COMPARATIVE STUDY OF THE ANTIOXIDANT ACTIVITIES OF LEAFY VEGETABLES (*SOLANUM NIGRUM, AMARANTHUS DUBIUS* and *CUCURBITA MAXIMA),* CONSUMED IN BUSHENYI DISTRICT, UGANDA

BY

KINYI HELLEN WAMBUI DCM, BSC. BIOCHEMISTRY

MSC. BCH/0001/102/DF

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN BIOCHEMISTRY OF KAMPALA INTERNATIONAL UNIVERISTY, UGANDA

DECEMBER 2013

DECLARATION

I, Kinyi Hellen Wambui, declare that this is my original work and has not been presented for any academic award in any other Institution.

Sign: ..... Date: 12/12/13.....

Kinyi Hellen Wambui DCM, B.Sc. (Honors)

CERTIFICATION

This is to certify that this thesis titled "Comparative Study of the antioxidant activities of leafy vegetables (*Solanum nigrum, Amaranthus dubius* and *Cucurbita maxima*), consumed in Bushenyi District, Uganda", submitted to the Directorate of Postgraduate and Research Kampala International University Western Campus, for the award of Master of Science in Biochemistry, is the original work done by Kinyi Hellen Wambui under our supervision.

Supervisor 1: Professor Wilson Byarugaba B.Sc. (UEA) MSc. Molecular Biology D.Sc (KÖln Germany), F.UNAS

Sign:..... Date: 07/12/2013

Supervisor 2: Professor O. A. Daini BSc. MSc. Biochemistry (Ife) PhD (Lagos)

Sign:..... Date: 10/12/13

DEDICATION

I dedicate this work to my parents Mr. Silvanus Kinyi Kihangu and Mrs. Jennifer Wanjiku Kinyi.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND ACRONYMS

ADP	Adenosine diphosphate
ALVs	African Leafy Vegetables
AOAC	Association of Analytical Chemists
ATP	Adenosine triphosphate
CO	Carbon monoxide
DNA	Deoxyribonucleic acid
DPPH	2, 2, Diphenyl-1-picrylhydrazyl
EDTA	Ethylene-diamine tetracetic acid
ET	Electron Transfer
FAO	Food and Agriculture Organization
HAT	Hydrogen Atom Transfer
HIV/AIDS	Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome
kcal	kilocalories
KIU-WC	Kampala International University Western Campus
MOH	Ministry of Health
PUFA	Polyunsaturated fatty acids
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
WHO	World Health Organization

LIST OF PLATES

LIST OF FIGURES

OPERATIONAL DEFINITIONS

African Leafy Vegetables: These were introduced in Africa over a hundred years ago and due to long use, have become part of the food culture in the continent.

Antioxidants: These are chemical substances that counteract free radicals or their actions. They minimize radical-caused damage by reducing the energy of the free radical, prevent the free radical from forming or interrupt the oxidation chain. They protect the cells against the effects of free radicals.

Free radical: This is an unstable atom or a group of atoms capable of independent existence and contains one or more unpaired electrons. Once formed these free radicals start oxidizing chain reactions that generate other radicals through either donating their unpaired electron to another chemical species or taking an electron from it.

Vegetable: A plant or part of a plant used as food, typically as an accompaniment to meat or fish, such as cabbage, potato, carrot or bean.

ACKNOWLEDGEMENT

I would first like to thank the NGO Sayansi for providing a research grant, which enabled the procurement of all the reagents and other material required for the study.

My Supervisors Prof. Wilson Byarugaba and Prof. O.A. Daini have been an overwhelming blessing. They have mentored, shaped and worked with me tirelessly to make this work what it is today. I am really grateful.

I am also thankful for my mentors Dr. Marta Vicente and Dr. Mohamed Balogun, who have constantly encouraged, pushed and sometimes bullied me to push harder and be the best I can be.

I acknowledge my family back home in Kenya, my Dad for constantly pushing me to finish up the Masters and my mum, for all her prayers and encouragement.

I am grateful to member of the University fraternity, whom I consulted once or twice to discuss different methods and helped me overcome some challenges I met.

Finally, I thank my classmates and friends whose encouragement helped push me to the end of this work.

ABSTRACT

This study explored the antioxidant potential of three vegetables, commonly consumed in Bushenyi District of Western Uganda. The vitamin C, reducing power, hydrogen peroxide scavenging activity and antihemolytic activity of the raw, steamed and boiled *Amaranthus dubius*, *Solanum nigrum* and *Cucurbita maxima* were investigated using *in vitro* methods. Spectrometry was used to evaluate the reducing power and antihemolytic activity, while titration using the 2, 6- indophenol method was used for vitamin C and replacement titration for hydrogen peroxide scavenging activity .The results from this study showed that vitamin C content differed among cultivars of the same vegetable. The vitamin C content of the raw vegetables ranged from 0.93mg/100g to 5.55mg/100g with the ranking *A.dubius*>*C.maxima*>*S.nigrum*. Cooking the vegetables by steaming or boiling caused 88- 99% reduction in the vitamin C content of the cooked vegetables. There was a significant increase in the vitamin C content of the water used for boiling. Cooking caused variable effects in the antioxidant activity of the three vegetables. There was decreased activity in the reducing and scavenging activity of *A.dubius* as the scavenging activity of *C.maxima* and *S.nigrum* increased on steaming. The Antihemolytic activity of *A.dubius* and *S.nigrum* increased on steaming while that of *C.maxima* was variable among the two cultivars. The results from this study indicate that the vegetables have the potential to offer antioxidant activity which, however, can be affected by various cooking methods. *A.dubius* is the vegetable with the highest antioxidant activity and steaming is recommended as the preferred method of cooking.

CHAPTER ONE

INTRODUCTION

1.1 Background

1.1.1 Historical background

Non-communicable diseases, including cardiovascular diseases, mental illnesses, trauma, cancer, and diabetes, are now major sources of morbidity and mortality in Sub-Saharan Africa, and are projected to overtake infectious diseases by 2030 (Mathers and Loncar 2006). For instance, the Uganda Heart Institute records demonstrated a 500% increase in outpatient attendance due to heart related conditions over 7 years (2002-2009; MOH 2012) and the Uganda Cancer Institute also reported an upward trend in cancer incidence over four years (2005-2009), particularly HIV infection related cancers (MOH, 2012). A study by Wasswa in 2006, reported that the number of people with diabetes was thought to have passed a million, in a population of 28 million. He reported that the incidence of diabetes was particularly high among the cattle-keeping peoples of the southwestern part of the country due to the changing lifestyles: people in the area are generally overweight, as they do not exercise. They also take a lot of milk, ghee, and animal products. The problem is particularly acute among women. This connotes that non-communicable diseases in Uganda threaten to overwhelm health systems; since about 2 to 7% of total healthcare costs are attributable to obesity. The problem is such that Businge Conan wrote on 6th October 2010 New Vision in his article: "Uganda: how diabetes will wreck the Economy", in which he opines that if left unchecked, non-communicable diseases like diabetes are set to cripple Uganda and the rest of Africa's overstretched healthcare services.

1.1.2 Theoretical background

Fortunately, the consumption of vegetables has been linked to reduction in the incidence of oxidative-stress related diseases such as cancer, diabetes, and neurodegenerative diseases, including Parkinson's and Alzheimer's diseases as well as inflammation and problems caused by cell and cutaneous aging (Ame *et al.*, 1993; Gerber *et al.*, 2002; Di

Matteo and Esposito, 2003). The protective action of fruits and vegetables has been attributed to the presence of antioxidant vitamins such as vitamin C, α-tocopherol and β-carotene, as well as compounds such as flavonoids, isoflavones, flavones, anthocyanins, catechin and isocatechin, (Kahkonen *et al.*, 1999; Prior and Cao, 2000). Consumption of food and beverages rich in phenolic content has been shown to reduce the risk of heart disease by slowing the progression of atherosclerosis by acting as antioxidants towards low-density lipoprotein (Bayili *et al.*, 2011).

1.1.3 Conceptual background

Antioxidants are substances that protect the cells against the effects of free radicals. The human body has endogenous antioxidants such as bilirubin, uric acid and protein thiols. Harmful effects resulting from the disequilibrium in the antioxidant-pro-oxidant balance can be largely prevented by the intake of antioxidant substances (Rattanachitthawat *et al.*, 2010). A great number of plant secondary metabolites that have antioxidant activity have been isolated (Shahvar *et al.*, 2010). These include vitamins C and E, as well as a number of food-derived poly-aromatic substances, belonging to stilbenes, flavonoids and phenolic acids as the main classes of nutritional antioxidants (Wayner *et al.*, 1987).

On the other hand, a free radical is an unstable form of any atom or group of atoms capable of independent existence that contains one or more unpaired electrons (Halliwell, 2006). Free radicals can be produced in animals and humans following normal metabolic reactions and pathological conditions (Fang *et al.*, 2002). They could be produced in different ways such as, breaking of covalent bonds by homoelitic cleavage, losing an electron or gaining an electron (Halliwell, 2006). Once formed these highly reactive chemical species can start a chain reaction, like dominoes. Their main danger comes from the damage they can cause when they react with important cellular components such as DNA, or the cell membrane (Pala and Tabakcioglu, 2007). Environmental sources of free radicals include: atomic hydrogen, many heavy-transition- metals (such as iron, copper, zinc and manganese), chlorine, many drugs, ionizing radiation and environmental wastes such as CO, asbestos, ozone and solvents (Pala and Tabakcioglu, 2007).

1.1.4 Contextual background

There are more than 45,000 species of plants in Sub-Saharan Africa of which 1000 can be eaten as green leafy vegetables, which happen to be the main stay of African diets (MacCalla, 1994). Green leafy vegetables are important components of African diets where they serve as a major ingredient of soups, or sauces that accompany carbohydrate staples (Chweya and Eyzaguire, 1999). These leafy vegetables are known to contain high levels of vitamin A, vitamin C, iron, calcium, protein and are a valuable source of nutrition in rural areas where they contribute substantially to protein, mineral and vitamin intake (Abukutsa-Onyango, 2003). Some are known to be rich in lysine, an essential amino acid that is lacking in diets based on cereal and fibres, while others are medicinal. The green, leafy vegetables contain polyphenols which have beneficial physiological effects on humans as antioxidants. They are also known to be anticarcinogenic and anti-arteriosclerotic (Imungi, 2002). A study carried out in Nairobi showed that consumption of African leafy vegetables is associated with the treatment of various diseases including therapy for patients with HIV/AIDS, diabetes, high blood pressure and other common ailments (Kimiywe, 2006).

In a case study of South Western Uganda, to determine the utilization of indigenous food plants Kiremere *et al.*, (2006) reported that among the leafy vegetables, *Amaranthus dubius* (*doodo*), *Solanum nigrum* (*eshwiiga*), pumpkin leaves (*ebishusha*) and *Amaranthus graecizans* (*enyabutongo*) are used by over 80 % of the respondents as food, in form of snacks, as a relish (sauce) and for medicinal purposes.

1.2 Statement of the Problem

Epidemiological data as well as *invitro* studies strongly suggest that foods containing phytochemicals with anti-oxidation potential have strong protective effects against major disease risks including cancer and cardiovascular diseases (Sun *et al.*, 2002). Exotic vegetables such as white cabbage, cauliflower, garlic, broccoli, kale, spinach, onion, eggplant, and cucumber have been shown to be rich sources of anti-oxidants (Gazzani *et al.*, 1998). In spite of a wide body of evidence confirming the nutritional contribution of African leafy vegetables to local diets, their health maintenance and protective properties

(Abukutsa-Onyango, 2003), there are no studies in Uganda to explore the antioxidant potential of these vegetables.

In addition, these vegetables are prepared and cooked in several ways. A survey conducted in Nairobi by Kimiye *et al.*, (2006) established that chopping before washing, repeated boiling and addition of sodium bicarbonate were the methods most commonly used to process vegetables. In Uganda Goode (1989), states that, 'traditional green leaves are steamed with the staple food, boiled and served on their own or either added to sauce just before serving". Kiremire *et al.*, (2006) also described steaming, mashing and boiling as methods used to process the vegetables in the rural setting but their study revealed an increase in the frying of vegetables. These cooking methods may either increase or reduce the bioavailability of bioactive compounds as well as the total antioxidant activity of the vegetables. A reduction of this activity of the vegetables will result to the loss of the protective activity against non-communicable diseases. It is therefore important to determine the potential of locally produced vegetables as a source of antioxidants.

1.3 Purpose of the Study

To compare the antioxidant activities of three locally consumed vegetables in Bushenyi District, Uganda

1.4 Research Objectives

1. To determine the amount of vitamin C in fresh, boiled and steamed extracts of *Amaranthus dubius*, *Solanum nigrum* and *Cucurbita maxima.*
2. To determine antioxidant activity in fresh, boiled and steamed extracts of *Amaranthus dubius*, *Solanum nigrum* and *Cucurbita maxima.*
3. To determine the anti-hemolytic activity of the raw, boiled and steamed extracts of *Amaranthus dubius*, *Solanum nigrum* and *Cucurbita maxima* on red blood cells of male wistar albino rats.

1.5 Research Questions

This study sought to answer the following research questions:

1. How much vitamin C is present in fresh, boiled and steamed extracts of *Amaranthus dubius*, *Solanum nigrum* and *Cucurbita maxima?*
2. How much antioxidant activity is present in fresh, boiled and steamed extracts of *Amaranthus dubius*, *Solanum nigrum* and *Cucurbita maxima?*
3. Do the raw, boiled and steamed extracts of *Amaranthus dubius*, *Solanum nigrum* and *Cucurbita maxima* offer a protective effect on erythrocytes against hemolysis?

1.6 Scope of the Study

1.6.1 Content scope

Although there are many vegetables consumed in Western Uganda, only three (*Amaranthus dubius*, *Cucurbita maxima* and *Solanum nigrum*) were chosen based on previous work (Kiremire *et al.*, 2006) that showed that these vegetables are the most commonly consumed in Western Uganda.

Several methods are used in processing the leaves post harvest before consumption. This study explored the effects of two processing methods boiling and steaming. This was based previous work, which describes boiling and steaming as the most common methods of food preparation in Western Uganda (Goode 1989).

This study determined the amount of vitamin C, as a substance with antioxidant activity. The biological antioxidant activity was demonstrated in an *in vitro* study to explore the protective effects of extracts from the three plant species against hemolytic damage on male Wistar rats' erythrocytes induced by hydrogen peroxide. Vitamin C was used as the standard against which the various antioxidant activities of the plants were compared.

1.6.2 Geographical scope

The study was limited to three vegetables consumed in Western Uganda. The vegetables were procured from Ishaka Municipal and Bushenyi Municipal markets in Bushenyi District. Pumpkin leaves were harvested from vegetable gardens in Ishaka and Bushenyi municipalities of Bushenyi District.

1.6.3 Time scope

The vegetables were purchased during the short rains in the month of October 2012 when they are plenty in the markets.

1.7 Justification of the Study

The very concept of food is changing from a past emphasis on health maintenance to the promising use of foods to promote better health and prevent chronic illnesses. 'Functional foods' are those that provide more than simple nutrition; they supply additional physiological benefit to the consumer (Devasagayam *et al.*, 2004).The growing reliance on and consumption of low quality cheap foods that are high in energy, is associated with a decline in the consumption of the local vegetables which have the potential to offer antioxidants that are protective against non-communicable diseases. The benefits of a diet rich in antioxidants will help in the fight against communicable diseases such as malaria and HIV which depend on an environment low in antioxidants for their pathogenesis (Clark *et al.*, 1989; Kashou *et al.*, 2011), as well as non-communicable diseases such as diabetes, hypertension and neurodegenerative diseases which have been associated with oxidative stress (Gerber *et al.*, 2002). This study sheds light on the antioxidant potential of local vegetables some of which are considered poor man's food, yet have the potential to be functional foods. Processing of foods is known to modify their organoleptic, physical and chemical composition and little information is available on the nutritional characteristic of cooked leaves (Woomer and Imbuni, 2005). This study provides important information on the antioxidant activity of the studied vegetables and the effect of commonly used cooking methods. It also provides relevant data on the validity of food based approaches to health problems, thus helping to find African solution to the potential epidemic of non-communicable diseases in Africa.

1.6 Conceptual Framework

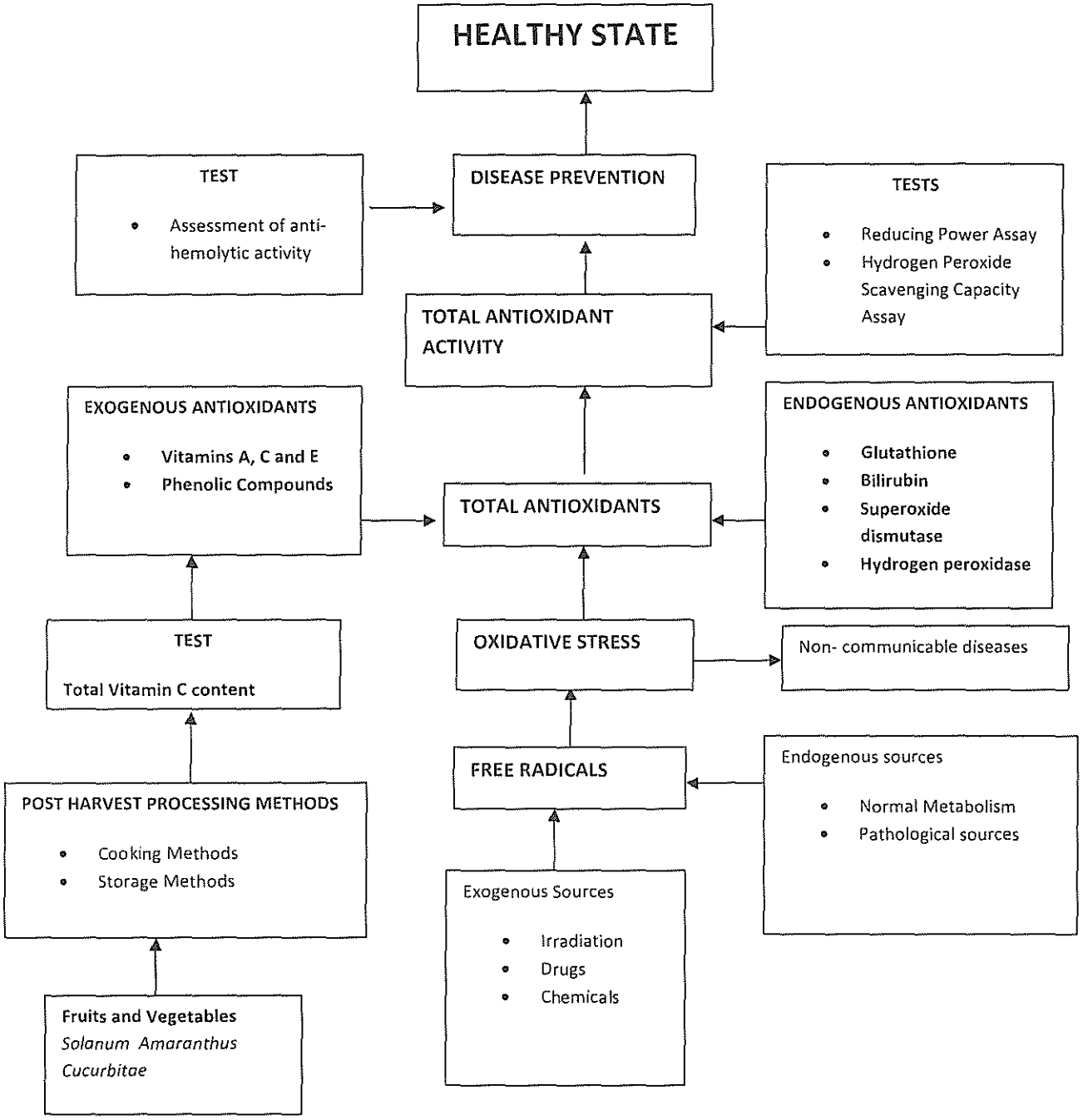


Figure 1: Conceptual framework

The concept of this study is portrayed in a sketch diagram in figure 1. Scientific evidence indicates that oxidative stress arising from the generation of free radicals contributes to ailments such as cancer, cardiovascular disease, cataract formation as well as accelerating the aging process (Fang *et al.*, 2002). Free radicals can enter the body from exogenous sources such as air pollution, cigarette smoke, drugs, pesticides, and exposure to ionizing radiation, heavy metals, alcohol, and various environmental chemicals, as well as be produced in physiological and pathological conditions (Pala and Tabakcioglu, 2007). Antioxidants which protect the cells against the effects of free radicals can be obtained from endogenous sources, such as bilirubin, or exogenous antioxidants such as vitamins C and E, as well as a number of food-derived (poly) aromatic substances, belonging to stilbenes, flavonoids and phenolic acids (Wayner *et al.*, 1987). The consumption of vegetables has been linked to reduction in the incidence of oxidative-stress related diseases. Majority of the antioxidant activity in vegetables may be from compounds such as flavonoids, anthocyanins, and isocatechins rather than from Vitamins C, E and β-carotene (Kahkonen *et al.*, 1999). This study determined the levels of vitamin C in 3 plant species consumed in Western Uganda, the effect of different cooking methods on the amounts of vitamin C and measured the total antioxidant activity of the three plant species following two cooking methods.

CHAPTER TWO

LITERATURE REVIEW

2.1 Vegetables

The term vegetable refers to a plant or part of a plant used as food, typically as accompaniment to meat or fish, such as a cabbage, potato, or bean. Vegetables are usually classified into: 1) green leafy vegetables such as amaranths and lettuce which have high health value; 2) roots and tubers for example potatoes and carrots and 3) other vegetables like beans and peas, (Bakhru, 2007). There has been a growing awareness in recent years of the health promoting and protecting properties of non-nutrient bioactive components in fruits and vegetables as well as epidemiological evidence linking the lack of dietary diversity, particularly of vegetables and fruits, to the growing incidence of chronic and non-communicable diseases in both developed and developing countries (Popkin, 2002). It was therefore no surprise when the 2003, "FAO/WHO Expert Consultation on Diet, Nutrition and Prevention of Chronic Diseases", recommended the intake of a minimum of 400g of fruits and vegetables per day (excluding potatoes and other starch tubers) for the prevention of chronic diseases such as heart disease, cancer, diabetes and obesity as well as for the prevention and alleviation of several micronutrients deficiencies especially in less developed countries (WHO, 2003).

2.1.1 African leafy vegetables

Indigenous leafy vegetables are those that have their natural habitat in Sub-Saharan Africa, while traditional leafy vegetables were introduced over a hundred years ago and due to long use, have become part of the food culture in the subcontinent (Smith and Eyzaguirre, 2007). Examples of African leafy vegetables found across Eastern Africa include: African nightshade (*Solanum scabrum)*, spider plant (*Cleome gynandra*), vegetable amaranth (*Amaranthus hybridus*), slenderleaf (*Crotalaria brevidens*), jutemallow (*Corchorus olitorius*), vegetable cowpea (*Vigna unguiculata*), pumpkin leaves (*Curcubita moshata*) and African kale (*Brassica carinata*) among many others (Abukutsa-Onyango, 2003).

2.1.2 Nutrient content of vegetables

Green leafy vegetables are known to be low in calories and fat, high in protein per calorie, dietary fiber and iron and calcium, and very high in micronutrients such as vitamin C, carotenoids, folate, magnesium as well as vitamin K. A good example is kale (*Brassica oleracea*) which was found to be a good source of vegetable protein (11.67%) and fiber (3.0%). Other proximate parameters included; moisture (81.38%), ash (1.33%), fat (0.26%), carbohydrate (2.36%) and energy (58.46 kcal/100 g). Observed minerals contained in the plant were: sodium (4.69 mg/100 g), potassium (7.03 mg/100 g), calcium (4.05 mg/100 g), iron (8.94 mg/100 g), zinc (2.16 mg/100 g) and magnesium (6.69 mg/100 g) (Embu and Anyika, 2011).

2.1.3 Phytochemical content of vegetables

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. There are over 10,000 of them, and they have effects such as antioxidant, boosting the immune system, anti-inflammatory, antiviral, antibacterial, and cellular repair. Reports show that the greatest sources of these phytochemicals are fruits and vegetables (Liu, 2004). These phytochemicals include polyphenols and tannins, flavonoids, anthracenosides, coumarins, steroids and triterpenes, iridoïds, cardenolids, carotenoids, saponins and alkaloids (Nana *et al.*, 2012).

2.1.4 Post harvest processing of vegetables

Processing of vegetables before consumption may have varying effects on the various nutritional components present. Preparatory methods such as sun drying, blanching and cooking reduce the moisture content of vegetables, cause different variations in the dry matter and result in significant losses in vitamin content especially vitamin C (Mepha *et al,* 2007). Phytochemicals are not spared either and they may also be reduced by cooking, sun and oven drying, steaming and shredding (Akubugwo *et al.*, 2008).

2.2 *Amaranthus* Species

Amaranths, which comprise the genus *Amaranthus*, are widely distributed, short-lived herbs, occurring in temperate and tropical regions. There are about 60 *Amaranthus* species, several of which are cultivated as leaf vegetables, grains or ornamental plants,

while others are weeds. The main species grown as vegetables are *A. tricolor, A. dubius, A. lividus, A. creuntus, A. palmeri* and *A. hybridus* while *A. hypochondriacus, A. cruentus* and *A. caudatus* are the main grain species (Teutonico and Knorr, 1985). *Amaranthus* species is one of the few plants whose leaves are eaten as a vegetable while the seeds are used in the same way as cereals; there is no distinct separation between the vegetable and grain types since the leaves of young plants grown for grain can be eaten as both human and animal food (Muyonga *et al.*, 2008).

Amaranthus dubius is a weedy plant widespread throughout the humid lowland tropics. It originates from tropical America, where it is common in the Caribbean region and from Southern Mexico to South America. It is a protected weed used as a pot herb in many African countries, and it possibly occurs in all African lowland areas. It is a cultivated vegetable in West Africa (Sierra Leone, Ghana, Benin, Nigeria), Central Africa (Cameroon, Democratic Republic of Congo), and East Africa (Kenya, Uganda) (Grubben 2004). *Amaranthus dubius* is widely referred to as "*Doodo*" by most communities in Uganda (Goode, 1989). Kiremire *et al.*, (2006) report the use of *Amaranthus* for income generation in communities in Western Uganda.

2.2.1 Morphological features

Amaranthus dubius is an erect annual herb, up to 150 cm tall. The stems are slender to stout, branched, glabrous or upwards, especially in the inflorescence, with short to rather long hairs. The leaves are arranged spirally, simple, without stipules; the petiole up to 8.5 to 12 cm long, with the lamina ovate or rhomboid-ovate, cuneate at the base, blunt or retuse at apex. The flowers are unisexual, subsessile, with 4 to 5 petals up to 2.5 mm long. The male flowers are usually near apex of inflorescences, with 5 stamens. The female flowers have a one-celled ovary crowned by three stigmas. The fruit is an ovoid-urceolate capsule, with a short inflated beak below the stigmas, dehiscing circularly, the lid strongly rugulose below the beak and 1-seeded (Grubben, 2004).

2.2.2 Nutritional uses

The main use of *Amaranthus dubius* is as a cooked leaf vegetable which is dark green, tender and tastes somewhat neutral. In Kenya and Uganda it is cooked with bitter leaf

vegetables such as nightshades (*Solanum* spp.), *Cleome gynandra* or *Launaea cornuta* to make it more palatable (Goode, 1989). The leaves easily become soft after 5–10 minutes cooking in lightly salted water. The composition of *Amaranthus dubius* leaves is comparable to *Amaranthus cruentus* and other amaranth leaves and is displayed in the table 1.

The leaves and stems contain nitrate, mostly in the stems, and oxalate at a level comparable to other green leafy vegetables. Most people cook *Amaranth* in ample water and discard the cooking water containing soluble nitrate and oxalate. The presence of a rather high content of hydrocyanic acid and oxalic acid makes *Amaranth* less suitable for fresh consumption by humans and as fodder for animals (Grubben, 2004).

Component	Composition/100g
Water	84.0g
Energy	176kJ (42kcal)
Protein	4.6g
Fat	0.2g
Carbohydrates	8.3g
Fiber	1.8g
Calcium	410g
Phosphorus	103mg
Iron	8.9mg
β-carotene	5716 µg
thiamin	0.05 mg
riboflavin	0.42 mg
niacin	1.2 mg
ascorbic acid	64 mg

Table 1: Average composition of Amaranth leaves (Leung *et al.*, 1968)

2.2.3 Other uses

Amaranthus dubius is a subsistence vegetable and a collected pot herb, often found in markets, and in Kenya it is grown on a commercial scale and sold in city markets. *Amaranth* leaves in general are recommended as a good food with medicinal properties for young children, lactating mothers and for patients with fever, hemorrhage, anemia, constipation or kidney complaints. In Tanzania the whole plant is used as a medicine against stomachache (Grubben, 2004).

2.3 *Cucurbita maxima* (Pumpkin leaves)

Pumpkin refers to certain varieties of *Cucurbita moschata*, *Cucurbita maxima*, and *Cucurbita mixta* which provide pumpkins and butternuts (*Cucurbita moschata*), squashes (*C. maxima*), gourds (*C. argyrosperma*), and zucchini or courgettes and ornamental gourds (*C. pepo*). Pumpkin of the species *Cucurbita maxima* also called squash guard, is known to have originated from South America, possibly Peru and is now widely distributed throughout the tropics (Tindall, 1983).

Pumpkins are very versatile in their uses for cooking. Most parts of the pumpkin are edible, including the fleshy fruit shell, the seeds, the leaves and even the flowers. Pumpkin leaves (*Cucurbita moshata*) are a popular vegetable in Western and Central regions of Kenya; where they are called "*seveve*" and are an ingredient in '*mukimo*', a mixture of mashed peas, maize and potatoes (Ngishili, 2009). In Uganda consumption of pumpkin leaves (*Cucurbita maxima*) occurs in different communities and regions as evidenced by the wide diversity of names; "*Ensujju*" among the Baganda (Central region), "*Kimisebebe*" or "*Mawondo*" in the Eastern region, "*Imunyuru*" among the Teso, "*Osusa*" in the West Nile region and "*Ebisunsa*" or "*Ebisusha*" in the West and South Western Uganda (Goode, 1989).

2.3.1 Morphological features

Pumpkins are angiosperms belonging to the *Cucurbitaceae* family that is characterized by prostrate or climbing herbaceous vines with tendrils. The root system is strongly advanced, the main taproot reaching 1 m length; 10-12 horizontal lateral roots go from it on the depth of 20-40 cm and may reach 4-5 m (Kirtikar and Basu, 2003). Leaves have long-leafstalk, alternate, smooth-margin or sinuate, pubescence in a various degree. They

have short tendrils mostly branched. Flowers are large, yellow or orange color, more often unisex, single; but bisexual flowers can be also present. *Cucurbita maxima* have large, fleshy fruits containing numerous seeds (Acquaah, 2004). Its fruits are large, variable in shape, round or oblong, covered with small raised spots weighing 2 – 5kg. The rind may be soft or hard, sometimes brightly colored. The flesh is yellow and the seeds are white or brown. Seeds are up to 2 cm in length, white, cream or yellow in color, oval. Weight of 1000 seeds is about 400 g. Seeds keep their germinating capacity during 6-8 years (Tindall, 1983).

2.3.2 Nutritional uses

The fruits, leaves and flowers of these cucurbits are used as vegetables, their seeds consumed roasted as a snack food (Fedha *et al.*, 2010) and the stem used as livestock feed. The younger leaves are collected and the outer tough skin of petioles (stalk of leaf) removed (together with the large leaf veins) then washed, chopped and boiled. They are used fresh and in some locations dried for use during the off-season.

The composition of pumpkin leaves and seeds are shown in Table 2 below. Pumpkin seeds also contain considerable amounts of vitamin E. Pumpkin fruit contains 1% protein and 8% carbohydrates, and the dried seeds contain 23% protein, 21% carbohydrates and up to 50% oil, but little information is available about the nutritional characteristics of cooked leaves (Woomer and Imbumi, 2005).

Component	Leaves/100g	Seeds/100g
Water	89.2g	5.5g
Energy	113kJ (27kcal)	2331kJ (555kcal)
Protein	4.0g	23.4g
Fat	0.2g	46.2g
Carbohydrates	4.4g	21.5g
Fiber	2.4g	2.2g
Calcium	477mg	57mg
Phosphorus	136mg	900mg
Iron	0.8mg	2.8mg
β-carotene	3600 µg	
thiamin	0.06mg	0.15 mg
riboflavin	0.32mg	0.42 mg
niacin	N/A	1.4 mg
ascorbic acid	80mg	64 mg

N/A-Not available

Table 2: Average composition of pumpkin leaves and seeds (Leung *et al.*, 1968)

2.4 *Solanum nigrum* (Eshwiga)

Solanum nigrum commonly known as black nightshade is a dicot weed in the *Solanaceae* family. It has over 1000 species worldwide with at least 100 indigenous species in Africa and adjacent islands which include a number of valuable crop plants and some poisonous ones (Jaeger and Hepper, 1986). This family also comprises many genera, well known for their therapeutic properties such as *Atropa belladonna* L. (deadly nightshade), *Datura stramonium* L. (Jimson weed), and *Hyoscyamus niger* L. (black henbane). The Solanaceae, to which the genus *Solanum* L. belongs, is a cosmopolitan family containing many essential vegetables and fruits such as potatoes, tomatoes, chillies, green and red peppers as well as ornamentals such as *Petunia, Schizanthus* and *Lycium* species.

In most parts of the world, the species *Solanum* are considered weeds and a problem for agriculture. This is not the case in Africa where they form one of the largest groups of leafy vegetables (Chweya and Eyzaguirre, 1999). Leaves and tender shoots are boiled or stewed and used as relish. The leaves of *Solanum nigrum* are widely consumed in Uganda where they are named differently by communities: "*Ensugga*" by the Baganda, "*Isufa*" by the Bagisu, "*Enmyorotin*" by the Sebei, "*Ocokock/Ocuga*" by the Lango, "*Enswiga*" by the Batoro and "*Eshwiga*" by the Banyankole (Goode, 1989). The vegetable is used in both urban and rural areas, but since it is often bitter it is mixed with *Amaranthus* spp., *Corchorus* spp. or with other green leafy vegetables

2.4.1 Taxonomy

Within *Solanaceae* family, *Solanum* constitutes the largest, variable and most complex genus. It consists of annual and perennial plants, forbs, vines, sub-shrubs, shrubs, and small trees. The family is widely distributed throughout tropical and temperate regions of the world, with centers of diversity occurring in Central and South America and Australia (D'Arcy 1991). The generic name *Solanum* is generally considered to be derived from the latin *solamen*, which refers to the quieting or sedative effects associated with many of the species (Edmonds and Chweya, 1997). *Solanum* is a taxonomically complex species due to the phenotypic plasticity of the species, genetic variation existing between populations of the same species, their ploidy levels ranging from diploid to hexaploid and their naturally occurring inter-specific hydridization (Manoko, 2007). The *Solanum nigrum* complex also known as *Solanum* L. section *Solanum* - is group of *Solanum* species with

general black nightshade characteristics. Some of the major species within the *Solanum nigrum* complex are: *Solanum nigrum*, *S. americanum*, *S. douglasii*, *S. opacum*, *S. ptychanthum*, *S.retroflexum*, *S. sarrachoides*, *S. scabrum*, and *S. villosum* (Edmonds and Chweya, 1997).

2.4.2 Morphological features

Solanum nigrum is an annual branched herb of up to 90 cm high, with dull dark green leaves, juicy, ovate or lanceolate, and toothless to slightly toothed on the margins. Flowers are small and white with a short pedicellate and five widely spread petals. Fruits are small, black when ripe (Cooper and Johnson, 1984). It is a popular plant in part due to its toxic content of solanine, a glycoalkaloid found in most parts of the plant, with the highest concentrations in the unripened berries. However, when ripe, the berries are the least toxic part of the plant and are sometimes eaten without ill effects. Similarly, the solanine increases in the leaves as the plant matures (Cooper and Johnson, 1984).

2.4.3. Nutritional uses

Leaves and tender shoots are widely boiled or stewed throughout the world and have provided a food source since early times, with *S. nigrum* being recorded as an ancient famine plant of the Chinese (Henderson 1974). In Kenya, boiled leaves of these *Solanums* are apparently recommended for pregnant women, since their consumption is believed to result in the birth of children with dark eyes and smooth skin and easy recuperation after delivery. It is also believed that children eating the vegetable do not get 'marasmus' or 'kwashiokor', especially if the vegetable is cooked with milk, groundnuts *(Arachis hypogaea* L.) or simsim *(Sesamum indicum* L.), (Opole *et al., 1991*). Five species of the larger *Solanum nigrum* complex are considered edible in Uganda (*S. amaericanum, S. scabrum, S. villosum, S. tardermotum Bitter and S. florulentum Bitte*r). Young leaves are picked, steamed and eaten like amaranth and spinach. Most people collect the leaves from wild or weedy plant; only in South-Western Uganda is *S. scabrum* cultivated as a commercial crop (Olet *et al.*, 2005).

The leaves can provide appreciable amounts of protein and amino acids, minerals including calcium, iron and phosphorus, vitamins A and C, fat and fiber, as well as appreciable amounts of methionine, an amino acid scarce in other vegetables (Fortuin and

Omta 1980). The nutrient values may, however, vary with soil fertility, plant age and type (variant or species) (Chweya 1985).

2.4.4 Medicinal uses

Although it is considered a rich source of one of the most popular plant poisons, *Solanum nigrum* has proven to be a reservoir of phytochemicals with pharmacological prospects (Lee and Lim, 2006). It has a long history of medicinal usage and has been used as a traditional folk medicine for treating various ailments such as pain, inflammation, fever and liver disorders. *Solanum nigrum* fruits in particular are an excellent remedy for liver disorders (Arulmozhi *et al.*, 2010). In East Africa the raw fruit, is chewed and swallowed for treatment of stomach ulcers or for general abdominal upsets which lead to continued stomach-ache while infusions of leaves and seeds are rubbed onto the gums of children who have developed false teeth. Pounded leaves are soaked in water, fermented and used for the treatment of boils, ulcers and swollen glands, unripe berries used to treat ring worms and various parts of the plant are believed to cure malaria, black fever, dysentery and urinary tract infection (Kokwaro 1976).

2.5 Free Radicals

Free radicals can be defined as group of atoms or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals (Halliwell & Gutteridge, 1999) and are very short lived, with half-lives in milli-, micro- or nanoseconds (Devasagayam *et al.*, 2004). In popular scientific/biomedical literature the term 'free radical' is used in a broad sense to include related reactive species such as 'excited states' that lead to free radical generation or those species that result from free radical reactions. Once free radicals are formed they can react with another radical or with another non radical molecule in various interactions. A feature that is clear is that a radical generates another radical through either donating its unpaired electron to another molecule or taking an electron from it, leading to a chain reaction (Nonhebel *et al.*, 1979). In the ensuing chain reaction, the cascading numbers of free radicals may overwhelm the body's defenses and inflict lethal damage.

Free radicals continually enter the body from exogenous sources such as air pollution (ozone, nitrous oxide), cigarette smoke (active or passive), drugs, pesticides, contaminated or rancid foods, unsaturated fats, exposure to ionizing radiation (ultraviolet light, x-rays, cosmic rays), heavy metals (mercury, cadmium, lead), alcohol, and various environmental chemicals (Pala and Tabakcioglu, 2007). Free radicals are also produced in humans and animals under physiological and pathological conditions (Fang *et al.*, 2002). They are continuously produced as intermediate products in various enzymatic pathways in the cell some of which leak from the active sites of enzymes and react with molecular oxygen. Enzymes such as xanthine oxidase, aldehyde oxidase, dihydroorotate dehydrogenase, flavoprotein dehydrogenase, aminoacid oxidase and tryptophan dioxygenase play a role in production of free radicals. Other sources include peroxisomes which are an important source of hydrogen peroxide in the cell and arachidonic acid and prostaglandin metabolism (Valko *et al.*, 2006).

2.5.1 Types of free radicals

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are the major components of the "free radical" system. ROS such as superoxide (O_2) and hydroxyl (OH) are derived from molecular oxygen during the normal oxygen metabolism. Molecular oxygen has two unpaired electrons that spin in the parallel direction and react with other radicals easily. It could be said, diradical molecular oxygen is the first step of the ROS formation and hydrogen peroxide (H_2O_2) that is derived from molecular oxygen, is not a free radical but it is the main source of other radicals (Halliwell 2006; Valko *et al.*, 2007). At the same time, ROS cause different free radicals such as carbon-centered organic radicals (R), peroxide radicals (ROO), alcoxy radicals (RO), thyil radicals (RS), thyil peroxide (RSO_2) and sulphenyl radicals (RSO) (Valko *et al.*, 2006).

The other major element of free radical system is reactive nitrogen species. Nitric oxide is a very important radical which acts as signaling molecule in different physiological and patho-physiological processes (Valko *et al.* 2007). Nitric oxide (NO) is synthesized by mammalian cells from L-arginine through a complex oxidation reaction catalyzed by the flavo-hemoprotein NO synthase. NO is also produced through reduction of nitrites by nitrite reductase whose activity in mammalian tissues has been linked to the

mitochondrial electron transport system, protonation, deoxyhemoglobin, and xanthine oxidase (Valko *et al.* 2006).

All transition metals, except copper, contain one electron in their outermost orbital and can be considered free radicals. Iron has the ability to gain and lose electrons (i.e. Fe^{2+}/Fe^{3+}) very easily. This property makes iron and copper (II) common catalysts of oxidation reactions. Break down of red blood cells releases free iron which can be detrimental to cellular membranes because of the pro-oxidation effect it may have (Pala and Tabakcioglu, 2007). On the other hand, copper has a full outer orbital, but can lose and can gain electrons very easily to make itself a free radical as iron (Pala and Tabakcioglu, 2007).

Most of the damage induced by ionizing radiations in biological systems is indirect and is mediated by products of radiolysis of water including hydrogen radical, hydrated electron, hydrogen peroxide, peroxyl radical (ROO•)and the superoxide anion (Devasagayam and Kesavan, 1996).

2.5.2 Free radicals in health, disease and aging

Biologically active free radicals have beneficial roles such as generation of ATP from ADP in the mitochondria (oxidative phosphorylation), detoxification of xenobiotics by Cytochrome P_{450}, apoptosis of defective cells, killing of micro-organisms and cancer cells by macrophages and cytotoxic lymphocytes, generation of prostaglandins and leukotrienes, which have many regulatory functions (Devasagayam *et al.*, 2004).

On the other hand, free radicals may affect important molecules of the cell such as lipids, proteins, DNA and carbohydrates. Cellular components involving polyunsaturated fatty acid residues of phospholipids are highly sensitive to peroxidation which causes increased membrane permeability and at the end, cellular damage (Valko *et al.*, 2007). Proteins are less sensitive to free radicals than lipids though effects of free radicals on protein can be harmful because of proteins' functional importance in the body. Amino acids which have sulphur (cysteine and methionine) and unsaturated bonds can be affected by free radicals (Valko *et al.*, 2007). While the effect of free radicals on

carbohydrates involves abstracting a hydrogen atom from one of the carbons, the effect on nucleic acids involves oxygen radicals which attack DNA bases. In both instances breaks in the carbohydrate and nucleic acid chains results in the loss of function (Cooke *et al.*, 2003, Devasagayam *et al.*, 2004).

In relation to disease, in a healthy human body, the generation of free radicals is effectively kept in check by the various levels of antioxidant defense. However, when the body gets exposed to adverse physicochemical, environmental or pathological agents this delicately maintained balance is shifted in favor of free radicals resulting in 'oxidative stresses (Sies, 1996). Oxidative stress has been implicated in various pathological conditions involving cardiovascular disease, cancer, neurological disorders, diabetes, ischemia/reperfusion, other diseases and ageing (Dalle- Donne *et al.*, 2006). The process of aging is to a large extent due to the damaging consequence of free radical action (lipid peroxidation, DNA damage, protein oxidation) as well as aging pigments (lipofusin granules) that accumulate in the subsarcosomal region of the muscle fibers and become more abundant with increasing age (Finkel *et al.*, 2000. Kumar, 2011).

2.6 Antioxidants

Antioxidants are substances that react with free radicals to neutralize their actions (Sies, 1996), minimize radical-caused damage by reducing the energy of the free radical and prevent the free radical from forming, or interrupting the oxidation chain reaction itself (Greenly, 2004). They work by donating an electron to a molecule that has been compromised by oxidation, bringing it back into a state of proper function (Zulkhairi *et al.*, 2010). Antioxidants are manufactured within the body and can also be extracted from the food humans eat such as fruits, vegetables, seeds, nuts, meats and oil.

There are at least four general sources of antioxidants in biological systems: Enzymatic antioxidant defenses which include superoxide dismutase, glutathione peroxidase and catalase, non-enzymatic antioxidants represented by small molecules such as ascorbic acid (Vitamin C), α-tocopherol (Vitamin E), glutathione (GSH), uric acid and bilirubin (Valko *et al.*, 2007) and polyphenols, large molecules with antioxidant activity such as

proteins like albumin, ceruloplasmin, and ferritin as well as hormones such as estrogen, angiotensin and melatonin (Prior et al., 2005). Under normal conditions, there is a balance between both the activities and the intracellular levels of these antioxidants. This balance is essential for the survival of organisms and their health.

Foods from plant origin usually contain natural antioxidants which had previously been attributed to the presence of antioxidant vitamins such as ascorbic acid, α- tocopherol and β-carotene however; several studies have shown that the majority of the antioxidant activity may be from phenolic compounds such as flavonoids, rather than from vitamins (Cao *et al.*, 1996, Sun *et al.*, 2002). Phenolic derivatives represent the largest group of secondary plant products, synthesized by higher plants, probably as a result of antioxidative strategies adapted in evolution (Robards and Antolovich, 1997). Significant antioxidant, antitumoral, antiviral and antibiotic activities are frequently reported for plant phenols and polyphenols due to their phenolic hydroxyl groups that have the ability to scavenge free radicals (Sawa et al., 1999). Currently, regular intake of fruits and vegetables is highly recommended, as the plant phenols and polyphenols they contain are thought to play important roles in long term health as well as reduce the risk of chronic and degenerative diseases (Apak *et al.*, 2007).

Finally, synthetic antioxidants are chemicals, approved by Food and Drug Administration for addition to foods, such as BHA (Butylated hydroxyl anisole), BHT (butylated hydroxy toluene), TVHQ (tertiary butylated hydroxy quinone) (Kumar, 2011). Due to their natural origin, the antioxidants obtained from plants are of greater benefit in comparison to synthetic ones. The use of natural antioxidants from plants does not induce side effects, while synthetic antioxidants have been found to have genotoxic effects (Kahl and Kappus, 1993).

2.7 Antioxidant Activity in Vegetables: Determination and Effect of Food Preparation

The diverse sources of antioxidants in biological systems create a challenge in the determination of total antioxidant activity of a vegetable. This is further complicated by the fact that individual antioxidants may, in some cases, act by multiple mechanisms in a

single system or by a different single mechanism depending on the reaction system and thus no single assay will accurately reflect all of the radical sources or all antioxidants in a mixed or complex system (Prior *et al.*, 2005).

Antioxidant assays can roughly be classified into two types: assays based on hydrogen atom transfer (HAT) reactions and assays based on electron transfer (ET) (Prior *et al.*, 2005). The majority of HAT-based assays apply a competitive reaction scheme, in which antioxidant and substrate compete for thermally generated peroxyl radicals through the decomposition of azo compounds. These assays include inhibition of induced low-density lipoprotein autoxidation, oxygen radical absorbance capacity (ORAC), total radical trapping antioxidant parameter (TRAP), and crocin bleaching assays. ET-based assays measure the capacity of an antioxidant in the reduction of an oxidant, which changes color when reduced. The degree of color change is correlated with the sample's antioxidant concentrations. ET-based assays include the total phenols assay by Folin-Ciocalteu reagent (FCR), Trolox equivalence antioxidant capacity (TEAC), ferric ion reducing antioxidant power (FRAP), and "total antioxidant potential" assay using a copper (II) complex as an oxidant. In addition, other assays intended to measure a sample's scavenging capacity of biologically relevant oxidants such as singlet oxygen, superoxide anion, peroxynitrite, and hydroxyl radical (Wright *et al.*, 2001).

The total antioxidant content of a vegetable may be influenced by pre-harvest, harvest and post-harvest factors. This study examined the effect of two cooking methods steaming and boiling.

Cooking has been shown to bring about changes in physical characteristics and chemical composition of the vegetables (Zhang and Hamauzu, 2004). A study by Sahlin *et al.*, in 2004, showed that boiling and baking had a small effect on the ascorbic acid, and total phenolics content of tomatoes, while studies by Zhang and Hamauzu in 2004, showed that cooking affects the antioxidant components and antioxidant activity of broccoli. Reduction in the total phenolic content of squash, pumpkins and leek after cooking was found by Nihal *et al.*, in 2005, while that of pepper, broccoli and green beans was

significantly increased. They attributed the reduction to breakdown of phenols and the increase due to the level of free flavonols.

Our study had two phases, the first phase determined the quantity of vitamin C and the second phase determined total antioxidant activity using three methods: Hydrogen peroxide radical scavenging activity, ferric reducing power and the protective action against hydrogen peroxide induced hemolysis of erythrocytes.

2.7. 1 Vitamin C (Ascorbic acid)

L-Ascorbic acid is the main biologically active form of vitamin C that is reversibly oxidized to form L-dehydroascorbic acid, which also exhibits biological activity. Further oxidation generates diketogulonic acid which has no biological function (Davey *et al.*, 2000). Ascorbic acid is widely distributed in plant cells where it plays many crucial roles in growth and metabolism. As a potent antioxidant, it has the capacity to eliminate several different reactive oxygen species, keeps the membrane-bound antioxidant α-tocopherol in the reduced state, acts as a cofactor maintaining the activity of a number of enzymes (by keeping metal ions in the reduced state), appears to be the substrate for oxalate and tartrate biosynthesis and has a role in stress resistance (Hernedez, 2006).

Since humans cannot synthesize ascorbate, the main source of the vitamin is dietary fruit and vegetables. Fruits (especially citrus) are the best sources of this vitamin. An accurate and specific determination of the nutrients content of vegetables is extremely important to understand the relationship of dietary intake and human health. More than 90% of the vitamin C in human diets is supplied by fruits and vegetables (Lee and Kader, 2000). The WHO recommends a daily intake of 45 mg/day with increased requirements among smokers, pregnant and lactating women and invalids (WHO, 2003).

Several studies indicate that tropical green leafy vegetables are very rich in vitamin C. A study undertaken by Adefegha and Oboh (2011) reveals vitamin C content of raw green leafy vegetables ranges from 321.4 mg/100 g *(A. hybridus)* to 842.0 mg/100g (*O. gratissimum).* However, cooking of the vegetables causes a significant decrease in the vitamin C content. Another study conducted by Mathooko and Imungi (1994) observed that ascorbic acid content of *Solanum nigrum* decreased with both an increase in the

cooking time and in the volume of water used for cooking. This loss could reach as much as 75-89% when boiling the vegetable for as long as 20 minutes.

2.7.2 Antioxidant activity: Reducing power and H_2O_2 scavenging activity

Since different antioxidant compounds may act *in vivo* through different mechanisms, no single method can fully evaluate the total antioxidant capacity of foods. For this reason, in this study two different assays were applied to obtain robust data on antioxidant activity of the selected plants. These assays included reducing capacity assay which evaluated the reducing potential of the samples and Hydrogen peroxide radical scavenging activity assay which measured the ability of antioxidants to quench a radical cation.

The reducing power assay assesses the ability to reduce iron (III) to iron (II). The amount of Fe^{2-} complex is monitored by measuring the absorbance of the Prussian blue formed of at 700nm (Valko et al., 2006). The absorption which is reflective of the reducing power usually increases with increase in concentration of antioxidants (Ebrahimzadeh *et al.*, 2009). Compounds with reducing power are capable of donating electrons and can thus reduce the effects of free radicals such as lipid peroxidation and modification of DNA bases (Yen and Chen, 1995). Hue et al., (2011) report an increase in the absorption at 700nm with an increase in the concentration of the leaf extract of *Ipomoea batatas var.*

Hydrogen peroxide is an important reactive oxygen species that is formed by the reaction catalyzed by superoxide dismutase as part of the antioxidant cascade, xanthine oxidase and phagocytes (Carter *et al.*, 1994). It has the capacity to damage cells and macromolecules such as proteins and DNA. It also generates hydroxyl radicals via the Fenton reaction with Fe. Though hydrogen peroxide can be reduced to water via catalase activity, excessive production could cause severe circumstances hence the need for other scavengers which could even be as supplements. Thus, removal of H_2O_2 is very important for protection of food systems. The scavenging of the hydrogen peroxide free radical has been used in several studies to determine the antioxidant capacity of different foods and the results are usually reported in terms of percent scavenged hydrogen peroxide (% H_20_2). One such study is the work of Okoko and Ere in 2012 where they

found that the leaf extract of *Solenostemon monostacgyus* has better H_20_2 scavenging effect (87.33%) than that of vitamin C (67.34%).

2.7.3 Antihemolytic effect

Erythrocytes are considered as prime targets for free radical attack owing to the presence of both high membrane concentration of polyunsaturated fatty acids (PUFA) and the oxygen transport associated with redox active hemoglobin molecules, which are potent promoters of reactive oxygen species. The inhibition of lipid peroxidation by antioxidants may be due to their free radical-scavenging activities.

This test is undertaken to determine the protective effect of the plant extracts against membrane damage of the erythrocytes by free radicals. Hydrogen peroxide is used as the free radical initiator. Ebrahimzadeh *et al.*, (2010) in their study to determine the antioxidant activity of leaves of *Laser trilobum L.*, found the aqueous-ethanolic extract had protective effects on erythrocytes against hydrogen peroxide induced hemolysis. This effect was dose dependent and increased with the concentration of the plant extract. They also hypothesized of a possible relationship between the reducing power activity of the extract and anti-hemolytic activity.

It is thus evident that green leafy vegetables are excellent sources of antioxidant compounds and activity. It is also evident that processing methods will influence not only the content of phytochemicals with antioxidant activity but also the antioxidant activity of the vegetables. There is a need to determine the antioxidant activity of vegetables locally consumed and to determine the influence of the local cooking methods on this activity.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Design

This study was of a laboratory based experimental design. It was a descriptive study in which quantitative data was collected. It involved the comparison of antioxidant activity of three vegetables commonly consumed green leafy vegetables in Bushenyi district, Uganda, following cooking by two methods; steaming and boiling.

3.2 Study Setting

The study was conducted at Kampala International University Western Campus, at the Institute of Biomedical Research (IBR).

3.3 Materials

3.3.1 Reagents

All Chemicals used were of analytical grade. They included: distilled water, Standard Ascorbic acid, Potassium ferricyanide, Dibasic sodium phosphate, Monobasic sodium phosphate, Trichloro acetic acid, Ferric chloride, Acetic acid, Dichloroindophenol (DCIP), Phosphate Buffered Saline (pH 7.4), Hydrogen peroxide, Methanol, and Chloroform.

3.3.2 Equipment

Milton Roy Spectronic 21D UV-visible spectrophotometer (Now Thermo-scientific Waltham, USA), mortar and pestle, hot plate, thermometers, timer, electronic weighing scale, oven, centrifuge, stainless steel sauce pan, aluminum foil, cutting board, kitchen knife, incubator, water bath, orbital shaker, tripod stand and gauge, micropipettes, test tube racks, disposable pipette tips, dissecting kit, needle and syringes, disposable gloves, EDTA containing vacutainers, and an animal cage.

3.3.3 Glassware

Burettes, Beakers, test tubes, boiling tubes, measuring cylinders, reagent bottles (amber and clear), stirring rod, conical flasks, cuvettes, centrifuge tubes

3.3.4 Animals

Five (5) Adult male Wistar albino rats weighing an average of 220grams were procured from the Department of Pharmacy, Kampala International University- Western Campus. The animals were kept in cages and fed with NUMA feeds (manufactured by Unga Manufacturers Uganda) once a day and provided with water *ad libitum.*

3.3.5 Plant material

The plant materials for the study were *Amaranthus dubius*, *Solanum nigrum* and *Cucubita maxima.*

3.4 Methods

3.4.1 Plant collection

Two of the plant samples (*Solanum nigrum* and *Amaranthus dubius*) were purchased from Bushenyi Market in Bushenyi Municipality and Ishaka Market in Ishaka Municipality, of Bushenyi district. Bushenyi Municipality is divided into the administrative Bushenyi and the commercial Ishaka. Bushenyi and Ishaka each possess a big market, in which produce from the surrounding villages is brought for sale. Pumpkin leaves are not a market commodity and so were harvested from private gardens in Kabare parish of Ishaka Municipality and Bushenyi town.

The samples were loosely packaged in large polythene papers to avoid compression of the leaves and transported immediately to the laboratory for processing. Meanwhile a sample had been taken for taxonomic identification at Mbarara University by Dr. Eunice Apio Opolot. Voucher specimens were deposited and given voucher numbers HWK 001 *Amaranthus dubius*, HWK 002 *Cucurbita maxima* and HWK 003 *Solanum nigrum* complex.

3.4.2 Sample processing

On arrival into the laboratory, the plant samples collected were divided into 3 groups A, B and C. The samples were washed with distilled water and chopped to small sizes 2-4 mm on a cutting board using a kitchen knife as it is done in the community. Sample A was analyzed as the fresh sample; B as the boiled sample and group C as the steamed sample.

3.4.2.1 Boiling

The method described by Prabnu and Barrett in 2009 was used for boiling evaluation (Appendix 1). One hundred grams (100 g) of the chopped vegetables (sample B) was placed in a stainless steel saucepan containing 500mls of distilled water. The saucepan was covered with aluminum foil to minimize vapor loss. The leaves were boiled by placing the saucepan on a hot plate and the water was brought to boil. Once the water had boiled, the vegetables were allowed to cook for 10 minutes. The boiled leaves were then allowed to drip on a sieve at room temperature (23^0C) for 10 minutes. The water used for boiling was collected in a beaker and analyzed for Vitamin C content.

The boiled leaves were then placed in a glass bottle capped and refrigerated at 4^0C for further analysis.

3.4.2.2 Steaming

The method of Akubugwo *et al.*, in 2008 [Appendix 2] which was slightly modified by placing the vegetables on a metal sieve instead of wire gauze and wrapping the vegetables in aluminum foil was used to steam the vegetables. One hundred grams of the chopped vegetables (sample C) were placed in a metal sieve wrapped with aluminum foil. The sieve was then placed on top of a stainless steel saucepan containing 500mls of boiling distilled water. The vegetables were then steamed for 10 minutes. The steamed vegetables were removed and cooled to room temperature, stored in capped glass bottles and refrigerated at 4^0C until further analysis.

3.4.3Vitamin C Determination

3.4.3.1 Extraction of vitamin C

Vitamin C was extracted according to the method used by Ogulensi *et al.*, (2009) [Appendix 3]. The samples A, B and C (2g) were separately extracted by use of a mortar and pestle in a 0.5% oxalic acid solution. Oxalic acid inhibits the rapid oxidative destruction of the vitamin; it also reduces the reducing action of phenols, tannins and glutathione which may be present in the extract (Burrel and Ebright, 1940). The mixture was then placed in a conical flask (wrapped with aluminum foil to prevent light destruction of vitamin C) and agitated at 1000 rotations per minute with the aid of an orbital shaker for 15 minutes at averages recorded

room temperature of 23^0C. The mixture was then filtered through Whatman filter paper No.4 to obtain a clear extract. All samples were extracted in triplicate.

3.4.3.2 Preparation of reagents

The oxalic acid was prepared by adding 100mls of distilled water to 0.5g of oxalic acid in a 250ml beaker. The mixture was refrigerated at 4^0C till use.

Standard ascorbic acid was prepared by weighing 50mg of ascorbic acid (Analytical grade, Sigma-Aldrich) and transferring it to a 50ml volumetric flask. It was then diluted to the 50ml mark with oxalic acid solution.

The indophenols solution dye was prepared by adding 42mg of sodium bicarbonate to 50ml of distilled water in a 150ml beaker. To this, 2, 6, dichloroindophenol (DCIP) sodium salt (50mg) was added and stirred. The mixture was diluted to 200ml with distilled water then filtered using filter paper into an amber bottle with a stopper and refrigerated at 4^0C until use.

3.4.3.3 Determination of vitamin C

The amount of Vitamin C was determined using dye titration AOAC procedure of 1990 as described by Nielsen in 2010. This method is based on the principle that ascorbic acid reduces the indicator dye to a colorless solution. At the end point of titrating an ascorbic acid containing sample with dye, excess unreduced dye is a rose-pink color in acid solution. The clear extract obtained above (14mls) was titrated with 2,6 dichloro-indophenol solution until a light but distinct rose pink color appeared and persisted for at least 5 seconds.

A blank containing 14mls of oxalic acid solution was also titrated with 2, 6 dichloroindophenol as above. Vitamin C standard (7mls) to which 7 mls of oxalic acid solution had been added was also titrated against the indophenol solution to standardize it. All determinations were conducted in triplicate.

The formula used to calculate amount of Vitamin C in the extract in mg/ml was:

$$\textbf{Concentration of Vitamin C in extract (mg/ml)} = \frac{(\text{mls of titrant extract- mls of titrant blank}) \times 0.05\text{mg/ml}}{(\text{mls of titrant standard-mls of titrant blank})}$$

And for ascorbic acid in 100g of vegetable:

$$\text{mg/100g} = \text{mg of ascorbic acid in aliquot} \times \frac{\text{total volume of extract}}{\text{Total volume of aliquot}} \times \frac{100}{\text{weight of vegetable (g)}}$$

3.4.4 Extraction for antioxidant activity

Plant sample extracts were used for determination of antioxidant activity by modification of the method of Katerere *et al.*, (2012) [Appendix 4]. The fresh, boiled and steamed samples were dried at 25^0C till a constant weight was achieved. The dried leaves were then ground to produce a homogenous powder using a mortar and pestle. The powder was weighed and soaked in an aqueous-methanol solvent (70% methanol) at a 1:10 ratio at 25^0C. The mixture was placed in the dark for three days after which, each sample was centrifuged for five minutes at 4000 rpm. The extracts were filtered using Whatman No. 1 filter paper and the aliquots used for the determination of antioxidant activity.

3.4.5 Reducing power assay

The reducing power was determined according to the method of Oyaizu (1986) as described in Jayanthi and Lalitha (2011) [Appendix 5]. It is based on the principle that antioxidant substances react with potassium ferricyanide (Fe3+) to form potassium ferrocyanide (Fe2+) which then reacts with ferric chloride to form ferrous complex that has an absorption maximum at 700nm.

The aliquots obtained from aqueous-methanolic extraction described above were used in this assay. Various concentrations of the plants extract (0, 0.25, 0.5, 1.0, 1.5, 2.0 and 3.0 mg/ml) were mixed with 2.5mls of phosphate buffer and 2.5ml of 1% potassium ferricyanide. This mixture was incubated in a 50^0C water bath for 20 minutes. After cooling to 25^0C, 2.5mls of 10% trichloroacetic acid was added and centrifuged at 3000rpm for 10 minutes. The upper layer of the solution (2.5ml) was mixed with 2.5mls of distilled water and 0.5ml of freshly prepared 0.1% ferric chloride solution. The absorbance reading was measured at 700nm. Ascorbic acid at various concentrations was used as a standard against which the reducing power of the extracts was compared. The phosphate buffer was prepared by mixing 37.50ml of 0.2 M dibasic sodium phosphate with 62.5ml of monobasic sodium phosphate.

To 100 µl of 5 %v/v suspension of erythrocytes in phosphate buffered saline, 50 µl of the extract at different concentrations (0.25, 0.5, 1, 1.5, 2 and 3 mg), was added. To this, 100 µl of 1 M H_2O_2 (in PBS, pH 7.4) was also added. The reaction mixtures were shaken gently in an incubator shaker at 37 °C for 3 h and then diluted with 8 ml of PBS and centrifuged at 2000 × g for 10 min. The absorbance of the resulting supernatant was measured spectrophotometrically at 540 nm to determine the level of hemolysis.

To obtain complete hemolysis, the erythrocytes were treated with 100 µM H_2O_2 without plant extract. The absorbance of the supernatant was measured at 540 nm. The inhibitory activity of the extract was compared with that of the standard antioxidant, vitamin C. To evaluate hemolysis induced by the extract, the erythrocytes were pre-incubated with 50 µl of the extract at a concentration of 10 mg/ml for 1 hour and the level of hemolysis was determined. Hemolysis was calculated by determining the hemolysis caused by 100 µM H_2O_2 as 100 %. The Inhibitory Concentration 50 (IC_{50}) values were calculated from the plots (concentration vs. absorbance) as the antioxidant concentration required for the inhibition of 50% hemolysis. The line of best fit was obtained from the plots and the IC_{50} calculated using linear regression as described below:

Equation for line of best fit is: **y=ax+b**

IC 50 = (0.5-b)/a

Key:
a= Gradient
b= Y-intercept
y= Absorbance at 540nm
x= Concentration in mg/ml

3.5 Data Analysis

Excel package was used to analyze the results obtained. For each test the overall means of the triplicates and standard deviation for the Vitamin C, reducing power, H_2O_2 radical scavenging capacity and anti-hemolyzing activity were compared. The difference between samples was determined by Student's T-test. P-value of <0.05 was regarded as significant. Students T-test was used to determine the significance of differences between the anti-hemolyzing effects of vitamin C and the different plant extracts.

3.6 Data Presentation

Data was presented in form of tables, graphs, pie charts and photographs.

3.7 Quality Control

Measures that were taken to ensure that quality results were obtained included:

1. All lab wares were washed with disinfectant and rinsed with distilled water to prevent contamination.
2. All chemicals used were of analytical grade.
3. A lab journal was opened and all the events on each day recorded in the journal to prevent missing any step or repeating tests that had already been conducted.
4. The experiments were conducted following established Standard Operating Procedures (SOPs).
5. All tests were conducted in triplicate and an average of the three results considered as the main result.

3.9 Ethical Consideration

The study did not involve human participants. Approval to conduct the research was sought from Kampala International University Institutional Ethics and Research Committee. Ethical principles regarding handling of experimental animals observed were as follows: The Wistar rats were kept in cages with adequate fresh air, and 12 hours of light by day and 12 hours of darkness by night. The beddings in the cages of fresh saw dust were changed every three days. Contamination was prevented by having the cage constructed with raised legs away from direct contact with the ground. The animals were provided with food and water *ad libitum*. The rats were sacrificed following inhalational anesthesia with chloroform.

3.10 Challenges and limitations

1. The vegetable samples were purchased from the markets and not grown for the study under controlled conditions due to time constraint. This was overcome by following laid out standard operating procedures to reduce margin of error and variability of results.
2. Destruction of vitamin C was kept at a minimum by foiling the glassware with aluminum to reduce exposure to light.

3. Power shortages interfered with the time schedule. This was overcome by working during weekdays when the University's generator was on standby.

CHAPTER FOUR

RESULTS

4:1 Photographs of vegetables used in the study

Plate 1: *Amaranthus dubius:* Purchased from Bushenyi Market

Plate 2: *Amaranthus dubius* purchased from Ishaka market

PLATE 3: Photographs of Cucurbita *maxima* leaves used in the study

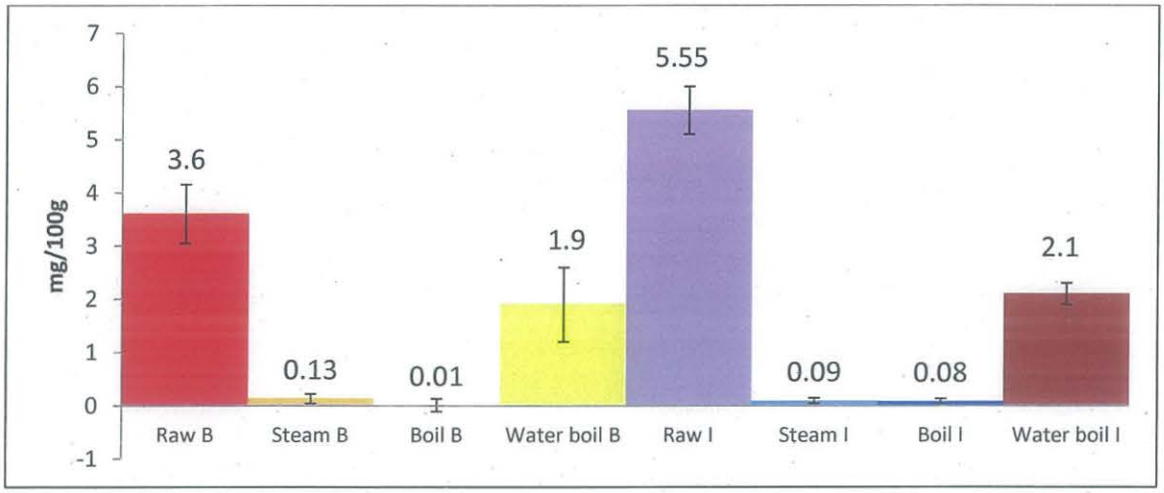
PLATE 4: Photographs showing *Solanum nigrum* purchased from Bushenyi market

4.2 Observations

On steaming the vegetables, the texture and consistency became softer than when they were raw. The color of *Amaranthus dubius* and *Solanum nigrum* turned to dark green while that of *Cucurbita maxima* lightened compared to that of the raw vegetables. On boiling, the vegetables softened to the extent of breaking down easily. In all the three samples, the intensity of green color was reduced and in *Amaranthus*, the vegetables turned to a dull shade of brown. The water used for boiling became green.

4.3 *Amaranthus dubius*

4.3.1 Vitamin C content



Key: B: Bushenyi **I:** Ishaka

Figure 2: Vitamin C content of *A. dubius*: The Vitamin C content of the raw, steamed and boiled vegetables purchased from Bushenyi and Ishaka markets is shown. The vitamin C content of the water used for boiling the vegetables is also plotted. The various samples are shown in X-axis while the Y-axis shows the Vitamin C present in mg/100g

The Vitamin C content of the raw vegetables purchased in Bushenyi (3.6mg/100mg) varied from those purchased from Ishaka (5.55mg/100mg). This variation was statistically significant (P-value = 0.009, one-tailed T-test). There were significant losses in the vitamin C content of the steamed and boiled vegetables. The loss was highest in the boiled sample from Ishaka (96.4%) and lowest in the steamed sample from Bushenyi (92.2%) as shown in Figure 2 above. The vitamin C content of the water used for boiling the vegetables increased to 2.1mg/100ml for the sample from Ishaka and 1.9mg/100mls for the sample from Bushenyi.

4.3.2 Reducing power

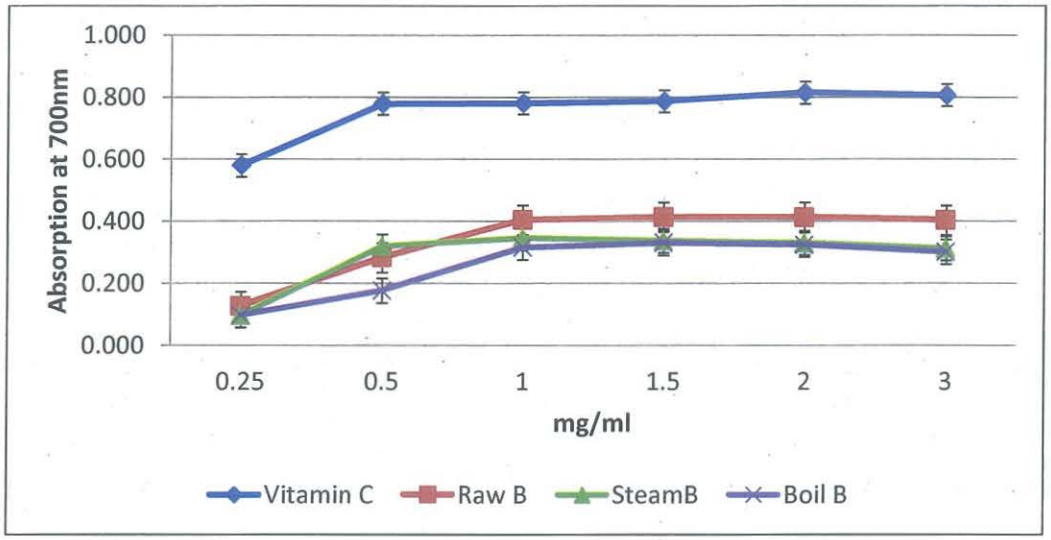


Figure 3: Reducing power of *A. dubius* Bushenyi: The reducing power of the raw, steamed and boiled vegetables purchased from Bushenyi is shown. The reducing power of the positive control Vitamin C is also plotted. The various concentrations are shown in X-axis while the Y-axis shows the absorbance at 700nm which is a measure of the reducing power.

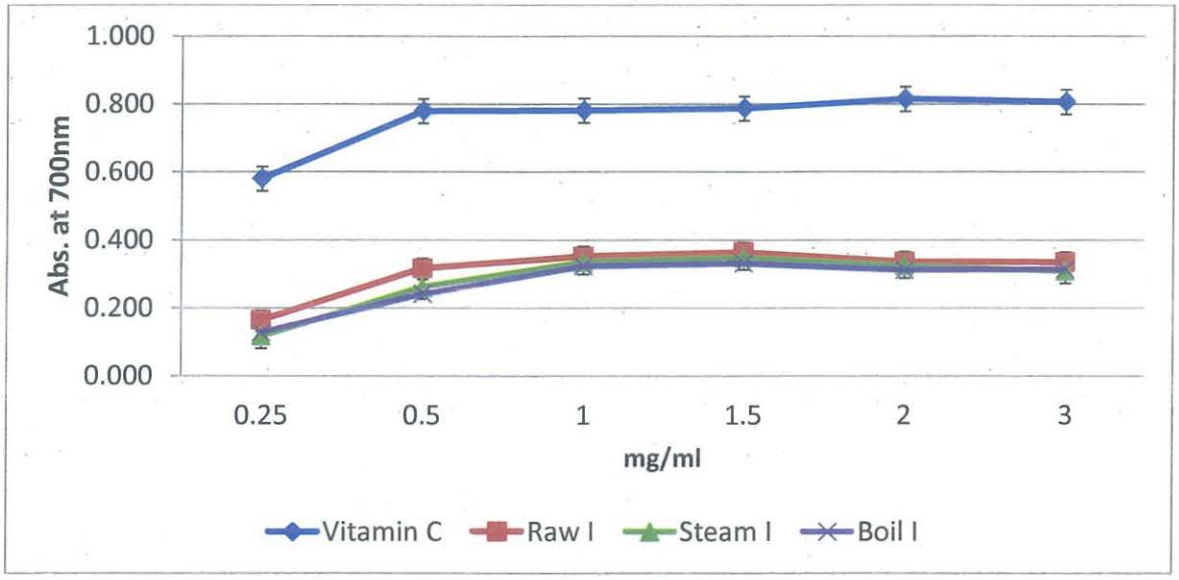


Figure 4: Reducing power of *A.dubius* Ishaka: The reducing power of the raw, steamed and boiled vegetables purchased from Ishaka is shown. The reducing power of the positive control Vitamin C is also plotted. The various concentrations are shown in X-axis while the Y-axis shows the absorbance at 700nm which is a measure of the reducing power.

The positive control Vitamin C had much higher reducing power than that of *A.dubius*. The reducing power increased with increase in the concentration of both the control and vegetable extract. The sample from Bushenyi had slightly higher reducing power than the sample from Ishaka, though this variation was not statistically significant (P-value = 0.215, one-tailed T-test). Among the three vegetable samples, the raw sample had higher reducing power which reduced when the sample was processed by either steaming or boiling as portrayed in Figure 3 and 4. The variation between the steamed and boiled vegetables is not statistically significant for the samples from Bushenyi (P-value=0.57, two-tailed T-test) and from Ishaka (P-value=0.87, two-tailed T-test).

4.3.3 Hydrogen Peroxide scavenging activity of *Amaranthus dubius*

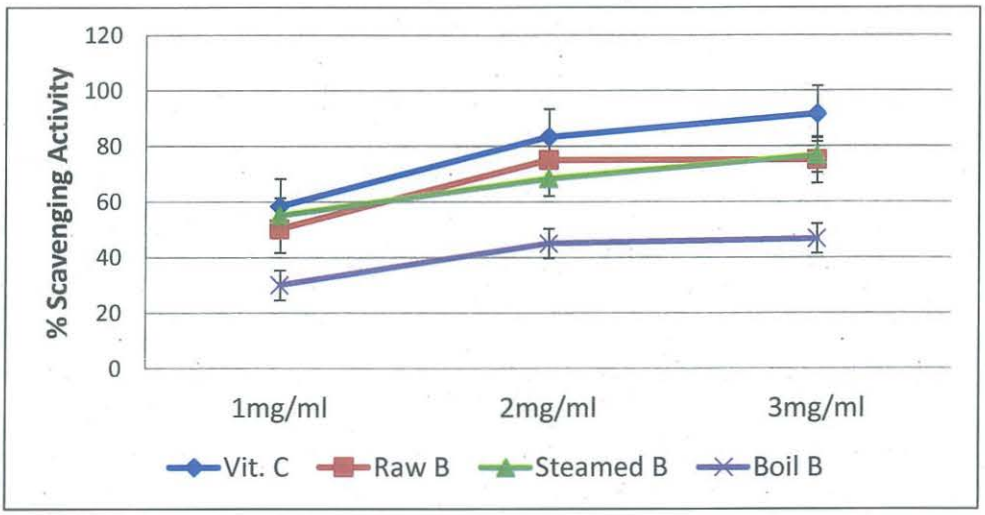


Figure 5: Hydrogen peroxide scavenging activity of *A. dubius* Bushenyi: The % scavenging activity of the raw, steamed and boiled vegetables purchased from Bushenyi is shown. The scavenging activity of the positive control Vitamin C is also plotted. The various concentrations are shown in X-axis while the Y-axis shows the % scavenging activity.

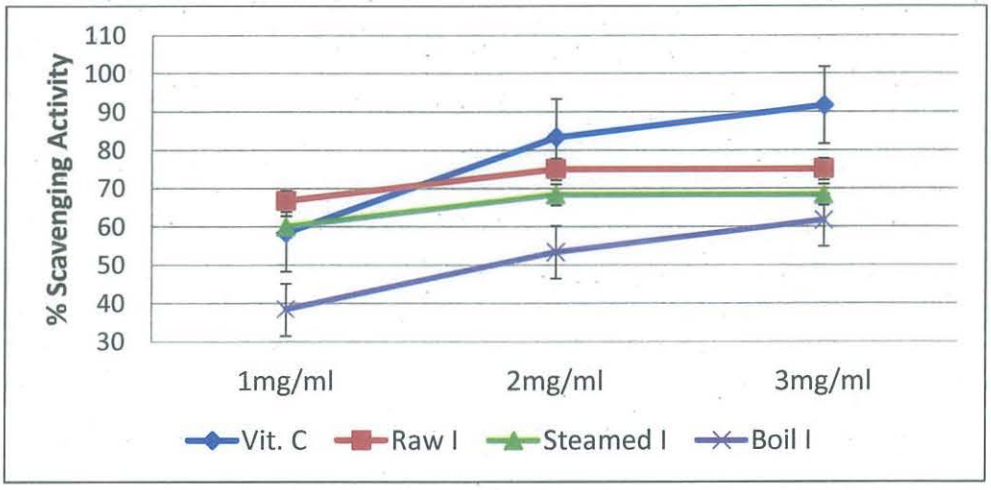


Figure 6: Hydrogen peroxide scavenging activity of *A. dubius* Ishaka: The % scavenging activity of the raw, steamed and boiled vegetables purchased from Ishaka is shown. The scavenging activity of the positive control Vitamin C is also plotted. The various concentrations are shown in X-axis while the Y-axis shows the % scavenging activity.

The % scavenging activity increased as the concentration of the control, Vitamin C and the various vegetable extracts increased as shown in Figure 5 and 6. There was no

significant variation in the scavenging activity of the extracts from the raw and steamed vegetables from Bushenyi (P-value=0.387, one-tailed T-test) and Ishaka (P-value=0.255, one-tailed T-test). There was significant variation between the boiled and raw samples from Bushenyi (P-value=0.001, one tailed T-test) and Ishaka (P-value=0.002, one tailed T-test). The variation between the scavenging activity of the boiled and steamed extracts from Bushenyi (P-value =0.006, two tailed T-test) and Ishaka (P-value =0.023, two-tailed T-test) was statistically significant.

4.3.4 Antihemolytic activity of *Amaranthus dubius*

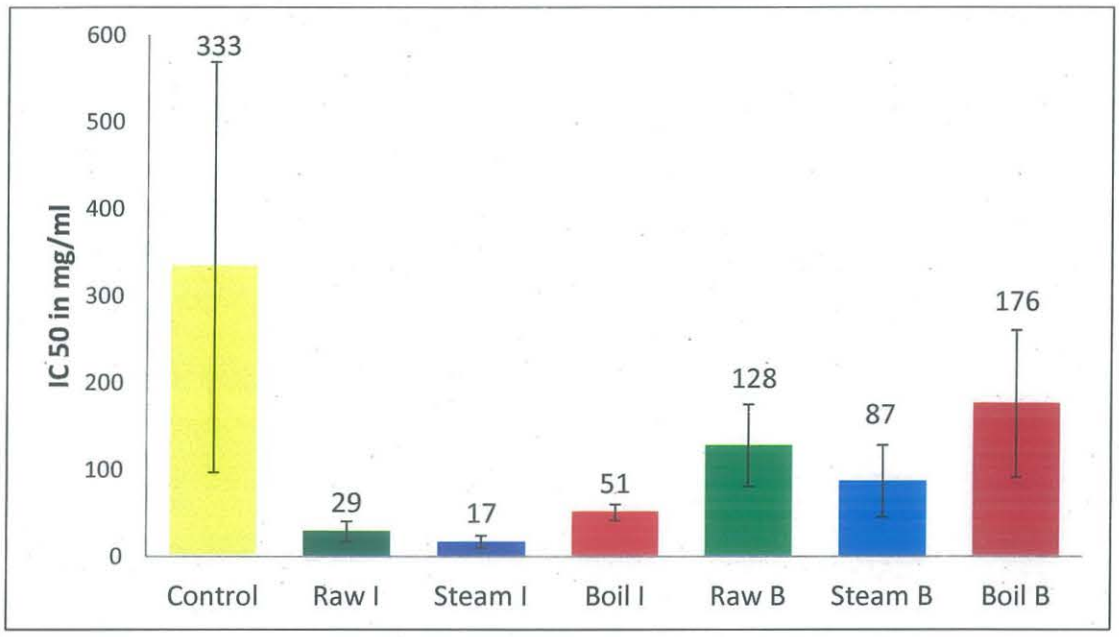
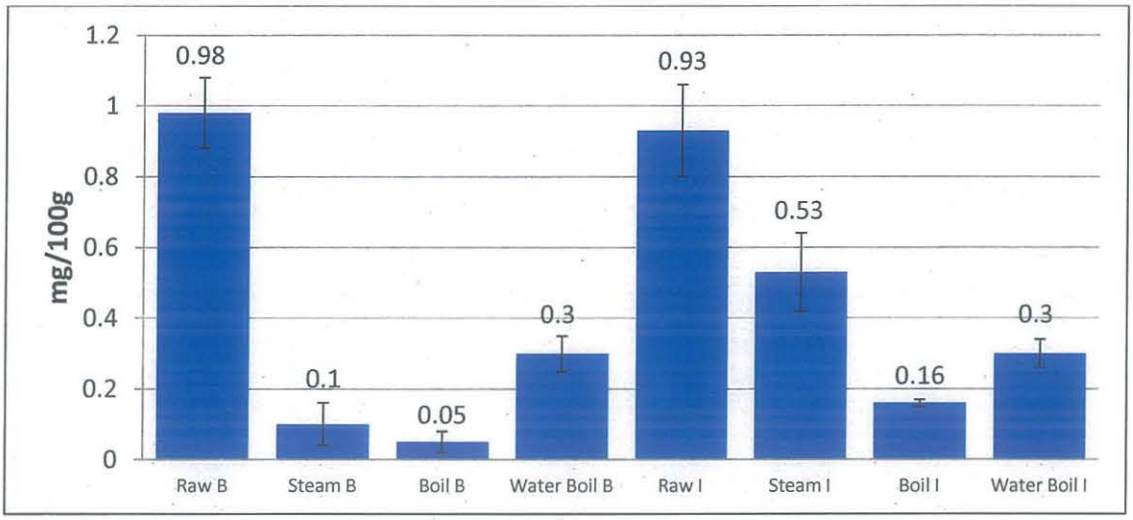


Figure 7: Antihemolytic activity of *A. dubius*: The IC_{50} of the raw, steamed and boiled vegetables purchased and the control Vitamin C is shown. As the IC_{50}, increases, Antihemolytic activity of the sample reduces. The various samples are shown in X-axis while the Y-axis shows IC_{50} in mg/ml.

As the protective effect of the extracts against hydrogen peroxide induced hemolysis of the red blood cells increases, the amount of extract calculated for 50% inhibition (IC_{50}) against hemolysis reduces. *A.dubius* had more antihemolytic activity than the positive control, Vitamin C as shown in figure 7. The sample purchased from Ishaka had higher antihemolytic activity compared with the sample from Bushenyi and this was statistically significant (P-value =0.0001, one-tailed T-test). The steamed samples had much higher antioxidant activity than both the raw and boiled extracts. Among the vegetable extracts, the boiled had the lowest antioxidant activity. The variation between the raw and steamed samples from Bushenyi (P-value = 0.002, one-tailed T-test) and Ishaka (0.005, one tailed T-test) was statistically significant as well as raw and boiled from Bushenyi (P-value =0.001, one tailed T-test).

4.4 *Solanum nigrum*

4.4.1 Vitamin C content of *Solanum nigrum*



Key B: Bushenyi **I:** Ishaka

Figure 8: Vitamin C content of *S. nigrum*: The Vitamin C content of the raw, steamed and boiled vegetables purchased from Bushenyi and Ishaka markets is shown. The vitamin C content of the water used for boiling the vegetables is also plotted. The various samples are shown in X-axis while the Y-axis shows the Vitamin C present in mg/100g

The variation between the Vitamin C content of the sample purchased from Bushenyi (0.98mg/100g) and that purchased in Ishaka (0.93mg/100g) was not statistically significant (P-value = 0.462, one-tailed T-test). There were significant losses in the vitamin C content of the steamed and boiled samples with the boiled sample from Bushenyi having the highest loss (87.5%) as shown in Figure 8 above. The water used to boil the vegetables also had an increment (0.3mg/100mls) in its Vitamin C content.

4.4.2 Reducing Power of *Solanum nigrum*

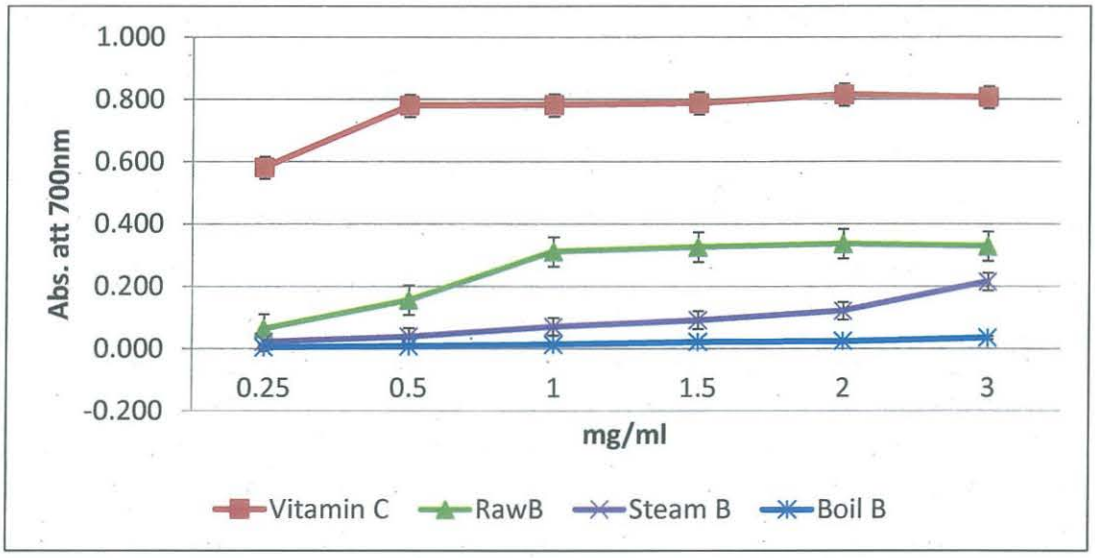


Figure 9: Reducing power of *S.nigrum* Bushenyi: The reducing power of the raw, steamed and boiled vegetables purchased from Bushenyi is shown. The reducing power of the positive control Vitamin C is also plotted. The various concentrations are shown in X-axis while the Y-axis shows the absorbance at 700nm which is a measure of the reducing power.

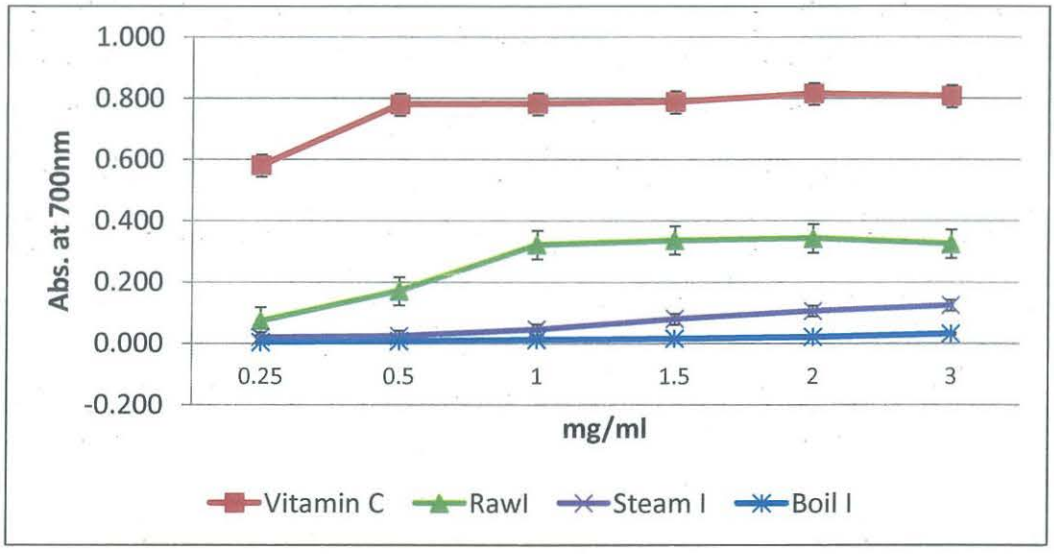


Figure 10: Reducing power of *S.nigrum* Ishaka: The reducing power of the raw, steamed and boiled vegetables purchased from Ishaka is shown. The reducing power of the positive control Vitamin C is also plotted. The various concentrations are shown in X-axis while the Y-axis shows the absorbance at 700nm which is a measure of the reducing power.

There was an increase in the reducing power as the concentration of the extracts and vitamin C increased as shown in Figure 9 and 10 above. The reducing power of the raw extracts was highest followed by that of the steamed, while the boiled sample had the lowest reducing power. The variation between the raw samples of *S.nigrum* from Bushenyi and Ishaka was statistically significant (P-value = 0.014, one-tailed T-test). There was significant variation (P-value = 0.019, two-tailed T-test) between the boiled and steamed extracts from Bushenyi.

4.4.3 Hydrogen peroxide activity of *Solanum nigrum*

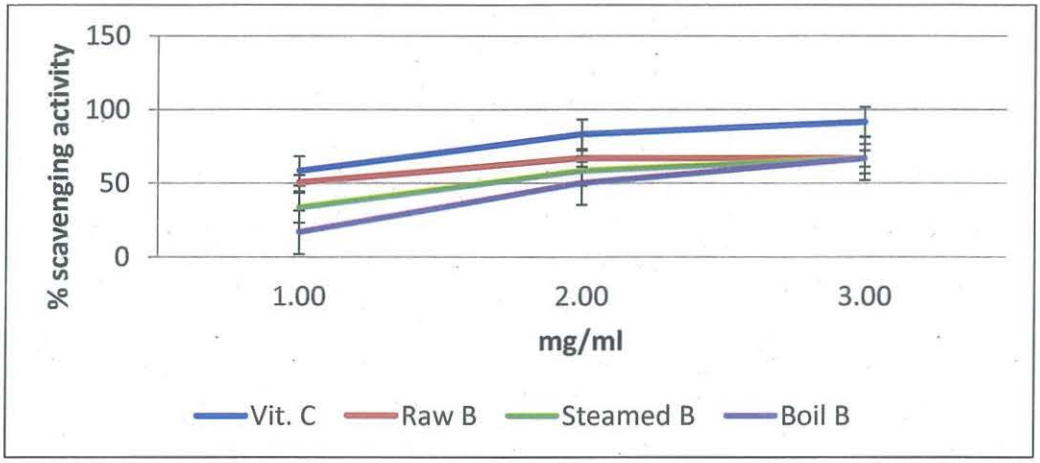


Figure 11: Hydrogen peroxide scavenging activity of *S.nigrum* Bushenyi: The % scavenging activity of the raw, steamed and boiled vegetables purchased from Bushenyi is shown. The scavenging activity of the positive control Vitamin C is also plotted. The various concentrations are shown in X-axis while the Y-axis shows the % scavenging activity.

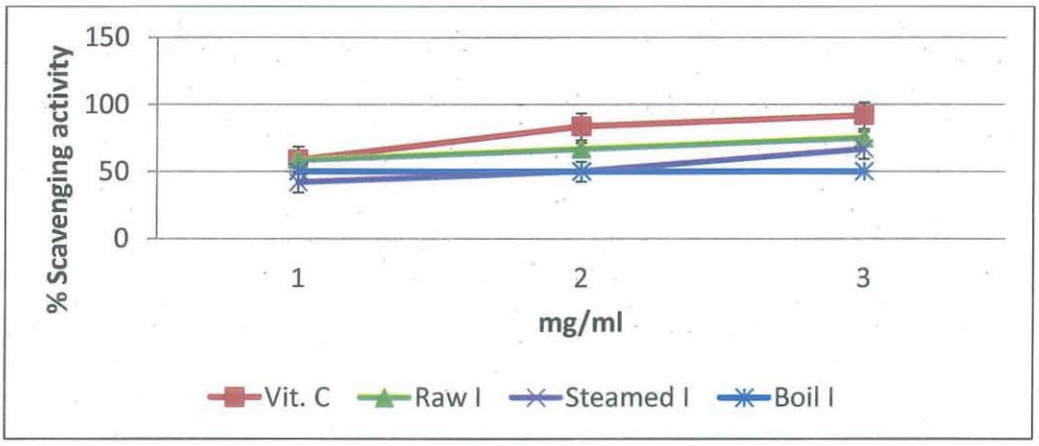


Figure 12: Hydrogen peroxide scavenging activity of *S.nigrum* Ishaka: The % scavenging activity of the raw, steamed and boiled vegetables purchased from Ishaka is shown. The scavenging activity of the positive control Vitamin C is also plotted. The various concentrations are shown in X-axis while the Y-axis shows the % scavenging activity.

The hydrogen peroxide scavenging activity increased with increase in concentration in Figure 11 and 12. The extracts from the raw vegetables had slightly higher scavenging activity compared to the extracts from steamed and boiled vegetables. This variation was higher at lower extract concentrations but narrowed as the concentration of the extract

increased. The difference in scavenging activity of the raw samples from Bushenyi was statistically significant (P-value=0.029, one tailed T-test) from that purchased from Ishaka. The difference between the raw and boiled extracts from Bushenyi (P-value =0.016, one-tailed T-test) and Ishaka (P-value= 0.010, one-tailed T-test) was statistically significant.

4.4.4 Antihemolytic activity of *Solanum nigrum*

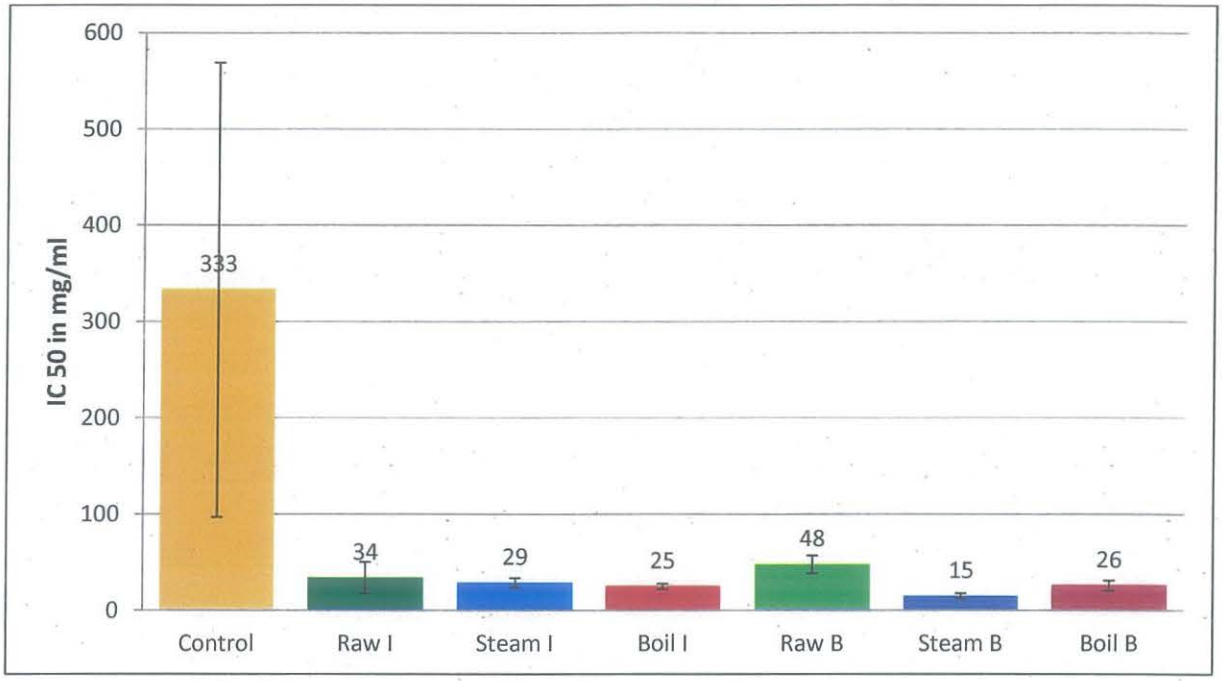
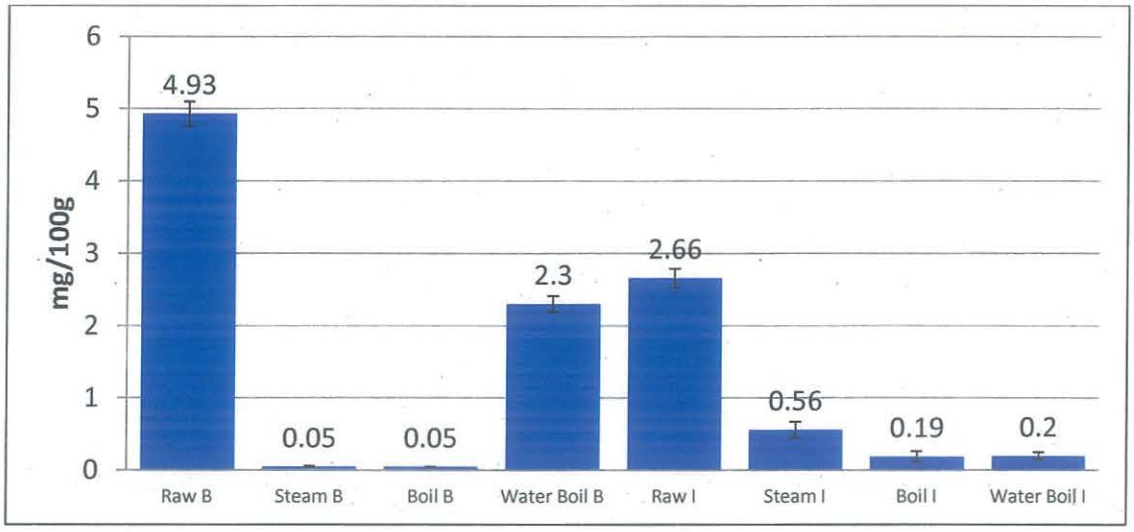


Figure 13: Antihemolytic activity of *S. nigrum:* The IC_{50} of the raw, steamed and boiled vegetables purchased and the control Vitamin C is shown. As the IC_{50}, increases, Antihemolytic activity of the sample reduces. The various samples are shown in X-axis while the Y-axis shows IC_{50} in mg/ml.

Extracts of *S. nigrum* had much higher protective activity on the red blood cells compared to Vitamin C. The variation between the extract obtained from the sample purchased from Bushenyi and that purchased from Ishaka was statistically significant (P-value=0.002, one-tailed T-test), with the raw sample from Ishaka having a higher protective effect. Processing the samples by steaming caused an increase in the protective activity as evidenced by the lower inhibitory concentration of the steamed samples. The variation between the raw/boiled for sample from Bushenyi (P-value=0.016, one tailed T-test) and Ishaka (P-value=0.025, one-tailed T-test) was statistically significant.

4.5 *Cucurbita maxima*

4.5.1 Vitamin C content of *Cucurbita maxima*



Key **B:** Bushenyi **I:** Ishaka

Figure 14: Vitamin C content of *C. maxima*: The Vitamin C content of the raw, steamed and boiled vegetables harvested from Bushenyi and Ishaka markets is shown. The vitamin C content of the water used for boiling the vegetables is also plotted. The various samples are shown in X-axis while the Y-axis shows the Vitamin C present in mg/100g

There was a marked variation in the Vitamin C content of the raw *C. maxima* sample collected from Bushenyi (4.93mg/100g) and that collected from Ishaka (2.66mg/100g) which was statistically significant (P-value=0.006, one-tailed T-test). There was significant loss in the Vitamin C content in both the boiled and steamed samples. This loss was highest in the boiled sample from Bushenyi (96.8%). There was an increase in the Vitamin C content of the water used to boil the vegetables

4.5.2 Reducing Power of *Cucurbita maxima*

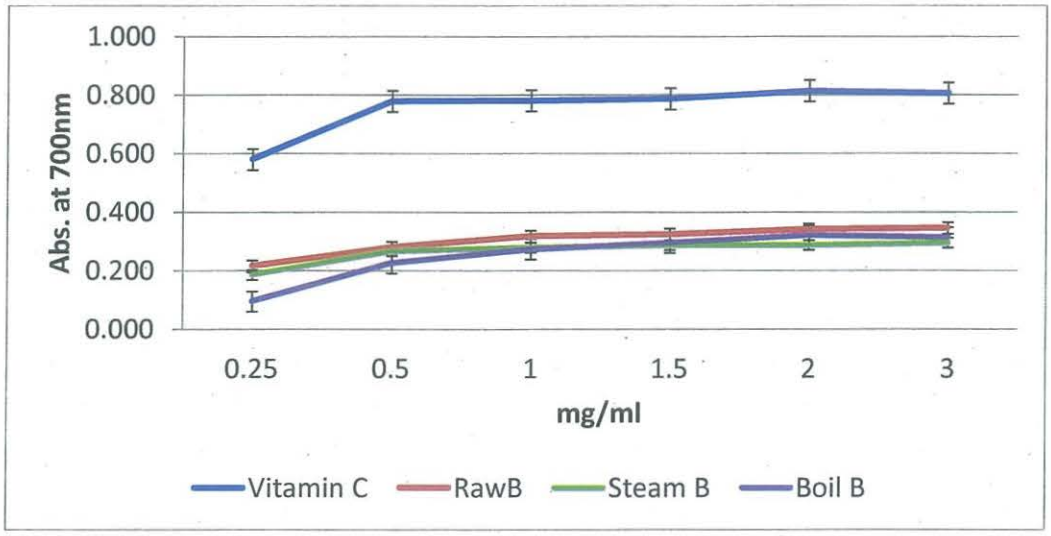


Figure 15: Reducing power of *C.maxima* Bushenyi: The reducing power of the raw, steamed and boiled vegetables harvested from Bushenyi is shown. The reducing power of the positive control Vitamin C is also plotted. The various concentrations are shown in X-axis while the Y-axis shows the absorbance at 700nm which is a measure of the reducing power.

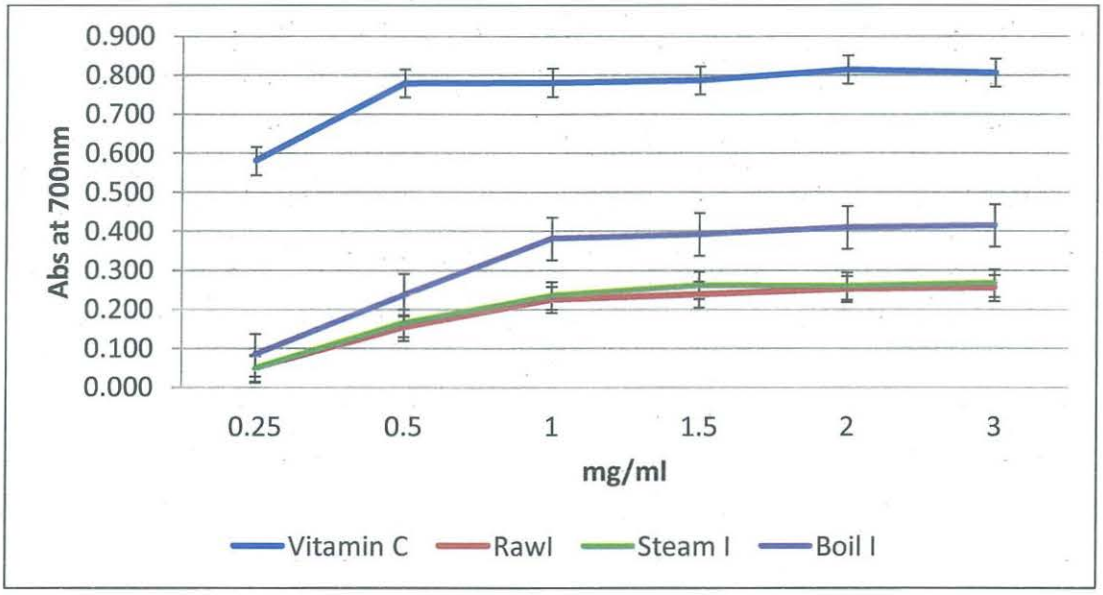


Figure 16: Reducing power of *C.maxima* Ishaka: The reducing power of the raw, steamed and boiled vegetables harvested from Ishaka is shown. The reducing power of the positive control Vitamin C is also plotted. The various concentrations are shown in X-axis while the Y-axis shows the absorbance at 700nm which is a measure of the reducing power.

The reducing power increased as concentration increased as shown in figure 15 and 16 above. Vitamin C had much higher reducing power than the raw extracts from both Ishaka and Bushenyi. The *C.maxima* extract derived from vegetables harvested in Bushenyi had much higher reducing power than the samples harvested from Ishaka, which was statistically significant (P-value = 0.0002, one-tailed T-test). There was a notable increase in the reducing power of the boiled extract from Bushenyi compared to both the steamed and raw extracts. The increase in reducing power of the boiled extract was not statistically significant for the sample from Bushenyi (P-value= 0.74, two-tailed T-test) and Ishaka (P-value=0.111, two-tailed T-test).

4.5.3 Hydrogen peroxide scavenging activity of *Cucurbita maxima*

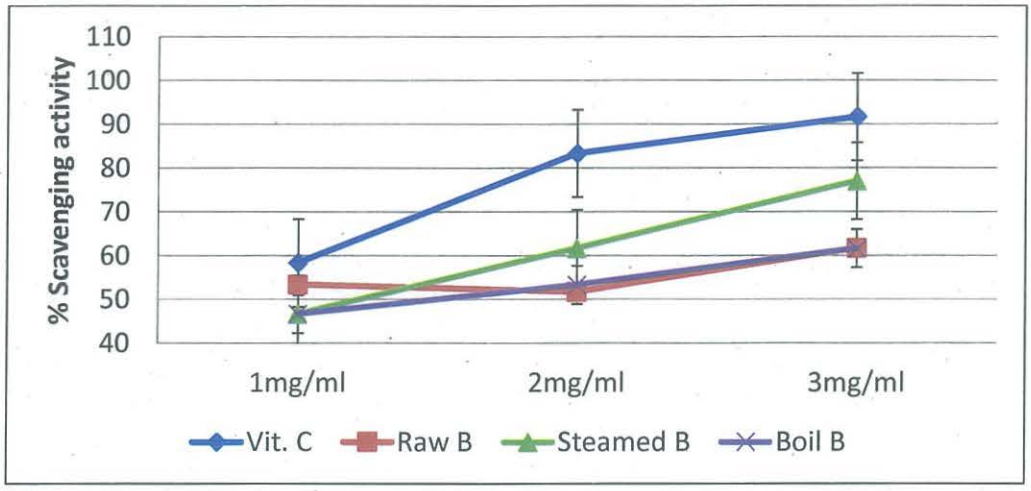


Figure 17: Hydrogen peroxide scavenging activity of *C. maxima* Bushenyi: The % scavenging activity of the raw, steamed and boiled vegetables harvested from Bushenyi is shown. The scavenging activity of the positive control Vitamin C is also plotted. The various concentrations are shown in X-axis while the Y-axis shows the % scavenging activity.

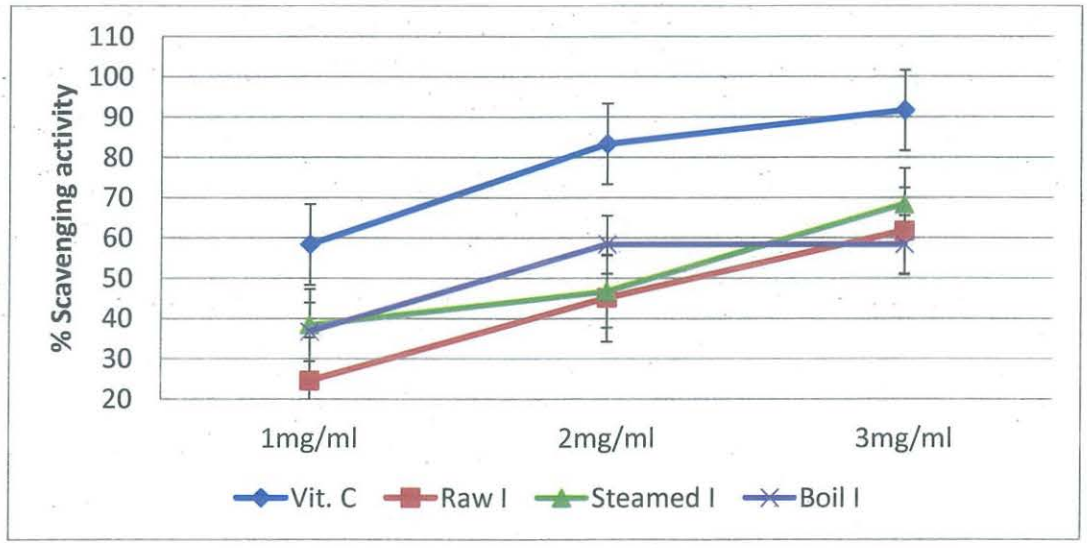


Figure 18: Hydrogen peroxide scavenging activity of *C.maxima* Ishaka: The % scavenging activity of the raw, steamed and boiled vegetables harvested from Ishaka is shown. The scavenging activity of the positive control Vitamin C is also plotted. The various concentrations are shown in X-axis while the Y-axis shows the % scavenging activity.

The scavenging activity increased with increasing concentration of the control, Vitamin C and extracts as shown in Figure 17 and 18. For the sample from Bushenyi, the steamed extract had higher scavenging activity than the raw, which was not statistically significant (P-value =0.344, one-tailed T-test). Boiling the sample from Bushenyi, did not result in any statistical significant (P-value =0.500, one-tailed T-test). Steaming the sample from Ishaka resulted in an increase in scavenging activity which was statistically significant (P-value=0.017, one-tailed T-test). Boiling also caused an increase in scavenging activity compared to the raw, which was also statistically significant (P-value=0.053, one-tailed T-test).

4.5.4 Antihemolytic activity of *Cucurbita maxima*

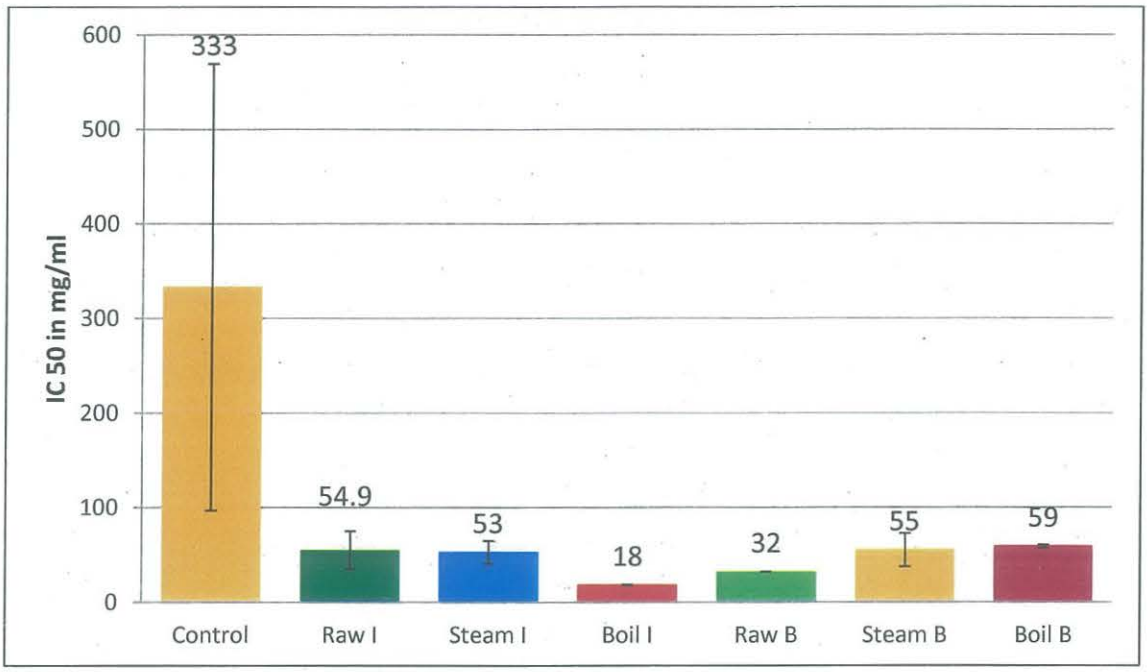


Figure 19:Anti-hemolytic activity of C. *maxima*: The IC_{50} of the raw, steamed and boiled vegetables and the control Vitamin C is shown. As the IC_{50}, increases, Antihemolytic activity of the sample reduces. The various samples are shown in X-axis while the Y-axis shows IC_{50} in mg/ml.

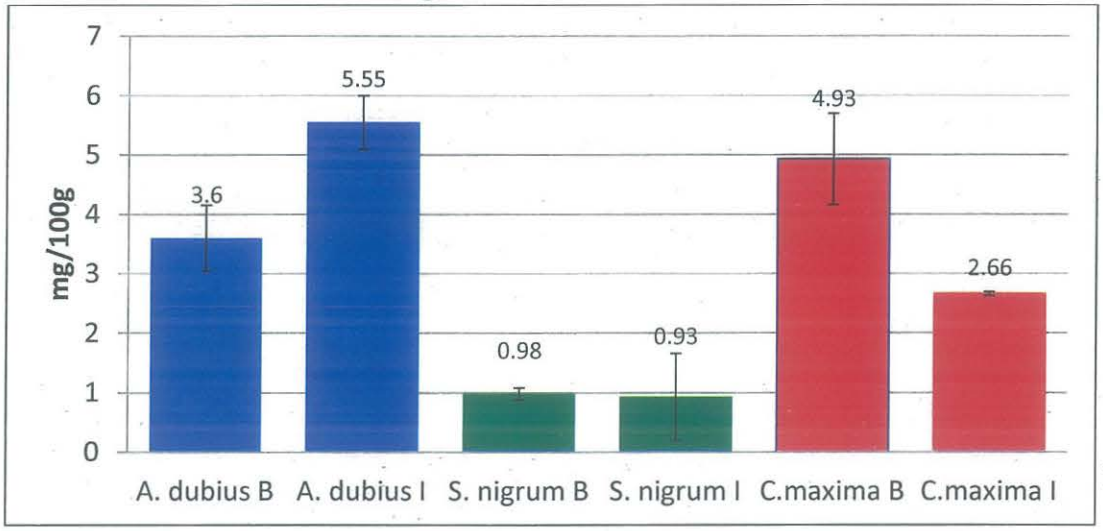
The vegetable extracts had lower inhibitory concentration compared to the positive control Vitamin C, signifying higher protective effects on the red blood cells against hemolysis. The raw extract from the sample harvested from Ishaka had higher inhibitory concentration than that harvested from Bushenyi as shown in figure 20, which was statistically significant (P-value=0.0001, one-tail T-test). Processing the sample from Ishaka improved its protective effect as evidenced by the lower IC_{50} of the boiled sample which was statistically significant (P-value=0.0003, one tail T-test) compared to the raw sample. For the sample from Bushenyi, the raw extract had higher antioxidant activity, which reduced as the vegetable was processed by either steaming or boiling, hence increasing the IC_{50}.

4.6 Comparative analysis

4.6.1 Comparative analysis of the Vitamin C content of the vegetables

The vitamin C content of all the vegetables was compared and one-tailed test was used to determine the statistical significance. All means, standard deviations and statistical significance are compiled in Appendix 6. Below is the analysis divided by cooking method.

Vitamin C content of the raw vegetables

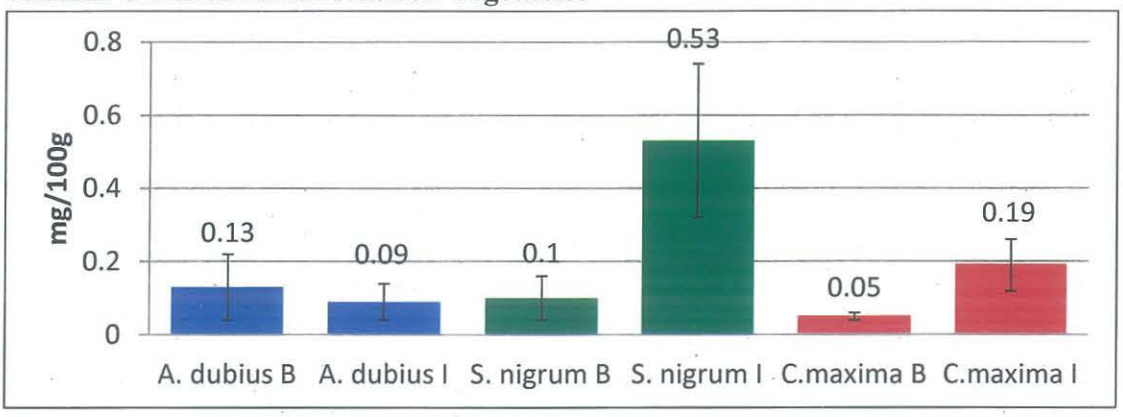


Key **B:** Bushenyi **I:** Ishaka

Figure 20: Vitamin C content of the raw vegetables: The Vitamin C content of the raw vegetables from Bushenyi and Ishaka markets is compared. The various samples are shown in X-axis while the Y-axis shows the Vitamin C present in mg/100g

Among the raw samples, *S.nigrum* had the lowest Vitamin C content, while *A. dubius* had the highest content as shown in Figure 20. The variation among the two cultivars of *A.dubius* (P-value= 0.009, one-tailed T-test) and *C. maxima* (P-value=0.006, one-tailed T-test) was statistically significant.

Vitamin C content of the steamed Vegetables



Key **B:** Bushenyi **I:** Ishaka

Figure 21: Vitamin C content of the steamed vegetables: The Vitamin C content of the steamed vegetables from Bushenyi and Ishaka is compared. The various samples are shown in X-axis while the Y-axis shows the Vitamin C present in mg/100g

Among the steamed samples, *S. nigrum* from Ishaka had the highest vitamin C content, followed by *C. maxima* from Ishaka as shown in Figure 21.

Vitamin C content of the boiled vegetables

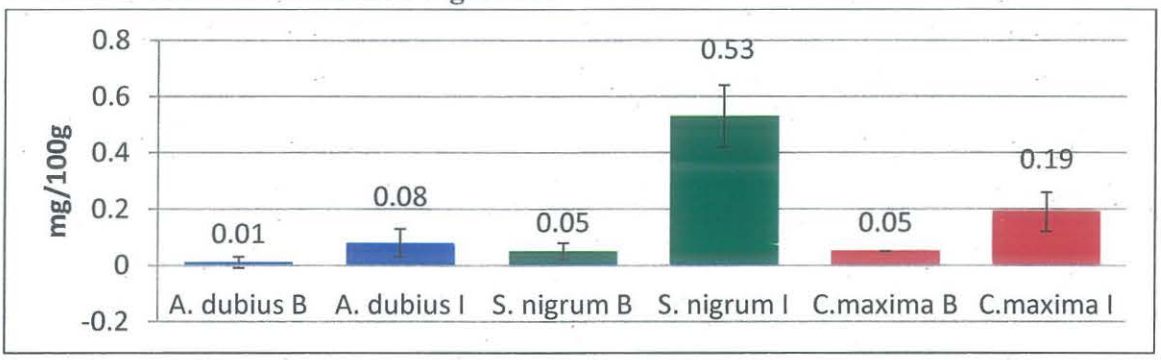


Figure 22: Vitamin C content of the boiled vegetables: The Vitamin C content of the boiled vegetables from Bushenyi and Ishaka is compared. The various samples are shown in X-axis while the Y-axis shows the Vitamin C present in mg/100g

The boiled samples from Ishaka had higher vitamin C content than the boiled samples from Bushenyi as shown in figure 22. On average *A. dubius* had the lower values while *C. maxima* had the highest values.

4.6. 2 Comparative analysis of the Reducing Power of the vegetables

Reducing power of the raw vegetables

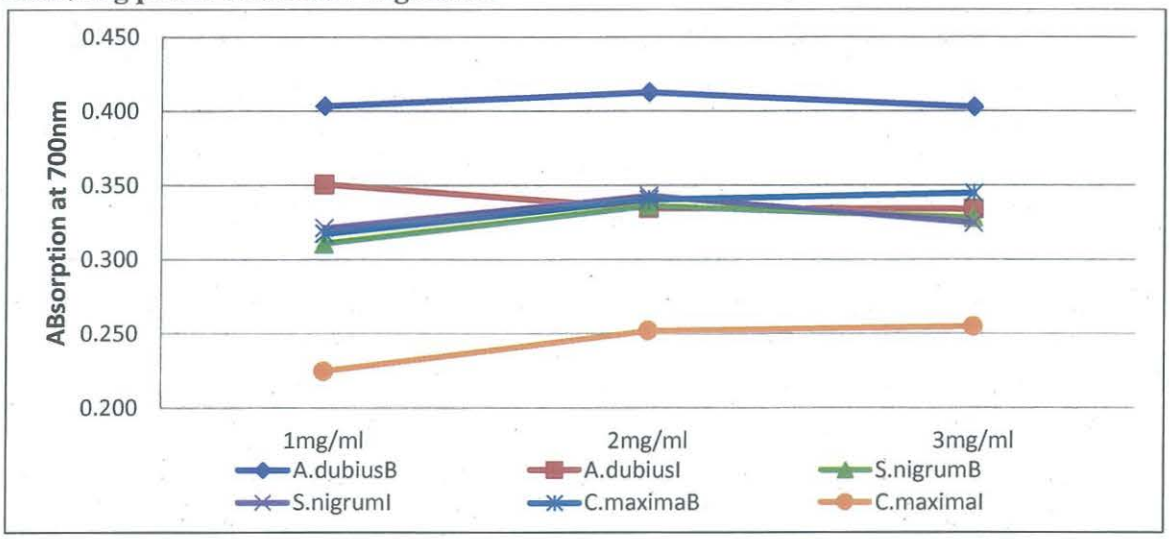


Figure 23: Reducing Power of the raw vegetables: The reducing power of the raw vegetables is compared. The various concentrations are shown in X-axis while the Y-axis shows the absorbance at 700nm which is a measure of the reducing power.

The extract of *A.dubius* Bushenyi had the highest reducing power, while that of *C. maxima* Ishaka had the lowest as shown in figure 23. The variation among the two cultivars of *S.nigrum* (P-value= 0.014, one-tailed T-test) and *C. maxima* (P-value=0.0001, one-tailed T-test) were statistically significant.

Reducing power of the steamed vegetables

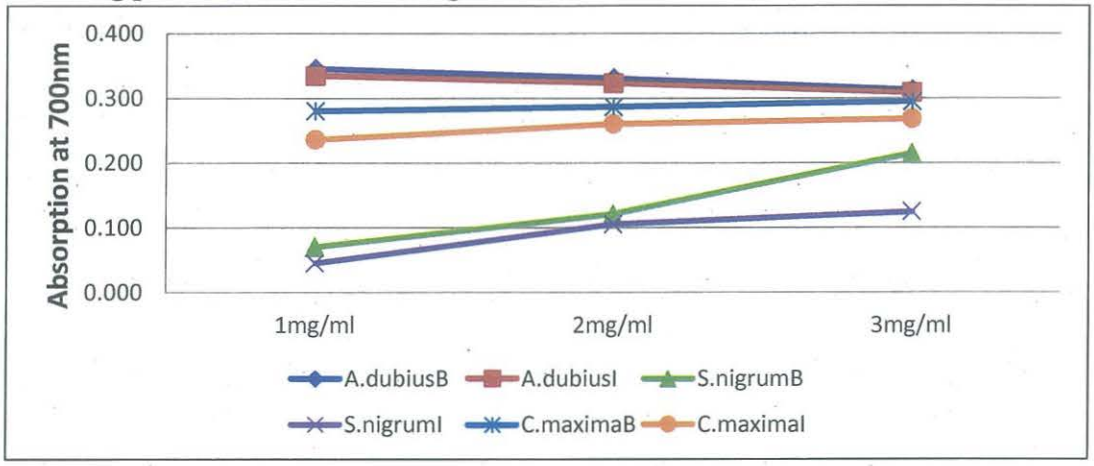


Figure 24: Reducing power of the steamed vegetables: The reducing power of the steamed vegetables is compared. The various concentrations are shown in X-axis while the Y-axis shows the absorbance at 700nm which is a measure of the reducing power.

The extract *A.dubius* had the highest reducing power while *S.nigrum* had the lowest reducing power as shown in figure 24. There was a statistically significant variation (P-value= 0.013, one-tailed T-test) of the two cultivars of *C.maxima*.

Reducing Power of the boiled vegetables

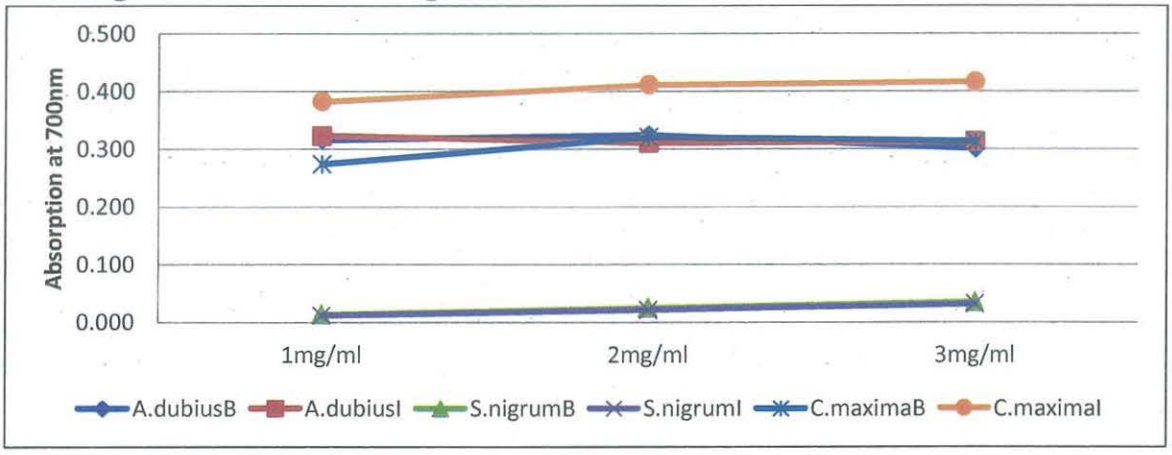


Figure 25: Reducing power of the boiled vegetables: The reducing power of the boiled vegetables is compared. The various extract concentrations are shown in X-axis while the Y-axis shows the absorbance at 700nm which is a measure of the reducing power.

Reducing power was lowest in the boiled *S.nigrum*, while it was highest in *C.maxima* from Ishaka as shown in figure 25. There was a statistically significant variation (P-value= 0.014, one-tailed T-test) of the two cultivars of *C.maxima*.

4.6.3 Comparative analysis of the Hydrogen peroxide scavenging activity of the vegetables

Hydrogen peroxide scavenging activity of the raw vegetables

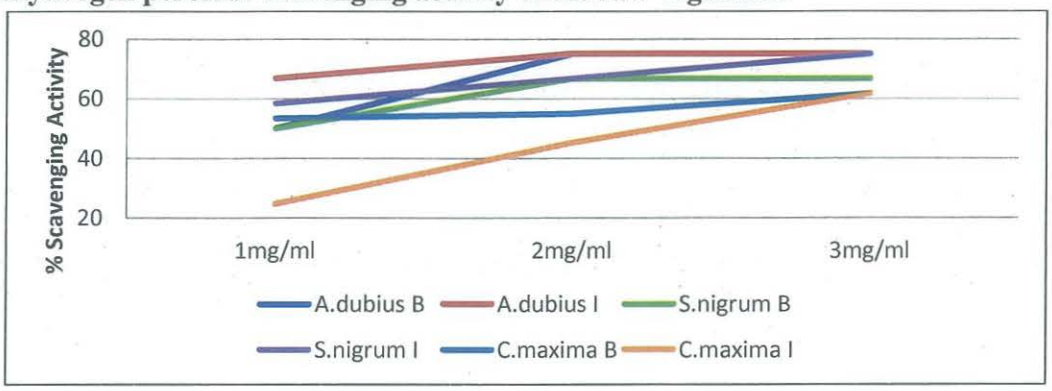


Figure 26: Hydrogen peroxide scavenging activity of the raw vegetables: The % scavenging activity of the raw vegetables is compared. The various extract concentrations are shown in X-axis while the Y-axis shows the % scavenging activity.

A.dubius from Ishaka had the highest scavenging activity while *C. maxima* from Ishaka had the lowest scavenging activity among the raw vegetables as depicted in figure 26 above.

Hydrogen peroxide scavenging activity of the steamed vegetables

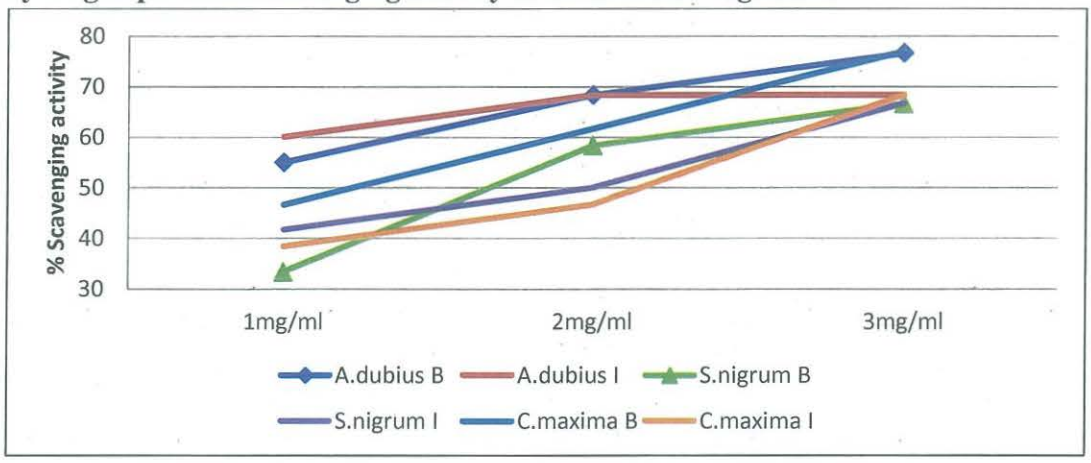


Figure 27: Hydrogen peroxide scavenging activity of the steamed vegetables: The % scavenging activity of the steamed vegetables is compared. The various extract concentrations are shown in X-axis while the Y-axis shows the % scavenging activity.

On average the steamed extracts of *A.dubius* had the highest scavenging activity as shown in figure 27. The variations between the cultivars of *A.dubius* (P-value= 0.410, one-tailed T-test), *S. nigrum* (P-value= 0.298, one-tailed T-test) and *C.maxima* (P-value= 0.253, one-tailed T-test) were not statistically significant.

Hydrogen peroxide scavenging activity of the boiled vegetables

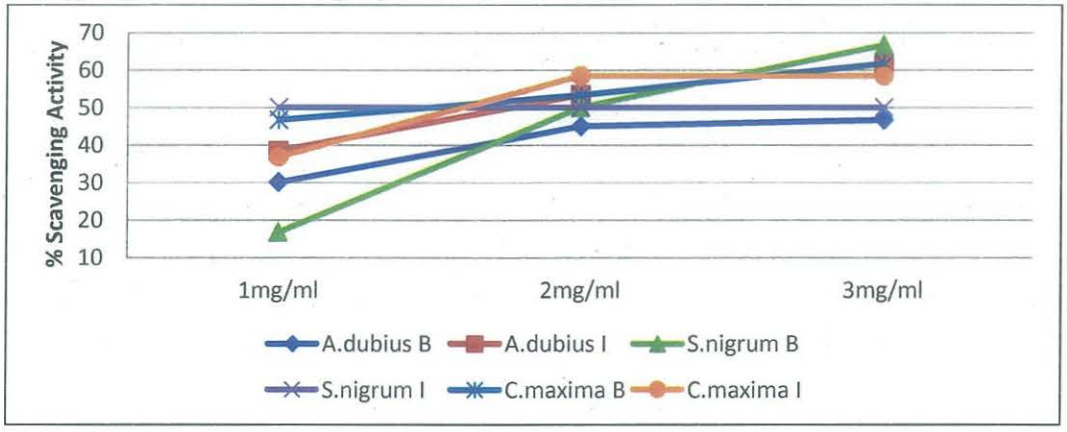


Figure 28: Hydrogen peroxide scavenging activity of the boiled vegetables: The % scavenging activity of the boiled vegetables is compared. The various extract concentrations are shown in X-axis while the Y-axis shows the % scavenging activity.

The extracts of boiled *C.maxima* had on average higher scavenging activity; compared to *A.dubius*, which was statistically significant (P-value=0.028, one-tailed T-test).

4.6. 4 Comparative analysis of the Antihemolytic activity of the vegetables

Antihemolytic activity of the raw vegetables

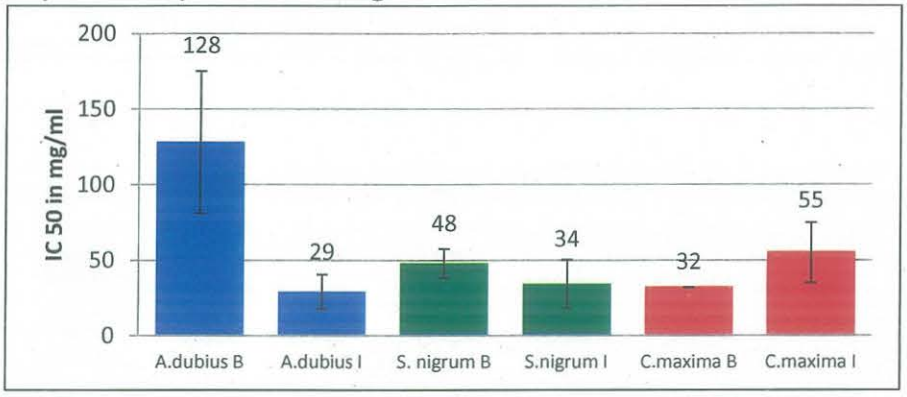


Figure 29: Antihemolytic activity of the raw vegetables: The IC_{50} of the raw vegetables and is compared. As the IC_{50}, increases, Antihemolytic activity of the sample reduces. The various samples are shown in X-axis while the Y-axis shows IC_{50} in mg/ml.

The extract of the raw *A.dubius* from Ishaka had the highest Antihemolytic activity while *A.dubius* from Bushenyi had the lowest activity as shown in Figure 29.

Antihemolytic activity of the steamed vegetables

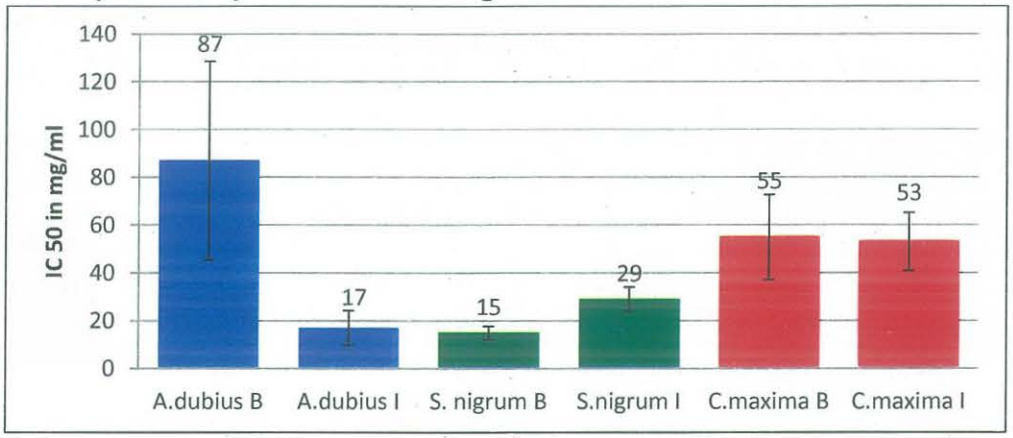


Figure 30: Antihemolytic activity of the steamed vegetables: The IC_{50} of the steamed vegetables and is compared. As the IC_{50}, increases, Antihemolytic activity of the sample reduces. The various samples are shown in X-axis while the Y-axis shows IC_{50} in mg/ml

The extracts of steamed *A.dubius* from Ishaka and *S.nigrum* from Bushenyi had the highest Antihemolytic activity while *A.dubius* Bushenyi had the lowest Antihemolytic activity

Antihemolytic activity of the boiled vegetables

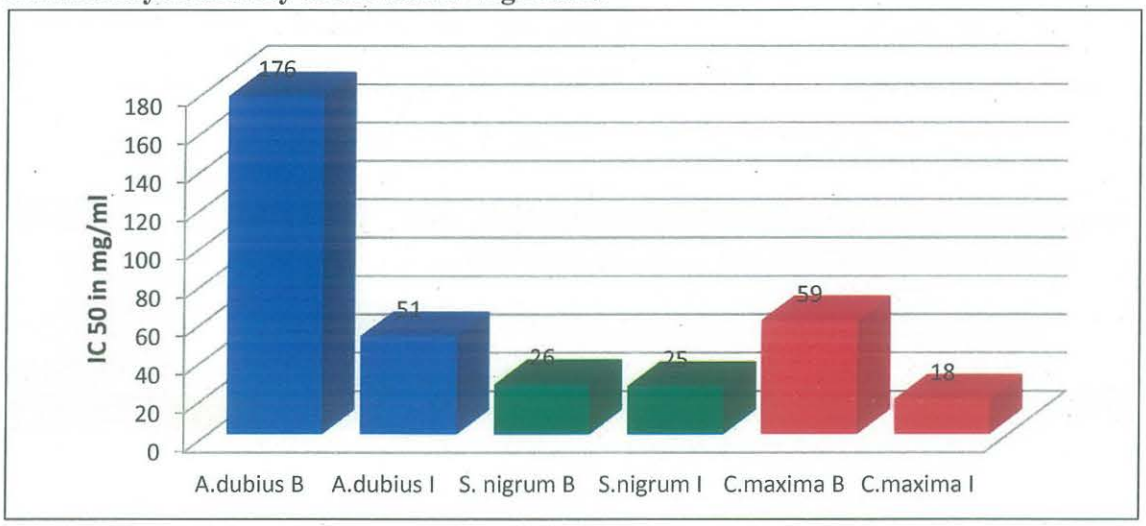


Figure 31: Antihemolytic activity of the boiled vegetables: The IC_{50} of the boiled vegetables and is compared. As the IC_{50}, increases, antihemolytic activity of the sample reduces. The various samples are shown in X-axis while the Y-axis shows IC_{50} in mg/ml

Among the boiled vegetables, extract of *C.maxima* Ishaka had the highest Antihemolytic activity followed by the extracts of *S.nigrum* as shown in figure 31.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

Vegetables are usually processed by application of dry or moist heat to improve their organoleptic properties or extend their shelf life, eliminate potential pathogens, neutralize poisonous or irritating substances as well as bring a halt to spoilage (Martin and Ruberte, 1998). Methods used to process vegetables such as drying, shredding, steaming, blanching, boiling, sterilizing and freezing are expected to affect the yield, composition and bioavailability of antioxidants (Amin *et al.*, 2006) and some nutritional antioxidants such as heat labile vitamin C.

This study sought to find out the effecting of cooking as done in Bushenyi district on the antioxidant activities of three commonly consumed vegetables in the District. This study ascertained that cooking affected the antioxidant activity of the vegetables which correlated with the review of Amin *et al.*, (2006). This implies that in Bushenyi District, where the vegetables are never eaten raw but are cooked by either steaming or boiling, the antioxidant activity is decreased for the vegetables, resulting in a reduction of the beneficial effects.

The study also revealed variation in vitamin C and antioxidant activity among the different cultivars of the vegetables. This could be explained by several factors. The differences may be due to genetic and environmental factors including the climate, the soil mineral composition and the carrying and storage conditions at the markets (Tomas-Barberan and Espin; 2001) Antioxidant potential varies depending on the maturity stage of the plant probably due to a more active plant metabolism which accompanies active or rapid growth in the first few months (Soengas *et al.*. 2011). Kim *et al.*. (2004) found that young cabbages had relatively higher amounts of flavonoids compared with mature cabbage, suggesting that maturity may affect the phenolic content. Several environmental factors may play a crucial role in the development and phytochemical content of vegetables. Kim *et al.*, (2004) found growing vegetables using organic fertilizers

increased their antioxidant capacity. These factors could thus explain the variation seen in the vitamin C, reducing power, hydrogen peroxide scavenging activity and Antihemolytic activity of the different samples obtained from Bushenyi and Ishaka of the same vegetable.

5.1.1 Vitamin C content

As much as households would like to include vitamin C in their menus, this study revealed that the source as well as the method of preparation is important. Cooking as done in Bushenyi has been shown to cause great reduction in the vitamin C content of the three vegetables. Vitamin C is heat labile and extremes of temperatures tend to destroy the vitamin (Nagy and Smooth, 1977, Adefegha and Oboh, 2009). Vitamin C is water soluble and thus losses could be due to leaching of the vitamin into the water used to boil (Oboh, 2005). Agbemafle *et al.*, (2012), reports similar findings in his study, and reports highest losses of vitamin C occur during the first 5 and 10 minutes of boiling. Funke, (2011) also reports similar findings among Amaranth leaves cooked by boiling and paraboiling. Destruction of enzymes and expulsion of oxygen from the water used to boil the vegetables due to high temperatures could explain the losses in vitamin C incurred by this method (Agbemafle *et al.*, 2012).

5.1.2 Antioxidant activity

Despite the great reduction in vitamin C levels of the steamed and boiled vegetables, they show significant antioxidant activity. This indicates that antioxidant activity in these vegetables may be due to compounds other than vitamin C. A great number of plant secondary metabolites that have antioxidant activity have been isolated (Shahvar *et al.*, 2010). These include vitamins C, A and E, as well as a number of food-derived poly-aromatic substances, belonging to stilbenes, flavonoids and phenolic acids as the main classes of nutritional antioxidants (Wayner *et al.*, 1987). A study on the evaluation of antioxidant potential in selected leafy vegetables by Routray *et al.*, (2013) revealed presence of phenolic compounds in raw methanolic and ethanolic extracts of several *Amaranth spp.* and *Cucubita maxima* leaves. Enzymic and non enzymic antioxidants

such as tannins, Flavonoids and phenolic have also been shown in *S.nigrum* (Maharana *et al.*, 2010, Jayachitra and Krithiga, 2012).

This study showed that the effect of cooking on antioxidant capacity of different vegetables maybe different. Different plants contain various compounds some of which are thermally labile and some are not and therefore the same cooking method may have different effects on different types of plants (Bernhardt and Schlich, 2006).

Vegetables in this study showed significant reducing capacity. This may be attributed to the presence of reductones which are terminators of free-radical chain reactions as suggested by Kumar and Kumar (2009).The increase in reducing power seeen with increase in concentration of the extract could be attributed to an increased concentration of reductones. The decrease of reducing power in the steamed and boiled vegetables may be due to breakdown of antioxidant compounds such as phenolics, tannins and flavonoids and their leaching into the surrounding water (Posedek, 2007). Some antioxidants compounds such as ascorbic acid and carotenoids are very sensitive to heat and storage and are lost during different vegetable processing steps (Zhang and Hamauzu, 2004). Akubugwo *et al.*, (2008) studied the effect of different processing methods on the mineral and phytochemical content of *A. hybridus* and *S.nigrum* leaves. They found a reduction in the alkaloid, flavonoids, saponins, tannins and phenols by the steaming method. This may as well support the reduced activity of the steamed and boiled extract of *A.dubius* and *S.nigrum* extracts in our study.

Surprisingly, there was increase in the reducing activity of *C. maxima* harvested from Ishaka after boiling. This increase may be due to production of redox active secondary plant metabolites or break down products, but is highly likely to be related to release of antioxidants from intracellular proteins, changes in plant cell wall structure, matrix modifications and more efficient release of antioxidants following boiling as reviewed by Rechkemmer (2007).

Hydrogen peroxide formed by the reaction catalyzed by superoxide dismutase as part of the antioxidant cascade, xanthine oxidase and phagocytes is an important reactive oxygen

species (Okoko and Ere, 2012). It has the capacity to damage cells and macromolecules such as proteins and DNA. Though hydrogen peroxide can be reduced to water via catalase activity, excessive production could cause severe circumstances hence the need for other scavengers. In this study steaming for 10 minutes had an overall increase in the hydrogen peroxide scavenging activity of *C.maxima* while boiling reduced the scavenging activity of all the three vegetables. The fact that steaming increased the hydrogen peroxide scavenging activity of *C.maxima* points to the pro-oxidant activity being due to peroxidase enzymes, which are inactivated by high temperatures (Gazzani *et al.*, 1998).

5.1.3 Antihemolytic activity

Erythrocytes are major targets for free radicals due to the presence of both high membrane concentrations of polyunsaturated fatty acids (PUFA) and the oxygen transport associated with redox active hemoglobin molecules (Ebrahimzadeh *et al.*, 2009). In this present study, the vegetables showed higher protective effect on erythrocytes than the positive control vitamin C. This may be due to the radical scavenging activity of the bioactive components present in the vegetable extracts showing potent antihemolytic nature of the vegetables. Flavonols and their glycosides are efficient antioxidants which guard erythrocytes from free radical mediated oxidative hemolysis (Dai *et al.*, 2006). This is because the binding of flavonoids to the red blood cell membranes significantly inhibits lipid peroxidation and at the same time, enhances their integrity against lysis (Chaudhuri et al., 2007). Thus in this study, inhibition of hydrogen peroxide mediated hemolysis could indicate presence of radical scavenging polyphenols especially flavonoids. The higher antioxidant activity of the steamed extracts may be due to greater release of the flavonoids. In contrast, *C.maxima* from Ishaka had higher Antihemolytic activity on boiling just as it was seen in reducing power. This sample may have been of older leaves which have been found to be tougher than younger leaves (Bittencourt-Rodrigues and Zucoloto, 2005) and may thus require higher temperatures to break down the tough cell membranes and release the intracellular antioxidant components.

5.2 Conclusions

The vitamin C content of *A.dubius, S. nigrum* and *C. maxima* on the average is 4.58mg/100g, 0.76mg/100g and 3.8mg/100g respectively.

Antioxidant activity of the cooked vegetables is due to other compounds in addition to vitamin C since they showed both reducing power and hydrogen peroxide scavenging activity in spite of great losses of vitamin C after cooking.

The antioxidant activity of the three vegetables is enough to confer some Antihemolytic activity.

Cooking the vegetables resulted in dramatic losses of vitamin C content when compared to the raw vegetables

Steaming may retain and in some instances promote the antioxidant activity of the vegetables while boiling reduces the antioxidant activity.

Finally, this study has a take home message that *Amaranthus dubius* is more reliable as a source of antioxidants second ranked by *Cucubita maxima* and lastly *Solanum nigrum.*

5.3 Recommendations

Individuals with increased requirement for antioxidation for example diabetics and hypertensive patients should consume *Amaranthus dubius.*

Consumers of the vegetables should consider other methods of cooking such as blanching or cook the vegetables in less water and consume the water used for cooking as part of the diet.

5.4 Further work

The role of various factors such as maturity during harvest, effect of different fertilizers and postharvest storage on the antioxidant activity should be further investigated

Phytochemical screening of the raw, steamed and boiled vegetables should be performed to determine which secondary plant metabolites are responsible for the antioxidant activity

REFERENCES

Abukutsa-Onyango M.O. (2003). Unexploited potential of indigenous African indigenous vegetables in Western Kenya. *Maseno Journal of Education, Arts and Science*; 4:103-122.

Acquaah G. (2004) Horticulture: Principles and Practices 3rd edition Upper saddle River: New Jersey, Pearson Education Inc. pp 33-35.

Adefegha S. A. and Oboh G. (2009). Cooking enhances the antioxidant properties of some tropical green leafy vegetables. *African Journal of Biotechnology* 10 (4):632-639.

Agbemafle R., Obodai E. A., Adukpo G. E., Amprako D. N. (2012). Effects of boiling time on the concentration of vitamin C and βeta carotene in five selected green vegetables consumed in Ghana. *Adv. Appl. Sci. Res.*, 3(5): 2815-2820

Akubugwo I.E., Obasi N. A., Chinyere G.C., and ugbogu A. E. (2008). Mineral and Phytochemical contents in leaves of *Amaranthus hybridus L.* and *Solanum nigrum L.* subjected to different processing methods. *Afr. J. Biochem. Res.* Vol 2(2): 040-044

Ame S.N., Shigrenaga M.K., Hagen T.M., (1993). Oxidants, antioxidants and degenerative diseases of ageing. *Proc Natl Acad Sci USA* 90: 7915-22.

Amin I., Norazaida K., Hainida K.I.E (2006). Antioxidant activity and phenolic content of raw and blanched Amaranthus species. *Food Chem.*, 94: 47-55

Apak Resat, Guclu Kubilay, Birsen D., Ozyurel M., Celik S.E., and Ozyurt D. (2007). Comparative evaluation of various Total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. Molecules. 12; 1496-1547

Arulmozhi V., Krishnaveni M., Karthishwaran K., Dhamodharan G., Mirunalini S. (2010) Antioxidant and antihyperlipidemic effect of *Solanum nigrum* fruit extract on the experimental model against chronic ethanol toxicity. *Phcog Mag; 6:42-50.*

Chweya J. A., (1985). Identification and nutritional importance of indigenous green leafy vegetables in Kenya. *Acta Hort.*; 153:99-108.

Chweya J.A. and Eyzaguire P.B. (eds) (1999) The biodiversity of traditional Leafy Vegetables. *International Plant Genetic Resources Institute. Rome. Italy.* Pp 34-38

Clark I. A., Chaudhri G., Cowden W. B., (1989). Some roles of free radicals in Malaria. *Free Radic. Biol Med.* 6(3) 315-21.

Cooke M.S., Evans M.D., Dizdaroglu M, Lunec J. (2003). Oxidative DNA damage: mechanisms, mutation and disease. *Faseb J.* 17:1195–1214.

Cooper M.R., Johnson A.W. (1984). Poisonous Plants in Britain and other effects on Animals and Man. *Ministry of Agriculture, Fisheries Food,* 161: 219-220.

D'Arcy, W.G. (1991). The Solanaceae since 1976, with a Review of its Biogeography. Pp.75-137 *in* Solanaceae III: Taxonomy, Chemistry and Evolution (J.G. Hawkes, R.N. Lester, M. Nee and N. Estrada-R., eds.). Academic Press, London. Pp 73-75

Dai FQ, Miao B., Zhou L., Liu Z. L. (2006). Protective effect of flavonols and their glycosides against free radical induced oxidative hemolysis of red blood cells. *Life Sci* 78: 2488-2493.

Dalle-Donne, I., Rossi, R., Colombo, R., Giustarini, D., & Milzani, A. (2006). Biomarkers of oxidative damage in human disease. *Clin. Chem., 52*: 601–623.

Davey M.W., Van M. M., Inze D., Sanmartin M., Kannelis A., Smirnoff N., Benzie I. J.J., Strain J. J., Favell D., Fletcher J. (2000). Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. *Journal of the Science of Food and Agriculture,* 80, 825-860.

Devasagayam T.P.A, Tilak J.C., Boloor K.K., Ketaki S. Sane, Saroj S. Ghaskadbi, R.D. Lele (2004). Free Radicals and Antioxidants in Human Health: Current Status and Future Prospects. *JAPI.* 52 (2): 36-37.

Devasagayam T.P.A. and Kesavan P.C. (1996) Radioprotective and antioxidant action of caffeine: Mechanistic considerations. *Indian J Expt Biol*: 34, 291-7.

Di Matteo V., Esposito E., (2003). Biochemical and therapeutic effects of antioxidants in the treatment of Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. Curr Drug Target CNS *Neurol Disord* 2: 95-107.

Ebrahimzadeh M.A., Nabavi S.M., Nabavi S.F. (2009). Correlation between the in vitro iron chelating activity and polyphenol and flavonoid contents of some medicinal plants. Pak J *Biol Sci*; 12(12): 934-938.

Ebrahimzadeh M.A., Nabavi S.M., Nabavi S.F., Eslami B., Rahmani Z. (2010). Antioxidant and antihemolytic activities of the leaves of Kefe cumin (Laser trilobum L) Umbelliferae. *Tropical Journal of Pharmaceutrical Research* 9 (5): 441-449.

Edmonds M. Jennifer and James A. Chweya. (1997). Black nightshades. *Solanum nigrum* L. and related species. Promoting the conservation and use of underutilized and neglected crops. 15. Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, Rome, Italy. Pp 35-38

Embu P.K. and Anyika J.U, (2011) Proximate and Mineral Composition of Kale (*Brassica oleracea*) Grown in Delta State. Nigeria. *Pakistan Journal of Nutrition* 10 (2): 190-194.

Fang Y., Yang S. and Wu G. (2002). Free radicals, antioxidants and nutrition. *Nutrition*, 18: 872-879

Fedha, M.S., Mwasaru, M.A., Njoroge, C. K,Ojijo, N. O and Ouma, G. O. (2010). Effect of drying on selected proximate composition of fresh and processed fruits and seeds of two pumpkin species *Agric. Biol. J. N. Am.*, 1(6): 1299-1302

Ferreira C.F.R., Baptista P., Vilas-Boas M. and Barros L. (2007). Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: Individual cap and stipe activity. *Food Chemistry 100*: 1511-1516. DOI: 10.1016/j.foodchem.2005.11.043.

Finkel T., and Holbrook N. J. (2000). Oxidants, oxidative stress and the biology of ageing. *Nature* 408, 239-247.

Fortuin, F.T.J.M. and S.W.P. Omta. (1980). Growth analysis and shade experiment with *Solanum nigrum* L., the black nightshade, a leaf and a fruit vegetable in West Java. *Neth. J. Agric. Sci.* 28(4):199-210.

Funke O.M., (2011). Evaluation of nutrient contents of Amaranth leaves prepared by different cooking methods. *Food and Nutrition Sciences*, 2, 249-252.

Gazzani, G., Papetti, A., Massolini, G. & Daglia, M. (1998). Antioxidative and pro-oxidant activity of water soluble components of some common diet vegetables and the effect of thermal treatment. *Journal of Food Chemistry*, 6: 4118- 4122.

Gazzani, G., Papetti, A., Massolini, G. & Daglia, M. (1998). Antioxidative and pro-oxidant activity of water soluble components of some common diet vegetables and the effect of thermal treatment. *Journal of Food Chemistry*, 6: 4118- 4122.

Gerber M, Boutron-Ruault MC, Hercberg S, Riboli E, Scalbert A, Siess M.H. (2002). Food and cancer: state of the art about the protective effect of fruits and vegetables. *Bull Cancer* 89: 293-312.

Goode P.M. (1989). Edible plants of Uganda. The value of wild and cultivated plants as food. FAO Food and Nutrition Paper 42/1. Food and Agriculture Organization of the United Nations, Rome, Italy. Pp 32-69

Greenly W. Larry (2004). A Doctor's Overview of Free Radicals and Four Synergistic Antioxidants, *Journal of Chiropractic Medicine.88:236-242*

Grubben, G.J.H. (2004). *Amaranthus dubius* Mart. ex Thell. [Internet] Record from Protabase. Grubben, G.J.H. & Denton, O.A. (Editors). PROTA (Plant Resources of Tropical Africa / Ressources végétales de l'Afrique tropicale), Wageningen, Netherlands. <http://database.prota.org/search.htm>. Accessed 03/07/2012 at 7:49 am.

Halliwell B. (2006) Oxidative stress and neurodegeneration: Where are we now? *J of Neurochemistry*. 97:1634–1658.

Halliwell, B. and Gutteridge, J. M. C. (1999). *Free radicals in biology and medicine* (3rd ed.). Oxford University Press. Pp72-80

Henderson, R.J.F. (1974). *Solanum nigrum* L. (Solanaceae) and related species in Australia. Contributions from the Queensland Herbarium No. 16:1-78.

Hernandez Y., Lobo M.G., Gonzalez M., (2006). Determination of vitamin C in tropical fruits: A comparative evaluation of methods. *Food Chem.* 96: 654-664.

Hue S., Boyce A. N., Somasundram C., (2011). Influence of growth stage and variety on the pigment levels in *Ipomoea batatas* (sweet potato) leaves. *Afr. J. Agric. Res.* 6(10) 2379-2385

Imungi J.K. (2002). The brighter side of Phenolic compounds abundance in African leafy vegetables Nairobi. *IPGRI Newsletter for Sub-Saharan Africa* Issue no. 17. Pp 17-20

Jaeger P.M.L. and Hepper F. N. (1986) A review of the genus Solanum in Africa, In: *Solanaceae: biology and systematics* (eds) W. G. D'Arcy (New York: Columbia University Press) pp 41–55.

Jayachitra A., Krithiga N. (2012). Study on antioxidant property in selected medicinal plant extracts. *International Journal of Medicinal and Aromatic plants* 2(3) 495-500.

Jayanthi P., and Lalitha P. (2011) Reducing power of the solvent extracts of *Eichornia crassipes (Mart) solms. Int.J Pharm Pharm. Sci.* Vol.3 Suppl. 126-128

Kähkönen M.P., Hopia A.I., Vuorela J.H., Rauha J.P., Pihlaja K., Kujala T.S., Heinonen M.(1999) "Antioxidant activity of plant extracts containing phenolic compounds" *J Agric Food Chem* 47: 3954-3962.

Kahl R., Kappus H. (1993). Toxicology of the synthetic antioxidants BHA and BHT in comparison with the natural antioxidant vitamin E. *Z. Lebensm Unters Forsch.* 196: 329-338.

Kashou A. H. and Agarwal A. (2011). Oxidants and Antioxidants in the pathogenesis of HIV/AIDS. *The Open Reproductive Science Journal.* Vol 3: 154-161

Katerere David R., Giulia Graziani, Kaizer M. Thembo, Norman Z. Nyazema and Alberto Ritieni (2012) Antioxidant activity of some African medicinal anddietary leafy African vegetables. *African Journal of Biotechnology 11(17):4103-4108* DOI : 10.5897/AJB11.3674

Kauffman, C.S.,Weber, L.E. (1990). Grain amaranth. In: *Advances in New Crops*, J. Janick & J.E. Simon (Eds). Timber Press, Portland, Oregon: 127-139.

Ke Cui, Xiaoling Luo, Keyi Xu, Ven Murthy M.R. (2004). Progress in neuropsychopharmacology and biological psychiatry, *Lebensm Unters Forsch* 28 (5): 771-799.

Kim D.O., Padilla-Zakour OI, Griffiths P.D. (2004). Flavonoids and antioxidant capacity of various cabbage genotypes at juvenile stage. *Journal of Food Science* 69, C685-C689

Kimiye J., Waundo J., Mbithe D. (2006). Reducing hidden hunger and malnutrition through traditional foods Nairobi. *IPGRI Newsletter for Sub- Saharan Africa* Issue No. 21.

Kimiywe J., Martorell R., Per P., Rubaihayo P. (2011). Mainstreaming Nutrition with Agriculture in Uganda; Role of Agriculture in Improving the Nutritional Status of Women and Children. *Uganda National Academy of Sciences*, Chapter 2 pp19 ISBN: 9970-866-08-4

Kiremire B.T., Kikafunda J. K., Musinguzi E. (2006). Utilization of indigineous food plants in Uganda: A Case study of South-Western Uganda. *African Journal of Food Agriculture Nutrition and Development.* 6 (6) Online Version ISSN 1684-5374

Kirtikar, K.R. and B.D. Basu, (2003). Indian Medicinal Plants. 2nd Edn., Oriental Enterprises, Uttaranchal, India, pp: 1606-1608.

Kokwaro, J.O. (1976). Medicinal plants of East Africa. East African Literature Bureau: Nairobi, Kampala, Dar-es-Salaam. Pp 48-72

Kumar S. and Kumar D. (2009). Antioxidant and free radical scavenging activity of edible weeds. *African Journal of food Agriculture nutrition and development* .Vol 9 (5) pp 1174-1190.

Kumar Shiv, (2011). Free Radicals and Antioxidants: Human and Food System. *Adv. Appl. Sci. Res., 2 (1):129-135*

Lee C.Y., Massey L.M. Jr, Van Buren, J.P., (1982). Effects of postharvest handling and processing on vitamin contents of peas. *J. Food Sci.* 47: 961–964.

Lee S.K. and Kader A.A. (2000). Preharvest and postharvest factors influencing Vitamin C content of horticultural crops. *Postharvest Biology and Technology 20 207-220*

Lee S-J, Lim K-T (2006). 150 kDa glycoprotein isolated from *Solanum nigrum* Linne stimulates caspase-3 activation and reduces inducible nitric oxide production in HCT-116 cells. Toxicol. *in Vitro*, 20(7): 1088- 1097.

Leung, W.-T.W., Busson, F. & Jardin, C. (1968). Food composition table for use in Africa. FAO, Rome, Italy pp 306.

Liu, R.H. (2004). Potential synergy of phytochemicals in cancer prevention: mechanism of action. *J. Nutrition.* 134: 34795-34855.

Liu, R.H. Dewanto, V., Wu, X. and Adorn, K. (2002). Effect of thermal processing on the nutritional values of tomatoes *http://ift.confex.com/ift/2002/techprogram/paper_14046.htm*

MacCalla A.F. (1994). Agriculture and Food Need in 2025; why we should be concerned. Consultative group on International Agricultural Research (CGIAR), Washington DC.

Maharana L., Kar D. M., Pattnail s., Sahu P. K., Si S. C. (2010) *Invitro* antioxidant activity of aqueous leaf extract of *Solanum nigrum Linn. Pharmacology online* 3: 333-345

Manoko, M.L. (2007). *A systematic study of African Solanum L. section Solanum* (*Solanaceae*). International Plant Genetic Resources Institute, Rome, Italy pp 4

Mathers C.D., Loncar D. (2006) Projections of Global Mortality and Burden of Disease from 2002 to 2030. *PLoS Med* 3(11): e442. doi:10.1371/journal.pmed.0030442

Mathooko, F.M. and J.K. Imungi. (1994). Ascorbic acid changes in three indigenous Kenyan leafy vegetables during traditional cooking. *Ecol. of Food and Nutr.* 32:239-245.

Maundu P. M. (1997). The status of traditional vegetable utilization in Kenya. In: L Guarino (Ed). Proceedings of the IPGRI International workshop on genetic resources of traditional vegetables in Africa: Conservation and use. ICRAFHQ, Nairobi, Kenya: 66-75.

Maundu P.M., Njiro E.I., Chweya J.A, Imungi J.K. and Seme E.N., (1999). The Kenyan Case Study in: Chweya J.A. and Eyazaguirre P.B. (eds), *The Biodiversity of Traditional Leafy Vegetables.* Rome IPGRI 51-84

Mepha H. D, Eboh L. and. Banigo D E.B (2007) Effects of processing treatments on the nutritive composition and acceptance of some Nigerian Leafy Vegetables.*African Journal of food Agriculture and Nutrition 7(1).*

MOH 2012: Ministry of Health, Republic of Uganda. http://health.go.ug/mohweb/?page_id=761 Accessed June 20th 2012 at 1:09 am.

Musana MS, Rubaihayo EB (2001). Horticulture.In: Mukiibi,J.K. (ed.).Agriculture in Uganda. Volume II. Crops. Fountain Publishers pp.532-555.

Muyonga H. J., Nabakaya D., Nakimbugwe D. and Masinde D. (2008). Chapter 8: Efforts to Promote Amaranth Production and Consumption in Uganda to fight Malnutrition. In Robertson G. L. and Lupien J.R (Eds) (2008) Using Food Science and Technology to improve Nutrition and Promote National Development. *Printed by International Union of Food Science and Technology. Pp99-107*

Nagy S, Smooth JM (1977). Temperature and storage effects on percent retention and percent U.S. recommended dietary allowances of vitamin C in canned single-strength orange juice. J. *Agric. Food Chem.* 25(1): 135-138

Nagy S. (1980). Vitamin C contents of citrus fruit and their products: a review. *J. Agric. Food Chem.* 28: 8–18.

Nana Fernand W, Adama Hilou, Jeanne F. Millogo2 and Odile G. Nacoulma (2012) Phytochemical Composition, Antioxidant and Xanthine Oxidase Inhibitory Activities of *Amaranthus cruentus* L. and *Amaranthus hybridus* L. Extracts. *Pharmaceuticals*, *5*, 613-628; doi: 10.3390/ph5060613

Ngishilli, (2009): Cook and Bull stories: Mukimo. http://ngishili.com/?p=282. Accessed on 13th February 2012.

Nielsen S. S. (2010). Food Analysis Laboratory Manual, Chapter 7: Vitamin C determination by Indophenol Method. Published by Springer Science + Business Media. Pp 57- 59. ISBN 978-1-4419-1462-0.

Nihal T., Sari F., Velioglus S. (2005). The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. *Food Chemistry* 93: 713-718

Nonhebel D.C, Tedder J.M., Walton J.C., Radicals (1979). Cambridge University Press; pp 200-231.

Oboh G (2005). Effect of blanching on the antioxidant property of some tropical green leafy vegetables. *Lebensm Wiss Technol* 38: 513-517.

Oboh G. and Akindahunsi A. A. (2004). Change in the Ascorbic acid, total phenol and antioxidant activity of sun-dried commonly consumed green leafy vegetables in Nigeria. *Nutrition and Health* 18:29 DOI: 10. 1177/026010600401800103

Ognjanovi B.I., Markovi S.D., Pavlovi S.Z., Žiki R.V., Štajn AŠ.., Saii Z.S. (2008). Effect of chronic cadmium exposure on antioxidant defense system in some tissues of rats: protective effect of selenium. *Physiol.Res.*, 57: 403-411.

Ogunlesi M., Okiei W., Ofor E., Awonuga O. (2009). Determinations of the concentrations of zinc and vitamin C in oysters and some medicinal plants used to correct male factor infertility. *Journal of Natural Products* Vol 2: 89-97

Okoko T. and Ere D. (2012) Antioxidant activities of Solenostemon monostachyus leaf extract using invitro methods. *Sci. Res. Essays* 7(6): 621-626. DOI: 10.5897/SRE 10.743

Olet E. A., Manfried Heun and Lye A. Kare, (2005). African crops or poisonous nightshade; the enigma of poisonous or edible black nightshade solved. *African Journal of Ecology 43. (2): 158-161.* DOI: 10.1111/j.1365-2028.2005.00556.x

Opole, M., J.A. Chweya and J.K. Imungi. (1991). Indigenous vegetables in Kenya. Field and Laboratory Experience Report. KENGO, Nairobi pp157-163

Oyaizu M., (1986) Studies on product of browning reaction prepared from glucose amine. *Jpn J Nutr* 07: 307-15.

Pala S. Funda and Tabakcioglu Kiymet (2007) Review: Free radicals: Our enemies or friends. *Advances in Molecular Biology* (1): 63-69.

Podesek A. (2007). Natural antioxidants and antioxidant capacity of Brassica vegetables: A review. *Lebensmittel-weissens und Technologie* 40, 1-11

Popkin B.M. (2002). An overview of the nutrition transition and its health implication: The Bellagio Meeting Public Health. *Nutr*; 5: 93-103

Prabhu S. and B. M. Diane, (2009). Effect of storage condition and domestic cooking on the quality and nutrient content of African leafy vegetables (*Cassia tora and Corchorus tridens*). *J Sci Food Agric* 2009; 89: 1709–1721 DOI 10.1002/jsfa.3644

Prior L. R.and G. Cao (2000), Analysis of Botanicals and Dietary Supplements for Antioxidant Capacity: A Review. *Journal of AOAC international* Vol 83, No.4. Downloaded from http://lib3.dss.go.th/fulltext/Journal/J.AOAC%201999-2003/J.AOAC2000/v83n4%28jul-aug%29/v83n4p950.pdf on 4th May 2012

Prior R. L., X. Wu, K. Schaich (2005) Standardized Methods for the Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements *J. Agric. Food Chem.*, 53, 4290-4302

Prior, R.L. and Cao, G. (2000). Antioxidant phytochemicals in fruits and vegetables. Diet and health implications. *Horticulture Science*, 35, 588±592.

Rattanachitthawat S, Suwannalert P, Riengrojpitak S, Chaiyasut C,Pantuwatana S (2010). Phenolic content and antioxidant activities in red unpolished Thai rice prevents oxidative stress in rats. *J. Med.Plants. Res.*, 4(9): 796-801.

Rechkemmer G. (2007). Nutritional aspects from thermal processing of food: Potential health benefits and risks In G. eisenbrand, K. H. Engel, W. Grunow, A. Hartwig, D. Knorr and I. B. Knudsen et al., (Eds),Symposium KGa.A Weinheim: Wiley VCH Verlag GmbH & Co.

Robards K., Antolovich M., (1997). Analytical chemistry of Fruit Bioflavonoids. A Review. *Analyst* 122: 11-34.

Routray R., Kar M., Sahu R. K (2013) Evaluation of antioxidant potential in selected leafy vegetables of Odisha India. *Int.J. Pharm Pharm Sci.* 5(1) 232-235.

Rubaihayo B. E., H. Tim, K. Edward, K. Achile, K. John, K. Flavia, M. Johnny, T. James and R. Patrick. (2004). Development of Mechanisms for Sustainable Production and Utilisation of Indigineous Vegetables and Management of their Genetic Diversity in Uganda. Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, Rome, Italy.

Rubaihayo, E.B. (1994). Indigenous (local) vegetables in Uganda. African Crop Science Conference Proceedings 1:120-124.

Rubaihayo, E.C. (1996). Local/indigenous vegetables in Uganda Research program. 1996 Annual report. Kawanda Agricultural Research Institute. Mimeo, 102pp.

Sahlin E., Savage G. P., & Lister C.e. (2004). Investigation of the antioxidant properties of tomatoes after processing. *Journal of Food composition and Analysis* 17; 635-637

Sawa T., Nakao M., Akaike T., Ono K., and Maeda H. (1999). Alkylperoxyl radical scavenging activity of various Flavonoids and other phenolic compounds: implications for the antitumor-promoter effect of vegetables. *Journal of Agricultural Food Chemistry* Vol 47;pp 397-402

Shahvar D., Rehman U.S., Raza A.M. (2010). Acetyl cholinesterase inhibition potential and antioxidant activites of ferulic acid isolated from *Impatiens bicolor* Linn. J. Med. Plants. Res., 4(3): 260-266.

Sies H. (ed.) (1996) Antioxidants in Disease, Mechanisms and Therapy, Academic Press, New York. Pp88-92

Smith I.F and Eyzaguirre P. (2007). "African leafy vegetables: their role in the World Health Organization's global fruit and vegetable initiative" *African Journal of Food Agriculture and Nutrition Development* Vol. 17 No. 3 ISSN 1684-5374.

Soengas P., Sotelo T., Velasco P., Cartea M.E. (2011) Antioxidant properties of *Brassica* vegetables. *Functional Plant Science and Biotechnology* 5 (Special Issue2); 43-55

Ssekabembe, C.K., Bukenya, C. and Nakyagaba, W. (2003). Traditional knowledge and practices in local vegetable production in central Uganda. *African crop science conference proceedings* 6:14-19.

Stewart MJ., Watson ID (1983). Standard units for expressing drug concentrations in biological fluids. *British Journal of Clinical Pharmacology* 16(1): 3-7

Sun, J., Chu, Y., Wu, X. and Liu, R. (2002). Antioxidant and antiproliferative activities of common fruits. *Journal of Agricultural and Food Chemistry.* 50, 7449-7454.

Sun, J., Chu, Y., Wu, X. and Liu, R. (2002). Antioxidant and antiproliferative activities of common fruits. *Journal of Agricultural and Food Chemistry,* 50:7449-7454.

Teutonico, R.A., Knorr, D. (1985). Amaranth: composition, properties and applications of a rediscovered food crop. *Food Tech.* 39(4): 49-61.

Tindall, H. D (1983) Vegetables in the Tropics Macmillan Hong Kong pp 146 – 167

Tomas-Barberan, Espin C.J., (2001). Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. *J. Sci. food Agric*; 81: 853-876

Valko M., Leibfritz D., Moncol J, Cronin MT, Mazur M, Telser J, (2007). Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol.*; 39(1):44-84.

Valko, M., Rhodes, C. J., Moncol, J., Izakovic, M.,and Mazur M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem. Biol. Interact.*, *160*, 1–40.

Vallejo, F; Tomás-Barberón, FA; García-Viguera, C. (2003). "Phenolic compound contents in edible parts of broccoli inflorescences after domestic cooking". *Journal of the Science of Food and Agriculture 83 (14): 1511–1516. doi:10.1002/jsfa.1585.*

Vorster H. J., (2007). The role and production of traditional leafy vegetables in three rural communities in South Africa [MSc thesis]. Pretoria: University of Pretoria; Available from http://upetd.up.ac.za/thesis/available/etd-02122009-115129/ (Accessed 22/06/2012).

Wasswa Henry (2006). Uganda struggles to cope with rise in diabetes incidence. BMJ; 333(7570): 672. PMCID: PMC1584399 doi: 10.1136/bmj.333.7570.672-c

Wayner D.D., Burton G.W., Ingold K.U., Barclay L.R., Locke S.J. (1987), The relative contributions of vitamin E, urate, ascorbate and proteins to the total peroxyl radical-trapping antioxidant activity of human blood plasma. *Biochim Biophys Acta*, 924:408-19.

WHO: World Health Organization (2003); Diet, Nutrition and prevention of Chronic Diseases. Report of joint FAO/WHO Expert Consultation Geneva, WHO (WHO technical report series No. 916). In WHO Fruit and Vegetables report. www.int/dietphysicalactivity/publications/fruit-vegetables-reportpdf Accessed on 23rd February 2012 11:58 am.

Woomer P. L. and Imbumi, M. (2005).Traditional Green Vegetables in Kenya. In Organic Resource Management in Kenya. Perspectives and Guidelines. Forum for Organic Resource Management and Agricultural Technologies (FORMAT). www.formatkenya.org Accessed on 28/06/2012 at 2:30pm

Wright J.S., Johnson E. R., Dilabio G. (2001). Predicting the activity of phenolic antioxidants: Theoretical method, analysis of substituent effects and application to major families of antioxidants. *J.Am. Chem. Soc.* 123, 1173-1183

Yang, J., Lin. H. and Mau, J. (2002). Antioxidant properties of several commercial mushrooms. *Food Chemistry*, 77: 229-235.

Yeng G. C. and Chen H. Y. (1995). Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J.Agri. Food Chem.*, 43(1): 27-32

Yuan X., Wang J., Yao H. Chen F. (2005). Free radical-scavenging capacity and inhibitory activity on rat erythrocyte hemolysis of feruloyl oligosaccharides from wheat bran insoluble dietary fiber. *LWT-Food Sci Technol,* 38, 877-883.

Zhang D., Hamauzu Y. (2004). Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during convectional and microwave cooking. *Food Chemistry*, 88 :503-509

Zulkhairi AH, Khairunnuur SM, Hafipah NRM, Azrina A, Rasadah AM, Kamilah KAK, Zamree SM, Shahidan AM (2010). An aqueous extract of Citrus mitis possesses antioxidative properties and improves plasma lipid profiles in rat induced with high cholesterol diet. *J. Med. Plants. Res.*, 4(1): 049-057.

APPENDICES

APPENDIX 1
Boiling as described by Prabnu and Barret, (2009).

For cooking evaluation, approximately 2 g of leaves were added to a beaker containing 50 mL of distilled water. The beaker was covered with aluminium foil to minimise vapour loss. The leaves were cooked by placing the beaker on a stirred heating plate (Model 51 450 Series, Cole & Parmer, Vernon Hills, IL, USA). It took approximately 10 min for the water to boil, and the leaves were boiled vigorously for 10 min, giving a total cooking time of 20 min. The cooked leaves were allowed to drain on a sieve at room temperature for 5 min following cooking. The drained water was collected in a beaker and also analysed. This cooking method was selected because it is typical of domestic cooking practices used in Africa.

APPENDIX 2
Steaming as described by Adefegha and Oboh (2009)

About 600 g each of the *A. hybridus L.* and *S. nigrum L.* leaves were collected were subjected to the following processing technique: Steaming (STM) of the leaf sample over wire gauze placed on top of a boiling water for 30 min.

APPENDIX 3
Vitamin C extraction as described by Ogunlesi, et al., (2012)

For determination of vitamin C, 2 g of the freshly powdered sample was homogenized with 100 cm3 0.5% oxalic acid, filtered through celite filter and the filtrate made up to 100 cm3 with 0.5% oxalic acid. The liquid obtained was filtered and aliquots titrated with freshly prepared 2, 6- dichlorophenol indophenol.

APPENDIX 4
Extraction for antioxidant activity as described by Katerere et al., (2012)

The plants were collected between March and April 2007, oven-dried at 50°C and separately ground into a fine powder using a Romer Labs Series II Grinding/Sub-sampling mill (Romer Labs, Tulln, Austria). Three grams (3 g) of each plant material were extracted with 30 ml of 70% aqueous methanol by sonication for 30 min and

centrifuged (Jouan CR3i of BICASA Spa, Italy) for 5 min at 4000 rpm. The extracts were filtered using Whatman no. 1 filter paper and the aliquots were analyzed for their antioxidant capacity and total phenol content.

APPENDIX 5
Reducing Power as described by Jayanthi and Lalitha (2011)

Principle

The reducing power of petroleum ether(PE), ethyl acetate(EA), acetone (Ac)and hydrolysed extract (Hy) of *Eichhornia crassipes* was determined by the slight modification of the method of Oyaizu, (1986). Substances, which have reduction potential, react with potassium ferricyanide (Fe3+) to form potassium ferrocyanide (Fe2+), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm.

Chemicals required

Potassium ferricyanide (1% w/v), phosphate buffer (0.2 M, pH 6.6), trichloro acetic acid (10%), ferric chloride (0.1%) and ascorbic acid (1%).

Phosphate buffer preparation

Dibasic sodium phosphate (37.50 ml of 0.2M) is mixed with 62.5 ml monobasic sodium phosphate and diluted to 100 ml with water.

Protocol for reducing power

Various concentrations of the plant extracts in corresponding solvents were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). This mixture was kept at 50°C in water bath for 20 minutes. After cooling, 2.5 ml of 10% trichloro acetic acid was added and centrifuged at 3000 rpm for 10 min whenever necessary. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. Control was prepared in similar manner excluding samples. Ascorbic acid at various concentrations was used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power.

APPENDIX 6

Comparative analysis of Vitamin C content of the vegetables

Sample	*Amaranthus dubius*		*Solanum nigrum*		*Curcubita maxima*	
	Bushenyi	**Ishaka**	**Bushenyi**	**Ishaka**	**Bushenyi**	**Ishaka**
Raw	3.6 ± 0.55	5.55 ± 0.45	0.98 ± 0.1	0.93 ± 0.73	4.93 ± 0.77	2.66 ± 0.03
Steamed	0.13 ± 0.09*	0.09 ± 0.05*	0.1 ± 0.06*	0.53 ± 0.21	0.05 ± 0.05*	0.56 ± 0.11*
Boiled	0.01 ± 0.12**	0.08 ± 0.05*	0.05 ± 0.03*	0.16 ± 0.01*	0.05 ± 0.05*	0.19 ± 0.17*
Water Boil	1.9 ± 0.7	2.1 ± 0.2	0.3 ± 0.05	0.3 ±0.04	2.3 ± 0.11	0.2 ±0.02

Values are presented as means of triplicates ± standard deviation. *denotes significant difference from raw at P<0.05. **denotes significant difference between the steamed and boiled sample at P<0.05

APPENDIX 7

Antihemolytic activity (IC_{50}) of the vegetables

Sample	*Amaranthus dubius*		*Solanum nigrum*		*Curcubita maxima*	
	Bushenyi	**Ishaka**	**Bushenyi**	**Ishaka**	**Bushenyi**	**Ishaka**
Raw	128 ± 47.1	29 ± 11.5	48 ± 9.4	34 ± 16.3	32 ± 0.1	54.9 ± 19.9
Steamed	87 ± 41.5*	17 ± 7.2*	15 ± 2.8*	29 ± 5.0	55 ± 17.7*	53 ± 12.1
Boiled	176 ± 84.5**	51 ± 9.2**	26 ± 5.1**	25 ± 2.9	59 ± 1.4*	18 ± 0.4*

Values are presented as means of triplicates ± standard deviation. *denotes significant difference from raw at P<0.05. **denotes significant difference between the steamed and boiled sample at P<0.05

KAMPALA
INTERNATIONAL
UNIVERSITY
EXPLORING THE HEIGHTS

Western Campus
P.O. Box [illegible]
Tel: [illegible]
E-mail: admin@kiu.ac.ug

Postgraduate Studies and Research Directorate (PGSRD)

28th August 2012

Kinyi Hellen Wambui
Msc. BCH/0001/102/DF

LETTER OF APPROVAL

This is to certify that the research proposal entitled **"A COMPARATIVE STUDY OF THE ANTIOXIDANT ACTIVITY OF Solanum nigrum, Amaranthus dubius and Curcubita maxima, VEGETABLES CONSUMED IN BUSHENYI DISTRICT, UGANDA"** was reviewed by the Board, Postgraduate Studies and Research; Research Subcommittee of Kampala International University-Western Campus (KIU-WC) in its meeting of 1st August, 2012 for its Scientific validity and Ethical appropriateness. The Committee approved that the investigator may start conducting her research.

Signed by: _____ 13 AUG 2012

Chairman, Research Sub-Committee

Date/Stamp