# CANCER SCREENING AND OCCURRENCE IN KANGEMI;POTENTIAL PREVENTION OF CANCER THROUGH CONSUMPTION OF AFRICAN NIGHTSHADE LEAFY VEGETABLE, USING A MICE MODEL

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**DEPARTMENT OF FOOD SCIENCE, NUTRITION, AND TECHNOLOGY**
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**UNIVERSITY OF NAIROBI**

**2020**

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This thesis is my original work and has not been submitted for the award of a degree in any other university.

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This thesis has been submitted with our approval as University supervisors.

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## Dedication

**I dedicate this thesis to my son:**

Jabali Gideon

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## Acronyms and abbreviations

| | |
|---|---|
| **ANLV** | African nightshade Leafy Vegetable |
| **BHT** | Butylated hydroxytoluene |
| **cDNA** | Complementary Deoxyribonucleic Acid |
| **CI** | Confidence Interval |
| **CS** | Cancer Screening |
| **DMEM** | Dulbecco's Modified Eagles Medium |
| **DNA** | Deoxynucleicacid |
| **DPPH** | 2,2 Diphenyl-1-picrylhydrazyl |
| **DPPH** | 2,2 diphenyl-1-picrylhydrazyl |
| **ECM** | Extracellular Membrane |
| **ELISA** | Enzyme-Linked ImmunoSorbent Assay |
| **Assay** | |
| **FFQ** | Food Frequency Questionnaire |
| **HPLC** | High -Performance Liquid Chromatograph |
| **ICNBS** | International Congress of national bibliographies |
| **IGF** | Insulin-like growth factor |
| **ILRI** | International Livestock Research Institute |

| | |
|---|---|
| **IHC** | Immunohisto Chemistry |
| **Kes** | Kenyan shillings |
| **KNH/UoN-ERC** | Kenyatta National Hospital/ University of Nairobi – Ethical Research Committee |
| **MTT assay** | The MTT assay is a colorimetric assay for assessing cell viability. |
| **MAPK** | Mitogen-Activated Protein Kinases |
| **PKC** | Protein Kinase C |
| **PI3K** | Phosphatidylinositol-3-Kinase |
| **NF-κβ** | Nuclear Factor-κβ |
| **RNA** | Ribonucleic acid |
| **ROS** | Radical Oxygen Species |
| **RT-PCR** | Real-time Polymerase Chain Reaction |
| **SPSS** | Statistical Package of Social Sciences |
| **qPCR** | Quantitative Polymerase Chain Reaction |
| **ALVs** | African leafy vegetables |
| **TGF** | Tumour Growth Factor |
| **uPA** | Urokinase Plasminogen Activator |

## Operational definitions

| | |
|---|---|
| **Anticancer** | This is an agent or substance that stops progression or prevents the formation of malignant tumors. |
| **Anti-mutagen** | This is a chemical or physical agent that prevents changes in the genetic material of an organism. Mutagens are carcinogenic. |
| **Antioxidants** | This are substances such as enzymes and vitamins that reduce or prevent damage caused by free radicals mostly formed by the body during metabolism |
| **Cancer** | This is the uncontrolled growth of cells that invade other normal cells and alter the functioning of a tissue or organ. |
| **Chemoprevention** | This is the use of the biological, natural or synthetic substance in order to prevent disease. |
| **Consumption patterns** | The usual eating habit of African leafy vegetables. |
| **Household** | Occupants of a house who live as a unit i.e. a family. |
| **Period prevalence or Lifetime prevalence** | **"**Is a compound measure and is constructed from prevalence at a point in time plus new cases and recurrences during the succeeding time period". (Mausner and Bahn 1985). Also referred to as lifetime prevalence. |
| **Phytochemicals** | oxidative or antimutagenic bioactive compounds |
| **Traditional leafy vegetable** | Leafy vegetables originally from a specific place (Kangemi) |
| **Transcription** | "Molecule that controls the activity of a gene by determining whether the |

| | |
|---|---|
| **factor** | gene's DNA (deoxyribonucleic acid) is transcribed into RNA (ribonucleic acid)." (Encyclopedia Britannica) |
| **Slum household** | as a group of individuals living under the same roof in an urban area who lack one or more of the following:<br>1. Durable housing of a permanent nature that protects against extreme climate conditions.<br>2. Sufficient living space which means no more than three people sharing the same room.<br>3. Easy access to safe water in sufficient amounts at an affordable price.<br>4. Access to adequate sanitation in the form of a private or public toilet shared by a reasonable number of people.<br>5. Security of tenure that prevents forced evictions. |

**Abstract**

Cancer is a disease that is steadily on the rise in Kenya despite the fact that two-thirds of the prevalence is due to preventable causes. One of these causes is poor dietary habits. Healthy diets include high levels of vegetable consumption. African leafy vegetables are easily available to both the urban and rural communities. The vegetables have high nutrient content and some of them have been implicated in the cure of chronic diseases. In the past, the vegetables were shunned in favor of their exotic counterparts. The African nightshade leafy vegetable is a side dish by communities in Kenya especially those in western and coastal regions. The vegetable has also been associated with medicinal properties to cure conditions such as stomach ache and inflammatory conditions in traditional medicine. It is presumed therefore that vegetable has the potential for conferring preventive properties against diseases such as cancer. This study was therefore designed to assess the preventive properties of African nightshade leafy vegetables against the expression of a tumor-activated protein. The study was carried out in two phases. In the first phase, a baseline survey was carried out in Kangemi a slum in Nairobi County. A sample of 439 households was calculated using Fischer formulae. The simple random sampling was used to select the households that would participate. The cross-sectional survey was an interviewer-administered questionnaire. In addition, the questionnaire included a food frequency section over a week's period and a demographics and economics section. It also included questions on cancer screening and the prevalence of leafy vegetable consumption. In the second phase, laboratory analysis used a factorial design. The two treatments assessed were, cooked vegetables, raw vegetables, against a control. The antioxidant activity of the leafy vegetable was determined using the Trolox standard. The expression of the tumor-activated protein (urokinase

plasminogen activator protein) was determined by a quantitative polymerase chain reaction. Analyzed inference variables that had a value $P \leq 0.05$ were considered as significant.

The association between gender and cancer screening was significant ($\chi^2$8.034, DF=1, P=0.005). There was a significant association between occupation and cancer screening ($\chi^2$28.158, DF=6, P=0.000). There was a significant relationship between knowing the benefits of leafy vegetable consumption and cooking time (U=33, p=0.008). In the second phase, the antioxidant activity of African nightshade leafy vegetables significantly increased when cooked (t=43.57, P= 0.000). In addition, expression of the urokinase plasminogen activator protein was reduced in mice fed with cooked vegetables by about 97% while the mice fed on raw vegetable expression was down-regulated by about 39 % as compared to the control.

In conclusion, gender and occupation are significant factors that determine cancer screening. The studies also established that residents have significant knowledge of the benefits of consuming leafy vegetables. Through laboratory analysis, the study concludes that the antioxidant activity of African nightshade leafy vegetables was significantly increased by steam blanching. There was a significant reduction of expression of urokinase plasminogen activator protein in mice fed with cooked African nightshade vegetables as compared to the mice fed on the control diet.

# CHAPTER ONE: GENERAL INTRODUCTION

## 1.1 Background

In most definitions, cancer is considered as the uncontrolled growth of a malignant cell that invades tissues and hinders the function of normal cells (Vineis, 2014). According to GLOBOCAN 2018, “one in five men and one in six women worldwide will develop cancer over the course of their lifetime.” In addition one in eight and one in eleven women will die from the disease. The estimated number of new cancer cases in 2018 worldwide is 18,078,957 people (Bray et al, 2018). The leading cancer incidences in the world including, lung, breast, and colon-rectal (Bray et al, 2018). African has a high number (57.3% )of cancer death compared to other parts of the world. “This has been attributed to poor prognosis and limited access to timely diagnosis and treatment (Bray et al, 2018).

Cancer is increasingly becoming a public health issue in Kenya and all over the world (Barkary and Christopher, 2013). It is the third leading cause of death after infectious and cardiovascular diseases (WHO, 2011). Cancer can affect any part of the body. Currently, in Kenya, cancer ranks as the third cause of death according to the Kenya Cancer Control Strategy (Opiyo et al., 2011). Data on cancer “annual incidence in Kenya is nearly 37,000 new cases with an annual mortality of over 28,000” (MOH, 2017).

The major cancers that have a high prevalence in Kenya among women include the cancer of the cervix and that of the breast, while among men they include the cancer of the esophagus, head, neck, and prostate (Muthike et al., 2015). Among children, blood cancers seem to be the most prevalent (Opiyo et al., 2011).

Consumption of fruits and vegetables as a means of reducing the risk of cancer has been advocated since the 20th century. It has been recommended that a person should take at least five servings of fruits and vegetables per day (Lewis et al., 2012). However, there has been conflicting evidence on how effective the vegetables are in reducing the cancer risk (Kothai et al., 2011).

Increasing evidence indicates that consumption of vegetables and fruits reduces the risk of chronic diseases such as cancer. From a nutrition point of view, vegetables are nutrient-dense rich in vitamins minerals and bioactive compounds (Lim, 2013). Epidemiological studies have shown that populations with a high prevalence of vegetable consumption have a low incidence of cancer (Bosetti et al., 2012). This is because vegetables, e.g. cruciferous vegetables, have been found to contain bioactive compounds called phytochemicals that are chemopreventive (Yaacob et al., 2010) and thus provide a protective effect against tumor formation.

According to a report by (Khire, 2013), Kenyan households are consuming less and less of the African leafy vegetables (ALVs), which include the African black nightshade, amaranth, spider plant, stinging nettle. This has led to several initiatives aimed at encouraging Kenyan households to grow and consume ALVs to ensure the preservation of biodiversity and health.

Currently, about 12 species of ALVs have been domesticated (Khire, 2013). Households that have grown these vegetables have been shown to have improved both their economic and nutrition status compared to before they started growing and consuming them. This has basically contributed to the economic empowerment of these households (Khire, 2013). In addition to ensuring that ALVs are grown and consumed more frequently, eight species of ALVs have been

identified for more research; they include *Cleome gynandra, Amaranthus species, Vigna unguiculata, Solanum species, Crotalaria species* (Khire, 2013). These are currently commercially grown and sold even in upstate supermarkets where they compete with their exotic counterparts. *Solanum nigrum* seems to top the list of popularity.

According to Otieno (2013) the Kenya Agricultural Research Institute (KARI), puts consumption of indigenous vegetables at 4% of all the greens consumed in the country. This figure is too low given their nutritional value as compared to exotic vegetables like cabbage, spinach, and kales which account for more than 90% of the total vegetable consumption.

In peri-urban Nairobi, production, and consumption of ALVs have been on the rise since the last decade. Sub-counties such as Kisii and Tharaka have one of the highest production rates where approximately 95% of households grow ALVs (Khire, 2013).

Consumption of ALVs is generally low in central Kenya most farmers in this region prefer exotic vegetables (Nyamongo and Silingi, 2010). Studies have shown that in areas where consumption of ALVs is low there is normally a high prevalence of micronutrient deficiencies (Nyamongo and Silingi, 2010). ALVs generally have a higher content of vitamins, minerals, and proteins compared to the exotic vegetables (Odhav et al., 2007). Moreover, ALVs have been proven to be resilient in hardship areas where the soils are poor and rainfall is low. In addition, ALVs are also more resilient to pests and diseases (Nyamongo & Silingi, 2010).

The indigenous vegetables have been found to have phytochemicals that are deemed to be chemopreventive to carcinogenesis. However, most households seldom consume these

vegetables (Kimiywe et al. 2007). In Slums, vegetables are normally grown not only to provide food but also as a supplementary source of income (Foeken and Mbogaine, 2000). However, the main types of vegetables grown in this form of urban agriculture (sack gardening) are the Kales (Gallaher et al, 2013).

The role of Indigenous vegetables in cancer prevention is inherent with the property that they have phytochemicals (Nyamongo and Silingi, 2010), that are effective in chemoprevention (Surh et al., 2003). The mechanism in which the dietary phytochemicals become chemopreventive is through activation or induction of expression of enzymes involved in cellular antioxidant defenses. These enzymes, in turn, eliminate electrophilic carcinogens (Surh et al., 2008). A wide variety of polyphenols and other phytochemicals induce cytoprotective enzymes that both eliminate electrophilic carcinogens and activate cellular antioxidant defenses. This essential property of edible phytochemicals has been recognized as one of the highly effective strategies for preventing cancer in the human population (Surh et al., 2008). This study, therefore, aims to evaluate the chemo-preventive properties of African black nightshade commonly available and consumed by the populations in Kenya as a leafy vegetable.

## 1.2 Statement of the problem

Cancer is the third leading cause of death in Kenya according to the National Cancer Control Strategy. Approximately 40,000 new cases of cancer every year and a mortality rate of 28,000 (MOH, 2017). Cancer is increasingly affecting more and more people in Kenya. In addition, Kenya is not adequately prepared to tackle the growing number of cancer patients using conventional methods. This is due to the lack of accessibility of diagnostic equipment that would enable early diagnosis for treatment and management.

The cancer increase in Kenya is probably due to the continued adaptation to an unhealthy lifestyle (Opiyo et al. 2011). According to the Kenya cancer control strategy, early detection is advocated as a means of control of cancer. It does not include advocacy in charge of lifestyles and dietary habits of consumption of vegetables and fruits. African leafy vegetables which contain the phytochemicals involved in cancer chemoprevention are shunned. An increase in cancer prevalence seems not to be given due attention because Kenya is still struggling with communicable diseases such as Malaria and maternal-child health to which a lot of resources have been invested (Opiyo et al. 2011). The majority (80%) of cancer diagnoses are made at the advanced stages where curative measures are not applicable (Opiyo et al. 2011). Modern cancer therapy is also expensive for most Kenyans especially those affected in the rural areas (Opiyo et al. 2011). Even when one is diagnosed with cancer at an early stage, the treatment cost is usually high and out of the affordable range for most Kenyans (Ginberg et al. 2012).

In the western world, the African black nightshade was viewed as a poisonous plant and was used by witchdoctors or fortune-tellers as they contain hallucinogens (Lee et al. 2004). The plant

has high alkaloid (atropine) which disrupts the central nervous system and causes death (Lee et al. 2004, Yang et al. 2010). However, studies on the entire herb have proved that the plant contains remarkable anti-tumor activity (Ding et al. 2013). A“Pharmacological experiment indicated that the total steroidal alkaloids from Solanum nigrum destroy sarcoma 180 in vivo” (Ding et al. 2013). There has been an increase in production and consumption of the African nightshade leafy vegetable due to the postulation that besides being a dietary the vegetable, it has healing properties. These properties have yet to be scientifically proven. It is still unclear how the consumption of African nightshade leafy vegetables contribute to human nutrition and health. (Odongo et al. 2018)

In addition, African nightshade leafy vegetables are usually poorly prepared for consumption. Most of the population still use old cooking methods to prepare the vegetables. This cooking method mostly involves boiling the vegetables and discarding the water. This probably leads to massive nutrient and health goodness loss.

### 1.3 Justification of the study

The African African black nightshade vegetable normally grows wildly. The vegetable can stand harsh conditions both biotic and abiotic conditions. In comparison to exotic vegetables, the African nightshade is more cost-effective. African black nightshade has high nutritional value and requires minimal maintenance. Since the vegetable can be easily assessable to many households, this leads to improved food security to the population. Moreover, improved nutritional status for the entire family and hence reduce the prevalence of malnutrition. This vegetable can also grow very easily in vegetable gardens around homesteads.

Plants have been used for medicinal purposes since the very beginning of time. In addition, the pharmaceutical industry uses extracts from plants to make drugs for treating various ailments. Most of the plant parts used are the fruits, roots, leaves and the stems (Mensah et al. 2008). African nightshade is among the plants considered to have medicinal value (Parikh et al. 2015).

African black nightshade has been reported to contain high levels of phytochemicals and micronutrients that could boost the immunity of an individual (Adefegha et al. 2011), (Tumwet et al. 2014). However, the majority of the population considers these vegetables as inferior foods and some communities do not even recognize them as food but as weeds (Rozy et al. 2016).
In addition, there has been a gradual loss of plant biodiversity (Rozy et al. 2016) and even the methods of cooking the African black nightshade vegetables have been forgotten in some communities (Taylor et al. 2012).

In recent times the African nightshade is one of the ALVs that has been domesticated. The vegetable sells in both the local and upstate markets. Currently, it is the most popular ALV. As research about its medicinal value is elucidated, consumption by the population should also be encouraged. In addition, the effective cooking method of the vegetable should be encouraged. African black nightshade leafy vegetables have been found to contain many phytochemicals including glycoalkaloids and antioxidants (Link et al. 2004). Which contribute to the medicinal properties of these vegetables. Hence, there is promise in the use of fruit and vegetable.

It has additionally been shown to contain high levels of glycoalkaloids and oxalates which are reduced to consumable levels during the processing phase (Akubugwo et al. 2007). It is also

been documented that the population that highly consumes this vegetable is much less likely to be affected by cardiovascular diseases (Kimiywe et al. 2007). The secondary metabolite glycol-alkaloid has been shown in several cases to have antiproliferative and cytotoxic effects against cancer cells (Mattew et al. 1995, Lim et al. 2013 and Kenny et al. 2013).

This study intends to show the potential of dietary glycol-alkaloids in fighting cancer cells. Moreover, most studies have only focused on checking the cytotoxic or apoptotic effect whereas the mechanisms of actions are normally not indicated clearly. Due to the content of phytochemicals in the African black nightshade, its consumption will attribute to the delay in the onset of cancer.

## 1.4 Objectives

### 1.4.1 Main Objective

The study will contribute towards lowering cancer occurrence through the consumption of African nightshade leafy vegetables.

### 1.4.2 Specific objectives

i. To determine the association of socioeconomic and demographic characteristics, consumption of leafy vegetables and cancer screening rate at Kangemi slum.
ii. To determine knowledge of the benefits of vegetable consumption and cooking time of leafy vegetables in peri-urban communities.
iii. To analyze the efficacy of chemo-preventive attributes of African nightshade leafy vegetables on cancer using mice model.

## 1.5 Hypotheses

i. The consumption patterns of African leafy vegetables are not associated with socioeconomic and demographic characteristics of the population or cancer screening in the household.

ii. Knowledge of the benefits of vegetable consumption is not associated with a cooking time of leafy vegetables in peri-urban communities.

iii. The African nightshade leafy vegetables have no chemo-preventive potential against the expression of urokinase plasminogen activator protein in mice.

# CHAPTER TWO: LITERATURE REVIEW

## 2.1. Types of cancer and their predisposing factors

Cancer is a disease that can affect any part of the body. The majority of cancer incidents are due to environmental and somatic factors (Grivennikov et al. 2010). Only 10% are due to genetic factors. Diet contributes approximately 35% of the total environmental and somatic factors (Grivennikov et al. 2010). Chronic inflammation due to infection, obesity or tobacco smoke increases cancer risk. During inflammation, tumor-promoting agents are basically produced such as Reactive Oxygen Species (ROS) and cytokines (Grivennikov et al. 2010).

Many hypotheses have been advanced to explain the varying cancer types in populations all over the world. It is postulated that diet has a major part to play (Shukla et al. 2004). It has been suggested that diets contain some chemoprevention properties that mitigate cancer. This is basically by "preventing mutagenesis and carcinogenesis due to their relatively non-toxic effects" to normal cells (Shukla et al. 2004). Head and neck cancers are mostly attributed to cigarette smoking. Tobacco smokers are three times more likely to develop these cancers than non- smokers (Clark et al. 2010). The risk of breast cancer is mainly associated with three major aspects, dietary patterns, reproductive patterns and genetic factors (Amir et al. 2010). Genetic predisposition is mainly by having either one of the mutated genes (Amir et al. 2010). Prostate and colon cancers are on the rise in Kenya (Gallaher et al.2013), due to a sedentary lifestyle and high consumption of calorie-dense foods. Cervical cancer is mainly due to an infectious agent (Human Papillomavirus) that is transmitted sexually and the late diagnosis of the infection (Jemal et al. 2010).

The Shukla and Pal (2004) review shows that lung cancer is initiated by tobacco smoke which has carcinogens (Shukla et al. 2004). Obesity is on the rise in Kenya, it is estimated that about 30% of Kenyans are obese (Hossain et al. 2007). Obesity increases the risk of both liver and pancreatic cancers. Most solid tumors occur in old age due to cell aging and in the process, the mutation occurs that leads to malignancy. Bacterial infections such as *Helicobacter pylori* cause stomach cancer and also mucosa-associated lymphomas. Hepatitis B and C increase the risk of hepatocellular carcinoma. In addition infections of Schistosoma or Bacteroides could lead to colon and bladder carcinomas (Hossain et al.2007).

### 2.1.1 Prevalent cancers in Kenya

Cancer is the third leading cause of death in Kenya (Opiyo et al. 2011). Unfortunately, the most affected age group is middle-aged unlike in developed countries where cancer is seen as a disease of the old above 70 years (Opiyo et al. 2011 and Muthike et al. 2013).

According to several studies done in Kenya (Opiyo et al. 2011 and Muthike et al. 2013), leading cancer among women is breast cancer followed closely by cervical cancer. Among men, the leading cause of cancer is prostate cancer. These cancers develop mainly due to individual lifestyles (Opiyo et al. 2011). Adopting a sedentary lifestyle, poor diets, and bacterial or viral infections should be treated on time, in order to forestall cancer occurrences (Bakary et al. 2013). Other cancers include oesophageal cancer, throat cancer, and skin cancer that have lower incidences.

## 2.2 Role of vegetables in diet and disease

Varying types of vegetables are grown and consumed all over the world. Vegetables consist of various plant parts including shoots and buds, leaves, seeds, stems pods flowers and roots. In Africa, African leafy vegetables are very important in the diet. In Kenya, more than forty plant leaves are used as vegetables. There has been controversy among researchers on the role of vegetable affect consumption and cancer prevention. According to a review by Willet (2010), there is no major effect of vegetable consumption on cancer prevention, and information on the specific vegetables in doing this is scare (Willet 2010).

A review by Shukla and Pal (2004), emphasizes that there is a decreased risk of cancer for persons who frequently consume vegetables and fruits. Vegetable constituents such as polyphenols and glycoalkaloids have been shown to exhibit pharmacological and biochemical activities (Shukla and Pal 2004).

Vegetables are known to be brightly colored mainly due to the presence of green, red and yellow pigments. The yellow to red colors are mainly due to carotenoids and anthocyanins both of which have been shown to inhibit human cancer cell proliferation. Another study has shown that the reason why individuals who drink alcohol and smoke cigarette have a high prevalence rate of cancer is due to the fact that they do not eat adequate amounts of fruits or vegetables (Key et al. 2010).

Cruciferous vegetables have been associated with decreased cancer risk due to the natural content of isothiocyanates. Isothiocyanates are able to inhibit the activation of P-450s that activate carcinogens and they also activate detoxifying enzymes that neutralize the carcinogens.

This eventually leads to cell apoptosis of cancer cells and the regulation of transcription factors (Yang et al. 2013).

Carrots are a common vegetable around the world with its origin in temperate regions such as Asia, Europe and North America (Arscott et al. 2010). The most consuming part is the root through in Asia the young shoots are eaten as herbs (Arscott et al. 2010). Carrots are not calorie-dense but have a high content of phytochemicals that include anthocyanins, carotenoids, and phenolic compounds. Moreover, the carrot root is high in fiber. According to a review, carrot roots also have macronutrients which include: 7% carbohydrate, 1% protein and 0.2% lipids (Arscott et al. 2010). Carrots owe their yellow color to carotenoids, some of which serve as a precursor of vitamin A (mainly beta-carotene) and possess very strong antioxidant properties.

The antitumor activity of carrots has been demonstrated in a study this is especially in the case of renal cancer. In an experimental study where men were taking carrot juice for 2 weeks, it showed that the men had reduced lymphocyte DNA strand breakage. There was also less oxidative base damage in the cells extracted from these men after the 2-week duration (Arsott et al. 2010).

**2.3 African leafy vegetables and their consumption in Kenya**

African leafy vegetables have been consumed for countless generations. Some are implicated in medicinal properties. For instance, spider plant vegetables (*Cleome gynandra*) were given to mothers who had just given birth to improve milk production. African nightshade was believed to solve stomach problems hence given to children with diarrhea. The traditional vegetables were consumed frequently due to their availability and even up to today some are used as symbols for certain cultures.

During colonial times, exotic vegetables were introduced to the African population. Most people then abandoned traditional African vegetables and replaced them for exotic vegetables such as spinach, cabbage, and broccoli.

In recent times, however, the demand for traditional African vegetables has greatly increased. This due to the health promotion effort by the Kenyan government and information emanating from research. Research has shown that traditional vegetables are important in solving the issues of malnutrition and non-communicable diseases in Kenya. Vegetables that have so far been domesticated include African nightshade, spider plant, cowpea, jute mallow African kale pumpkin leaves, and amaranths. Of this vegetable, the most popular in production and consumption is the African nightshade.

### 2.4. Consumption of African nightshade leafy vegetable in Kenya

African black nightshade leaves are consumed as a side dish when cooked. In Kenya, they have been domesticated and will be found selling not only in open-air markets but also in the supermarkets where the middle-class families shop. The vegetables are on the way to becoming the most consumed in the country, next to the kales (local name *Sukuma wiki*) (Odhav et al. 2007). In most communities, Africa nightshade leafy vegetables are considered inferior such that they are poorly utilized in the form of food. However, in times of drought poor farmers also depend on these vegetables as a source of income (Olet et al. 2005).

In most parts of the world, nightshade grows like a weed but, in Kenya, it is one of the ALV species that has been domesticated (Rozy et al. 2016). In Asia, nightshade leaves are eaten for curing ailments such as inflammation and fever (Ramya et al. 2011). The plant is said to have

several bioactive compounds, which include glycoalkaloids, glycoproteins, polyphenols, and other polysaccharides that when extracted have been found to be effective in immune modulation[21]. Immune modulation is basically the process of altering the immune response. The African black nightshade extracts have been shown to boost immunity by increasing the T-cells and CD-4 cells which are essential against tumor formation (Ramya et al.2011). In addition, leaves of African nightshade have also been used to treat worm infestation, gastrointestinal infections and the management of chronic diseases (Kimiywe et al. 2007).

In a study to test for a cytotoxic effect on several human cancer cell lines, African nightshade ethanolic extracts showed cytotoxic effects on the cancer cells (Putri et al. 2011).

Aqueous plant extracts have been found to have inhibitory effects against cervical cancer cell lines. Organic extracts have anti-aging effects due to the presence of antioxidants (Ramya et al.2011). In spite of all this benefits African black nightshade has not been accepted as contemporary medicine.

Commonly known as *Solanum scabrum* (African nightshade) is a vegetable as well as a medicinal plant. A member of the Solanaceae family of plants that are well known for their therapeutic properties (Tiossi et al. 2012). The genus is commonly known for the production of alkaloids which further form glycoalkaloids when combined to sugar moieties. The glycoalkaloids are responsible for the bitter taste of these vegetables (Tiossi et al. 2012). In vitro studies show that three steroidal glycosides β2 solamargine, solamargine and degalactotigonin extracted from African black nightshade have shown cytotoxicity on six cultured human solid tumor cell lines of the colon, prostate, and breast[17]. The cytotoxic effects have lately been

attributed to secondary metabolites that have steroidal compounds (Tiossi et al. 2012). The mechanism resulting in the apoptosis of tumor cells in the production of caspase protease and cell cycle regulation (Ding et al. 2013).

### 2.4.1. Chemical composition of African nightshade leafy vegetable

Proximate and elemental composition of African nightshade *Solanum nigrum* leaves is shown in Figures 1 and 2 respectively.

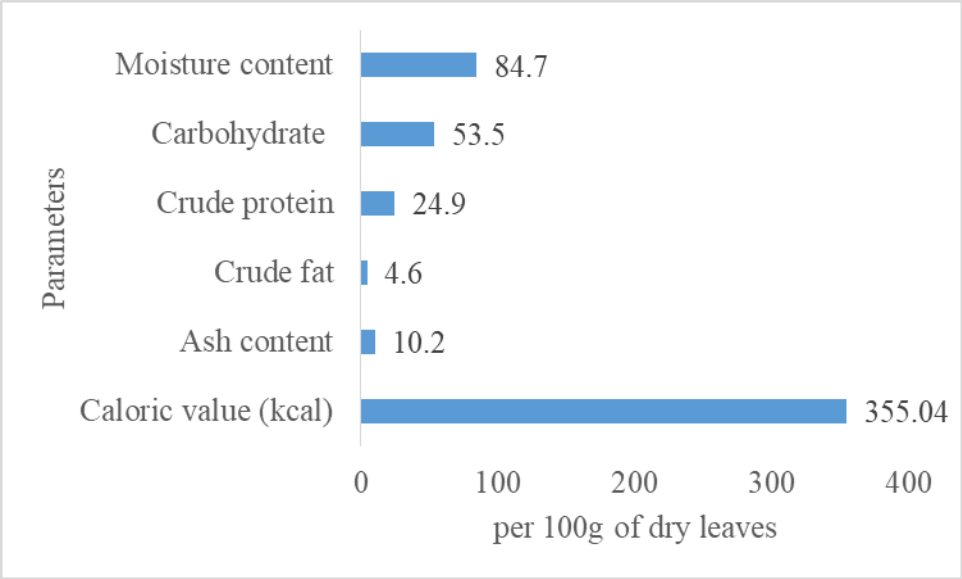


**Figure 1: Proximate composition of African nightshade leafy vegetable: Source Akubugwo et al., 2007**

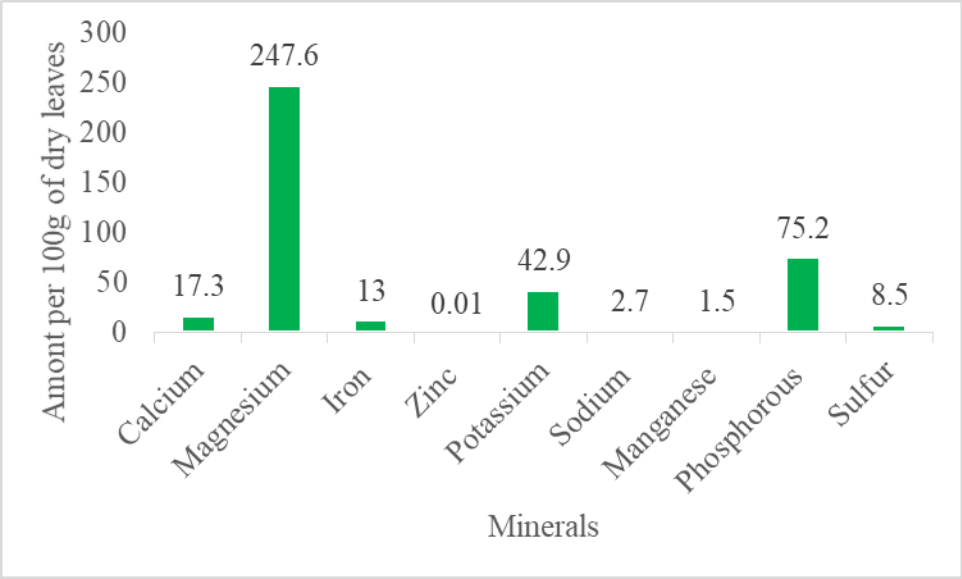


**Figure 2: Mineral contents of African nightshade leafy vegetables: Source Akubugwo et al., 2007**

### 2.5 Role of vegetables in the prevention of cancer

The best way to deal with any disease is by preventing it (Shukla et al. 2004). Many strategies of chemoprevention have been put forward this includes use of blocking agents which inhibit the activation of tumor formation, by use of agents that make the cell resist attacks from carcinogens hence preventing tumor formation, lastly are the suppressing agents such as selenium that suppress the cell from becoming malignant (Shukla et al. 2004).

In populations that consume vegetables frequently, the cancer risk is significantly reduced. Vegetables contain many bioactive compounds in the form of antioxidants and anti-mutagens. These compounds have been associated with their chemopreventive properties, as shown in figure 3.

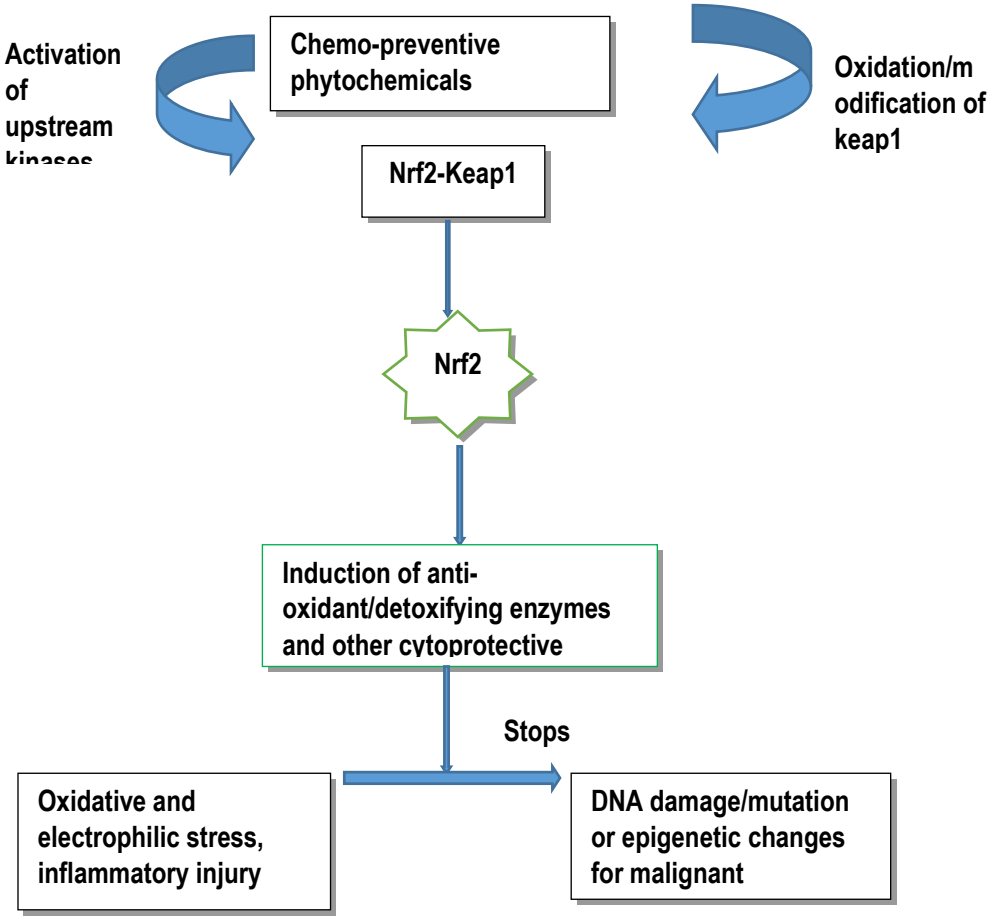


**Figure 3: Figure 3: Mechanism of chemoprevention by dietary phytochemicals through Nrf2 signaling source: (Surh et al., 2008)**

Nrf2 signaling is activated by chemopreventive phytochemicals through phosphorylation either by upstream protein kinases or direct interaction with keap1 cysteine thiols. Subsequently, cytoprotective enzymes can abrogate oxidative stress and inflammatory tissue injury, thereby blocking DNA damage or suppressing proliferation of initiated cells and malignant transformation (Surh et al. 2008).

The high mortality rates associated with cancer are caused by the metastatic spread of tumor cells from the site of their origin. This causes about 90% of all cancer deaths (de Geus et al.

2017). Tumor cells invade either the lymphocyte vessels or blood vessels to access general circulation and then establish themselves in other tissues. Molecular steps of cancer metastasis are generally similar in all solid tumors (de Geus et al.2017).

This classical metastatic cascade encompasses intra-vasculation by tumor cells, their circulation in lymph and blood vascular systems to distant organs. Accumulating evidence indicates several mechanisms of metastasis. One of these systems involves the urokinase plasminogen activator (uPA) (de Geus et al.2017).

### 2.5.1 Effects of phytochemicals on tumor aggravating proteins

Phytochemicals play a key role in inhibiting and delaying cancer progression. Species such as cardamom mainly used in Asia and Russia have many health benefits such as a cure to flu. The main ingredient responsible for this is monoterpene compounds. Phytochemicals have been shown to down-regulate the nuclear factor κB (NF-κβ) pathway which is important in tumor genesis and inflammation (Das et al. 2012).

Chemoprevention against cancer using dietary phytochemicals basically means providing prophylaxis's which arrests initiation and promotion of a tumor. In recent times, phytotherapy is preferred as an alternative to contemporary drugs as they are less aggressive and do not create resistance. Phytochemicals mainly block and suppress the production of Reactive Oxygen Species (ROS) (Moongkarndi et al. 2007).

Dietary plants are the main source of phytochemicals in the world (Su et al. 1992). Essential phytochemicals include selenium in garlic, genistein in soy proteins and many others. However,

the studies of any anti-cancer properties for plants found originally in Africa is rare if not absent (Su et al. 1992).

#### 2.5.1.1 Urokinase-type plasminogen activator

Urokinase plasminogen activator (uPA) is an enzyme of the family of a serine protease and it binds to a receptor uPAR, which is a protein usually found anchored at the cell membrane. The binding of the enzyme to the receptor ensures the activation of proteolytic activity (De Geus et al. 2017). The main function of uPA is the cleavage of the inactive serine protease plasminogen to active plasmin. Plasmin in return has a broad substrate first, it can degrade extracellular protein (ECM) such as fibronectin, vitronectin, fibrin, and laminin. Secondly, uPA further stimulates tumor growth by proteolytically activating latent forms of several growth factors such as SF/HCF, BFGF, and TGF-β (De Geus et al. 2017).

Urokinase plasminogen activator is normally regulated by two plasminogen activator inhibitors which are PAI-1 and PAI-Q. Enhanced expression of components of uPA has been found in many carcinomas (such as colon breast, stomach, and prostate) and melanomas. Urokinase plasminogen activator (uPA) is expressed usually in myofibroblasts at the invasion front. Increased uPA activity has been correlated with tumor invasiveness and a prognostic indicator of disease recurrence and metastasis in multiple cancer types (De Geus et al. 2017).

In experimental tumor models, invasion and metastasis could be inhibited by the treatment of tumor cells with antibodies or pharmacologic agents that inhibit uPA activity (de Geus et al. 2017).

UPA and PAI-1assay formats have been evaluated and the Enzyme-linked immunosorbent assay (ELISA) was deemed to be the best method to evaluate cancer prognosis (Taubert et al.2010).

Other methods that were tested include Immunohistochemistry (IHC), qualitative real-time reverse transcriptase (RT)-PCR. The uPA enzyme inhibitor PAI-1 has been found to having tumor supporting functions hence has been used to be a prognostic indicator of the worsening situation in patients with tumor (Taubert et al. 2010).

A study done on human breast cancer cell line MCF-7, to investigate the effects of curcumin on the breast cancer cells on how they respond to in terms of cell adhesion and invasion showed that suppression of nuclear factor ϰB (NF-ϰB), lead to a reduction of uPA secretion which controls cell motility, invasiveness and metastatic spread of cancer (Zong et al. 2012).

NF-ϰB is a responsive element that is present in the promoter region of uPA and hence mediates uPA secretion and regulates cell migration (Zong et al. 2012).

In addition, tumor growth factor-β1 has also been shown to be a pro-oncogenic factor that occurs in the late stages of tumor progression (Tobar et al. 2010). The main function of TGF-β1 is to enhance the capacity to degrade the cell invasiveness and epithelial-mesenchymal transition (EMT).TGF-β1 activates NF-κB which then acts as a promoter for the expression of the serine protease uPA, which when produced causes cell invasiveness of tumor cells (Tobar et al. 2010).

The uPA/uPAR also plays a role in monocyte and macrophage chemotaxis. In the tumor microenvironment, inflammatory components present as a large number of infiltrating macrophages (tumor-associated macrophages).These TAMs are increasingly recognized as important contributors to cancer progression and metastasis (Zhang et al. 2011).

## 2.6. Role of National Cancer Control

One of the guiding principles of Kenya National cancer control strategy (KNCC), its prevention, early detection, and cancer screening. According to the strategy, prevention offers the most cost-effective long-term strategy for the control of cancer. The strategy acknowledges that 50% of cancer can be prevented by adopting a healthy lifestyle. KNCC intends to reduce cancer through primary prevention and early detection. The KNCC intends to carry out activities such as setting guidelines on diet, nutrition, and physical activity. On diet guidelines, fruits, vegetables, and unrefined foods are encouraged. Other ways in which the KNCC has proposed to reduce the risk of cancer include, promoting safe cooking and preservation methods, encourage physical activities and healthy diets.

## 2.7 Conceptual framework

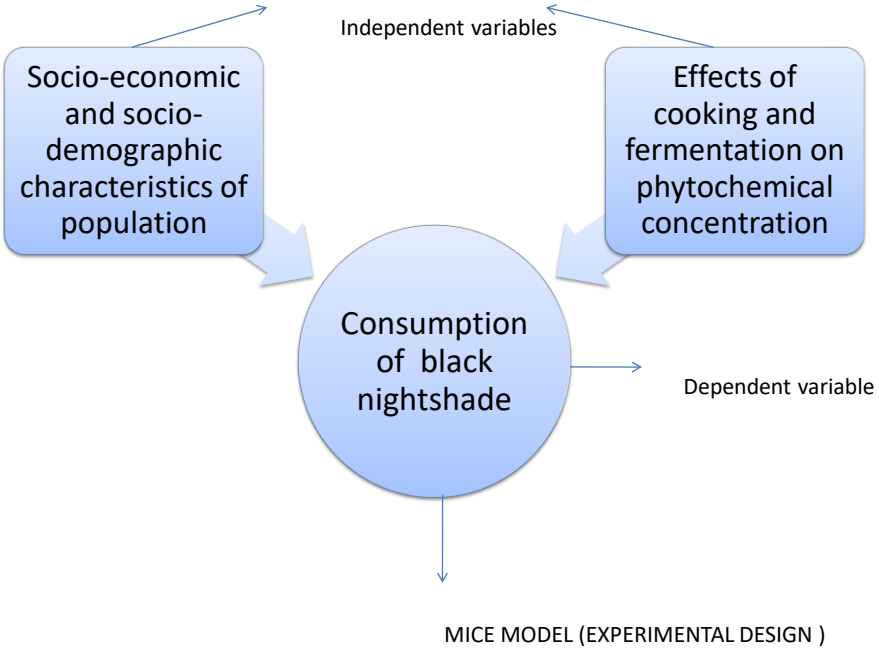


**Figure 4: Conceptual framework**

The conceptual framework shows that phytochemicals contained in the African black nightshade possess potential chemopreventive effects. The frequency of consumption of the vegetables could be affected by the socio-demographics/ economics of the individual. In addition, even when consumed frequently the method of preparation may affect the concentration of phytochemicals that are essential for fighting against carcinogens. To demonstrate the chemopreventive potential of phytochemicals in black nightshade a mice model was used.

## CHAPTER THREE: CANCER SCREENING IN ASSOCIATION WITH CONSUMPTION OF LEAFY VEGETABLES IN KANGEMI SLUM


### Abstract

Cancer is an increasing health burden in the developing world. Diet and early cancer screening are among the few measures that could either prevent or enable early detection hence enhancing higher chances of cure. Consumption of at least five servings of vegetables per day has been recommended as effective in combating cancer and other non-communicable diseases. African leafy vegetables are rich in phytochemicals and that enhance the management of cancer. Some of the vegetables that have been domesticated in Kenya include *Solanum scrabrum,* Amaranths species, and *Corchorus olitorius L*. Consumption of the African leafy vegetables has been on a gradual rise in the recent past. The rise is due to health promotion efforts by governments to emphasize the higher nutritional and health benefits they confer to the body. The objective of this study was to assess the prevalence of consumption of green leafy vegetables and in association with cancer screening in the slum community. The study was cross-sectional in design and used a structured questionnaire to get quantitative information. Random sampling was used to select 439 households for the study. The method used was an interviewer-administered questionnaire. The questionnaire included a food frequency questionnaire over a seven day period and a section on demographic and economic characteristics. Association was done using chi-square analysis. The significance level was at a $P \leq 0.05$. The prevalence of cancer in the area was at 4%. More females (23%) than males (14%) were found to have been screened for. The study established was a significant association between gender and cancer screening ($\chi^2$8.034, DF=1, P=0.005). There was a significant association between occupation and cancer screening ($\chi^2$28.158, df = 6, P=0.000). The most daily consumed leafy vegetables were kales (18.72%), spinach (16.44%) and

cabbages (5.71%). Consumption frequency of vegetables that were associated with cancer screening included: broccoli (P=0.00, Cl (0.000-0.001), kales (P=0.01, Cl (0.009-0.015), mushroom P=0.02, Cl (0.014-0.021), Spinach P=0.00, Cl (0.001-0.003) and pumpkin P=0.02, Cl (0.019-0.027). The demographic characteristics (gender and occupation) associated with cancer screening. Consumption frequency of broccoli, kales, mushroom, spinach, and pumpkin leaves was associated with cancer screening. The study recommends the introduction of nutrition education in cancer screening campaigns.

### 3.1 Introduction

Cancer is a disease that can affect any area and any person all over the globe. Cancer screening has been shown to be one of the ways in which mortality due to cancer can be significantly reduced (Cookle et al. 2004) and (Kris et al. 2002). Primary cancer prevention such as acquiring a vaccination or banning of smoking has been shown to be gold standards for cancer prevention and has also been indicated to be a cost-effective way to deal with cancer menace. Early detection and screening is a secondary cancer prevention measure that is used in addition to primary measures to ensure increased awareness (McKee 2004).

In low-income groups, cancer screening was shown to be very low (Islam et al. 2017). Normally in these areas, cancer is diagnosed at a late stage which often leads to the high mortality rates in these areas (Islam et al. 2017).

In Kenya many interventions have been done in order to increase the levels of cancer screening in the county, these include free medical checkups in hospitals, churches and even in the office. This form of intervention is mostly focused on the middle class because the low-income group

hardly go to hospitals and work mostly at home or at informal employment where current interventions are not targeting. This study, therefore, intended to establish the link between the frequency of consumption of leafy vegetables and cancer screening in Slums in Kangemi.

Cancer is increasingly being demystified and it has been shown that up to 10% of the disease is due to genetic causes while 90% is due to lifestyle or environmental exposures (Drewnowski et al. 2015). The diet of an individual can contribute up to a 35% risk of cancer occurrence (Drewnowski et al. 2015 and Seguin et al. 2016). “Most carcinogens in the diet are in the form of nitrates, nitrosamines, pesticides, and dioxins come from food, food additives or from cooking” (Donkin et al. 1998).

High fruits and vegetable consumption (five servings per day) has been shown to reduce the risk of cancer occurrence (Donkin et al. 1998). “The type of vegetable that has the most protective effect are raw vegetables followed by allium vegetable, carrots, green vegetables, cruciferous vegetables and tomatoes”(Ochieng et al. 2018)

African leafy vegetables (ALVs) have been associated with nutritious and immune-boosting properties. This indicates that they can, therefore, be categorized as functional foods. Moreover, studies have found ALVs to be having bioactive compounds that play crucial roles in the body when consumed (Gupta et al. 2009 and Adefegha et al. 2018). The roles include prevention from chronic diseases.

There is limited information on the consumption of ALVs in Kenya. However, indications are that the vegetables are superior to their exotic counterparts in the provision of micronutrients.

The ALVs that are found in Kenya are diverse. The vegetables, for a long time, we're growing wildly but in recent times most have been domesticated and commercialized. These vegetables include amaranths leaves, spider plant leaves, African nightshade leaves, cowpeas leaves and jute mallow leaves among others. The object of this study was to, therefore, the association between the consumption pattern of green leafy vegetables and cancer screening in peri-urban Nairobi.

### 3.2 Study design and methodology

#### Study design

The study was cross-sectional and descriptive in design. Data was collected and analyzed per objective on consumption pattern of African black nightshade as a leafy vegetable and the socio-economic and socio-demographic characteristics of households in Kangemi, a slum area of Nairobi Metropolis

Data on dietary consumption patterns of leafy vegetables were collected using a food frequency questionnaire. In addition data on cancer screening which also included socio-demographic and socio-economic characteristics of respondents were collected using a semi-structured questionnaire.

#### Methodology

#### 3.2.1. Study Site

The study was carried out in the Kangemi slum. Kangemi is located on the western outskirts of Nairobi County. It has a population of over 100,000 people (KNBS) The population is multi-ethnic although the majority are from the Luhya community. The slum is surrounded by middle-

class dwellings, but to the southern end, it borders another slum called Kawangware. Kangemi hosts one of the largest open-air markets for fruits, vegetables, and other farm produce. About one-quarter of the population is Catholic by religion (KNBS). Appendix 3 shows a map of Westlands constituency which has five wards among which Kangemi the study site is highlighted.

### 3.2.2. Sample size calculation

Sample size (N) will be calculated based on Fischer formulae (1991):

$N= Z^2PQ/D^2$

P =which is the proportion of population Consuming indigenous vegetables 50%.

Z= is the standard normal deviation set at 1.96 (for 95% confidence interval).

Q= is 1- P

D= is the desired degree of precision set at 5% and a 1.1% design effect was considered.

A sample size of **423** households was calculated using the formula considering attrition at 10%.

#### 3.2.2.1 Inclusion criteria

The inclusion criteria for individuals include residents of the Kangemi ward. One individual per household will be interviewed. In addition, only residents who consent to undertake the interview will participate in the study. To be involved in the study the respondent will be required to be above 18 years of age (adult).

#### 3.2.2.2. Exclusion criteria

This will involve those individuals who are not willing to participate in the study or respondent not a resident of Kangemi. The study will also exclude households that do not have respondents at that particular time of interview.

### 3.2.3 Sampling procedure

Multistage sampling was used: Nairobi County was chosen through purposive sampling as the decision to reach the place did not use any probability. In Nairobi County, there are 18 constituencies and Westlands constituency was chosen through purposive sampling. Kangemi ward was chosen by purposive sampling and through simple random selection, the four villages were selected out of eight. Further through simple random sampling, households were selected for an interview (Figure 5). A sampling frame of 100,000 households in Kangemi was used. The sampling frame was divided by eight to get a sample of 12,500 households in every village. Using a calculator 106 households were randomly selected in the respective villages. Equal distribution households within the area necessitated the equal distribution of sampled households.

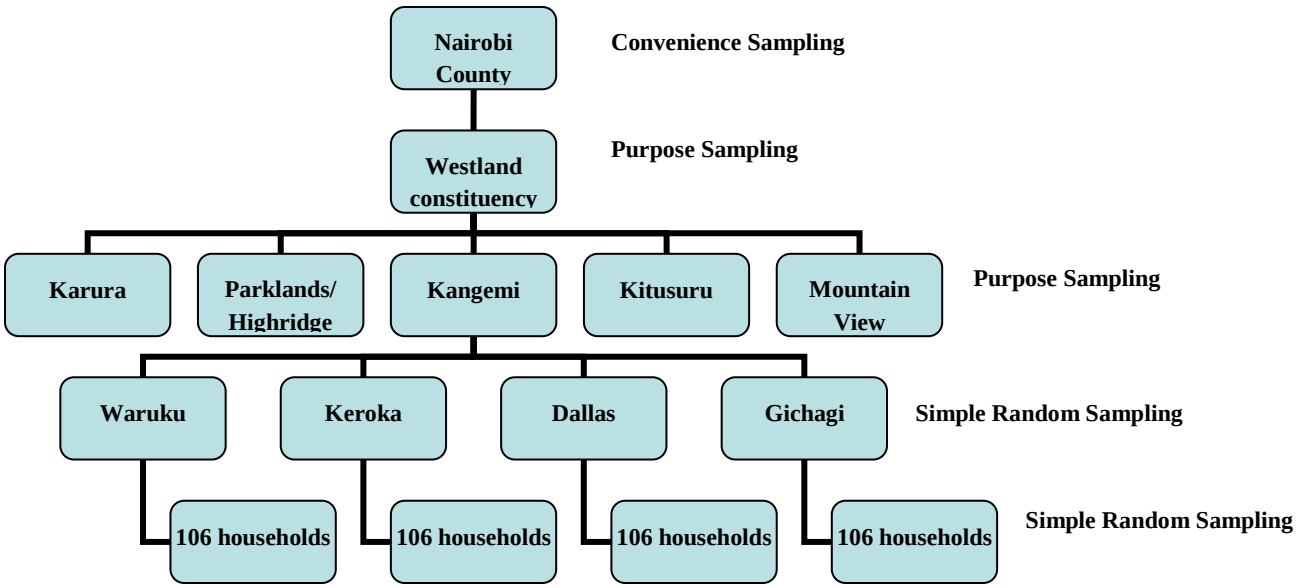


**Figure 5: Sampling Schema**

### 3.2.3 Recruitment and Training of Research Assistants

An advertisement was done in the study area for research assistants through word of mouth. Five research assistants from genders were recruited. This included three ladies and two gentlemen. The Criteria for qualification as a research assistant were that: one must have completed Secondary education and must be knowledgeable in English and Swahili languages. Research assistants were trained for two days, the training was conducted at Kihumbuini area. Training module attached in appendix 5.

### 3.2.4 Pretesting of study tools

The Structured Questionnaire was pre-tested on thirty households in Kihumbuini village, one week before the study. The questionnaire was revised and coding was done based on the responses from the pre-test exercise. Thirty households are chosen as the minimum sample size for a pre-test because it can uncover common problems with the tools (Perneger et al 2015).

### 3.2.5 Data Collection Tools

Data was collected in August 2015 using an individual questionnaire and food frequency questionnaire.

**Individual questionnaire**

This was to determine the socio-demographic and socio-economic characteristics of respondents at the household level. This included age, marital status, religion and level of education for demographics while economic include occupation, income, and housing facilities.

To ensure that the information collected was accurate, various interview techniques were used; the question asked probed the respondent to get more information. The interviewer listened carefully, maintain eye contacts and give maximum attention when conducting an interview.

**Food frequency questionnaire**

Individuals were interviewed on their levels of consumption of leafy vegetables using a semi-qualitative food frequency questionnaire as recommended by Willett, 2012. Food frequency was used to assess the frequency with which vegetables were consumed during a specified period of time (7days). The food frequency questionnaire (FFQ) took approximately 20 minutes to administer. Food scores were calculated from the FFQ of consumption of certain food groups. A score of one was given to the respondent who had consumed a specific vegetable. During analysis the most frequently and least frequently consumed vegetable was highlighted.

### 3.2.6 Data quality control and analysis

The data from the structured food frequency questionnaire were pre-tested to ensure any information that is not clear to the respondents is rectified before going for the actual data

collection. To ensure validity there was the double-entry of data. Questionnaires were checked for completeness, data cleaning was done during data entry and during analysis, and normality test was done to check for outliers.

The data were analyzed using statistical analysis such as chi-square. The SPSS V. 20.0 was used as a tool for data management and statistical analysis. The statistical significance of results was fixed at a 95% confidence level ($P \leq 0.05$).

### 3.2.7 Ethical considerations and consent to participate

Before beginning the study ethical clearance was obtained from the Kenyatta National Hospital/ University of Nairobi Ethical research committee (KNH-UoN ERC) reference number P705/12/2014**.** During the study, voluntary consent was obtained from all respondents**.** Every individual qualified for the study had an equal opportunity to participate in the study. The appropriate methodology was used both in the survey. The study is non-invasive and informed consent was obtained from the respondents. All the information obtained was handled in confidence. Before respondents could give information for an interview they were taken through a consent form. The consent form (See appendix 1) informed the respondents on the following aspects; study questions or objectives, the voluntariness of their participation, benefits and risks of the study and information on contacts of the investigator and the KNH/UoN- ERC. After accepting to carry on with the interview the respondent was requested to sign the consent form in the presence of a witness who also signed.

### 3.3 Results

#### 3.3.1 Sociodemographic characteristics

The majority of the respondents were females at 88%. The majority age group was the youth at 76%. About three-quarters of the respondents were married (78%) with only 1.6% of the respondents being divorced. Most of the respondents were of the Christian religion (99%). Approximately a third of the respondents had secondary school level education (32%). About a quarter of respondents had primary school level education (24%). Households which were headed by males were 81%. Table 1 shows a summary.

**Table 1: Socio-Demographic characteristics**

| **Total (N)** | **439** | **Frequency** | **Percentage** |
|---|---|---|---|
| **Sex** | Male | 52 | 11.8 |
| | Female | 387 | 88.2 |
| **Age** | 18-35yrs | 333 | 75.9 |
| | 36-50yrs | 74 | 16.9 |
| | 51-65yrs | 30 | 6.8 |
| | Above 65yrs | 2 | 0.5 |
| **Marital status** | Married | 343 | 78.1 |
| | Single | 71 | 16.2 |
| | Separated | 11 | 2.5 |
| | Divorced | 7 | 1.6 |
| | Widow/widower | 7 | 1.6 |
| **Religion** | Christian | 434 | 98.9 |

| | | | |
|---|---|---|---|
| | Muslim | 4 | 0.9 |
| | Other | 1 | 0.2 |
| **Education level** | Never | 14 | 3.2 |
| | Dropped at primary | 62 | 14.1 |
| | Completed primary | 105 | 23.9 |
| | Dropped at secondary | 69 | 15.7 |
| | Completed secondary | 140 | 31.9 |
| | Dropped at tertiary | 16 | 3.6 |
| | Completed tertiary | 33 | 7.5 |
| **Household head** | Father | 355 | 80.9 |
| | Mother | 83 | 18.9 |

### 3.3.2 Socioeconomic Characteristics

The economic status of the households was quite divergent with the least earning KES 400 per month and the most earning KES 60,000. The median monthly income was 9,000, the mean was 10,002 and the standard deviation of 6,179. All the respondents were in the low-income bracket as indicated in Table 2.

**Table 2: Occupation of respondents**

| **Total N=435** | | **Frequency** | **Percentage** |
|---|---|---|---|
| **Occupation** | Casual | 59 | 13.4 |
| | unemployed | 93 | 21.2 |
| | Salaried employees | 51 | 11.6 |
| | Housewife | 148 | 33.7 |
| | Farmer | 5 | 1.1 |
| | Self-employed | 79 | 18.0 |
| **The income per month (KeS)** | | | |
| | 400- 15,000 | 342 | 77.9 |
| | 15,001- 30,000 | 60 | 13.7 |
| | 30,001- 60,000 | 7 | 1.6 |

### 3.3.3 Cancer Screening and Occurrence

A total of 439 individuals participated in the study. Out of 387 females who had participated in the study, 23% gone for cancer screening. On the other hand, only 14% of the 57 males had participated in screening. Only approximately 4% of the households interviewed had cases of cancer patients as shown in Table 3.

**Table 3: Cancer Occurrence distribution by Type and Gender**

| Types of cancer (N=18) | Males | Females |
|---|---|---|
| **Breast cancer** | 0% | 28% |
| **Cervical cancer** | 0% | 6% |
| **Liver cancer** | 0% | 11% |
| **Neck cancer** | 6% | 0% |
| **Stomatch cancer** | 0% | 22% |
| **Throat cancer** | 6% | 22% |

Females were most affected by breast cancer (28%), stomach cancer (22%) and throat cancer (22%). Males were affected by neck cancer (6%) and throat cancer (6%).

### 3.3.4 Frequency of Consumption of Green Leafy Vegetables by Respondents

The green leafy vegetables that were consumed on a daily basis were kales where about 82 (18.72%) respondents said to be consuming them. The second most consumed leafy vegetable on a daily basis was spinach 72 (16.44%). The cabbage was the third most consumed leafy vegetable at 25(5.71%). African black nightshade leafy vegetable was shown to be the most daily consumed ALVs at 15(3.42%). While the vegetable that was rarely consumed was indicated as the stinging nettle at 94.1% of the respondents indicating that they have never consumed it. Table 4 shows a summary of the frequencies.

**Table 4: Frequency of vegetable consumption**

| Weekly vegetable consumption | Never % | Once – 3times % | 4times- daily % | Chi-square |
|---|---|---|---|---|
| **African leafy vegetables (ALVs)** | | | | |
| Amaranth | 32.2 | 59.6 | 8.2 | 0.09 (0.08-0.10) |
| African nightshade (*Solanum scrabrum)* | 20.1 | 68.3 | 11.6 | 0.22 (0.21-0.23) |
| Cowpea (*Vigna unguiculata*) | 40.4 | 53.0 | 6.6 | 0.17 (0.16-0.18) |
| Spider plant *(Chlorophytum comosum)* | 49.8 | 42.7 | 7.5 | 0.05 (0.04-0.05) |
| Stinging nettle *(Urtica dioica*) | 94.0 | 5.0 | 0.9 | 0.08 (0.07-0.084) |
| Jute Mallow (*Corchorus olitorius)* | 52.5 | 42.7 | 4.8 | 0.14 (0.135-0.153) |
| Pumpkin (*Telfairia occidentalis*) | 56.6 | 39.0 | 4.3 | 0.02 (0.019-0.027) |
| **Exotic leafy vegetables** | | | | |
| Kales | 18.3 | 46.1 | 35.6 | 0.01 (0.009-0.015) |
| Spinach | 24.9 | 40.6 | 34.5 | 0.00 (0.001-0.003) |
| Cabbage | 33.3 | 51.1 | 15.5 | 0.11 (0.10-0.12) |

The most commonly consumed ALV (with consumption 4 and more times per week) is African nightshade leafy vegetables with a total of 11.6%, followed by amaranths species with a total of 8.2%. The least consumption is with stinging nettle. Compared to exotic vegetables, the most commonly consumed are kales with a total of 35.6%, followed by spinach 34.5%, cabbage total of 15.5%. Still, exotic vegetables are more commonly consumed than ALVs.

### 3.3.5 Association among variables Demographic characteristics, Consumption of Leafy Vegetables and Cancer Screening

To show the association between cancer screening and different variables, the chi-square test, was carried out. The Phi- Cramer's V test was also analyzed to show the strength of the association. Table 5 shows the demographic characteristics associated with cancer screening.

**Table 5: Demographic characteristics association to cancer screening**

| **Total (N)** | **439** | **Frequency** | **Percentage** | **$\chi^2$ to (cs)** |
|---|---|---|---|---|
| | Male | 52 | 11.8 | $\chi^2$ =8.034,df=1 |
| **Sex** | Female | 387 | 88.2 | P=0.005* |
| | 18-35yrs | 333 | 75.9 | |
| **Age** | 36-50yrs | 74 | 16.9 | $\chi^2$ =2.961,df=3 |
| | 51-65yrs | 30 | 6.8 | P=0.398 |
| | Above 65yrs | 2 | 0.5 | |
| **Marital status** | Married | 343 | 78.1 | |
| | Single | 71 | 16.2 | $\chi^2$ =7.404,df=4, |

| | | | | |
|---|---|---|---|---|
| | Separated | 11 | 2.5 | P=0.116 |
| | Divorced | 7 | 1.6 | |
| | Widow/widower | 7 | 1.6 | |
| **Religion** | Christian | 434 | 98.9 | $\chi^2$ =0.507,df=2, |
| | Muslim | 4 | 0.9 | P=0.776 |
| | Other | 1 | 0.2 | |
| **Education level** | Never | 14 | 3.2 | |
| | Dropped at primary | 62 | 14.1 | $\chi^2$ =3.511,df=6 |
| | Completed primary | 105 | 23.9 | P=0.742 |
| | Dropped at secondary | 69 | 15.7 | |
| | Completed secondary | 140 | 31.9 | |
| | Dropped at tertiary | 16 | 3.6 | |
| | Completed tertiary | 33 | 7.5 | |
| **Household head** | Father | 355 | 80.9 | $\chi^2$ =0.607,df=2 |
| | Mother | 83 | 18.9 | P=0.738 |

Cs- cancer screening

### 3.3.6 Association among consumption of nightshade vegetables, demographic and economic variables

The variables that had a significant association with consumption of nightshade leafy vegetables included: marital status and household head (socio-demographic variables). In addition, the occupation was another variable with association with African nightshade leafy vegetable consumption. Table 6 shows a summary of the significant associations.

**Table 6: Association among consumption of African nightshade vegetables, demographic and economic variables**

| Consumption of African nightshade leavy vegetable | | Yes | No | Test value | p-value | Phi and Cramer's V test |
|---|---|---|---|---|---|---|
| **Marital status** | Married | 284 | 59 | 12.57 | P=0.006 | 0.179 |
| | Single | 45 | 26 | | | |
| | Separated | 9 | 2 | | | |
| | Divorced | 6 | 1 | | | |
| | Widow/widower | 6 | 1 | | | |
| **Occupation** | Casual | 50 | 9 | 21.77 | P=0.001 | 0.225 |
| | Unemployed | 60 | 33 | | | |
| | Salaried employees | 42 | 9 | | | |
| | Housewife | 131 | 17 | | | |
| | Farmer | 4 | 1 | | | |
| | Self-employed | 60 | 19 | | | |
| **Household head** | Male | 293 | 62 | 9.27 | P=0.006 | 0.149 |
| | Female | 56 | 27 | | | |

## 3.4 Discussion

### 3.4.1 Socio-demographic and socio-economic characteristics

In Kenya, there has been an influx of people migrating to the urban areas in search of employment and a better life. This has resulted in the creation of Slums. It is estimated that over two-thirds of the urban population live in slums or slum-like residence. Slums are characterized by high unemployment, social fragmentation, high levels of morbidity and poor living conditions. From the finding of the study most respondents were either staying at home or unemployed. This concurs with most studies done in Slums (Waila et al 2018). Findings showed that women were left at home with the children while the men went to look for work or money to meet the family's needs. This is evidenced by the high percentage of respondents being women yet the households' heads are males.

Most young people migrate to urban settings to look for work compared to the older. Over three-quarters of the population is below thirty-five years of age and unemployed. This indicates that most of the population are dependents. This highlights the plight of many slum dwellers who are likely to be exploited by unscrupulous people who lure them to harmful practices such as drugs, alcohol and violent gang that lead to insecurity in such neighborhoods.

The education level for most of the respondents was up to secondary school only. The lack of tertiary education in most of the residents is an indicator of the underemployment rate in the area.

The average income of the respondents per household from the study is approximately about three dollars per day. In relation to the housing situation where most houses are rented and the average rent is $30 per month. Considering the average income, it is quite difficult to get decent amenities such as good schools and hospitals. This causes the informal settlement dwellers to rely on free services that the government offers. However, government facilities are often underfunded and lack enough personnel.

### 3.4.2 Prevalence and Cancer Screening

Cancer incidence or occurrence studies have been done in developed nations in Europe, North America and parts Asia (Kamboh et al. 2018). In Africa, little has been done due to the lack of equipment and resources. From the findings of this study, cancer affected 4% of the interviewed households in Kangemi. Being a dwelling place for the low-income population.

The county council clinic in the area offered free screening services for cervical, breast and prostate cancers. However, only 23% of the female and 14% of male respondents stated having been screened for cancer. This indicates low levels of lack of awareness for the seriousness of screening for early detection to increase the chances of cure. The residents only go to the clinic when sick. A study done in India showed screening improved when mobile than stationary clinics were used (Killip et al. 2007). It is possible that the accessibility of the clinics was poor and that influenced the popularity to go for screening.

Breast cancer was the most reported form of cancer followed by stomach and throat cancers. Liver cancer incidents were also reported. Results compare well with other studies, breast cancer is one of the most prevalent cancers that affect women worldwide (Philips et al 2018,

Chandarlapty et al 2016). In the developing nations where most people living in urban settings live in slums, it is important to note the disparities in terms of the type of cancer prevalent. Breast cancer for instance in the developed world is mostly relating to lifestyle factors such as diets and lack of physical activity (Rudolph et al 2018), while in developing nations cancer of the liver, stomach and throat seem to be more prevalent due to environmental exposures, malnutrition and infections (viral or bacterial) the lead to formation of cancer (Bernhardt et al. 2006).

Comparing the demographic characteristics and cancer screening there was a significant association in the sex of respondents indicating that females were more willing to undertake the screening as compared to males. This could also be translated to mean that women are more available to go for the screening as compared to men because the females stay home, while the latter is busy in casual jobs trying to make ends meet for their families.

It was observed from the study that occupation had a statistically significant association with cancer screening. This is because most people who are working have limited time to go to the clinic for screening as compared to those who were unemployed or housewives. This should also inform policy where employers should give time off even to the casuals for medical screening in order to ensure a healthy and productive individual. Results indicate that all respondents were in the low-income bracket. This is indicated as true since the Kangemi area is a slum and the population residing there is poor.

### 3.4.3 Frequency of consuming leafy green vegetables

The recommended daily intake of vegetables is about three servings (Hasnain et al. 2014). The increased consumption of vegetables has shown a correlation with a decrease in non-communicable diseases. Moreover, the intake of leafy vegetables has also shown a decrease in mortality especially with patients with chronic diseases (Donnelly et al. 2015). In addition, low consumption leafy vegetables have been evidenced to cause the death of approximately 2.7 million people worldwide annually (Vineis et al. 2013).

ALVs consumption is still low compared to exotic counterparts. However, recently ALVs are gaining popularity due to health benefits they have been associated with (Dietrich et al. 2006). From the study, African nightshade leafy vegetable was most consumed compared to other indigenous vegetables. this was followed by amaranth, spider plant, and cowpea respectively. The ALVs are slowly gaining popularity even being sold in upscale supermarkets. Generally, it is seen that ALVs are eaten less frequently compared to their exotic counterparts such as cabbage, kales, and spinach. A previous study had indicated that the most consumed African leafy vegetables include the amaranths and African African black nightshade (Aggarwal et al. 2009). Mostly exotic leafy vegetables were consumed frequently because most respondents know how to cook them and the vegetables are easily available throughout the year. African leafy vegetables, on the other hand, take a long time to process before cooking and individuals mostly consume the vegetables based on ethnic backgrounds (Aggarwal et al. 2009). For example, jute mallow will be mostly consumed by individuals from the Luhya community. The other communities are not familiar with the methods of preparation for consumption.

The frequency of consuming traditional vegetables also differs from exotic vegetables. Most individuals preferred to consume African leafy vegetables about once or twice a week. Consumption of exotic leafy vegetables was more frequent. This is because of the easy availability of exotic vegetables. Moreover, the processing time of exotic vegetables is shorter as compared to ALVs. Individuals preferred exotic as they are very easy to cook as compared to African leafy vegetables.

Until about a decade ago, ALVs especially African nightshade leafy vegetables were regarded as a diet for the poor. They were substituted with exotic vegetables such as kales, spinach, and cabbage by the colonizers. Due to promotion ALVs are slowly being reintroduced into the diets of Kenyans of all socioeconomic levels. Currently, several ALVs have been domesticated, including *Vigna unguiculata, Amaranthus species,* and *Solanum scrabrum.* The domesticated vegetables are sold in open and upstate markets. Moreover, African nightshade vegetables often outsell the exotic vegetables due to the gain of popularity.

### 3.5 Conclusion

Cancer screening was done by a quarter of the respondents. Only 4% of the population had reportedly been diagnosed with cancer. African nightshade leafy vegetable was the most consumed ALV. Kale was the most consumed exotic leafy vegetable. There was a relationship between demographic characteristics (marital status, occupation, and household head) and consumption pattern of nightshade leafy vegetables. There was a significant association between cancer screening and consumption frequency of kales, pumpkin, and amaranth. Hence it was concluded that there was a relationship between the respective consumption of vegetables and cancer screening. Further research is recommended to unravel the nature of the relationship

between cancer screening and vegetable consumption. In addition, nutrition counseling sessions to include cancer screening programs.

# CHAPTER FOUR: KNOWLEDGE ON BENEFITS OF VEGETABLE CONSUMPTION AND COOKING TIME OF LEAFY VEGETABLES IN A PERI-URBAN COMMUNITIES


## Abstract

Leafy vegetables are widely available in sub-Saharan Africa, their consumption offers many benefits to the body. Nutritionists and other health professionals encourage clients and patients respectively to consume them. The increase of non-communicable diseases despite the increased knowledge of the benefits of leafy vegetable consumption is worrying. Currently, leafy vegetables have been shown to boost immunity due to the high ascorbic acid levels. Phytochemicals and other active compounds found in them. Phytochemicals use different mechanisms to reduce the risk of chronic diseases and generally improve human health. Leafy vegetables are also high in fiber that is said to control the blood sugar levels. This helps in the prevention and management of diabetes. Cooking time of leafy vegetables is important to ensure maximum benefits from leafy vegetables. Sometimes the cooking of particular African leafy vegetables is community-specific. The more leafy vegetables are cooked the more the nutrients degrade. Hence cooking methods such as steaming greatly encouraged, while boiling is discouraged. This study aims at providing baseline information on the association between knowledge of benefits and cooking methods.

This study was carried out in the informal settlement of Kangemi in Nairobi- Kenya. The cross-sectional study design was used to collect information from randomly selected 439 respondents. The tools for collecting data were interviewer-administered questionnaires. Most respondents were women. However, proportionally more men were knowledgeable about benefits than

women. From the study, most respondents had knowledge of the benefits of vegetables. The major benefits given by the respondents included: immunity boosting, improve blood production and cures stomach alignment. Moreover, the most currently employed cooking method is boiling and stewing. The results also showed that there is a significant relationship between knowledge of the benefits of consumption of leafy vegetables and cooking time. However, those who have knowledge of benefits cooked the vegetables for longer periods. This indicates that knowledge of the benefits of leafy vegetables alone is inadequate. Clients need to be sensitized on the benefits of cooking the vegetables for a short period.

**4.1 Introduction**

Vegetable consumption is associated with health benefits that reduce the risk of chronic diseases (Anad et al. 2008). In particular cancer risk has been shown to significantly reduce due to increased consumption of fruits and vegetables (Key 2011). Generally, it is recommended that an individual is supposed to have at least five servings of fruit and vegetables per day (Steinmetz et al. 1996) Research has found a strong link between increased vegetable consumption and decreased risk in chronic diseases. Consuming approximately 400g/day of vegetables can prevent 20% of all cancer incidence (Van Duyn et al. 2000).

The protective role of vegetable consumption against chronic diseases such as cancer and coronary heart disease is due to phytochemicals. Phytochemicals are active compounds found in vegetables that help in promoting the health of an individual. Table 7 shows examples of this phytochemical and the mechanism of action against carcinogenesis.

**Table 7: Common phytochemicals in vegetables and the mechanism of action**

| Phytochemicals | Mechanisms of action |
|---|---|
| **Sulfides eg Dithiolthiones** | Increases enzyme activity that detoxifies carcinogens |
| **Carotenoids (β-carotene antioxidant )** | Helps in the differentiation of normal epithelial cells and inhibits cell proliferation |
| **Flavonoids (quercetin)** | This an antioxidant that reduces cell proliferation |
| **Glucosinolates** | Induces protective enzymes against cancer |
| **phytoestrogens** | An antioxidant that inhibits the growth of cancer cells |
| **Phenols** | An antioxidant that protects DNA from damage by carcinogens. |
| **Capsain** | The antioxidant prevents carcinogens from binding to DNA |
| **Anthocyanins** | Antioxidant |
| **Tannins** | Prevents carcinogens from binding to target sites |

Source: Van Duyn et al. 2000

Socio-economic status and education level of individuals have shown a relationship with vegetable consumption (Imungi et al. 2002 and Smith et al. 2005). Gender has shown a relationship in vegetable consumption where men tend to consume less (Ang et al. 2019). “The Health Eating Index (HEI) a measure of diet quality”, indicates that high consumption of vegetables is associated with better quality diets (Parsons et al. 2018).

Leafy vegetables are considered side dishes in most households in Africa. The African leafy vegetables (ALVs), have been shown to have high nutritional quality. For example, the amaranths have flavonoids which scavenge oxygen radicals and prevent oxidation of low-density lipoproteins (Onyango et al. 2012). This because they provide proteins, essential minerals and also provide vitamins (Rastogi et al 2004).

Cooking is one of the factors that affect the nutritional content of leafy vegetables (Donta et al. 2012). B-carotene (vitamin A precursor) is normally lost when cooking by 11% to 43% margin (de Moura et al. 2005. Antioxidant activity shows mixed results. For example in broccoli after cooking antioxidant activity remained the same (Parikh et al. 2015). Baking and microwave cooking of vegetables has been shown to preserve antioxidants while boiling results in the greatest loss of antioxidant (Stewart et al. 2017).

The objective of this study was to determine whether there was an association among knowledge on benefits, cooking method and cooking time of leafy vegetables.

## 4.2 Study design and methodology

### 4.2.1 Study design

The study was cross-sectional and descriptive in design. The study used interviewer-administered questionnaires. The respondents gave information on the benefits of consumption of leafy vegetables and the method of cooking leafy vegetables.

### 4.2.2 Methodology

#### 4.2.2.1 Study site

The data was collected from the Kangemi ward. Kangemi is located in a small valley on the western outskirts of Nairobi city. It is home to approximately 100,000 people (KNBS). See appendix 3

#### 4.2.2.2 Sample size calculation

The sample size (N) was calculated based on Fischer formulae· The sample size was calculated using the formula considering attrition at 10.38%. A total of 439 households were randomly selected.

Sample size (N) was calculated based on Fischer formulae (Fischer et al. 1991)

$N= z^2pq/d^2$

p =which is the proportion of population Consuming indigenous vegetables 50%.

z= is the standard normal deviation set at 1.96 (for 95% confidence interval).

q= is 1- p

d= is the desired degree of precision set at 5% and a 1.1% design effect was considered.

A sample size of 384 households was calculated using the formula. Attrition of 10% was considered and the total calculated sample size was 439 households.

#### 4.2.2.3 Inclusion and exclusion

The respondents had to be above 18 years old. In which age was verified from their national identification cards. Any individual who was not a resident of the Kangemi ward was excluded.

A resident was considered to a person who lives there permanently or has been living there for the past six months.

#### 4.2.2.4 Sampling procedure

Kangemi ward was chosen by purposive sampling. Through simple random selection, five villages were selected. These villages are Kihumbuni, Gitoka, Gichagi, Sodom, and Waruku. Further through the spinning of a pen method, households were selected for an interview. This is as shown in figure 3.

#### 4.2.2.5 Individual questionnaire

This sought to determine the knowledge on the benefits of vegetables, the type of cooking method and estimated time taken to cook the leafy vegetables. The question on knowledge was "do you know any benefit of eating leafy vegetables in your body?" The answer was "yes" or "no". 'Give one benefit of eating a leafy vegetable to your body?" The respondent gave only one benefit per respondent.

#### 4.2.2.6 Data quality control and analysis

The questionnaire was pre-tested to ensure any information that is not clear to the respondents is rectified before going for the actual data collection. Moreover, supervision from the supervisors' enhanced the validity of the data.

The data collected were analyzed using, SPSS 16.0 software which was used as a tool for data management and statistical analysis. The statistical significance of results was considered at a 95% confidence interval.

### 4.3 Results

#### 4.3.1 Benefits of Consumption of African leafy vegetables as Perceived by Respondents

A total of 439 individuals participated in the study. Total men respondents were 52 of whom 19% did not know the benefits of consuming green leafy vegetables. Women respondents who did not know the benefits of green leafy vegetables were 22% of the total 387 who participated. This is shown in figure 6.

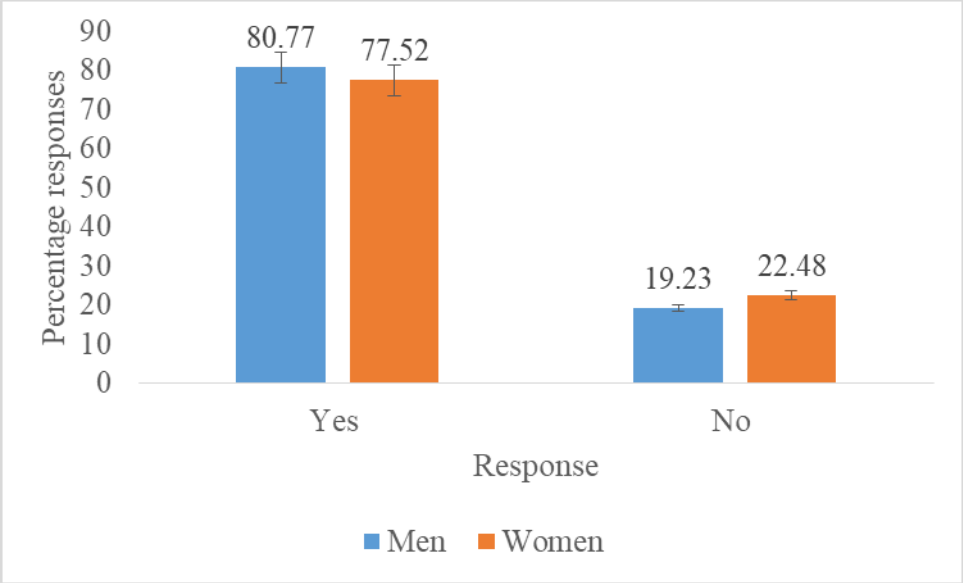


**Figure 6: Proportion of respondents in relation to knowledge on the benefits of vegetables**

The benefits that the respondents thought that were attributed to consumption of vegetables were as shown in Table 8. Respondents indicated immunity boosting (37%) and improving hemoglobin (35%) levels as the main benefits of leafy green vegetable consumption. Based on gender 55% of men knew that leafy vegetables boost immunity while 40% of the women knew that leafy vegetables improve hemoglobin levels. These benefits are, however, not medically authenticated.

**Table 8: Benefits of consuming vegetables as given by respondents**

| Benefits of consuming vegetables | Frequency (N) | Percentage% |
|---|---|---|
| Boosts immunity | 121 | 33 |
| Improve blood production | 127 | 35 |
| Cabs stomach ailments | 18 | 5 |
| Skin | 10 | 3 |
| Provides the body with energy | 44 | 12 |
| Strengthen bones | 8 | 2 |
| Provide protein in diet | 10 | 3 |
| Provide a balanced diet | 2 | 0.6 |
| Enhance the taste of food | 2 | 0.6 |
| Helps in controlling blood sugar | 3 | 0.8 |

### 4.3.2 Methods of cooking and cooking time

The following cooking methods as indicated in Table 9. The most common cooking method was a combination of boiling and stewing (72%). Stewing only (20%) was the second most used method of cooking.

The mean cooking time of the respondents was 41 ± 21 minutes while the cooking time range was from nine minutes to a maximum of 102 minutes.

**Table 9: Cooking Methods for leafy vegetables**

| Method of cooking | Frequency | Percentage n (403) |
|---|---|---|
| Boiling and stewing | 290 | 72.0 |
| Stewing only | 81 | 20.0 |
| Boiling only | 25 | 6.0 |
| Steaming | 6 | 1.5 |
| Boiling and fermentation | 1 | 0.5 |

### 4.3.3 Knowledge of benefits and time of cooking

Using a Mann-whitey U test, those who had knowledge of the benefits of leafy green vegetables had a significantly higher cooking time than those who did not have knowledge (U=33,p=0.008).

## 4.4 Discussion

The majority of respondents knew of at least one benefit that green leafy vegetables confer to the body. Proportionally more men had knowledge of the benefits of vegetable consumption than women. This is an interesting result since the previous study has shown that men consume fewer vegetables compared to women (De Moura et al. 2005). Studies on vegetable consumption have majored on women and children due to the importance of nutrition in the lifecycle. However, studies on men, in particular, are limiting.

The study showed that the majority of men know that consumption of leafy vegetables leads to improved immunity and prevention of diseases. Leafy vegetables are high in anti-oxidants (Stewart et al. 2014) and antioxidants have been associated with building the body's immunity

(Lieberman, 2003). Many women knew that leafy vegetables improve hemoglobin levels. This is reflective of nutrition education programs that encourage women of reproductive age to consume leafy vegetables as a source of iron. Leafy vegetables have been shown to have high iron levels (Sanoussi et al. 2015). Iron deficiency anemia is the leading micronutrient deficiency in the world (Yang et al. 2013). In Keny, iron deficiency anemia among pregnant women is 55.1% (Ministry of Health 2013). Consumption of green leafy vegetables has been shown to be one of the ways to combat iron deficiency in developing countries. Iron is important in blood formation since it is involved in erythropoiesis. Iron deficiency results in other serious consequences such as heart failure, and angina (Moliner et al. 2019). Eventually, iron deficiency anemia could lead to death. Other benefits that respondents attributed to consumption of leafy vegetables were: provision of energy, provision of proteins, smoothen skin and increase in bone density. Only a few respondents attributed leafy vegetable consumption to control blood sugar. This indicates that the population has not yet realized, the crucial part that vegetables play in the prevention and management of diseases such as diabetes. These allegations about the vegetables have, however, to a large extent not been scientifically authenticated.

The majority of respondents gave boiling and stewing as a method to prepare leafy vegetables for consumption. This method of preparation results in loss of water-soluble nutrients. This is aggravated by the fact that the boiling water is often discarded. Ascorbic acid is particularly lost during cooking and other water-soluble vitamins and minerals (Cilla et al. 2018). Ascorbic acid is important for immune building in the body. Minerals are also decreased by cooking by this method; because of the mineral leach into the boiling water which is often drained (Davidson and Manulu 2018. The method of boiling then stewing leafy vegetables comes from the culture.

Some vegetables, like African leafy vegetables (*Solanum nigrum*), are bitter. In order to remove the bitter taste, the vegetables are first boiled and the water discarded then stewed. Stewing only was the second most popular method of cooking. Stewing is mostly used to cook exotic vegetables such as kale, spinach, and cabbage.

Steaming has been shown to preserve most nutrients in the vegetables (Bernhardt et al 2006). However, only a few respondents use it as a method to cook leafy vegetables.

From the Mann Whitney test, respondents who had knowledge of the benefits of vegetables used more time to cook leafy vegetables. This was a significant result. This indicates that these respondents were using the traditionally established methods, while the ones who were not familiar with the vegetables cooked them by methods that are taught in nutrition. The traditional methods lead to a higher loss of nutrients (Santibanez et al. 2017). Educating the population on proper cooking of leafy vegetables for consumption is good. However, informing them on how to maximize the nutrients from the consumption of leafy vegetables is even more important.

### 4.5 Conclusion

Proportionally more men had knowledge of the benefits of leafy vegetable consumption than women. The most common method of cooking vegetables was boiling then stewing. There was a significant relationship between knowledge of the benefits of vegetables and cooking duration. A significant relationship indicated those who knew the benefits of consuming the vegetables, spent more time cooking vegetables

## CHAPTER FIVE: THE EFFICACY OF CHEMO-PREVENTIVE ATTRIBUTES OF AFRICAN NIGHTSHADE LEAFY VEGETABLE ON CANCER USING MICE MODEL


### Abstract

Human cancers are on the steady increase in the world yet, two-thirds of cancers are due to preventable causes. Diet is one major aspect that can be modified to lower the risk of cancer. The objective of this study was first, to assess the antioxidant potential of African nightshade vegetables and formulations in mice feed. Second, to evaluate the effect of consumption of vegetables on the expression of a tumor marker (urokinase plasminogen activator protein) in mice. The antioxidant activity of African nightshade leafy vegetables, when cooked and raw was determined using the Trolox standard. Using an experimental design, mice were divided into three groups. Group one was fed on a control diet. Group two was fed on a diet formulation containing cooked vegetables. Group three was fed on a diet formulation containing raw vegetables. Then the expression of urokinase plasminogen activator protein was determined by a quantitative polymerase chain reaction. The study was conducted at a 95% confidence interval. The results indicated that the antioxidant activity of African nightshade leafy vegetables increased with steam cooking. The statistical significance was established between cooked and raw vegetables. The expression of urokinase plasminogen activator protein is reduced to 3% in mice fed with cooked vegetable formulation compared to the mice fed with control diet formulation. Expression of uPA is reduced to 61% in mice fed with raw vegetable formulation compared to the mice fed with the control diet formulation. The study concludes that steam cooking was effective in increasing the antioxidant activity of African nightshade leafy vegetables. The tumor marker urokinase plasminogen activator protein expression was shown to decrease with the consumption of African nightshade vegetables.

## 5.1 Introduction

Cancer is a major health problem around the world. However, about 67% of human cancers are due to preventable causes by lifestyle modification (Borek 1997, Gao et al. 2018). In Kenya cancer ranks as the third leading cause of death after infectious and cardiovascular diseases (MoH 2017) "Chemoprevention refers to the use of agents to inhibit, reverse or retard tumor genesis" (Borek 1997, Gao et al. 2018). In recent years, ongoing research on modified diets. Vegetable consumption is greatly encouraged because the food contains phytochemicals that offer chemopreventive potential. Green leafy vegetables especially contain numerous phytochemicals that are yet to be explored in terms of cancer chemoprevention (Onyango et al. 2012). Phytochemicals are broadly defined as, "non-nutritive compounds in the plant-based diet that possesses substantive anti-carcinogenic and anti-mutagenic properties" (Santibanez et al. 2017, Tsai et al. 2014). Population-based research has shown that cancer risk is reduced by about 50% by consuming fruits and vegetables (Borek 1997, Rahmani et al. 2015). According to WHO at least five servings (one serving is about 75g cooked vegetable) of and vegetables are recommended by day (Borek 1997, Rahmani et al. 2015).

Vegetables are excellent sources of phytochemicals. "In numerous animal model studies, the evaluation of the edible parts of a plant to prevent cancer has been done" (Borek 1997). Dark green leafy vegetables have shown the potential to be chemopreventive due to the high levels of antioxidants (Borek 1997, Rahmani et al. 2015). The most important antioxidants are ascorbic acid, carotenoids which are also precursors of vitamin A, Vitamin E and phenolic compounds (Onyango et al. 2012)

Cancer growth has three stages, initiation, promotion, and progression (Borek 1997, Gao et al. 2018). Tumor initiation involves the process of being exposed to the carcinogen. Tumor promotion is the stage where there is an accumulation of pre-neoplastic cells accumulate. This stage is normally reversible. The last stage is the progression stage where there is tumor growth leading to metastasis.

On the other hand, chemo-preventive properties are divided into blocking agents and suppressing agents. Blocking agents "prevent carcinogens from reaching the target sites, from undergoing metabolic activation or from subsequently interacting with DNA, RNA or protein" (Borek 1997). Suppression agents inhibit the transformation of initiated cells in either promotion or progression (Borek 1997, Gao et al. 2018).

Phytochemicals, which include antioxidants, are able to alter tumor formation (Borek 1997, Gao et al. 2018). An antioxidant is a molecule that defends the cells against free radicals that damage cellular molecules (Santiabanez et al. 2017). Alterations that are associated with tumor formation mainly occur in the cell signaling pathways (Kaltnmeier et al. 2017). Signaling pathways regulate cell proliferation and differentiation (Kaltnmeier et al. 2017).

Mitogen-activated protein kinases (MAPK), are intracellular signaling pathways that maintain homeostasis (Kaltnmeier et al. 2017). Any alterations to the MAPK pathway leading to the uncontrolled growth of cells. This results in tumor formation. Phytochemicals have shown to have the ability to switch off and on specific signaling molecules (Kaltnmeier et al. 2017).

Protein kinase C (PKC) and phosphatidylinositol-3-kinase (PI3K) have been singled out to be targets of chemopreventive phytochemicals. The two kinases activate transcription factors such as nuclear factor-κB (NF-κB) (Borek 1997). Abnormal activation of NF-κB provides protection to malignant cells against apoptosis (Borek 1997). Overexpression of NF-κB causes the neoplastic transformation of cells (Borek 1997). Chemopreventive phytochemicals derived from the diet have been shown to suppress NF-κB activation (Borek 1997).

Urokinase plasminogen activation (uPA) is a serine protease that is 53kDa (Tsai et al 2014). The protein has a major role in tumor progression. In tumor cells, uPA expression is not properly regulated which increases the capacity for the cells to metastasis (Tsai et al. 2014) Studies have indicated that the transcription factor NF-κB regulates the transcription of uPA genes (Borek 1997, Tsai et al. 2014, Kapinova et al. 2018).

“Chemoprevention by dietary phytochemicals is acceptable cost-effective (Holzscheiter et al. 2008). However, there is little evidence that plant antioxidants derived from the diet can decrease risk or improve the prognosis of cancer (Holzscheiter et al. 2008). Animal models (mice) were used in order to have a clear control and treatment group.

According to the National cancer control and management strategy, to reduce the risk posed by unhealthy diets and physical inactivity, one of the activities is to encourage the consumption of vegetables. This study seeks to find evidence that the expression of uPA can be reduced by the consumption of African nightshade leafy vegetables.

## 5.2 Study design and methodology

### 5.2.1 Experimental design

A factorial experiment (Figure 7) was used to determine the total antioxidant activity and evaluate the effect of African nightshade leaf vegetable consumption on the expression of urokinase plasminogen activator protein in mice model. The vegetables were analyzed both when raw, and when cooked.

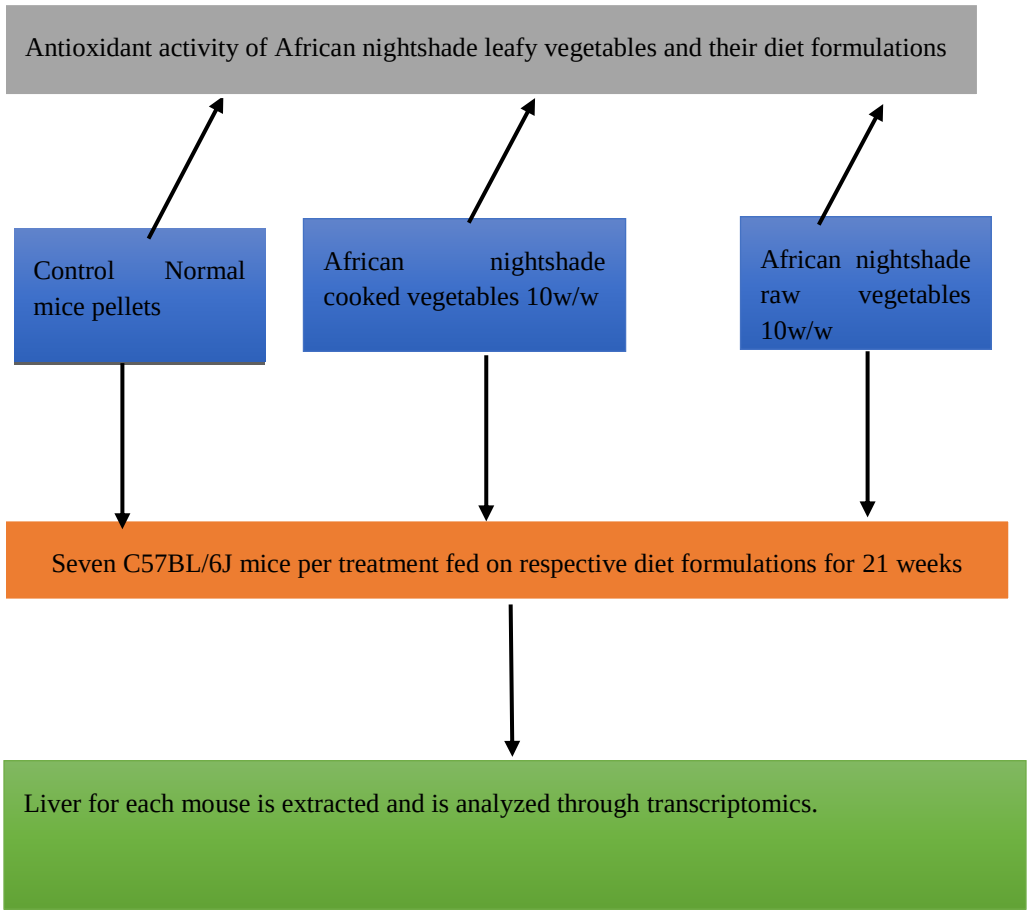


**Figure 7: Experimental design workflow**

### 5.2.2 Preparation of the vegetable extracts

The vegetables were purchased from an open-air market in Nairobi and taken to the Department of Food Science, Nutrition and Technology laboratory within hours. The vegetables were then

washed and macerated. The vegetables were then separated into two batches. One batch was cooked by steam cooking and the other was left. The steam blanching procedure was:

A selected batch of vegetables was placed in a metal basket and the basket placed in a steam blancher. At a saturated steam temperature of 151.9◦c for five minutes. The vegetables were then cooled to about $40^0$c for another five minutes.

The two samples (cooked and raw) were then blended to homogeneity in a blender then freeze-dried to ensure equal moisture content.

**Extraction:** Freeze-dried samples (0.3g) were weighed accurately into propylene tubes. Then 10ml of 80% methanol was added. The tubes placed in a mechanical shaker (Thomas scientific-Innova 26 incubator shaker) at 25◦c for 24 hours. The mixture was then centrifuged at 4000rpm (1792G) for 10min. An aliquot of the supernatant was used for the analysis of antioxidant activity determination.

### 5.2.3 Determination of antioxidant activity

Antioxidant activity of the extract was determined against a Trolox standard, according to the standard operating procedure of Biosciences Eastern and Central Africa (BECA)-ILRI hub Kenya campus. In a 100ml volumetric flask, 0.103g of Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid, 97% pure) was dissolved in10ml of absolute methanol and made up to volume with absolute methanol. It was then transferred to an amber-colored bottle. The stock solution was then stored at -$20^o$c. Trolox calibration standards were prepared by pipetting 0, 0.125, 0.25, 0.5, 0.75, 1.0, 1.25ml of the Trolox stock solution into clean 25ml volumetric flasks and diluted to volume with 80% methanol. The solutions were stored in amber-

colored bottles for a period of two weeks in a freezer at (-20$^{o}$c). To prepare 2, 2 diphenyl-1-picrylhydrazyl (DPPH) stock solution, 0.2g of DPPH was dissolved in 100ml of absolute ethanol. The DPPH solution was stored in the -20$^{o}$c freezer. To prepare DPPH working solution was an aliquot of 1ml of the stock solution into the 100ml volumetric flask with ethanol added to top up.

50ul of each standard and unknown sample was pipetted into a microtitre. Using a plate shaker, the microtiter plate was incubated in the dark for 20minutes. Absorbance was read at 515nm in a microtiter plate spectrophotometer reader (ELISA microplate reader). The standard calibration curve was plotted. The results were then expressed as total antioxidant content as mg of Trolox equivalents per 100g of dry sample. The results were calculated and expressed as follows:

Total antioxidant activity in mg of Trolox equivalent per 100g of dry sample

$$\frac{C*DF*100}{W*1000}$$

Where: C= concentration obtained from the calibration in ug/ml

DF= total dilution factor

100= conversion factor to results in mg/100g

W=weight of the sample in grams

1000= conversion from ug/ml to mg/ml

### 5.2.4 Evaluation of the cancer chemo-prevention in mice models

The chemoprevention properties of the vegetables were determined using the C57BL/6J mice model. This is recognized as a genetically relevant animal model that mimics human carcinogenesis.

Eight female and two males C57BL/6J mice were purchased from the International Livestock Research Institute (ILRI) Kenya. All mice used in this study were bred and housed in the animal facility at the school of medicine, clinical pharmacology unit, University of Nairobi.

#### 5.2.4.1 Housing for mice

All of the mice were housed in IVC 420 cages, with a flooring area of 420cm2, made up of polysulphone/polycarbonate structural material. The nesting material was sourced from a local construction shop, and the mice were in cages containing 1-5 individuals. A 12 hour light: dark cycle was maintained; with the light period from7: 00 am to7: 00 pm. Room temperature was 20° ± 2°C, and humidity 50-65%.

#### 5.2.4.2 Vegetable material

The African nightshade vegetables were washed, then shredded, and oven-dried at 50°C for eight hours, and packed in sealed polyethylene bags for storage until required for use. The dried vegetables were then used to prepare the experimental diet.

#### 5.2.4.3 Mice experimental diet

The mice pellet was bought from the Unga group limited Kenya. This diet for the control group was relatively rich in animal fat, protein, and sucrose, and consists of 64% cereal products (wheat, barley), vegetable protein 16.5% (extracts of soya beans, and dried yeast), 2% soy oil, 15% animal protein (fish meal, whey powder), and 2.5% supplements (vitamins, minerals, amino acids).

Diet type 1: dried raw African black nightshade leafy vegetables mixed with powdered mice pellet mouse diet at 10% (w/w).

Diet type 2: dried steam-cooked African black nightshade leafy vegetables mixed with powdered mice pellet diet at 10% (w/w).

Diet type 3: Control diet, pure powdered mice pellet diet

The sample size was determined on the basis of the inhibition effect observed from (-)-epigallocatechin-3-gallate, the major catechin in green tea in the ApcMin stain using the same standard deviation. (Yang et al. 2009) It is estimated that 21 mice in each group would be sufficient to detect a difference in means across the group.

#### 5.2.4.4 Breeding of mice

Mouse C57BL/6J is recognized as a genetically relevant animal model mimicking human carcinogenesis. Eight wild-types (C57BL/6J) female mice were divided into two groups through simple random sampling; each group was added to a male mouse. For breeding purposes, the mice were given the mice pellets in order to breed optimally. Between the age of six and 28 weeks, the mice pups started being fed the experimental diet. Seven mice were randomly assigned to the three different treatments. The mice were separated into three different cages according to their treatments. Mice were daily fed on 500g of diet formulation daily and water bottle for the three cages was changed every day during the cause of the experiment.

#### 5.2.4.5 Necropsy of mice

Mice of 28 weeks of age were sacrificed by cervical dislocation. Each mouse was weighed before a midline section was made and the liver removed

### 5.2.5 Determination of expression of uPA by Quantitative Real-Time Polymerase Chain Reaction (QPCR)

Total RNA from liver tissues was extracted using a Qiagen kit. For quantification of uPA mRNA, highly sensitive qPCR assays (Thermofisher 7900) were established. Based on the cDNA sequences of uPA (GenBank accession no. NM_002658). Primers for amplification for real-time monitoring of PCR reactions were obtained from Holzscheiter et al 2008; the amplicon lengths were 103 bases, uPA Forward primer AGT GTC AGC AGC CCC ACT, Reverse primer CCC CCT GAG TCT CCC TGG. The assay was performed with an optimized sybr green master mixture containing 0.5 µM of primer, 5 mM $MgCl_2$ in a total volume of 20 µl. The amplification program started with pre-denaturation at 95˚C for 10 min, followed by 45 cycles of amplification: denaturation for 10 sec at 95˚C, annealing for 10 sec at 62˚C (uPA) and elongation for 5 sec at 72˚C. Finally, the amplification products were cooled down to 40˚C for 30 sec. To verify the results, randomly selected samples were run on 1.5% agarose gels.

Mice Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was the housekeeping gene, it was chosen for normalization of the data. The primer sequence was GAPDH forward 5'-CCCCCAATGTATCCGTTGT-3', GAPDH reverse 5'-TAGCCCAGGATGCCCTTTAG- 3' (GenBank accession no. NM_01008).

Analysis of uPA expression in 21 mice liver tissue samples was done. “The mRNA concentrations were determined after extensive optimization of PCR conditions, including primer and MgCl2 concentrations and reaction temperatures and times. This permitted for obtaining a highly sensitive, specific, and reproducible quantitative real-time RT-PCR for

specific detection of these mRNAs. Single and sharply defined melting curves with narrow peaks were obtained for PCR products of the analyzed gene.

### 5.2.5 Data analysis

To ensure that accuracy was maintained, the data from the laboratory analysis was done in triplicate. For data analysis, SPSS 20.0 program was used. The levels of significance in correlations between continuous variables were calculated using an unpaired t-test and the analysis of variance (ANOVA). P-values $\leq 0.05$ were used for statistical significance. All calculations were performed using the SPSS statistical package, release 20.0 (SPSS Inc., Chicago, IL, USA).

## 5.3 Results

### 5.3.1 Antioxidant activity of raw and cooked vegetable

The antioxidant activities of the vegetable cooked and raw, as well as their respective diet formulations. To point some highlights include cooked vegetables and the cooked formulation had a much higher antioxidant activity as compared to the raw formulation. As Table 10 shows the control diet had the least antioxidant activity.

**Table 10: Antioxidant activity of African nightshade vegetables**

| **Vegetables** | **Antioxidant activity (mg/100g)of dry matter** | **Statistical test** |
|---|---|---|
| **Cooked** | 2.48 | |
| **Cooked** | 2.35 | 2.4±0.07 |
| **Cooked** | 2.37 | |
| | | **t-test, t=43.57 P=0.000** |
| **Raw** | 0.39 | |
| **Raw** | 0.39 | 0.37±0.04 |
| **Raw** | 0.32 | |
| **Formulations** | | |
| **Cooked formulation** | 0.32 | |

| | | | |
|---|---|---|---|
| **Cooked formulation** | 0.31 | 0.31±0.01 | |
| **Cooked formulation** | 0.30 | | |
| **Raw formulation** | 0.13 | | **ANOVA,F=1348.00,P= 0.000** |
| **Raw formulation** | 0.14 | 0.136±0.00 | |
| **Raw formulation** | 0.14 | | |
| **Control formulation** | 0.03 | | |
| **Control formulation** | 0.03 | 0.03±0.00 | |
| **Control formulation** | | 0.03 | |

### 5.3.1 Quantitative real-time polymerase chain reaction

The PCR efficiency was determined both for the target gene (uPA) and the reference gene (Glyceraldehyde-3- phosphate dehydrogenase- GAPDH) by qPCR analysis of serial dilutions from cDNA. The threshold crossing point values (Ct or CP) were linearly correlated with the logarithmic value of the DNA amount. The slope of this line gave the PCR efficiency; this is shown in Figures 8 and 9.

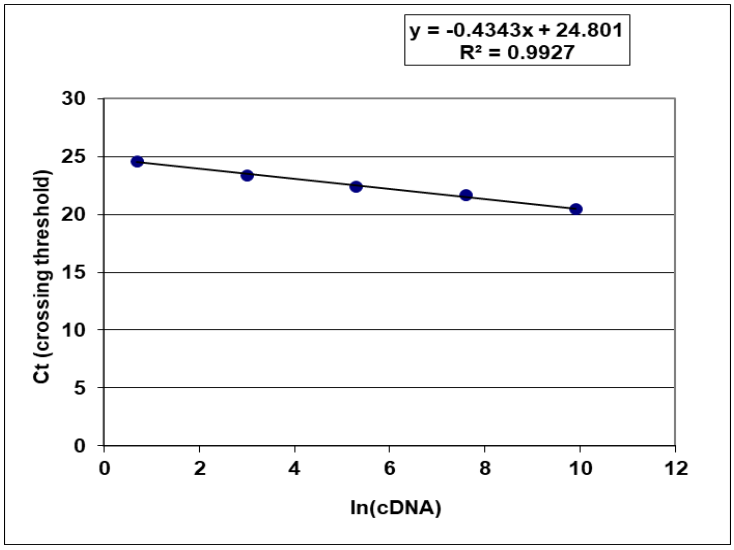


**Figure 8: PCR efficiency of the uPA gene**

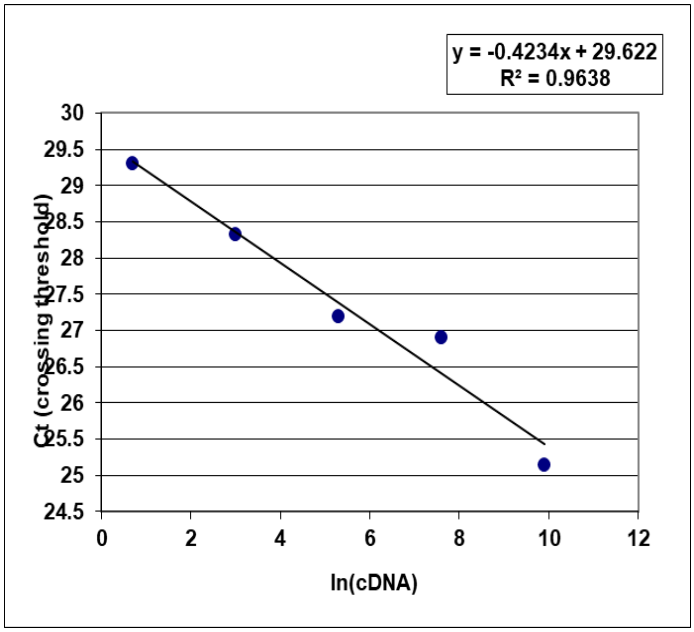


**Figure 9: PCR efficiency of reference gene**

The PCR efficiency calculated for the uPA and GAPDH standard curve was used to calculate up- or down-regulation of a certain uPA gene (normalized to GAPDH gene) for the cooked and raw treatments as compared to the control treatment. Figure 10 below shows the n-fold of control. The expression of urokinase plasminogen activator (uPA) protein is reduced to 3% in mice fed with cooked vegetables compared to the mice fed with the control diet. The expression of uPA is reduced to 61% in mice fed with raw vegetables compared to the mice fed with the control diet.

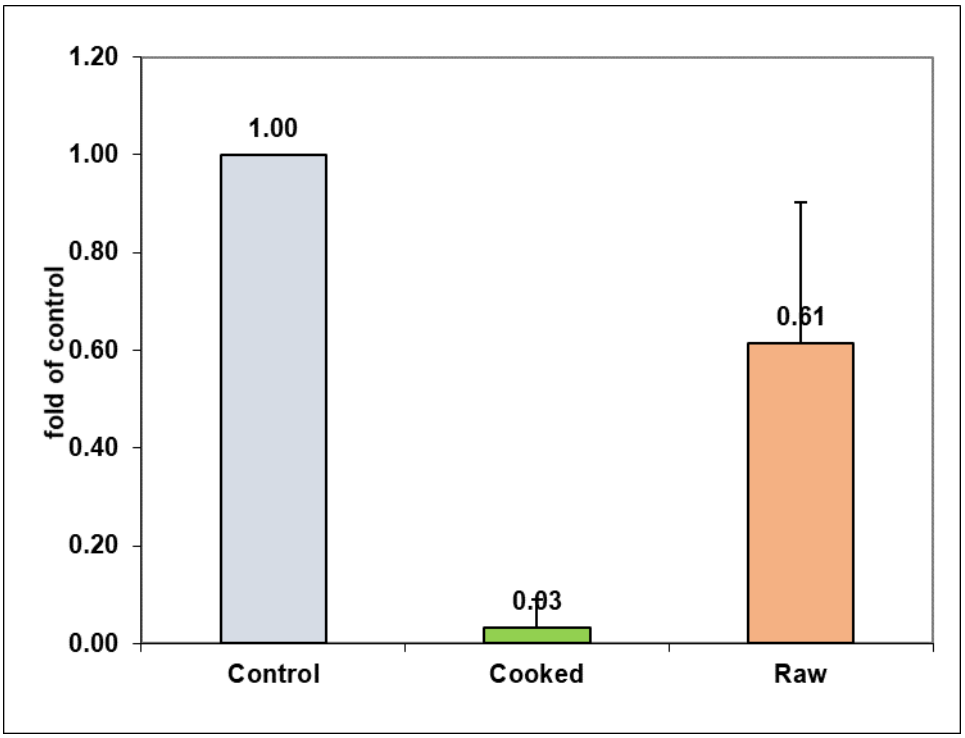


**Figure 10: Expression of uPA as n-fold of the control**

**5.4 Discussion**

Studies on nightshade have shown that the plant is medicinal (Ronoh et al. 2018, Ngwene et al. 2017). African nightshade plant, in particular, contains high levels of phytochemicals (Ivan et al. 2018, Mwona et al. 2017). The vegetable also contains essential vitamins that help to build the body's immunity (Gogo et al. 2017, Ochieng et al. 2018).

In this study African Nightshade, Leafy Vegetable (ANLV) was in focus. ANLV has always been consumed by rural African communities (Kansiime et al. 2018, Musotsi et al. 2017). However, after the introduction of exotic vegetables such as kales, cabbage, and spinach, most urban communities stopped consuming them (Demmler et al. 2017). Now consumption of ANLV is on the steady rise due to their implication in health benefits (Kansiime et al. 2018).

This is in the light that, in Kenya, non-communicable diseases are on the rise due to poor lifestyle and diet (Onyango et al. 2018, Azzini et al. 2017).

Antioxidants that are part of phytochemicals, (Moyo et al. 2018) are essential especially in stopping cell invasion and tumor progression. This study sought to find out how the expression of a tumor marker uPA, is affected by the consumption of ANLV using a mice model. This study also sought to find out the antioxidant activity of the ANLV.

The ANLV vegetables were cooked in steam. This method is recommended because it ensures that most of the essential nutrients and phytochemicals are optimally preserved (Fortes et al. 2018). Form the results of the antioxidant activity of the vegetables was higher in the cooked than the raw vegetables. Moreover, there was a statistically significant difference between cooked and raw vegetables. These results indicate that ANLVs are better consumed when cooked than when raw.

Studies have encouraged eating vegetables raw (Taborelli et al. 2017, Nobosse et al. 2017). Generally, the nightshade vegetables are prepared for consumption by cooking. The antioxidant activity of cooked vegetables was twice as high as compared to raw vegetables. This is probably explained by the ability of the steam heat to break the cell wall and release the nutrients. The cooking method (steam cooking) used has been reported to lead to high antioxidant activity (Benchimol et al. 1989, Murador et al. 2016) as compared to boiling in water or stewing.

The study also investigated the antioxidant activity in the feed formulation for the mice study. The cooked formulation had the highest, then the raw formulation and the control formulation had the lowest antioxidant activity.

Each group of mice was fed on one of the three different formulations for 21 weeks. After sacrificing the animals, the RNA from liver tissue extracted and converted to cDNA. From the results, uPA was least expressed in mice group fed on the cooked formulation as compared to the control. The mice group fed with raw vegetable formulation also had a decrease in expression of uPA by about 40% compared to the control. Urokinase plasminogen activator (uPA) protein is a tumor marker that determines the aggressiveness of cancer and guide treatment (Mahmood et al. 2018). This shows that the consumption of African nightshade leafy vegetables significantly down-regulates the expression of the uPA gene.

The tumor marker (uPA gene) is used to show the prognosis of cancer (Castello et al. 2002), the more highly expressed it is, the poorer the prognosis. From this study, the group that ate cooked ANLV had the best prognosis.

"The plasminogen activation system plays a role in cancer progression" (Dolcet et al. 2005). The mechanism is whereby plasminogen is activated to plasmin by transcriptional factors such as NF-κB (Mckay et al. 2008). The activated plasmin then causes the degradation of the extracellular matrix. In addition, there is tumor cell invasion and metastasis of the cancer cells to the whole body. Studies have also shown that uPA is an indicator of patients' survival rates (Dass et al. 2008).

Antioxidants have been closely linked to transcription factor NF-KB. The 7dietary antioxidants prevent the transcription factors NF-KB from being expressed in the body. This later prevents uPA also from being expressed and activated to plasmin. This indicates that the levels of antioxidants in a diet are correlated to the expression of uPA.

In this study, the animal model was used in order to ascertain the control of the experiment. Mice models have been used in cancer clinical studies because they can be easily extrapolated to humans.

**5.5 Conclusion**

From the study, Cooked (steam cooked) ANLV have significantly higher antioxidant activity than raw ANLV. Consumption of cooked (steam cooked) African nightshade leafy vegetables reduced the expression of urokinase plasminogen activator protein.

# CHAPTER SIX: GENERAL DISCUSSION CONCLUSION AND RECOMMENDATIONS

## 6.1 General discussion

Cancer incidences are increasing steadily in Kenya among people from all social-economic backgrounds. On one hand, the population of the increasingly growing middle class is quickly adopting westernized dietary practices and sedentary lifestyles. Given the low-income socio-economic groups are trying to catch up to satisfying the desire to be sophisticated. Air pollution, stress, smoking and currently climate change all contribute to exposure to increased risk of cancer. One of the cost-effective ways to manage cancer is early detection. In this study, cancer screening rates were low at less than a quarter of the population had been screened, despite the campaigns to encourage the population to go for cancer screening. Women were going for cancer screening as compared to men. Given the cancers diagnosed, it was too late to assure any significant hope for a cure. The most prevalent cancer was breast cancer in women while liver and throat cancers affected males. The cancers prevalent, it has been reported, are caused by environmental carcinogens. Moreover, on the economic characteristics of the population, the occupation of the respondents had a significant association with cancer screening. This aspect is seen where a third of respondents were housewives and less than a quarter were unemployed. Cancer is an ”expensive“ disease because it drains the finances of a household very fast. In Kenya chemotherapy is approximately KES 30,000 per session. Considering the low-income population, the treatment and management of cancer are impossible.

Vegetable consumption is considered to have protective effects against non-communicable diseases (Sasazuki et al. 2018). ALVs have a high-level of nutrients (Akubugwo et al. 2007).

They have also been reported to have high levels of antioxidants which are alleged to assist with the management of cancer (Sasazuki et al.2018). some ALVs including nightshade has been implicated in the cure of certain ailments although the information has not been scientifically authenticated. Consumption of ALVs in the country is on the rise. The population has been sensitized on the benefits associated with their consumption. The increase of interest to ALVs can be evidenced in the increase of both the production and demand of these vegetables. From the market observations, ANLVs were the most popular ALVs vegetables. This can be evidenced even from the food frequency questionnaire, where they were the most frequently consumed even more than the exotic vegetables such as cabbages. The commonly consumed ALVs included cleome gynandra, Amaranthus spp. Since food frequency shows the pattern of consumption, the study indicated that the population normally consumes vegetables based on accessibility and availability. Accessibility in terms of financial ability. This means the cheaper the vegetable the more it is consumed. Moreover, the availability aspect came to play as some vegetables are seasonal. However, this is quickly being bridged through the use of irrigated production to make the ALVs available throughout the year.

On knowledge of benefits of leafy vegetables, the Majority of respondents knew at least one benefit of consuming leafy vegetables. Respondents from the study mainly boiled and stewed vegetables for consumption. Boiling is one of the harsh methods of cooking vegetables. Boiling causes leaching of water-soluble nutrients. Boling also causes the degradation of heat-labile nutrients such as vitamins. Interestingly there was a statistically significant association between cooking time and knowledge of the benefits of consumption. However, those who had knowledge of the benefits of vegetables cooked vegetables for a longer time than those who had

little or no knowledge. This shows that the knowledge of the importance of consumption of the vegetables was not integrated with the detrimental effects of cooking on the retention of nutrients. Steam cooking was not a common method of cooking although the method better spares the nutrients than the method of boiling and stewing that was used. The results indicated that the antioxidant activity of cooked ANLV vegetables was higher than that of the raw ANLV vegetables. This result is very interesting, indicating that the antioxidant activity of ANLV vegetables can be increased by cooking. It is also possible the cooking caused autolysis of plant cell wall thereby releasing the fiber-bond phenols with high antioxidant activity. (Moyo et al. 2018). Moreover, it possible that some water-soluble components were leached out during cooking so that the matrix of the vegetables left a higher proportion of the antioxidants than the raw. Antioxidants assist in building the body's immunity. Antioxidants reduce oxidative oxygen radicals that cause inflammation and cause the formation of cancer cells. This study, research to find the effect fed mice on cooked and raw African nightshade leafy vegetables. After 22 weeks of feeding the mice were sacrificed and the liver harvested. Using QPCR, the expression of tumor marker uPA was examined. The results showed that the expression of the uPA was higher in the mice fed with the control and raw ANLV diet. The mice that consumed cooked ANLV diet had the least expression of uPA. The vegetables are usually eaten cooked. The expression of tumor marker uPA was higher in raw than the cooked vegetable. The expression of urokinase plasminogen activator (uPA) protein is reduced to 3% in mice fed with cooked vegetables compared to the mice fed with the control diet. The expression of uPA is reduced to 61% in mice fed with raw vegetables compared to the mice fed with the control diet.

## 6.2 Conclusion

This research study concludes there was a relationship between demographic characteristics (marital status, occupation, and household head) and consumption pattern of nightshade leafy vegetables. From the food frequency analysis most daily consumed vegetables include kales, cabbages, spinach, and amaranth. There was a significant association between cancer screening and consumption frequency of broccoli, kales, mushroom, pumpkin, and amaranth. African nightshade is one most daily eaten indigenous vegetable.

Proportionally more men had knowledge about the benefits of leafy vegetable consumption than women. The most common method of cooking vegetables was boiling then stewing. There was a significant relationship between knowledge of the benefits of vegetables and cooking duration. A significant relationship indicated those who knew the benefits of consuming the vegetables, spent more time cooking vegetables.

Cooked ANLV has higher antioxidant activity than raw ANLV. Therefore concluding that cooking ANLV by steam blanching significantly increases the antioxidant activity of the vegetables. Lastly, the study also concludes that the consumption of cooked ANLV reduces the expression of urokinase plasminogen activator protein in mice models.

## 6.3 Recommendations

1. Men should be made aware of the importance of going for cancer screening. The community health workers should encourage and enforce cancer screening in the community. In addition, during cancer screening session's nutrition counseling on good dietary habits should be included.

2. Policymakers should from policies that contribute to improving the consumption of African leafy vegetables.

3. Proper methods of cooking vegetables such as steaming be encouraged in nutrition education forums.

4. Other tumor markers may be investigated in regard to African nightshade leafy vegetables.

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## Appendix1: Introduction and Consent form

### *A study on: "Consumption Patterns and anti-cancer properties of African leafy vegetables in Kagemi ward Nairobi-County Kenya"."*

Hello. My name is Caroline Wakuthie Muthike and I am from the University Of Nairobi, Department of Food Science, Nutrition and Technology, Applied Human Nutrition Programme. I am seeking to find out the consumption patterns and chemopreventive potential that African black nightshade in Kangemi.

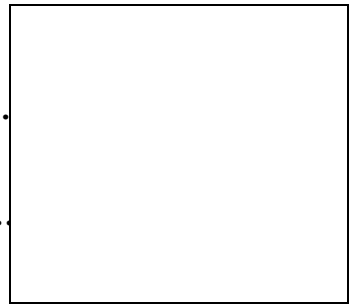
The information you provide will be only used to shed light on the research subject. The interview may take about 30 minutes. The study questions include personal questions in the first section on socio-economic and socio-demographic section, frequency of consuming (African leafy vegetables) ALVs and the recipe used to prepare African black nightshade leafy vegetables. Information provided will be confidential and will only be used to make a Thesis and publications. Your name will therefore not feature in any writing. However, Reference numbers will be used to connect your name and your answers without identifying you.

Your participation in this study is absolutely voluntary, and also if you have any issue concerning the study that you don't wish to raise with me you can contact KNH/UON-ERC. However, I hope that you will participate in this survey since your view is important.

It will be beneficial for you to participate in the study since we are increasing awareness about ALVs which grow wildly and some are growing at home. ALVs have high nutritional benefits as well as medicinal. Hence providing evidence of this will encourage more people to consume them. Therefore more health benefits and even the household food security situation could improve if embraced in your family diet.

The interview involves asking questions and the only risk is not answering truthfully as it will produce wrong results. However, if you wish to stop at any time kindly inform me. By Signing or approving this consent indicates that you understand what will be expected of you and are willing to participate in this survey.

**May I begin the interview now?**



**Signature of the respondent (for literate): ……**

**Signature of interviewer……**

**Date: …….**

**Signature of witness……**

**Date ……..**

Thump print for illiterate respondents.

Contact of KNH-UON-Ethical research committee Email: uonknh_erc@unobi.ac.ke, phone: 254) 020 2726300 Ext 44355

The study will run for a duration of one month.

Contact of researcher: Muthike Caroline Wakuthie On: 0713817478

**"JINSI WAKAZI WA KANGEMI WARD WANAJVYO KULA MBOGA ZA KIENYENJI NA CHEMICHEMI ZINAZO KINGA SARATANI"**

Mimi Ni Caroline Wakuthie Muthike kutoka chou kikuu cha Nairobi. Departmenti ya Food Science Nutrition and Technology. Nataka kufanya utafiti jinsi unavyo kula mboga za kienyenji na kama mboga hizi zina chemichemi ya kutukinga na saratani.

Maoni unayo yatuwa hapa yatatumiwa tu kutengeneza Thesis na Publication. Utaojiwa kwa dakika therathini kuhusu saratani, jamill yako na jinsi unavyokula mboga ze kienyeji. Pia jina lako halitatumiwa kuzanza form ila numbari itaandikwa.

Kuhusika na utafiti huu ni huru na usipotaka kushiliki pia inakubalika. Ingawanje tunakusihi kushiliki kwani kuna nanufaa kama kujielimisha kuhushu mboga za kienyenji na umuhimu waka katika afya ya familia. Kama kuna kitu chochote ungetaka kulalamikia au kusema bila ya kunuhusisha una weza zungumza na KNH/UoN- ERC.

Interview hill inahusisha kujibu maswali kwa kweli kwani uwongo utapotosha utafiti wetu. Ukihisi kuwa huna ardhi ya kujibu masawali tafadhali tufaamishe mara moja.

Sahini utakatupa hapa jinni ya thibitisha kuwa una jibu maswali haya kwa ihali yako.

Naweza anazainterview hii?----------

Signature yako----------

Tarehe----------

Signature ya mshaidi----------…

Tarehe ……

KNH/UON-ERC, Email: uonknh_erc@unobi.ac.ke

Numbari ya simu: 254) 020 2726300 Ext 44355

Utafiti huu utendela kwa muda wa mwezi moja

Nambari zangu az simu ni +2540713817478

## Appendix 2: Questionnaires

**A STUDY ON "Consumption pattern and chemoprevention properties of African African black nightshade against cancer in Kangemi area"**

**Q/NO----------**

**Part 1: Interviewer's section**

Date of interview----------Name of the Interviewer----------

Location/ Ward of respondent/village----------

Respondents Sex----------

**Part 2 Respondent's Section**

Date of Birth (Mwaka wa kuzaliwa)----------

Marital status ( hali yako ya ndoa?)----------

Religion (Ndini yako)----------

Education Level (Kiwango cha masomo)----------

Occupation (Riziki yako)----------

Household head (Nani mkubwa wa hill familia?)----------

What is your average household income per month? (Ni pesa ngapi unapata za nyumbani kwa mwezi?)----------

**Part 3 individual cancer status**

Is there anyone in your household who has been diagnosed with cancer? (kuna yeyote kwa familia ambaye ana ungua ungonjwa wa saratani?)----------

Within the last 10yrs is there anyone who has been diagnosed or died from cancer in your household? ( kwa miaka 10 iliyopita kuna yeyote kwa famila ambaye ameungua ungonjowa wa saratani?) ----------

If yes which year was it diagnosed? (Ungonjwa huu ulugunduliwa mwaka upi?)----------

What treatments were involved?----------

**Part 4: Frequency of consuming ALVs (kiwango cha kula mboga ze kienyeji) Q/NO---------**

| Vegetable Name | Portion size ½ cup of cooked vegs | Once a week | 2 times a week | 3times a week | 4 times a week | 5 times a week | 6times a week | 7times a week | More than onces a day | Never eat |
|---|---|---|---|---|---|---|---|---|---|---|
| Amaranthus ( terere) | | | | | | | | | | |
| Night shade (Managu) | | | | | | | | | | |
| Cowpea (Nthoroko) | | | | | | | | | | |
| Stinging Nettle ( Thaa) | | | | | | | | | | |
| Spider plant (munyugunyugu) | | | | | | | | | | |
| Commelina benghalens (Kimore) | | | | | | | | | | |
| Oxyganum Sinuatum (Cong'e) | | | | | | | | | | |
| Edible Fungi (Makunu) | | | | | | | | | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Kales (Sukuma wiki) | | | | | | | | | | |
| | | | | | | | | | | |
| Cabbage | | | | | | | | | | |
| Spinach | | | | | | | | | | |

## Part 5 Cooking Method

| **Portion size/ weight (grams)/number** | **Materials** |
|---|---|
| | Vegetables |
| | Oil |
| | Salt |
| | Spices |
| | Water |
| | Onion |

**Qn/ No----------**

**METHOD (must indicate duration (time) for each step)**

| |
|---|
| 1. |
| |
| |

| |
|---|
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| |
| |

## Method for cooking fermented African black nightshade vegetables

| Portion size/ Number/ weight in grams | Materials |
|---|---|
| | vegetables |
| | Oil |
| | Salt |
| | Species |
| | Water |
| | Other |
| | Other |

## Method

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**Qn/no----------**

**Production**

1. Do you grow African leafy vegetables (ALVs) on your farm?

2. What size of the land is used for production

- Less than 1/8 acre
- 1/8 acre
- ¼ acre
- ½ Acre
- 1 acre
- More than 1 Acre

3. Do you grow the ALVs throughout the year?
4. What types of ALVs do you produce?

5. Who grows the ALVs?
6. Why do you grow the ALVs
    - For household purposes
    - For ornamental purposes
    - For commercial purposes
7. Are you aware of any health benefits
    - Yes
    - No
8. What inputs do you use?
    - Inorganic fertilizers
    - Organic fertilizers
    - Pest and control
    - Soil fertility improvement
9. How do you handle the vegetables after harvesting
    - Storage
    - Preservation
    - cooking

## Appendix 3: Map of Westlands constituency


N
Karura
Karura
Kitisuru
Kitisuru
Muthaiga
Loresho
Spring Valley
Parklands/Highridge
Kyuna
Upper Parklands
Highridge
Mountain View
Kangemi
Kangemi
Mountain View
Gichagi

## Appendix 4 Data Analysis Matrix

**Table 11: Data analysis matrix**

| Data | Data type | Descriptive | Measures of central tendency | Measures of deviation | Inference |
|---|---|---|---|---|---|
| Socio-demographic, socio-economic and consumption pattern of ALVs | | | | | |
| Age | Continuous | Frequencies | Mean, mode | Standard derivation | |
| Marital status | Categorical | Percentages | | | Chi-square |
| Education level | Categorical | Percentages | | | Chi-square |
| Religion | Categorical | Percentages | | | Chi-square |
| Occupation | Categorical | Percentages | | | Chi-square |
| Sex | Categorical | percentages | | | |
| Household head | Categorical | Percentages | | | |
| Household income | Continuous | Frequencies | | | |
| Cancer Screening and occurrence | | | | | |
| Cancer screening | Categorical | Percentages | | | Chi-square |
| Cancer occurrence at the household level | Categorical | Percentages | | | Chi-square |
| Determination of consumption pattern of leafy vegetables in Kagemi | | | | | |
| Frequency of consuming vegetables | Categorical | Percentage | | | Chi-square association between demographics/ economics and consumption of ALVs |
| Determination of effects of cooking and fermentation on phytochemicals properties of African black nightshade leafy vegetables | | | | | |
| Concentration of total flavonoids | Continuous | Frequencies | Mean mode | Std deviation and variance | ANOVA |
| Concentration of total phenolics | Continuous | Frequencies | Mean mode | Std deviation and variance | ANOVA |
| Concentration of glycol-alkaloids | Continuous | Frequencies | Mean mode | Std deviation and variance | ANOVA |
| Determination of chemopreventive potential of African black nightshade leaf extracts using APC $^{MIN}$ mice model | | | | | |
| ImmnoHisto chemistry | Categorical | Percentage | | | $X^2$ Regression |

| | | | | | |
|---|---|---|---|---|---|
| uPA-RPCR | Continuous | | Mean,mode,median | Std deviation | $X^2$ |

**Table 12: Table on activities to be carried out**

| Chemoprevention against cancer through Consumption indigenous vegetables in Imenti North District Meru county | | | |
|---|---|---|---|
| Objectives | Indicators | Means of verification | Assumptions |
| Main objective | | | |
| Assess the consumption patterns and the anti-cancer properties of African leafy vegetables | G1% consumption of African leafy vegetables<br>G2 % decrease in cancer cells due to exposure to ALVs extracts. | • Household survey<br>• Laboratory experiment | |
| SO1<br>The determination of Socio demographic socio-economic status. | • The socio-demographic and socio-economic status of the population | • H/H Survey | • Support from community members |
| consumption pattern of ALVs | • Number of people consuming ALVs | • H/H Survey | • Cooperation from both community and enumerators |
| SO3The determination of effects cooking and fermentation on phytochemical | • % cons of β-carotene in the ALVs.<br>• % conc of total phenolics in the ALVs<br>• % conc of antioxidant activity | • Laboratory analysis | • Well organized and equipped laboratory |
| SO4 The determination of the chemopreventive potential of African black nightshade leafy vegetables. Using APC $^{MIN}$ mice model | • Number of mice without intestinal adenomas<br>• %conc. of uPA protein after diet to African black nightshade leafy vegetables | • Laboratory analysis | • Well equipped molecular laboratory |

## Appendix 5: Training Module

**Trainer/ Investigators Guide**

**Training Objectives**

1. To familiarize the recruited enumerators with the survey protocols
2. To explain good enumerator habits and
3. To explain effective data collection techniques

**Methods of training**

The training methods will include lectures, discussions and role play

**Table 5: Table Showing the Training module (adapted from WFP)**

| **Day 1** | **Activity** | **Time** | **Materials** |
|---|---|---|---|
| | • Introduction,<br>• objectives,<br>• expectations and<br>• ground rules | 40 minutes @ session | Sample questionnaire<br>Flip chart<br>Variety of colored marker pens<br>Note books<br>Pens, Pencils. File folders |
| **Day 2** | • good enumerator habits<br>• effective data collection techniques<br>• practice data collection instruments in workshop setting | 1hr @ session | |
| **Day 3** | • practice using data collection instrument in a field setting (pre-test) | 4hrs | Sample questionnaire, pencil |