand the circumstances under which they are synthesized and accumulated in these plants. Spider plant accumulates high levels of anti-nutritional factors such as nitrates in addition to their high nutritional value. This has long been a concern to human nutrition and health (Franceschi and Nakata, 2005).

Research studies (Kopsell *et al.*, 2003; Aires *et al.*, 2006) have shown that availability of plant nutrients can be an important factor in determining the activity of secondary metabolism within plants. Nitrogen is one of the most important factors controlling the yield and quality of vegetables (Juan *et al.*, 2008). In developing countries, such as Kenya, nitrogen is often supplied as manure because of the high cost of synthetic fertilizers. However information on the effect of manure or inorganic fertilizers on the polyphenols contents of vegetables is still scanty. Only a few studies (Stout *et al.*, 1998; Juan *et al.*, 2008) have investigated the effect of different levels of nitrogen on the total phenolics.

Secondary metabolites are also known to vary in amount and content depending on the age of the plant (Dumas *et al.*, 2003). Although it has been reported that there exists a large variability in the levels of phenolic compounds at various stages of

maturation (Ellnain-Wojtaszek *et al.*, 2001) of some leafy vegetables, the changes that occur in the content of these chemicals at different growth stages of traditional leafy vegetables found in Sub-Saharan Africa is poorly understood. Even so one needs to know the best time to harvest. Nutritional quality of vegetables is dependent on factors like variety, degree of maturity and the weather conditions during growth (Lee and Kader, 2000; Olsson *et al.*, 2004). It can also be influenced by post harvest handling and storage (Gil *et al.*, 1999; Howard *et al.*, 1999; Patil *et al.*, 1995). There is higher amount of vitamin C present in ripe mature vegetables, whose content increases progressively in advance stages of maturity according to Maroun *et al.*, (2009).

The present project was therefore designed to determine the levels of total phenolic, for total phenolic, total flavonoids, tannins, alkaloids, nitrates, proximate composition, minerals and vitamin C content in five accessions of spider plant grown using a mineral fertilizer calcium ammonium nitrate (CAN) and cattle manure, during different stages of maturity.

1.2 Problem Statement

Surveys have shown that spider plant is among the traditional leafy vegetables whose consumption is on the increase in Kenya (IPGRI, 2003). However, due to lack of improved varieties and lack of fertilizer use has led to low yields of the crop (Ekpong, 2009; Madisa *et al.*, 1997). Since the vegetable is a C₄ plant it is expected to do very well in both the tropics and sub-tropics (Brown *et al.*, 2005). But this has not been achieved due to poor agronomic practices like lack of research information

regarding various varieties together with their optimum handling and nutrient handling during cultivation to optimize phytonutrient quality. Presently, there are many genotypes of spider plant at offer (IPGRI, 1997). However, majority of these varieties are yet to be characterized in terms of their nutritional and phytochemical quality in relation to nitrogen application or different stages of harvest. The organoleptic properties like taste, appearance and texture differ widely among varieties and across stages of development due to accumulation of phenolics and fibre which influence them (Chweya and Eyzaguirre, 1999).

Nitrogen application of between 100-250 Kg N/ha has been shown to increase fresh and dry above ground biomass in leafy vegetables such as *Solanum retroflexum* and Brassica raph (Van Aeverbeke *et al.*, 2007) in comparison to vegetable which did not receive any nitrogen. With this information, commercial farmers may be prompted to apply nitrogen indiscriminately to boost leaf yields (Agong and Masinde, 2006). This might negatively impact on their phytochemical, nutritional and sensory qualities. It can also lead to accumulation of nitrates which can cause a health risk to consumers. The response to nitrogen application could be genetically moderated. However, there is scarcity of data on response to spider plant accession to both nitrogen application and stage of maturity at harvest.

1.3 Justification

Poverty and the insufficient supply of nutritious foods are hindrances to an adequate and balanced diet which is essential for health. This affects majority of people in developing countries (Negi and Roy, 2003). This issue may be solved in part by

increasing the consumption of locally available foods like *Cleome gynandra* because these are inexpensive and can be highly nutritious. Because many of the households frequently consume these vegetables several strategies have been adopted by farmers to meet the rising demand. Leafy vegetables like *Cleome gynandra* are an excellent source of protein, vitamins and minerals, and dietary fibre (Orech *et al.*, 2005) and being familiar and inexpensive these can be used by large segments of the population to meet essential dietary requirements. Spider plant vegetables are one of the most cost-effective and sustainable solutions to counter micronutrient deficiencies, which affect far more people than hunger alone and are widespread in most of sub-Saharan Africa (Prabhu and Barrett, 2009).

1.4 Objectives

1.4.1 Main objective

To determine the influence of nitrogen application and time of harvest on the level of phytochemicals, nutrients and sensory attributes of five selected accessions of spider plant

1.4.2. Specific objectives

1. To evaluate the type and amount of phytochemicals and the changes caused by nitrogen and stage of harvest
2. To determine the influence of nitrogen application and harvest stage on nutritional composition of the five accessions of spider plant (*Cleome gynandra*)
3. To determine the effect of nitrogen application on the sensory attributes of spider plant accessions

4. Evaluate the *in vitro* antioxidation and antimicrobial properties of the various cultivars of spider plant
5. Determine the effect of boiling on phytochemical and nutrient retentions.

1.5 Null Hypotheses

1. Application of nitrogen and stage of maturity do not influence the phytochemicals and nutritional compositions of the five accessions of spider plant
2. Application of nitrogen does not influence the sensory properties of the five accessions of spider plant.
3. The five accessions of spider plant do not have antioxidant and antimicrobial activity.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Description of Spider plant

Spider plant (*Cleome gynandra* L.) (appendix VI) belongs to the botanical family Capparaceae which contains between 700 and 800 species, divided into 45 genera (Kuhn 1988; Kokwaro *et al.*, 1990). The vegetable is an erect herbaceous annual herb, which is branched and rather stout. Favourable agronomic and environmental conditions, it can grow up to 1.5 m in height, and is usually 0.5-1.0 m tall (Chweya and Eyzaguirre, 1999). Locally it is known as *Chinsaga* in Kisii, *Adek* in Dholuo and *Tsisaka* in Luhya (Chweya and Eyzaguirre, 1999).



Spider plant photo (Kebwaro, 2012)

It has a long tap root, with a few secondary roots with root hairs. Stems and leaf petioles are thickly glandular and rarely glabrous. They exhibit variable pigmentations, from green to pink to purple. Leaves are alternate, digitately palmate and petiolate. Each leaf has 5-6 leaflets, but most commonly 5 (rarely 3-4), which are pinnately dissected and sessile. They vary from obovate to elliptic in shape, and are usually 2-10 cm long and 2-4 cm wide. They are sparsely hairy, but this is variable, and they have finely toothed margins or round ends. The petioles are 3-23 cm long, the cotyledonary leaves have single leaflets, and leaves are oppositely arranged on the stem. (Kuhn, 1988).

2.2 Influence of agronomic practices

Choice of proper planting material, field preparation, fertilizer application, weeding and use irrigation in absence of rain are some of the agronomic practices in cultivation of vegetables (Leskovar *et al.*, 2009).

2.2.1 Application of fertilizer

Application of nitrogen is a common practice that has shown positive results. Nitrogen being one of the most important essential plant nutrients it controls quality and yield of vegetables (Opiyo, 2004). Moreover, nitrogen modulates the biosynthesis of secondary metabolites (like phenolic compounds, glucosinolate, carotenoid, among others) (Maria *et al.*, 2010). According to Rossini *et al.*, (2011) nitrogen application led to 40% increase in maize plant biomass. On the same note Ruiz Diaz *et al.*, (2009) realized a 16% increase in soybean dry weight by nitrogen application as opposed to plants which did not receive any nitrogen at all.

A number of studies have reported that an increase in fertilization significantly promotes the total phenolic contents and the antioxidant activity in leaf mustard (Li *et al.*, 2008), broccoli (Vallejo *et al.*, 2003; De pascale *et al.*, 2007) and ‘friarello’, a local *B. rapa* crop widely grown in Southern Italy (Pereira, 2009). Li *et al.*, (2008) also determined the effect of nitrogen supply on leaf mustard and found that the total phenolic content was considerably decreased by increasing nitrogen fertilization. These studies provide clear evidence that nitrogen and sulfur nutrition can be used to manipulate total phenolic concentrations of spider plants with potential benefits to human health and as a result, it can be concluded that sulphur fertilization may improve the nutritional value of these crops.

Several studies have compared the contents of certain phytochemicals between organic and conventional fruits and vegetables (Sousa, 2005; Zhao *et al.*, 2009; Young *et al.*, 2005). Sousa *et al.*, (2005) studied the content of phenolic compounds in tronchuda cabbage under organic and conventional agriculture and they conclude that generally, leaves from organic culture have higher amounts of phenolics, probably due to the interference of mineral fertilizers and pesticides with the biosynthetic pathway of phenolic compounds (Leskovar *et al.*, 2009).

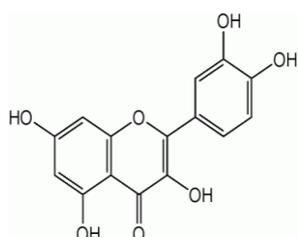
In another study, Young *et al.*, (2005) found that pak choi samples grown organically had higher levels of total phenolics than conventional samples, but these differences among organic and conventional growing were not found in collards. They concluded that the production method did not increase the biosynthesis of phenolics but the organic system provided an increased opportunity for insect attack, resulting

in a higher level of total phenolic agents in pak choi. Thus, insect attack might be a biotic stress factor contributing to higher levels of total phenolic agents in some vegetables from organic production systems (Maria *et al.*, 2010).

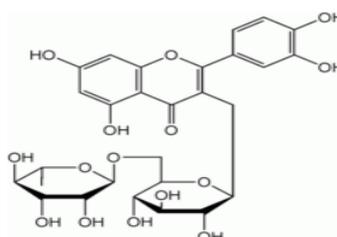
In another experiment to determine the production of alkaloids in *Datura innoxia* in the different plant parts at different increasing fertilization ratios, total alkaloid in most plant parts were consistent and positively correlated with the ratio of fertilizer application up to 600 kg per hectare and then tended to decrease at 800 kg per hectare. The maximum contents of total alkaloids were found in the leaves and fruits as compared to other organs while the maximum alkaloid content was recorded at 600 kg N per hectare (Al-Humaid, 2003).

Flavonoids are also said to be affected by availability of nutrients during growth (Sousa *et al.*, 2008). Flavonoids are polyphenolic compounds comprising fifteen carbons with two aromatic rings connected by a three-carbon bridge, hence C₆-C₃-C₆ (Figure 1). They are the most numerous of the phenolics and are found throughout the plant kingdom (Crozier *et al.*, 2009). They are present in high concentrations in the epidermis of leaves and fruits and have important and varied roles as secondary metabolites, being involved in processes like UV protection, pigmentation, stimulation of nitrogen-fixing nodules and disease resistance (Cushnie and Lamb, 2005).

Quercetin
(Quercitrin)



Quercetin-3-rutinoside



Quercetin-3-rhamnose

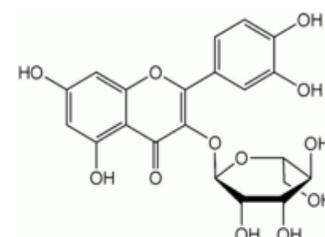


Figure 1: The free aglycone and the glycosylated form of quercetin

On the contrary nitrogen at high rates tends to decrease vitamin C content while it can be increased by lower to moderate rates (Seung and Adel, 2011).

2.3 Different agroecological zones

Depascale *et al.*, (2007) stated that location influence the mineral and trace element compositions of rice, wheat, oats and barley and these are mainly attributed to the altered soil conditions and that the nature and chemical composition of the soil are also involved in location differences in mineral elements. Mogren *et al.*, (2006) stated that variations in the chemical compositions of leafy vegetables, including quantity of compounds that are useful and detrimental to humans are influenced by environmental conditions and the age of plants at harvest.

Phytochemicals present in most vegetables crops are very susceptible to changes in environmental conditions. Phenolic contents are affected by biotic stresses (insect

attack and pathogen infection) and abiotic stresses (light, temperature, nutrient supplies, water availability, growing conditions and UV radiation) besides storage conditions, post-harvest treatments and the estimation methods (Depascale *et al.*, 2007; Gawlik-Dziki, 2008).

These conditions, besides the biosynthesis of phenolic antioxidant compounds, affect the final concentration of polyphenols in plant tissues. Phenolics are produced in plants as secondary metabolites via the shikimic acid pathway. Phenylalanine ammonialyase (PAL), the key enzyme catalyzing the biosynthesis of phenolics from the aromatic amino acid phenylalanine, has been found to be responsive to biotic and abiotic stresses (Maria *et al.*, 2010).

2.4 Stage of crop maturity

Age of the plant at which harvesting is done is also proved to affect vegetable composition. For instance secondary metabolites are also known to vary in amount and content depending on the age of the plant (Dumas *et al.*, 2003). Although it has been reported that there exists a large variability in the levels of phenolic compounds at various stages of maturation (Ellnain-Wojtaszek *et al.*, 2001) of some leafy vegetables, the changes that occur in the content of these chemicals at different growth stages of traditional leafy vegetables found in Sub-Saharan Africa is poorly understood. Adouko *et al.*, (2008) reported that a study on okra fruits showed that lipid concentration increased with age. At the same time it was observed that sugar and protein concentrations were highest in fruits that were 5 to 9 days-old on the plant before harvest.

A study on different stages of maturity of an *Amaranthus* species (*Koyyathotakura*) in India indicated that phosphorus content was more at 15 days of age (63 mg/100 g) and decreased to (60 mg/100 g) at 30 days of age. At 45 days from planting, the phosphorus level further decreased to (56 mg/100 g). Calcium content of the vegetable at 15 days of age was (105 mg/100 g) and increased to (136 mg/100 g) at 30 days of age. It however decreased to (121 mg/100 g) at 45 days old. Magnesium content of the vegetable studied was (42 mg/100 g) at 15 days old, increased to (77 mg/100 g) at 30 days old and then decreased to (72.33 mg/100 g) at 45 days from planting (Khader and Rama, 2003). In the same study, it was shown that as the plant matured from 15 days to 30 days; iron and manganese contents increase whereas zinc and copper contents decrease. Barillari *et al.*, (2006) noted that can also be affected by not only the genotype, pre-harvesting climatic conditions, harvesting methods, post harvest handling procedures but also maturity.

The stage of maturity can seriously influence sensory quality of vegetable crops (Mattheis and Fellman, 1999). According to Gajewski and Arasimowicz (2004) eggplant cultivars grown in a PE foil tunnel differed in some sensory traits when they were evaluated immediately after harvest with the maturity stage of fruits playing a key role in affecting their quality. Because of differences in maturity, fruits obtained from plants grown in a greenhouse with controlled climate differed in sensory traits from fruits of plants grown in a foil tunnel (Gajewski *et al.*, 2009).

2.5 The effect of postharvest practices on vegetable quality

2.5.1 Influence of processing and cooking

Phenolics in vegetables exist in both free and conjugated forms. Generally, only conjugated flavonoids are present in fresh vegetables, but aglycones (Figure 1) may be found as a result of food processing (Maria *et al.*, 2010). It is known that processing may affect the concentration and biological activities of different compounds present in plants to a significant extent (Ganiyu, 2005). This aspect seems to be very important taking into account that only some vegetables are consumed in a raw state and most of them are processed before consumption (Maria *et al.*, 2010).

The recent literature data show a consistent trend for the effects of thermal processing on the total antioxidant activity in vegetable *Brassica* crops when comparing to other vegetable crops; however, when compare to the total flavonoid or total phenolic content, the results did not show such consistency. This suggests that the effect of thermal processing on phenolic, flavonoid or total antioxidant activities is different in different products and deserve further research. In addition, differences in processing methods may have different effects on the content of distinct phytochemicals (Zhang and Hamauzu, 2004; Racchi *et al.*, 2002).

Industrial processing such as blanching, canning, sterilizing and freezing, as well as cooking methods are expected to affect the yield, chemical composition and bioavailability of antioxidants (Podsdek, 2008). Operations such as cutting and slicing may cause a rapid enzymatic depletion of several naturally occurring antioxidants as a result of the cellular disruption, which allows contacts of substrates

and enzymes. During vegetable cooking, qualitative changes, antioxidant breakdown and their leaching into surrounding water may influence the antioxidant activity of the vegetables (Podsedeck *et al.*, 2008).

Some antioxidant compounds like ascorbic acid and carotenoids are very sensitive to heat and storage and are lost during different vegetable processing steps (Zhang *et al.*, 2004). However, flavonoids and some phenolic compounds are quite stable at high temperature and over long periods of storage (Vallejo *et al.*, 2003). Several studies have shown that blanching has a significant effect on the contents of ascorbic acid and total phenolics, and on the antioxidant activity of green leafy vegetables (Gliszczynska-Swiglo *et al.*, 2006).

Blanching of vegetables does not necessarily cause the loss of antioxidant properties. In some vegetables, blanching might actually increase the availability of the natural occurring antioxidant components besides improving the palatability of vegetable crops (Lin and Chang (2005; Zhang *et al.*, 2004).

The studies provide information that the loss of dietary antioxidants is caused by the cooking conditions, such as the type of cooking (conventional, steaming, microwaving, just to name a few), cooking time and amount of water. A loss of antioxidant capacity after boiling has been observed for several vegetables (Zhang *et al.*, 2004). It is well known that the cooking process drastically reduces the vitamin C content of vegetables and several other authors report a loss in the phenolic content of vegetables after cooking (Natella *et al.*, 2010).

The overall loss of antioxidants (for oxidation, as in the case of vitamin C or for a simple diffusion in the cooking water, as in the case of phenolics) results in the decrease of antioxidant capacity. In another study, Vallejo *et al.*, (2003) compared the losses in phenolics compounds when broccoli was submitted to high pressure boiling, low-pressure boiling, steaming and microwaving. The authors found clear disadvantages when microwave cooking was used, noticing losses of 97, 74 and 87% in flavonoids, sinapic acid derivatives and caffeoylquinic acid derivatives, respectively. To this respect, Zhang and Hamauzu (2004) reported losses in the total phenolics of 62% in broccoli florets and of 43% in broccoli stems. Similar results were found by Francisco *et al.*, (2010) who reported a loss of 65–75% of flavonoids and 70–80% of hydroxycinnamic acids under conventional and high-pressure cooking in turnip tops. Nevertheless, losses were reduced to 20–30% by steaming cooking, showing that this is the ideal method to preserve secondary metabolites in *Brassica* crops.

Lin and Chang (2005) evaluated the antioxidant activity of broccoli under different cooking treatments and found that a precooking and/or cooking treatment had no profound effect on the antioxidant properties of broccoli. In another study, Sultana and Anwar (2008) reported the effects of different cooking methods (boiling, frying and microwave cooking) on the antioxidant activity of some selected vegetables including cabbage, cauliflower, yellow turnip and white turnip and concluded that all the cooking methods affected the antioxidant properties of these vegetables; however, microwave treatment exhibited more deleterious effects when compared to

those of other treatments. Most phenolic compounds are water soluble and they are recovered in the water after cooking (Zhang *et al.*, 2004).

The studies established that the steam-cooking of broccoli results in an increase in the content of flavonoids and phenolic acids as compared to fresh broccoli, whereas cooking in water has the opposite effect. The increase in the content of polyphenols and carotenoids is related to their enhanced availability whereas the observed losses of the compounds are mainly due to their leaching into the cooking-water (Sousa *et al.*, 2008).

However, other studies reported very slight losses of total flavonoids and caffeoylquinic derivatives in broccoli (11% and 8% respectively), while no loss of total sinapic and feruloyl derivatives occurred (Zhang *et al.*, 2004). During steaming, phenolic compounds can remain in the edible part of broccoli, probably owing to the inactivation of oxidative enzymes (Zhang *et al.*, 2004). Natella *et al.*, (2010) concluded that microwave and pressure cooking are less detrimental than boiling to the phenolics content of several vegetables.

2.5.2 How storage affect quality of vegetables

Several studies indicate an increasing content of polyphenols for material stored under different modified atmosphere packaging for few days at low temperature. This might be an indication of further biosynthesis of polyphenols for plant protection in the first days after harvest (Starynska *et al.*, 2003), presumably triggered as a reaction to stress in the plants. Furthermore, it has been reported that

longer storage times resulted in a decreased phenolic content, like flavonol glucosides in several vegetable crops (Price *et al.*, 1997).

In *Brassica* crops for example, the influence of storage on free phenolic content was recently studied in different cultivars of Chinese cabbage cultivated in Germany under field conditions (Harbaum *et al.*, 2008). These authors found that storage at 20 °C resulted in rapid yellowing and floppy leaves which resulted in an undesirable appearance and sensory quality for consumers. The increasing levels of polyphenols observed in different works in the plant from post-harvest treatments (storage) open up possibilities for increased phenolic content in vegetables and foods. Also postharvest storage can influence sensory characteristics of eggplant fruits, as it was reported by Gajewski *et al.*, (2006). Overall sensory quality of the fruits was found being significantly lower after 3-week storage than that of freshly harvested ones.

2.6 Genetic diversity and its effect on the quality of vegetables

Phenolics are distributed differently depending on the crop and on the plant part evaluated. External and internal leaves of different *B. oleracea* crops like tronchuda cabbage (Ferrerres *et al.*, 2005) savoy cabbage (Martinez *et al.*, 2010) were found to be different in terms of total phenolic content. Quercetin, kaempferol and phenolic acids derivatives from the external and internal leaves, seeds and sprouts leaves of tronchuda cabbage have been reported by several researchers (Ferrerres *et al.*, 2005; Sousa *et al.*, 2007) and the different composition seems to be determinant for the antioxidant activity displayed by each.

Biosynthesis and concentration of phenolic compounds in plants depends on genetic and environmental factors. Several studies have demonstrated that there is a substantial and significant variation for the antioxidant phytochemicals in vegetables. For instance, *Brassica* species, exhibit variation both within and among species, and even among crops of the same species; thus, the potential health benefits provided by cruciferous crops will depend firstly on the genotype. The phenolic compound composition may differ between cultivars, as well as among parts within the individual plant as shown in several crops like turnip greens and turnip tops (Francisco, 2010) and tronchuda cabbage according to Fernandes *et al.*, (2007).

2.7 Uses of *Cleome gynandra* (spider plant)

Spider plant is consumed in most African and several South Asian countries. The plant is nutritious and is known to contain high levels of β -carotene, vitamin C, and moderate levels of calcium, magnesium, and iron (Silué, 2009). Throughout Africa, the tender leaves or young shoots, and often the flowers as well, are eaten boiled as a pot herb, tasty relish, stew or side dish. The leaves and shoots are gathered from the wild or are cultivated.

In East Africa, fresh leaves are used as ingredients in other mashed foods, and the dried leaves are ground and incorporated in weaning foods (Mathenge, 1995). The leaves are rather bitter, and for this reason are cooked with other leafy vegetables such as cowpea (*Vigna* spp.), amaranths (*Amaranthus* spp.) and black nightshade (*Solanum nigrum* L.). To reduce the bitterness, cream may be added to the boiled leaves, and the mixture should preferably be left overnight in a cooking pot. The leaves could also be boiled and the water discarded and then combined with other

ingredients in stew. The leaves and tender shoots are boiled whole, or chopped, and may be mixed with other ingredients. In Zambia, pounded groundnuts are often added to dishes to enhance flavour.

The high fibre content (1.3-1.4% -fresh weight) of the leaves enables them to be dried and stored. The leaves may be blanched, made into small balls and sun-dried. These balls can be stored for more than 6 months, and are reconstituted by soaking in water before being used in cooking. In several African countries, the vegetable is an important food in rural areas. In some countries, only this leafy vegetable is available during the relish-gap period, and therefore plays a significant role in household food security during drought (Chweya and Mnzava, 1997).

The seeds are oleiferous, containing polyunsaturated oil, which is extracted by pressure and does not need refining. They are used as bird food. The seed cake has an excellent acid spectrum and can therefore be utilized in animal feeds. In some African countries (e.g. Zambia, Zimbabwe, Botswana, Malawi, Uganda, Tanzania and Kenya), during periods of abundance, the leaves and young tender shoots are sold in rural and urban markets by gatherers and growers, who are mostly rural women. The vegetable can therefore provide a source of income for rural areas, especially for the poor and the unemployed (Chweya and Mnzava, 1997).

Seed of spider flower has high levels of polyunsaturated oils that can reach up to 29.6 %. The oil can be extracted by simple pressing and does not require refining. The seed cake can be used for animal feed, and the seed itself for feeding birds.

Spider flower remedies are used to alleviate migraine, vomiting, diphtheria, vertigo, headache, pneumonia, septic ears, and stomach ailments. Spider plant has insecticidal and insect repellent properties. Spraying an aqueous extract of spider flower can considerably reduce aphid and thrip populations. Intercropping spider plant with cabbage also reduces diamondback moth as well as thrip attacks. Intercropping spider plant in rose-producing greenhouses at 8.3plants/M² was reported to reduce red spider mite populations in Kenya. The plant also was shown to have anti-tick properties (Silué, 2009). *Cleome gynandra* L. has traditionally been used for the treatment of rheumatic and other inflammatory conditions according to Narendhirakannan *et al.*, (2005). In recent years, phenolic compounds, which are in abundance in spider plants, have been intensively investigated because of their potential health-promoting effects (Jahangir *et al.*, 2009; Vallejo, 2002).

They have been reported to possess many useful properties for human health, including anti-inflammatory, enzyme inhibition, antimicrobial, antiallergic, vascular and cytotoxic antitumor activity, but the most important action of phenolics is their antioxidant activity (Cushnie and Lamb, 2005; Podosedek, 2007). Furthermore, phenolic compounds possess other properties such as hydrogen peroxide production in the presence of certain metals, the ability to scavenge electrophiles and inhibit nitrosation reactions and chelate metals and, therefore, they act by blocking the

initiation of several human diseases (Ackland *et al.*, 2005; Fresco *et al.*, 2010; Skandrani *et al.*, 2010).

The antioxidant activity of phenolic compounds is related with its chemical structure that confers them redox properties. They can play an important role in adsorbing and neutralizing reactive oxygen species (ROS), quenching singlet and triplet oxygen, or decomposing peroxides. Reactive oxygen species, derived from oxidation processes, are an important part of the defense mechanisms against infection, but excessive generation of free oxygen radicals may damage the tissue.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Project site

The field experiments were conducted at Ruiru (1° 9' 0" S, 36° 58' 0" E) situated in Central Province, Kenya. The first trial was conducted from March to June, 2011 while the second trial from March to June, 2012. Another trial was set up at the Department of Horticulture, Jomo Kenyatta University of Agriculture and Technology (JKUAT) (latitude. 1° 10' 48' S, long. 37° 07' 12' E) JKUAT farm between June and September, 2012. Greenhouse experiments were conducted at JKUAT green houses from March to June, 2012 and from June to September, 2012. Five accessions of spider plant from the World Vegetable Centre (WVC) in Arusha, Tanzania were grown and were subjected to three nitrogen treatments. All experiments were replicated three times.

The nitrogen treatments were 3.3KgM⁻² of decomposed cattle manure, 2.6g N/plant and 5.2g N/plant of calcium ammonium nitrate (CAN with 26% N). These treatments were selected bearing in mind what the local farmers apply. The treatments were applied in 4 splits, with first quarter of the nitrogen level at three weeks after planting, and the rest 4 days apart after planting. Watering was done by horse pipe daily to keep the soil moist. Aphids were controlled by spraying with Pirimor® (2-Dimethylamino-5,6-dimethylpyrimidin-4-yl dimethyl- carbamate), at 2.5mg/litre of water. During flowering, the flowers were removed daily to encourage vegetative growth.

3.2 Experimental design

The project was designed as a randomized split plot design with three blocks to take care of the replication, as provided in Table 1. The accessions were assigned to the main plots while the nitrogen treatments were assigned to the sub-plots. The vegetables were harvested at 4, 5, 6, 7, and 8 weeks after planting and prepared for analysis. The vegetables were then analysed for total phenolic, total flavonoids, tannins, alkaloids, nitrates, proximate composition, minerals (calcium, iron, magnesium and zinc) and vitamin C content. Samples from the 6th week after planting were also subjected to sensory analysis. Antimicrobial analysis and antioxidation activity were conducted to evaluate the health claims on spider plant while blanching was done to determine retention of nutrients and phytochemicals during processing.

Table 1: Field experimental layout with different nitrogen treatments

Main plot	Sub plot			Main plot	Sub plot			Main plot	Sub plot		
5	T3	T1	T2	3	T1	T3	T2	1	T2	T1	T3
2	T2	T1	T3	5	T3	T1	T2	3	T1	T2	T3
4	T1	T2	T3	2	T2	T3	T1	4	T3	T2	T1
3	T1	T3	T2	1	T2	T3	T1	2	T3	T1	T2
1	T1	T2	T3	4	T2	T3	T1	5	T1	T2	T3

Key: Accessions

TREATMENT

1. Control
2. MLSF 17
3. UGSF 9
4. UGSF 36
5. UGSF 14

T1- 3.3Kg/M² decomposed cattle manure

T2- 2.6g CAN/plant

T3- 5.2g CAN/plant

3.3 Sampling

About 200g of samples from the different treatments were harvested weekly. This was done by destructive sampling. Sampling commenced four weeks after the emergence of seedlings and was done weekly for the next five weeks. The samples were brought to the laboratories in the Department of Food Science and Technology, JKUAT and were immediately prepared for moisture content and ascorbic acid determination.

3.3.1 Cooking of spider plant leaves

The fresh leaf samples were sorted and weighed as per variety and treatment. About 80 grams was washed with running tap water to remove soil particles then rinsed with distilled water and finally boiled in 200mL of distilled water for 15 minutes.

The mixture was then macerated in a Warring blender for 10 minutes and then centrifuged at 4000g for 30 min. The supernatant was then filtered through Whatman No.1 filter paper and the extract was finally preserved aseptically in an airtight bottle at 5°C for later use. This was meant to determine retention of nutrients and phytochemicals. The rest of the vegetable samples were oven dried at 40°C for 24 hours and stored at room temperature for further analysis.

3.3.2 Preparation of samples and extraction of *Cleome gynandra*

Vegetable (*Cleome gynandra*) samples were ground into moderately fine powder using an electric grinder (model M10R Japan) and stored until needed for use (Onoruvwe and Olorunfemi, 1998). A 50g portion of each of the dried vegetable sample were taken and cold extracted with 80% ethanol, using the method of Regnier and Macheix, (1996), with slight modifications. The samples were completely immersed in the solvent and the container shaken for 30 minutes to ensure sufficient contact with the solvent using a Kika Labortechnik Shaker, (Model KS 250 Basic, Staufen, Germany). The mixture was left to stand for 72 hours in an enclosure at $25 \pm 2^\circ\text{C}$. The mixture was then centrifuged at 4,000rpm for 10 minutes at a temperature of 4°C using a Kokusan Centrifuge from Kokusan Corporation (Model 2000C, Tokyo Japan). The supernatant was later filtered using No. 1 Whatman paper filter. The solvent was evaporated to dryness under vacuum at 70°C using a rotary evaporator (Model RE 100B, Bibby Sterilin Ltd, Staffordshire, England). The dry extract obtained was put in a glass light proof container and store at 4°C till a time when it would be used as described by Alanis *et al.*, (2005).

3.4 Proximate analysis

Moisture content, crude fibre, crude fat, crude protein and total ash of the harvested vegetables leaves were determined using the AOAC methods (2000) as described by Indrayan *et al.*, (2005).

3.4.1 Moisture content

A moisture dish was dried in an oven at 105 - 110°C for 1 - 2 h and then cooled to room temperature in a desiccator. About 5g of fresh sample was weighed into the moisture dish and heated in the oven at 105°C for 2 h. It was then cooled to room temperature in the desiccator and the final weight of the sample was taken (AOAC, 2000). The moisture content was then calculated as follows:

$$\% \text{ Moisture} = \frac{(\text{Wt of sample before drying} - \text{Wt of sample after drying}) \times 100}{\text{Wt of sample before drying}}$$

3.4.2 Ash content

For determination of ash content, method of AOAC (2000) was followed. Briefly, the silica crucibles were heated at 550°C for 1 h to obtain the constant weight. They were then cooled to room temperature in a desiccator and weighed. Five grams of the dried sample was weighed into the crucible. It was first heated on a heating mantle till all the material was completely charred, followed by incineration in a muffle furnace at 550°C for 3 - 5 h. It was cooled in a desiccator and weighed. To ensure complete ashing, it was heated again in the furnace for half an hour, cooled and weighed. This was repeated consequently until constant (ash became white or greyish white). The percentage ash content was calculated as follows:

$$\text{Crude ash \%} = \frac{\text{Weight of Ash} \times 100}{\text{Weight of sample}}$$

3.4.3 Crude fat content

Fat extraction was determined using soxhlet method and hexane (b.p 65 - 70°C) was used. The extraction flask was heated to constant weight at 105°C for 1 h then cooled to room temperature in a desiccator. About 5g of sample was then weighed into the extraction thimble and stoppered with cotton wool. The thimble containing the sample was dried at 105°C for 1 h then placed in the extraction apparatus. Hexane was filled to two thirds of the extraction flask. The apparatus was set up and extraction started by heating the flask in a water bath 65°C for 4 hours. The temperature was controlled so that about 80 condensed droplets fell into the thimble per minute. After extraction, hexane was allowed to drain down the flask then the thimble was quickly retrieved with forceps. The collected hexane was then evaporated in a water bath using rotary vacuum evaporator (Bibby Sterling Ltd, RE 100B, UK). The flask containing the extracted fat was then dried at 105°C for 1 hour, and then cooled to room temperature in a desiccator. The weight was finally taken and the % fat calculated as follows:

$$\text{Fat \%} = \frac{\text{Weight of fat extracted} \times 100}{\text{Weight of sample}}$$

3.4.4 Crude protein

The crude protein was determined using micro Kjeldahl method described in AOAC (2000). About 1 g of the sample was weighed accurately and transferred to a digestion flask. Five grams of catalyst K₂SO₄ and 0.5g CuSO₄ was then added.

Fifteen millilitres of concentrated H_2SO_4 was added and heated in a fume hood first with a small flame then increasing the temperature gradually. This converted any organic nitrogen to ammonium sulphate, $(\text{NH}_4)_2\text{SO}_4$, in solution. The contents were then heated until the liquid was colourless. After digestion, the contents of the flask were cooled to room temperature then transferred to a 100 ml volumetric flask and filled up to the mark with distilled water. A 10ml aliquot of the digestion solution was taken into a distilling flask and 15ml of 40% NaOH was added to decompose $(\text{NH}_4)_2\text{SO}_4$ to alkaline.

The solution was distilled using distillation apparatus. Approximately 25 ml of 4% boric acid (H_3BO_3) containing 2 drops of phenolphthalein indicator was placed in the receiver flask below the delivery tube. The burner was then placed under the distilling flask and adjusted so that 60-80ml of the distillate collected in about 10-20 minutes. Ammonia was liberated and it changed the solution in the receiver flask from red (acid) to colourless (neutral) to blue (alkaline). The distillate was then titrated with 0.02N HCl solution. The colour changed from blue to dirty green to orange, which was the end point. Reagent blank determination was also done. The percentage nitrogen was calculated as follows:

$$\text{Nitrogen (\%)} = (V1 - V2) \times N \times f \times 0.014 \times 100/v \times 100/w$$

Where:

V1 = Titre for sample (ml)

V2 = Titre for blank (ml)

N = Normality of standard HCl solution (0.02)

F = factor of standard HCl solution

v = volume of diluted digest taken for distillation (10ml)

w = weight of sample taken.

Crude protein content was obtained by multiplying the percentage nitrogenous matter by a factor of 6.25 (James, 1995).

3.4.5 Crude fibre

Fibre content was obtained from the loss in weight on ignition of dried residue remaining after digestion of fat-free samples under specified condition. This imitates the gastric and intestinal action in the process of digestion. Two grams of moisture and fat free material was refluxed for 30 minutes with 200ml of 1.25% H₂SO₄ in a reflux condenser. It is then removed and filtered using a Whatman filter paper No. 54. The insoluble matter is then washed with boiling water. After filtration and washing, the residue was treated with boiling 1.25% NaOH and again boiled under the reflux condenser for another 30 minutes. It was then filtered, washed with hot water and then 1% HCl finally washed with diethyl ether. The residue was finally ignited in a muffle furnace and the ash weighed. The percentage crude fibre was obtained as follows (AOAC, 2000):

$$\% \text{ Crude fibre} = \frac{W_1 - W_2}{W} \times 100$$

Where W_1 = weight of acid and alkali digested sample, W_2 = weight of incinerated sample after acid and alkali digestion and W = weight of sample taken.

3.4.6 Carbohydrate content

Carbohydrate was determined by difference of the total ash content, crude fat, crude protein and crude fibre from 100%

3.5 Determination of mineral composition

Minerals were determined after dry ashing according to the method described by the AOAC (2000). The total ash obtained after ashing was boiled with 10ml of 20% hydrochloric acid in a beaker and then filtered into a 100ml standard flask. It was then made up to the mark with distilled water. The minerals Na and K were determined from the resulting solution using emission flame photometer. The standard solutions of 0, 2, 4, 6, 8 and 10 ppm of Na and K were prepared from NaCl and KCl salt solutions and aspirated into the flame photometer. Absorbance was then recorded to prepare a standard curve. The levels of Zn, Mn, Ca, Mg, and Fe were determined through atomic absorption spectrophotometer (AAS, Model A 6200, Shimadzu, Corp, Kyoto, Japan) using standard methods. Working standards of 0, 0.5, 1.0, 1.5, 2.0 and 2.5 ppm were prepared from the standard solution by serial dilution. Each standard was aspirated into AAS and its emission and absorption, respectively was recorded to prepare a standard curve. The same procedure was applied for the prepared sample solutions for each extract and results recorded. The mineral concentrations were calculated from the standard curve

3.6 Determination of Vitamin C

The amount of vitamin C in a sample was determined by redox titration method according to AOAC methods (1998). This involved the reaction between ascorbic acid in the sample and 2, 6-dichloroindophenol (DCIP). Trichloroacetic acid (TCA) reagent was prepared by dissolving 10g of TCA in 100ml of distilled water. Standard ascorbic acid (1 mg/ml) was then prepared.

The DCIP solution was prepared by dissolving 0.25g of 2, 6-dichloroindophenol in about 500ml of distilled water. Sodium bicarbonate (0.21g) was then added and dissolved. The resulting solution was finally diluted to 1L with distilled water to make approximate concentration of 250mg DCIP/L. A portion of 5g of the sample was ground in a mortar with acid washed sand using a suitable volume of 10% TCA. It was then transferred into a 100ml volumetric flask and made up to the mark with the TCA reagent, then immediately filtered through a fluted filter paper.

A sample of 10ml of each sample (0.05 mg/ml) was pipetted into a 100ml conical flask and titrated with the DCIP solution until a permanent light red or pink color appeared. The volume of DCIP needed to oxidize all of the ascorbic acid was recorded and the procedure repeated. A blank determination was also carried out with TCA. The ascorbic acid content was calculated using the dye factor determined by the titration of standard ascorbic acid solution with DCPIP dye using the balanced equation for the oxidation-reduction reaction between ascorbic acid and DCIP. Vitamin C content was then obtained as follows:

$$\text{Vitamin C content (mg/100g)} = \frac{(A - B) C}{W} \times 100$$

W

Where:

A = Volume in ml of the indophenol solution used for sample titration

B = Volume in ml of the indophenol solution used for sample blank titration

C = Mass in mg of ascorbic acid equivalent to 1ml of indophenol standard solution

W = weight in g of sample taken for sample preparation.

3.7 Detection of Phytochemicals

Qualitative analysis was carried out to ascertain the presence of the different phytochemicals as described by Trease and Evans (1989) and Harborne, (1998) before quantitative analysis was done. All chemicals used in the study were analytical grade (Sigma-Aldrich, St. Louis, MO, USA). Qualitative analysis of phytochemicals was also done by separation using thin layer chromatography (TLC) (Mallikharjuna *et al.*, 2007).

3.7.1 Determination of alkaloids

2 g portions of extract of each sample were further extracted by warming it for 2 minutes with 20ml of 1% sulphuric acid in a 50ml conical flask in a water bath, with intermittent shaking. It was then centrifuged and the supernatant pipetted off into a small conical flask. One drop of Meyer's reagent was added to 0.1ml supernatant in a semi-micro tube. A cream precipitate indicated the presence of alkaloids (Trease and Evans, 1989).

Quantification of alkaloids was done by the alkaline precipitation gravimetric method described by Harborne, (1998). To 5g of the sample in 250ml beaker, 200ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 4 h at 28°C. It was later filtered via Whatman No 42 filter paper. The filtrate was then concentrated to one quarter of its original volume by evaporation. Concentrated ammonium hydroxide was added drop wise to the extract until the alkaloid precipitated. The alkaloid precipitated was received in a weighed filter paper, washed with 1% ammonia solution and dried in the oven at 80°C. Alkaloid content was calculated and expressed as a percentage of the weight of sample analyzed.

3.7.2 Determination of flavonoids

A 5 ml measure of dilute ammonia solution was added to a portion of the aqueous filtrate of the extract followed by addition of concentrated H₂SO₄. A yellow coloration observed indicated the presence of flavonoids. The yellow coloration disappeared on standing. Total flavonoids were then determined by colorimetric aluminium chloride method (Ebrahimzaded *et al.*, 2008). Briefly, 0.5 mL solution of each plant extracts in methanol were separately mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water, and left at room temperature for 30 minutes.

The absorbance of the reaction mixture was measured at 417 nm with a double beam Perkin Elmer UV/Visible spectrophotometer (USA). Total flavonoid content was calculated both as quercetin and catechin from a calibration curve. The calibration

curve was prepared by preparing quercetin and catechin solutions at concentrations 12.5 to 100 mg/ml in ethanol (appendix II).

3.7.3 Determination of tannins

Tannin was determined by the Folin-Denis colorimetric method described by Kirk and Sawyer (1998). About 0.5 g of the dried powdered sample was boiled in 20ml of water in a test tube and then filtered through Whatman No. 42 filter paper. A few drops of 0.1% ferric chloride were added. A brownish green or a blue-black coloration indicated the presence of tannins. Total tannins were determined by a method by Kirk and Sawyer, (1998). A 5 g portion of the sample was dispersed in 50ml of distilled water and shaken. The mixture was then allowed to stand for 30 min at 28°C before it was filtered through Whatman No. 42 filter paper. Two millilitres of the extract and standard tannin solution (tannic acid) 0, 0.1, 0.2, 0.3, 0.4 and 0.5 mg/ml was dispersed into a 50ml volumetric flask. Similarly 2ml of distilled water was put in separate volumetric flasks as a blank to calibrate the instrument to zero. Two millilitres of Folin-Denis reagent was added to each of the flasks followed by 2.5ml of saturated Na_2CO_3 solution.

The content of each flask was made up to 50ml with distilled water and allowed to incubate at 28°C for 90 min. Their respective absorbance was measured in a UV-vis spectrophotometer (UV mini 1240 model, Shimadzu Corp., Kyoto, Japan) at 760nm. Calculations were done based on the tannins standard curve (appendix I).

3.7.4 Determination of phenolic compounds

To determine presence of phenolic compounds, ferric chloride test were carried out where the extract were diluted to 5ml with distilled water. To this, a few drops of neutral 5% Ferric chloride solution were added. A dark green or a blue-black color indicated the presence of phenolic compounds.

Total phenolic content in the vegetables was estimated spectrophotometrically using Folin Ciocalteu reagent, as described by Spanos and Wrolstad (1990) with slight modification, using gallic acid as a standard. An accurately weighed quantity (2-5 g) of the sample was homogenized or blended into a puree and passed through a cheese cloth to remove debris. It was then centrifuged at 4°C and 12,000g for 20 minutes and the supernatant preserved. The sample was then passed through a 0.45 µL membrane filter. To 0.1ml of the sample extract, 5.0ml of 0.2 N Folin-Ciocalteu reagent and 4.0ml of saturated Na₂CO₃ solution were added. The standard curve was prepared using gallic acid (0, 0.1, 0.2, 0.3, 0.4 and 0.5 mg/ml). The mixture was allowed to stand for 90 minutes and absorbance measured at 765 nm using UV-vis spectrophotometer (UV mini 1240 model, Shimadzu Corp., Kyoto, Japan). The amount of total phenolics was expressed as mg gallic acid equivalents per 100 g sample.

3.7.5 Determination of steroids

Two millilitres of acetic anhydride were added to 0.5ml of vegetable extract of each sample followed by 2ml of concentrated sulphuric acid. Change of colour from violet to blue or to green indicated the presence of steroids (Harborne, 1998).

3.7.6 Determination of saponins

To qualitatively determine saponins, about 2 g of the powdered sample were boiled in 20ml of distilled water in a water bath and filtered. Ten millilitres of the filtrate was mixed with 5ml of distilled water and shaken vigorously to form a stable persistent froth. The froth was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion (Obadoni and Ochuko, 2001).

3.7.7 Test for terpenoids

To confirm the presence of terpenoids, about 5ml of each extract was mixed with 2ml of chloroform, and concentrated sulphuric acid was then carefully added to form a layer. A reddish brown coloration that formed at the interface indicated presence of terpenoids (Harborne, 1998).

3.7.8 Test for anthraquinones

Powdered plant material was boiled with 10% HCl for a few minutes, then filtered and allowed to cool. This was then partitioned against equal volume of chloroform. Formation of rose-pink color upon addition of 10% aqueous ammonium solution indicated the presence of anthraquinones (Harborne, 1998).

3.8 Determination of Nitrates

Nitrates were determined by Salicylic acid method according to Cotaldo (1975). Nitrate stock standard was prepared from potassium nitrate, KNO_3 . This was diluted with distilled water to obtain working standard solutions of 10, 20, 30, 40, 50, 60, 80, and 100mg/L. 5% salicylic acid solution (m/v) in concentrated H_2SO_4 was also prepared. Aliquots (0.1 mL) of working standard solutions (10–100 mg/L NO_3^- -N)

in a test tube were mixed thoroughly with 0.4 mL salicylic acid solution. After 20 min at room temperature, 9.5 mL 2N NaOH solution was slowly added to obtain 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, and 1.0 mg/L NO₃ –N solutions.

Portions (0.1000 g) of vegetable tissues (spider plant ground samples) were suspended in 10mL hot distilled water, kept at 45°C for 1 hour, and then filtered through Whatman No. 42 filter paper. A 0.1 mL volume of the preceding extract was thoroughly mixed in a 30 mL tube with 0.4 mL salicylic acid solution. After 20 min at room temperature, 9.5 mL 2N NaOH solution was slowly added. Samples were extracted and analysed immediately by reading their absorbance at 410 nm with the UV-VIS spectrophotometer.

3.9 Determination of antioxidation and antimicrobial activities

3.9.1 Determination of antioxidant activity

The radical-scavenging activity was determined using diphenyl picryl hydrazyl (DPPH) radical according to Ayoola *et al.*, (2006). This provides information on the reactivity of the test compounds with a stable free radical and gives a strong absorption band at 517nm in the visible region. 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 cuvette placed in the spectrophotometer (Analar grade). Vitamin C was used as the antioxidant standard at the same concentrations as the extract. One ml of the extract was placed in a test tube, and 3ml of methanol added followed by 0.5ml 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 UV-Vis spectrophotometer (UV mini 1240 model, Shimadzu Corp., Kyoto, Japan). All tests were run in triplicate and the radical scavenging activity was then calculated using the following formula:

$$\% \text{ inhibition} = \{[Ab-Aa]/Ab\} \times 100$$

Where: Ab = absorption of the blank sample, Aa = absorption of the extract.

3.9.2 Antimicrobial analysis

Collection and screening of test micro-organisms

Isolates of the following organisms, bacteria *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* as well as fungus *Candida albicans* were obtained from Food Microbiology Laboratory, Department of Food Science & Technology, JKUAT. The bacteria and fungi had been stored in glycerol solution at -20°C prior to use.

Antibacterial assay of spider plant extracts

Pure cultures of the test microorganisms were inoculated with the broth (Oxoid, England), incubated at 37°C for 24 hours, diluted with sterile nutrient broth to a density of 9×10^8 cfu/ml by serial dilution. Sterile disposable plates were used and appropriate media (Table 2) prepared and poured into the sterile displaceable plates as per AOAC method 966.23 (AOAC, 1995). Inoculation of the prepared plates with the organism was done using a sterilized pipette to transfer 0.1ml of the suspensions into the plate followed by spreading with a Canards rod to achieve uniform spread on the plate. Sensitivity of all the micro-organisms to the various extracts was done

using the cork and bore diffusion method by Rojas *et al.*, (2003) and according to Shahidi (2004) with slight modifications.

Using a sterile cork borer of 6 mm diameter, three holes were made into the already set agar in the Petri-dishes containing the bacterial culture. Then 0.1 ml of each of the concentrations 0.1g/ml, 0.2g/ml and 0.3g/ml of the extracts was poured into the wells in triplicates. Standard drugs were used as control. A second control was set up to check the viability of the micro-organisms. The micro-organisms were inoculated in the corresponding agar media and placed in the incubator at 37°C overnight with pure distilled water in the holes.

The controls without vegetable extract were examined for growth and those with the vegetable extract were examined for zones of inhibition of growth. This was estimated by measuring the linear diameter of the inhibition zone. Antibacterial activity was recorded if the zone of inhibition was greater than 9mm (Hassan *et al.*, 2006).

Antibacterial activity was evaluated by measuring the inhibition zone formed around the discs using a ruler calibrated in millimetres. The assay was replicated thrice and the mean values calculated as provided by Junaid *et al.*, (2006). Since the discs used were 6 mm in diameter, mean zone-sizes less than 6 mm meant that the micro-organisms were resistant to the extracts, while greater than that were considered to be due to antimicrobial activity. Standard antimicrobial sensitivity discs containing two antimicrobials, one being antibiotic and the other antimycotics were used as controls. Nalidixic acid (30µg/disc) was used as the standard antibiotic.

Table 2: Microorganisms, agar and incubation time and temperature employed for analysis

Microorganisms	Agar	Incubation time and temperature
<i>Bacillus subtilis</i>	Nutrient agar	37°C for 24 hours
<i>Pseudomonas aeruginosa</i> (27853 ATCC)	Pseudomonas agar	37°C for 24 hours
<i>Escherichia coli</i> (25922 ATCC)	Violet-Red Bile Glucose agar	37°C for 24 hours
<i>Staphylococcus aureus</i> (25923 ATCC)	Baird Parker with egg yolk Tellurite	37°C for 24 hours
<i>Candida albicans</i> (90028)	Potato Dextrose agar with 10% tartaric acid	25°C for 72 hours

Antifungal activity assay

The antifungal activity assay was carried out using the disc diffusion agar method (Ajaiyeoba *et al.*, 2000). Sabouraud dextrose agar (SDA) was poured in the sterile petri dishes and left to dry. About 100µl of the inoculum was introduced into the agar and spread evenly. Sterile extract impregnated discs were placed on fungal seeded plates and incubated at 28°C for 48 hours. As a control, clotrimazole (50µg/disc) was used.

Following an incubation period of 72 hours, plates were removed from the incubator and antifungal activity was evaluated by measuring the zones of inhibition of fungal growth. Clear zones or mean zone-sizes less than 6 mm meant that the fungi were resistant to the extracts, while greater than 6mm meant that the extracts were active

that were considered to be due to antimicrobial activity. The experiment was performed three times and antifungal activity test results were statistically analysed.

3.10 Sensory evaluation

Samples were harvested during the 6th week after planting only. This was due to the logistics involved as samples were grown in pots and their yields were not enough to carry sensory analysis weekly. Vegetables were cooked by steaming and samples were assigned random numbers and subjected to sensory evaluation to determine if they were different. The influence of accession and nitrogen application on the difference in sensory attributes was also underscored. This was done by a team of 28 untrained panellists. Each recorded their responses of likes and dislikes on the taste, colour, texture, aroma and overall acceptability of the vegetables basing on a nine point hedonic scale (Ihekoronye and Ngoddy, 1985; Lawless and Heymann, 1998; Stone and Sidel, 2004). Before each sample testing, the panelists rinsed their mouth with water. The assessment was carried out under natural light at room temperature. A copy of the questionnaire used is attached in Appendix III.

3.11 Statistical analysis

Data obtained from various laboratory analyses of these samples were subjected to the general analysis of variance (ANOVA) employing split plot design, using Genstat statistical software, version 14 (Payne et al., 2006) to check for main effects of treatments. Mean values obtained for nitrate levels were first subjected to logarithmic (base 10) transformation to achieve the assumption of normality, before analysis of variance was carried out.

Duncan's Multiple Range tests (Steel and Torrie, 1980) was used to identify and separate significant differences among treatment means ($P < 0.05$)

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 The Composition of spider plant in terms of accessions

4.1.1 Differences of accessions on proximate composition of spider plant

With the five accessions in experiment, MLSF 17 recorded significantly ($p \leq 0.05$) the lowest moisture content as shown in Tables 3 and 4. This translates to more dry matter than the rest. This observation was true for both JKUAT and Ruiru field trials. The moisture content of fresh samples of *Cleome gynandra* leaves ranged between 80.75 and 92.06. This range in moisture content of *Cleome gynandra* under different harvesting stages might be as a result of structural changes in plants as they grow older. Florkowski *et al.*, (2009), attests that maximum water content of vegetables varies among vegetable types and is dependent upon cultivation conditions and structural differences.

The results obtained in this study compared well with the 81.8-89.6 %, 86.6 % and 90 % reported in *Cleome gynandra* L. in earlier studies by Chweya and Mnzava (1997), Silue (2009) and Hassan *et al.*, (2007) respectively. High moisture content in vegetables is indicative not only of its freshness but also easy perishability (Adepoju and Oyewole, 2008). On the same attribute George *et al.*, (2003) observed that moisture content makes a significant contribution to the texture of the leaves and helps in maintaining the protoplasmic content of the cells; besides making them perishable and susceptible to spoilage by micro-organism during storage. MLSF 17 was also recorded significantly ($p \leq 0.05$) lowest amounts of crude fat. UGSF 9 recorded significantly ($p \leq 0.05$) the highest amount of crude protein at 33.65% on dry

weight basis for all the field trials, while UGSF 36 yielded the significantly highest amounts of both carbohydrates and crude fibre.

Table 3: Percentage proximate composition as influenced by accession of spider plant for Ruiru, March-June, 2012

	Control	MLSF 17	UGSF 9	UGSF 36	UGSF 14
Crude ash	2.82 ^e	2.77 ^d	2.62 ^c	2.50 ^a	2.53 ^b
Crude fat	0.52 ^c	0.51 ^c	0.44 ^a	0.47 ^b	0.48 ^b
Crude fibre	2.16 ^b	2.53 ^e	2.26 ^c	2.41 ^d	2.08 ^a
Crude protein	4.53 ^b	5.70 ^e	5.55 ^d	4.45 ^a	4.73 ^c
Carbohydrates	4.89 ^a	6.03 ^e	5.62 ^c	5.71 ^d	5.23 ^b
Moisture	85.08 ^d	82.32 ^a	83.52 ^b	84.44 ^c	84.95 ^d

Values followed by the same letter within rows are not significantly different according to Duncan's Multiple Range Tests at 5% probability level.

Table 4: Percentage proximate composition of the accessions of spider plant at JKUAT, June-September, 2012

	Control	MLSF 17	UGSF 9	UGSF 36	UGSF 14
Crude ash	3.03 ^c	3.80 ^e	2.62 ^b	2.50 ^a	3.42 ^d
Crude fat	0.45 ^a	0.44 ^a	0.44 ^a	0.46 ^b	0.55 ^c
Crude fibre	2.64 ^e	2.59 ^d	2.26 ^a	2.44 ^c	2.41 ^b
Crude protein	4.12 ^a	4.43 ^c	4.91 ^d	4.31 ^b	4.33 ^b
Carbohydrates	4.46 ^a	4.84 ^b	4.48 ^a	5.07 ^c	5.21 ^d
Moisture	85.30 ^c	83.40 ^a	85.3 ^c	85.22 ^c	84.08 ^b

Values followed by the same letter within rows are not significantly different ($p>0.05$) according to Duncan's Multiple Range Test at 5% probability level.

4.1.2 Variations of accession in minerals and vitamin C content

The various accessions responded differently to mineral and vitamin C composition. For calcium, MLSF 17 recorded significantly ($p \leq 0.05$) the highest amount in JKUAT field trial, UGSF 14 recorded significantly ($p \leq 0.05$) the highest amount in Ruiru second trial (March to June, 2012) (Tables 5 and 6). On the other hand, UGSF 9 recorded significantly the lowest amount in both field and greenhouse trials.

On the case of magnesium, the control accession recorded the highest amount in JKUAT field trial while there was no significant ($p \geq 0.05$) difference between UGSF 9 and UGSF 14 in Ruiru season 1. In the same way, no significant difference was observed between MLSF 17 and UGSF 9 in green house 1 with 39.59 Mg/100g and 39.09 Mg/100g dry sample respectively. With zinc, UGSF 14 was significantly ($p \leq 0.05$) highest than all the other accessions in both JKUAT and Ruiru filed trials, while MLSF 17 was significantly highest in green house trials. On the same note all the accessions were significantly ($p \leq 0.05$) higher than the control in all trials.

In JKUAT and Ruiru field trials all the accessions recorded significantly ($p \leq 0.05$) lower amounts than MLSF 17. In Ruiru, March-June, 2012 alone MLSF 17 recorded 20.84 Mg/100g dry sample of iron. UGSF 14 on the other, remained significantly ($p \leq 0.05$) lower than all the rest in the field trials, while there was no significant difference between UGSF 9 and UGSF 36 in green house 1 trials. In JKUAT field trial MLSF 17 recorded significantly ($p \leq 0.05$) the highest amount followed by UGSF 14. In Ruiru, March-June 2011, the results for the two accessions were reversed. However, the two did show any significant ($p \geq 0.05$) difference in green house trials.

UGSF 36 remained significantly ($p \leq 0.05$) lower than any of the other accessions in the field trials but it had no significant difference with UGSF 9 in the green house trials.

Table 5: Minerals and Vitamin C content of the accessions in field trials (Mg/100g)

	Accession	Ca	Mg	Zn	Fe	Vit C
JKUAT, Jun-Sept, 2012	CONTROL	370.2 ^c	54.2 ^e	0.575 ^a	19.17 ^{ab}	322.2 ^c
	MLSF 17	393.8 ^e	53.21 ^d	0.725 ^d	21.16 ^c	360.8 ^e
	UGSF 9	343.4 ^a	49.32 ^a	0.617 ^b	19.65 ^b	291.1 ^b
	UGSF 36	351.3 ^b	50.41 ^b	0.63 ^{bc}	19.78 ^{ab}	277.7 ^a
	UGSF 14	380.5 ^d	51.5 ^c	0.687 ^c	19.39 ^a	342.5 ^d
Ruiru March-June, 2011	CONTROL	263.1 ^d	51.15 ^c	0.554 ^a	19.45 ^b	288.7 ^c
	MLSF 17	211.4 ^b	50.01 ^{bc}	0.675 ^d	20.84 ^e	305.9 ^d
	UGSF 9	159.9 ^a	49.41 ^b	0.617 ^b	19.77 ^d	260.7 ^b
	UGSF 36	267.1 ^d	47.14 ^a	0.631 ^c	19.71 ^c	248.6 ^a
	UGSF 14	253.7 ^c	49.21 ^b	0.717 ^e	19.41 ^a	343.2 ^e
Ruiru March-June 2012	CONTROL	378.7 ^c	50.75 ^c	0.587 ^a	19.68 ^{ab}	216.7 ^c
	MLSF 17	389.5 ^d	51.01 ^c	0.594 ^{ab}	20.96 ^c	242.6 ^e
	UGSF 9	151.3 ^a	48.41 ^b	0.633 ^c	19.87 ^b	195.5 ^b
	UGSF 36	258.2 ^b	46.76 ^a	0.621 ^{bc}	19.82 ^{ab}	186.8 ^a
	UGSF 14	409.8 ^e	48.01 ^b	0.728 ^d	19.46 ^a	231.1 ^d

Values followed by the same letter within columns are not significantly different ($p > 0.05$) according to Duncan's Multiple Range Test at 5% probability level.

Table 6: Minerals and Vitamin C compositions of the accessions in green house trials (Mg/100g)

	Accession	Ca	Mg	Zn	Fe	Vit C
GREEN	CONTROL	208.17 ^d	40.47 ^d	0.721 ^a	14.85 ^a	154.3 ^b
HOUSE	MLSF 17	167.27 ^b	39.59 ^c	0.909 ^e	16.39 ^d	166.3 ^c
March-June, 2012	UGSF 9	126.51 ^a	39.09 ^c	0.774 ^b	15.23 ^c	139.1 ^a
	UGSF 36	211.35 ^e	37.29 ^a	0.788 ^c	15.32 ^c	138.6 ^a
	UGSF 14	200.77 ^c	38.94 ^b	0.861 ^d	15.02 ^b	168.3 ^c
GREEN	CONTROL	211.11 ^d	41.27 ^d	0.722 ^a	17.89 ^a	155.9 ^b
HOUSE	MLSF 17	176.27 ^b	38.35 ^c	0.911 ^e	19.75 ^d	170.8 ^c
June-	UGSF 9	132.52 ^a	38.02 ^b	0.775 ^b	18.34 ^{bc}	142.7 ^a
September,	UGSF 36	221.45 ^e	37.49 ^a	0.786 ^c	18.47 ^c	141.6 ^a
2012	UGSF 14	206.21 ^c	37.53 ^a	0.863 ^d	18.10 ^b	172.6 ^c

Values followed by the same letter within columns are not significantly different according to Duncan's Multiple Range Test at 5% probability level.

4.1.3.1 Types of phytochemicals present in selected accessions of spider plant

The present study revealed the presence of a wide array of phytochemicals including alkaloids, flavonoids, tannins, saponins, steroids and phenols (Table 7). Alkaloids, flavonoids, saponins, phenols and amino acids were most common and present with almost all the accessions, whereas terpenoids, steroids and anthraquinones were absent in most accessions.

Of paramount importance are their antioxidant activity and their wide range of pharmacologic properties of the spider plant (Wargovich, 2000). They have been associated with anticarcinogenic and antiarteriosclerotic properties and they have the ability to fight dental carries and diarrhoea (Hertog *et al.*, 1992; Prior and Cao, 2000;

Imungi, 2002). The polyphenols are also considered to have potential for the management of HIV/AIDS. For instance, caffeic acid derivatives (such as dicaffeoylquinic and dicaffeoyltartaric acids) have been shown to selectively inhibit Human Immunodeficiency Virus type1 (HIV-1) integrase (Robbins, 2003). In addition, *in vitro* studies have shown that quercetin prevents oxidation of low density lipoprotein (Kandaswami and Middleton, 1994), a process which is thought to be an intermediate step in the formation of atherosclerotic plaques (Hollman, *et al.*, 1997). It is possible that the preference for spider plant vegetables shown by some communities arises not only from desire to enrich their diet, but also from their ability to prevent and cure diseases.

Table 7: Phytochemicals detected in selected accessions of spider plant

	Control	MLSF 17	UGSF 9	UGSF 36	UGSF 14
Alkaloids	++	++	++	++	++
Flavonoids	++	++	++	++	++
Terpenoids	++	-	++	-	++
Phenolics	++	++	++	++	++
Saponins	++	++	-	++	++
Steroids	-	-	++	++	-
Tannins	++	++	++	++	++
Anthraquinones	++	-	-	-	++

Key: ++ Present, - Absent

4.1.3.2 The differences of accessions on phytochemical composition

The JKUAT field trial any show accession UGSF 14 record significantly the highest amounts of both total phenolic content and tannins, UGSF 36 recorded significantly ($p \leq 0.05$) the highest amount of both alkaloids and total flavonoid content in the same

field trial (Table 8). This observed were as well observed in green house season 1 trial (Table 9).

Table 8: Phytochemical content of the accessions (Mg/g) in green house trials

	Accession	Total Phenolics (GAE)	Alkaloids	Total Flavonoids (QE)	Tannins (TAE)
GREEN HOUSE	CONTROL	9.51 ^a	14.43 ^a	13.19 ^a	8.798 ^a
	MLSF 17	9.98 ^{ab}	19.36 ^b	17.71 ^b	9.233 ^b
March- June, 2012	UGSF 9	10.35 ^b	28.31 ^d	25.9 ^d	9.576 ^c
	UGSF 36	11.73 ^c	31.76 ^e	29.06 ^e	10.85 ^e
	UGSF 14	10.61 ^c	26.31 ^c	24.07 ^c	9.816 ^d
GREEN HOUSE	CONTROL	9.881 ^a	14.88 ^a	13.78 ^a	9.14 ^a
	MLSF 17	10.377 ^{ab}	19.11 ^b	18.71 ^b	9.59 ^b
June- September 2012	UGSF 9	10.75 ^b	27.93 ^c	29.25 ^e	9.95 ^c
	UGSF 36	12.72 ^c	30.79 ^e	24.96 ^d	11.76 ^e
	UGSF 14	11.02 ^b	26.56 ^d	21.45 ^c	10.20 ^d

Values followed by the same letter within columns are not significantly different according to Duncan's Multiple Range Test at 5% probability level. GAE= Gallic acid equivalent, QE=quercetin equivalent, TAE=Tannic acid equivalent

However, in green house trial season 2, UGSF 9 recorded significantly ($p \leq 0.05$) highest total flavonoid content than the rest of the accession. In Ruiru field trials there was no significant difference in total flavonoid and total phenolic content for LMSF 17 and UGSF 14. UGSF 14 further recorded a significantly highest amount of alkaloids in Ruiru season 1 and highest tannins in Ruiru, March-June, 2012 at 28.87 Mg/g and 11.62 Mg/g DMB respectively.

Table 9: Phytochemical composition (Mg/g) in accessions from field trials

	Accession	Total Phenolics (GAE)	Alkaloids	Total Flavonoids (QE)	Tannins (TAE)
JKUAT, June-September 2012	CONTROL	10.46 ^c	27.51 ^b	27.16 ^b	16.56 ^c
	MLSF 17	10.92 ^d	28.99 ^c	28.63 ^c	17.29 ^d
	UGSF 9	10.03 ^a	26.38 ^a	26.05 ^c	15.88 ^a
	UGSF 36	10.26 ^b	26.97 ^{ab}	26.63 ^{ab}	16.24 ^b
	UGSF 14	14.07 ^e	37.56 ^d	37.09 ^d	22.27 ^e
RUIRU, March-June, 2011	CONTROL	12.70 ^b	24.37 ^a	22.18 ^a	11.75 ^b
	MLSF 17	13.55 ^c	27.06 ^d	25.12 ^d	12.53 ^d
	UGSF 9	12.10 ^a	25.78 ^c	23.62 ^c	11.20 ^a
	UGSF 36	12.90 ^{bc}	24.45 ^b	21.41 ^b	11.84 ^c
	UGSF 14	13.81 ^c	28.87 ^e	27.24 ^e	12.65 ^e
RUIRU, March-June, 2012	CONTROL	11.35 ^{ab}	24.47 ^a	22.38 ^a	10.51 ^b
	MLSF 17	12.21 ^c	26.56 ^d	24.92 ^d	11.31 ^d
	UGSF 9	11.15 ^a	25.69 ^c	23.50 ^c	10.32 ^a
	UGSF 36	11.73 ^b	24.72 ^b	22.61 ^b	11.12 ^c
	UGSF 14	12.55 ^c	28.47 ^e	26.04 ^e	11.62 ^e

Values followed by the same letter within columns are not significantly different according to Duncan's Multiple Range Test at 5% probability level. GAE= Gallic acid equivalent, QE=quercetin equivalent, TAE=Tannic acid equivalent

4.1.4 Variations of different accessions in terms of of antioxidation

The results for the influence of accessions on the radical scavenging activity, is presented in figure 2 below. At low concentrations up to 1.5 Mg/ml accession UGSF 14 remained significantly higher before it started to reduce. This was followed by MLSF 17. UGSF 36 remained significantly lower though it had no significant difference with UGSF 9 at the concentration of 1 Mg/ml.

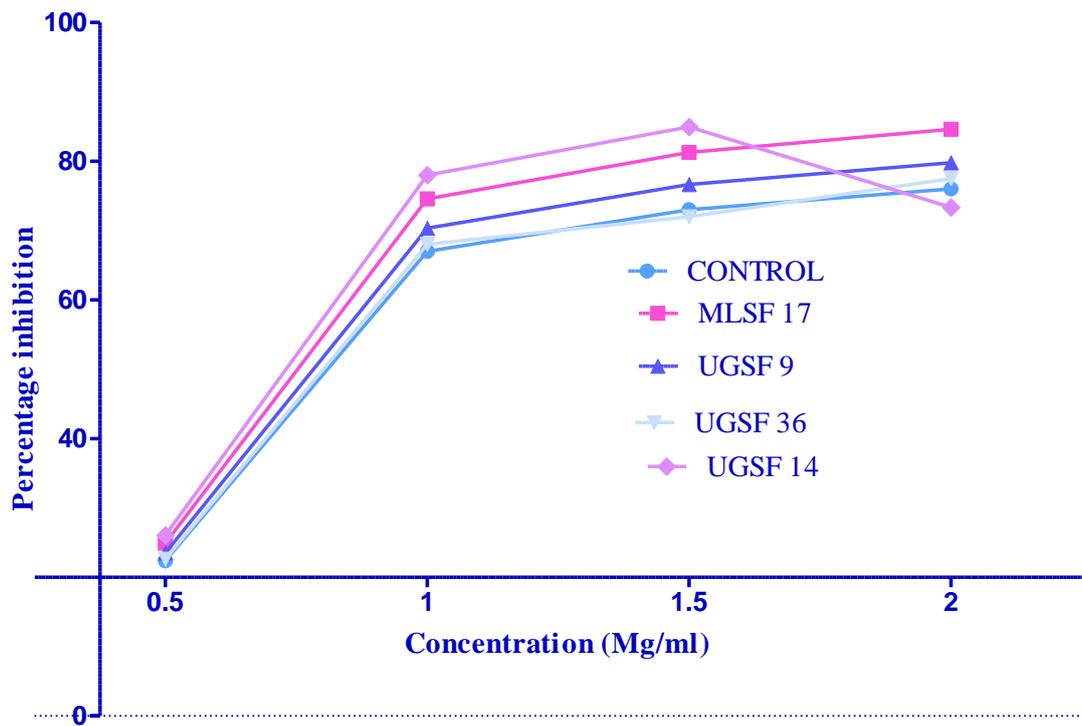


Figure 2: Percentage radical (DPPH) scavenging activity of the accessions

4.1.5 Microbial growth inhibition by the different accessions

The accessions had no significant difference in microbial growth inhibition on *Pseudomonas aeruginosa* ($p \geq 0.05$) while UGSF 14 show significantly ($p \leq 0.05$) highest growth inhibition on *E. coli*, while UGSF 14 and MLSF 17 had no significant difference with the control for growth inhibition on *Bacillus subtilis* (Table 10).

Table 10: Extent of microbial growth inhibition (mm) as influenced by accessions

Accession	<i>Bacillus subtilis</i>	<i>E. coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Candida albicans</i>
Control	12.73 ^b	13.88 ^{bc}	11.50 ^a	11.40 ^b	11.22 ^b
MLSF 17	13.18 ^b	12.28 ^{bc}	10.40 ^a	12.85 ^b	7.62 ^a
UGSF 9	9.45 ^a	11.98 ^b	10.85 ^a	10.92 ^a	7.95 ^a
UGSF 36	9.53 ^a	9.53 ^a	11.08 ^a	10.92 ^a	11.25 ^b
UGSF 14	12.60 ^b	14.15 ^c	11.50 ^a	14.55 ^b	12.18 ^b

Values followed by the same letter within columns are not significantly different according to Duncan's Multiple Range Test at 5% probability level. The extracts were at a concentration of 150Mg/ml

4.1.6 Effect of accession on the organoleptic properties of spider plant

Sensory analysis results of the various spider plant dishes are presented in Table 11. There was no significant difference in the parameters of appearance, aroma, texture, flavour and overall acceptance. So neither the level of nitrogen employed in agronomy nor the accessions proved to be significance. In this case, the four new accessions are much like the control variety and hence consumers can readily consume them as they could the control. On the same note, accept the null hypothesis that nitrogen fertilization or genotype as no influence on the sensory attributes of this vegetable.

Table 11: Sensory attributes as influenced by accession

Attribute	Appearance	Aroma	Taste	Texture	Overall Preference
Control	6.370 ^a	6.556 ^a	6.235 ^a	6.049 ^a	6.420 ^a
MLSF 17	6.691 ^a	6.407 ^a	6.111 ^a	6.333 ^a	6.420 ^a
UGSF 9	6.543 ^a	6.753 ^a	6.309 ^a	6.321 ^a	6.605 ^a
UGSF 36	6.741 ^a	6.630 ^a	6.296 ^a	6.457 ^a	6.605 ^a
UGSF 14	6.748 ^a	6.753 ^a	6.185 ^a	6.383 ^a	6.420 ^a
C.V %	27.1	25.4	27.9	26.4	22.1

Values followed by the same letter within columns are not significantly different according to Duncan's Multiple Range Test at 5% probability level.

4.1.7 Influence of accession on nitrate accumulation

UGSF 36 accumulated the highest significant ($p \leq 0.05$) amount of nitrate in both JKUAT and Ruiru, first (March-June, 2011) field trials as presented in Table 12. On the same note, the control had the highest significant amount of nitrate in Ruiru (March-June, 2012) second field trial. Generally, green house samples accumulated the highest amount of nitrate than samples from the field. In both green house trials the highest significant amount was recorded in UGSF 9, while there was no significant ($p \geq 0.05$) difference between MLSF 17 and UGSF 14.

Table 12: Log transformed Nitrates as influenced by accession (Mg/Kg)

	JKUAT, Jun- Sept, 2012	Ruiru Mar-June, 2011	Ruiru Jun-Sept, 2012	Greenhouse Mar-June, 2012	Greenhouse Jun-Sept, 2012
ACCESSION					
CONTROL	1.2342 ^d	1.1141 ^c	1.1747 ^c	2.0862 ^b	2.0854 ^b
MLSF 17	1.0224 ^a	0.9367 ^a	0.9369 ^a	2.0086 ^{ab}	2.0587 ^{ab}
UGSF 9	1.1568 ^c	1.1821 ^d	1.1132 ^d	2.2432 ^c	2.2534 ^c
UGSF 36	1.7834 ^e	1.9481 ^e	1.0160 ^b	1.9597 ^a	1.9327 ^a
UGSF 14	1.089 ^b	1.082 ^b	1.072 ^c	2.0634 ^{ab}	2.0175 ^{ab}

Values followed by the same letter within columns are not significantly different according to Duncan's Multiple Range Test at 5% probability level.

4.2 Influence of nitrogen application on composition of selected spider plant

accessions

4.2.1 Influence of nitrogen on proximate composition

Tables 13 and 14 present the effect of nitrogen on proximate composition. Application of nitrogen influenced proximate composition differently. Application of N led to significant ($p \leq 0.05$) increase in crude ash and a significant reduction in crude fat. This suggests that applying could increase the amount of mineral composition of the spider plant vegetables. Application of 2.6g N/plant led to significant reductions in crude fibre, crude proteins and carbohydrate content of the vegetable samples. However, the application of N did not significantly increase the moisture content ($p \geq 0.05$).

Table 13: Proximate composition as influenced by nitrogen application at Ruiru, March-June, 2012

N	Crude Ash	Crude Fat	Crude Fibre	Crude Protein	Carbohydrate	Moisture
N1	2.26 ^a	0.62 ^b	2.17 ^b	4.77 ^b	5.33 ^c	84.84 ^a
N2	2.40 ^b	0.49 ^a	1.93 ^a	4.24 ^a	4.39 ^a	85.16 ^a
N3	2.72 ^c	0.43 ^a	2.37 ^c	4.74 ^b	5.06 ^b	84.68 ^a

Values followed by the same letter within columns are not significantly different according to Duncan's Multiple Range Test at 5% probability level. N1=3.3Kg/M² of decomposed cattle manure, N2=2.6g CAN/plant, N3=5.2g CAN/plant.

Table 14: Proximate composition as influenced by nitrogen application at JKUAT, June-September, 2012

N	Crude Ash	Crude Fat	Crude Fibre	Crude Protein	Carbohydrate	Moisture
N1	2.17 ^a	0.62 ^b	2.19 ^b	4.92 ^b	5.38 ^c	84.71 ^a
N2	2.28 ^b	0.44 ^a	1.98 ^a	4.25 ^a	4.55 ^a	85.12 ^a
N3	2.68 ^c	0.44 ^a	2.40 ^c	4.86 ^b	5.11 ^b	84.51 ^a

Values followed by the same letter within columns are not significantly different according to Duncan's Multiple Range Test at 5% probability level. N1=3.3Kg/M² of decomposed cattle manure, N2=2.6g CAN/plant, N3=5.2g CAN/plant.

4.2.2 Influence of nitrogen application on mineral and vitamin C content

Application of calcium ammonium nitrate (CAN) significantly ($p \leq 0.05$) increased the calcium content (Tables 15 and 16). However, there was no notable trend in the case of rate applied as applying 5.2g N/plant was accompanied with significant ($p \leq 0.05$) decrease in calcium from application of 2.6g N/plant in Ruiru first trial (2011). This was however, reversed in Ruiru second trial (March-June, 2012). JKUAT (Jun-Sept, 2012) trials did not record any significant difference. In green house trials application of calcium significantly increased calcium content, with no

difference in the first trial (March-June, 2012) between 2.6 and 5.2g N/plants but 5.2g N/plant being significantly higher in the second green house trial (June-September, 2012).

Table 15: Minerals and Vitamin C compositions as influenced by N application in field trials

	Nitrogen	Ca	Mg	Zn	Fe	Vitamin C
JKUAT, Jun- Sept, 2012	N1	353.4 ^a	47.72 ^a	0.626 ^a	17.23 ^a	330.1 ^b
	N2	369.1 ^b	53.11 ^b	0.702 ^b	23.15 ^c	344.3 ^c
	N3	373.34 ^b	59.47 ^c	0.794 ^c	22.53 ^b	287.4 ^a
Ruiru Mar- Jun 2011	N1	200.8 ^a	49.38 ^a	0.659 ^a	20.10 ^a	292.6 ^b
	N2	268.8 ^c	55.15 ^b	0.738 ^b	26.19 ^c	305.9 ^c
	N3	223.5 ^b	61.75 ^c	0.836 ^c	25.41 ^b	255.5 ^a
Ruiru, March-Jun, 2012	N1	362.2 ^a	43.37 ^a	0.542 ^a	16.73 ^a	221.0 ^b
	N2	379.8 ^b	49.33 ^b	0.675 ^b	21.79 ^c	230.7 ^c
	N3	390.6 ^c	54.26 ^c	0.681 ^c	21.36 ^b	191.9 ^a

Values followed by the same letter within columns are not significantly different according to Duncan's Multiple Range Test at 5% probability level. N1=3.3Kg/M² of decomposed cattle manure, N2=2.6g CAN/plant, N3=5.2g CAN/plant

A clear quadratic trend was observed in the case of both magnesium and zinc contents throughout the field and green house trials. In all cases application of nitrogen led to significant increase in the amount of the two elements. Applying 5.2g N/plant yielded significantly ($p \leq 0.05$) higher magnesium and zinc than application of 2.6g N/plant. With iron content, addition of N fertilizer significantly ($p \leq 0.05$) increased iron in the vegetables. On the same note, application of excess N beyond 2.6g N/plant led to significant reduction in the amount of iron throughout the field and green house trials. For vitamin C, application of nitrogen in moderate (2.6g

N/plant) amounts significantly increased vitamin C content throughout the field and green house trials. However, addition of 5.2g N/plant led to significant reductions in vitamin C content.

Vitamin C, including ascorbic acid and dehydroascorbic acid, is one of the most important nutritional quality factors in many horticultural crops and has many biological activities in the human body. The content of vitamin C in fruits and vegetables can be influenced by various factors such as genotypic differences, pre-harvest climatic conditions and cultural practices, maturity and harvesting methods, and postharvest handling procedures. There was more vitamin C from field trials than from green house trials. This may be as a result differences in the intensity of light during growth. Seung and Kader (2000) noted that the higher the intensity of light during the growing season, the greater is vitamin C content in plant tissues.

Vitamin C recorded in this study within the ranges of 127-484Mg/100g early reported by (Chweya and Mnzava, 1997) in Spider plant and also 330-350Mg/100g on fresh weight, reported in fenugreek by Singh *et al.*, (2010). Nitrogen fertilizers at high rates tend to decrease the vitamin C content in many fruits and vegetables (Seung and Kader,2000). In another study, Lisiewska and Kmiecik (1996) reported that increasing the amount of nitrogen fertilizer from 80 to 120 kg per hectare decreased the vitamin C content by 7% in cauliflower. Based on these reports, nitrogen fertilizers, especially at high rates, seem to decrease the concentration of vitamin C in many fruits and vegetables. Plant growth is generally enhanced by the nitrogen fertilization so that a relative dilution effect may occur in the plant tissues.

Nitrogen fertilizers are also known to increase plant foliage and thus may reduce the light intensity and accumulation of ascorbic acid in shaded parts (Mozafar, 1993). Reduction in ascorbic acid content in leaf tissue resulting from higher fertilizer application rates has also been reported in other crops (Barker, 1999).

Table 16: Minerals and Vitamin C compositions as influenced by N application in green house trials

	N	Calcium	Magnesium	Zinc	Iron	Vitamin C
GREEN	N1	265.8 ^a	35.89 ^a	0.826 ^a	15.57 ^c	174.6 ^c
HOUSE	N2	277.6 ^b	39.94 ^b	0.925 ^b	20.28 ^a	154.6 ^b
Mar-Jun, 2012	N3	280.8 ^b	44.73 ^c	1.04 ^c	19.68 ^b	130.7 ^a
GREEN	N1	255.3 ^a	36.2 ^a	0.828 ^a	15.87 ^a	177.9 ^c
HOUSE	N2	281.8 ^b	40.1 ^b	0.927 ^b	20.68 ^c	157.8 ^b
Jun-Sept, 2012	N3	293.6 ^c	43.23 ^c	1.05 ^c	20.06 ^b	134.5 ^a

Values followed by the same letter within columns are not significantly different according to Duncan's Multiple Range Test at 5% probability level. N1=3.3Kg/M², N2=2.6g CAN/plant, N3=5.2g CAN/plant

4.2.3 The influence of nitrogen application on phytochemical composition

Application of nitrogen fertilizer led to significant reductions in total phenolic content, flavonoids and tannins for both green house and field trials (Tables 17 and 18). Alkaloids content showed a linear increase up to 2.6g N/plant application before starting to decline.

Total phenolics

As the carbon/nutrient balance theory predicts, in terms of a high plant photosynthetic activity relative to the nitrogen supply, the excess of carbon is allocated to phenolics, a nitrogen-free defense substance (Yongke *et al.*, 2005).

Recent work by Sousa *et al.*, (2008) demonstrated an overall trend of higher total phenolics concentration in organically grown tronchuda cabbage, accompanied by lower plant fresh weight, as compared with conventionally fertilized samples. They suggested that the lack of nutrients, particularly insufficient nitrogen supply as a result of a low mineralization rate under organic production, could have boosted synthesis of phenolic compounds while limiting rapid growth of new leaves.

These results are in agreement with those of Li *et al.*, (2008) who found that the total phenolics concentrations of leaf mustard (*Brassica juncea* Coss) were considerably decreased by increasing nitrogen supply. This negative correlation between nitrogen application and total phenolics could be explained by the protein competition model (PCM) (Jones and Hartley, 1999). The PCM hypothesis makes a contingent prediction: when biomass increases in response to elevated nitrogen nutrition, phenolic concentrations will decline because increased protein demand for growth will decrease partitioning of carbon skeletons to phenolics (Jones and Hartley, 1999).

Additionally, water availability, mineral and organic nutrients of the soil have been found to have a marked effect on the phenolic contents of plants (Barberan and Espin, 2001). Under conditions of abundant insulation normally encountered in the

tropics, the rate of photosynthesis of carbohydrates may be so high that if the levels of available nitrogen and minerals are low, they get quickly depleted by synthesis of primary metabolites. The excess carbohydrates can then only be shunted to production of nitrogen- and mineral-free molecules such as the phenolic compounds (Waterman *et al.*, 1984).

Flavonoids

There were significant differences in total flavonoid content between the different sites of study. There were clear differences in flavonoid content across the sources of and amount of N. Just as observed for the total phenolics, the quercetin content decreased with increasing rates of N supplied as CAN. The marked differences in quercetin content between the field trials may be attributable to sunshine hours the crop was exposed to during the growing season and the cooler air temperatures in. Patil *et al.*, (1995) have shown that metrological factors such as temperature and rainfall patterns have a stronger influence on quercetin concentration in onion cultivars than soil factors or plant maturity. Therefore, the most critical factors for the accumulation of flavonoids like quercetin (rainfall and temperature) are also the ones that are more difficult to control in open air.

Alkaloids

Green house and field experiments proved that there was a significant ($p \leq 0.05$) reduction in increasing the N rate from 2.6g N/plant to 5.2g N/plant, as far as the alkaloids were concerned. This observation was in agreement with other researchers. In analysis involving the herb periwinkle, Ziba *et al.*, (2011), noted that increasing rate of nitrogen applied increased the concentration of vinblastine (alkaloid) linearly

till a maximum value at 150Kgha⁻¹. Nitrogen being a constituent of alkaloids plays an important role in synthesis of alkaloids, hence increasing nitrogen leads to an increase in alkaloid content. It is assumed that this increase in alkaloid content is attributed to increase in yield due to fertilization. Lata and Sadowsika (1996) reported that the concentration and total yield of alkaloids increased linearly by increasing the rate of nitrogen. Al-Humaid (2004) found out that increase in nitrogen leads to increase in alkaloid content in datura.

Table 17: Influence of Nitrogen on phytochemical composition Mg/g) in green house trials

	Nitrogen	Total phenolics (GAE)	Alkaloids	Total Flavonoids (QE)	Tannins (TAE)
GREEN	N1	10.89 ^b	11.44 ^b	25.16 ^b	10.56 ^c
HOUSE	N2	10.10 ^a	13.61 ^c	23.33 ^a	9.797 ^a
Mar-Jun, 2012	N3	10.32 ^a	10.84 ^a	23.84 ^a	10.01 ^b
GREEN	N1	11.81 ^c	11.54 ^b	24.61 ^c	10.31 ^c
HOUSE	N2	10.21 ^a	12.47 ^c	22.11 ^a	9.532 ^a
Jun-Sept, 2012	N3	10.46 ^b	10.71 ^a	22.48 ^b	9.774 ^b

Values followed by the same letter within columns are not significantly different according to Duncan's Multiple Range Test at 5% probability level. N1=3.3Kg/M², N2=2.6g CAN/plant, N3=5.2g CAN/plant. GAE= Gallic acid equivalent, QE=quercetin equivalent, TAE=tannic acid equivalent.

Table 18: Influence of Nitrogen on phytochemical composition in (Mg/g) from field trials

Site	Nitrogen	Total phenolics(GAE)	Alkaloids	Total flavonoids(QE)	Tannins (TAE)
RUIRU	N1	12.84 ^c	25.93 ^b	27.28 ^c	11.79 ^c
MAR-	N2	12.01 ^a	29.67 ^c	22.12 ^a	10.23 ^a
JUN, 2011	N3	12.31 ^b	23.86 ^a	23.31 ^b	10.56 ^b
RUIRU,	N1	12.03 ^b	25.37 ^b	26.94 ^c	11.29 ^c
MAR-	N2	11.56 ^a	31.92 ^c	21.92 ^a	10.70 ^a
JUN, 2012	N3	11.80 ^{ab}	21.93 ^a	22.81 ^b	10.93 ^b
JKUAT	N1	10.92 ^a	30.02 ^b	29.65 ^c	18.08 ^c
JUN-	N2	11.10 ^b	32.07 ^c	28.71 ^a	17.29 ^a
SEPT, 2012	N3	11.42 ^c	29.35 ^a	28.98 ^b	17.58 ^b

Values followed by the same letter within columns are not significantly different according to Duncan's Multiple Range Test at 5% probability level. N1=3.3Kg/M², of decomposed cattle manure, N2=2.6g CAN/plant, N3=5.2g CAN/plant. GAE= Gallic acid equivalent, QE=quercetin equivalent, TAE=tannic acid equivalent

4.2.4 Influence of Nitrogen application on antioxidation capacity

Impact of nitrogen application antioxidation capacity (Fig 3) show nitrogen application significantly reduce scavenging capacity. This may be due to the fact that excess nitrogen significantly reduce vitamin C, polyphenols and flavonoids all of which have antioxidant activity, hence a decline in antioxidation activity.

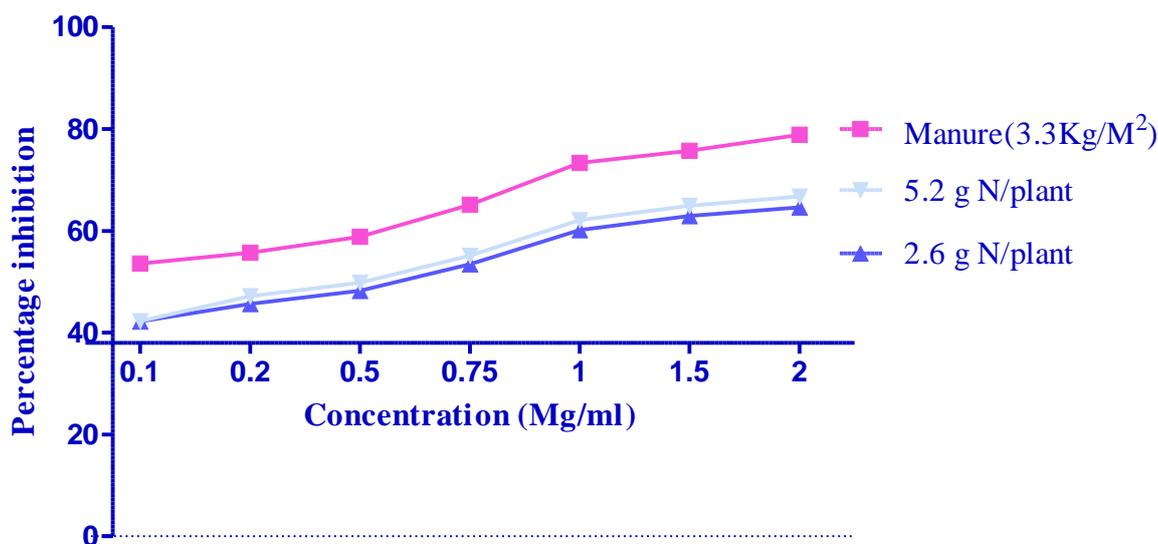


Figure 3: Radical scavenging capacity as influenced by nitrogen application

In Table 19, the minimum concentration needed to scavenge 50% of the DPPH radicals (IC_{50}) is presented. Application of nitrogen is shown to increase the IC_{50} .

Table 19: Maximum inhibition and IC_{50} as per nitrogen treatment

Treatment	Maximum inhibition (%)	IC_{50} (Mg/ml)
N1	92.26±3.33	0.01±0.02
N2	91.5±1.87	0.015±0.04
N3	89.05±2.21	0.014±0.06
Ascorbic acid	65.3±1.14	0.085±0.17

The results are the means of IC_{50} and maximum percentage inhibition values of two replicates \pm SEM and their respective concentrations. SEM= Standard error of the mean. IC_{50} value - the concentration, which scavenged 50% of the DPPH radicals. N1=3.3Kg/M² of decomposed cattle manure, N2=2.6g CAN/plant, N3=5.2g CAN/plant

4.2.5 Sensory properties as a result of nitrogen application

Sensory analysis results of the various spider plant dishes are presented in Table 20.

There was no significant difference in the parameters of appearance, aroma, texture, flavour and overall acceptance. So neither the level of nitrogen employed in

agronomy nor the accessions proved to be significance. In this case, the four new accessions are much like the control variety and hence consumers can readily consume them as they could the control. On the same note, we fail to reject the null hypothesis that nitrogen fertilization or genotype as no influence on the sensory attributes of this vegetable.

Table 20: Sensory attributes as influenced by nitrogen application

Sensory attribute					
Nitrogen	Appearance	Aroma	Taste	Texture	Overall preference
N1	6.630 ^a	6.533 ^a	6.193 ^a	6.341 ^a	6.570 ^a
N2	6.452 ^a	6.607 ^a	6.163 ^a	6.267 ^a	6.393 ^a
N3	6.763 ^a	6.719 ^a	6.326 ^a	6.319 ^a	6.519 ^a
C.V %	27.0	25.3	27.8	26.4	22.1

Values followed by the same letter within columns are not significantly different according to Duncan's Multiple Range Test at 5% probability level. N1=3.3Kg/M² of decomposed cattle manure, N2=2.6g CAN/plant, N3=5.2g CAN/plant

4.2.6 Influence of nitrogen application on accumulation of nitrates

Application of nitrogen led to significant accumulation of nitrates irrespective of the harvest time or the accession concerned (Table 21). Green house trials generally recorded the highest amount of nitrate accumulation.

The coupling of nitrogen assimilation and photosynthetic electron transport in leaves normally imply that light intensity is the key factor in determining nitrate concentrations in leaf crops. Month to month differences in light intensity caused as much as threefold variations in nitrate concentrations in lettuce grown in Western

Europe. Winter-sown crops have generally higher nitrate concentration than summer crops in the same environment and Northern European crops have higher nitrate levels compared to Southern European crops. These differences can be explained by higher irradiance in summer which tends to reduce nitrate, and also to higher growth rates which coincide with periods of high irradiance and warmer temperatures (Kanaan and Economakis 1992). Current UK crop assurance protocols therefore suggest that growers should avoid sampling lettuce during dull weather conditions or during a particular time of the day (Anonymous, 2002). The maximisation of light availability influences also the level of nitrate when crops are produced under glasshouse conditions (Premuzic *et al.*, 2002).

These results in this study were similar to those reported by Ali and Mohamad (2012) that the use of chemical fertilizer may increase nitrate content in crops and hence decrease the quality. However, this response to nitrogen was genotype dependent. In their study of four genotypes of onion, with different rates of nitrogen, nitrate accumulation was significantly increased from 0-300Kg N/ha. Ahmed (2009) also noted that nitrogen fertilizer in excess levels cause significant increment in nitrate accumulation of crops. Several other studies confirmed that the nitrate content of organically grown vegetables is often lower than of vegetables treated with mineral-N fertiliser (Leclerc *et al.*, 1991). An increased level of all fertiliser types tended to result in increased nitrate content. In the summer trial, lettuce treated with mineral-N fertiliser had significantly higher nitrate content than organically fertilised lettuce. The increased level of mineral N-fertiliser resulted in significantly higher nitrate content, while that of organic fertiliser (composted manure only) resulted in

significantly higher nitrate content. On the other hand, increased levels of fresh manure and nettle extract tended to lead to higher nitrate contents.

Though *Cleome gynandra* remains a valuable source of nutrients, the consumption of it may pose some health risk due to the accumulation of anti-nutritional factors such as nitrates. As a consequence if nitrogenous fertilization is that if supplied in excess some of the N taken up will accumulate as nitrate in the vacuoles instead of being converted to amino-nitrogen (Demsar *et al.*, 2004; Santamaria *et al.*, 2001; Santamaria *et al.*, 1999a). This accumulated foliar nitrate poses a health risk and is subject to regulation as it is known to interfere with the blood haemoglobin in the oxygen transport. If consumed, nitrate can have toxic effects by two main routes.

First, in the saliva and the gastrointestinal tract, nitrate is reduced to nitrite which can then be re-oxidized to nitrate by oxyhemoglobin in the bloodstream with the resultant formation of methemoglobin (in which the central iron of the haem group is Fe³⁺). Unlike hemoglobin, methemoglobin has no ability to bind oxygen (Santamaria, 2006) so the capacity of the blood to deliver oxygen to the body tissues is impaired (Hill, 1999). This condition is referred to as methemoglobinemia and is more serious with infants than with grown children or adults. Second, nitrites react with secondary amino compounds commonly present in diets to form nitrosamines, which have been implicated in carcinogenesis (Hanssen and Marsden, 1987). The nitrate content of vegetables is therefore a determinant of their nutritional quality and hence the recommended limits for the nitrate content of vegetables (Food Standards Agency, 2001) need to be strictly adhered to.

The World health organization recommends 220 mg maximum safe daily intake of nitrate for adults of (WHO, 2000). Therefore the benefits of increased yield, protein and vitamins contents of leafy vegetables arising from nitrogenous fertilization need to be balanced against the risk of excessive nitrate contents. This is because vegetables are an outstanding source of vitamins, minerals and biologically active compounds (Kmiecik, Lisiewska, and Slupski, 2004). About 87% of the total nitrate concentration in a normal diet is believed to be a direct result of vegetable intake (Huarte- Mendicoa *et al.*, 1997).

Table 21: Log transformed Nitrates as influenced by Nitrogen application (Mg/Kg)

Nitrogen	JKUAT, Jun- Sept, 2012	Ruiru Mar-Jun, 2012	Ruiru Jun-Sept, 2012	Greenhouse Mar-Jun, 2012	Greenhouse Jun-Sept, 2012
N1	1.0567 ^a	1.0018 ^a	1.0442 ^a	1.900 ^a	1.8967 ^a
N2	1.5698 ^b	1.059 ^b	1.068 ^b	2.1622 ^b	2.1879 ^b
N3	1.9827 ^c	1.088 ^c	1.075 ^c	2.1548 ^b	2.2112 ^c

Values followed by the same letter within columns are not significantly different according to Duncan's Multiple Range Test at 5% probability level. N1=3.3Kg/M² of decomposed cattle manure, N2=2.6g CAN/plant, N3=5.2g CAN/plant for field trials

4.3 Effect of harvest time on composition of selected spider plant accessions

4.3.1 Influence of harvest time on proximate composition

There was a significant ($p \leq 0.05$) gradual reduction in the amount of both protein and crude fat from the 6th to the 8th week (Table 22). Both fibre and carbohydrate recorded significant ($p \leq 0.05$) increases from the 6th to the 8th week. On ash content, the 6th week recorded a significantly ($p \leq 0.05$) high ash content which took a

declining trend as it got to the 8th week. Moisture content was significantly ($p \leq 0.05$) decreased from the 4th week to the 8th week even as the vegetables were approaching senescence.

Table 22: Percentage proximate composition as influenced by harvest time

		WAP	Crude protein	Crude fat	Crude ash	Crude fibre	Carbo Hydrate	Moisture
Ruiru 2011	Mar-Jun,	4	3.69a	0.42a	1.81a	1.24a	2.41a	90.56c
		6	4.70b	0.55c	2.92c	2.42b	3.35b	86.18b
		8	5.20c	0.51b	2.73b	3.07c	4.66c	83.86a
Ruiru 2012	Mar-Jun	4	3.78a	0.43a	1.85a	1.31a	2.46a	90.42c
		6	5.01b	0.50b	2.85b	3.37c	3.39b	85.05b
		8	5.29c	0.57c	2.87b	3.05b	4.79c	83.34a
JKUAT Sept 2012	Jun-	4	3.37a	0.26a	1.64a	1.14a	2.23a	91.35c
		6	5.60c	0.52b	2.83b	2.42b	3.49b	85.14b
		8	4.92b	0.61c	3.14c	3.23c	5.06c	83.05a

Values followed by the same letter within columns are not significantly different according to Duncan's Multiple Range Test at 5% probability level. WAP=weeks after planting.

4.2.3 Influence of harvest time on mineral and vitamin C content

Calcium showed no particular trend. In JKUAT field trial calcium content significantly ($p \leq 0.05$) right from 4 weeks after planting to 8 weeks (Tables 23 and 24). In Ruiru trials, the 8th week yielded significantly highest amount of calcium with significant increases from the 6th to the 8th week, having been a significant drop in the 5th week. But on the green house trials there was a specific trend of gradual significant increase ($p \leq 0.05$) right from the 4th week through the 8th. In the green

house trial season week 4 and 5 recorded no significant difference in terms of calcium.

However, vitamin C did not take any particular trend with increase in maturity as far as maturity is concerned. For instance, in JKUAT the 4th week produced highest significant ($p \leq 0.05$) amount at 245.3Mg/100g of fresh sample. It then reduced in the 5th week, before significantly increasing again in the 6th week. This may be attributed to the influence of other factors like nitrogen and light intensity which also affect vitamin C content. This same trend has been observed in spinach. There are differences in ascorbic acid content during plant growth in spinach although there appears to be no consistent pattern to this variation. Bergquist *et al.*, (2007) working with immature spinach found a higher ascorbic acid content in younger leaves (stage I) than in older leaves (stage II and III). In other leafy vegetables, ascorbic acid content has been found to change during growth, but with no consistent trend to increase or decrease (Guil-Guerrero *et al.*, 2003; Sørensen *et al.*, 1994; Weston and Berth, 1997).

Table 23: Minerals and Vitamin C compositions as influenced by Harvest time in field trials (Mg/100g)

	Time	4	5	6	7	8
		(WAP)				
JKUAT, Jun-Sept, 2012	Ca	293.4 ^a	285.7 ^b	346.3 ^c	400.6 ^d	456.9 ^e
	Mg	76.21 ^e	51.06 ^d	42.33 ^c	30.47 ^b	21.75 ^a
	Zn	0.894 ^d	0.45 ^b	0.528 ^c	0.851 ^e	0.405 ^a
	Fe	15.66 ^c	22.61 ^d	29.16 ^e	17.52 ^b	13.43 ^a
	Vit C	245.3 ^d	213.4 ^a	233.1 ^c	212.0 ^a	226.4 ^b
RUIRU Mar-Jun, 2011	Ca	219.6 ^b	182.1 ^a	244.7 ^c	218.5 ^b	291.4 ^d
	Mg	57.22 ^e	45.92 ^d	38.8 ^c	28.76 ^b	22.67 ^a
	Zn	0.674 ^d	0.385 ^b	0.533 ^c	0.815 ^e	0.276 ^a
	Fe	13.57 ^c	17.38 ^d	24.48 ^e	12.46 ^b	11.95 ^a
	Vit C	198.65 ^e	172.89 ^b	187.4 ^d	169.2 ^a	184.03 ^c
RUIRU, Mar-Jun, 2012	Ca	314.2 ^b	306.8 ^a	367.2 ^c	421.5 ^d	477.9 ^e
	Mg	80.94 ^e	55.06 ^d	39.33 ^c	29.37 ^b	20.75 ^a
	Zn	0.793 ^d	0.453 ^b	0.627 ^c	0.959 ^e	0.325 ^a
	Fe	16.96 ^c	21.72 ^d	30.6 ^e	15.57 ^b	14.94 ^a
	Vit C	233.7 ^e	203.4 ^b	220.0 ^d	199.0 ^a	216.5 ^c

Values followed by the same letter within columns are not significantly different according to Duncan's Multiple Range Test at 5% probability level. WAP=weeks after planting

With magnesium there was a significant ($p \leq 0.05$) reduction in magnesium content from the 4th to 8th week in both field and green house trials. Zinc recorded the highest significant ($p \leq 0.05$) amount in the 7th week after planting, while for the green house trails it was in the 5th week. In the case of iron, there was a significant increase till the 6th week before taking a significant drop in the 7th week and further drop in the 8th week. This proved true for both green house and field trials.

Table 24: Minerals and Vitamin C compositions as influenced by harvest time in greenhouse trials (Mg/100g)

	Time(WAP)	Ca	Mg	Zn	Fe	Vit C
GREENHOUSE Mar- Jun, 2012	4	266.8 ^a	55.89 ^e	0.812 ^c	16.53 ^c	160.7 ^b
	5	277.6 ^b	44.73 ^d	0.911 ^d	17.28 ^d	138.8 ^a
	6	283.8 ^c	39.9 ^c	1.04 ^e	20.68 ^e	154.2 ^b
	7	290.3 ^d	35.6 ^b	0.754 ^b	16.51 ^b	137.8 ^a
	8	300.5 ^e	28.4 ^a	0.655 ^a	14.12 ^a	175.1 ^c
GREEN HOUSE Jun- Sept, 2012	4	268.8 ^a	54.89 ^e	0.806 ^c	15.77 ^b	165.2 ^c
	5	279.6 ^b	48.94 ^d	0.915 ^d	18.48 ^c	142.7 ^a
	6	275.8 ^b	43.73 ^c	1.04 ^e	21.38 ^e	155.6 ^b
	7	293.5 ^c	36.53 ^b	0.743 ^b	19.32 ^d	140.7 ^a
	8	304.2 ^d	27.76 ^a	0.643 ^a	14.45 ^a	179.4 ^d

Values followed by the same letter within columns are not significantly different according to Duncan's Multiple Range Test at 5% probability level. WAP is weeks after planting.

4.3.3 Influence of harvest time on phytochemical composition

Ruiru, March-June, 2011 (Tables 25 and 26) recorded significantly ($p \leq 0.05$) highest amount of total phenolics in the 5th week while alkaloids were significantly ($p \leq 0.05$) at the 6th week.

Table 25: Effect of harvest time on phytochemical composition (Mg/g) of field trials

	TIME (WAP)		4	5	6	7	8
Ruiru	Total phenolics	(GAE)	13.24 ^c	15.20 ^e	14.41 ^d	11.06 ^a	11.39 ^b
Mar-Jun,	Alkaloids		23.42 ^a	24.57 ^b	29.15 ^e	24.57 ^c	26.13 ^d
2011	Total flavonoids	(QE)	21.56 ^a	22.40 ^b	27.24 ^e	22.69 ^c	23.93 ^d
	Tannins	(TAE)	12.31 ^b	14.06 ^d	13.31 ^c	10.15 ^a	10.46 ^a
RUIRU,	Total phenolics	(GAE)	10.22 ^a	11.33 ^b	11.80 ^c	10.39 ^a	13.67 ^d
March-	Alkaloids		20.00 ^a	24.16 ^b	29.45 ^e	24.37 ^c	25.93 ^d
June,	Total flavonoids	(QE)	19.81 ^a	22.60 ^c	26.94 ^e	22.29 ^b	23.72 ^d
2012	Tannins	(TAE)	8.76 ^a	10.49 ^c	10.93 ^d	9.83 ^b	12.66 ^e
JKUAT,	Total phenolics	(GAE)	9.887 ^a	10.59 ^c	11.06 ^d	10.14 ^b	12.81 ^e
Jun-Sept,	Alkaloids		26.14 ^a	27.83 ^b	29.06 ^c	27.36 ^b	33.68 ^d
2012	Total flavonoids	(QE)	26.71 ^a	27.48 ^b	28.69 ^c	27.02 ^b	33.26 ^d
	Tannins	(TAE)	15.61 ^a	16.76 ^c	17.51 ^d	16.05 ^b	20.68 ^e

Values followed by the same letter within the same column are not significantly different according to Duncan's Multiple Range Test at 5% probability level. WAP is weeks after planting. GAE= Gallic acid equivalent, QE=quercetin equivalent, TAE=Tannic acid equivalent

However, for Ruiru, March-June, 2012 trials the 8th week recorded the highest amount of both tannins and total phenolic, while 6th week recorded the significantly highest amount of both alkaloids and total flavonoids. Flavonoids showed a quadratic trend in Ruiru, March-June, 2012. The flavonoid concentrations increased significantly ($P < 0.05$) as you get to the 6th in all field trials. Onyango *et al.*, (2003) working with amaranthus, observed that the flavonoids rutin are mostly produced in the stage of blossoming. It is further confirmed that rutin can be changed into quercetin or other compounds during leaf senescence. In this study, the spider plants

started flowering at week around week 6 since planting and this might explain the increase in total flavonoids during this time period.

Table 26: Phytochemical composition (Mg/g) of green house trials as a result of harvest time

	TIME (WAP)	Total phenolics (GAE)	Alkaloids	Total flavonoids (QE)	Tannins (TAE)
GREENHOUSE	4	9.54 ^b	22.51 ^a	20.15 ^a	8.78 ^{bc}
Mar-Jun, 2012	5	9.39 ^b	23.22 ^b	21.72 ^c	8.639 ^b
	6	11.12 ^c	24.27 ^c	22.75 ^d	10.23 ^d
	7	8.77 ^a	24.39 ^c	22.82 ^e	8.068 ^a
	8	12.47 ^d	22.08 ^a	20.65 ^b	11.47 ^e
GREENHOUSE	4	9.34 ^{ab}	20.93 ^b	19.92 ^a	8.50 ^b
Jun-Sept, 2012	5	8.55 ^a	21.59 ^c	21.54 ^c	8.79 ^c
	6	12.23 ^b	22.57 ^d	23.10 ^e	9.56 ^d
	7	8.98 ^a	22.69 ^d	22.67 ^d	7.98 ^a
	8	13.24 ^c	20.53 ^a	20.32 ^b	10.51 ^c

Values followed by the same letter within columns are not significantly different according to Duncan's Multiple Range Test at 5% probability level. WAP= Weeks after planting. GAE= Gallic acid equivalent, QE=quercetin equivalent, TAE=Tannic acid equivalent

4.3.4 Influence of stage of harvest on nitrate accumulation

The 8th after planting accumulated significantly the most amount of nitrate in Ruiru trials (Table 27). This may be due to the fact that the plant has hit senescence and no more need of nitrogen for growth which just accumulates in the edible parts.

Table 27: Log transformed Nitrates as influenced by Harvest time (Mg/Kg)

HARVEST TIME(WAP)	4	5	6	7	8
JKUAT, Jun-Sept, 2012	1.1430 ^b	1.2265 ^c	1.2240 ^c	0.5301 ^a	1.7821 ^d
Ruiru Mar-Jun,2011	1.0876 ^b	1.1156 ^c	1.1521 ^d	0.6431 ^a	1.2769 ^e
Ruiru Mar-Jun,2012	1.0965 ^b	1.1386 ^c	1.1494 ^d	0.6379 ^a	1.2899 ^e
Greenhouse Mar-Jun,2012	2.1430 ^b	2.2659 ^c	2.2400 ^c	1.5301 ^a	2.1821 ^b
Greenhouse Jun-Sept,2012	2.1615 ^b	2.2398 ^c	2.2714 ^c	1.6210 ^a	2.1922 ^b

Values followed by the same letter within the same row are not significantly different according to Duncan's Multiple Range Test at 5% probability level. WAP= Weeks after planting.

4.4 Correlation between the antioxidation and the total flavonoids

The total radical scavenging activity (RSA) had strong positive correlation with both the total flavonoids and total phenolics. This suggests that antioxidation is influenced by polyphenols especially flavonoids and not just ascorbic acid, tocopherols and carotenoids. Their correlation coefficient (r) values in Ruiru, March to June, 2011 were 0.8926 for flavonoids and 0.8729 for polyphenols while for Ruiru, March-June, 2012 had 0.8835 for flavonoids and 0.8495 for polyphenols. Results for this correlation from Ruiru March-June, 2011 and Ruiru, March-June, 2012 are shown in the scatter plots in figures 4 and 5 respectively.

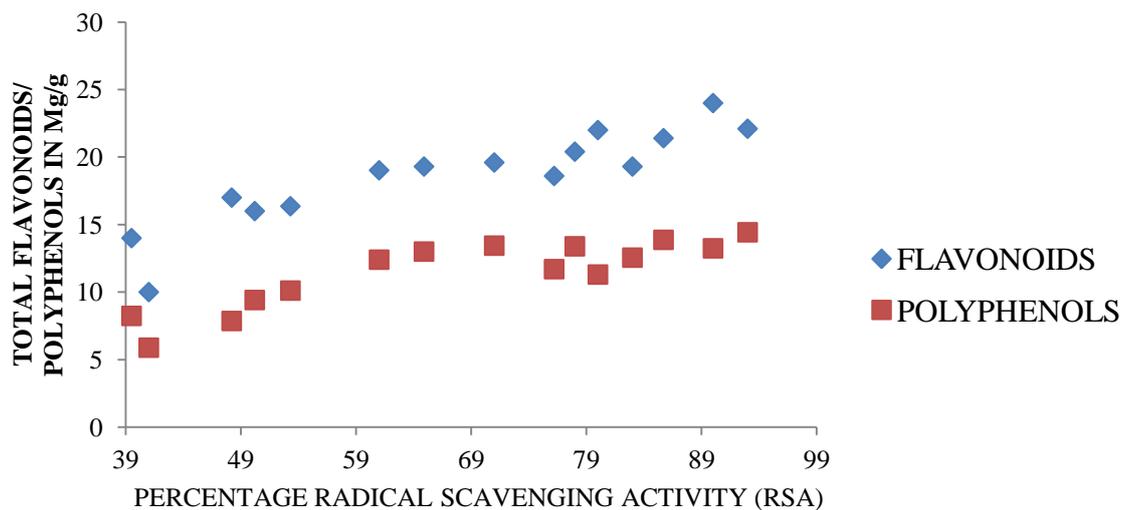


Figure 4: Correlation between antioxidation (RSA) and total flavonoids and total polyphenols in Ruiru, March-June, 2011

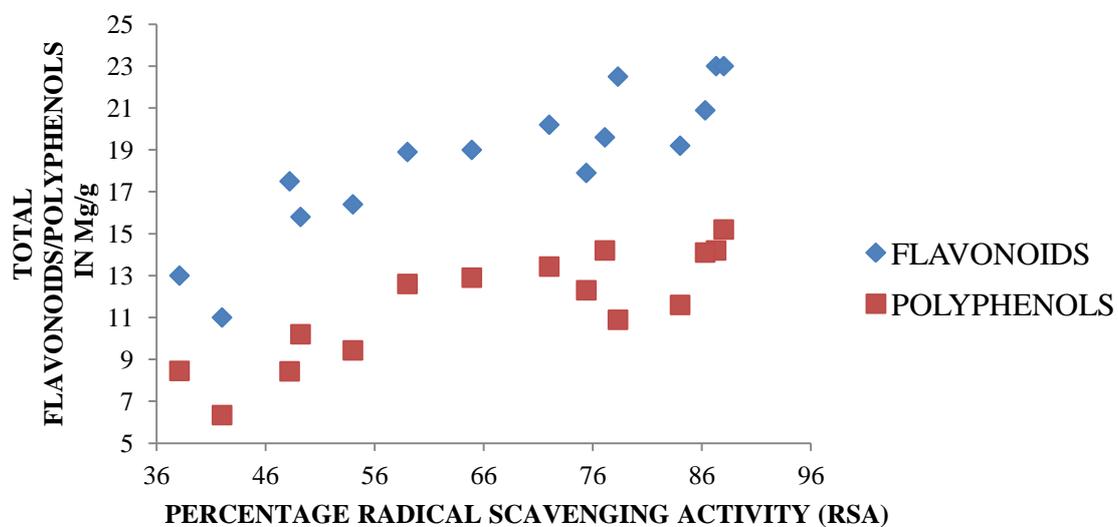


Figure 5: Correlation between antioxidation (RSA) and total flavonoids and total polyphenols in Ruiru, March-June, 2012

4.5 Effect of concentration on antimicrobial activity of the control accession

As the concentration increases inhibition increases. At 100Mg/ml *Bacillus subtilis* was inhibited by 6.8mm which was significantly different when the concentration was increased to 150, 200 or 250 Mg/ml. Increase in concentration led to increase in the inhibition (Table 28). These concentrations of the control showed significant differences in inhibitions. There were no significant differences among the accessions in terms of inhibition.

According to the results, Nalidixic acid proved effective against all bacteria and fungi despite not being an antimycotic. On the other hand Clotrimazole was very effective in inhibiting growth of *Candida albicans* (being an antimycotic) but still had some activity against *E.coli*. The extracts from *Cleome gynandra* compared well with the activity of the standard antimicrobials but at higher concentrations compared to low concentrations of the antimicrobials.

Table 228: Extent of microbial growth inhibitions (mm) by various concentrations of extract of the control accession

Sample Extract	Concentration (Mg/ml)	<i>Bacillus subtilis</i>	<i>E. coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Candida albicans</i>
	100	6.88 ^a	7.02 ^a	5.40 ^a	6.02 ^a	4.60 ^a
	150	10.52 ^b	10.28 ^b	8.32 ^b	9.46 ^b	8.12 ^b
	200	12.54 ^b	13.22 ^c	12.94 ^c	13.16 ^c	11.08 ^c
	250	16.04 ^c	18.92 ^d	17.60 ^d	22.04 ^d	16.38 ^d
Control	Nalidixic acid (30 µg/disc)	21.5	17.9	19.5	20.2	19.6
	Clotrimazole (50 µg/disc)	–	9.10	–	–	31.0

Values followed by the same letter within columns are not significantly different according to Duncan's Multiple Range Test at 5% probability level. Results are given as mean of zone of inhibitions in mm (n=3). Values < 6 mm show resistant while > 6 are sensitive. _ means there was no antimicrobial activity

4.6 Retention of nutrients and phytochemicals during thermal processing

The effect thermal processing, in this case blanching, on the retention of nutrients and phytochemicals is shown in Table 29. These values are likely to change depending on the how long and at what temperature the vegetables are subjected to. Micronutrients like zinc and iron were so much affected by cooking temperatures. This calls for shortened time during cookery to most of these nutrients. All phytochemicals and nutrients recorded reductions after boiling. Tannin was effectively reduced by about 51%. This agrees with other research findings. Zhang and Hamauzu (2004) and Racchi *et al.*, (2002) reported that tannic acid or phenolic compounds in vegetables showed heavy loss during prolonged cooking time, whereas in other processes, such as blanching or stir-frying, percent tannin loss is lower. This would explain why tannic acid is not easily destroyed or eliminated in a short time period of a heating process. It might need a longer heating time of cooking or being largely leached into the cooking water in order to reduce tannin content.

Vitamin C losses of more than 80% was recorded which agrees with Mathooko and Imungi (1994) have reported loss of 80% ascorbic acid in cooked drained *Amaranthus hybridus* while Yadav and Sehgal (1995) reported ascorbic acid losses of 93% in *Amaranthus tricolor*. So these loses can be reduced with reduced water and boiling time. As the spider plants recording high vitamin C contents of up to 300Mg/100 it is possible to meet the RDA of 75Mg per day for an average adult person.

Table 229: Percentage retentions of nutrients and phytochemicals after boiling

Nutrient/Phytochemical	Fresh	Boiled	Percentage retention
Vitamin C (Mg/100g)	210.1±5.52	33.6±1.67	15.99±2.18
Calcium (Mg/100g)	249.6±3.21	188.24±2.12	75.54±2.2
Magnesium (Mg/100g)	44.28±1.34	22.49±0.87	51.02±1.5
Zinc (Mg/100g)	1.03±0.01	0.08±0.01	7.77±1.12
Iron (Mg/100g)	14.85±1.22	7.53±0.56	50.71±2.3
Alkaloids (Mg/g)	63.68±1.32	44.17±0.27	69.36±1.3
Flavonoids (Mg/g)	63.37±0.28	45.26±0.22	71.42±4.32
Total phenolics (Mg/g)	29.56±0.41	11.12±0.32	37.62±3.65
Total tannins (Mg/g)	36.32±0.33	18.54±0.24	51.05±5.31

Values are given as means of three replicates ± SEM. SEM= Standard error of the mean.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Nitrogen application is very crucial in boosting the nutritional quality of spider plant both in their mineral and proximate compositions. The phenolic content including that of quercetin, are also enhanced by use of manure, low levels of N as CAN and with advancing maturity of the vegetables at harvest. Polyphenols, principally the group of flavanols consisting of kaempferol, quercetin and myricetin, have been implicated in playing an important role in human health.

MLSF 17 and UGSF 14 exhibited superior characteristics in proximate, vitamin C, mineral and phytochemical compositions. On the other, UGSF 9 tended to accumulate more nitrates than the rest of the cultivars. It was also recorded lowest in terms of phytochemical, proximate and mineral compositions. Moreover, the various sample varieties assessed were effective against all the bacterial and fungal strains tested. It should be noted the results were comparable to those of the standard antimycotics and antibiotics. The accession did not influence the sensory properties; therefore we accept the null hypothesis that accession does not influence sensory properties of spider plant.

Use of CAN as opposed to manure led to significant increases in accumulation of nitrates. So farmers need to be careful in applying mineral fertilizer as this will led to

health risks. So higher rates like 5.2 g N/plant needs to be avoided. On the other hand, nitrogen application led to significant reductions in the amount of total phenolics, total flavonoids and tannins. It however, had a significant increase in amount of alkaloids before showing a significant reduction at 5.2 g N/plant. Antioxidant activity positively correlated with the total flavonoids recorded. This tends to prove that ascorbic acid, beta carotene and vitamin E are not the only factors responsible for antioxidation but also phenolics.

Application of mineral nitrogen fertilizer (CAN) significantly increased amount of crude ash while crude fat reduced, something not serious as vegetables are not primarily consumed because of their fat content. Moisture on the other hand, was not affected by the application of nitrogen. All minerals analysed increased with increase in rate of CAN nitrogen applied. However, the most optimum rate was 2.6g N/plant, as excess led to significant reduction in calcium, iron and zinc. Vitamin C was significantly increased by application of 2.6g N/plant while significantly reduced by application of 5.2 g N/plant. Application of CAN led to significant reductions in polyphenols as compared to application of manure. So it calls for reduction of the rate of CAN nitrogen to maximise both the nutritional and phytochemical contents of the vegetables.

Proteins and crude fat reduced as the vegetables approached senescence while fibre and carbohydrates recorded significant increases during the same period. The 6th week after planting proved the most optimum harvesting period to derive the maximum nutrition. Magnesium had a quadratic trend with significant gradual

reductions from the 4th to the 8th week after planting. Iron increased significantly till the 6th week then started dropping while vitamin C and both calcium and zinc had no particular trend. In all the 6th week yield the highest average amount in nutritional composition.

All field trials show the 8th week record the highest significant amounts in nitrates accumulation while the 5th and 6th recorded the highest for the green house trials. Alkaloids show a highest record in the 6th week, while total phenolics in the 5th week. All phytochemicals and nutrients reduce drastically during thermal processing (boiling). This calls for shortened processing time for maximum retention. Green housing led to reductions in amount of phytochemical and nutritional compositions which suggest that open field conditions are the most appropriate for maximising on both phytochemical and nutritional composition.

5.2 Recommendations

Further research on the response of vegetable spider plant accessions to continuous use of manure as well as the different sources of manure is recommended as this is normally the case with small scale farmers in developing countries. Breeding programs to come up with more varieties of spider plants that accumulate less nitrates will help in solving the nitrate toxicity problem in immature leaves. Traditional preservation methods such as boiling for limited periods of time followed by sun drying should be evaluated to determine the quality of the vegetables because these methods compared to refrigeration are more affordable to majority of the people living in developing countries. To investigate, isolate and elucidate the

compounds responsible for antioxidant and antimicrobial activity in the spider plant accessions using spectroscopic and chromatographic studies and carry out metabolomic fingerprinting of these varieties to access the profiles of secondary metabolites in them.

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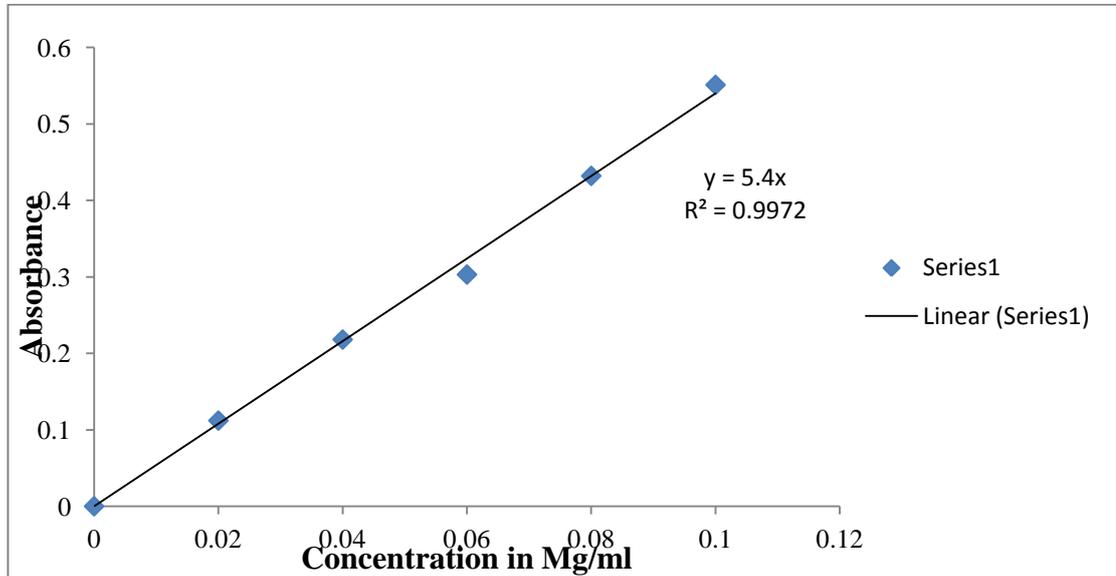
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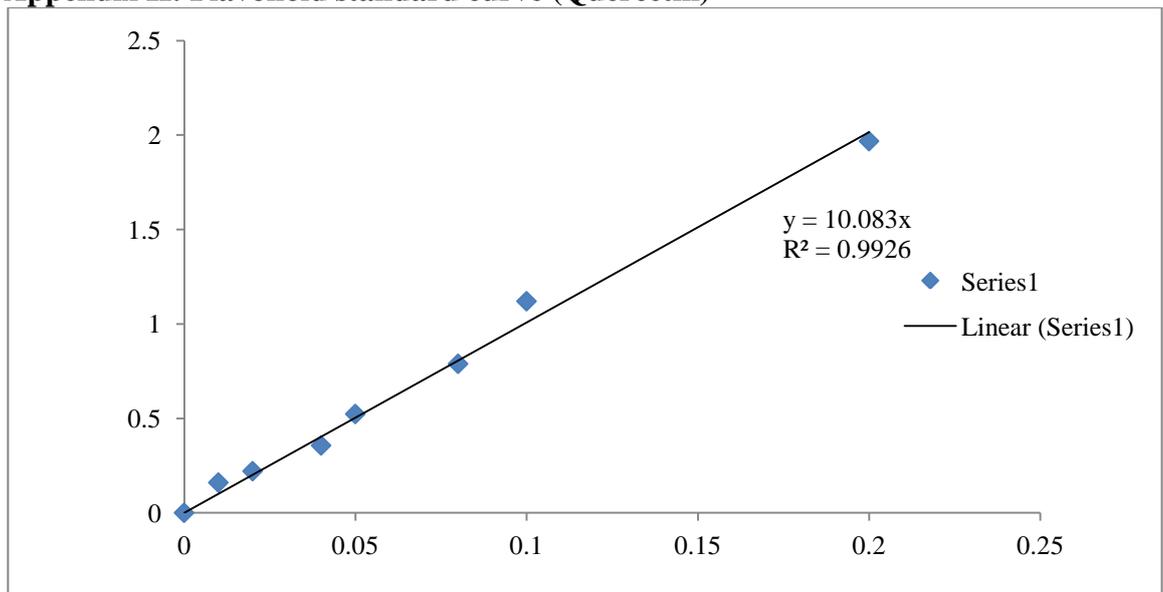
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APPENDICES

Appendix I: Tannins standard curve



Appendix II: Flavonoid standard curve (Quercetin)



Appendix III: Sensory evaluation questionnaire

Date.....Time.....

Instructions

You are provided with cooked vegetables for sensory evaluation. Please taste each sample and express how much you like/ dislike the product using the criteria provided. Please rinse your mouth after tasting each sample.

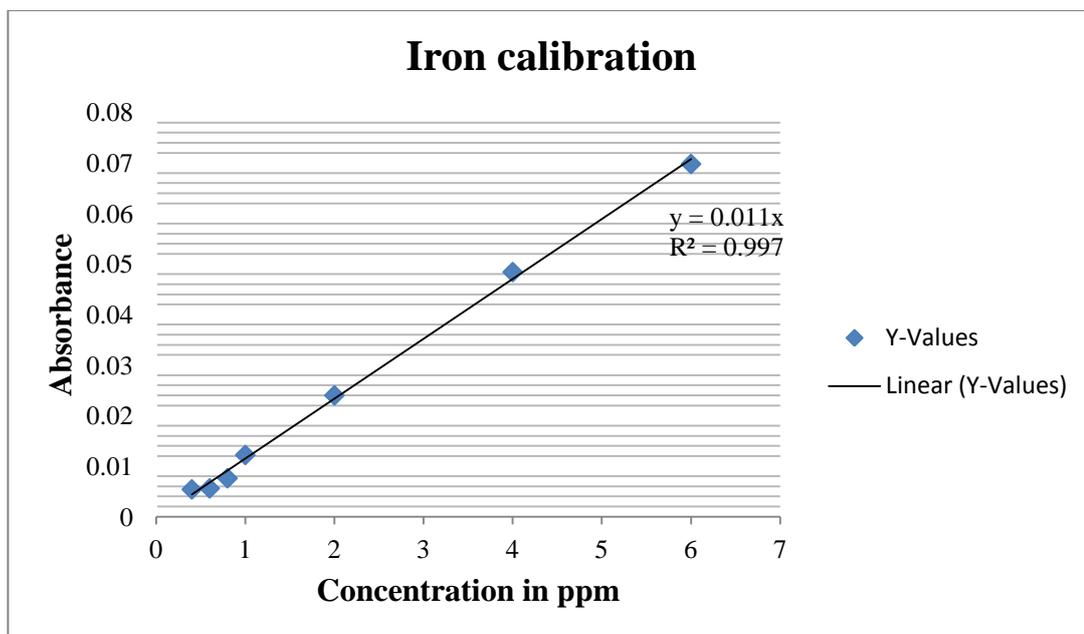
Attributes to be assessed: colour, taste, flavour and general acceptability. Give any other comment about the products and please be honest

Description	Score
Dislike extremely	1
Dislike very much	2
Dislike moderately	3
Dislike slightly.....	4
Neither like nor dislike.....	5
Like slightly	6
Like moderately	7
Like very much.....	8
Like extremely.....	9

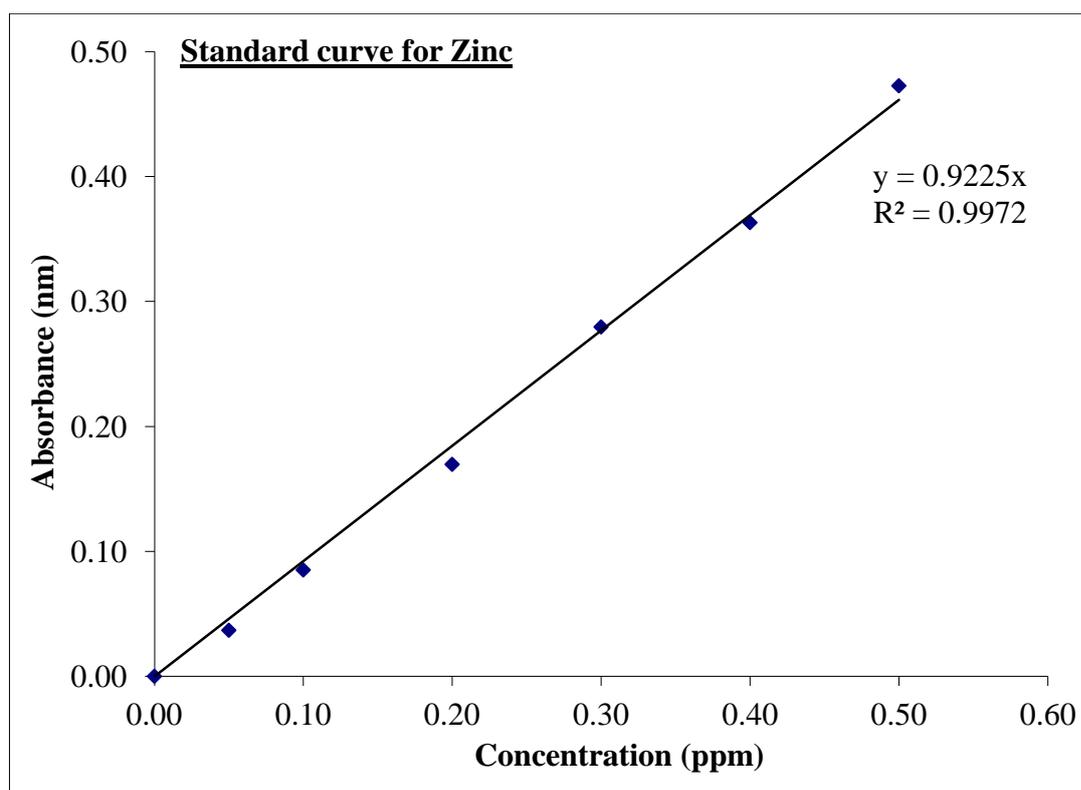
Remarks

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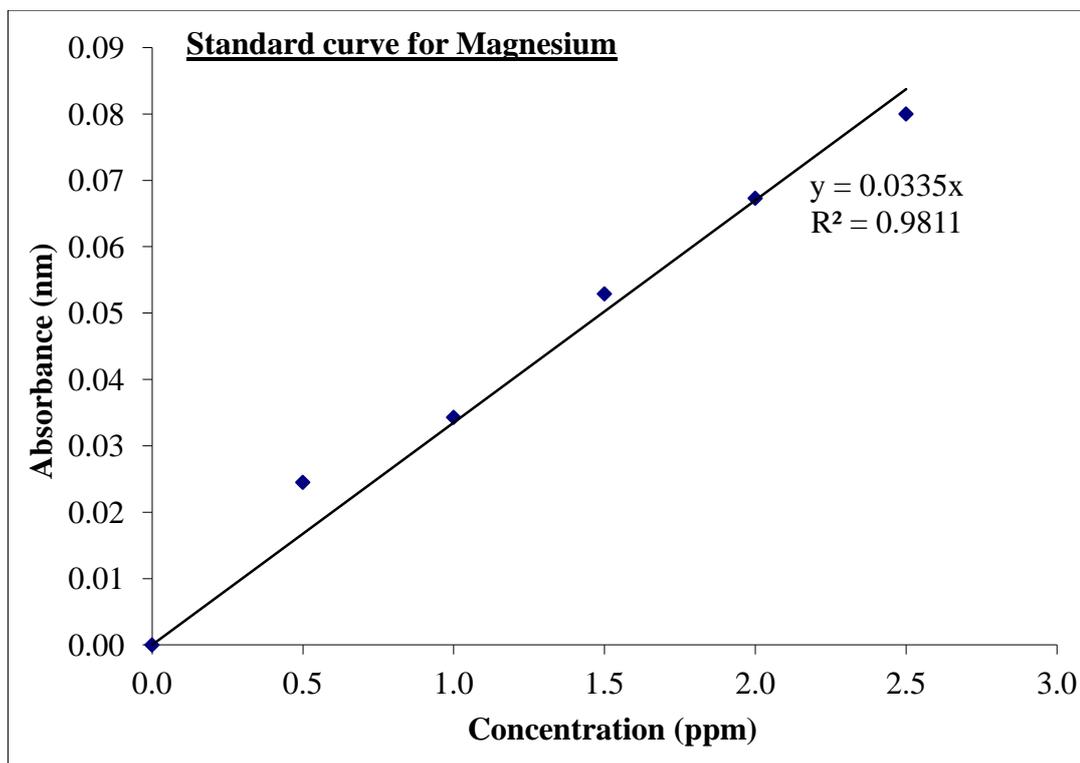
Appendix IV: Iron calibration curve



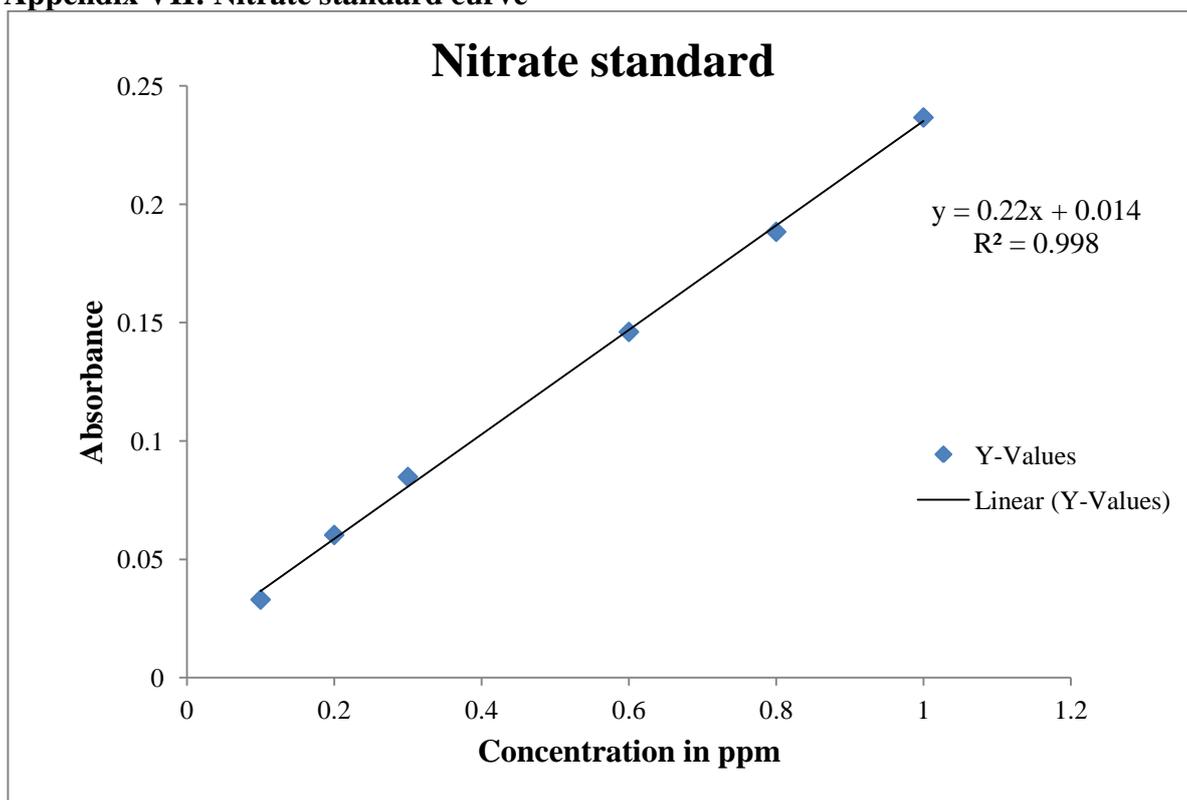
Appendix V: Zinc standard curve



Appendix VI: Magnesium standard curve



Appendix VII: Nitrate standard curve



Appendix VIII: JKUAT out door application of CAN



Appendix IX: *Cleome gynandra* (Spider plant) accessions

