THE ROLE OF AFRICAN INDIGENOUS LEAFY VEGETABLES IN IMMUNE BOOSTING

BY

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A thesis submitted in fulfillment for the degree of Doctor of Philosophy in Applied Human Nutrition

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DECLARATION

I, Teresa Ngeywa Tumwet certify that the thesis comprises only my original work towards the PhD except where indicated, due acknowledgement has been made in the text to all other materials used. This work has not been submitted in any university for a degree.

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PUBLICATIONS

During the course of this research work, a number of articles were generated which are based on the work of this thesis and submitted for publication. They are listed here for reference.

1. **Tumwet TN, Kang'ethe EK, Kogi-Makau W and Mwangi AM (2014)** Diversity and Immune boosting claims of some African indigenous leafy vegetables in Western Kenya. *African Journal of Food, Agriculture, Nutrition and Development* **14(1)** : 8529-44**(Chapter 3)**

2. **Proceedings at the 11th African Crop Science Society Conference held in Uganda, 13th-17th October 2013** Effect of different cooking methods on the nutritional value of some African indigenous leafy vegetables in Kenya**(Chapter 5)**

3. **Proceedings at the 11th African Crop Science Society Conference held in Uganda, 13th-17th October 2013** The impact of *Amaranthus hybridus* on the immunity of white albino rats**(Chapter 6)**

DEDICATION

I dedicate this thesis to my late husband, Benson Kerry Tumwet (posthumous) for the encouragement he gave me to further my education. This gave me the strength to continue even after he passed away just as I was starting the PhD course.

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and strengthen you and may He give you many more years to live for him as you serve others.

ABSTRACT

The immune system is a collection of biological processes within an organism that protects against disease. A healthy/strong immune system ensures that one is less susceptible to infections and ill health. Factors affecting the proper functioning of the immune system are many and they include micronutrient deficiencies, infections, illnesses, major burns, medications and emotional and physical stress. Micronutrient deficiencies are of concern worldwide and especially in the developing countries. The main deficiencies of public health importance are Vitamin A, iron, iodine and zinc. African Indigenous Leafy Vegetables are very rich in vitamins, minerals and other nutrients. There have been allegations of immunity boosting of individuals with infections, particularly HIV infection, through consumption of different indigenous vegetables such as spiderplant, African nightshade, stinging nettle and amaranthus. This study investigates the contribution of African Indigenous Leafy Vegetables in immune boosting using immune suppressed white albino laboratory rats.

A structured questionnaire and focused group discussions were used to document the diversity of African Indigenous Leafy Vegetables in the study area, Maseno division of Kisumu West district, and to rank them according to immune boosting and health claims, and popularity in terms of production and consumption in comparison with literature. The three African Indigenous Leafy Vegetables

significant in terms of contributing to healthy functioning of the body, immune boosting and good nutrition amaranth (*Amaranthus hybridus),* African nightshade (*Solanum nigrum)*and spiderplant (*Gynandropsis gynandra*) were further studied. They were planted at a plot in the College of Agriculture and Veterinary Sciences, University of Nairobi and nutritional value in terms of beta carotene, ascorbic acid, and minerals iron, zinc, copper, magnesium, manganese and calcium determined at both vegetative and flowering stages. Different vegetable preparation methods standardized from the communitiesø practices were also employed and the nutrients compared in these different methods across the three AILVs. The preparation methods were boiling for 5 minutes, boiling for 5 minutes and a further 3 minutes with milk, frying for 5 minutes, frying for 10 minutes and raw. The eight nutrients of immune boosting importance mentioned earlier were determined. The contribution of African Indigenous Leafy Vegetables to immune boosting was investigated using immune suppressed White Albino rats by measuring C-reactive protein, CD3%, T-Killer cells, CD^+4 counts and CD^+8 counts. In this, thirty female albino rats were divided into four groups A, B, C and D. The immunity of groups A, B and C was suppressed using Cyclosporine A, thereafter groups A and B were given raw and cooked *A. hybridus* respectively. Group C was the positive control while group D was the negative control.

The results of the survey confirm that there is diversity of AILVs in the study area with nine such vegetables. Three of these were rich in the eight selected micronutrients and were therefore studied further. The vitamin content of the vegetables reduced with flowering while mineral content increased. *Amaranthus hybridus* was in overall of higher nutritional value than *Solanum nigrum* and *Gynandropsis gynandra* and boiling or steaming for five minutes was the best cooking method for nutrient retention. *Amaranthus hybridus* boosted the immunity of White albino rats as was seen in the ratio of CD^+4/CD^+8 counts.

ABBREVIATIONS

AGRA – Alliance for Green Revolution in Africa

AILV – African Indigenous Leafy Vegetable

CAADP – Comprehensive Africa Agriculture Development Program

CRP – C-Reactive Protein

EDTA – Ethylene Diamine Tetra-acetic Acid

FANTA – Food and Nutrition Technical Assistance Project

FAO – Food and Agriculture Organization

GOK – Government of Kenya

NAAIAP – National Accelerated Agricultural Input Access Project

PBS –Phosphate Buffered Saline

Spp. – Species

SRA – Strategy to Revitalize Agriculture

UNICEF – United Nations Children's Fund

VAD – Vitamin A Deficiency

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

THESIS LAYOUT

Chapter 1 - This chapter discusses the economic situation worldwide which has led to widespread food and nutrition insecurity and therefore micronutrient deficiencies. The diversity and importance of the African Indigenous Leafy Vegetables (AILVs) in Sub-Sahara Africa is discussed as a cheap and easily available resource to combat these deficiencies. Finally the justification of this research, its objectives and research questions are presented.

Chapter 2 ó This chapter discusses the immune boosting claims of AILVs in view of the impact of the various micronutrients in the vegetables on the immune system of humans and laboratory rats. It also discusses the vegetable micronutrient content and factors that affect this.

Chapter 3- This chapter looks at the findings of the baseline survey carried out in Maseno division of Kisumu West district to document the diversity and immune boosting claims of some AILVs. Information on diversity of AILVs, demographic data and nutritional values placed on the AILVs by the two main communities (Luo and Luhya) in the study area is presented. The results of the data analysis which identified the vegetables with most health and immune boosting claims are presented. It also outlines how the three AILVs were selected for nutritional value analysis.

Chapter 4 - This chapter discusses the nutritional value of the three AILVs selected in chapter 2 and planted at a plot within the College of Agriculture and

Veterinary Sciences, *Amaranthus hybridus, Solanum nigrum and Gynandropsis gynandra.* Data on the nutritional value at both vegetative and flowering stages of these vegetables is discussed.

Chapter 5 - This chapter discusses the effect of different cooking methods on the nutritional value of the three AILVs at vegetative stage. Four cooking methods were employed and the raw vegetable used as a control.

Chapter 6 - This chapter discusses the impact of *A. hybridus* on the immunity of White albino rats. Information on how the rats were assigned different groups and the different treatments is presented. The four immune indicator parameters across the groups over time are also discussed.

Chapter 7- This chapter discusses the whole work generally, gives conclusions and recommendations

CHAPTER 1: INTRODUCTION

1.1 Background information

Combination of different crises of economic, political and environment in the world today has led to increased poverty (Onyango, 2010). These have pushed more and more people into food and nutrition insecurity thus widespread malnutrition (Nairobi, 2011). This leads to loss of economic productivity at the individual, household, community and national levels due to lack of adequate energy, protein and micronutrients. About 2 billion people worldwide have multiple micronutrient deficiencies of mainly vitamin A, iron, iodine or zinc (WHO, 2007). Most of them live in low income countries and children under five, lactating or pregnant women and those challenged especially the HIV/AIDS patients are the most vulnerable.

Micronutrient deficiencies in Kenya are not different from the world trends. In 1999, 89% of children under 6 years and 56% of women of child bearing age had iron deficiency anemia, 50% of children under 6 years and 50% of women were zinc deficient and 84% of children under 6 years were vitamin A deficient (GOK and UNICEF, 2002). An earlier survey carried out in 1994 indicated an iodine deficiency of 16% in 8-10 years old children (Gitau, 1995).

Micronutrient deficiencies have been seen to reduce with increased consumption of fruits and vegetables (Anderson et al., 2004; Heber, 2004; Gupta and Prakash, 2009) since they contain high amounts of micronutrients, vitamins and minerals which are also important in enhancing the absorption of other nutrients in the body (Funke, 2011; Saliu et al., 2012).Vegetables are vital components of a daily diet and form a major source of essential nutrients (Smith and Eyzaguirre, 2007). Most vitamins and minerals reported to improve the immune system are found in vegetables and fruits (Gibson, 2005; Shankar, 2001).These micronutrients are normally required in small quantities yet must be supplied in foods because they cannot be made by the human body, or if they are made, not in adequate amounts.

Vegetable production and consumption has been seen to bring about nutritional and economic improvements especially if the production is for income generation (Legwaila et al., 2011). Increased demand for vegetables in the urban and peri-urban areas of Lusaka Zambia provided a market for the vegetables and therefore increased production **(**Nguni and Mwila, 2007). Such an activity makes micronutrients more available *(*Ali and Abedullah, 2002*)*.

Vegetables are either exotic or indigenous. Exotic vegetables in Kenya are those which have recently been introduced and they include cabbages, carrots and spinach. Indigenous vegetables on the other hand are either those which were

originally in an area (Abukutsa-Onyango et al.,2006) or introduced and have been used over a long period of time until they form part of the culture and tradition of a community (Maundu, 1997). Indigenous vegetables include nightshade (*Solanum species*), spiderplant (*Cleome species*), amaranth *(Amaranthus species)*, cowpea (*Vigna species*), sweet potato leaves (*lpomeas species*), pumpkin leaves (*Cucurbita species*), jute mallow (*Corchorus olitorius*) and cassava leaves (*Manihot esculenta*) among others (Lyatuu and Lebotse, 2010). The different types of indigenous vegetables that are available in Kenya are listed in Appendix 4.

In some parts of Africa and within some ethnic groups, indigenous vegetables play an important role in nutrition and employment creation in both urban and peri-urban areas (Gockowki et al., 2003). Despite their overwhelming superiority in terms of food security, employment creation and healing traits among others, indigenous vegetables have been neglected in research and development by most governments worldwide (Schippers, 2000; Abukutsa-Onyango, 2007; Onyango, 2010).They are considered old fashioned, poor man's food (Gotor and Irungu, 2010) and therefore shameful to consume. Little is therefore known about them and they are threatened with genetic erosion due to change in land use and eating habits (Gudrun et al., 2004).   In the recent past, however, interest in these vegetables has arisen due to claims on immune boosting properties (Kimiywe et al., 2007) and other health benefits.

Indigenous vegetables are increasingly getting popular for their contribution in food and nutrition security to millions of Africans in rural and urban areas (Rubaihayo, 2002; Lyatuu and Lebotse, 2010). They are regarded as having medicinal values with some believed to cure multiple illnesses(Kimiywe et al., 2007). For instance, amaranthus is believed to cure malaria, AIDS, colds and flu and diarrhea while African night shade is believed to cure malaria, diabetes and high blood pressure among others. Stinging nettle on the other hand is believed to cure anemia, backache, colds and coughs among the urban and peri-urban residents of Nairobi (Kimiywe et al., 2007).The renewed interest in indigenous vegetables has come along with many claims on their immune boosting properties and those who are in dire need of boosting their immunity like the HIV infected and other vulnerable groups are fast adopting their use.

1.2 Immunity

Immunity is a medical term that describes a state of having sufficient biological defenses to avoid infection, disease, or other unwanted biological invasion (WIKIPEDIA: Free Encyclopedia, 2008). The immune system is therefore a collection of biological processes within an organism that protects against disease (Stewart and Edward, 2001).A healthy/strong immune system therefore ensures that one is less susceptible to infections and ill health (Rabson et al, 2005). Some infections, and in particular HIV infection, result in further compromise of the

immunity. There have been allegations of immunity boosting of the HIV infected through consumption of different indigenous vegetables such as the spider plant, African nightshade, stinging nettle and amaranthus (Kimiywe et al., 2007).In Kenya in 2008, the HIV infected were at 1.9million and the anti-retroviral (ARVs) drugs need was at 470,000 persons yet only 38% of these were reached (The Henry Kaiser Family Foundation, 2009).There is therefore fear that those not benefitting from ARVs are being taken advantage of by those who are marketing anything and everything in the name of immune boosting.

Innate immunity is the non-specific immune system which constitutes the first line defense against an invasion. It comprises of anatomical barriers, secretory molecules and cellular components such as skin, lungs, digestive system and serum. This differs from adaptive immunity which is the second line of defense and is pathogen specific(Rabson et al., 2005).Acquired (adaptive) immunity being pathogen specific is therefore developed after exposure to a foreign invader (pathogen) through illness or immunization (Rabson et al., 2005). In this case when an invading organism enters body tissues it causes illness and white blood cells, monocytes, macrophages, neutrophils and natural killer cells move into action to attack the invader. Later during recovery, lymphocytes become active and create antibodies. These and the activated lymphocytes become part of the acquired immunity.

Immune protection normally starts by first recognizing potentially harmful invading organisms through signatures. This process is very important because if the immune system gets confused and identifies its own cells as foreign it may react against itself as in the case of autoimmune illness in rheumatoid arthritis and most thyroid illnesses (Rabson et al., 2005).

The immune system which consists of both innate and adaptive immunity is divided into two branches, the cell-mediated T cells which are thymus dependent and the humoral component in which B lymphocytes produce anti-bodies in response to an antigen (Gibson, 2005). The main isotypes of antibody molecules are immunoglobulin M, A, 1, 2, 3 and 4 (IgM, IgA, IgG1, IgG2, IgG3, IgG4) *(*Shankar, 2001).

The cell mediated T-cells are further divided into two, the CD^+4 and CD^+8. CD^+8 lymphocytes effect destruction of virus-infected cells, bacteria and some malignant cells, while the CD^+4 cells function by secreting immuno-modulatory cytokines which promote T-cell growth, facilitate anti-body production by B-cells and activate microbicidal functions of macrophages. Both branches of the immune system are therefore under the control of CD^+4 T-cells which play the role of activating the immune system (Gibson, 2005).

C-reactive protein is a sensitive and specific kind of protein produced during acute infection (Gibson, 2005). Its decrease in blood reflects improved immune strength. The normal range of this protein in the blood is 1.0-3.0mg/l with 1.0mg/l being considered as low risk and 3.0mg/l as high risk. CD^+4 cells measure the strength of the immune system while CD^+8 measures the amount of cells circulating in the blood to destroy infected cells. In a strong immune system CD^+4 cell counts increase while CD^+8 cell counts decrease and the reverse is true in a poor immune system. The normal range of CD^+4 cells in humans is 500-1500 while that of CD^+8 is 150-1000 (Gibson, 2005).

1.3 Justification

The claims on the African indigenous leafy vegetables of immune boosting properties have resulted in many entrepreneurs taking up packaging of the vegetables in many forms, including powder, and marketing them as immune boosters. The products have become very attractive to those whose immunity has been compromised.

While the use of African indigenous leafy vegetables as immune boosters may be very helpful, the problem is that there is no scientific backing and evidence for this. Proof of concept studies are lacking and therefore the vegetables cannot be

exploited fully. Isolated work on African indigenous leafy vegetables shows that they might have high potential in improving the immunity of people (Kimiywe et al., 2007) due to their nutrient and non-nutrient bioactive properties (Smith and Eyzaguirre, 2007). Such work has however, not been ‡ied∅ in terms of showing which of the vegetables have the highest immune boosting properties and which vegetable processing methods retain most of the immune boosting components.

The purpose of this research was therefore to contribute to scientific basis for the immune boosting claims on African indigenous leafy vegetables in Kenya for evidence based promotion and improvement of production as well as consumption of the vegetables.

1.4 Research Objectives

Main objective: The main objective of this research was to establish the role of African indigenous leafy vegetables in immune boosting for food and nutrition security

Specific objectives

1. To document the diversity of African indigenous leafy vegetables and their immune boosting claims in Maseno division of Kisumu West district

2. To compare the levels of beta carotene, ascorbic acid and minerals iron, magnesium, manganese, copper, calcium and zinc in each of the selected African indigenous leafy vegetables at vegetative and flowering stages

3. To determine the effect of different cooking methods on the levels of beta carotene, ascorbic acid and minerals iron, magnesium, manganese, copper, calcium and zinc in the selected African indigenous leafy vegetables

4. To determine the impact of *Amaranthus hybridus* vegetable on the immunity of White albino rats.

1.5 Research Questions

1. How diverse are the AILVs in Maseno division of Kisumu West district? What are the immune boosting, health or medicinal claims attached to them?

2. What are the levels of the eight micro-nutrients of immune importance, beta carotene, ascorbic acid and minerals iron, magnesium, manganese, copper, calcium and zinc in the selected AILVs?

3. How do these micro-nutrient levels vary with different cooking methods and growth stage?

4. Do these micro-nutrients in *A. hybridus* have any impact on the immunity of white albino rats?

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CHAPTER 2: LITERATURE REVIEW

2.1 Impact of micronutrients on immunity

Immunity level of humans and animals is affected by several factors including the health status, micronutrient intake and food preparation methods among others. Some foods have gained popularity due to claims of immune boosting properties. For instance the gourd in India has historically been used as a vegetable yet it is preferred in case of ailments because of its cooling effect to the stomach and digestibility (Singh, 2004). An analysis of leaves of some under-utilized plants revealed that they were rich in micronutrients associated with healing effects (Sunmola et al., 2012). The process of healing infers improvement of immune system.

Vitamins and minerals are important micro-nutrients for good nutrition and protection from disease and ill health. These micronutrients boost immunity through synthesis of the immune cells, repair, maintenance and proper functioning of the immune system (Shanker, 2001). They can be supplied through consuming a variety of foods that are rich in these nutrients. The micro-nutrients of importance in immune functioning include calcium, magnesium, manganese, zinc, copper, iron, and vitamins A and C. It is known that most of the African indigenous leafy vegetables contain these immune boosting vitamins and minerals. The main roles and food sources of these nutrients are summarized in Table 2.1 and the dark green

leafy vegetables indicated include amaranth (*Amaranthus hybridus),* African

nightshade (*Solanum nigrum)* and Spider plant (*Gynandropsis gynandra).*

Table 2.1: Food sources and functions of some micronutrients

Micronutrient	Role	Deficiency symptoms	Food source
Vitamin A	Growth and function of T and B cells for immunity	Increased adult and infant mortality Failure growth in children	Dark green leafy vegetables
Vitamin C	Immune function, protein metabolism, iron absorption, increases resistance to infections	Tiredness, bleeding gums	Citrus fruits, tomato, guava, baobab, green leafy vegetables
Calcium	Immune defenses, builds strong bones and teeth, functioning of heart and muscles, blood clotting and pressure	Osteoporosis, tooth decay, periodontal disease	Dark green leafy vegetables, shrimp, dried fish, beans, lentils
Iron	Transports oxygen to blood, eliminates old red blood cells, build new cells, required for utilization of energy and metabolism by cells	Anemia, depression	Leafy vegetables, red meat, poultry, shellfish, egg, peanut, groundnut, lentils, beans, dried fruits
Magnesium	Strengthen the muscles, important for nervous system function, bone and teeth development	Anxiety, heart attack, muscular irritability	Dark green vegetables, cereals, seafood, nuts, legumes, groundnuts
Manganese	Activates various enzymes	Elevated cholesterol, dizziness	Green leafy vegetables
Copper	Activates various enzymes	Anemia, fragile bones, weakness	Green leafy vegetables

Micronutrient	Role	Deficiency symptoms	Food source
Zinc	Proper functioning of immune system	Immune impairment, brittle nails, memory impairment	Pumpkin seeds, sunflower seeds, beans, meat, seafood, green leafy vegetables

(FANTA, 2004; Gibson, 2005; Shankar, 2001*)*

Vitamin A is the most important immune boosting nutrient because it strengthens many of the first line defenses (innate immunity) like the skin, lungs and digestive tract (Gibson, 2005). Vitamin C found in most fruits and vegetables is another immune booster that maintains and repairs body tissues. It helps in the absorption of calcium and iron from food, resistance from infections and lowers levels of blood cholesterol (Gibson, 2005).

Micronutrients in the food do not always act independently. One nutrient may affect the outcome of another nutrient. Hence knowledge of nutrient interaction is important to guard against any negative effects. Song et al. (2006) demonstrated in chicken that iodine and selenium interaction affects immunity and when iodine levels in blood are lower than 0.2mg/kg; the additional effect of higher levels of selenium does not always result in significant changes in blood lymphocytes.

Experimental studies in humans have shown that there is an inhibitory effect of zinc on iron absorption and combined supplementation of the two nutrients was

less efficacious than single supplementation with iron (Bodwell and Erdman, 1988; Olivares et al., 2007). Bodwell and Erdman (1988) have also shown that large doses of zinc inhibit copper absorption and may cause copper deficiency which would indirectly affect iron status leading to anemia in humans. This is because these nutrients are ÷similarø and an overdose of one ÷blocksø the absorption of the other (Bodwell and Erdman, 1988).This in turn exerts a negative effect on immunity because these trace elements serve many metabolic functions in the body (Olivares et al., 2007).

2.2 Vegetable micronutrient content

Micro-nutrient is the collective term used to describe vitamins and trace elements required in only small amounts in the body (Gibson, 2005). They have a role in growth and proper functioning of the immune system.

African indigenous leafy vegetables are known to be good sources of micro-nutrients and of higher nutritional value than exotic vegetables (FAO, 1990)and could be very important in immune boosting. Vitamins and minerals contained in these vegetables such as vitamins E, C, beta carotene, lutein and selenium are also anti-oxidants.

A study conducted in Kwa-Zulu Natal, South Africa to assess the mineral content (Fe, P, Na, Zn, Mg, Mn and Ca) and anti-oxidant levels of 20 such vegetables showed that 12 of the vegetables had mineral concentrations exceeding 1% of plant dry weight which is much higher than the typical mineral concentration of other edible leafy vegetables. High levels of anti-oxidant activity (96%) were noticed in *Justiciaflava* and *Portulaca Oleracea* (Odhav et al., 2007). The high mineral concentrations and anti-oxidant activity showed that the local vegetables can contribute to improving nutritional value of the diets of rural and urban people.

Another investigation to test the popularity of African indigenous leafy vegetables carried out in Cameroon revealed that African indigenous leafy vegetables were more popular than exotic ones both in urban and peri-urban areas because they played a role in nutritional contribution and employment creation (Gockowki et al., 2003).

2.3 Factors affecting vegetable micronutrient content

Several factors affect the vegetable micronutrient content including plant species, postharvest handling, the growing environment, cookware and cooking methods. Water and type of soil for growing vegetables provide its nutrient source and therefore affect the vegetablesø nutrient content. An experiment designed to test the nutrient content of soils irrigated with treated sewage water and uptake by vegetables was carried out in India (Saraswat et al., 2005). The results showed that

vegetables grown on soils irrigated with treated sewage water had high levels of extractable copper, iron, manganese and zinc.

Type of utensils used in cooking food may affect its mineral content. In a bid to determine the bioavailability of iron in green leafy vegetables, five species of leafy vegetables were cooked in iron and aluminum utensils (Kumari et al., 2004). Both the fresh and cooked vegetables were analyzed for moisture, total and bio-available iron, ascorbic acid, dietary fiber, tannins, total oxalates and soluble oxalates. The results showed that the iron content of greens cooked in iron utensils was higher therefore the actual amount of available iron also increased(Kumari et al., 2004).

Vegetable cooking method may affect retention of nutrients. Cooking leafy vegetables in a pressure cooker and open pan has revealed that pressure cooking retained more beta carotene than open pan cooking. In addition, combination of acidulants and anti-oxidant spices in food during cooking improved the retention of beta carotene (Gayathri et al., 2004). Boiling, stir frying, micro-waving and steaming reduced chlorophyll content and caused vitamin C loss in varying amounts in vegetables and boiling caused the highest losses (Gao-feng et al., 2009). Cooking in 2% brine before freezing for preservation retained higher minerals than the blanched and frozen vegetables (Lisiewska et al., 2009).

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CHAPTER 3: DIVERSITY AND IMMUNE BOOSTING CLAIMS OF SOME AFRICAN INDIGENOUS LEAFY VEGETABLES IN WESTERN KENYA

3.1 Abstract

A survey was carried out to document the diversity and immune boosting claims of African indigenous leafy vegetables (AILVs) in Western Kenya. Both qualitative (focus group discussion, key informant interviews) and quantitative (interviews) methods of data collection were used. The results showed that there is diversity of AILVs in the study area with nine popular and frequently consumed. Seven of these are cultivated but two, stinging nettle (*Urtica massaica*) and vine spinach (*Basella alba*) grow wildly. The AILVs are cultivated at subsistence level in home gardens with minimal inputs and only excess of this is sold. The religion one belonged to was significantly ($p < 0.05$) associated with consumption or non-consumption of some of the vegetables. The vegetables are rain fed and the process of harvesting is by first uprooting during thinning followed by breaking the main stem and finally plucking off the leaves with maturity. Vegetable preparation in most households was mainly by women. There was no processing and preservation of the AILVs for use during the dry season. The communities rely on wild weeds during such seasons. The AILVs though consumed for good nutrition are also associated with various medicinal and immune boosting claims. Out of the nine, five are known for various health benefits, African nightshade and spider plant for good nutrition by 31.8% and 25.1% of the respondents,

respectively, slender leaf for healing power by 34%, cowpea leaves and slender leaf for anti-aging by 50% and 43.8%, respectively, and cowpea leaves (43.6%) and amaranthus (53%) for smooth skin and adding blood, respectively. Chi square analysis indicated that African night shade, spider plant and amaranthus are statistically significantly ($p<0.05$) associated with the claims of being contributors to good nutrition, healthy functioning of the body and immune boosting. Further analysis showed that spider plant and amaranthus are significantly ($p<0.05$) associated with claims on immune boosting. Further research on these two AILVs should be carried out to isolate the active ingredients in immune boosting.

Key words: indigenous vegetables, diversity, immune boosting

3.2 Introduction

The definition of vegetables varies from culture to culture depending on food selection and preparation (Katz and Weaver, 2003). Different vegetables have different edible parts, for example flower buds (broccoli, cauliflower), seeds (peas, sweet corn), leaf sheaths (leeks), leaves (kales, cabbage), buds (brussels, sprouts), stems of shoots (ginger, asparagus) and bulbs (onions, garlic) among others (WIKIPEDIA, 2009).

There are about 800 to 1000 species of edible leafy vegetables in Sub-Sahara Africa referred to as indigenous although only a small percentage of these are utilized as food (Bioversity, 2009). The high diversity of these vegetables shows their importance in adaptation to the environment and consumer preference.

Indigenous vegetables have been used as a side food with the staples in the African culture for a long time and have been an integral part of agricultural systems (Adebooye et al., 2003). They have been an important contributor to micronutrient intake and food and nutrition security. Production of indigenous vegetables does not require high amounts of resources such as fertilizer and pesticides. In fact where resources are limiting, farmers have been known to use indigenous vegetables both to meet their food and nutrition security as well as improve the soil structure (Tim, 2005).

Western Kenya is known for its high consumption of diverse African indigenous leafy vegetables (AILVs) for a long time (Abukutsa-Onyango, 2007). This practice is passed on from generation to generation. Most people start consuming the AILVs from childhood and only realize their health benefits later in life. According to a survey conducted in 2003 in six districts of Nyanza and Western provinces, ten AILVs representing eight botanical families were found (Abukutsa-Onyango, 2007).

AILVs in the past have widely been underutilized and neglected in research, breeding and modern production methods (Gudrun et al., 2004). They have however received a lot of interest in the recent past due to their contribution to food and nutrition security and have also been regarded as having medicinal and immune boosting values (Abukutsa-Onyango, 2007; Kimiywe et al., 2007; Lyatuu and Lebotse, 2010). Despite the interest, their cultivation has remained at subsistence level and traditional where only minimal inputs are used.

3.3 Objective

The objective of the baseline survey was to document the diversity of African indigenous leafy vegetables and their immune boosting claims in Maseno division of Kisumu West district.

3.4 Materials and methods

The study used both qualitative and quantitative methods of data collection. A pretested structured questionnaire (Appendix 3) was administered to 420 respondents who were mainly the women of the households (with an allowance of about 10% attrition rate).Sample size was calculated using Fisher et al., (1998) formula $\mathbf{N=z^2pq/d^2}$ where N is the sample size, z is the normal deviation (1.96) corresponding to 95% confidence interval, p and q are each 50% estimated proportion of those who believe AILVs either have immune boosting properties or not respectively, d is the degree of accuracy set at 5%. Themes of interest to this

study were developed and formed the checklist for discussion in 4 focus groups and among 10 key informants. Two enumerators were recruited and trained on data collection and ethics of the fieldwork. They then translated the questionnaire and themes of discussion into appropriate languages of the respondents(Luo, Luhya) with the guidance of the principal researcher as they administered.

The respondents were drawn from the 4 locations of Maseno division, Kisumu West district and were equitably distributed across the four locations and also across each sub-location in these locations. Transect method of sampling as described by Adebo, (2000) was used to have as much a representation of the location as possible. In this method, the center of each of the sub-locations in the four locations of the division was identified and representative respondents interviewed towards the four corners opposite each other. A randomized cluster sampling method (Fisher et al., 1998) was used to identify the respondents so as to include households that produced and consumed the AILVs, those who produced and sold, those who purchased and consumed and those who sold but did not consume. It had been realized during the pretesting of the tools that these groups were in existence. A respondent was anybody in a household who was charged with the responsibility of preparing and cooking vegetables which included AILVs and/or exotic.

The structured questionnaire covered three main sections; demographic data which included tribe, denomination, education level and occupation of both the respondent and spouse; economic status which was determined through observation on type of housing and ownership of Radio/Television set and consumption patterns which covered all types of vegetables consumed. The themes of discussion with FGDs covered information and knowledge of any health claims and benefits of indigenous vegetables (Appendix 3).

There was diversity of AILVs in the study area with nine such vegetables. Preliminary analysis of the quantitative data revealed that five AILVs were popular in the area in terms of production, consumption and associated with different medicinal and immune boosting claims by majority of the respondents across the division. These were photographed and used as a visual aid during four (one per location) focus group discussions. Proportionate piling method as described by Adebo, (2000) was used to ‑rank‑ the five vegetables as per the claims. The 5 vegetables were spider plant (*Gynandropsis gynandra*), amaranthus (*Amaranthus hybridus*), African nightshade (S*olanum nigrum*), slender leaf (C*rotalaria brevidens*) and cowpea leaves (V*igna unguiculata*).

In proportionate piling method (Adebo, 2000), participants of the focus group discussion were given one hundred (100) bean seeds. The beans were distributed

among the photographs of the 5 AILVs to show the relative importance of each vegetable as far as the health claim was concerned (Adebo, 2000). The seeds were collected and again distributed among the photographs of the 5 AILVs when discussing another health claim as per the communitiesø understanding. This process was repeated until all the three main claims, immune boosting, õadding bloodö and having healing power were exhausted.

Individual discussions were also held with 10 key informants drawn from Agricultural officers, farmers, traders and promoters of AILVs to get an insight into the key issues coming out of the survey.

Statistical analysis

Statistical analysis was performed using SPSS version 16 (SPSS Inc., Chicago, USA). Data were entered in excel then exported to SPSS and cleaned before analysis. Analysis was by one-way analysis of variance (ANOVA) followed by Chi-square. Differences were considered significant at 95% confidence interval (p<0.05).

3.5 Results

Demographic characteristics

Table 3.1 shows the demographic data. The ages of the respondents ranged from 15 to 103 years and majority (77%)were below 50 years. They were either Luo or

Luhya by tribe with Luo making the majority (85.5%). Most of them were farmers (82.6%) and had attained primary (72.1%) level of education. Most of the respondents were married and monogamous marital status was the most common (58.4%). Majority of the respondents were Christians and belonged to various denominations but especially Hera (22.4%), Apostolic (21.7%), Catholic (16.3%) and Anglican (14.2%). A few belonged to Roho, Nomia, Legio, Israel and Muslim.

Table 3.1: Demographic data

Variable name	Sub-variable	%
Age	15-50yrs	77
	Above 50yrs	23
Tribe	Luo	85.5
	Luhya	14.5
Occupation	Farmers	82.6
	Business	8.4
	Employed	5.9
	None	3.1
Education	Primary	72.1
	Secondary	13.3
	None	13.3
	College	1.2
Marital status	Monogamous	58.4
	Widowed	27.0
	Polygamous	12.7
	Separated	1.6
	Divorced	0.3
Denomination	Hera	22.4
	Apostolic	21.7
	Catholic	16.3
	Anglican	14.2
	Roho	9.8
	Nomia	9.5
	Legio	3.3
	Israel	2.6
	Muslim	.2

(N=420)

A chi square test [value, df] showed that denomination(Nomia and Legio Maria sects) [67.81, 7], p=0.0 was significantly important in negating AILVs consumption (p<0.05) while age [45.59, 64], p=0.96; tribe [0.02, 1], p=0.612; occupation [4.68, 4], p=0.322 and education level [1.04, 3], p=0.79 of the respondents were not significant (p>0.05).

Diversity of African indigenous leafy vegetables consumed in the study area

Almost all (99.8%) the respondents reported that they had heard of African indigenous leafy vegetables (AILVs) and all had heard of more than one AILV. They were able to list nine AILVs as shown in Table 3.2. Out of these, five AILVs were well known by majority of the respondents, spider plant (98.6%), slender leaf (98.1%), African nightshade (97.1%), cowpea leaves (97.1%) and amaranthus (92.1%). The least known AILV was Russian comfrey, only 0.2% of the respondents had heard of it. Majority (415 i.e.98.8%) of the respondents grew and consumed AILVs while some (273 i.e. 65%) grew, consumed and sold them. Further discussion with the respondents indicated that the older women could remember many more AILVs which were extinct. They also had more information on the health benefit claims of the AILVs.

Table 3.2: Diversity of AILVs listed

Vegetable	No. of respondents	% of respondents
Spider flower	414	98.6
Slender leaf	412	98.1
African nightshade	408	97.1
Cowpea leaves	408	97.1
Amaranthus	387	92.1
Vine spinach	31	7.4
Cassava leaves	27	6.4
Stinging nettle	12	2.9
Russian comfrey	1	0.2

(N=420)

Table 3.3 shows the distribution of study respondents by the vegetables they reported to consume. Majority of them consumed five AILVs, slender leaf (94.5%), African nightshade (94.3%), cowpea leaves (91.2%), spiderplant (91.2%) and amaranthus (77.6%). Only one (0.2%) respondent reported to consume Russian comfrey.

Table 3.3: Distribution of respondents by AILV consumed

Vegetable	No. of respondents	% of respondents
Slender leaf	397	94.5
African nightshade	396	94.3
Cowpea leaves	383	91.2
Spider flower	383	91.2
Amaranthus	326	77.6
Vine spinach	89	21.2
Cassava leaves	20	4.8
Stinging nettle	12	2.9
Russian comfrey	1	0.2

(N=420)

Almost all the respondents (94.4%) obtained advice on the consumption of the indigenous vegetables from their parents as shown in Figure 3.1. Other sources of advice were health workers, extension workers, neighbors, friends and the media.
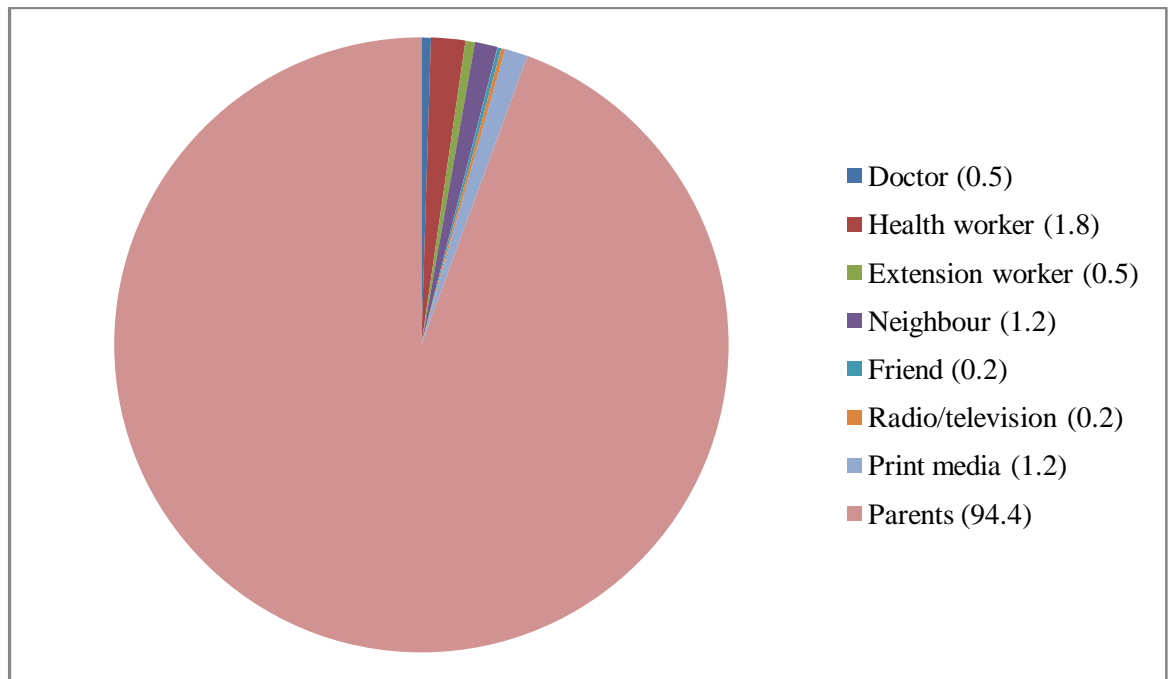
Figure 3.1: Percent distribution of AILV consumers by source of advice on consumption

Immune boosting claims

Table 3.4 shows the percent distribution of the respondents by health benefits they said were associated with the various AILVs. The five AILVs consumed by a majority of respondents were associated with different health benefits. About one third and one quarter of the respondents associated African nightshade (31.8%) and spiderplant (25.1%) respectively with good nutrition. Slender leaf was however associated with having healing power (stomachache, malaria) by 34% of the respondents, cowpea leaves and slender leaf associated with anti-aging properties by 50% and 43.8% of the respondents respectively. Most respondents

associated cowpea leaves (43.6%) and amaranthus (53%) with smooth skin and õadding bloodö respectively. Association of a health benefit to an AILV was not exclusive of each other. Many respondents associated a health benefit to more than one vegetable and vice versa.

Table 3.4: Percent distribution of respondents by health claims on different AILVs

Vegetable	Health claim (percent of respondents)							
	None	**Good Nutrition**	**Immune Boosting**	**Healthy body function**	**Healing Power**	**Anti-aging agent**	**Smooth skin**	**Adds blood**
African nightshade	26.1	31.8	19.0	12.5	19.4	0	3.6	26.0
Spider plant	21.7	25.1	24.0	19.0	21.4	6.2	9.1	7.0
Amaranthus	13.0	16.1	25.3	22.0	11.3	0	7.3	53.0
Slender leaf	12.0	15.2	19.0	20.7	34.0	43.8	29.1	5.0
Cowpea leaves	26.1	11.6	11.8	25.5	13.5	50.0	43.6	9.0
Total	100	100	100	100	100	100	100	100

Five major AILVs were mentioned for various health benefits: Cowpea leaves, Amaranthus, African nightshade, Slender leaf and Spiderplant. Stinging nettle was less associated with the various health benefits, only 1.8% of the responses made smooth skin claims on cassava leaves, 0.5% claimed that vine spinach had immune boosting properties while no claims were made on Russian comfrey.

It emerged that a high proportion of respondents believed that amaranthus (*Amaranthus hybridus*), African nightshade (*Solanum nigrum*) and spider plant (*Gynandropsis gynandra*) contributed to immune boosting, good nutrition and healthy functioning of the body. Further analysis of the responses indicated that amaranthus and spiderplant are statistically significantly ($p<0.05$) associated with immune boosting.

Although majority of the respondents (86%) had heard of AILVs from their parents, information on the benefits of these vegetables was mainly received from the health facility (54.5%) and only 22.5% from parents as shown in figure 3.2. The other sources of information on benefits and general awareness of AILVs were extension workers, social workers, school and the media.
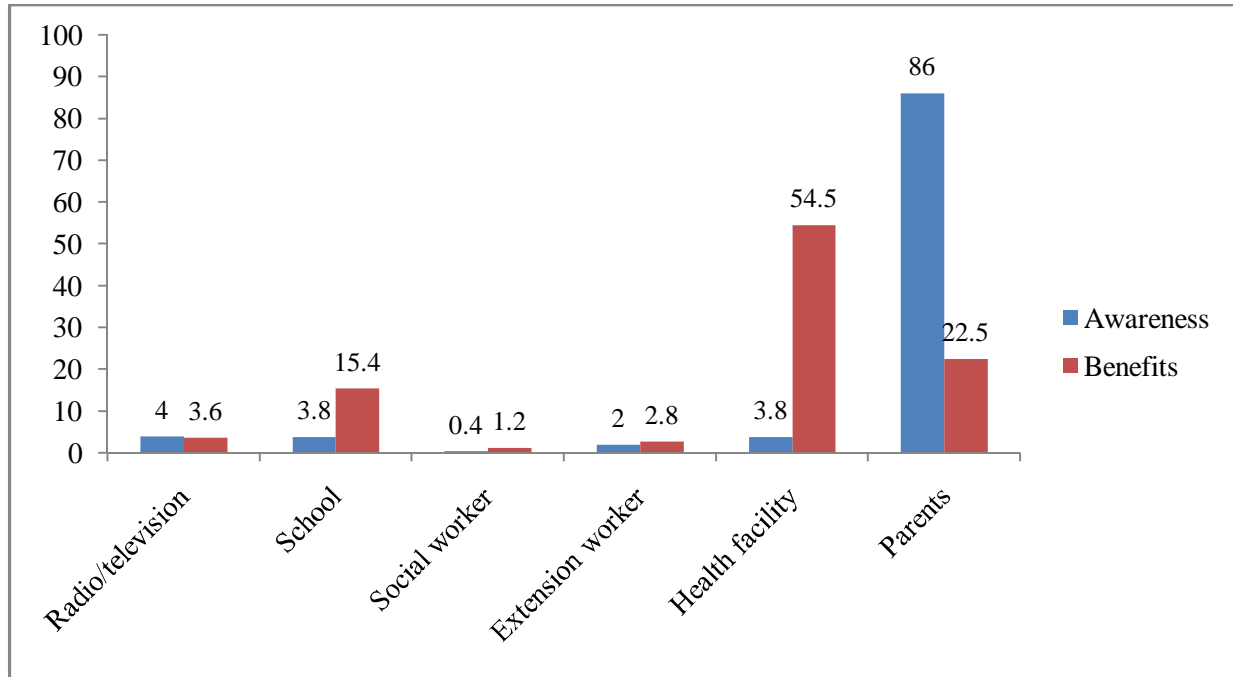
Figure 3.2: Sources of Information on AILVs awareness and benefits(% of responses)

Majority of the responses were an indication of feeling changes after consuming AILVs (Figure 3.3). The type of changes expressed after consuming AILVs were varied and were grouped into strong immunity (676) which made up73.31% of the responses and rejuvenated (233) which was 25.28% of the responses. Strong immunity according to the communities meant where one did not have frequent illnesses due to consuming AILVs. The respondents also gave responses on behalf of someone else they knew had been consuming AILVs and the responses were those who had felt similar changes, 627(73.42%) for strong immunity and 200(23.42%) for rejuvenated health. Responses for not feeling any changes were very few for both the respondents and someone else known to them.
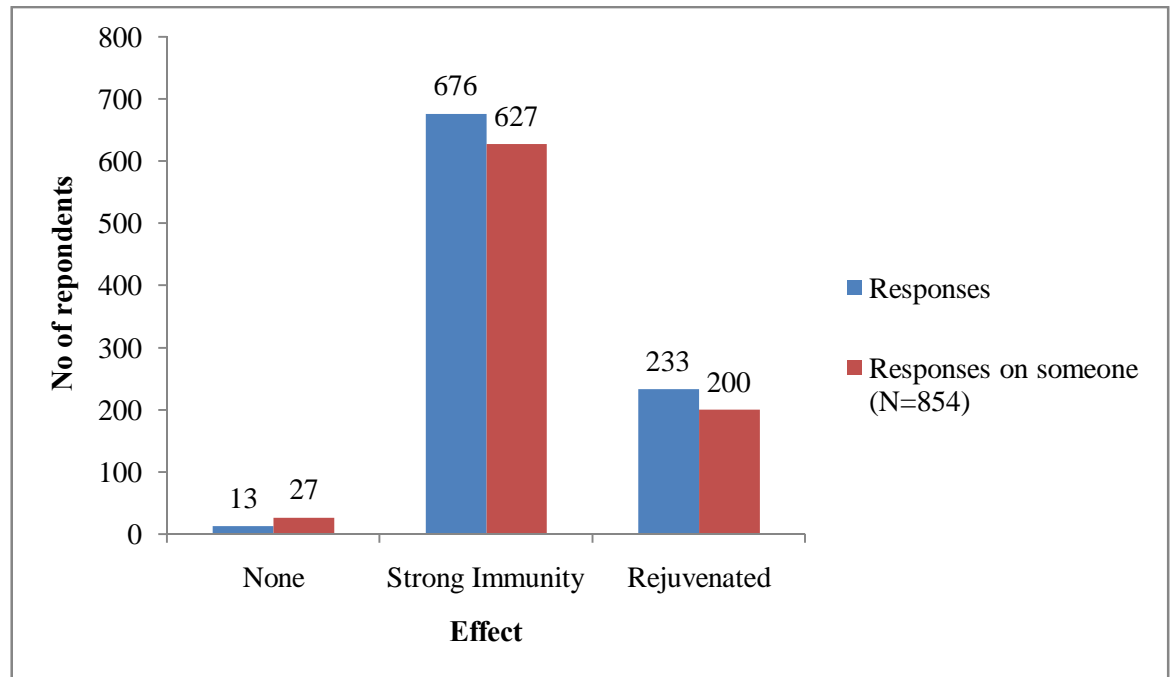
Figure 3.3: Changes felt after consuming AILVs

Information from the four focus group discussions (Table 3.5), gave an indication of how the five main AILVs were associated with immune boosting, õadding bloodö and healing power across the four locations. In three locations, North West Kisumu, East Seme and West Kisumu, African nightshade (*Solanum nigrum*) was known for immune boosting as was shown by 30%, 58% and 49% of the allocation by respondents. In all the four locations, amaranthus (*Amaranthus hybridus*) was known for õadding bloodö as shown by 100%, 45%, 100% and 86% indications in Otwenya, N/W Kisumu, East Seme and West Kisumu respectively. Slender leaf (*Crotalaria brevidens*) was known for having healing power (malaria and stomachache) as shown by 100% of the bean seeds in all the four locations. There were also claims that African nightshade (*Solanum nigrum*) and spider plant

(*Gynandropsis gynandra*) could be used to treat ulcers and stomachache. Stinging nettle (*Urtica massaica*) though not known to many was said to be good for õadding bloodö and immune boosting (12 respondents).

Table 3.5: Distribution of 100 bean seeds per health benefit across the four locations

	Immune boosting				"adding blood"				Healing power			
	a	b	c	d	a	b	c	d	a	b	c	d
African nightshade	8	30	58	49	0	0	0	0	0	0	0	0
Spider plant	41	24	7	16	0	26	0	14	0	0	0	0
Amaranthus	5	27	22	0	100	45	100	86	0	0	0	0
Slender leaf	10	10	13	35	0	0	0	0	100	100	100	100
Cowpea leaves	36	9	0	0	0	29	0	0	0	0	0	0

Where, a is Otwenya location;   b is North West Kisumu location;   c is East Seme location;   d is West Kisumu location

Amaranthus was attributed to õadding bloodö and Slenderleaf to healing power in all the 4 locations. African nightshade was attributed to immune boosting in 3 of the locations. Information from the key informants indicated that AILVs were gaining interest in the study area because of the immune boosting claims. Farmers were cultivating the vegetables on kitchen gardens and more and more was finding its way into the markets.

3.6 Discussion

Diversity of African indigenous leafy vegetables consumed in the study area

Africa is well known for its diversity of vegetables and plant species (Adebooye and Opabode, 2004) especially because of the different ecosystems and vegetation zones. Only a small percentage of these species are cultivated (Bioversity, 2009).

The fact that a vegetable in Africa is consumed as an accompaniment for the main staple has helped in biodiversity of vegetables both wild and cultivated (Maundu et al., 2009; Smith and Eyzaguirre, 2007).

Like Botswana, Kenya and especially the western region is endowed with a variety of African indigenous leafy vegetables like *Cleome, Amaranthus, Corchorus* and *Vigna* species and other medicinal plants. Most of them grow naturally and are plenty during the rainy seasons (Abukutsa- Onyango, 2007; Legwaila et al., 2011). They are better adapted to the environment because of long usage than the introduced exotic ones (Keding et al., 2007).

The results of this study indicate that there is a diversity of AILVs in the study area. However, indications are that the diversity is declining. The older people could remember some AILVs that are extinct and no longer in use. These findings corroborate with those of Van Rensburg and colleagues, (2007) who attributed this to increased promotion and use of exotic vegetables and the negative image towards AILVs (Marshall, 2001; Van Rensburg et al.,2007).

The findings that out of the nine AILVs mostly grown and consumed in the study area, five appear more popular in terms of production, consumption, marketing

and medicinal claims are in line with those of another survey carried out in Western Kenya (Abukutsa-Onyango, 2007) in which these five vegetables were among the ten highly ranked AILVs. Consumption of AILVs increases during the rainy seasons since they are normally plenty and cheap (Kimiywe et al., 2007). The communities in the study area depend on wild weeds as vegetables during the dry season; only identified by their local names as *Adongonyayuora*, *Nyadekdani*, *Okuoro* (Pappea capensis*), Atipa* (Asytasia mysorensis), *Achak* (Launaea cornuta), *Nyawendagwata, Osieko, Odielo* (Commelina Africana), *Nyabondo* (Mimusops kummel) and *Ogundu* (Sida tenuicarpa) among others (Maundu et al., 1999). This indicates that vegetables which provide important nutrients to the body are an ever present component in the meals in this area.

Immune boosting claims

The two main ethnic communities in the study area, the Luo and Luhya, have been placed on agricultural and mixed farming economic systems. This kind of system encourages utilizing both cultivated and wild plants which increases diversity of food consumption (Abukutsa-Onyango, 2007). The AILVs are associated with many health benefits but mainly immune boosting, good nutrition and healthy functioning of the body (Kimiywe et al., 2007).

Vegetables contain high micronutrients which perform many functions in the body and mainly improve the immune system. Several isolated research work done on

AILVs to determine their nutritional value (Maundu et al., 1999; Odhav et al., 2007; Weinberger and Msuya, 2004) indicate that they have a high potential in improving the immunity of people due to their nutrient and non-nutrient bioactive properties (Smith and Eyzaguirre, 2007). A large number of the AILVs have been reported to have health protecting properties and uses (Kimiywe et al., 2007; Maundu et al., 1999; Okeno et al., 2003). Orech et al., (2005) on the other hand observed that some phytochemicals in some of the AILVs consumed in Western Kenya may pose toxicity problems when consumed in large quantities or over a long period of time. There is however not enough evidence on the toxicity of these AILVs.

The results of the present study indicate that majority of the respondents knew of AILVs at childhood from their parents (86%). Inclusion of AILVs in the diet of these communities is therefore ingrained deep into their cultures. Information on health benefits of the AILVs is however mainly received from the health facility (54.5%) and only 22.5% from parents. Education level influences the awareness on the health benefits of AILVs thus implying that education is a significant factor in deciding whether or not to consume the AILVs.

The immune boosting, õadding bloodö and healing power claims from the focus group discussions on the African nightshade, amaranthus and slenderleaf

respectively corroborates with the findings of a survey in Tanzania (Keding et al., 2007) and another in Kenya (Kimiywe et al., 2007). Different AILVs have been associated with different health benefits by different groups and the AILVs are normally eaten in combination of many species at ago (Marshall, 2001) depending on cultural background, economic status and seasonality of the AILVs (Smith and Eyzaguirre, 2007) among others.

The older women seem to be more aware of the health benefits and diversity of AILVs than the younger ones. In fact their vegetable preparation methods vary greatly with the older women putting a lot of effort and time to prepare quality vegetables õbelievedö to be of a higher nutritional value than that prepared in a hurry by the younger women. These findings corroborate with those of Keding et al., (2007) in Tanzania. The difference in vegetable preparation between the older and younger women was attributed to the problem of not being able to pass on indigenous knowledge on production, consumption, processing and preservation of AILVs from generation to generation due to modernization and urbanization (Weinberger and Msuya, 2004). Through observations, the Luhya community who formed 14.5% of those interviewed value the health benefits of the vegetables more than the Luo community (85.5%) though there is no significant difference between the two tribes when it comes to AILVs consumption (p>0.05).

In conclusion, the AILVs consumed in the study area are diverse. The AILVs are known for different health benefits. Amaranthus and spider plant are claimed to significantly contribute to immune boosting. An indepth research of trials on these AILVs is recommended to ascertain the immune boosting and other health claims identified in this study. This would give them a scientific backing in exploiting their potential.

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CHAPTER 4: THE NUTRITIONAL VALUE OF SELECTED AFRICAN INDIGENOUS LEAFY VEGETABLES IN KENYA AT VEGETATIVE AND FLOWERING STAGES

4.1 Abstract

This study was conducted to determine the nutritional value of three African indigenous leafy vegetables, *Amaranthus hybridus*, *Gynandropsis gynandra* and *Solanum nigrum* both at vegetative (5-7weeks) and flowering (8-11 weeks) stages harvested from an experimental plot where they had been planted at the College of Agriculture and Veterinary Sciences, University of Nairobi. The parameters determined during both harvest periods were moisture content, beta carotene, ascorbic acid, calcium, copper, magnesium, manganese, iron and zinc. The vegetables had high moisture content, and at vegetative stage it ranged from 87.6% (*G. gynandra*) to 90.5% (*A. hybridus*) while at the flowering stage the moisture content ranged from 82.3%(*G. gynandra)* to 89.4% *(A. hybridus)*. *A. hybridus* was nutritionally superior to the other two vegetables at both vegetative and flowering stages. At the vegetative stage it was of higher nutritional value than the other two African indigenous leafy vegetables in four out of the eight nutrients analyzed (ascorbic acid, manganese, magnesium and calcium) while *S. nigrum* (Beta-carotene and iron) and *G. gynandra* (copper and zinc) were each nutritionally superior in two out of the eight nutrients. At the flowering stage, *A. hybridus* was still nutritionally superior in four nutrients out of the eight (beta carotene, manganese, magnesium and calcium), *G. gynandra* was superior in three (ascorbic acid, zinc and iron) while *S. nigrum*was only superior incopper. The nutrients in

the three vegetables at vegetative level in mg/100g ranged as follows, copper 0.57±0.01 (*A. hybridus*) to 0.79±0.02 (G. gynandra), zinc 4.37±0.026 (*S. nigrum*) to 14.9±0.29 (*G. gynandra*), manganese 16.0±0.09 (*G. gynandra*) to 32.5±1.15 (*A. hybridus*), iron 23.5±1.364 (*G. gynandra*) to 42.4±0.21 (*S. nigrum*), magnesium 252.8±2.18 (*S. nigrum*) to 734.0±6.1(*A. hybridus*), calcium 1370±5.6 (*S. nigrum*) to 3153±51.3 (*A. hybridus*), beta carotene 23.8±1.1 (*A. hybridus*) to 30.6±0.82 (*S. nigrum*) and ascorbic acid 749±14.7 (*S. nigrum*) to 3014.6±14.2 (*A. hybridus*). At flowering stage, the eight nutrients in mg/100g in the three vegetables ranged as follows; beta carotene15.7±0.4 (*G. gynandra*) to 30.4±0.8*(A. hybridus)*, ascorbic acid 464.8±0.4 (*A. hybridus*) to 525.8±9.04 (*G. gynandra*), copper 0.20±0.10 (*G. gynandra*) to 0.51±0.044 (*S. nigrum*), zinc 5.28±0.24 (*S.nigrum*)to 15.2±0.67 (*G. gynandra*), manganese 8.66±0.32 (*S. nigrum*) to 46.9±1.24 (*A. hybridus*), iron 11.5±1.3 (*S. nigrum*) to 73.7±8.5 (*G. gynandra*), magnesium 223.3±14.3 (*S. nigrum*) to 597.9±10.1 *(A. hybridus)*, calcium 710.5±43.9 (*S. nigrum*) to 3556.5±135.5 (*A. hybridus*). Data analysis using ANOVA indicated that there is significant ($P<0.05$) difference across the vegetables in all the eight nutrients determined at both vegetative and flowering stages.

Key words: Nutritional value, vegetative, flowering, nutrients

4.2 Introduction

Vegetables are vital components of a daily diet and form a major source of essential nutrients (Aregheore, 2012; Funke, 2011). Most vitamins and minerals said to improve the immune system are found in vegetables and fruits. These are also rich in antioxidants and therefore when consumed the antioxidants eliminate the free oxidative radicals as a result of normal metabolism (Wang et al., 2008). A high intake of fruits, vegetables, whole grains and plant proteins has been associated with a reduced risk of cancer, heart disease and some chronic diseases of ageing (Heber, 2004).

In Sub-Sahara Africa, the cheapest easily available vegetables are the indigenous ones yet information on their production, consumption and nutritional value among others is scarce (Abukutsa-Onyango, 2007; Smith and Eyzaguirre, 2007) and are threatened by genetic erosion due to changing eating habits (Gudrun et al., 2004). Regular releases of the improved varieties of routinely cultivated vegetables replace traditional varieties and their wild relatives contributing further to the scarcity of information on African indigenous leafy vegetables (AILVs) (Adebooye et al., 2005) yet they are very rich in micronutrients. Micronutrient deficiency, though a global health problem, is worse in the developing countries (WHO, 2007) which ironically are endowed with AILVs that are rich in micronutrients. Increased production and consumption of AILVs can ensure availability of micronutrients (Ali and Abedullah, 2002; Nguni and Mwila, 2007).

In the recent past, however, a lot of interest in these vegetables has arisen due to claims on medicinal and immune boosting properties (Kimiywe et al., 2007). Many factors affect the proper functioning of the immune system. They include micronutrient deficiencies, infections, illnesses, major burns, medications and emotional and physical stress (Gibson, 2005). The micronutrients known to boost immunity can be supplied through consuming a variety of foods rich in these nutrients such as African indigenous leafy vegetables which are known to contain these nutrients in higher amounts compared to the exotic ones like spinach, kales and cabbage (FAO, 1990; Legwaila et al., 2011; WHO, 2000; WHO, 2001).

The three AILVs which were significant in terms of contributing to healthy functioning of the body, immune boosting and good nutrition from the baseline survey were selected, planted and nutritional value compared at vegetative and flowering stages in the current chapter. The nutritional values were also compared across four cooking methods in the next chapter (chapter 5). The AILVs are amaranth (*Amaranthus hybridus),* African nightshade (*Solanum nigrum)* and spider plant (*Gynandropsis gynandra*).The eight micronutrients known for general good health and immune boosting (beta carotene, ascorbic acid and minerals iron, zinc, magnesium, manganese, copper and calcium) were determined in these three AILVs.

The three AILVs have featured in several studies from Western Kenya and other parts of East Africa as priority vegetables for consumption (Abukutsa-Onyango, 2007; Kimiywe et al., 2007; Maundu et al., 1999; Weinberger and Msuya, 2004) but few of these studies have compared them as relates to the eight micronutrients at vegetative and flowering stages which is an important step to be able to advice accordingly on when to consume.

4.3 Objective

The objective of the study was to compare the levels of vitamins A and C and minerals iron, magnesium, manganese, copper, calcium and zinc in each of the selected African indigenous leafy vegetables at vegetative and flowering stages

4.4 Materials and methods

The vegetables were planted on the same location within the campus and similar agronomic practices were applied to the three AILVs. This created uniformity and reduced the effect of confounding factors like climate and agro-ecological zones on the levels of the eight nutrients determined.

Seeds of *Amaranthus hybridus, Solanum nigrum and Gynandropsis gynandra* were purchased from a market in Maseno Division Kisumu West district. They

were planted at a well prepared fine tilth plot at the field station of College of Agriculture and Veterinary Sciences, University of Nairobi between October 2011 and March 2012. The seed was mixed with soil at a ratio of 1:4 and planted in 4 rows per vegetable. The rows were 30cm wide and the seed planted 0.5cm deep. The vegetables were in blocks of 1m apart from one vegetable type to another. A distance of 1m was also maintained on each side of the research block. Farmyard manure was used and the vegetables were basically rainfed but watering was practiced three times per week during the dry season. Weeding was done shallowly from time to time to ensure no weeds grew. Once grown the vegetables were identified scientifically by the Botanists at the National Museums of Kenya. Two rows of each vegetable were randomly selected and harvested at vegetative (5-7 weeks after planting) stage and the remaining two at flowering (8-11 weeks after planting). This was done early in the morning by breaking the main shoot which consisted of the stalk and leaves at the vegetative stage and flowers stalk and leaves at the flowering stage. The harvested vegetables were quickly transported to the Food Science and Nutrition Laboratory, University of Nairobi in black polythene bags. They were then cleaned by removing any foreign bodies and damaged vegetables ensuring quality and uniformity. The vegetables were thereafter washed three times in previously boiled and cooled tap water. They were then drained of the water and thoroughly mixed before preparing the samples within one hour of harvesting.

Levels of beta carotene and ascorbic acid were determined at the Food Science and Nutrition laboratory at College of Agriculture and Veterinary sciences, University of Nairobi and minerals calcium, copper, manganese, magnesium, iron and zinc were analyzed at the Department of Mines and Geology, Ministry of Environment and Natural Resources laboratories.

Preparation of vegetables for analysis

About 300g of the cleaned, washed and drained vegetables were thoroughly mixed and a representative sample of 150g was selected and finely chopped using a knife and chopping board and mixed before weighing 2g each of the triplicate samples for beta carotene and ascorbic acid determination and 2.5g each of the duplicate samples for moisture content determination. The remaining chopped vegetables were used for mineral determination.

Analytical methods

Moisture content determination

Moisture content of the fresh vegetables was determined by drying the 2.5g of the fresh vegetables in an air oven at 105^0C overnight to a constant weight (AOAC, 1980). Moisture content was calculated from the loss in weight..

Vitamin determination

Beta carotene was determined using Method No. 44 of International Federation of Fruit Juice Producers adopted in 1972. About 2g of the vegetable was ground using a mortar and pestle and total carotenoids extracted completely using acetone then topped to 50ml mark. Half of this was dried in a rotary evaporator at 60^0C until all the liquid had evaporated and the residue deposited on the inside wall of the volumetric flask. The concentrated extract was then dissolved in 1ml petroleum spirit. The separation of beta carotene from the total carotenoids was done using a chromatographic column packed with silica gel as the fixed media and petroleum spirit as the mobile media. Beta carotene which is usually the first yellow color elute was received in a 25ml volumetric flask. The absorbance was read in a Spectrophotometer (Cecil 4400, England) at 450nm wave length. The amount of beta carotene was calculated using the standard curve and the following formula:-Concentration/absorbancex spectrophotometer reading

Ascorbic acid was determined by titration (AOAC, 1980). Two grams of the vegetable were extracted in 50ml of 10% Trichloroacetic acid (TCA) using a mortar and pestle. The solution was filtered then 5ml of the filtrate was added to 5ml of 4% Potassium iodide (KI) solution. Two drops of starch solution was added as an indicator. The same was titrated with N-bromsuccinimide solution to a

purple-blue color for 5 seconds. The amount of Ascorbic acid in mg/100g was calculated using the following formula (AOAC, 1980):

Ascorbic acid=V.C x 176/178 (mg)

Where,

178 is the molecular weight of N-bromsuccinimide, and,

176 is the molecular weight of ascorbic acid

V=volume of the N-bromsuccinimide (ml)

C=concentration of N-bromsuccinimide (mg/ml) which was 10%

Mineral determination

The six minerals, copper, calcium, iron, magnesium, manganese and zinc were determined by use of Atomic Absorption Spectrophotometer (AAS) method and each mineral had its own lamp(AOAC, 1980). The standards for each mineral were used from time to time during the determination for calibration and to ensure the readings were within the normal curves. The sample was dried at 110^{0} C for 1 hour then ground. 1 gram was digested with 20ml concentrated nitric acid on a hot plate at 60^{0} C until nitrogen dioxide (brown gas) disappeared and the volume was reduced to about 10ml. The mixture was cooled slightly then 2ml of hydrogen peroxide was added and returned to the hot plate for a few minutes when the solution was clear after any remaining solids had been oxidized. The same was

filtered and topped to 50ml with distilled water then read on the Atomic Absorption spectrophotometer.

Statistical analysis

Statistical analysis was performed using SPSS version 16 (SPSS Inc., Chicago, USA). Data were entered in excel then exported to SPSS and cleaned before analysis. Analysis was by one-way analysis of variance (ANOVA) followed by Kruscal Wallis multiple comparisons post hoc test. Results are expressed as means±SD of triplicate samples. Differences were considered significant at 95% confidence interval (p<0.05).

4.5 Results

Beta carotene levels at vegetative stage were highest in *S. nigrum* and lowest in *A. Hybrids* while that of ascorbic acid at the same stage was highest in *A. hybridus* and lowest in *S. nigrum*. At the flowering stage on the other hand, beta carotene was highest in *A. hybridus* and lowest in *G. gynandra.* Ascorbic acid at the same flowering stage was highest in *G. gynandra* and lowest in *A. hybridus* (Table 4.1).

Table 4.1: Vitamin content of three AILVs on dry weight basis (mg/100g) at both vegetative and flowering stages

	Amaranthus hybridus	*Solanum nigrum*	*Gynandropsis gynandra*
Beta carotene			
-vegetative	23.84±1.07	30.59±0.82	29.39±0.53
-flowering	30.40±0.78	23.24±0.49	15.70±0.40
Ascorbic acid			
-vegetative	3014.60±14.180	748.99±14.74	1193.70±0.91
-flowering	464.79±0.40	466.65±13.66	525.79±9.04

These are means of triplicate samples (means±SD)

Table 4.2 shows the levels of minerals at vegetative and flowering stages for the three AILVs. At vegetative stage, copper was in very small quantities. The mineral which had the highest amounts in the three AILVs at this stage was calcium. *A. hybridus* had the highest amounts in three out of the six minerals. Two out of six minerals were highest in *G. gynandra*. *S. nigrum* had the highest amounts in iron. *S. nigrum* also had the lowest levels in three out of the six minerals. *G. gynandra* had the lowest amounts of manganese and iron. The levels of the other minerals in the AILVs varied.

At the flowering stage, the levels of copper reduced from the vegetative stage in all the three AILVs (Table 4.2). The levels of zinc however increased in all the three AILVs. Manganese increased in *A. hybridus* and *G. gynandra* but decreased in *S. nigrum*. The same trend was true for iron which increased in *A. hybridus* and *G.*

gynandra and reduced in *S. nigrum.* Magnesium on the other hand decreased in *A.*

hybridus and *S. nigrum* but increased in *G. gynandra*. Calcium increased only in *A.*

hybridus and reduced in the other two AILVs.

Table 4.2: Mineral content of three AILVs (mg/100g)dry weight basis at both vegetative and flowering stages

Minerals (stage)	Amaranthus hybridus	Solanum nigrum	Gynandropsis gynandra
Copper(vegetative)	0.56±0.01	0.60±0.02	0.78±0.02
(flowering)	0.47±0.09	0.51±0.04	0.20±0.10
Zinc (vegetative)	7.80±0.22	4.37±0.03	14.77±0.29
(flowering)	9.76±0.67	5.28±0.24	15.23±0.672
Manganese(vegetative)	32.45±1.15	19.69±0.06	15.96±0.09
(flowering)	46.89±1.24	8.66±0.32	22.66±1.27
Iron (vegetative)	31.43±0.58	42.44±0.21	23.54±1.36
(flowering)	37.33±2.38	11.48±1.29	73.73±8.48
Magnesium(vegetative)	733.96±6.14	252.80±2.18	266.28±5.82
(flowering)	597.93±10.07	223.30±14.26	271.43±6.44
Calcium(vegetative)	3153.20±51.29	1369.70±5.59	1627.30±61.89
(flowering)	3556.46±135.52	710.55±43.91	1441.82±58.92

The values are means of triplicate samples (mean±SD)

Comparison between the three vegetables using ANOVA at both vegetative and

flowering stages indicated that all the eight nutrients, beta carotene, ascorbic acid,

zinc, manganese, iron, magnesium, copper and calcium were significantly different

($p < 0.05$). Multiple comparison between the vegetables indicated that there was

significant difference in all except between *S. nigrum* and *G. gynandra* as related

to beta carotene (p>0.05) at vegetative stage and between *A. hybridus* and *S. nigrum* as related to copper and ascorbic acid at flowering stage (Appendix 7).

Moisture content

The moisture content at the vegetative stage was 87.6% (*G. gynandra*), 90.3% (*S. nigrum*) and 90.5% (*A. hybridus*), while at the flowering stage it was 82.3% (*G. gynandra)*, 89.2% (*S. nigrum)* and 89.4% (*A. hybridus)*. The difference in the moisture content in each of the three AILVs at vegetative and flowering stage was not significant (p>0.05).

4.6 Discussion

As a vegetable matures, different nutrients reach peak at different maturity stages (Makobo et al., 2010) and as it starts flowering to develop seed, the leaves reduce in growth (Akubugwo et al., 2007) therefore vitamin content. This corroborated the findings of the nutritional value analysis in the current study where the eight nutrients determined either reduced or increased with maturity depending on the vegetable. The two vitamins determined reduced with maturity except for beta carotene in *A. hybridus.* The minerals on the other hand increase in *A. hybridus* and *G. gynandra* each in four out of the six minerals at flowering. In *S. nigrum* at flowering stage, only one mineral (zinc) increased while the other five minerals reduced. It is known that *S. nigrum* first flowers then forms ‒bigger‖ fruits before

the seeds develop, but the other two AILVs flower, form ₌smallerø fruits and develop seed immediately. This therefore implies that when harvesting the vegetables at flowering stage, *A. hybridus* and *G. gynandra* had some seed. It is known that seed accumulate minerals more than any part of a vegetable (Akubugwo et al., 2007).

It is also known that the levels of the eight micro-nutrients in AILVs vary from one location of growing to another and across varieties, species (Weinberger and Msuya, 2004) and different stages of vegetable growth (Makobo et al., 2010). The amounts of the eight nutrients in the three AILVs in this study would generally meet the recommended daily requirements of an adult (Blake, 2008). AILVs are very rich in micronutrients compared to the exotic vegetables and are a very good resource against micronutrient deficiencies (Kimiywe et al., 2007; Gotor and Irungu, 2010; Miguel and Ivanovic, 2011). Increased production and consumption of African indigenous leafy vegetables would contribute to alleviating these deficiencies but due to nutrient interaction in the body which affects bioavailability (Lonnerdal, 1988); it is recommended that a variety of these vegetables are consumed to meet the daily requirements.

The three AILVs in this study have been ranked as priority among AILVs in the East African region by several studies(Abukutsa-Onyango, 2007; Gotor and Irungu, 2010; Kimiywe et al., 2007; Maundu et al., 1999; Weinberger and Msuya,

2004). They have also been associated with health promoting claims (Gotor and Irungu, 2010; Kimiywe et al., 2007; Smith and Eyzaguirre, 2007). Studies on nutritional value of AILVs have indicated that *S. nigrum, A. hybridus* and *G. gynandra* are of high value especially as relates to the eight micronutrients (Maundu et al., 1999; Odhav et al., 2007; Weinberger and Msuya, 2004) determined in this study. These micronutrients are essential in immune boosting and proper functioning of the body (Blake, 2008). The amounts of these micronutrients in the three vegetables at both vegetative and flowering stages in the present study are in higher values than those in the findings of Maundu et al., (1999), Weinberger and Msuya, (2004) and Odhav et al., (2007). The difference is attributable to the fact that the current study used the stalk, leaves and the main shoot commonly consumed in the study area for analysis while the above studies used only the leaves. It could also be due to different analytical methodologies as is noted that the samples in Weinberger and Msuya (2004) were raised to 700^0C and those in Odhav et al., (2007) were analyzed in an oven. It is also known that locational and varietal differences of the AILVs bring about differences in the amounts of nutrients.

The findings of the nutritional analysis in this study indicate that the eight micronutrients vary with growth stage of the vegetables. *A. hybridus* is more nutritious compared to the other two AILVs in most of the micronutrients

determined and therefore important in promoting good health. This corroborates with the findings of Weinberger and Msuya (2004) where three African indigenous vegetables were analyzed and amaranthus was nutritionally superior followed by African nightshade. Funke, (2011) reported that doctors recommended consumption of amaranth for patients who were anemic. Amaranthus is very rich in among others, ascorbic acid which is an important nutrient for iron absorption and therefore hemoglobin synthesis (Lonnerdal, 1988).

In conclusion therefore, the three AILVs used in the present study had high levels of vitamins at vegetative stage and high minerals at flowering stage. The minerals are especially in higher values for *A. hybridus* and *G. gynandra* at flowering stage than *S. nigrum.* It is therefore advisable to consume the three AILVs at both stages of growth since they are mainly consumed as sources of vitamins and minerals as well as an accompaniment for most staples.

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CHAPTER 5: EFFECT OF DIFFERENT COOKING METHODS ON THE NUTRITIONAL VALUE OF SOME AFRICAN INDIGENOUS LEAFY VEGETABLES IN KENYA

5.1 Abstract

This study was carried out to determine the effect of different cooking methods on the nutritional value of three African indigenous leafy vegetables, *Solanum nigrum, Gynandropsis gynandra* and *Amaranthus hybridus* between October 2011 and March 2012. They were cooked using four different methods; boiled for 5 minutes, boiled for 5 minutes and a further 3 minutes with fresh milk, fried in salad oil for 5 minutes and fried in salad oil for 10 minutes. The moisture content and levels of eight nutrients, beta carotene, ascorbic acid, calcium (Ca), copper (Cu), magnesium (Mg), manganese (Mn), zinc (Zn) and iron (Fe) were determined both in the raw and variously cooked samples. The raw samples were generally of higher nutritional value than the cooked samples. In *A. hybridus*, the best cooking method with the highest nutrient retention was boiling for 5 minutes while the worst cooking method was frying for 10 minutes. In *G. gynandra*, the best cooking method was boiling for 5 minutes while the worst was boiling for 8 minutes with milk. In *S. nigrum*, boiling for 5 minutes was the best cooking method with the highest nutrient retention while boiling for 8 minutes with milk was the worst. One way ANOVA indicated a significant difference between the three vegetables across the various cooking methods (p<0.05). *Amaranthus hybridus* had an overall

higher nutritional value across the different cooking methods. Boiling vegetables for 5 minutes was the best cooking method.

5.2 Introduction

Most vegetables worldwide are cooked before consuming to improve palatability, texture and taste (Migliot et al., 2008). Cooking also eliminates potential pathogens and neutralizes poisonous or irritating substances while bringing spoilage to a halt (Martin and Meitner, 1998). Various cooking methods are used based on convenience and taste preference rather than nutrient retention yet cooking induces significant changes in chemical composition affecting concentration and nutrient bioavailability (Gao-feng et al., 2009). Some cooking methods may oxidize anti-oxidants (Shahnaz et al., 2003) and affect the vegetable nutrient retention. It has also been revealed that adding spices (Gayathri et al., 2004) and cooking leafy vegetables in a specific utensil (Kumari et al., 2004) may have an effect on the nutrient content. It is therefore important to choose a cooking method that leads to optimal nutrient retention and bioavailability to get maximum nutrients from a vegetable (Funke, 2011).

The three African indigenous leafy vegetables which were of significance in contributing to immune boosting, healthy functioning of the body and good nutrition from the baseline survey were selected. They were cooked using various preparation methods standardized from the communitiesø practices and the levels of beta carotene, ascorbic acid, and minerals iron, zinc, calcium, copper, magnesium and manganese determined. The raw samples of these vegetables were used as a control. The levels of these nutrients were compared both within each vegetable and across the three vegetables for the various cooking methods.

5.3 Objective

The objective of the study was to determine the effect of various cooking methods on the levels of vitamins A and C and minerals iron, magnesium, manganese, copper, calcium and zinc of the selected African indigenous leafy vegetables.

5.4 Materials and methods

The three African indigenous leafy vegetables were *Solanum nigrum, Gynandropsis gynandra,* and *Amaranthus hybridus.* They were harvested at vegetative stage early in the morning by breaking the main shoot and cleaned as described on section 4.4.

To obtain homogenous samples, each vegetable was harvested in batches of about 1kg then each batch was thoroughly mixed before selecting a representative sample of 750g. The 750g sample was divided into five equal samples. Each sample was washed, drained and finely chopped and thoroughly mixed again before being subjected to its preparation method.

Sample preparation

Sample 1-the vegetable was boiled for 5 minutes while covered but frequently stirring and then rapidly cooled by spreading on a large surface area (tray) and placing in a refrigerator.

Sample 2-the vegetable was boiled for 5 minutes while covered and frequently stirring and a further 3 minutes with fresh milk then rapidly cooled as sample 1.

Sample 3-the vegetable was fried with salad oil for 5 minutes covered, while frequently stirring. It was then rapidly cooled as sample 1.

Sample 4-the vegetable was fried with salad oil for 10 minutes covered while frequently stirring. It was rapidly cooled as sample 1.

Sample 5- the raw vegetables.

Cooking of samples was done at 93^0C while distilled water was only added if it seemed like the vegetable was burning. Five milliliters of salad oil was used in frying for every 150grams of fried samples of vegetable. On the other hand, no additional water was added to the different cooked samples of *A. hybridus* and *S. nigrum* during cooking. During cooking of *G. gynandra* samples however, for every 150g of vegetable, 28ml of distilled water was added to each of the samples boiled for 5minutes, fried for 5 minutes and boiled for 8 minutes while 112ml was added to the sample fried for 10 minutes. As regards the samples boiled for 8 minutes where fresh pasteurized milk was added for further boiling, only the amount of milk required for the vegetable to continue boiling for a further three minutes was added. The amount of milk for every 150g of vegetable was 27mleach for *A. hybridus* and *S. nigrum*, and 73ml for *G. gynandra.*

Moisture content and levels of beta carotene and ascorbic acid were determined immediately after sample preparation. Thereafter, the minerals zinc, iron, copper, calcium, magnesium and manganese were determined. The duration of sample preparation from harvesting was a maximum of one hour.

Analytical methods

Procedures of moisture content, beta carotene, ascorbic acid and mineral determination were carried out as described in section 4.4.

Statistical analysis

Statistical analysis was performed using SPSS version 16 (SPSS Inc., Chicago, USA). Data was entered in excel then exported to SPSS. Analysis was by one-way analysis of variance (ANOVA) followed by Kruscal Wallis multiple comparisons post hoc test. Results are expressed as means±SD of triplicate samples. Differences were considered significant at 95% confidence interval ($p<0.05$).

5.5 Results

The nutritional value of the three vegetables was generally higher in the raw samples for all the eight nutrients (Tables 5.1, 5.2, 5.3). Boiling for 5 minutes was the best cooking method for nutrient retention across all the three vegetables.

The raw form of *A. hybridus* had higher nutritional value in three (Mn, Mg and ascorbic acid) out of the eight nutrients compared to the forms prepared in the different ways (Table 5.1). On comparing the four cooking methods, boiling for 5 minutes was the best one with the highest nutrient retention in five (Zn, Mn, Mg, beta carotene and ascorbic acid) out of the eight nutrients. Frying for 10 minutes was the worst cooking method and retained the least amount of calcium, beta carotene, and ascorbic acid.

Table 5.1: Micronutrient content(mg/100g dry weight) of *Amaranthus hybridus* by cooking method

	Raw	Boiled 5mins	Boiled/milk 8mins	Fried 5 mins	Fried 10 mins
Cu	0.56±0.01	0.51±0.07	0.25±0.01	0.56±0.56	0.37±0.19
Zn	7.80±0.22	8.47±1.40	7.79±0.11	7.10±0.27	7.43±0.29
Mn	32.45±1.15	28.90±0.78	22.31±1.24	25.65±0.59	23.87±0.41
Fe	31.43±0.58	30.07±0.72	25.27±1.14	32.71±1.00	30.42±0.90
Mg	733.96±6.14	557.67±13.07	549.77±6.00	476.47±8.71	546.35±3.54
Ca	3153.20±51.29	3065.40±199.90	4765.00±55.28	2756.10±127.86	2644.50±79.27
Beta carotene	23.84±1.07	46.58±0.61	36.53±0.811	36.45±0.66	35.90±1.49
Ascorbic acid	3014.60±14.18	1590.10±0.92	1431.10±0.82	1394.90±69.13	1229.0±8.25

The values in the table are means of triplicate samples of *Amaranthus hybridus* (mean±SD)

Data analysis using ANOVA showed that there was significant ($p < 0.05$) difference between all the nutrients across the five preparation methods in *A.hybridus* except in zinc ($p > 0.05$). Multiple comparisons using Kruscal Wallis post hoc test however revealed that there was significant difference ($p < 0.05$) in most of the comparisons except for copper between samples boiled 5 minutes and fried 5 minutes; boiled 5 minutes and raw; fried 5 minutes and raw (Appendix 7). Differences in zinc content were significant only between the samples boiled 5 minutes and fried 5 minutes ($p < 0.05$) but not in the rest of the samples. Manganese content on the other hand was significantly different ($p < 0.05$) across all the samples except those boiled for 8 minutes with milk and those fried for 10 minutes. Iron levels across the five preparation methods were significantly different ($p < 0.05$) in all the comparisons except for samples boiled for 5 minutes and fried for 10 minutes; boiled for 5 minutes and raw; fried for 5 minutes and raw; fried for 10 minutes and

raw. As for magnesium, multiple comparison between all the samples showed significant difference ($p<0.05$) except for samples boiled for 5 minutes and boiled for 8 minutes with milk; boiled for 5 minutes and fried for 10 minutes; boiled for 8 minutes with milk and fried for 10 minutes. Comparison between the 5 preparation methods for calcium were significantly different ($p<0.05$) except for samples boiled for 5 minutes and raw; fried for 5 minutes and fried for 10 minutes. Multiple comparisons for beta carotene indicated significant difference ($p<0.05$) across the five preparation methods except between samples boiled for 8 minutes with milk and fried 5 minutes; boiled for 8 minutes with milk and fried 10 minutes; fried for 5 minutes and fried for 10 minutes. Ascorbic acid content was significantly different ($p<0.05$) in all the multiple comparisons except in samples boiled for 8 minutes with milk and fried for 5minutes.

The raw sample of *G. gynandra* had the highest values in six out of the eight nutrients, copper, zinc, manganese, magnesium, calcium and ascorbic acid (Table 5.2). On comparing the four cooking methods, boiling for 5 minutes was the best cooking method and it best retained five nutrients out of the eight, zinc, manganese, magnesium, calcium and beta carotene. The worst cooking method was boiling for 8 minutes with milk and retained the least nutrients in six out of the eight nutrients; zinc, manganese, magnesium, calcium, beta carotene and ascorbic acid.

Table 5.2: Micronutrient content(mg/100g dry weight) of *Gynandropsis gynandra* by cooking method

	Raw	Boiled 5mins	Boiled/milk 8mins	Fried 5 mins	Fried 10 mins
Cu	0.78±0.02	0.20±0.01	0.37±0.04	0.46±0.05	0.39±0.05
Zn	14.77±0.29	13.56±0.57	7.70±0.05	8.61±0.08	9.31±0.17
Mn	15.96±0.09	10.70±0.97	5.42±0.16	9.27±0.24	9.11±0.29
Fe	23.54±1.36	17.32±0.48	19.61±0.71	20.23±0.95	24.94±0.95
Mg	266.28±5.82	217.05±3.99	113.95±1.31	185.96±2.41	180.20±3.48
Ca	1627.30±61.89	1244.60±5.27	839.37±8.04	1056.30±85.49	1066.40±10.42
Beta carotene	29.394±0.53	32.00±0.52	18.37±0.72	31.39±0.36	28.29±0.57
Ascorbic acid	1193.70±0.91	687.78±7.02	615.50±6.13	720.13±0.75	730.68±0.92

The values in the table are means of triplicate samples (mean±SD)

Multiple comparisons using Kruscal Wallis showed that there was significant difference ($p < 0.05$) between the levels of nutrients across the 5 preparation methods except between the following samples (Appendix 7):

boiled 8 minutes with milk/fried 10 minutes (Cu);

fried 5 minutes/fried 10 minutes (Mn);

boiled 8 minutes with milk/fried 5 minutes (Fe);

fried 10 minutes/raw (Fe);

fried 5 minutes/fried 10 minutes (Mg);

fried 5 minutes/fried 10 minutes (Ca);

boiled 5 minutes/boiled 10 minutes with milk(Beta carotene)

Nutrient values in the raw sample of *S. nigrum* had higher nutrient values in seven out of the eight nutrients, except for beta carotene which was highest in the sample fried for 10 minutes (Table 5.3). On comparing the four cooking methods, boiling for 5 minutes was the best cooking method and retained the highest nutrients in five out of the eight zinc, manganese, iron, magnesium and ascorbic acid. The worst cooking method was boiling for 8 minutes with milk and retained the least nutrients for six out of the eight; zinc, manganese, iron, calcium, beta carotene and ascorbic acid.

Table 5.3:Micronutrient content(mg/100g dry weight) of *Solanum nigrum* by cooking method

	Raw	Boiled 5mins	Boiled/milk 8mins	Fried 5 mins	Fried 10 mins
Cu	0.60±0.02	0.25±0.04	0.35±0.02	0.38±0.02	0.49±0.01
Zn	24.37±0.03	4.24±0.17	3.73±0.12	3.83±0.11	3.80±0.01
Mn	19.69±0.06	17.21±0.35	8.84±0.42	15.64±0.08	14.11±0.44
Fe	42.44±0.21	39.61±0.87	32.87±0.63	38.35±0.84	36.53±0.93
Mg	252.80±2.18	197.88±4.44	165.43±3.60	154.50±3.53	170.62±0.73
Ca	1369.70±5.59	880.89±8.28	758.08±15.26	866.76±26.06	991.71±6.16
Beta carote	30.59±0.82	23.57±0.37	23.51±0.28	34.62±0.53	39.86±0.34
Ascorbic acid	748.99±14.74	684.23±14.56	425.04±5.18	509.39±6.63	561.79±7.78

The values in the table are means of triplicate samples (mean±SD)

Multiple comparisons using Kruscal Wallis showed that there was significant difference ($p < 0.05$) between the levels of nutrients across the 5 preparation methods except between the following samples (Appendix 7),

boiled 8 minutes with milk/fried 5 minutes (Cu);

boiled 5 minutes/raw (Zn);

boiled 8 minutes with milk/fried 5 minutes (Zn);

boiled 8 minutes with milk/fried 10 minutes (Zn);

fried 5 minutes/fried 10 minutes (Zn);

boiled 5 minutes/fried 5 minutes (Fe);

boiled 8 minutes with milk/fried 10 minutes (Mg);

boiled 5 minutes/fried 5 minutes (Ca);

boiled 5 minutes/boiled 10 minutes with milk (Beta carotene)

Multiple comparisons between the levels of the nutrients across the three vegetables and the five preparation methods showed that there was significant difference ($p<0.05$) within and between most of the groups except for the following samples (Appendix 7);

boiled for 5 minutes *S. nigrum/G. gynandra* (Cu and ascorbic acid)
boiled 8 minutes with milk *S. nigrum/G. gynandra* (Cu)
boiled for 8 minutes with milk *A. hybridus/g. gynandra* (Zn)
fried for 5 minutes *S. nigrum/G. gynandra* (Cu)

fried 10 minutes *A. hybridus/G. gynandra* (Cu)

fried 10 minutes *S. nigrum/G. gynandra* (Ca)

raw *S. nigrum/G. gynandra* (Beta carotene)

A. hybridus was of higher nutritional value than the other two ALVs across the preparation methods. On comparing nutrient retention in the four cooking methods in the three vegetables and the eight micronutrients, boiling for 5 minutes had the highest nutrient retention. This method of cooking had the highest nutrient retention in 5 nutrients out of the eight in each of the three vegetables, *A. hybridus* (Zn, Mn, Mg, Beta carotene and Ascorbic acid), *G. gynandra* (Zn, Mn, Mg, Ca and Beta carotene) and *S. nigrum* (Zn, Mn, Fe, Mg and Ascorbic acid). The next important cooking method for *S. nigrum* and *G. gynandra* was frying for 10 minutes with the highest nutrient retention of Cu, Ca, Beta carotene and Fe, Ascorbic acid respectively. As for *A. hybridus*, the other three nutrients were Ca which was best retained in samples boiled for 8 minutes with milk and Fe and Cu in samples fried for 5 minutes. One nutrient in *G. gynandra*, Cu was best retained in samples fried for 5 minutes.

Percentage moisture content was highest in the raw forms of the three vegetables and ranged from 87.6% (*G. gynandra*) to 90.5% (*A. hybridus*) as indicated in Table 5.4.The percentage moisture content in *Solanum nigrum* and *Gynandropsis gynandra* followed the same trend with reducing amounts in raw, boiled 5

minutes, fried 10 minutes, fried 5 minutes and boiled 8 minutes with milk respectively. The percentage moisture content in *Amaranthus hybridus* on the other hand did not follow this order. Its percentage moisture content was in reducing trend from raw, boiled 5 minutes, boiled 8 minutes/milk, fried 5 minutes and fried 10 minutes.

Table 5.4: Percent moisture content of the variously cooked AILVs

Vegetable	Raw	Boiled 5mins	Boiled 8mins/milk	Fried 5mins	Fried 10mins
Amaranthus hybridus	90.5	87.5	85.2	85.2	84.6
Solanum nigrum	90.3	83.0	72.4	78.5	81.6
Gynandropsis gynandra	87.6	81.4	73.2	78.4	78.7

The values are arithmetic average of duplicate samples per each vegetable and cooking method

5.6 Discussion

As expected, raw vegetables are generally of a higher nutritional value than the variously cooked within the same vegetable. It would appear that raw vegetables are of more benefit to consume than cooked, yet cooking of vegetables is important to soften the matrix of cells and increase extractability of nutrients (Migliot et al., 2008) while destroying anti-nutritional factors (Martin and Meitner, 1998). Cooking for a longer time however leads to a higher loss of most of the nutrients (Mathooko and Imungi, 1994; Funke, 2011) especially if cooking water is discarded since most nutrients leach into it (Jimenez-Monreal et al., 2009). The

same is observed in the current study whereby most nutrients are retained when the vegetables are boiled for only 5 minutes in its water with no water discarded, further cooking leads to loss of most nutrients.

The nutrients across the raw samples of the three vegetables are higher in *Amaranthus hybridus* for four out of the eight nutrients determined. These findings corroborate with those of Funke, (2011) in which Amaranth had high values of micronutrients including Ascorbic acid which is an important nutrient in the absorption of iron from food thus red blood cell formation (Lonnerdal, 1988). Though boiling is the most common method of cooking vegetables worldwide, boiling in excess water has led to leaching of some nutrients especially ascorbic acid (Gao-feng et al., 2009). The vegetables in the current study were boiled in own water and extra water only added to avoid burning and no excess water was discarded. Boiling for five minutes has the highest nutrient retention across the three vegetables and frying on the other hand was not the best method of cooking for nutrient retention since some nutrients are oxidized during frying (Shahnaz et al., 2003) though some oil in the vegetables during cooking enhances absorption of beta carotene and other nutrients.

The high moisture content of the raw vegetables of *Amaranthus hybridus* (90.5%) and *Solanum nigrum* (90.3%) corroborate with the findings of Fuglie, (2001) and

FAO, (2006). This high moisture content helps with faster and easier assimilation of nutrients with less pressure on the digestive system (Lussier, 2010).The moisture content of raw *Gynandropsis gynandra* is however lower (87.6%). The reduction of the moisture content of the three vegetables variously cooked to less than 90% indicates that some water is lost during cooking.

The findings of the current study indicate that different cooking methods have different effects on different nutrients. Vegetables worldwide are consumed for the high micronutrient content therefore it is important to choose a cooking method which best retains most nutrients in a vegetable. In this case, boiling for 5 minutes retains most nutrients across the three vegetables. The issues of bioavailability were, however, not considered in this study.

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CHAPTER 6: EFFECT OF *Amaranthus hybridus* ON THE IMMUNITY OF LABORATORY WHITE ALBINO RATS

6.1 Abstract

The efficacy trial tested the role of *A. hybridus* in boosting the immunity of 30 female laboratory albino rats. This vegetable was of higher nutritional value compared to the other two AILVs analysed in the preceding chapters of this thesis. The ratsø ages ranged from 13-20 weeks and weighed 160-250 g. They were randomly assigned to four groups, A to D of 10 rats each for groups A and B, and 5 rats each for groups C and D. An effort was made to ensure the different ages were evenly distributed in the four groups. The rats were followed for 16 weeks during which groups A to C had their immunity suppressed with an initial daily dose of 10mg/kg body weight of Cyclosporine A for 22 days and for a further 45 days at 30mg/kg body weight. Thereafter, groups A (raw) and B (cooked)were given the vegetable at a rate of 1-2% of their body weight as a supplement to the rat pellet every other day for 24 days. The rats were bled four times, (T1 to T4) whereT1 was the baseline, T2 was22 days after T1, T3 was45 days after T2 and T4 was24 days after T3. Levels of the four immune indicators;CD3%, CD^+4 and CD^+8 counts, CRP were determined during each bleeding. The results indicated that the positive control (group C) was worse off than the groups given vegetables after suppressing immunity (groups A and B) in terms of CD^+4/CD^+8 ratio. There was however no significant difference (p>0.05) within the groups given raw or cooked vegetable. The negative control group (D) was the best performing group

as per this indicator. The change of C-reactive protein levels between T3 and T4 when the vegetable was introduced was positive for the groups receiving the vegetable (A and B) and negative for those not receiving (C and D). There was however no significant difference ($p > 0.05$) between all the groups as per CRP. The vegetables may have introduced anti-nutritional factors.

Key words: Albino rats, *A. hybridus,* immune indicators, Cyclosporine A

6.2 Introduction

Amaranth is an indigenous African leafy vegetable that is consumed the world over and especially in Africa. In Kenya it is consumed in every part of the country by all the people. It grows fast especially after the rains and is diverse with different varieties and species (Abukutsa-Onyango, 2007). This vegetable has a high density of vitamins, minerals and other bioactive ingredients and there have been a lot of claims on immune boosting and other health benefits about it (Kimiywe et al., 2007) though such claims have not been authenticated.

The preceding work of this research analysed the eight micronutrients of immune importance for the three AILVs selected from the baseline survey which contributed significantly to immune boosting, healthy functioning of the body and good nutrition. *Amaranthus hybridus* was selected to be used in the efficacy since

it was of higher nutritional value in most nutrients determined and boiling for 5 minutes adapted since it retained most of the nutrients. The vegetable was fed to the rats as a supplement to the rat pellet feed.

The albino rats were used in this efficacy trial since they proliferate very fast and have a reasonable size. They also quickly adapt to different environmental factors and have been used in laboratories for different studies ranging from immunological, toxicological to nutrition (Benevenga et al., 1995).

Since several factors affect the proper functioning of the immune system including micronutrient deficiencies, infections, illnesses, major burns, medications and emotional and physical stress, there is no single test that can measure immune response (Gibson, 2005). CD3 constitutes CD^+4 and CD^+8 T-killer cells. CD^+4 counts indicate the strength of the immune system, the higher the counts the stronger the immunity, while the higher the CD^+8 counts the weaker the immune strength. The two indicators are inversely related to each other and should be considered together for a clearer picture of the impact of any treatment.

The 30 white albino rats in this study were used to test the immune boosting claims of *Amaranthus hybridus* with a view to possibly exploiting this potential and using the vegetable as an immune booster.

6.3 Objective

The objective of the efficacy trial was to determine the impact of *Amaranthus hybridus* vegetable on the immunity of white albino rats.

6.4 Materials and methods

Materials

Animals

The white albino rats were all female weaners acquired from the Animal Unit of the College of Biological and Physical Sciences, University of Nairobi and were acclimatized for one month before deworming. They were housed in spacious and high cages allowing free movement as described by Lawlor M (Guttman 1990). There were six cages of 5 rats each. The Animal House for the cages was kept clean, well ventilated with temperature/humidity maintained at $19\text{-}21^0\text{C}/61\text{-}66\text{g/m}^3$. Lighting was at 12hr light/12hr dark and the rats fed rat pellets and tap water ad libitum. The rats were kept clean by changing beddings (wood shavings) every third day or earlier as was necessary. A veterinary doctor was always on call

and from time to time monitored the health of the rats throughout the experimental period. The weights of the rats were taken every third day throughout the experiment period to ensure dosage of drug and vegetable is per weight and also to monitor the general health and wellbeing of the rats.

Cyclosporine A

Cyclosporine A (CyA)drug was used to suppress the immunity of the rats. The drug binds to cyclophilin protein of lymphocytes especially the T-cells to form a complex. The complex inhibits calcineurin enzyme which under normal circumstances would activate T-cell proliferation. Cyclosporine A thus lowers the activity of cells and their immune response. CyA was packaged in capsules of 50mg each and was diluted in virgin olive oil initially to 1ml equivalent to 10mg and during the second regimen to 1ml equivalent to 30mg.

Vegetables

About 600g leaves of *Amaranthus hybridus* was harvested at vegetative stage from a plot in the Field Station of College of Agriculture and Veterinary Sciences, University of Nairobi early morning of every day of feeding and cleaned as described in section 4.4. A representative homogeneous sample of 500g was picked from this and washed three times using tap water then rinsed twice with distilled water. The leaves were then finely chopped and divided into two equal portions after further mixing. One portion was prepared as raw while the other was boiled in its water, stirring frequently for 5 minutes. Half of each of the two

samples was pound separately using mortar and pestle and passed through a sieve to make juice for the rats. The other half was used to determine vitamins A and C and minerals iron, zinc, copper, calcium, magnesium and manganese using the analytical methods described in section 4.4. The same vegetables fed to the rats was sampled twice per week, therefore vegetables were sampled for analysis 6 times during the vegetable feeding regimen (appendix 2). The vegetable juice was fed to the animals by oral tubation.

Blood

The rats were bled four times by retro- orbital bleeds. Each time 1ml of blood per rat was drawn, 0.5ml for T-cell determination was collected in EDTA (1ml tubes and capillary tubes) and 0.5ml blood for serum C-reactive protein determination collected in non-EDTA (1ml tubes and capillary tubes).

Methods

In this research, the CD3% in the blood and T-killer cells, CD^+4, CD^+8 counts and C-reactive protein were determined to give an indication of immune strength in 30 White Albino rats.

Table 6.1:Date of birth of experimental White albino rats by treatment group

GROUP	18/2/2012	13/3/2012	21/3/2012	4/4/2012
A	4	5		1
B	3	6	1	
C	1	2	1	1
D	1	2		2

During the 16 weeks, the immunity of the albino rats in groups A, B and C was suppressed for 22 days using cyclosporine A at a rate of 10mg/kg body weight, then blood was drawn from all the rats. This was used to check the levels of the four parameters (T2). Immunity was immediately thereafter suppressed for a further 45 days at 30mg/kg body weight. The drug was given orally and daily (every 24 hours).Then blood was drawn from all the rats. This was used to check the levels of the four parameters (T3). Thereafter rats in the two groups, A and B were given *Amaranthus hybridus* orally in juice form every other day for 24 days. This was given to supplement their normal diet, rat pellets. Group A was given the raw vegetable; while group B was given the cooked at a rate of 1-2% of the body weight which translated to 20% of the daily feed. Rats eat feed of 5-10% of their body weight daily (Wolfensohn and Lloyd, 2003) and the volume of vegetable juice used in this efficacy trial was the maximum amount that the rats could take comfortably as was established during the pre-trial on non-experimental rats.

Group C was the positive control and was not given any vegetable after immunity was suppressed while group D was the negative control(no treatment). At the end of the 24 days all the rats were bled for final checking of levels of the four parameters (T4). The treatments during the experiment period are summarized in the table6.2:-

Table 6.2:Treatment of the thirty white albino rats over time

Group	No. of rats	T1	Suppress immunity 22days	T2	Suppress immunity 45 days	T3	Give vegetable	T4
A	10	√	√	√	√	√	√	√
B	10	√	√	√	√	√	√Cooked	√
C	5	√	√	√	√	√	×	√
D	5	√	×	√	×	√	×	√

A- Suppressed immunity, raw vegetables.   T1- 1st bleedingç- administered
B- Suppressed immunity, cooked vegetables.T2- 2nd bleeding×- not administered
C- Suppressed immunity, no vegetables.   T3- 3rd bleeding
D- No suppressed immunity, no vegetables. T4- 4th bleeding

Analytical methods

CD3, CD^{+}4, CD^{+}8 determinations

0.5ml (500µl) of blood drawn from the rats was used to determine T-cell counts using the anti-rat antibodies (catalogue no. 551397, Belgium) method and read on the Facs Calibar.

80µl of blood was put in a falcon tube of 1ml volume. The white blood cells were then stained using antibodies, 2µl each of anti-body for CD3, CD^{+}4 and CD^{+}8. These antibodies were added following each other ensuring mixing after each addition. The sample was incubated for 20minutes.1000µl of lysing buffer was added and mixed in thoroughly to destroy any red blood cells and also to clean the

blood sample. The sample was then incubated for a further 10 minutes then span at 1500rpm for 5 minutes. Any unbound antibodies were then washed off using Phosphate Buffered Saline (PBS) washing buffer for 2-3 times until the sample was ─clean╛ Then 500µl of washing buffer was added to suspend the T-cells for reading in the Facs callibar, Flow Cytometry

C - reactive protein (CRP) determination

The other 0.5ml (500µl) blood was used to determine the CRP concentration by using the anti-rat CRP from BD (catalogue no. 557825, Belgium) method and read on Elisa Reader.

The 500µl of blood was transferred to a falcon tube and allowed to clot for 30 minutes then span on a centrifuge at 3000rpm for 5 minutes. Serum was collected using a pipette and diluted using the provided stock to 1:4000. Thereafter, 100µl of diluted serum was transferred into a micro-well of a plate and incubated for 30 minutes at room temperature. The same was washed off 4-5 times with gentle stream of wash buffer, PBS. To each micro-well was added100µl of detection antibody. The plate was covered and incubated for 30 minutes at room temperature then washed 4-5 times with washing buffer. Thereafter, 100µl of Tetramethylbenzidine (TMB) substrate solution was added and incubated for 5-10 minutes at room temperature. The reaction was stopped by adding 100µl of stop solution and absorbance read at 630 nm wavelengths on an Elisa reader within 30

minutes of stopping reaction.CRP concentration in mg/ml was obtained by multiplying the absorbance with the dilution factor (which was 4,000).

Statistical analysis

Statistical analysis was performed using SAS Software. To compare the different treatment groups, one-way analysis of variance (ANOVA) and t-test were carried out (Appendix 7). One way analysis of variance is robust for unequal observations as was the case in this study where the four groups A, B, C and D had varying number of rats.. Results are expressed as means±SD of samples and differences were considered significant at 95% confidence interval ($p<0.05$).

6.5 Results

During the 16 weeks, some rats died both during bleeding or feeding and at T3 and T4 there were a total of 27 and 25 rats respectively. The total number of rats who had died by the end of the experiment were 5, two from group A and one each from groups B, C and D.

The trend of CD3% was the same for groups A and B and also the same for groups C and D (table 6.3) over time. The trend of $CD^+4\%$ and $CD^+8\%$ across the groups over time was however varied.

Table 6.3: CD3%, CD+4% and CD+8%values in the different groups across time

Treatment	Time	CD3%	CD+4%	CD+8%
Raw (A)	**T1**	59.61Ñ11.57	38.53±6.22(10)	28.31±8.61(10)
	T2	74.92±9.78(10)	42.79±4.99(10)	27.87±4.79(10)
	T3	42.61±5.88(8)	44.14±6.56(8)	20.62±6.23(8)
	T4	62.11±11.88(8)	33.47±6.90(8)	30.83±8.30(8)
Cooked (B)	**T1**	62.72±14.21(10)	36.43±4.78(10)	26.15±8.49(10)
	T2	75.79±12.50(10)	41.83±5.55(10)	21.77±6.78(10)
	T3	35.38±9.55(10)	43.16±5.77(10)	18.43±8.85(10)
	T4	60.08±8.22(9)	39.09±4.46(9)	25.48±6.71(9)
Postcontrol(C)	**T1**	68.24±7.81(5)	43.33±3.14(5)	21.31±4.94(5)
	T2	64.03±17.18(5)	38.86±5.51(5)	19.62±7.38(5)
	T3	26.52±7.15(4)	43.59±1.87(4)	12.10±6.86(4)
	T4	66.28±5.19(4)	42.78±5.59(4)	27.05±11.30(4)
Negcontrol(D)	**T1**	74.40±6.42(5)	42.50±4.35(5)	29.88±4.50(5)
	T2	68.18±9.43(5)	38.72±4.80(5)	22.37±11.55(5)
	T3	60.65±9.55(5)	40.63±4.25(5)	29.45±7.78(5)
	T4	70.99±4.79(4)	50.02±3.35(4)	24.16±3.52(4)

The results are expressed as mean±SD (n)

Analysis of the data indicated that there was significant differences ($p < 0.05$) in a few parameters in the different groups over time as indicated in the table (Appendix 7). The group which was given the raw vegetable had significant difference before (T3) and after introduction of the vegetable (T4) in CD3%, CD4% and CD8% while that given the cooked showed the same trend only for CD3%.There was significant difference ($p < 0.05$) for the positive control group for CD3% between T3 and T4, also for the negative control between T3 and T4 for CD4%.

The CD$^+$4 and CD$^+$8 immune indicators had varied trends across the groups (Table 6.4) over time. When the vegetable was introduced at T3 the changes of the ratio CD$^+$4/CD$^+$8, between T3 and T4 was negative for groups A, B and C and positive change for group D (negative control)

Table 6.4: CD$^+$4 and CD$^+$8 Counts and Ratio

Treatment	Time	CD$^+$4	CD$^+$8	CD$^+$4/CD$^+$8
Raw (A)	**T1**	23.37±7.22(10)	17.62±7.87(10)	1.36
	T2	32.26±7.16(10)	20.76±3.83(10)	**1.54**
	T3	18.68±3.13(8)	9.03±3.75(8)	**2.14**
	T4	20.77±5.82(8)	19.71±8.35(8)	**1.09**
Cooked (B)	**T1**	23.03±6.29(10)	16.95±8.32(10)	**1.39**
	T2	31.65±6.66(10)	16.65±6.82(10)	**1.92**
	T3	14.90±3.26(10)	6.93±3.98(10)	**2.34**
	T4	23.58±4.63(9)	15.63±5.53(9)	**1.53**
Postcontrol (C)	**T1**	29.74±5.23(5)	14.38±2.84(5)	**2.03**
	T2	24.58±6.69(5)	13.21±8.08(5)	**1.98**
	T3	11.65±3.60(4)	3.55±2.94(4)	**3.60**
	T4	28.18±2.22(4)	18.35±9.07(4)	**1.58**
Negcontrol (D)	**T1**	31.59±3.95(5)	22.27±4.16(5)	**1.42**
	T2	26.35±4.64(5)	15.88±8.86(5)	**1.73**
	T3	24.80±5.73(5)	18.30±6.75(5)	**1.38**
	T4	35.57±4.30(4)	17.17±2.93(4)	**2.07**

The results are expressed as **mean±SD(n)**
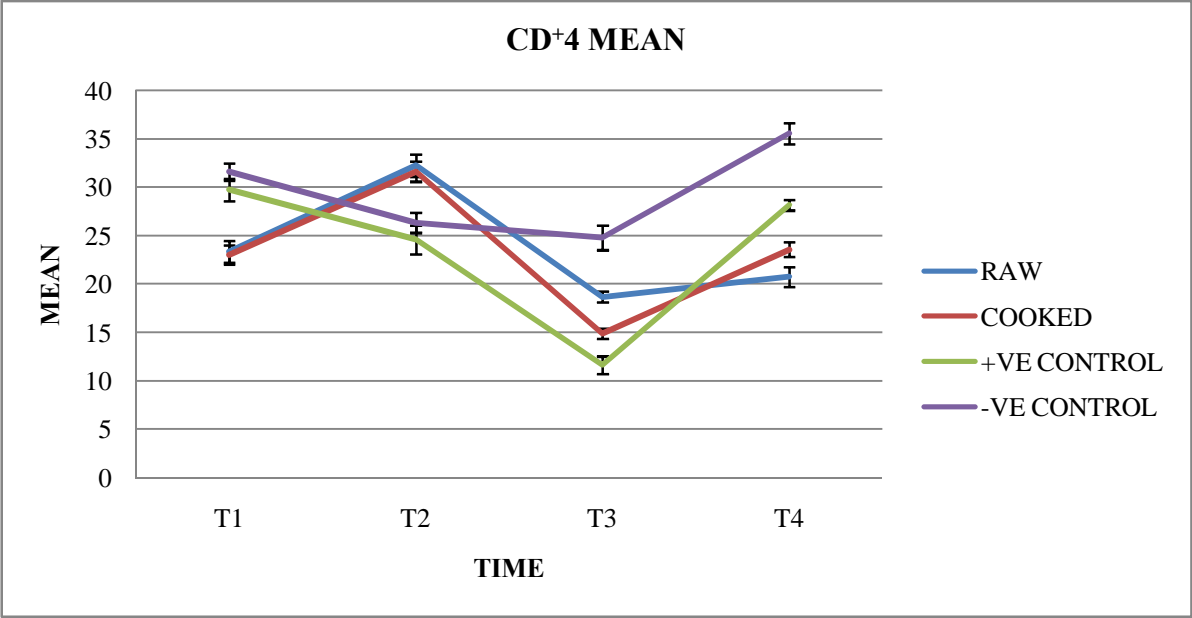
Figure 6. 1: CD$^+$4 trend over time
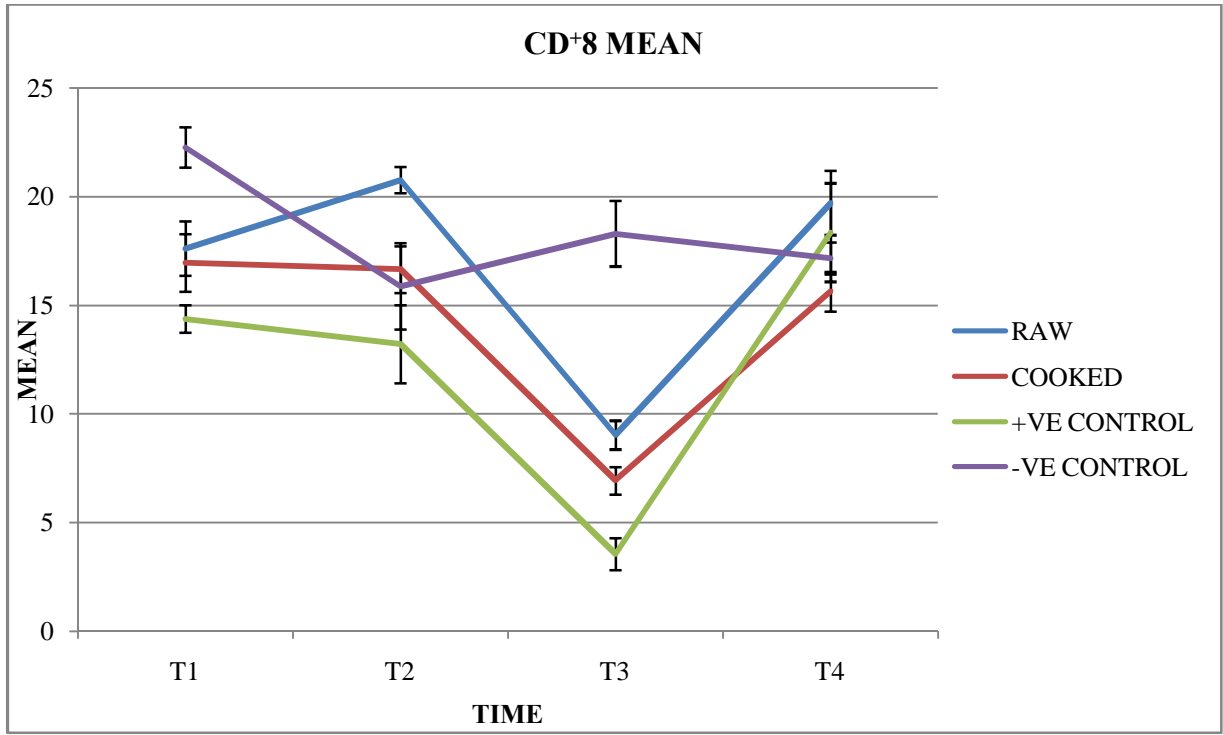
Figure 6. 2: CD$^+$8 trend over time

Analysis of the data showed that there was significant difference (p<0.05) for CD^+4 counts at T1 between groups A/D, B/D; at T3between groups A/B, A/D, B/D, A/C, C/D and at T4 between A/D, A/C, B/D and C/D. As for CD^+8 counts, there was significant difference (p<0.05) at T1 for the groups C/D and at T3 for the groups A/D, B/D and C/D. There was also significant difference (p<0.05) for $CD+4/CD^+8$ ratio between groups A/C at T1, and A/C, B/D at T3; also at T4 for groups A/B and A/D (Appendix 7).

The means of CRP varied across groups over time (table 6.5). When the vegetable was introduced at T3, these means increased in groups A and B but reduced in groups C and D at T4.

Table 6.5: C-Reactive Protein (mg/ml)

Treatment	T1	T2	T3	T4
Raw (A)	0.29±0.21 (7)	0.32±0.22 (7)	0.29±0.14 (7)	0.37±0.07 (7)
Cooked (B)	0.34±0.15 (8)	0.27±0.07 (8)	0.24±0.12 (8)	0.27±0.17 (8)
Postcontrol (C)	0.31±0.20(4)	0.14±0.16(4)	0.34±0.24(4)	0.33±0.06(4)
Negcontrol (D)	0.37±0.06(3)	0.32±0.02(3)	0.20±0.17(3)	0.15±0.25(3)

The results are expressed as mean±SD(n)

Analysis of the data showed that there was no significant difference between and within the groups as relates to C-reactive protein over time (Appendix 7).
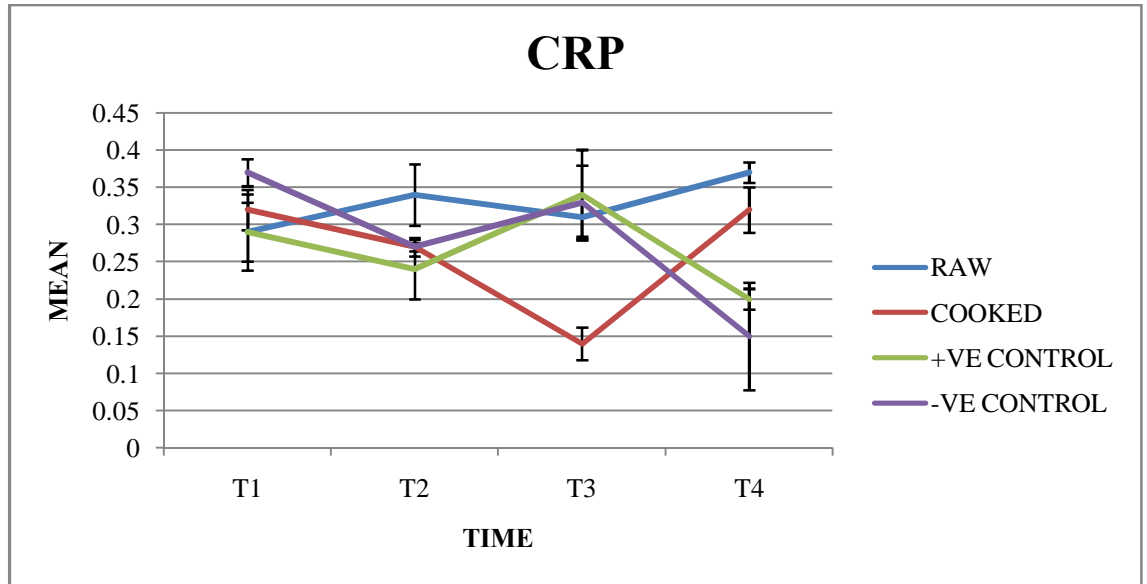
Figure 6. 3: CRP trend over time

6.6 Discussion

Different diets have different impacts on the immunity of laboratory animals. Vitamin A deficiency (VAD) diet in rats induces anatomic and metabolic changes comparable to those associated with neurodegenerative disorders (Rahab et al., 2009) but it does not affect body weight and longitudinal growth in mice (Sagazio et al., 2007). Protein energy malnutrition on the other hand causes a long lasting oxidative damage to the brain resulting to reduced anti-oxidant activity (Feoli et al., 2006) in rats.

The ill health of laboratory animals may be restored with supplementation of an anti-oxidant rich diet (Gupta and Prakash, 2009). Dietary supplementation with

garlic, ginger and cabbage in male Wister rats reversed anemia caused by the toxic effect of cadmium (Eteng et al., 2012) and orchidectomized rats had improved anti-oxidant activity with orange pulp (Deyhim et al., 2007) and reduced osteoporosis due to improved femoral density with citrus juice (Deyhim et al., 2006). Pomegranate seed oil improved the immunity of mice (Yamasaki et al., 2006) while introduction of an extract from a plant traditionally used for medicinal purposes, *Alstonia boonei,* had beneficial effect in rats by lowering the lipid profile (Gabriel et al., 2008).

Vegetables are very rich in micronutrients and have been known to bring about various positive changes in laboratory rats in both hematological parameters and immune boosting. Supplementing the diet of rats with an African leafy vegetable has been seen to have remarkable increase in weight, hemoglobin and white blood cells, and reduction in serum protein and lipid peroxidation (Iweala et al., 2009) and diabetic laboratory rats showed increased total red blood cells and white blood cells(Saliu et al., 2012). These changes were an indication of immune stimulation. These findings corroborate with those of the present efficacy trial which indicates that *A. hybridus* boosts the immunity of white albino rats as seen in the changes of $CD^{+}4/CD^{+}8$ ratios between T3 and T4 after the introduction of *Amaranthus hybridus*. The positive control group is worse than the other 3 experimental groups because they needed the vegetable to boost immunity after its suppression.

Nevertheless, the boosted immunity due to the vegetable does not measure up to the negative control group whose immunity had not been previously suppressed. This could be due to nutrient binding factors in the vegetable. It is known that AILVs are high in phytates and other anti-nutritional factors that bind divalent metal ions like calcium, iron, zinc and copper forming chelates which make the metal ions unavailable (Uusiku et al., 2010; Ademoroti, 1996 as cited by Agbaire and Emoyan, 2012). These phytates and other anti-nutritional factors are destroyed by cooking. That is possibly why the group given raw vegetable (A) are worse than those who got the cooked vegetable (B) as seen in the changes of CD^+4/CD^+8 ratio between T3 and T4 which was more towards the positive for those who received the cooked vegetable thus stronger immunity.

The concentration of several serum proteins are affected by an infection or inflammation, these include Erythrocyte Sedimentation Rate (ESR), C-reactive protein (CRP) and Plasma Viscosity (PV). CRP changes fast with an inflammation and is not affected by many other factors like ESR and PV. During an inflammation therefore, it is recommended to measure the levels of CRP (Gibson, 2005) which changes fast and is more stable than the other two, ESR and PV.CRP levels in this study show variation without any specific pattern across the groups during the experimental period (T1 to T3). This however increases with the introduction of the vegetable at T3 while it reduces without the vegetable. The

changes in CRP concentrations between T3 and T4 when the vegetable is introduced are positive for the two groups (A and B) that received the vegetable. While that of positive control (C) and negative control (D) which did not receive the vegetable are negative. This could be due to the presence of anti-nutritional factors in the vegetable which made the animals in groups A and B ÷unhealthyø and increase in CRP concentration. While the vegetable is rich in micronutrients, it could also introduce some anti-nutritional factors which therefore increased CRP.

A healthy functioning immune system requires a variety of vitamins and minerals including anti-oxidants which are abundant in AILVs. The *A. hybridus* given to the white albino rats in this study provided such necessary nutrients. In conclusion therefore *A. hybridus* boosts the immunity of white albino laboratory rats and there is no significant difference ($p<0.05$) within and between the groups receiving raw and those receiving cooked vegetables.

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CHAPTER 7: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATION

General discussion

Consumption of a diversity of whole foods brings about food and nutrition security (GOK, 2011). This improves the health, nutritional status and productivity of a person especially if these foods are rich in micronutrients (fruits and vegetables).

The recommended daily requirement for consumption of fruits and vegetables is 400g (FAO, 1990). In the developing world however, the quantities of fruits and vegetables consumed varies depending on the season, rural or urban areas, culture and tradition among others but most populations are not able to meet the recommended daily intake of fruits and vegetables (Smith and Eyzaguirre, 2007; Shackleton et al., 2009).

Africa is however endowed with a cheap source of micronutrients in form of a diversity of African indigenous leafy vegetables (AILVs) which are very rich in micronutrients compared to exotic vegetables (FAO, 1990). African indigenous leafy vegetables have been known for good health, immune boosting and disease control (Kimiywe et al., 2007). They are therefore important for food and nutrition security. These vegetables have however been neglected in research and development and under-utilized, considered old fashioned, poor man's food and

therefore shameful to consume (Gotor and Irungu, 2010). Information on their agronomic practices, quality seed, and extension messages among others is lacking (Abukutsa-Onyango,

2007). Little is therefore known about them and are threatened with genetic erosion due to change in land use and eating habits (Saliu et al., 2012; Gudrun et al., 2004). In the recent past, however, a lot of interest in these vegetables has arisen due to claims on immune boosting properties. They have also been known to have medicinal values (Kimiywe et al., 2007).

In Kenya, Agriculture is the mainstay of the economy and the government has put in place several policies and programmes to address food and nutrition insecurity. The strategies in the recent past include, Strategy to Revitalize Agriculture (SRA), Vision 2030 and National Accelerated Agricultural Input Access Project(NAAIAP) and in the region, Alliance for Green Revolution in Africa (AGRA) and Comprehensive African Agricultural Development Programme (CAADP) among others (Nairobi, 2011). All these are aimed at increasing agricultural productivity and reduce poverty and therefore improve food and nutrition security. Most of these efforts however have concentrated on cereals (especially maize) productivity (Nairobi, 2011). Yet the Kenya government policy is to ensure safe and high quality foods by setting, promoting and enforcing appropriate guidelines, standards and regulatory framework.

Poverty and food insecurity is still a force to reckon with in Kenya despite these efforts undertaken by the government. Ten million people suffer from chronic food and nutrition insecurity annually and 2 to 4 million require food emergency at any given time (Nairobi, 2011). The problem of food and nutrition insecurity is worst among those whose immunity is compromised because of HIV/AIDS and/or other health conditions. The rich diversity of AILVs (chapter 3) is only profitable if most of them are utilized as food and a source of income (Lyatuu and Lebotse, 2010).

Increased consumption of AILVs leads to increased demand and therefore production especially in the urban and peri-urban areas (Nguni and Mwila, 2007). The fear of extinction of AILVs because of the changing farming practices and eating habits (Gudrun et al., 2004) and inability to pass the indigenous knowledge of their production, preparation, consumption and medicinal/health claims among others from generation to generation due to modernization and movement to urban centers (Weinberger and Msuya, 2004) makes researchers and other players very concerned about this resource. The findings of the baseline survey in this study (chapter 3) indicated that the older women had more medicinal/health value for the AILVs and consumed a higher variety than the younger women. Other factors which affected consumption of a variety of AILVs included seasonality, availability in the market, ethnicity and denomination one belonged to. Those who

belonged to Nomia and Legio Maria sects in the study area for example, did not consume most of the AILVs. The AILVs were known for different health benefits and three of these, *A. hybridus, G. gynandra* and *S. nigrum* were significant in contributing to good health, good nutrition and immune boosting. Further analysis of the survey data indicated that *A.hybridus* and *G. gynandra* were significant in contributing to immune boosting.

It is known that as a vegetable grows the nutritional value of the different parts change (Akubugwo et al., 2007) and vitamins which are mainly found in the leaves reduce with maturity while minerals increase. The findings of the present study (chapter 4) indicate high levels of vitamins at vegetative stage and high minerals at flowering stage. The minerals are especially in higher values for *A. hybridus* and *G. gynandra* at flowering stage than *S. nigrum. A. hybridus*is more nutritious compared to the other two AILVs in most of the micronutrients determined both at vegetative and flowering stages and for different cooking methods. This vegetable is therefore important in promoting good health and it was selected for use in the efficacy trial to confirm the immune boosting claims.

A cooking method is very important in ensuring nutrient retention in vegetables. Boiling in excess water leaches micronutrients (Gao feng et al., 2009) while frying may oxidize nutrients (Shahnaz et al., 2003). Cooking methods vary from

community to community and this was one of the findings in the baseline survey. Several ingredients were added to the vegetables during cooking including salt, onions, tomatoes, milk and groundnuts among others. Most of the AILVs were mixed, two or more types, during cooking and the duration of cooking varied greatly, some even going to three days! Frying of the vegetables by the communities was optional. All the practices above had different effects on the nutritional value of the AILVs. The methods of cooking used in this study, chapter 5, were standardized from the baseline survey findings and did not have any other additives other than those indicated. The findings of the present study (chapter 5) are that boiling the vegetable in its water for 5 minutes has the highest nutrient retention though it was not possible in this study to consider the issue of bioavailability. This cooking method was however used during the efficacy trial.

The findings of the efficacy trial (chapter 6) indicate that *A. hybridus* boosts immunity of White albino rats as seen in the four indicators (T-killer cells CD^+4 and CD^+8 counts, CD3% and C - reactive protein levels). The rats which receive the vegetable (A and B) show a more positive response than the positive control (C) whose immunity had been suppressed but not given the vegetable as related to CD^+4/CD^+8 ratio. That is the rats whose immunity is suppressed and not given the vegetable (C) are worse off than those whose immunity is suppressed and given the vegetable (A and B). On the other hand, the improvement of those who have

suppressed immunity and given vegetable is still worse off than the negative control D (no suppression, no vegetable).The vegetable however seems to have nutrient binding factors which might be destroyed with cooking. Thus the rats receiving the cooked vegetable, B, are better than those receiving the raw, A as indicated by the CD^+4/CD^+8 ratio across time. The positive control, C, are worse than the other animals receiving the vegetable. The negative control continues to be stronger and stronger all through, that is why it is the best performing group in terms of the four indicators of immunity. The rats on a scale of worst to best in immunity levels are rated as C, A, B and D as per the changes of the ratios of CD^+4/CD^+8 before and after introduction of the vegetable.

CRP is a remote indicator of immune status, presence of this protein in the serum only shows presence of an infection but not how bad it is. On comparing performance of the four groups in respect to CRP levels, the changes between T3 and T4 when the vegetable is introduced are positive for the groups receiving vegetables (groups A and B) except the positive control (C) and the negative control (D) where the changes are negative. This indicates that CRP concentration rises in the groups receiving the vegetable as opposed to those not receiving. The vegetable seemed to introduce some anti-nutritional factors in those receiving it thus increased CRP compared to those not receiving.

Conclusions and recommendations

The diversity of AILVs is narrowing and the older women could remember some extinct species that were known for their medicinal/health benefits. There is therefore need to conserve and re-introduce these AILVs to the community. This will also require more research on the same and aggressive promotion campaigns including nutrition education in schools, churches, colleges and institutions of higher learning through print and electronic media. There should also be a concerted effort to promote proper preparation, processing and preservation methods of AILVs for nutrient retention and bioavailability, and availability throughout the year for food and nutrition security. Such value addition will also ensure increased incomes for the farmers.

AILVs are harvested throughout the growth stage yet the micronutrient levels vary at each of these stages, there is therefore need to advice the consumers on the optimal period of consuming the AILVs since most diets in the developing world are micronutrient deficient.

In conclusion therefore, AILVs are very rich in micronutrients and *A.hybridus* boosts the immunity of white albino rats. Further research on the three AILVs in this study should be carried out and especially on *A.hybridus* with a view to isolate

the active ingredients in immune boosting. Confounding factors like anti-nutritional factors and effect of preparation method on bioavailability of some nutrients should be researched further. This would be useful in exploiting their nutritional potential especially for those whose immunity is compromised such as HIV/AIDS patients and other health conditions and for those who are in more need of the nutrients like the under-fives and lactating and pregnant mothers. Also, the post-harvest losses and perishability of AILVs is known to be very high (Onyango, 2010) therefore strategies to appropriately process, preserve and distribute for utilization (Chavasit, 2002) in the shortest time possible are very key. This will ensure availability throughout the year and proper cooking methods promoted will also ensure maximum retention of the nutrients.

The African governments should ensure better gain from this resource which has been around for a long time by supporting development of more research interests by all players to correctly identify and document AILVs by their vegetable species and cultivars, health promoting and protecting traits. Also standardized and certified seeds, proper agronomic practices and extension messages among others should be availed for informed public awareness campaigns. The awareness campaigns should target the younger generation who may not be familiar with the taste, preparation and nutritional value of AILVs. The young generation and

especially in schools have also been seen as good change agents for eating habits

(Anderson et al., 2004) in homes and other institutions.

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APPENDICES

Appendix 1: Changes in weight over time

ANIMAL	17TH JULY	20TH JULY	23RD JULY	26TH JULY	29TH JULY	1ST AUG	5TH AUG	13TH AUG	16TH AUG	19TH AUG	22ND AUG	25TH AUG
A1	234.50	240.60	226.10	237.90	214.50	227.70	228.30	246.78	245.30	229.70	DIED-	
A2	224.40	227.90	219.90	209.80	192.90	208.00	218.90	216.10	215.20	189.10	171.70	182.70
A3	238.20	233.70	224.60	235.90	232.80	231.20	239.20	243.10	245.20	240.90	227.00	221.60
A4	243.40	228.80	220.70	227.00	233.50	230.70	238.20	248.70	244.70	241.20	225.30	226.90
A5	206.60	202.10	203.80	204.60	206.30	206.60	203.20	209.70	209.90	206.00	206.10	208.50
A6	226.90	222.10	212.10	211.10	190.70	199.80	204.20	217.30	217.50	213.60	215.70	218.50
A7	201.20	202.60	198.10	205.30	184.10	200.40	199.50	210.80	213.40	191.00	173.80	177.00
A8	193.70	194.80	181.60	202.90	194.30	187.60	191.30	195.70	200.30	194.20	198.30	193.80
A9	186.40	186.10	174.60	191.60	185.90	177.50	186.60	200.30	189.30	185.40	173.50	169.10
A10	206.90	209.50	199.50	210.60	211.90	202.80	217.40	220.00	216.90	213.60	207.30	210.50
B1	239.90	237.30	226.10	228.90	208.30	212.70	219.30	234.30	228.20	225.20	220.10	220.10
B2	186.20	185.60	178.40	187.90	169.40	182.00	189.40	193.30	195.10	167.50	160.10	155.50
B3	185.20	183.20	172.40	181.10	181.50	180.40	184.70	189.60	176.60	172.90	173.80	176.30
B4	165.80	163.90	153.40	157.10	187.80	154.50	154.50	161.20	157.20	155.70	144.80	147.30
B5	215.30	210.10	202.90	207.20	209.20	209.70	224.90	228.60	226.20	215.30	214.60	205.80
B6	222.50	228.50	215.10	207.10	197.90	214.40	214.10	228.20	230.30	202.90	186.60	183.00
B7	206.40	199.60	188.10	192.20	200.00	201.70	207.70	209.30	215.10	204.60	196.90	198.60
B8	208.60	197.60	190.90	192.50	200.80	202.40	209.30	218.30	216.60	205.20	207.10	200.60
B9	204.30	184.60	187.40	186.00	194.40	197.00	203.00	216.40	213.30	198.00	203.90	195.40
B10	227.70	214.50	218.50	213.50	220.80	221.10	224.70	239.00	236.70	230.30	228.80	231.50
C1	211.00	219.20	204.90	207.00	184.30	185.40	206.30	207.80	215.50	203.40	195.60	196.20
C2	225.50	214.10	211.20	218.00	230.00	227.90	229.30	234.30	236.50	225.20	228.50	228.50

C3	227.80	222.80	220.40	220.00	228.60	226.20	224.40	226.00	224.50	219.50	220.20	215.30
C4	241.30	217.90	215.60	215.80	222.50	218.90	225.80	224.10	225.60	215.40	217.00	220.50
C5	193.90	185.20	181.10	176.50	183.40	192.80	197.70	194.00	194.00	186.70	179.20	172.90
D1	236.40	234.50	240.60	238.90	242.80	237.20	238.80	232.60	249.70	254.50	253.50	247.10
D2	262.60	260.80	263.20	265.70	268.50	267.10	270.40	265.80	279.70	282.20	279.40	282.60
D3	213.50	212.60	208.30	214.50	217.00	213.40	220.50	216.70	219.00	227.10	229.00	221.90
D4	189.30	185.80	191.80	190.90	190.00	190.50	192.60	189.50	195.60	195.00	195.60	193.90
D5	183.80	180.40	182.60	184.20	185.10	184.80	186.80	177.10	181.40	181.90	184.50	182.20

ANIMAL	28TH AUG	31ST AUG	3RD SEPT	6TH SEPT	10TH SEPT	15TH SEPT	18TH SEPT	25TH SEPT	1st OCT	12TH OCT	18TH OCT
A1											
A2	173.70	152.00	161.70	179.70	DIED-						
A3	211.60	203.80	208.30	209.00	223.40	234.50	255.30	247.90	255.30	258.10	260.30
A4	225.10	215.30	209.10	206.60	223.70	231.50	245.60	249.50	257.90	276.70	275.10
A5	210.60	201.00	216.20	218.20	215.20	213.90	214.10	222.80	223.50	212.30	209.90
A6	217.70	201.60	215.50	212.50	202.30	214.80	217.70	226.30	221.40	216.60	228.50
A7	174.70	164.20	171.50	186.70	208.20	201.90	206.20	215.20	217.80	219.90	218.70
A8	192.80	186.40	207.50	206.30	216.40	213.40	217.90	220.80	216.30	225.60	227.50
A9	162.30	160.60	173.60	176.00	189.00	198.20	191.70	198.20	193.40	205.70	210.10
A10	201.30	198.80	207.00	221.10	232.60	229.90	250.60	244.80	251.20	246.50	250.50
B1	203.20	194.60	191.70	190.20	211.70	228.20	246.00	253.70	253.10	251.80	248.60
B2	160.60	164.10	187.70	196.00	202.80	212.10	222.10	230.60	230.90	233.10	237.40
B3	167.30	165.00	167.30	177.60	198.70	191.10	200.70	204.30	201.10	205.50	205.40
B4	146.50	143.20	147.70	152.50	160.20	163.50	168.30	168.40	164.90	170.70	178.30
B5	193.20	192.50	169.40	169.60	198.60	208.10	220.40	232.30	239.30	247.30	258.80
B6	188.70	182.30	175.00	188.30	205.90	214.20	214.80	222.40	224.60	230.70	239.70
B7	191.80	174.80	186.80	194.30	204.30	203.60	197.00	208.10	208.20	DIED-	

B8	191.60	177.70	171.80	174.30	187.40	204.30	202.40	213.70	216.50	223.60	234.50
B9	191.50	191.40	199.70	212.00	209.90	215.50	216.00	210.50	218.40	222.70	228.40
B10	235.40	218.70	233.50	234.30	240.10	239.30	239.80	228.90	229.80	237.40	236.60
C1	189.70	186.20	196.00	203.20	213.70	234.70	231.40	232.70	240.60	234.70	244.90
C2	218.80	210.70	206.10	212.80	232.60	222.10	223.00	210.40	216.70	238.10	260.60
C3	221.40	209.60	227.40	229.60	238.50	248.80	241.80	235.60	236.70	234.70	228.30
C4	224.90	205.90	202.50	206.10	213.40	186.70	178.60	166.90	DIED		
C5	171.60	168.70	171.40	167.40	164.70	173.00	178.30	196.00	200.90	212.20	220.60
D1	235.40	245.40	233.60	243.10	252.70	253.90	260.30	260.40	254.70	259.20	256.30
D2	271.40	280.40	272.20	281.30	285.30	297.20	300.30	304.70	DIED		
D3	215.00	223.30	215.90	226.50	236.30	244.20	245.20	249.30	247.40	246.80	252.00
D4	183.00	190.40	193.60	193.40	201.10	207.30	202.70	209.30	206.30	210.60	208.50
D5	183.30	184.40	187.00	185.20	190.30	197.80	198.30	201.90	201.70	201.60	205.80

Appendix 2: Nutritional Value of sampled vegetables during feeding (4th-24th Oct.2012)

Dates	Cu	Zn	Mn	Fe	Mg	Ca	Beta Carotene	Ascorbic Acid
4/10R1	0.90	19.04	43.45	32.09	697.13	2,399.44	1.83	108.40
4/10R2	0.99	19.17	49.57	31.09	689.95	2,431.55	2.04	107.06
4/10R3	0.79	19.92	48.36	30.22	643.58	2,489.69	1.90	108.62
4/10C1	1.08	19.79	41.35	33.96	540.95	2,606.42	2.07	84.36
4/10C2	1.00	19.79	45.17	68.76	559.16	2,730.96	1.81	86.20
4/10C3	0.89	21.44	46.81	34.82	520.75	2,687.45	1.80	86.37
8/10R1	1.00	23.10	29.57	28.47	507.27	1,814.62	0.25	16.63
8/10R2	0.99	21.61	26.86	30.23	544.71	1,781.92	0.30	21.00
8/10R3	1.00	28.31	29.90	31.30	557.72	1,836.72	0.29	19.30
8/10C1	1.87	21.20	21.30	27.21	319.96	1,856.24	0.51	23.97
8/10C2	1.60	23.07	23.87	26.37	319.12	1,830.40	0.55	27.04
8/10C3	1.29	23.24	23.24	28.21	317.34	1,939.41	0.46	21.19
10/10R1	0.70	26.26	34.02	26.56	596.40	2,066.85	0.52	8.22
10/10R2	0.60	29.45	38.54	29.35	573.58	2,019.37	0.67	10.26
10/10R3	0.50	24.80	32.04	29.76	545.14	1,937.10	0.40	5.84
10/10C1	0.39	29.78	31.76	26.33	369.33	2,043.98	0.42	2.84
10/10C2	0.50	29.95	30.05	26.17	392.54	2,112.04	0.40	2.35
10/10C3	0.30	28.77	29.36	26.50	359.11	2,037.04	0.38	2.49
12/10R1	0.10	21.43	27.56	25.68	478.57	1,745.95	0.17	9.66
12/10R2	0.20	23.69	29.79	27.49	489.30	1,746.90	0.15	9.02
12/10R3	0.20	23.52	31.10	28.01	477.97	1,761.96	0.15	9.76
12/10C1	0.20	25.72	23.83	25.12	277.56	1,844.69	0.41	19.85
12/10C2	0.30	23.15	24.33	26.61	276.46	1,941.25	0.33	18.77
12/10C3	0.60	26.40	26.99	25.31	292.28	1,878.32	0.35	17.72
18/10R1	0.00	19.89	27.29	36.89	539.28	1,736.91	0.19	8.21
18/10R2	0.00	20.31	29.34	35.72	519.63	1,695.39	0.19	10.20
18/10R3	0.00	20.76	30.35	34.80	503.76	1,747.68	0.16	8.88
18/10C1	0.00	19.63	22.91	28.69	328.25	1,785.81	0.33	16.14
18/10C2	0.00	18.49	19.18	30.21	302.62	1,707.02	0.33	16.10
18/10C3	0.00	19.12	18.82	36.25	276.84	1,597.27	0.30	13.78
24/10R1	0.00	20.00	28.25	33.03	511.84	1,788.30	0.36	14.76
24/10R2	0.00	25.00	29.76	32.84	520.34	1,753.57	0.32	14.88
24/10R3	0.00	19.29	27.14	33.01	501.59	1,702.72	0.39	13.82
24/10C1	0.00	19.06	18.46	24.92	257.59	1,749.65	0.59	20.21
24/10C2	0.00	21.60	21.60	33.30	274.50	1,772.60	0.59	20.30
24/10C3	0.00	18.34	18.93	28.35	257.24	1,603.49	0.64	19.49
RAT PELLETS	4.10	23.19	51.87	109.35	147.43	717.24		
	4.50	23.40	49.29	104.98	149.47	717.46		
	4.00	23.49	50.88	108.57	150.45	732.38		

R is raw; C is cooked

Appendix 3: Data collection tools

A. Discussion guide for qualitative data

1. List all the vegetables grown and/or consumed in your area
2. Who is responsible for growing them?
3. Which ones are for ;-
   - Home consumption only
   - Home consumption and sale
   - Sale only
   - For ornamental purposes
   - For herbal/medicinal purposes
4. What are indigenous vegetables?
5. What are exotic vegetables?
6. Of the above vegetables, which ones would you consider indigenous?
7. Why?
8. Do you have any beliefs or attitudes as relates to these indigenous vegetables?
9. Are these beliefs and attitudes confined to some ailments, cultural and/or vulnerable groups?
10. How would you rank these vegetables based on these beliefs and attitudes?
11. Which ones do people like consuming?
12. Why?
13. How are the vegetables prepared?
14. Are there people who consume the vegetables but they do not grow it?
15. How much money do they spend in purchasing the vegetables in a given time?
16. What could be the preference, growing the vegetables and consuming or purchasing them for consumption?
17. Are there people who just grow the vegetables for sale?
18. Were these vegetables introduced/promoted or they have always been around?
19. If introduced/promoted, by who and why?
20. Did the introduction/promotion improve consumption of the vegetables?

B. Questionnaire for quantitative data

1. Name of respondent---------------------- Age-------------
   Denomination --------------------------------

2. Occupation ---------------------------
   1=farmer   2=casual worker   3=employed(permanent)

98=other(specify)

3. Level of education ---------------------
   1= None   4= college

   2= primary   5= university

   3= secondary   8=other (specify)

4. Marital status

   1= married (monogamous)   5=divorced

   2=married (polygamous)   6=widowed

   3=single   98=other (specify)

   4=separated

5. Name of the head of household ----------------------------- Age--------
   Denomination ---------------------------

   (If head of household is the mother then fill 5b)

5b. Name of spouse----------------- Age -----------------

Denomination -------------------------

6. Education level of head of household
   1= None   4= college

   2= primary   5= university

   3= secondary   98=other (specify)

7. Occupation of spouse
   1=farmer   2=casual worker   3=employed(permanent)

   98=other(specify)

8. Observe the type of housing
   1=Iron sheet roof and mud wall

   2= Iron sheet roof and brick wall

   3= Iron sheet roof and stone wall

   4= Iron sheet roof and timber wall

   5=Grass roof and mud wall

   6= Grass roof and brick wall

7= Grass roof and stone wall

8= Grass roof and timber wall

98=other(specify)

9. Do you own any television or radio?   TV--------------
   1=yes   2=no   Radio----------

10. Have you ever heard of traditional/indigenous vegetables?
    1=yes   2=no

11. Where did you hear of them?
    1=radio/television   4=newspaper

    2=school   5=extension workers(agric)

    3=social worker   6=health facility

    98=other (specify)

12. Which traditional/indigenous vegetables have you heard of?
    1=managu   6=vine spinach

    2=saga   7=stinging nettle

    3=amaranthus   8=Russian comfrey

    4=mitoo   98=other (specify)

    5=cassava leaves

13. Have you heard of any benefits of these vegetables?

    1=yes   2=no

14. If yes, what benefits

    1=none   5=healing power

    2=good nutrition   6=anti-aging nutrients

    3=immune boosting   7=smooth skin

    4=healthy functioning of body   98=other (specify)

15. Where did you get the information on the benefits?
    1=radio/television   4=newspaper

    2=school   5=extension workers(agric)

3=social worker   6=health facility

98=other (specify)

16. Do you grow any of the traditional/indigenous vegetables?
    1=yes   2=no

17. If yes, which ones do you grow?
    1=managu   5=saga

    2=mitoo   6=stinging nettle

    3=cassava leaves   7=amaranthus

    4=Russian comfrey   8=vine spinach

    98=other (specify)

18. How do you harvest the vegetables?
    1=pluck the leaves   4=cut the branches

    2=break the stems   5=pluck the flowers

    3=uproot   98=other (specify)

19. What do you use the traditional/indigenous vegetables for?
    1=consume   5=animal feed

    2=for sale   6=cover crop

    3=for donation   7=keep busy

    4=for ornamental   98=other (specify)

20. If consume the traditional/indigenous vegetables, which ones?
    1=managu   5=saga

    2=mitoo   6=stinging nettle

    3=cassava leaves   7=amaranthus

    4=Russian comfrey   8=ndaramiandetø

    98=other (specify)

21. If you do not grow the vegetables but you consume, where do you get them?

    1=donation   5=health facility

2=neighbor   6=agric demonstration plot

3=market   7=show/field-day

4=pick weeds   98=other (specify)

22. When did you start consuming the traditional vegetables?

1=when I fell sick   5=after knowing the benefits

2=after giving birth   6=after joint pains

3=after an operation   7=when started having grey hair

4=when visited upcountry   98=other (specify)

23. How do you prepare the vegetables before cooking?

1=chop and wash   3=dry

2=wash and chop   98=other(specify)

24. How do you cook the vegetables?

1=boil   5=Fry

2=boil and fry   6=boil with soda ash

3=boil and add fermented milk   7=boil with ashes

4=boil, add milk and ferment   98=Other(specify)

25. Who advised you to start consuming the traditional/indigenous vegetables?

1= doctor   5= friend

2= health worker   6=heard on radio/TV

3= extension worker   7=read on newspaper/book

4= neighbor   98=other (specify)

26. Have you seen/felt any changes since started consuming?

1=yes   2=no

27. If yes, what changes?

1=none   5=healed

2=strong   6=feel younger

3= stronger immunity   7=smooth skin

4=healthy   98=other (specify)

28. Do you know of somebody else who has been consuming traditional vegetables?

   1=yes   2=no

29. What is their experience of the vegetables?

    1=none   5=healed

    2=strong   6=feel younger

    3= stronger immunity   7=smooth skin

    4=healthy   98=other (specify)

Appendix 4: Indigenous vegetables commonly grown in Kenya

English name	Swahili name	Family	Scientific name	Health claims
Black Nightshade	Mnavu	Solanaceae	*Solanum nigrum*	-Unripe fruits treat toothache -Leaves treat stomachache -Pounded leaves and fruits treat tonsillitis
Spider plant	Mgagani	Capparaceae	*Cleome gynandra*	-Leaves treat constipation -Roots treat chest pain -Water after boiling leaves treats diarrhea -Used for deworming
Amaranth	Mchicha	Amaranthaceae	*Amaranthus species*	-Treats anemia -Plant ashes used as salt
Cowpea	Kunde	Papilionaceae	*Vigna*	Treats skin disease

English name	Swahili name	Family	Scientific name	Health claims
			unguiculata	
Sweet potato leaves	Majani ya viazi	Convolvulaceae	*Ipomea batatas*	
Pumpkin leaves	Maboga	Cucurbitaceae	*Cucurbita pepo*	
Ethiopian cabbage	Sarato	Brassicaceae	*Brassica carinata*	-Water obtained after boiling leaves used to treat diarrhea -seed oil used for birth control
Vine spinach	Nderema	Basellaneae	*Basella alba*	Treats constipation in humans and animals
Stinging nettle	Thabai	Urticaceae	*Urtica massaica*	Treats arthritis
Jute/Jew's mallow	Mlenda	Tiliaceae	*Corchorus olitorius*	-root treats toothache -leaves treats abdominal pains
Sunnhemp	Kimiro	Papilionaceae	*Crotalaria brevidens*	
Black jack	kishonanguo	Compositae	*Bidens pilosa*	
Cassava leaves	Kiamvu muhogo		*Manihot esculenta*	
Bitter lettuce	Mchunga	Compositae	*Launaea cornuta*	-Treats malaria -leaves given to chicken for lung diseases

Source: Traditional food plants of Kenya; Maundu et al (1999)

Appendix 5: Nutritional value of some indigenous vegetables mg/100g edible portion

Vegetable	Beta carotene	iron	zinc
Amaranth [a]	329	37.05	0.433
[b]	172.9	3.0	-
[c]	-	34	15
Nightshade [a]	3.23	8.9	0.261
[b]	3.7	0.3	-
[c]	-	3.0	0.5
Spider plant [a]	2.10	49.95	0.407
[b]	-	6.0	-
[c]	-	22	0.8
Cowpea leaves [a]	4.45	17.9	0.304
[b]	-	1.9	0.3
[c]	-	1.9	0.6
Pumpkin leaves [a]	5.34	26.65	0.196
[b]	-	104	0.2
[c]	-	1.1	0.5

[a] Weinberger K and Msuya J (2004). Indigenous vegetables in Tanzania. Significance and prospects AVRDC Technical bulletin no.3

[b] Grubben G.J.H and Denton O.A (2004). Vegetables. Plant Resources of Tropical Africa 2. Wageningen Netherlands (668)

[c] Shackleton C.M, Pasquini M.W and Drescher A.X (2009). African Indigenous vegetables in urban agriculture

Appendix 6: Photos of indigenous vegetables commonly consumed

Stinging nettle

Ethiopian cabbage

Vine spinach

Jute/Jew's mallow

Sunnhemp

Pumpkin leaves

Cowpea leaves

Spider plant

Amaranth

African nightshade

Appendix 7: By-product of statistical analysis

i) Nutritional analysis of vegetables with different cooking methods (vegetative stage)

Amaranthus hybridus

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Cu	Between Groups	.220	4	.055	32.234	.000
	Within Groups	.017	10	.002		
	Total	.237	14			
Zn	Between Groups	3.165	4	.791	1.814	.203
	Within Groups	4.363	10	.436		
	Total	7.528	14			
Mn	Between Groups	198.817	4	49.704	62.875	.000
	Within Groups	7.905	10	.791		
	Total	206.722	14			
Fe	Between Groups	95.939	4	23.985	30.230	.000
	Within Groups	7.934	10	.793		
	Total	103.873	14			
Mg	Between Groups	110132.854	4	27533.213	413.461	.000
	Within Groups	665.921	10	66.592		
	Total	110798.775	14			
Ca	Between Groups	8836641.065	4	2209160.266	161.775	.000
	Within Groups	136557.223	10	13655.722		
	Total	8973198.288	14			
Vit_A	Between Groups	781.099	4	195.275	201.971	.000
	Within Groups	9.668	10	.967		
	Total	790.767	14			
Vit_C	Between Groups	6366823.885	4	1591705.971	1.576E3	.000
	Within Groups	10100.268	10	1010.027		
	Total	6376924.153	14			

Post Hoc Tests

Multiple Comparisons

LSD

Dependent Variable	(I) Method	(J) Method	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Cu	1	2	.25182*	.03370	.000	.1767	.3269
		3	-.05773	.03370	.117	-.1328	.0174
		4	.13962*	.03370	.002	.0645	.2147
		5	-.05258	.03370	.150	-.1277	.0225
	2	1	-.25182*	.03370	.000	-.3269	-.1767
		3	-.30955*	.03370	.000	-.3846	-.2345
		4	-.11220*	.03370	.008	-.1873	-.0371
		5	-.30440*	.03370	.000	-.3795	-.2293
	3	1	.05773	.03370	.117	-.0174	.1328
		2	.30955*	.03370	.000	.2345	.3846
		4	.19734*	.03370	.000	.1223	.2724
		5	.00514	.03370	.882	-.0699	.0802
	4	1	-.13962*	.03370	.002	-.2147	-.0645
		2	.11220*	.03370	.008	.0371	.1873
		3	-.19734*	.03370	.000	-.2724	-.1223
		5	-.19220*	.03370	.000	-.2673	-.1171
	5	1	.05258	.03370	.150	-.0225	.1277
		2	.30440*	.03370	.000	.2293	.3795
		3	-.00514	.03370	.882	-.0802	.0699
		4	.19220*	.03370	.000	.1171	.2673
Zn	1	2	.68466	.53931	.233	-.5170	1.8863
		3	1.37749*	.53931	.029	.1758	2.5791
		4	1.04771	.53931	.081	-.1539	2.2494
		5	.67148	.53931	.241	-.5302	1.8731
	2	1	-.68466	.53931	.233	-1.8863	.5170
		3	.69283	.53931	.228	-.5088	1.8945
		4	.36305	.53931	.516	-.8386	1.5647
		5	-.01318	.53931	.981	-1.2148	1.1885
	3	1	-1.37749*	.53931	.029	-2.5791	-.1758
		2	-.69283	.53931	.228	-1.8945	.5088

140

		4	-.32977	.53931	.555	-1.5314	.8719
		5	-.70601	.53931	.220	-1.9077	.4956
	4	1	-1.04771	.53931	.081	-2.2494	.1539
		2	-.36305	.53931	.516	-1.5647	.8386
		3	.32977	.53931	.555	-.8719	1.5314
		5	-.37623	.53931	.501	-1.5779	.8254
	5	1	-.67148	.53931	.241	-1.8731	.5302
		2	.01318	.53931	.981	-1.1885	1.2148
		3	.70601	.53931	.220	-.4956	1.9077
		4	.37623	.53931	.501	-.8254	1.5779
Mn	1	2	6.59580*	.72596	.000	4.9783	8.2133
		3	3.25294*	.72596	.001	1.6354	4.8705
		4	5.02957*	.72596	.000	3.4120	6.6471
		5	-3.54596*	.72596	.001	-5.1635	-1.9284
	2	1	-6.59580*	.72596	.000	-8.2133	-4.9783
		3	-3.34285*	.72596	.001	-4.9604	-1.7253
		4	-1.56622	.72596	.056	-3.1838	.0513
		5	-10.14176*	.72596	.000	-11.7593	-8.5242
	3	1	-3.25294*	.72596	.001	-4.8705	-1.6354
		2	3.34285*	.72596	.001	1.7253	4.9604
		4	1.77663*	.72596	.034	.1591	3.3942
		5	-6.79891*	.72596	.000	-8.4164	-5.1814
	4	1	-5.02957*	.72596	.000	-6.6471	-3.4120
		2	1.56622	.72596	.056	-.0513	3.1838
		3	-1.77663*	.72596	.034	-3.3942	-.1591
		5	-8.57554*	.72596	.000	-10.1931	-6.9580
	5	1	3.54596*	.72596	.001	1.9284	5.1635
		2	10.14176*	.72596	.000	8.5242	11.7593
		3	6.79891*	.72596	.000	5.1814	8.4164
		4	8.57554*	.72596	.000	6.9580	10.1931
Fe	1	2	4.80449*	.72729	.000	3.1840	6.4250
		3	-2.64062*	.72729	.005	-4.2611	-1.0201
		4	-.34819	.72729	.642	-1.9687	1.2723
		5	-1.35806	.72729	.091	-2.9786	.2624
	2	1	-4.80449*	.72729	.000	-6.4250	-3.1840
		3	-7.44511*	.72729	.000	-9.0656	-5.8246
		4	-5.15268*	.72729	.000	-6.7732	-3.5322
		5	-6.16255*	.72729	.000	-7.7830	-4.5421

	3	1	2.64062*	.72729	.005	1.0201	4.2611
		2	7.44511*	.72729	.000	5.8246	9.0656
		4	2.29243*	.72729	.010	.6719	3.9129
		5	1.28256	.72729	.108	-.3379	2.9031
	4	1	.34819	.72729	.642	-1.2723	1.9687
		2	5.15268*	.72729	.000	3.5322	6.7732
		3	-2.29243*	.72729	.010	-3.9129	-.6719
		5	-1.00987	.72729	.195	-2.6304	.6106
	5	1	1.35806	.72729	.091	-.2624	2.9786
		2	6.16255*	.72729	.000	4.5421	7.7830
		3	-1.28256	.72729	.108	-2.9031	.3379
		4	1.00987	.72729	.195	-.6106	2.6304
Mg	1	2	7.89315	6.66294	.264	-6.9528	22.7391
		3	81.20252*	6.66294	.000	66.3566	96.0485
		4	11.31717	6.66294	.120	-3.5288	26.1631
		5	-176.29008*	6.66294	.000	-191.1360	-161.4441
	2	1	-7.89315	6.66294	.264	-22.7391	6.9528
		3	73.30936*	6.66294	.000	58.4634	88.1553
		4	3.42402	6.66294	.618	-11.4219	18.2700
		5	-184.18324*	6.66294	.000	-199.0292	-169.3373
	3	1	-81.20252*	6.66294	.000	-96.0485	-66.3566
		2	-73.30936*	6.66294	.000	-88.1553	-58.4634
		4	-69.88535*	6.66294	.000	-84.7313	-55.0394
		5	-257.49260*	6.66294	.000	-272.3386	-242.6467
	4	1	-11.31717	6.66294	.120	-26.1631	3.5288
		2	-3.42402	6.66294	.618	-18.2700	11.4219
		3	69.88535*	6.66294	.000	55.0394	84.7313
		5	-187.60725*	6.66294	.000	-202.4532	-172.7613
	5	1	176.29008*	6.66294	.000	161.4441	191.1360
		2	184.18324*	6.66294	.000	169.3373	199.0292
		3	257.49260*	6.66294	.000	242.6467	272.3386
		4	187.60725*	6.66294	.000	172.7613	202.4532
Ca	1	2	-1699.56149*	95.41391	.000	-1912.1569	-1486.9660
		3	309.26686*	95.41391	.009	96.6714	521.8623
		4	420.94105*	95.41391	.001	208.3456	633.5365
		5	-87.77455	95.41391	.379	-300.3700	124.8209
	2	1	1699.56149*	95.41391	.000	1486.9660	1912.1569
		3	2008.82835*	95.41391	.000	1796.2329	2221.4238

		4	2120.50254*	95.41391	.000	1907.9071	2333.0980
		5	1611.78694*	95.41391	.000	1399.1915	1824.3824
	3	1	-309.26686*	95.41391	.009	-521.8623	-96.6714
		2	-2008.82835*	95.41391	.000	-2221.4238	-1796.2329
		4	111.67419	95.41391	.269	-100.9213	324.2696
		5	-397.04142*	95.41391	.002	-609.6369	-184.4460
	4	1	-420.94105*	95.41391	.001	-633.5365	-208.3456
		2	-2120.50254*	95.41391	.000	-2333.0980	-1907.9071
		3	-111.67419	95.41391	.269	-324.2696	100.9213
		5	-508.71561*	95.41391	.000	-721.3111	-296.1202
	5	1	87.77455	95.41391	.379	-124.8209	300.3700
		2	-1611.78694*	95.41391	.000	-1824.3824	-1399.1915
		3	397.04142*	95.41391	.002	184.4460	609.6369
		4	508.71561*	95.41391	.000	296.1202	721.3111
Vit_A	1	2	10.04938*	.80285	.000	8.2605	11.8382
		3	10.13462*	.80285	.000	8.3458	11.9235
		4	10.68179*	.80285	.000	8.8929	12.4707
		5	22.74722*	.80285	.000	20.9584	24.5361
	2	1	-10.04938*	.80285	.000	-11.8382	-8.2605
		3	.08524	.80285	.918	-1.7036	1.8741
		4	.63241	.80285	.449	-1.1564	2.4213
		5	12.69784*	.80285	.000	10.9090	14.4867
	3	1	-10.13462*	.80285	.000	-11.9235	-8.3458
		2	-.08524	.80285	.918	-1.8741	1.7036
		4	.54718	.80285	.511	-1.2417	2.3360
		5	12.61260*	.80285	.000	10.8237	14.4015
	4	1	-10.68179*	.80285	.000	-12.4707	-8.8929
		2	-.63241	.80285	.449	-2.4213	1.1564
		3	-.54718	.80285	.511	-2.3360	1.2417
		5	12.06542*	.80285	.000	10.2766	13.8543
	5	1	-22.74722*	.80285	.000	-24.5361	-20.9584
		2	-12.69784*	.80285	.000	-14.4867	-10.9090
		3	-12.61260*	.80285	.000	-14.4015	-10.8237
		4	-12.06542*	.80285	.000	-13.8543	-10.2766
Vit_C	1	2	158.94267*	25.94901	.000	101.1247	216.7607
		3	195.18096*	25.94901	.000	137.3630	252.9990
		4	361.08908*	25.94901	.000	303.2711	418.9071
		5	-1424.47988*	25.94901	.000	-1482.2979	-1366.6619

2	1	-158.94267*	25.94901	.000	-216.7607	-101.1247
	3	36.23829	25.94901	.193	-21.5797	94.0563
	4	202.14641*	25.94901	.000	144.3284	259.9644
	5	-1583.42254*	25.94901	.000	-1641.2405	-1525.6045
3	1	-195.18096*	25.94901	.000	-252.9990	-137.3630
	2	-36.23829	25.94901	.193	-94.0563	21.5797
	4	165.90812*	25.94901	.000	108.0901	223.7261
	5	-1619.66083*	25.94901	.000	-1677.4788	-1561.8428
4	1	-361.08908*	25.94901	.000	-418.9071	-303.2711
	2	-202.14641*	25.94901	.000	-259.9644	-144.3284
	3	-165.90812*	25.94901	.000	-223.7261	-108.0901
	5	-1785.56895*	25.94901	.000	-1843.3870	-1727.7510
5	1	1424.47988*	25.94901	.000	1366.6619	1482.2979
	2	1583.42254*	25.94901	.000	1525.6045	1641.2405
	3	1619.66083*	25.94901	.000	1561.8428	1677.4788
	4	1785.56895*	25.94901	.000	1727.7510	1843.3870

*. The mean difference is significant at the 0.05 level.

Gynandropsis gynandra

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Cu	Between Groups	.535	4	.134	91.034	.000
	Within Groups	.015	10	.001		
	Total	.550	14			
Zn	Between Groups	119.864	4	29.966	333.090	.000
	Within Groups	.900	10	.090		
	Total	120.764	14			
Mn	Between Groups	175.014	4	43.753	196.790	.000
	Within Groups	2.223	10	.222		
	Total	177.237	14			
Fe	Between Groups	113.903	4	28.476	32.263	.000
	Within Groups	8.826	10	.883		
	Total	122.730	14			
Mg	Between Groups	37231.302	4	9307.826	671.091	.000
	Within Groups	138.697	10	13.870		
	Total	37369.999	14			
Ca	Between Groups	1042850.195	4	260712.549	114.957	.000

	Within Groups	22679.066	10	2267.907		
	Total	1065529.260	14			
Vit_A	Between Groups	366.461	4	91.615	299.483	.000
	Within Groups	3.059	10	.306		
	Total	369.520	14			
Vit_C	Between Groups	636833.247	4	159208.312	8.947E3	.000
	Within Groups	177.953	10	17.795		
	Total	637011.200	14			

Post Hoc Tests

Multiple Comparisons

LSD

Dependent Variable	(I) Method	(J) Method	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Cu	1	2	-.17279*	.03130	.000	-.2425	-.1030
		3	-.26254*	.03130	.000	-.3323	-.1928
		4	-.19076*	.03130	.000	-.2605	-.1210
		5	-.57666*	.03130	.000	-.6464	-.5069
	2	1	.17279*	.03130	.000	.1030	.2425
		3	-.08975*	.03130	.017	-.1595	-.0200
		4	-.01797	.03130	.579	-.0877	.0518
		5	-.40387*	.03130	.000	-.4736	-.3341
	3	1	.26254*	.03130	.000	.1928	.3323
		2	.08975*	.03130	.017	.0200	.1595
		4	.07178*	.03130	.045	.0020	.1415
		5	-.31412*	.03130	.000	-.3839	-.2444
	4	1	.19076*	.03130	.000	.1210	.2605
		2	.01797	.03130	.579	-.0518	.0877
		3	-.07178*	.03130	.045	-.1415	-.0020
		5	-.38590*	.03130	.000	-.4556	-.3162
	5	1	.57666*	.03130	.000	.5069	.6464
		2	.40387*	.03130	.000	.3341	.4736
		3	.31412*	.03130	.000	.2444	.3839
		4	.38590*	.03130	.000	.3162	.4556
Zn	1	2	5.85354*	.24490	.000	5.3079	6.3992
		3	4.94980*	.24490	.000	4.4041	5.4955
		4	4.24849*	.24490	.000	3.7028	4.7942
		5	-1.20947*	.24490	.001	-1.7551	-.6638

145

	2	1	-5.85354*	.24490	.000	-6.3992	-5.3079
		3	-.90374*	.24490	.004	-1.4494	-.3581
		4	-1.60505*	.24490	.000	-2.1507	-1.0594
		5	-7.06301*	.24490	.000	-7.6087	-6.5173
	3	1	-4.94980*	.24490	.000	-5.4955	-4.4041
		2	.90374*	.24490	.004	.3581	1.4494
		4	-.70132*	.24490	.017	-1.2470	-.1556
		5	-6.15927*	.24490	.000	-6.7049	-5.6136
	4	1	-4.24849*	.24490	.000	-4.7942	-3.7028
		2	1.60505*	.24490	.000	1.0594	2.1507
		3	.70132*	.24490	.017	.1556	1.2470
		5	-5.45795*	.24490	.000	-6.0036	-4.9123
	5	1	1.20947*	.24490	.001	.6638	1.7551
		2	7.06301*	.24490	.000	6.5173	7.6087
		3	6.15927*	.24490	.000	5.6136	6.7049
		4	5.45795*	.24490	.000	4.9123	6.0036
Mn	1	2	5.28597*	.38500	.000	4.4281	6.1438
		3	1.43348*	.38500	.004	.5756	2.2913
		4	1.58835*	.38500	.002	.7305	2.4462
		5	-5.26076*	.38500	.000	-6.1186	-4.4029
	2	1	-5.28597*	.38500	.000	-6.1438	-4.4281
		3	-3.85250*	.38500	.000	-4.7103	-2.9947
		4	-3.69763*	.38500	.000	-4.5555	-2.8398
		5	-10.54674*	.38500	.000	-11.4046	-9.6889
	3	1	-1.43348*	.38500	.004	-2.2913	-.5756
		2	3.85250*	.38500	.000	2.9947	4.7103
		4	.15487	.38500	.696	-.7030	1.0127
		5	-6.69424*	.38500	.000	-7.5521	-5.8364
	4	1	-1.58835*	.38500	.002	-2.4462	-.7305
		2	3.69763*	.38500	.000	2.8398	4.5555
		3	-.15487	.38500	.696	-1.0127	.7030
		5	-6.84911*	.38500	.000	-7.7069	-5.9913
	5	1	5.26076*	.38500	.000	4.4029	6.1186
		2	10.54674*	.38500	.000	9.6889	11.4046
		3	6.69424*	.38500	.000	5.8364	7.5521
		4	6.84911*	.38500	.000	5.9913	7.7069
Fe	1	2	-2.28972*	.76707	.014	-3.9989	-.5806
		3	-2.91093*	.76707	.004	-4.6201	-1.2018

		4	-7.62137*	.76707	.000	-9.3305	-5.9122
		5	-6.21948*	.76707	.000	-7.9286	-4.5103
	2	1	2.28972*	.76707	.014	.5806	3.9989
		3	-.62121	.76707	.437	-2.3304	1.0879
		4	-5.33166*	.76707	.000	-7.0408	-3.6225
		5	-3.92976*	.76707	.000	-5.6389	-2.2206
	3	1	2.91093*	.76707	.004	1.2018	4.6201
		2	.62121	.76707	.437	-1.0879	2.3304
		4	-4.71044*	.76707	.000	-6.4196	-3.0013
		5	-3.30855*	.76707	.002	-5.0177	-1.5994
	4	1	7.62137*	.76707	.000	5.9122	9.3305
		2	5.33166*	.76707	.000	3.6225	7.0408
		3	4.71044*	.76707	.000	3.0013	6.4196
		5	1.40190	.76707	.098	-.3073	3.1110
	5	1	6.21948*	.76707	.000	4.5103	7.9286
		2	3.92976*	.76707	.000	2.2206	5.6389
		3	3.30855*	.76707	.002	1.5994	5.0177
		4	-1.40190	.76707	.098	-3.1110	.3073
Mg	1	2	103.10685*	3.04080	.000	96.3315	109.8822
		3	31.09265*	3.04080	.000	24.3173	37.8680
		4	36.85233*	3.04080	.000	30.0770	43.6277
		5	-49.22377*	3.04080	.000	-55.9991	-42.4484
	2	1	-103.10685*	3.04080	.000	-109.8822	-96.3315
		3	-72.01420*	3.04080	.000	-78.7895	-65.2389
		4	-66.25452*	3.04080	.000	-73.0298	-59.4792
		5	-152.33062*	3.04080	.000	-159.1059	-145.5553
	3	1	-31.09265*	3.04080	.000	-37.8680	-24.3173
		2	72.01420*	3.04080	.000	65.2389	78.7895
		4	5.75968	3.04080	.087	-1.0156	12.5350
		5	-80.31642*	3.04080	.000	-87.0917	-73.5411
	4	1	-36.85233*	3.04080	.000	-43.6277	-30.0770
		2	66.25452*	3.04080	.000	59.4792	73.0298
		3	-5.75968	3.04080	.087	-12.5350	1.0156
		5	-86.07610*	3.04080	.000	-92.8514	-79.3008
	5	1	49.22377*	3.04080	.000	42.4484	55.9991
		2	152.33062*	3.04080	.000	145.5553	159.1059
		3	80.31642*	3.04080	.000	73.5411	87.0917
		4	86.07610*	3.04080	.000	79.3008	92.8514

147

Ca	1	2	405.26993*	38.88364	.000	318.6318	491.9081
		3	188.32239*	38.88364	.001	101.6842	274.9605
		4	178.25490*	38.88364	.001	91.6167	264.8931
		5	-382.65734*	38.88364	.000	-469.2955	-296.0192
	2	1	-405.26993*	38.88364	.000	-491.9081	-318.6318
		3	-216.94754*	38.88364	.000	-303.5857	-130.3094
		4	-227.01504*	38.88364	.000	-313.6532	-140.3769
		5	-787.92727*	38.88364	.000	-874.5654	-701.2891
	3	1	-188.32239*	38.88364	.001	-274.9605	-101.6842
		2	216.94754*	38.88364	.000	130.3094	303.5857
		4	-10.06749	38.88364	.801	-96.7056	76.5707
		5	-570.97972*	38.88364	.000	-657.6179	-484.3416
	4	1	-178.25490*	38.88364	.001	-264.8931	-91.6167
		2	227.01504*	38.88364	.000	140.3769	313.6532
		3	10.06749	38.88364	.801	-76.5707	96.7056
		5	-560.91223*	38.88364	.000	-647.5504	-474.2741
	5	1	382.65734*	38.88364	.000	296.0192	469.2955
		2	787.92727*	38.88364	.000	701.2891	874.5654
		3	570.97972*	38.88364	.000	484.3416	657.6179
		4	560.91223*	38.88364	.000	474.2741	647.5504
Vit_A	1	2	13.62839*	.45160	.000	12.6222	14.6346
		3	.61052	.45160	.206	-.3957	1.6167
		4	3.71183*	.45160	.000	2.7056	4.7181
		5	2.60770*	.45160	.000	1.6015	3.6139
	2	1	-13.62839*	.45160	.000	-14.6346	-12.6222
		3	-13.01787*	.45160	.000	-14.0241	-12.0116
		4	-9.91656*	.45160	.000	-10.9228	-8.9103
		5	-11.02069*	.45160	.000	-12.0269	-10.0145
	3	1	-.61052	.45160	.206	-1.6167	.3957
		2	13.01787*	.45160	.000	12.0116	14.0241
		4	3.10131*	.45160	.000	2.0951	4.1075
		5	1.99718*	.45160	.001	.9910	3.0034
	4	1	-3.71183*	.45160	.000	-4.7181	-2.7056
		2	9.91656*	.45160	.000	8.9103	10.9228
		3	-3.10131*	.45160	.000	-4.1075	-2.0951
		5	-1.10413*	.45160	.035	-2.1104	-.0979
	5	1	-2.60770*	.45160	.000	-3.6139	-1.6015
		2	11.02069*	.45160	.000	10.0145	12.0269

		3	-1.99718*	.45160	.001	-3.0034	-.9910
		4	1.10413*	.45160	.035	.0979	2.1104
Vit_C	1	2	72.28138*	3.44435	.000	64.6069	79.9559
		3	-32.34405*	3.44435	.000	-40.0185	-24.6696
		4	-42.89958*	3.44435	.000	-50.5741	-35.2251
		5	-505.92473*	3.44435	.000	-513.5992	-498.2503
	2	1	-72.28138*	3.44435	.000	-79.9559	-64.6069
		3	-104.62543*	3.44435	.000	-112.2999	-96.9509
		4	-115.18096*	3.44435	.000	-122.8554	-107.5065
		5	-578.20611*	3.44435	.000	-585.8806	-570.5316
	3	1	32.34405*	3.44435	.000	24.6696	40.0185
		2	104.62543*	3.44435	.000	96.9509	112.2999
		4	-10.55553*	3.44435	.012	-18.2300	-2.8811
		5	-473.58068*	3.44435	.000	-481.2552	-465.9062
	4	1	42.89958*	3.44435	.000	35.2251	50.5741
		2	115.18096*	3.44435	.000	107.5065	122.8554
		3	10.55553*	3.44435	.012	2.8811	18.2300
		5	-463.02515*	3.44435	.000	-470.6996	-455.3507
	5	1	505.92473*	3.44435	.000	498.2503	513.5992
		2	578.20611*	3.44435	.000	570.5316	585.8806
		3	473.58068*	3.44435	.000	465.9062	481.2552
		4	463.02515*	3.44435	.000	455.3507	470.6996

*. The mean difference is significant at the 0.05 level.

Solanum nigrum

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Cu	Between Groups	.211	4	.053	110.747	.000
	Within Groups	.005	10	.000		
	Total	.215	14			
Zn	Between Groups	1.009	4	.252	22.818	.000
	Within Groups	.111	10	.011		
	Total	1.120	14			
Mn	Between Groups	197.851	4	49.463	492.830	.000
	Within Groups	1.004	10	.100		
	Total	198.855	14			
Fe	Between Groups	152.796	4	38.199	69.147	.000

		Sum of Squares	df	Mean Square	F	Sig.
	Within Groups	5.524	10	.552		
	Total	158.320	14			
Mg	Between Groups	18689.645	4	4672.411	463.808	.000
	Within Groups	100.740	10	10.074		
	Total	18790.385	14			
Ca	Between Groups	671010.663	4	167752.666	799.153	.000
	Within Groups	2099.131	10	209.913		
	Total	673109.794	14			
Vit_A	Between Groups	604.196	4	151.049	589.346	.000
	Within Groups	2.563	10	.256		
	Total	606.759	14			
Vit_C	Between Groups	205729.157	4	51432.289	458.607	.000
	Within Groups	1121.489	10	112.149		
	Total	206850.646	14			

Post Hoc Tests

Multiple Comparisons

LSD

Dependent Variable	(I) Method	(J) Method	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Cu	1	2	-.09921*	.01780	.000	-.1389	-.0595
		3	-.12766*	.01780	.000	-.1673	-.0880
		4	-.23560*	.01780	.000	-.2753	-.1959
		5	-.34482*	.01780	.000	-.3845	-.3052
	2	1	.09921*	.01780	.000	.0595	.1389
		3	-.02845	.01780	.141	-.0681	.0112
		4	-.13639*	.01780	.000	-.1761	-.0967
		5	-.24560*	.01780	.000	-.2853	-.2059
	3	1	.12766*	.01780	.000	.0880	.1673
		2	.02845	.01780	.141	-.0112	.0681
		4	-.10794*	.01780	.000	-.1476	-.0683
		5	-.21715*	.01780	.000	-.2568	-.1775
	4	1	.23560*	.01780	.000	.1959	.2753
		2	.13639*	.01780	.000	.0967	.1761
		3	.10794*	.01780	.000	.0683	.1476

		5	-.10922*	.01780	.000	-.1489	-.0695
	5	1	.34482*	.01780	.000	.3052	.3845
		2	.24560*	.01780	.000	.2059	.2853
		3	.21715*	.01780	.000	.1775	.2568
		4	.10922*	.01780	.000	.0695	.1489
Zn	1	2	.50422*	.08585	.000	.3129	.6955
		3	.41195*	.08585	.001	.2207	.6033
		4	.43824*	.08585	.000	.2469	.6295
		5	-.13441	.08585	.149	-.3257	.0569
	2	1	-.50422*	.08585	.000	-.6955	-.3129
		3	-.09226	.08585	.308	-.2836	.0990
		4	-.06598	.08585	.460	-.2573	.1253
		5	-.63863*	.08585	.000	-.8299	-.4473
	3	1	-.41195*	.08585	.001	-.6033	-.2207
		2	.09226	.08585	.308	-.0990	.2836
		4	.02628	.08585	.766	-.1650	.2176
		5	-.54637*	.08585	.000	-.7377	-.3551
	4	1	-.43824*	.08585	.000	-.6295	-.2469
		2	.06598	.08585	.460	-.1253	.2573
		3	-.02628	.08585	.766	-.2176	.1650
		5	-.57265*	.08585	.000	-.7639	-.3814
	5	1	.13441	.08585	.149	-.0569	.3257
		2	.63863*	.08585	.000	.4473	.8299
		3	.54637*	.08585	.000	.3551	.7377
		4	.57265*	.08585	.000	.3814	.7639
Mn	1	2	8.36544*	.25867	.000	7.7891	8.9418
		3	1.56837*	.25867	.000	.9920	2.1447
		4	3.09778*	.25867	.000	2.5214	3.6741
		5	-2.48332*	.25867	.000	-3.0597	-1.9070
	2	1	-8.36544*	.25867	.000	-8.9418	-7.7891
		3	-6.79708*	.25867	.000	-7.3734	-6.2207
		4	-5.26766*	.25867	.000	-5.8440	-4.6913
		5	-10.84877*	.25867	.000	-11.4251	-10.2724
	3	1	-1.56837*	.25867	.000	-2.1447	-.9920
		2	6.79708*	.25867	.000	6.2207	7.3734
		4	1.52942*	.25867	.000	.9531	2.1058
		5	-4.05169*	.25867	.000	-4.6280	-3.4753
	4	1	-3.09778*	.25867	.000	-3.6741	-2.5214

		2	5.26766*	.25867	.000	4.6913	5.8440
		3	-1.52942*	.25867	.000	-2.1058	-.9531
		5	-5.58111*	.25867	.000	-6.1575	-5.0048
	5	1	2.48332*	.25867	.000	1.9070	3.0597
		2	10.84877*	.25867	.000	10.2724	11.4251
		3	4.05169*	.25867	.000	3.4753	4.6280
		4	5.58111*	.25867	.000	5.0048	6.1575
Fe	1	2	6.74114*	.60687	.000	5.3890	8.0933
		3	1.25452	.60687	.066	-.0977	2.6067
		4	3.07302*	.60687	.000	1.7208	4.4252
		5	-2.83424*	.60687	.001	-4.1864	-1.4821
	2	1	-6.74114*	.60687	.000	-8.0933	-5.3890
		3	-5.48662*	.60687	.000	-6.8388	-4.1344
		4	-3.66812*	.60687	.000	-5.0203	-2.3159
		5	-9.57538*	.60687	.000	-10.9276	-8.2232
	3	1	-1.25452	.60687	.066	-2.6067	.0977
		2	5.48662*	.60687	.000	4.1344	6.8388
		4	1.81850*	.60687	.013	.4663	3.1707
		5	-4.08877*	.60687	.000	-5.4409	-2.7366
	4	1	-3.07302*	.60687	.000	-4.4252	-1.7208
		2	3.66812*	.60687	.000	2.3159	5.0203
		3	-1.81850*	.60687	.013	-3.1707	-.4663
		5	-5.90726*	.60687	.000	-7.2594	-4.5551
	5	1	2.83424*	.60687	.001	1.4821	4.1864
		2	9.57538*	.60687	.000	8.2232	10.9276
		3	4.08877*	.60687	.000	2.7366	5.4409
		4	5.90726*	.60687	.000	4.5551	7.2594
Mg	1	2	32.45021*	2.59153	.000	26.6759	38.2245
		3	43.37154*	2.59153	.000	37.5973	49.1458
		4	27.25243*	2.59153	.000	21.4781	33.0267
		5	-54.92495*	2.59153	.000	-60.6992	-49.1507
	2	1	-32.45021*	2.59153	.000	-38.2245	-26.6759
		3	10.92133*	2.59153	.002	5.1470	16.6956
		4	-5.19778	2.59153	.073	-10.9721	.5765
		5	-87.37516*	2.59153	.000	-93.1494	-81.6009
	3	1	-43.37154*	2.59153	.000	-49.1458	-37.5973
		2	-10.92133*	2.59153	.002	-16.6956	-5.1470
		4	-16.11911*	2.59153	.000	-21.8934	-10.3448

		5	-98.29649*	2.59153	.000	-104.0708	-92.5222
	4	1	-27.25243*	2.59153	.000	-33.0267	-21.4781
		2	5.19778	2.59153	.073	-.5765	10.9721
		3	16.11911*	2.59153	.000	10.3448	21.8934
		5	-82.17738*	2.59153	.000	-87.9517	-76.4031
	5	1	54.92495*	2.59153	.000	49.1507	60.6992
		2	87.37516*	2.59153	.000	81.6009	93.1494
		3	98.29649*	2.59153	.000	92.5222	104.0708
		4	82.17738*	2.59153	.000	76.4031	87.9517
Ca	1	2	122.81278*	11.82971	.000	96.4545	149.1710
		3	14.13640	11.82971	.260	-12.2218	40.4946
		4	-110.81107*	11.82971	.000	-137.1693	-84.4528
		5	-488.79253*	11.82971	.000	-515.1508	-462.4343
	2	1	-122.81278*	11.82971	.000	-149.1710	-96.4545
		3	-108.67638*	11.82971	.000	-135.0346	-82.3181
		4	-233.62385*	11.82971	.000	-259.9821	-207.2656
		5	-611.60531*	11.82971	.000	-637.9635	-585.2471
	3	1	-14.13640	11.82971	.260	-40.4946	12.2218
		2	108.67638*	11.82971	.000	82.3181	135.0346
		4	-124.94746*	11.82971	.000	-151.3057	-98.5892
		5	-502.92892*	11.82971	.000	-529.2872	-476.5707
	4	1	110.81107*	11.82971	.000	84.4528	137.1693
		2	233.62385*	11.82971	.000	207.2656	259.9821
		3	124.94746*	11.82971	.000	98.5892	151.3057
		5	-377.98146*	11.82971	.000	-404.3397	-351.6232
	5	1	488.79253*	11.82971	.000	462.4343	515.1508
		2	611.60531*	11.82971	.000	585.2471	637.9635
		3	502.92892*	11.82971	.000	476.5707	529.2872
		4	377.98146*	11.82971	.000	351.6232	404.3397
Vit_A	1	2	.06210	.41336	.884	-.8589	.9831
		3	-11.04242*	.41336	.000	-11.9634	-10.1214
		4	-16.28908*	.41336	.000	-17.2101	-15.3681
		5	-7.01292*	.41336	.000	-7.9339	-6.0919
	2	1	-.06210	.41336	.884	-.9831	.8589
		3	-11.10452*	.41336	.000	-12.0255	-10.1835
		4	-16.35118*	.41336	.000	-17.2722	-15.4302
		5	-7.07502*	.41336	.000	-7.9960	-6.1540
	3	1	11.04242*	.41336	.000	10.1214	11.9634

		2	11.10452*	.41336	.000	10.1835	12.0255
		4	-5.24666*	.41336	.000	-6.1677	-4.3256
		5	4.02950*	.41336	.000	3.1085	4.9505
	4	1	16.28908*	.41336	.000	15.3681	17.2101
		2	16.35118*	.41336	.000	15.4302	17.2722
		3	5.24666*	.41336	.000	4.3256	6.1677
		5	9.27616*	.41336	.000	8.3551	10.1972
	5	1	7.01292*	.41336	.000	6.0919	7.9339
		2	7.07502*	.41336	.000	6.1540	7.9960
		3	-4.02950*	.41336	.000	-4.9505	-3.1085
		4	-9.27616*	.41336	.000	-10.1972	-8.3551
Vit_C	1	2	259.18636*	8.64673	.000	239.9202	278.4525
		3	174.83995*	8.64673	.000	155.5738	194.1061
		4	122.44065*	8.64673	.000	103.1745	141.7068
		5	-64.75812*	8.64673	.000	-84.0242	-45.4920
	2	1	-259.18636*	8.64673	.000	-278.4525	-239.9202
		3	-84.34641*	8.64673	.000	-103.6125	-65.0803
		4	-136.74570*	8.64673	.000	-156.0118	-117.4796
		5	-323.94448*	8.64673	.000	-343.2106	-304.6784
	3	1	-174.83995*	8.64673	.000	-194.1061	-155.5738
		2	84.34641*	8.64673	.000	65.0803	103.6125
		4	-52.39929*	8.64673	.000	-71.6654	-33.1332
		5	-239.59807*	8.64673	.000	-258.8642	-220.3320
	4	1	-122.44065*	8.64673	.000	-141.7068	-103.1745
		2	136.74570*	8.64673	.000	117.4796	156.0118
		3	52.39929*	8.64673	.000	33.1332	71.6654
		5	-187.19877*	8.64673	.000	-206.4649	-167.9327
	5	1	64.75812*	8.64673	.000	45.4920	84.0242
		2	323.94448*	8.64673	.000	304.6784	343.2106
		3	239.59807*	8.64673	.000	220.3320	258.8642
		4	187.19877*	8.64673	.000	167.9327	206.4649

*. The mean difference is significant at the 0.05 level.

ANOVA TABLES FOR THE 3 VEGETABLES AT VEGETATIVE STAGE

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Cu	Between Groups	.080	2	.040	157.596	.000
	Within Groups	.002	6	.000		
	Total	.081	8			
Zn	Between Groups	168.323	2	84.161	1.898E3	.000
	Within Groups	.266	6	.044		
	Total	168.589	8			
Mn	Between Groups	448.356	2	224.178	507.578	.000
	Within Groups	2.650	6	.442		
	Total	451.006	8			
Fe	Between Groups	540.711	2	270.356	362.506	.000
	Within Groups	4.475	6	.746		
	Total	545.186	8			
Mg	Between Groups	450418.260	2	225209.130	8.864E3	.000
	Within Groups	152.440	6	25.407		
	Total	450570.700	8			
Ca	Between Groups	5575472.840	2	2787736.420	1.288E3	.000
	Within Groups	12983.712	6	2163.952		
	Total	5588456.552	8			
Vit_A	Between Groups	77.891	2	38.945	55.919	.000
	Within Groups	4.179	6	.696		
	Total	82.070	8			
Vit_C	Between Groups	8646082.042	2	4323041.021	3.094E4	.000

Within Groups		838.450	6	139.742		
Total		8646920.492	8			

Post Hoc Tests

Multiple Comparisons

LSD

Dependent Variable	(I) Vegetable	(J) Vegetable	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Cu	AA	BB	-.21688*	.01300	.000	-.2487	-.1851
		CC	-.04014*	.01300	.021	-.0719	-.0083
	BB	AA	.21688*	.01300	.000	.1851	.2487
		CC	.17673*	.01300	.000	.1449	.2085
	CC	AA	.04014*	.01300	.021	.0083	.0719
		BB	-.17673*	.01300	.000	-.2085	-.1449
Zn	AA	BB	-6.96405*	.17195	.000	-7.3848	-6.5433
		CC	3.43086*	.17195	.000	3.0101	3.8516
	BB	AA	6.96405*	.17195	.000	6.5433	7.3848
		CC	10.39491*	.17195	.000	9.9742	10.8157
	CC	AA	-3.43086*	.17195	.000	-3.8516	-3.0101
		BB	-10.39491*	.17195	.000	-10.8157	-9.9742
Mn	AA	BB	16.48357*	.54262	.000	15.1558	17.8113
		CC	12.75809*	.54262	.000	11.4303	14.0858
	BB	AA	-16.48357*	.54262	.000	-17.8113	-15.1558
		CC	-3.72548*	.54262	.000	-5.0532	-2.3977
	CC	AA	-12.75809*	.54262	.000	-14.0858	-11.4303

156

		BB	3.72548[*]	.54262	.000	2.3977	5.0532
Fe	AA	BB	7.88874[*]	.70512	.000	6.1634	9.6141
		CC	-11.01162[*]	.70512	.000	-12.7370	-9.2862
	BB	AA	-7.88874[*]	.70512	.000	-9.6141	-6.1634
		CC	-18.90036[*]	.70512	.000	-20.6257	-17.1750
	CC	AA	11.01162[*]	.70512	.000	9.2862	12.7370
		BB	18.90036[*]	.70512	.000	17.1750	20.6257
Mg	AA	BB	467.67953[*]	4.11556	.000	457.6091	477.7499
		CC	481.15742[*]	4.11556	.000	471.0870	491.2278
	BB	AA	-467.67953[*]	4.11556	.000	-477.7499	-457.6091
		CC	13.47789[*]	4.11556	.017	3.4075	23.5483
	CC	AA	-481.15742[*]	4.11556	.000	-491.2278	-471.0870
		BB	-13.47789[*]	4.11556	.017	-23.5483	-3.4075
Ca	AA	BB	1525.87247[*]	37.98203	.000	1432.9338	1618.8112
		CC	1783.48575[*]	37.98203	.000	1690.5471	1876.4244
	BB	AA	-1525.87247[*]	37.98203	.000	-1618.8112	-1432.9338
		CC	257.61328[*]	37.98203	.001	164.6746	350.5520
	CC	AA	-1783.48575[*]	37.98203	.000	-1876.4244	-1690.5471
		BB	-257.61328[*]	37.98203	.001	-350.5520	-164.6746
Vit_A	AA	BB	-5.55774[*]	.68140	.000	-7.2251	-3.8904
		CC	-6.75116[*]	.68140	.000	-8.4185	-5.0838
	BB	AA	5.55774[*]	.68140	.000	3.8904	7.2251
		CC	-1.19342	.68140	.130	-2.8607	.4739
	CC	AA	6.75116[*]	.68140	.000	5.0838	8.4185
		BB	1.19342	.68140	.130	-.4739	2.8607
Vit_C	AA	BB	1820.85253[*]	9.65200	.000	1797.2349	1844.4701

		CC	2265.56946[*]	9.65200	.000	2241.9519	2289.1871
	BB	AA	-1820.85253[*]	9.65200	.000	-1844.4701	-1797.2349
		CC	444.71693[*]	9.65200	.000	421.0993	468.3345
	CC	AA	-2265.56946[*]	9.65200	.000	-2289.1871	-2241.9519
		BB	-444.71693[*]	9.65200	.000	-468.3345	-421.0993

*. The mean difference is significant at the 0.05 level.

AA is *Amaranthus hybridus*

BB is *Gynandropsis gynandra*

CC is *Solanum nigrum*

ANOVA TABLES FOR THE 3 VEGETABLES AT FLOWERING STAGE

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Cu	Between Groups	.176	2	.088	13.401	.006
	Within Groups	.039	6	.007		
	Total	.215	8			
Zn	Between Groups	148.837	2	74.419	233.445	.000
	Within Groups	1.913	6	.319		
	Total	150.750	8			
Mn	Between Groups	2244.760	2	1122.380	1.037E3	.000
	Within Groups	6.493	6	1.082		
	Total	2251.253	8			
Fe	Between Groups	5867.890	2	2933.945	111.119	.000
	Within Groups	158.422	6	26.404		
	Total	6026.313	8			
Mg	Between Groups	249267.403	2	124633.702	1.080E3	.000

		Sum of Squares	df	Mean Square	F	Sig.
	Within Groups	692.384	6	115.397		
	Total	249959.788	8			
Ca	Between Groups	1.311E7	2	6552828.892	827.217	.000
	Within Groups	47529.229	6	7921.538		
	Total	1.315E7	8			
Vit_A	Between Groups	324.269	2	162.135	488.234	.000
	Within Groups	1.993	6	.332		
	Total	326.262	8			
Vit_C	Between Groups	7221.956	2	3610.978	40.340	.000
	Within Groups	537.085	6	89.514		
	Total	7759.041	8			

Post Hoc Tests

Multiple Comparisons

LSD

Dependent Variable	(I) Vegetable	(J) Vegetable	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Cu	AA	BB	.2791855*	.0660941	.006	.117459	.440912
		CC	-.0317418	.0660941	.648	-.193468	.129985
	BB	AA	-.2791855*	.0660941	.006	-.440912	-.117459
		CC	-.3109273*	.0660941	.003	-.472654	-.149201
	CC	AA	.0317418	.0660941	.648	-.129985	.193468
		BB	.3109273*	.0660941	.003	.149201	.472654

Zn	AA	BB	-5.4643487*	.4610019	.000	-6.592380	-4.336318
		CC	4.4806128*	.4610019	.000	3.352582	5.608644
	BB	AA	5.4643487*	.4610019	.000	4.336318	6.592380
		CC	9.9449615*	.4610019	.000	8.816931	11.072992
	CC	AA	-4.4806128*	.4610019	.000	-5.608644	-3.352582
		BB	-9.9449615*	.4610019	.000	-11.072992	-8.816931
Mn	AA	BB	24.2303939*	.8493729	.000	22.152053	26.308734
		CC	38.2312054*	.8493729	.000	36.152865	40.309546
	BB	AA	-24.2303939*	.8493729	.000	-26.308734	-22.152053
		CC	14.0008116*	.8493729	.000	11.922471	16.079152
	CC	AA	-38.2312054*	.8493729	.000	-40.309546	-36.152865
		BB	-14.0008116*	.8493729	.000	-16.079152	-11.922471
Fe	AA	BB	-36.3940180*	4.195532	.000	-46.660117	-26.127919
		CC	25.8546844*	4.195532	.001	15.588586	36.120783
	BB	AA	36.3940180*	4.195532	.000	26.127919	46.660117
		CC	62.2487024*	4.195532	.000	51.982604	72.514801
	CC	AA	-25.8546844*	4.195532	.001	-36.120783	-15.588586
		BB	-62.2487024*	4.195532	.000	-72.514801	-51.982604
Mg	AA	BB	326.5024782*	8.771066	.000	305.040453	347.964504
		CC	374.6295738*	8.771066	.000	353.167548	396.091599
	BB	AA	-3.2650248E2*	8.771066	.000	-347.964504	-305.040453
		CC	48.1270956*	8.771066	.002	26.665070	69.589121
	CC	AA	-3.7462957E2*	8.771066	.000	-396.091599	-353.167548
		BB	-48.1270956*	8.771066	.002	-69.589121	-26.665070
Ca	AA	BB	2.1146359E3*	7.267066	.000	1936.817203	2292.454623
		CC	2.8459110E3*	7.267066	.000	2668.092300	3023.729719

	BB	AA	-2.1146359E3*	7.267066	.000	-2.292455E3	-1.936817E3
		CC	731.2750963*	7.267066	.000	553.456387	909.093806
	CC	AA	-2.8459110E3*	7.267066	.000	-3.023730E3	-2.668092E3
		BB	-7.3127510E2*	7.267066	.000	-909.093806	-553.456387
Vit_A	AA	BB	14.7013496*	.4705200	.000	13.550029	15.852670
		CC	7.1574795*	.4705200	.000	6.006159	8.308800
	BB	AA	-14.7013496*	.4705200	.000	-15.852670	-13.550029
		CC	-7.5438701*	.4705200	.000	-8.695191	-6.392549
	CC	AA	-7.1574795*	.4705200	.000	-8.308800	-6.006159
		BB	7.5438701*	.4705200	.000	6.392549	8.695191
Vit_C	AA	BB	-60.9999847*	7.725030	.000	-79.902454	-42.097515
		CC	-1.8603478	7.725030	.818	-20.762818	17.042122
	BB	AA	60.9999847*	7.725030	.000	42.097515	79.902454
		CC	59.1396369*	7.725030	.000	40.237167	78.042107
	CC	AA	1.8603478	7.725030	.818	-17.042122	20.762818
		BB	-59.1396369*	7.725030	.000	-78.042107	-40.237167

*. The mean difference is significant at the 0.05 level.

AA is *Amaranthus hybridus*

BB is *Gynandropsis gynandra*

CC is *Solanum nigrum*

ii) <u>Efficacy data analysis with different treatments over time</u>

COMPARISON OF RAW AT TIME 1 AND 2 FOR THE VARIABLES CD3 CD4 CD8 CD4COUNTS CD8COUNTS
CRPNG RCD4CD8 CRPNG2

```
                              T-Tests

Variable      Method         Variances      DF     t Value    Pr > |t|

CD3%          Pooled         Equal          18     -3.20      0.0050***
CD3%          Satterthwaite  Unequal        17.5   -3.20      0.0051
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CD4%         Pooled         Equal       18    -1.69    0.1079
CD4%         Satterthwaite  Unequal   17.2    -1.69    0.1087
CD8%         Pooled         Equal       18     0.14    0.8890
CD8%         Satterthwaite  Unequal   14.1     0.14    0.8894
CD4COUNTS    Pooled         Equal       18    -2.77    0.0127
CD4COUNTS    Satterthwaite  Unequal     18    -2.77    0.0127
CD8COUNTS    Pooled         Equal       18    -1.13    0.2715
CD8COUNTS    Satterthwaite  Unequal     13    -1.13    0.2770
RCD4CD8      Pooled         Equal       18    -0.70    0.4953
RCD4CD8      Satterthwaite  Unequal     18    -0.70    0.4953
CRPng2       Pooled         Equal       12    -0.31    0.7641
CRPng2       Satterthwaite  Unequal     12    -0.31    0.7641
```

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
CD3%	Folded F	9	9	1.40	0.6242
CD4%	Folded F	9	9	1.56	0.5199
CD8%	Folded F	9	9	3.23	0.0954
CD4COUNTS	Folded F	9	9	1.02	0.9795
CD8COUNTS	Folded F	9	9	4.23	0.0429
RCD4CD8	Folded F	9	9	1.01	0.9926
CRPng2	Folded F	6	6	1.09	0.9223

COMPARISON OF RAW AT TIME 1 AND 3 FOR THE VARIABLES CD3 CD4 CD8 CD4COUNTS CD8COUNTS CRPNG RCD4CD8 CRPNG2

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
CD3%	Pooled	Equal	16	3.77	0.0017
CD3%	Satterthwaite	Unequal	13.9	4.04	0.0012
CD4%	Pooled	Equal	16	-1.86	0.0817
CD4%	Satterthwaite	Unequal	14.8	-1.85	0.0849
CD8%	Pooled	Equal	16	2.12	0.0504
CD8%	Satterthwaite	Unequal	15.9	2.20	0.0433
CD4COUNTS	Pooled	Equal	16	1.70	0.1076
CD4COUNTS	Satterthwaite	Unequal	12.8	1.85	0.0878
CD8COUNTS	Pooled	Equal	16	2.83	0.0122
CD8COUNTS	Satterthwaite	Unequal	13.4	3.04	0.0091
RCD4CD8	Pooled	Equal	16	-2.82	0.0124
RCD4CD8	Satterthwaite	Unequal	9.56	-2.62	0.0267
CRPng2	Pooled	Equal	12	-0.05	0.9582
CRPng2	Satterthwaite	Unequal	10.4	-0.05	0.9584

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
CD3%	Folded F	9	7	3.88	0.0877
CD4%	Folded F	7	9	1.11	0.8627
CD8%	Folded F	9	7	1.91	0.4073
CD4COUNTS	Folded F	9	7	5.33	0.0383
CD8COUNTS	Folded F	9	7	4.40	0.0636
RCD4CD8	Folded F	7	9	4.35	0.0448
CRPng2	Folded F	6	6	2.27	0.3415

COMPARISON OF RAW AT TIME 1 AND 4 FOR THE VARIABLES CD3 CD4 CD8 CD4COUNTS CD8COUNTS CRPNG RCD4CD8 CRPNG2

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
CD3%	Pooled	Equal	16	-0.45	0.6586
CD3%	Satterthwaite	Unequal	15	-0.45	0.6600
CD4%	Pooled	Equal	16	1.63	0.1224
CD4%	Satterthwaite	Unequal	14.3	1.61	0.1289
CD8%	Pooled	Equal	16	-0.63	0.5386
CD8%	Satterthwaite	Unequal	15.4	-0.63	0.5373
CD4COUNTS	Pooled	Equal	16	0.82	0.4217
CD4COUNTS	Satterthwaite	Unequal	16	0.85	0.4103
CD8COUNTS	Pooled	Equal	16	-0.55	0.5930
CD8COUNTS	Satterthwaite	Unequal	14.7	-0.54	0.5961
RCD4CD8	Pooled	Equal	16	1.59	0.1317
RCD4CD8	Satterthwaite	Unequal	15.8	1.61	0.1269
CRPng2	Pooled	Equal	12	-1.05	0.3159
CRPng2	Satterthwaite	Unequal	7.45	-1.05	0.3280

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
CD3%	Folded F	7	9	1.05	0.9196
CD4%	Folded F	7	9	1.23	0.7542
CD8%	Folded F	9	7	1.07	0.9462
CD4COUNTS	Folded F	9	7	1.54	0.5817
CD8COUNTS	Folded F	7	9	1.12	0.8495
RCD4CD8	Folded F	9	7	1.27	0.7682
CRPng2	Folded F	6	6	8.16	0.0219

COMPARISON OF RAW AT TIME 2 AND 3 FOR THE VARIABLES CD3 CD4 CD8 CD4COUNTS CD8COUNTS CRPNG RCD4CD8 CRPNG2

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
CD3%	Pooled	Equal	16	8.20	<.0001
CD3%	Satterthwaite	Unequal	15	8.67	<.0001
CD4%	Pooled	Equal	16	-0.50	0.6262
CD4%	Satterthwaite	Unequal	12.8	-0.48	0.6385
CD8%	Pooled	Equal	16	2.79	0.0130
CD8%	Satterthwaite	Unequal	12.9	2.71	0.0179
CD4COUNTS	Pooled	Equal	16	4.98	0.0001
CD4COUNTS	Satterthwaite	Unequal	12.9	5.39	0.0001
CD8COUNTS	Pooled	Equal	16	6.51	<.0001
CD8COUNTS	Satterthwaite	Unequal	15.3	6.53	<.0001
RCD4CD8	Pooled	Equal	16	-2.40	0.0290
RCD4CD8	Satterthwaite	Unequal	9.57	-2.23	0.0512
CRPng2	Pooled	Equal	12	0.31	0.7642
CRPng2	Satterthwaite	Unequal	10.2	0.31	0.7651

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
CD3%	Folded F	9	7	2.77	0.1930
CD4%	Folded F	7	9	1.73	0.4361
CD8%	Folded F	7	9	1.70	0.4518
CD4COUNTS	Folded F	9	7	5.23	0.0401
CD8COUNTS	Folded F	9	7	1.04	0.9817
RCD4CD8	Folded F	7	9	4.32	0.0457
CRPng2	Folded F	6	6	2.47	0.2962

COMPARISON OF RAW AT TIME 2 AND 4 FOR THE VARIABLES CD3 CD4 CD8 CD4COUNTS CD8COUNTS CRPNG RCD4CD8 CRPNG2

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
CD3%	Pooled	Equal	16	2.51	0.0231
CD3%	Satterthwaite	Unequal	13.6	2.46	0.0282
CD4%	Pooled	Equal	16	3.33	0.0043
CD4%	Satterthwaite	Unequal	12.4	3.21	0.0073
CD8%	Pooled	Equal	16	-0.95	0.3546
CD8%	Satterthwaite	Unequal	10.6	-0.90	0.3889
CD4COUNTS	Pooled	Equal	16	3.67	0.0021
CD4COUNTS	Satterthwaite	Unequal	16	3.76	0.0017
CD8COUNTS	Pooled	Equal	16	0.36	0.7270
CD8COUNTS	Satterthwaite	Unequal	9.35	0.33	0.7495
RCD4CD8	Pooled	Equal	16	2.27	0.0370
RCD4CD8	Satterthwaite	Unequal	15.8	2.31	0.0349
CRPng2	Pooled	Equal	12	-0.61	0.5563
CRPng2	Satterthwaite	Unequal	7.34	-0.61	0.5633

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
CD3%	Folded F	7	9	1.47	0.5747
CD4%	Folded F	7	9	1.92	0.3583
CD8%	Folded F	7	9	3.01	0.1271
CD4COUNTS	Folded F	9	7	1.51	0.5978
CD8COUNTS	Folded F	7	9	4.76	0.0341
RCD4CD8	Folded F	9	7	1.28	0.7617
CRPng2	Folded F	6	6	8.87	0.0178

COMPARISON OF RAW AT TIME 3 AND 4 FOR THE VARIABLES CD3 CD4 CD8 CD4COUNTS CD8COUNTS CRPNG RCD4CD8 CRPNG2

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
CD3%	Pooled	Equal	14	-4.16	0.0010
CD3%	Satterthwaite	Unequal	10.2	-4.16	0.0019
CD4%	Pooled	Equal	14	3.17	0.0068
CD4%	Satterthwaite	Unequal	14	3.17	0.0069
CD8%	Pooled	Equal	14	-2.78	0.0147
CD8%	Satterthwaite	Unequal	13	-2.78	0.0155
CD4COUNTS	Pooled	Equal	14	-0.90	0.3859
CD4COUNTS	Satterthwaite	Unequal	10.7	-0.90	0.3903
CD8COUNTS	Pooled	Equal	14	-3.30	0.0053
CD8COUNTS	Satterthwaite	Unequal	9.72	-3.30	0.0083
RCD4CD8	Pooled	Equal	14	3.52	0.0034
RCD4CD8	Satterthwaite	Unequal	9.45	3.52	0.0061
CRPng2	Pooled	Equal	12	-1.39	0.1890
CRPng2	Satterthwaite	Unequal	9.1	-1.39	0.1968

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
CD3%	Folded F	7	7	4.08	0.0832
CD4%	Folded F	7	7	1.11	0.8959

164

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CD8%          Folded F       7        7       1.77     0.4674
CD4COUNTS     Folded F       7        7       3.46     0.1240
CD8COUNTS     Folded F       7        7       4.95     0.0513
RCD4CD8       Folded F       7        7       5.53     0.0381
CRPng2        Folded F       6        6       3.59     0.1448
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COMPARISON OF COOKED AT TIME 1 AND 2 FOR THE VARIABLES CD3 CD4 CD8 CD4COUNTS CD8COUNTS CRPNG RCD4CD8 CRPN

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
CD3%	Pooled	Equal	18	-2.19	0.0423
CD3%	Satterthwaite	Unequal	17.7	-2.19	0.0426
CD4%	Pooled	Equal	18	-2.33	0.0316
CD4%	Satterthwaite	Unequal	17.6	-2.33	0.0319
CD8%	Pooled	Equal	18	1.28	0.2184
CD8%	Satterthwaite	Unequal	17.2	1.28	0.2192
CD4COUNTS	Pooled	Equal	18	-2.98	0.0081
CD4COUNTS	Satterthwaite	Unequal	17.9	-2.98	0.0081
CD8COUNTS	Pooled	Equal	18	0.09	0.9308
CD8COUNTS	Satterthwaite	Unequal	17.3	0.09	0.9309
RCD4CD8	Pooled	Equal	18	-1.70	0.1071
RCD4CD8	Satterthwaite	Unequal	16.5	-1.70	0.1086
CRPng2	Pooled	Equal	14	1.20	0.2489
CRPng2	Satterthwaite	Unequal	9.89	1.20	0.2570

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
CD3%	Folded F	9	9	1.29	0.7082
CD4%	Folded F	9	9	1.35	0.6632
CD8%	Folded F	9	9	1.57	0.5139
CD4COUNTS	Folded F	9	9	1.12	0.8662
CD8COUNTS	Folded F	9	9	1.49	0.5629
RCD4CD8	Folded F	9	9	1.85	0.3722
CRPng2	Folded F	7	7	4.63	0.0607

COMPARISON OF COOKED AT TIME 1 AND 3 FOR THE VARIABLES CD3 CD4 CD8 CD4COUNTS CD8COUNTS CRPNG RCD4CD8 CRPN

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
CD3%	Pooled	Equal	18	5.05	<.0001
CD3%	Satterthwaite	Unequal	15.8	5.05	0.0001
CD4%	Pooled	Equal	18	-2.84	0.0109
CD4%	Satterthwaite	Unequal	17.4	-2.84	0.0112
CD8%	Pooled	Equal	18	1.99	0.0621
CD8%	Satterthwaite	Unequal	18	1.99	0.0621
CD4COUNTS	Pooled	Equal	18	3.63	0.0019
CD4COUNTS	Satterthwaite	Unequal	13.5	3.63	0.0029
CD8COUNTS	Pooled	Equal	18	3.43	0.0030
CD8COUNTS	Satterthwaite	Unequal	12.9	3.43	0.0045
RCD4CD8	Pooled	Equal	18	-2.17	0.0435
RCD4CD8	Satterthwaite	Unequal	10.4	-2.17	0.0540
CRPng2	Pooled	Equal	14	1.48	0.1613
CRPng2	Satterthwaite	Unequal	13.4	1.48	0.1623

165

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
CD3%	Folded F	9	9	2.21	0.2521
CD4%	Folded F	9	9	1.46	0.5834
CD8%	Folded F	9	9	1.09	0.9026
CD4COUNTS	Folded F	9	9	3.72	0.0636
CD8COUNTS	Folded F	9	9	4.37	0.0388
RCD4CD8	Folded F	9	9	12.73	0.0008
CRPng2	Folded F	7	7	1.54	0.5836

COMPARISON OF COOKED AT TIME 1 AND 4 FOR THE VARIABLES CD3 CD4 CD8 CD4COUNTS
CD8COUNTS CRPNG RCD4CD8 CRPN

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
CD3%	Pooled	Equal	17	0.49	0.6323
CD3%	Satterthwaite	Unequal	14.7	0.50	0.6238
CD4%	Pooled	Equal	17	-1.25	0.2290
CD4%	Satterthwaite	Unequal	17	-1.25	0.2273
CD8%	Pooled	Equal	17	0.19	0.8516
CD8%	Satterthwaite	Unequal	16.7	0.19	0.8497
CD4COUNTS	Pooled	Equal	17	-0.22	0.8317
CD4COUNTS	Satterthwaite	Unequal	16.4	-0.22	0.8290
CD8COUNTS	Pooled	Equal	17	0.40	0.6928
CD8COUNTS	Satterthwaite	Unequal	15.7	0.41	0.6868
RCD4CD8	Pooled	Equal	17	-0.24	0.8129
RCD4CD8	Satterthwaite	Unequal	16.5	-0.24	0.8102
CRPng2	Pooled	Equal	14	0.85	0.4086
CRPng2	Satterthwaite	Unequal	13.8	0.85	0.4087

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
CD3%	Folded F	9	8	2.99	0.1380
CD4%	Folded F	9	8	1.15	0.8572
CD8%	Folded F	9	8	1.60	0.5176
CD4COUNTS	Folded F	9	8	1.84	0.4016
CD8COUNTS	Folded F	9	8	2.27	0.2635
RCD4CD8	Folded F	9	8	1.76	0.4380
CRPng2	Folded F	7	7	1.25	0.7759

COMPARISON OF COOKED AT TIME 2 AND 3 FOR THE VARIABLES CD3 CD4 CD8 CD4COUNTS
CD8COUNTS CRPNG RCD4CD8 CRPN

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
CD3%	Pooled	Equal	18	8.12	<.0001
CD3%	Satterthwaite	Unequal	16.8	8.12	<.0001
CD4%	Pooled	Equal	18	-0.52	0.6066
CD4%	Satterthwaite	Unequal	18	-0.52	0.6066
CD8%	Pooled	Equal	18	0.95	0.3568
CD8%	Satterthwaite	Unequal	16.9	0.95	0.3577
CD4COUNTS	Pooled	Equal	18	7.14	<.0001
CD4COUNTS	Satterthwaite	Unequal	13.1	7.14	<.0001
CD8COUNTS	Pooled	Equal	18	3.89	0.0011
CD8COUNTS	Satterthwaite	Unequal	14.5	3.89	0.0015
RCD4CD8	Pooled	Equal	18	-1.36	0.1917
RCD4CD8	Satterthwaite	Unequal	11.6	-1.36	0.2008
CRPng2	Pooled	Equal	14	0.62	0.5479
CRPng2	Satterthwaite	Unequal	11.2	0.62	0.5503

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
CD3%	Folded F	9	9	1.71	0.4353
CD4%	Folded F	9	9	1.08	0.9096
CD8%	Folded F	9	9	1.70	0.4393
CD4COUNTS	Folded F	9	9	4.17	0.0447

```
CD8COUNTS      Folded F          9          9          2.93    0.1245
RCD4CD8        Folded F          9          9          6.87    0.0084
CRPng2         Folded F          7          7          3.01    0.1691
```

COMPARISON OF COOKED AT TIME 2 AND 4 FOR THE VARIABLES CD3 CD4 CD8 CD4COUNTS CD8COUNTS CRPNG RCD4CD8 CRPN

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
CD3%	Pooled	Equal	17	3.20	0.0053
CD3%	Satterthwaite	Unequal	15.7	3.27	0.0050
CD4%	Pooled	Equal	17	1.18	0.2553
CD4%	Satterthwaite	Unequal	16.8	1.19	0.2500
CD8%	Pooled	Equal	17	-1.20	0.2478
CD8%	Satterthwaite	Unequal	16.8	-1.20	0.2476
CD4COUNTS	Pooled	Equal	17	3.03	0.0076
CD4COUNTS	Satterthwaite	Unequal	16.1	3.09	0.0070
CD8COUNTS	Pooled	Equal	17	0.36	0.7267
CD8COUNTS	Satterthwaite	Unequal	16.8	0.36	0.7237
RCD4CD8	Pooled	Equal	17	1.57	0.1342
RCD4CD8	Satterthwaite	Unequal	14.3	1.62	0.1271
CRPng2	Pooled	Equal	14	-0.04	0.9686
CRPng2	Satterthwaite	Unequal	9.35	-0.04	0.9689

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
CD3%	Folded F	9	8	2.31	0.2523
CD4%	Folded F	9	8	1.55	0.5499
CD8%	Folded F	9	8	1.02	0.9855
CD4COUNTS	Folded F	9	8	2.07	0.3193
CD8COUNTS	Folded F	9	8	1.52	0.5652
RCD4CD8	Folded F	9	8	3.26	0.1107
CRPng2	Folded F	7	7	5.79	0.0337

COMPARISON OF COOKED AT TIME 3 AND 4 FOR THE VARIABLES CD3 CD4 CD8 CD4COUNTS CD8COUNTS CRPNG RCD4CD8 CRPN

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
CD3%	Pooled	Equal	17	-6.01	<.0001
CD3%	Satterthwaite	Unequal	17	-6.06	<.0001
CD4%	Pooled	Equal	17	1.70	0.1066
CD4%	Satterthwaite	Unequal	16.7	1.73	0.1025
CD8%	Pooled	Equal	17	-1.94	0.0695
CD8%	Satterthwaite	Unequal	16.6	-1.97	0.0662
CD4COUNTS	Pooled	Equal	17	-4.76	0.0002
CD4COUNTS	Satterthwaite	Unequal	14.2	-4.68	0.0003
CD8COUNTS	Pooled	Equal	17	-3.97	0.0010
CD8COUNTS	Satterthwaite	Unequal	14.4	-3.90	0.0015
RCD4CD8	Pooled	Equal	17	2.01	0.0605
RCD4CD8	Satterthwaite	Unequal	9.89	2.12	0.0608
CRPng2	Pooled	Equal	14	-0.45	0.6589
CRPng2	Satterthwaite	Unequal	12.7	-0.45	0.6596

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
CD3%	Folded F	9	8	1.35	0.6833
CD4%	Folded F	9	8	1.67	0.4804
CD8%	Folded F	9	8	1.74	0.4457

169

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CD4COUNTS    Folded F          8        9      2.02    0.3160
CD8COUNTS    Folded F          8        9      1.93    0.3474
RCD4CD8      Folded F          9        8     22.40    0.0002
CRPng2       Folded F          7        7      1.92    0.4076
```

COMPARISON OF POSCONTROL AT TIME 1 AND 2 FOR THE VARIABLES CD3 CD4 CD8 CD4COUNTS CD8COUNTS CRPNG RCD4CD8

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                              T-Tests

Variable     Method          Variances      DF    t Value    Pr > |t|

CD3%         Pooled          Equal           8      0.50      0.6309
CD3%         Satterthwaite   Unequal      5.58      0.50      0.6365
CD4%         Pooled          Equal           8      1.58      0.1532
CD4%         Satterthwaite   Unequal      6.35      1.58      0.1629
CD8%         Pooled          Equal           8      0.43      0.6806
CD8%         Satterthwaite   Unequal      6.99      0.43      0.6822
CD4COUNTS    Pooled          Equal           8      1.36      0.2113
CD4COUNTS    Satterthwaite   Unequal      7.56      1.36      0.2134
CD8COUNTS    Pooled          Equal           8      0.31      0.7673
CD8COUNTS    Satterthwaite   Unequal      4.97      0.31      0.7719
RCD4CD8      Pooled          Equal           8      0.01      0.9916
RCD4CD8      Satterthwaite   Unequal         8      0.01      0.9916
CRPng2       Pooled          Equal           6      1.28      0.2470
CRPng2       Satterthwaite   Unequal      5.67      1.28      0.2496


                       Equality of Variances

Variable     Method      Num DF     Den DF     F Value    Pr > F

CD3%          Folded F       4          4        4.84      0.1557
CD4%         Folded F        4          4        3.08      0.3010
CD8%         Folded F        4          4        2.23      0.4574
CD4COUNTS    Folded F        4          4        1.63      0.6462
CD8COUNTS    Folded F        4          4        8.10      0.0672
RCD4CD8      Folded F        4          4        1.05      0.9643
CRPng2       Folded F        3          3        1.63      0.6974
```

COMPARISON OF POSCONTROL AT TIME 1 AND 3 FOR THE VARIABLES CD3 CD4 CD8 CD4COUNTS CD8COUNTS CRPNG RCD4CD8

```
                              T-Tests

Variable     Method          Variances      DF    t Value    Pr > |t|

CD3%         Pooled          Equal           7      8.26     <.0001
CD3%         Satterthwaite   Unequal      6.81      8.35     <.0001
CD4%         Pooled          Equal           7     -0.14      0.8904
CD4%         Satterthwaite   Unequal      6.61     -0.15      0.8839
CD8%         Pooled          Equal           7      2.35      0.0510
CD8%         Satterthwaite   Unequal      5.32      2.26      0.0703
CD4COUNTS    Pooled          Equal           7      5.86      0.0006
CD4COUNTS    Satterthwaite   Unequal      6.91      6.13      0.0005
CD8COUNTS    Pooled          Equal           7      5.60      0.0008
CD8COUNTS    Satterthwaite   Unequal      6.45      5.58      0.0011
RCD4CD8      Pooled          Equal           7     -2.47      0.0430
RCD4CD8      Satterthwaite   Unequal       3.4     -2.21      0.1032
CRPng2       Pooled          Equal           6     -0.20      0.8513
CRPng2       Satterthwaite   Unequal      5.83     -0.20      0.8515
```

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
CD3%	Folded F	4	3	1.19	0.9228
CD4%	Folded F	4	3	2.81	0.4231
CD8%	Folded F	3	4	1.93	0.5343
CD4COUNTS	Folded F	4	3	2.12	0.5644
CD8COUNTS	Folded F	3	4	1.07	0.9107
RCD4CD8	Folded F	3	4	11.94	0.0366
CRPng2	Folded F	3	3	1.41	0.7857

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
CD3%	Pooled	Equal	7	0.43	0.6805
CD3%	Satterthwaite	Unequal	6.85	0.45	0.6660
CD4%	Pooled	Equal	7	0.19	0.8558
CD4%	Satterthwaite	Unequal	4.49	0.18	0.8677
CD8%	Pooled	Equal	7	-1.03	0.3368
CD8%	Satterthwaite	Unequal	3.92	-0.94	0.3993
CD4COUNTS	Pooled	Equal	7	0.55	0.5983
CD4COUNTS	Satterthwaite	Unequal	5.63	0.60	0.5705
CD8COUNTS	Pooled	Equal	7	-0.94	0.3801
CD8COUNTS	Satterthwaite	Unequal	3.47	-0.84	0.4537
RCD4CD8	Pooled	Equal	7	0.47	0.6509
RCD4CD8	Satterthwaite	Unequal	4.52	0.44	0.6784
CRPng2	Pooled	Equal	6	-0.24	0.8195
CRPng2	Satterthwaite	Unequal	3.45	-0.24	0.8251

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
CD3%	Folded F	4	3	2.26	0.5290
CD4%	Folded F	3	4	3.17	0.2940
CD8%	Folded F	3	4	5.22	0.1441
CD4COUNTS	Folded F	4	3	5.53	0.1915
CD8COUNTS	Folded F	3	4	10.20	0.0481
RCD4CD8	Folded F	3	4	3.09	0.3037
CRPng2	Folded F	3	3	13.37	0.0610

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
CD3%	Pooled	Equal	7	4.05	0.0049
CD3%	Satterthwaite	Unequal	5.57	4.43	0.0053
CD4%	Pooled	Equal	7	-1.62	0.1484
CD4%	Satterthwaite	Unequal	5.1	-1.79	0.1316
CD8%	Pooled	Equal	7	1.57	0.1614
CD8%	Satterthwaite	Unequal	6.77	1.58	0.1595
CD4COUNTS	Pooled	Equal	7	3.46	0.0106
CD4COUNTS	Satterthwaite	Unequal	6.32	3.70	0.0091
CD8COUNTS	Pooled	Equal	7	2.25	0.0594
CD8COUNTS	Satterthwaite	Unequal	5.24	2.47	0.0539
RCD4CD8	Pooled	Equal	7	-2.47	0.0431
RCD4CD8	Satterthwaite	Unequal	3.42	-2.21	0.1027
CRPng2	Pooled	Equal	6	-1.36	0.2229
CRPng2	Satterthwaite	Unequal	5.2	-1.36	0.2300

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
CD3%	Folded F	4	3	5.76	0.1818
CD4%	Folded F	4	3	8.65	0.1073
CD8%	Folded F	4	3	1.16	0.9426
CD4COUNTS	Folded F	4	3	3.45	0.3363

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CD8COUNTS    Folded F        4          3        7.56    0.1282
RCD4CD8      Folded F        3          4       11.38    0.0398
CRPng2       Folded F        3          3        2.30    0.5126
```

COMPARISON OF POSCONTROL AT TIME 2 AND 4 FOR THE VARIABLES CD3 CD4 CD8 CD4COUNTS CD8COUNTS CRPNG RCD4CD8

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
CD3%	Pooled	Equal	16	2.51	0.0231
CD3%	Satterthwaite	Unequal	13.6	2.46	0.0282
CD4%	Pooled	Equal	16	3.33	0.0043
CD4%	Satterthwaite	Unequal	12.4	3.21	0.0073
CD8%	Pooled	Equal	16	-0.95	0.3546
CD8%	Satterthwaite	Unequal	10.6	-0.90	0.3889
CD4COUNTS	Pooled	Equal	16	3.67	0.0021
CD4COUNTS	Satterthwaite	Unequal	16	3.76	0.0017
CD8COUNTS	Pooled	Equal	16	0.36	0.7270
CD8COUNTS	Satterthwaite	Unequal	9.35	0.33	0.7495
RCD4CD8	Pooled	Equal	16	2.27	0.0370
RCD4CD8	Satterthwaite	Unequal	15.8	2.31	0.0349
CRPng2	Pooled	Equal	12	-0.61	0.5563
CRPng2	Satterthwaite	Unequal	7.34	-0.61	0.5633

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
CD3%	Folded F	7	9	1.47	0.5747
CD4%	Folded F	7	9	1.92	0.3583
CD8%	Folded F	7	9	3.01	0.1271
CD4COUNTS	Folded F	9	7	1.51	0.5978
CD8COUNTS	Folded F	7	9	4.76	0.0341
RCD4CD8	Folded F	9	7	1.28	0.7617
CRPng2	Folded F	6	6	8.87	0.0178

COMPARISON OF POSCONTROL AT TIME 3 AND 4 FOR THE VARIABLES CD3 CD4 CD8 CD4COUNTS CD8COUNTS CRPNG RCD4CD8

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
CD3%	Pooled	Equal	6	-9.00	0.0001
CD3%	Satterthwaite	Unequal	5.47	-9.00	0.0002
CD4%	Pooled	Equal	6	0.27	0.7933
CD4%	Satterthwaite	Unequal	3.67	0.27	0.7989
CD8%	Pooled	Equal	6	-2.26	0.0644
CD8%	Satterthwaite	Unequal	4.95	-2.26	0.0738
CD4COUNTS	Pooled	Equal	6	-7.82	0.0002
CD4COUNTS	Satterthwaite	Unequal	5	-7.82	0.0005
CD8COUNTS	Pooled	Equal	6	-3.10	0.0210
CD8COUNTS	Satterthwaite	Unequal	3.62	-3.10	0.0413
RCD4CD8	Pooled	Equal	6	2.26	0.0644
RCD4CD8	Satterthwaite	Unequal	4.46	2.26	0.0797
CRPng2	Pooled	Equal	6	0.05	0.9645
CRPng2	Satterthwaite	Unequal	3.32	0.05	0.9656

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
CD3%	Folded F	3	3	1.90	0.6119
CD4%	Folded F	3	3	8.90	0.1057
CD8%	Folded F	3	3	2.71	0.4341
CD4COUNTS	Folded F	3	3	2.62	0.4506

```
CD8COUNTS     Folded F           3          3         9.53     0.0965
RCD4CD8       Folded F           3          3         3.86     0.2968
CRPng2        Folded F           3          3        18.81     0.0379
```

**COMPARISON OF NEGCONTROL AT TIME 1 AND 2 FOR THE VARIABLES CD3 CD4 CD8 CD4COUNTS
CD8COUNTS CRPNG RCD4CD8**

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
CD3%	Pooled	Equal	8	1.22	0.2579
CD3%	Satterthwaite	Unequal	7.05	1.22	0.2624
CD4%	Pooled	Equal	8	1.31	0.2278
CD4%	Satterthwaite	Unequal	7.93	1.31	0.2282
CD8%	Pooled	Equal	8	1.35	0.2130
CD8%	Satterthwaite	Unequal	5.19	1.35	0.2319
CD4COUNTS	Pooled	Equal	8	1.93	0.0903
CD4COUNTS	Satterthwaite	Unequal	7.8	1.93	0.0912
CD8COUNTS	Pooled	Equal	8	1.46	0.1823
CD8COUNTS	Satterthwaite	Unequal	5.68	1.46	0.1972
RCD4CD8	Pooled	Equal	8	-1.00	0.3473
RCD4CD8	Satterthwaite	Unequal	4.09	-1.00	0.3734
CRPng2	Pooled	Equal	4	1.48	0.2133
CRPng2	Satterthwaite	Unequal	2.37	1.48	0.2583

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
CD3%	Folded F	4	4	2.16	0.4746
CD4%	Folded F	4	4	1.21	0.8555
CD8%	Folded F	4	4	6.58	0.0953
CD4COUNTS	Folded F	4	4	1.38	0.7632
CD8COUNTS	Folded F	4	4	4.53	0.1723
RCD4CD8	Folded F	4	4	89.18	0.0007
CRPng2	Folded F	2	2	10.69	0.1711

**COMPARISON OF NEGCONTROL AT TIME 1 AND 3 FOR THE VARIABLES CD3 CD4 CD8 CD4COUNTS
CD8COUNTS CRPNG RCD4CD8**

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
CD3%	Pooled	Equal	8	2.67	0.0283
CD3%	Satterthwaite	Unequal	7	2.67	0.0320
CD4%	Pooled	Equal	8	0.69	0.5123
CD4%	Satterthwaite	Unequal	8	0.69	0.5123
CD8%	Pooled	Equal	8	0.11	0.9178
CD8%	Satterthwaite	Unequal	6.41	0.11	0.9185
CD4COUNTS	Pooled	Equal	8	2.18	0.0607
CD4COUNTS	Satterthwaite	Unequal	7.1	2.18	0.0649
CD8COUNTS	Pooled	Equal	8	1.12	0.2948
CD8COUNTS	Satterthwaite	Unequal	6.66	1.12	0.3011
RCD4CD8	Pooled	Equal	8	-0.07	0.9429
RCD4CD8	Satterthwaite	Unequal	7.13	-0.07	0.9431
CRPng2	Pooled	Equal	4	1.68	0.1679
CRPng2	Satterthwaite	Unequal	2.54	1.68	0.2073

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F

```
CD3%         Folded F        4        4        2.21     0.4607
CD4%         Folded F        4        4        1.05     0.9643
CD8%         Folded F        4        4        2.98     0.3150
CD4COUNTS    Folded F        4        4        2.11     0.4885
CD8COUNTS    Folded F        4        4        2.63     0.3713
RCD4CD8      Folded F        4        4        2.08     0.4967
CRPng2       Folded F        2        2        7.22     0.2432
```

COMPARISON OF NEGCONTROL AT TIME 1 AND 4 FOR THE VARIABLES CD3 CD4 CD8 CD4COUNTS CD8COUNTS CRPNG RCD4CD8

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
CD3%	Pooled	Equal	7	0.88	0.4085
CD3%	Satterthwaite	Unequal	6.99	0.91	0.3924
CD4%	Pooled	Equal	7	-2.83	0.0253
CD4%	Satterthwaite	Unequal	7	-2.92	0.0222
CD8%	Pooled	Equal	7	2.07	0.0767
CD8%	Satterthwaite	Unequal	7	2.14	0.0698
CD4COUNTS	Pooled	Equal	7	-1.45	0.1912
CD4COUNTS	Satterthwaite	Unequal	6.27	-1.43	0.2003
CD8COUNTS	Pooled	Equal	7	2.06	0.0779
CD8COUNTS	Satterthwaite	Unequal	6.94	2.15	0.0686
RCD4CD8	Pooled	Equal	7	-2.52	0.0400
RCD4CD8	Satterthwaite	Unequal	5.97	-2.47	0.0490
CRPng2	Pooled	Equal	4	1.50	0.2080
CRPng2	Satterthwaite	Unequal	2.26	1.50	0.2587

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
CD3%	Folded F	4	3	1.80	0.6582
CD4%	Folded F	4	3	1.69	0.6962
CD8%	Folded F	4	3	1.64	0.7152
CD4COUNTS	Folded F	3	4	1.19	0.8411
CD8COUNTS	Folded F	4	3	2.01	0.5917
RCD4CD8	Folded F	3	4	1.39	0.7373
CRPng2	Folded F	2	2	15.60	0.1205

COMPARISON OF NEGCONTROL AT TIME 2 AND 3 FOR THE VARIABLES CD3 CD4 CD8 CD4COUNTS CD8COUNTS CRPNG RCD4CD8

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
CD3%	Pooled	Equal	8	1.25	0.2452
CD3%	Satterthwaite	Unequal	8	1.25	0.2452
CD4%	Pooled	Equal	8	-0.67	0.5223
CD4%	Satterthwaite	Unequal	7.89	-0.67	0.5225
CD8%	Pooled	Equal	8	-1.14	0.2888
CD8%	Satterthwaite	Unequal	7.01	-1.14	0.2933
CD4COUNTS	Pooled	Equal	8	0.47	0.6520
CD4COUNTS	Satterthwaite	Unequal	7.67	0.47	0.6525
CD8COUNTS	Pooled	Equal	8	-0.49	0.6406
CD8COUNTS	Satterthwaite	Unequal	7.47	-0.49	0.6415
RCD4CD8	Pooled	Equal	8	0.98	0.3563
RCD4CD8	Satterthwaite	Unequal	4.19	0.98	0.3807
CRPng2	Pooled	Equal	4	1.21	0.2925
CRPng2	Satterthwaite	Unequal	2.05	1.21	0.3469

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
CD3%	Folded F	4	4	1.03	0.9813
CD4%	Folded F	4	4	1.27	0.8206
CD8%	Folded F	4	4	2.20	0.4626
CD4COUNTS	Folded F	4	4	1.53	0.6916

177

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CD8COUNTS    Folded F        4        4        1.72    0.6114
RCD4CD8      Folded F        4        4       42.96    0.0031
CRPng2       Folded F        2        2       77.20    0.0256
```

COMPARISON OF NEGCONTROL AT TIME 2 AND 4 FOR THE VARIABLES CD3 CD4 CD8 CD4COUNTS
CD8COUNTS CRPNG RCD4CD8

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
CD3%	Pooled	Equal	16	2.51	0.0231
CD3%	Satterthwaite	Unequal	13.6	2.46	0.0282
CD4%	Pooled	Equal	16	3.33	0.0043
CD4%	Satterthwaite	Unequal	12.4	3.21	0.0073
CD8%	Pooled	Equal	16	-0.95	0.3546
CD8%	Satterthwaite	Unequal	10.6	-0.90	0.3889
CD4COUNTS	Pooled	Equal	16	3.67	0.0021
CD4COUNTS	Satterthwaite	Unequal	16	3.76	0.0017
CD8COUNTS	Pooled	Equal	16	0.36	0.7270
CD8COUNTS	Satterthwaite	Unequal	9.35	0.33	0.7495
RCD4CD8	Pooled	Equal	16	2.27	0.0370
RCD4CD8	Satterthwaite	Unequal	15.8	2.31	0.0349
CRPng2	Pooled	Equal	12	-0.61	0.5563
CRPng2	Satterthwaite	Unequal	7.34	-0.61	0.5633

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
CD3%	Folded F	7	9	1.47	0.5747
CD4%	Folded F	7	9	1.92	0.3583
CD8%	Folded F	7	9	3.01	0.1271
CD4COUNTS	Folded F	9	7	1.51	0.5978
CD8COUNTS	Folded F	7	9	4.76	0.0341
RCD4CD8	Folded F	9	7	1.28	0.7617
CRPng2	Folded F	6	6	8.87	0.0178

COMPARISON OF NEGCONTROL AT TIME 3 AND 4 FOR THE VARIABLES CD3 CD4 CD8 CD4COUNTS
CD8COUNTS CRPNG RCD4CD8

T-Tests

Variable	Method	Variances	DF	t Value	Pr > |t|
CD3%	Pooled	Equal	7	-1.96	0.0912
CD3%	Satterthwaite	Unequal	6.11	-2.11	0.0785
CD4%	Pooled	Equal	7	-3.59	0.0088
CD4%	Satterthwaite	Unequal	7	-3.70	0.0077
CD8%	Pooled	Equal	7	1.25	0.2516
CD8%	Satterthwaite	Unequal	5.8	1.36	0.2249
CD4COUNTS	Pooled	Equal	7	-3.11	0.0171
CD4COUNTS	Satterthwaite	Unequal	6.99	-3.22	0.0147
CD8COUNTS	Pooled	Equal	7	0.31	0.7672
CD8COUNTS	Satterthwaite	Unequal	5.69	0.34	0.7494
RCD4CD8	Pooled	Equal	7	-1.97	0.0893
RCD4CD8	Satterthwaite	Unequal	6.98	-2.02	0.0830
CRPng2	Pooled	Equal	4	0.27	0.8006
CRPng2	Satterthwaite	Unequal	3.52	0.27	0.8023

Equality of Variances

Variable	Method	Num DF	Den DF	F Value	Pr > F
CD3%	Folded F	4	3	3.97	0.2862
CD4%	Folded F	4	3	1.61	0.7258
CD8%	Folded F	4	3	4.88	0.2238
CD4COUNTS	Folded F	4	3	1.78	0.6651

```
CD8COUNTS    Folded F      4      3      5.30    0.2019
RCD4CD8      Folded F      4      3      1.50    0.7705
CRPng2       Folded F      2      2      2.16    0.6329
```

Where RCD4CD8- Ratio of CD4/CD8; CRPng2- levels of CRP