MAKERERE UNIVERSITY

Nutritional content of Indigenous leafy vegetables and their use in human nutrition in South-Kivu, DR Congo

BY

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DECLARATION

I, **Diana AMINI BAHATI,** declare to the best of my knowledge that this study is my original work, and has never been submitted to Makerere University or any other University/Institution for the any award.

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DEDICATION

To my Father KAJUNJU BAHATI Mushule,

To my Mother MUNYERENKANA LUBASI Charlotte,

To my Aunty NAKAHYA Justine and her Husband “Mzee” BAHANULE Wenceslas,

To my Sisters and Brothers, the KAJUNJUs and BAHANULEs,

To the disciples of The WAY INTERNATIONAL around the world.

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LIST OF ABBREVIATIONS

AA	: Amino Acids
ILVs	: Indigenous Leafy Vegetables
ANOVA	: Analyses of Variance
AOAC	: Association of Official Analytical Chemists
CVDs	: Cardiovascular Diseases
DRC	: Democratic Republic of the Congo
DCPIP	: 2,6_dichlorophenol_indophenol
FAO	: Food and Agriculture Organization
FIDA	: Fonds International de Développement Agricole
GAE	: Gallic Acid Equivalent
IFAD	: International Fund for Agricultural Development
ITFCs	: Indigenous and traditional food crops
LV	: Leafy vegetables
MONUSCO	: Mission de l'Organisation des Nations Unies pour la Stabilisation en République Démocratique du Congo
PAM	: Programme Alimentaire Mondial
RDA	: Recommended Dietary Intakes
TCA	: Trichloro acetic acid
UNICEF	: United Nations Child Fund
UK	: United Kingdom
USAID	: United States Agency for International Development
USDA	: United States Department of Agriculture
WHO	: World Health Organization

ABSTRACT

Many indigenous food species are nutrient dense; with nutritional potential to alleviate hunger and malnutrition. Ethnic communities in South Kivu province (Eastern DR Congo), especially in Walungu territory, know a numerous species of indigenous vegetables. Consumption of these vegetables has been associated with local nutritional and medicinal benefits. However, limited scientific evidence on their nutritional profile and potential contribution has led to their underutilization and devalorization. This study assessed the nutritional content and the use of indigenous leafy vegetables known and consumed by households in Walungu territory in the Eastern region of the Democratic Republic of the Congo. A cross-sectional and descriptive study including households' interviews and laboratory analysis of eight selected indigenous leafy vegetables was employed. The results revealed that 20 species of Indigenous Leafy Vegetables (ILVs) were commonly known and consumed in Walungu District. Majority of respondents claimed that these ILVs were consumed because they were available, tasty and nutritious. Washing vegetables with clean water before boiling was the common (98.8%) pre-processing treatment while boiling was the predominant (93.2%) preparation and cooking method used. In addition, cooking was done for one hour in the majority of households (58%). Air drying was the predominant (32.7%) preservation technology applied on vegetables in Walungu. Other alternatives included sun drying (26.2%) or no preservation at all (40.1%). Results further indicated that out of the 20 AILs commonly known and consumed in Walungu, *Mushaka (Cleome schimperi)*, *Lushenda (Capsicum frutescens)* and *Bishogolo (Phaseolus vulgaris)* leaves were the most nutritious due to their protein content (5-28%), dietary fiber (8-38%), Iron content (81-177mg/kg), carotenoids (29.9-132.1µg/g) and vitamin C (42.2-56.8mg/100g) concentration. These results suggested that indigenous leafy vegetables consumption and utilization should be promoted for their availability and nutritional value. Further investigations should be undertaken to establish the impact of a daily consumption of these ILV on the health status of individuals across different age groups and gender; and to assess the nutritional value of varieties among species of indigenous leafy vegetables and the effect of processing patterns of them.

Key words: Indigenous vegetables, utilization, food and nutrition security

CHAPTER ONE: INTRODUCTION

1.1 Background

Food availability, accessibility, and utilization have emerged as the three dimensions of food security (Jones, Ngure, Pelto, and Young, 2013). However, Bashir and Schilizzi (2013) reported that utilization domain is the most neglected component in studies conducted in Asia and Africa. Utilization reflects food consumption and use at household and individual level (Pangaribowo, Gerber, and Torero, 2013).

According to (FAO, 2012), nutrition security is the need to secure access to an appropriate nutritious diet, comprising all essential nutrients and water, coupled with a sanitary environment and adequate health services and care to ensure a healthy and active life for household members. Human needs can only be satisfied through a diversity of macro- and micronutrients to promote good health and prevention from disease (Hwalla, Labban, and Bahn, 2016). Failure to meet these macro and micronutrients needs leads to malnutrition. As malnutrition is an outcome of food insecurity and nutrition insecurity, the causes of food insecurity and nutrition insecurity are interconnected and are rooted in political and cultural environment, in poverty, and disempowerment of women (Ruel, 2013).

Many aspects such as habits and knowledge about nutritional value, food processing and feeding practices (all of which are shared at the household level) influence the diet composition of the individuals as well as the biological utilization of the food (Pangaribowo et al., 2013). Nutrition security leads to malnutrition (over- or under-nutrition) which can have short-term consequences such as mortality; and long-term consequences such as adult size, intellectual ability, economic productivity, reproductive performance, metabolic and cardiovascular disease (Levine and Chastre, 2011).

About 805 million of the 7 billion (11.5%) people globally suffer from chronic undernutrition (Fram, Bernal, and Frongillo, 2015, IFPRI, 2016). In the developing regions of the world, the proportion of undernourished people reached 13%, in African countries 20%, and in Asian countries 13% of the population (Prosekov & Ivanova, 2018). In Central African Countries, including the Democratic Republic of the Congo (DRC), malnutrition is still a widespread problem despite global improvements in recent decades (UNSCN, 2010). The causes of the poor nutritional situation in the DRC are complex and linked to several factors (FAO, 2010), including inappropriate eating habits and practices (Programme National de Securite

Alimentaire, 2010). In DRC, nearly 2 million children (12 % of cases in the world) are suffering from severe acute malnutrition, with the eastern provinces of North Kivu, South Kivu, and Tanganyika being the most affected regions of the country (OCHA, 2017). In DRC, while about 15 percent of households suffer from protein deficiency, at least 50 percent of the population is deficient in vitamin B12, calories, riboflavin, iron, vitamin E, folate, and zinc (Koffi AKAKPO, Josée RANDRIAMAMONY, 2014)

The interventions to prevent mortality and health disorders related to malnutrition include community nutrition programs, micronutrient supplementation, deworming, biofortification, water and sanitation sector infrastructure improvements (Shekar et al., 2015). However, these interventions are not generalized to all populations worldwide. Many indigenous cultures across the globe use their foods as medicine to maintain the health and well-being of their community. Indigenous foods in various cultures across African countries have been studied (Oloko, 2016). Indigenous foods are healthier compared to highly processed foods (Kimani, Mayer, and Swiderska, 2020; Reardon et al., 2021). Traditional food systems, from forests (plant foods) to nutrient rich local fish and meat, involve the production of diverse foods which ensures adequate dietary diversity (IFAD, 2017). However, many of those indigenous food species are neglected and underutilized despite their nutritional potential which is a response to malnutrition (FAO, IFAD, UNICEF, WFP, & WHO, 2017).

The utilization/consumption of indigenous leafy vegetables (traditional, wild or leafy greens) as an important source of nutrients is attracting a greater deal of attention as a way of alleviating hunger and malnutrition (Kimiywe, Waudo, Mbithe, and Maundu, 2005). Among the rural poor, indigenous leafy vegetables often contribute to 27% of their recommended daily requirements of essential vitamins and minerals. The vegetables have been used as medicine forming part of local health care systems (Fanzo, Hunter, Borelli, and Mattei, 2013). Lack of nutritional information and evidence, a negative attitude towards traditional indigenous foods, particularly indigenous vegetables labelled as weeds by most researchers, policies that do not recognize sufficiently the important role of these foods in food and nutrition security and health, lack of advocates and champions to promote traditional and indigenous foods are all constraints to effective utilization of indigenous vegetables (FAO, 2014).

1.2 Problem Statement

Indigenous foods' knowledge has the potential to promote food and nutrition security with their content of essential vitamins and minerals as well as supplementary protein and calories (Rampa, Lammers, Linnemann, Schoustra, and Winter, 2020). The DRC is one of the richest countries in the world in terms of biodiversity in general and in terms of phytogenetic resources for food and agriculture in particular. Sustainable management and utilization of these resources remains a priority to ensure the food and nutritional security of the population (FAO, 2009).The rural population (70% of the total population) in DRC depends on the indigenous foods which come mostly from the forests for their livelihoods (vegetables, fishery resources, wild fruits, meats) (Kuamba, 2016).

Despite a diverse occurrence of many indigenous vegetables species, most urban and rural population of the DRC does not consume a significant volume of vegetables (Line Termote et al., 2012). Significant consumption is mainly concentrated among communities living close to forests or in the bush, but these communities represent a very small part of the country population. Limited consumption of the indigenous vegetables by the population is due to lack of information and scientific evidence on the nutritional profile of indigenous vegetables available in DRC, because the indigenous leafy vegetables have been labelled in many cases as weeds (D'Haese, Mayambu, & Winter, 2013; Daudet, 2012). Until now, the Indigenous plant foods have been little studied in South Kivu province which has numerous species of indigenous (wild) leafy vegetables consumed by some ethnic communities where they have been recognized as having some therapeutic benefits. Furthermore, less information is known on their nutritional value and their potential to sustain nutrition security (Birhashwirwa, 2015).

The aim of this study was to address the knowledge gap (awareness, attitudes and practices in the use) by characterizing the taxonomy, morphology and nutritional content of indigenous leafy vegetables grown by households in Walungu territory of South Kivu, DRC.

1.3 Research Objectives

The overall objective of the study was to assess the nutritional content and the use of indigenous leafy vegetables known and consumed by the households in South-Kivu

The specific objectives were:

i. To determine awareness, attitudes, and practices by households regarding use of indigenous leafy vegetables in Walungu territory of South-Kivu;

ii. To characterize taxonomically the major leafy vegetables available in Walungu territory of South-Kivu;

iii. To determine the nutritional composition and some bioactive compounds of the most consumed indigenous leafy vegetables in Walungu territory, South-Kivu.

1.4 Research questions

The study was guided by the following research questions:

i. What are the attitudes and practices applied on the indigenous vegetables?

ii. What are the major indigenous leafy vegetables consumed in Walungu Territory of South-Kivu Province and factors influencing their consumption?

iii. What is the nutritional content of selected indigenous vegetables?

1.5 Significance of the study

In DRC, traditional/indigenous leafy vegetables are underutilized, and their profitability is low compared to exotic vegetables (Fanzo et al., 2013). Insufficient data on the chemical, nutritional and medicinal properties of traditional or indigenous leafy vegetables commonly grown in DRC is one of the factors contributing to underutilization of indigenous leafy vegetables by the Congolese population (USAID, 2015). On the other hand, lack of satisfactory assessment of the nutritional value of indigenous leafy vegetables and lack of their knowledge by the youth (which composes about 50% of the entire population) have limited profitability of those vegetables. As a result, indigenous leafy vegetables are not promoted.

This study aimed at addressing the knowledge gap by characterizing indigenous vegetables commonly grown in Walungu territory/South Kivu in DRC. Information on the phytochemical and nutritional properties of the indigenous leafy vegetables commonly grown in Walungu is the cornerstone for formulating important decisions to promote the indigenous leafy vegetables in South Kivu/DRC.

Promoting indigenous leafy vegetables consumption grown in Walungu South Kivu will supplement efforts initiated by the National Programme of Nutrition and humanitarian organizations to support local community fighting against food and nutrition insecurity.

CHAPTER TWO: LITERATURE REVIEW

2.1. Indigenous foods

An erosion of indigenous and traditional foods crops (ITFCs) and agricultural production in the Global South has dramatically changed the global food system in the last 50 years (Menendez-Baceta, Aceituno-Mata, Tardío, Reyes-García, and Pardo-de-Santayana, 2012). Since the Green Revolution in the 1960s, agriculture has mainly focused on developing conventional cereal and horticultural crops, and as a result, these foods became more popular and replaced many locally produced crops, leaving the development and cultivation of ITFCs to be severely undervalued (Zonneveld, Kindt, Solberg, N'Danikou, and Dawson, 2021). Traditional and indigenous foods are native foods or were introduced a long time ago, whether locally produced or accessed from the wild (Kasimba, Covic, Motswagole, Laubscher, and Claasen, 2019). Most of indigenous food systems are typically biodiversity-rich, climate resilient and environmentally sustainable, and produce nutritious indigenous foods. Promoting indigenous plant and animal foods is a means to enhance nutrition and resilience to climate change (Kimani, Mayer, and Swiderska, 2020). Indigenous vegetables form a significant and inexpensive source of a balanced diet for poor rural households in Africa where their vegetable consumption is often regarded as a poor man's diet and their nutrients are destroyed during cooking, reducing their effectiveness in ensuring food security (Bua and Onang, 2017).

Vegetables are an important part of the human's diet. Not only vegetables are a potential source of important nutrients, but also they constitute important functional food components by contributing protein, vitamins, iron, calcium, iodine and zinc which have marked health effects (Arai, 2002). Depending on vegetable species, variety and tissue, high levels of health-protecting antioxidants, such as vitamin C, phenolic compounds including phenyl-propanoids and flavonoids, and or carotenoids can be found. The entire tissue of vegetables is rich in bioactive compounds, such as phenolic compounds, carotenoids, vitamins and may contribute to protect against many diseases (Szabo, Cătoi, and Vodnar, 2018). Overall, it is estimated that up to 2.7 million lives could potentially be saved each year if vegetable consumption, which are sources of minerals and vitamins were sufficiently increased. Vegetable consumption has been associated with higher bone mineral density, reduction of cardiovascular disease risks, cancer prevention and a decrease in all mortality (Asano et al., 2008).

2.2. Diversity of indigenous foods in DRC

In DRC, household life in rural and urban areas is supported by a mosaic of plants and crops for consumption. Hundreds of species of indigenous/wild plant products intended for human consumption are known (Ombeni and Munyuli, 2016). For the indigenous vegetable foods, it has been shown that fruits (45%) were commonly sought after and consumed by the Congolese population, followed by leaves (38%) which are prepared as vegetables (Ombeni and Munyuli, 2016).

Moyene, (2009) findings on indigenous and traditional vegetable foods in the Batéké plateau showed that 169 species of indigenous traditional vegetable foods belonging to 65 plant families are valued. In Bandundu, in addition to cultivated vegetables (cassava leaves, sweet potatoes or amaranth), children sometimes collect wild leaves in the forest which develop in abundance at certain times of the year (Biloso and Lejoly, 2006). Termote and Van Damme, (2012) documented 166 indigenous plant species and 2 varieties in 71 plant families from which 198 plants parts are used for 228 different specific food uses.

Nutritional diversity finds its foundations in the general field of biodiversity, and agricultural diversity encompasses ecosystems, animals, plants and microorganisms related to food and agriculture (Malaisse, 2004). Food biodiversity is important by including wild, indigenous and traditional foods that contribute to nutritional security as well as the conservation and sustainable use of this biodiversity (Kuamba, 2016). Indigenous food plants and other wild and traditional foods like hunting animals or collected insects play a crucial role in many food systems by improving the resiliency of the food system and the diversity and quality of the diet (Malaisse, 1997).

2.3. Indigenous vegetables

Indigenous vegetables are sometimes referred to as wild vegetables or plants whose leaves, roots or fruits are acceptable and used as vegetables by rural and urban populations through tradition, custom and habit (Towns and Shackleton, 2018). For most species, the young growth points and tender leaves are the plant parts that are used in the preparation of vegetable dishes. Petioles and in some cases young tender stems are also included, but old, hard stems are discarded (Redzic, 2006; Rensburg et al., 2004). These vegetables are widely consumed, especially during famines and natural disasters when cultivation of other vegetables is not possible. Those indigenous vegetables include the vegetables that are both domesticated or grown wild in areas with disturbed soils or agricultural activity (Assan, 2014). The wild varieties grow naturally in the bush whereas Semi-wild varieties or semi-cultivated on the other hand are vegetables that are protected when they grow near homes or in the home gardens (Venter, Rensburg, Vorster, Heever, and Zijl, 2007).

The production of Indigenous leafy vegetables is still at subsistence level and is more common in rural areas. Indigenous leafy vegetables are still mostly treated as weeds by researchers and extension personnel who also criticize farmers for not keeping this weed population under control, thus labeling this important food as not worthy of the space it occupies in the fields (Mayekiso and Mditshwa, 2017). Indigenous vegetables could make a positive contribution to world food production system because they adapt easily to harsh or difficult environments (Maseko et al., 2017). The introduction of exotic vegetables has caused under-utilization of these indigenous vegetables. There has been a great decline in the availability of indigenous leafy vegetables which has also been caused by an excessive cultivation of field crops, change of habitat, as well as chemical eradication of wild vegetables (Mathaba, 2017). Indigenous Leafy Vegetables (ILVs) play an important role in the food security strategies of many rural households and their utilization and production lie within the domain of the older women (Vorster, Stevens, and Steyn, 2008).

2.4. Awareness, attitudes and practices of smallholder farmers on ILVs

Food habits are complex and are influenced by a range of factors. A number of interrelated factors can influence the use and choices of food items and these factors can be external or internal. Food habits are dynamic and are maintained because they are effective, practical and meaningful behaviours in a particular culture. Food choices can also vary from one generation to another over time (Dlamini, 2017).

The acceptance and utilization of indigenous vegetables is currently constrained by limited knowledge about their nutritional composition, their methods of preparation and preservation as well as their importance as source of food in the community (Mayekiso and Mditshwa, 2017). The indigenous vegetables are generally labelled as invasive weeds and old fashioned, hence they are regarded as low status food which is only consumed by the poor (Shackleton, Pasquini, and Drescher, 2009). This unfortunate label has stigmatized these healthy crops, especially among the youth. Parents have found it difficult to motivate their children to learn something about their traditional food crops and the effect of these labels are apparent in the youth. The decrease in the use of indigenous vegetables by several rural communities has caused a greater incidence of poor diets and nutritional deficiency diseases (Mathaba, 2017; Voster, Rensburg, Zijl, and Venter, 2007).

The indigenous vegetables seemed to serve first as food, but are also used as medicine in traditional health system. The leafy vegetable dishes are prepared from single specie or from a combination of different species (Marshall, 2001), however other ingredients, such as tomatoes, onions, peanut flour and spices are eventually added to enhance their taste (Smith, 2013). Cooking methods also vary from thorough boiling and steaming to partial frying after boiling, which may include the replacement of the first cooking water with fresh water in the case of bitter-tasting species (Remans et al., 2011). Besides their use as food, indigenous vegetables have various uses and preparation methods in medicine as well as in cosmetics depending on the diversity in culture, the gender and the ethnicity (Mathaba, 2017). In Kenya for example, consumption of the indigenous leafy vegetables (ILVs) is only significant amongst the agricultural communities and hunter-gatherers while among the pastoral communities, ILVs are occasionally used in soups and milk for flavour and good health (Ntawuruhunga, 2016).

2.5. Consumption patterns of ILVs in Sub Saharan Africa

Dietary patterns have varied over time depending on the agricultural practices and the climatic, ecologic, cultural, and socio-economic factors, which influence food availability. To date, virtually all dietary patterns adequately satisfy or even exceed the nutritional needs of population groups (FAO/WHO, 2001). It has been found that consumption patterns have been affected globally by rising incomes, changing prices, urbanization, globalization, demographic shifts, improved transportation, and changing consumer tastes and preferences (Rampal, 2018). Indigenous vegetables form a significant part of the traditional diets of cultivators communities in Africa, while their consumption is usually less significant among cattle keepers communities (Shackleton et al., 2009). It was reported that consumption of vegetables in Sub-Saharan countries is very low as compared to other countries like those in Asia, and Latin America (Onim and Mwaniki, 2008; Ruel, Minot, and Smith, 2004). According to WHO and FAO (2001), a person should consume 400 grams of vegetable daily, or 146 kg per year. However, most African countries do not meet this requirement except Kenya which clearly stands out with an average vegetable consumption of 147 kg/person/year in urban areas and 73 kg/person/year in the rural areas (FAO, 2017). In DR Congo, at the national level vegetables are consumed at an average of 5.7 days a week (Akakpo et al., 2014).

The rural exodus of the population and the culture, as well as, the weak domestication of indigenous leafy vegetables has contributed to the reduction of their consumption. Also, much of the knowledge about the use of indigenous leafy vegetables is detained by the older generations and is not fully incorporated into the education system, thus remains inaccessible to the younger generations and could be lost (Mumbi, 2004). Indigenous leafy vegetables contribute significantly to household food security and add variety to cereal-based staple diets. These vegetables are traditionally cooked and eaten as a relish together with a starchy staple food (Heever, 1995; Vainio-Mattila, 2000). When the indigenous vegetables are cooked, salt is usually added to enhance the taste whereas oil, butter, groundnuts, coconut, milk, bicarbonate of soda, tomato and onion are added depending on availability and preference (Nguni and Mwila, 2007; Ogoye-Ndegwa and Aagaard-Hansen, 2003).

2.6. Contribution of ILVs to household health, food and nutrition security

Most population groups afflicted by micronutrient deficiency largely subsist on refined cereal grain or tuber-based diets, which provide energy and protein (with improper amino acid balance) but are insufficient in critical micronutrient (FAO/WHO, 2001). Including vegetables in the diet, which have high micronutrient density is a potential way of ensuring optimal nutrition with adequate micronutrient for most population groups. Insufficient vegetable and fruit consumption belongs to the top ten risk factors contributing to mortality and causes 2.7 million deaths annually worldwide (Ezzati, Lopez, Rodgers, Vander Hoorn, and Murray, 2002).

In the tropics, malnutrition is rampant, where per capita vegetable supplies in most countries fall far short of the minimum recommended 146 kg per year. The recent interest in the role of phyto-chemicals and antioxidants on health and their presence in plant foods lend further support to the recommendation for increasing vegetables and fruit consumed in the diet (FAO/WHO, 2001). Micronutrient deficiencies mainly causing vitamin A, iron and iodine disorders remain widespread while the chronic diseases, including cardiovascular disease, cancer, chronic respiratory diseases and diabetes are increasing globally (WHO, 2005). Increased fruit and vegetable consumption has been widely promoted because of the health benefits. Their micronutrients and non-nutrient phytochemicals have been associated with health maintenance and prevention of chronic diseases (Steinmetz and Potter, 1996).

Besides being nutritious, indigenous vegetables are grown locally on a small scale, often resistant to diseases and tolerant of environmental stresses, thus available throughout the year (Shackleton et al., 2009). A quite large number of indigenous leafy vegetables have long been known and reported to have health protecting properties and uses (Ayodele, 2005; Malgras, 1992; Okeno, Chebet, and Mathenge, 2003). Several of these indigenous leafy vegetables continue to be used for prophylactic and therapeutic purposes by rural communities. This indigenous knowledge of the health promoting and protecting attributes of indigenous leafy vegetables is clearly linked to their nutritional and non-nutrient bioactive properties. More recent reports have shown that indigenous leafy vegetables also contain non-nutrient bioactive phytochemicals that have been linked to protection against cardiovascular and other degenerative diseases, protein, vitamin and minerals (Akenga, Ochora, Friis, and Box, 2005; Ayodele, 2005; Malgras D, 1992).

2.6.1. Proximate composition and human nutrition and health

Proteins contain various amounts of amino acids and are essential for the health, growth, development, reproduction, lactation, and survival of organisms. Metabolic disorders and kwashiorkor in particular, are caused by a severe deficiency of protein whereas marasmus is caused by the severe deficiency of both protein and energy in humans, particularly in many children of developing nations (Wu, 2016). Carbohydrates are the most important source of calories for the world's population because of their relatively low cost and wide availability (US-Food and Nutrition Board, 1989). The primary role of carbohydrates is to provide energy to the cells in the body. Grains and certain vegetables are major contributors. Fruits and darkly colored vegetables contain little or no starch (Otten, Hellwig, and Linda, 2006). Indigenous Vegetables are sources of important macronutrients such as proteins and energy and can provide approximately 10% of the recommended daily allowance (RDA) of protein (Abukutsa-Onyango, Kavagi, Amoke, and Habwe, 2010; Rensburg et al., 2007).

Dietary fiber is a nutritional concept based not on physiological functions but on defined chemical and physical properties. Dietary fiber is a broad category of non-digestible food ingredients with an associated health benefit (Papathanasopoulos & Camilleri, 2010; Raninen, Lappi, Mykkänen, and Poutanen, 2011). Dietary fiber intake has many functions in diet and provides many health benefits such as reducing the risk for developing coronary heart disease, stroke, hypertension, diabetes, obesity, and certain gastrointestinal diseases. Increasing fiber intake lowers blood pressure and serum cholesterol levels (Anderson et al., 2009; Burton-

Freeman, 2000; Gordon, 1992). The current recommendations for dietary fiber intake are related to age, gender, and energy intake, and the general recommendation for adequate intake (AI) is 14 g/1000 kcal (USDA, 2005). The total dietary fiber content of ILVs depends on differences in stages of plant maturity, seasonal variation, fertilizers or chemicals used, variety of plant, geographical location and the method used for analysis (Aletor, Oshodi, and Ipinmoroti, 2002; Punna and Paruchuri, 2004)

2.6.2. Micronutrients and Human nutrition and health

A number of findings have shown that utilization of indigenous vegetables is highly valuable in its nutrition aspect (Mnzava, 1997). Indigenous vegetables contain high levels of minerals and significant amounts of vitamins. On average 100g of fresh vegetable contain levels of iron, zinc and vitamins that would provide 100% of the daily requirement (Abukutsa-onyango, 2003). Recommended nutrient intakes for vitamins and minerals were initially established on the understanding that they are meant to meet the basic nutritional needs of over 97% of the population. The micronutrient insufficiency and inappropriate diet prepare the body for infection (Byrd and David, 1944; FAO and WHO, 1998). Indigenous vegetables have long been, and continue to be reported to significantly contribute to the dietary vitamin and mineral intakes of local populations (Mulokozi, Hedrén, and Svanberg, 2004; Osifo, 1970).

Vitamin A is an essential nutrient needed in small amounts by humans for the normal functioning of the visual system; growth and development; and maintenance of epithelial cellular integrity, immune function, and reproduction (FAO and WHO, 1998). Many health aspects can be impacted by deficiency in vitamin A, such as defects in immune responses and development. Extreme deficiency in this vitamin leads to xerophthalmia (dry eyes), corneal ulceration, blindness, and increased mortality, especially in children (Fitzpatrick et al., 2012). The vitamin A content of most staple diets can be significantly improved with the addition of a relatively small portion of plant foods rich in carotenoids, the precursors of vitamin A (FAO/WHO, 2001). The vitamin A content of ILVs is highly species dependent and varies from 99 mg RE to 1970 mg RE (per 100 g edible portion) in some indigenous leafy vegetables (FAO/WHO, 2001; Uusiku, Oelofse, Duodu, Bester, and Faber, 2010).

Vitamin C is an electron donor (reducing agent or antioxidant). When there is insufficient vitamin C in the diet, humans suffer from the potentially lethal deficiency disease scurvy. Three important manifestations of scurvy gingival changes, pain in the extremities, and haemorrhagic manifestations precede oedema, ulcerations, and ultimately death (FAO/WHO, 2001; McLaren, 1981). Haytowitz, (1995); Mascotti, Rup, and Thach, (1995) indicated that ascorbate is found in many fruits and vegetables. In unprocessed indigenous leafy vegetables, vitamin C range from 2 to 311 mg per 100 g edible portion (Uusiku et al., 2010). In sub-Saharan Africa, the intake of ascorbic acid from leafy vegetables is often determined by seasonal factors, as well as postharvest storage, time and temperature of storage, cooking practices and chlorination of water (FAO/WHO, 2001).

Iron functions as a component of a number of proteins, including hemoglobin and enzymes, the former being important for the transport of oxygen to tissues throughout the body for metabolism (FAO and WHO, 1998). According to (Odhav, Beekrum, Akula, and Baijnath, 2007) some indigenous leafy vegetables are excellent sources of iron , but the levels are influenced by factors such as soil and climatic conditions as well as plant variety, plant age and the use of fertilizers. Populations most at risk for iron deficiency are infants, adolescents, and women of childbearing age, especially pregnant women (FAO and WHO, 1998).

Zinc is present in all body tissues and fluids. The total body zinc content has been estimated to be 30mmol (2g). Skeletal muscle accounts for approximately 60% of the total body content and bone mass, with a zinc concentration of 1.5–3mmol/g (100–200mg/g), for approximately 30% (FAO and WHO, 1998; WHO, 1996). Zinc plays a central role in the immune system, affecting a number of aspects of cellular and humoral immunity (Shankar and Prasad, 1998).The clinical features of severe zinc deficiency in humans are growth retardation, delayed sexual and bone maturation, skin lesions, diarrhoea, alopecia, impaired appetite, increased susceptibility to infections mediated via defects in the immune system, and the appearance of behavioural changes (WHO, 1996). Zinc deficiency is associated with impaired gastrointestinal and immune functions which is of vital importance (Welch, 1993). Excellent sources of Zinc include lean red meat, whole-grain cereals, pulses, and legumes which provide the highest concentrations (25–50mg/kg raw weight). Fish, roots and tubers, green leafy vegetables, and fruits are only modest sources of zinc, having concentrations less than 10mg/kg (Sandström, 1989).

Iodine is unique among the required trace elements because it is the constituent of the thyroid hormones. Iodine deficiency is a common cause of preventable mental defects (Ujowundu et al., 2011; Zimmermann, 2009). The intake of iodine generally corresponds to the amount entering the local food chain from geochemical environment and it is normally low from natural foods (US-Institute of Medicine FNB, 2002). According to Vanderpas et al., (1990) iodine ingestion have to be regulated as deficiency can lead to extreme fatigue, endemic goiter, cretinism and recurrent miscarriages. Gaitan, (1988); Osman and Fatah, (1981) reported that mineral nutrient deficiencies such as zinc, copper, iron, also contribute to inability to use iodine well and this may lead to the development of Goiter. Fortunately, vegetables have higher concentrations than cereal crops (Fordyce, 2010)

2.6.3. Importance non-nutritional components of ILVs

Indigenous Leafy Vegetables are rich sources of bioactive compounds with beneficial effects on health. Understanding the role of these components in the maintenance of health is crucial for managing protein-energy malnutrition (PEM) and chronic diseases of lifestyle (Uusiku, Oelofse, Duodu, Bester, and Faber, 2010b). According to Njume et al. (2014), when included in human diet, indigenous leafy vegetables are also known to play a role in reducing the incidence of oxidative stress-related diseases due to beneficial health functionality of their phenolic constituents which include flavonoids hydrolysable and condensed tannins, coumarins, phenolic acids. As antioxidants, the phenolic compounds of ILVs protect cells from the damaging effects of free radicals arising from cellular redox reactions (Njume, Goduka, & George, 2014).

Oxalic acid is present in the cell sap of many of the green leafy vegetable. Depending on plant species, oxalates can occur as insoluble salts of calcium, magnesium and iron, and soluble salts of potassium and sodium or as a combination of these two forms (Champ, 2002; Noonan, 1999). Some research findings reported that oxalates causes irritation and swelling in the mouth and throat (Ladeji, Ahin, and Aduwamai, 2004). And other study (Radek and Savage, 2008) indicated that addition of a source of calcium to vegetables containing high levels of soluble oxalate has shown to reduce intestinal available oxalate content in such foods.

Phytic acid is a natural substance that acts as a major storage of phosphorus in all leafy vegetables. Phytic acid is found in plant tissues as salts of cations such as potassium, magnesium and calcium (Harland and Oberleas, 1986; Reddy and Shridhar Sathe, 2001). Agbaire and Emoyan, (2012); Agbaire PO,(2012); Nkafamiya, Osemeahon, Modibbo, and Aminu, (2010) reported that although phytate is an antioxidant, it has been shown to inhibit absorption of minerals. Hence, diet high in phytate content reduces bioavailability of zinc and iron.

2.7. Challenges in the use of Indigenous Leafy Vegetables

The traditional vegetables are becoming scarce and their utilization is majorly declining. The decrease in the consumption of these vegetables might be caused by modernization, as the younger people desire more fatty tastes associated with fast food and snacks (Ozturk, Hakeem, Ashraf, and Ahmad, 2018). Another possible reason of the decline in the consumption of these vegetables could be that people are not familiar with their taste, thus they claim that they taste bad, this is due to the blandness of their preparation and the use of limited ingredients when they are prepared (Rensburg et al., 2004). The changing taste preferences and people's perceptions also attribute to the decline of traditional vegetables (Chivenge, Mabhaudhi, Modi, and Mafongoya, 2015). The process of collecting traditional vegetables in the garden or field has also attributed to the decline of the utilization of traditional vegetables and most households prefer the ones which they buy. People also prefer commercial vegetables since they are available in the market in all seasons, while traditional vegetables have seasonal availability, most of them are not available in dry season (Mathaba, 2017). But, major challenges include the unavailability of quality-certified seed to enhance productivity, undeveloped seed systems depriving farmers' choices of varieties, limited preservation methods given their high perishability, resulting in poor quality, processed products, and negative perceptions among some of the young generation, educated elite, and urban dwellers (Dube, Heijman, Ihle, and Ochieng, 2017).

CHAPTER THREE: MATERIAL AND METHODS

3.1. Study area

The study was carried out in Walungu Territory/District of South Kivu Province in Democratic Republic of the Congo. South Kivu is located in the Eastern part of the Democratic Republic of the Congo approximately between 1° 36 'South Latitude and 5° South Latitude, and 26° 47' East Longitude and 29 ° 20' East longitude. It has an estimated area of 65,070 km² with a population of 4,944,662 inhabitants (76 persons per km²). South Kivu province shares borders with the Republic of Rwanda from which it is separated by the Ruzizi River and Lake Kivu, Burundi, and Tanzania separated by Lake Tanganyika. South-Kivu has internal borders with North Kivu, Maniema, and Katanga provinces. Bukavu is the capital of the province. Baraka and Uvira are the two other major cities. The province has eight territories including Fizi, Idjwi, Kabare, Kalehe, Mwenga, Shabunda, Uvira and Walungu. The landscape includes mountains (eastern north part), the Central Basin (western part) and a vast plain (southern east part). Agriculture is a primary livelihood for many households in South Kivu, and there are commercial plantations of cash crops such as cinchona, coffee, sugar cane, tea, cacao, etc. (Birhashwirwa, 2015; MONUSCO, 2015; Murphy, Glaeser, Maalouf-Manasseh, Collison, & Sethuraman, 2015).

Walungu territory (Area: 1,800 km², total population of 368,857 inhabitants) has two sectors including: *Kaziba* and *Ngweshe*. *Ngweshe-Sector* has sixteen counties, namely Burhale, Ikoma, Irongo, Izege, Lubona, Luchiga, Lurhala, Kamanyola, Kamisimbi, Kaniola, Karhongo, Mulamba, Mushinga, Nduba, Rubimbi, and Walungu. The sector of Kaziba possesses fifteen counties, namely; Bulumbwa, Butuzi, Chibanda, Chiburhi, Chihumba, Chirimiro, Kahungwe, Kashanga, Kashozi, Lukube, Muhumba, Mulambi, Mushingwa and Ngando. Walungu Territory experiences two seasons: the dry season with 3 months from June to September and the rainy season with 9 months. The dry season is characterized by high temperature and a scarcity of rains, forcing farmers to cultivate in marshy places and wet land. The soil in this area is clayey and poorer due to erosion and overpopulation (Birhashwirwa, 2015).

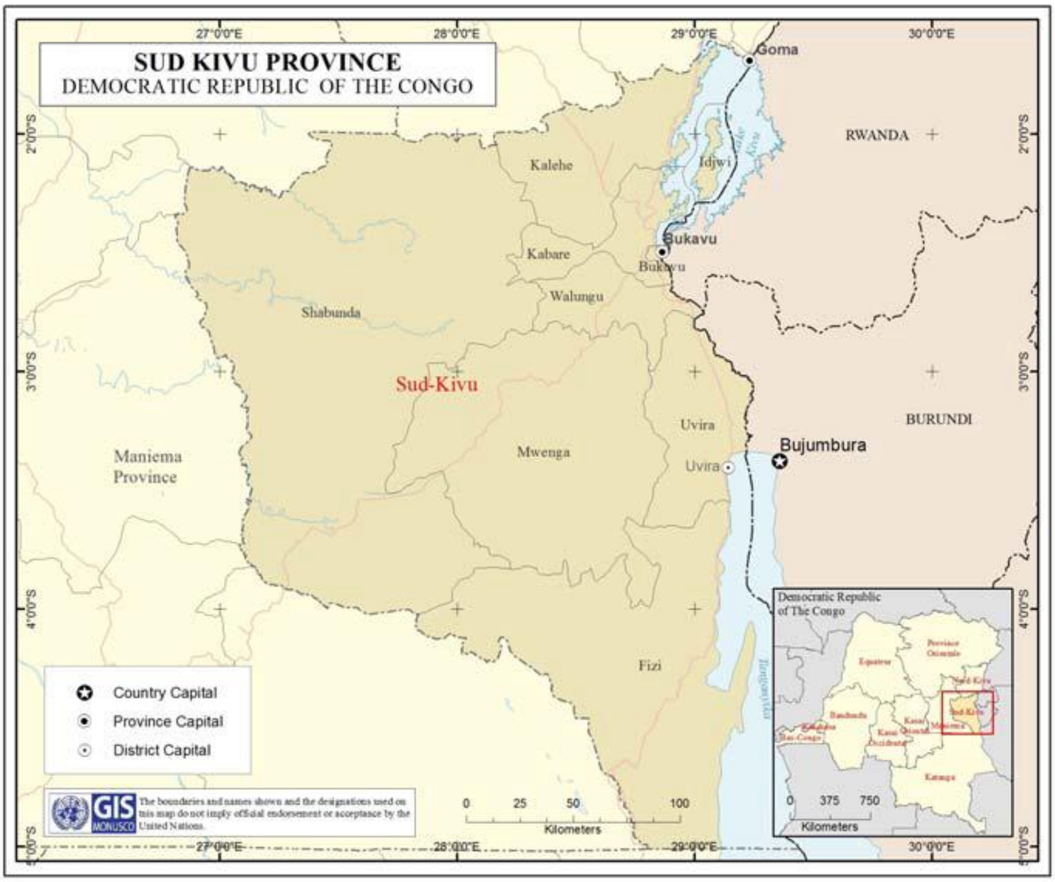

Figure 1: South-Kivu Province map and its territories (MONUSCO, 2015)

3.2. Research Design

A cross-sectional study including household interviews and laboratory analysis was employed. The study involved two phases namely: I) Assessing the awareness, habits, and practices of households in the South-Kivu regarding the indigenous leafy vegetables and II) Nutritional profiling of 8 major leafy vegetables commonly known and consumed in the South-Kivu, DR-Congo.

Phase I: A structured questionnaire was developed (Appendix 1). Eight field assistants, who had been trained for two days, then administered the questionnaire to female or male household heads, or spouses of household heads. Where they were unavailable, an adult female or male member of the household was interviewed. The information recorded included demographic information, indigenous vegetables known and consumed, frequency of their consumption, source of supply, and methods of preparation, conservation and their use and/or consumption patterns. Focus group discussion was conducted in each of the four counties. Each discussion comprised 10-12 people: men and women (heads of household) and lasted

50-60 minutes. As per the guidelines, information collected included perceptions on production, types of indigenous leafy vegetables and their local names, the mode of use of indigenous leafy vegetables, perceived health benefits and sources of these vegetables as well as the perceived problems associated with their use

Phase II: A collection of 20 indigenous leafy vegetables known and consumed by the households in Walungu territory and their taxonomic characterization using the identification keys on herbarium used by (Balagizi, 2010).

Phase III: Descriptive statistics were used to screen 8 major leafy vegetables commonly known and consumed in the South-Kivu for their proximate composition and chemical properties. In this phase, only 8 leafy vegetables most known and consumed were selected from the 20 species. The parameters used for screening the leafy vegetables include moisture content, carbohydrates, proteins, dietary fibers, vitamins (A and C), minerals (Fe, Zn and I), flavonoids, phenolic compounds, tannins, antioxidants and anti-nutrient (Phytates and Oxalates).

3.3. Sampling method and sample size

The study was conducted in four counties purposively selected in the Walungu territory/District (South Kivu Province) namely *Ikoma*, *Lurhala*, *Mulamba* and *Walungu*. Counties were selected based on their status of vegetable production and existence of indigenous plants growing in backyard gardens or as wild plants. Households were selected using simple random sampling method. The number of households included in the survey was determined using the following formula by Magnani (Magnani, 1997).

$$n = \frac{t^2 \times p(1-p)}{m^2}$$

Where: n = required sample size, t = Confidence level at 95% (standard value of 1.96), p= Estimated percentage of households producing and consuming vegetables (Ruel et al., 2004), m= Margin of error at 5% (standard value of 0.05)

Therefore: $n = \frac{1.92^2 \times 0.88(1-0.88)}{0.05^2} = 162.3 \simeq 163$

A total number of 165 households were interviewed in the four counties/sites namely Ikoma, Lurhala, Mulamba and Walungu; making an overall total of 660 households (n = 660) for the entire study.

3.4. Collection and characterization of Indigenous leafy vegetables known and consumed in Walungu

A collection of 20 indigenous leafy vegetables known and consumed by the households in Walungu territory was made from the farms and the bush. Their taxonomic characterization was made using the identification keys on herbarium used by (Balagizi, 2010)

3.5. Laboratory analysis

Based on the survey results, eight indigenous leafy vegetables known and consumed in South Kivu DR-Congo (*Bishusha, Lujinji, Lushenda, Madekere, Muhole, Mushaka, Nderema* and *Ngaingai*) were collected from a few randomly selected farms in the four counties in Walungu Territory/South Kivu (DR Congo) where they were produced under standard agronomic practices. The leafy vegetables were harvested at their respective mature stage, put in zip polythene bags, dated and coded. They were then kept in cool boxes containing ices and transported by car within 17 hours to the laboratory.

3.5.1. Proximate analysis

3.5.1.1. Moisture Content

Moisture content was determined using the method of AOAC, (2016). About five grams of sample was weighed onto a pre-conditioned petri-dish and dried in a hot air oven (Gallenkamp, UK) at 100oC for about 16 h. Dry sample was cooled in a desiccator for about 5 min and weighed. The percentage moisture content was calculated as shown below.

Moisture (%) $= \frac{(W2-W3)}{(W2-W1)} * 100$

Where W1= Initial weight of the empty dish

W2= Weight of the dish plus (+) wet sample before drying

W3= Final weight of dish plus sample after drying

3.5.1.2. Determination of Carbohydrates

- Extraction of carbohydrates by boiling in hot water bath

Carbohydrates was determined according to the method of Nielsen, (2010). About half a gram of sample was weighed into a beaker and 100 ml of hot water added to provide medium for extraction. One milliliter (1ml) of 5% lead acetate was added to precipitate out the proteins. The mixture was incubated in water bath (Grants OLS 200, England) at 80oC for 45 min to extract the carbohydrates. The mixture was allowed to cool and neutralized excess acetate with sodium hydrogen carbonate. The mixture was quantitatively transferred to 100 ml volumetric flask and made up to mark with distilled water.

- Hydrolysis of carbohydrates using concentrated hydrochloric acid

Five milliliters (5 ml) of the mixture was pipetted out into a dry conical flask and was added 50 ml of distilled water followed by one milliliter of concentrated hydrochloric acid to hydrolyze the carbohydrates to simple sugars. The flask was heated in hot water bath to boil for 20 min. After 20 min, the mixture was allowed to cool and then was neutralized the excess dilute hydrochloric acid with sodium hydrogen carbonate. Distilled water was added to the mixture to make up to the 100 ml mark in the volumetric flask. A two tenth of milliliters (0.2ml) of diluted hydrolyzed sample were pipetted into a test tube followed by 0.8 ml of distilled water and 1ml of 5% phenol reagent. Carefully and gently, 5 ml of concentrated Sulphuric acid was added, vortexed carefully and left to stand for 10 minutes. After 10 minutes, the solution was taken to the spectrophotometer (spectroquant Pharo 300M, EU) to read absorbance at 470 nm. Standard glucose calibration curve was made by plotting absorbance against standard glucose concentration (0, 10, 20, 30 and 40 mg). Concentration of total sugars was calculated using equation below.

$$Concentration\ (g/100g) = \frac{(\text{Absorbance - Intercept on vertical axis}) \times \text{D} \times \text{Vo} \times \text{Vi}}{\text{Sample weight (g)} \times \text{Slope} \times \text{V} \times 10}$$

Where D = Dilution factor; 10 = a conversion factor from mg/100g to g/100g; V= volume of sample pipetted in ml; V0 = final volume of sample diluted up to 100 ml mark, Vi = Total volume.

3.5.1.3. Determination of protein content

The Kjeldahl method was used as described by Kirk and Sawyer (1991). It is assumed that all protein in the sample contains nitrogen which has an atomic mass of 14. About 0.5gram of sample was weighed out in triplicates into clearly labeled digesting Kjeldahl tubes. An aliquot of 10 ml of concentrated sulphuric acid and one spatula of mixed complex Kjeldahl catalyst was added to each tube. The samples in each tube were digested at 360°C on a digesting block to clear solution; this converted the nitrogen in the protein to ammonium sulphate. The digested samples were then cooled down and diluted to exactly 50 ml with distilled water. An aliquot of 5ml of diluted samples were taken for distillation in Markham distillation apparatus to distill off ammonia which was trapped in calibrated 2% boric acid solution. Three drops of

1% phenolphthalein were added & about 5 ml of 40% sodium hydroxide to neutralized the acid and facilitate liberation of ammonia gas. The distillate was titrated against 0.05 M hydrochloric acid to determine the ammonia absorbed by boric acid. Protein content was calculated as shown in equation 1.

$$\% \text{ Crude protein } = \frac{(V_2 - V_1)M_{HCl}}{W} \text{ X } 14 \text{ X } 6.25\text{X}100$$

Where,

V_2 = Volume (ml) of hydrochloric acid solution required for the test sample

V_1 = Volume of hydrochloric acid required for the blank test

M_{HCL} = Morality of Hydrochloric acid

W = Weight in grams of test sample

6.25 = Nitrogen conversion factor of protein

14 = Atomic mass of Nitrogen

3.5.1.4. Determination of dietary fiber

Dietary fiber of food matrix was determined using the method described by Kirk and Sawyer (1991). About one gram of the sample was weighed out in triplicate into a 600 ml conical flask, 100 ml of acid detergent fiber were added and boiled for one hour in fiber analyzer (Labconco, Uk). The digest was filtered through glass sinter crucible connected to a vacuum pump (Charles Austen pumps ltd, UK) to collect the residues which were then taken to the oven for drying at 100oC for 45 min to drive off the moisture. Dietary fiber was calculated using equation below.

$$\text{Dietary fibre } (\%) = \frac{W_1 - W_2}{W_s}\text{X}100$$

Where: W_2 = Weight of dry sinter-glass (g)

W_1= Weight of dry sinter-glass and sample (g) and W_s = Weight of the sample

3.5.2. Phenolic compounds

3.5.2.1. Determination of Total phenolic compounds

The phenolic content was determined according to the Folin-Ciocalteu spectrophotometric method as described by (Singleton et al., 1999). Five (5) g of the homogenized sample were extracted with 50 ml of ethanol water solution (80%) in a conical flask with a magnetic stirrer (magnet 4.0×0.5 cm) at 700 rpm for 1 h at room temperature (20±1 °C). The leafy vegetable extracts were then filtered through a filter paper No 89.

About 0.5 ml of the sample extract was added to 2.5 ml of Folin-Ciocalteu reagent (diluted 10 times with water) and, after 3 minutes 2 ml of sodium carbonate (Na2CO3) (75 g/l) were added. The sample was mixed and incubated for 30 minutes at room temperature. The absorbance was determined using a spectrophotometer at 765 nm. The same procedure was repeated for the standard solution of gallic acid and the calibration line was constructed. Based on the measured absorbance, the concentration of phenolics was read from the calibration line; then the content of phenolics in extracts was expressed in terms of Gallic Acid Equivalent (mg of GAE/100g of vegetable).

3.5.2.2. Determination of flavonoid

The total flavonoid content was estimated by aluminum chloride method (Quettier-deleu *et al.*, 2000). Leafy vegetable extract samples (0.5 ml) were mixed with 2.5 ml of distilled water and 150µl $NaNO_2$ solution (5%). The contents were vortexed for 10 sec and left at room temperature for 5 min. Then, 300 µl $AlCl_3$ (10%) were added and contents vortexed and incubated at room temperature for 6 minutes. One (1) ml NaOH (1 M) and 550 µl of distilled water were added. The solution was mixed well and allowed to stand for 15 min. The absorbance for each sample was measured at 510 nm. Total flavonoid content was expressed as quercetin equivalent in mg/100g

3.5.2.3. Determination of tannins

The Tannins (tannic acid) in vegetables were estimated according to a method described by Saxena *et al.* (2013). 0.2 g of dried powdered vegetables was weighed into 250 ml conical flasks and 35 ml water added. The flask was heated gently and allowed to boil for 30 min. The resultant solution was transferred into 50 ml polypropylene tube and topped to 50 ml using deionized water and centrifuged at 1902 x g for 10 min. The supernatant was collected into separate vials. Into a 96 well microtiter plate, 50 µl of sample (supernatant), standards (tannic acid) and blank solution was added followed by addition of 50 µl of Folin–Denis reagent and 100 µl of 7% sodium carbonate solution before mixing by priming using multichannel pipette. The absorbance reading obtained at 700 nm after 30 min. A standard calibration curve of Tannic acid was used to calculate the concentration of total tannins in mg per 100 g of the dry sample.

3.5.3. Vitamins

3.5.3.1. Determination of total carotenoids

The raw vegetables were analyzed for total carotenoids using AOAC methods (AOAC, 1984). Five grams of each sample was accurately weighed into a mortar and pestle in duplicate and mixed with 1milliliter of water and macerated into fine paste. Ten milliliters of acetone (cold) were added into a mortar and a pestle to extract the greenish yellow pigments containing the total carotenoid. Quantitatively, the extract was transferred into a 50mililiters volumetric flask through a funnel with a piece of glass wood. The above procedure was repeated until the residues were colorless. The total volume of acetone used for extraction was 50 milliliters. Then 30 milliliters of Petroleum Ether were put into the separating funnel and added the Acetone Carotenoid extract. Adding 300ml portion of distilled water to the acetone carotenoid extract letting it flow along the walls of the funnel and avoids formation of an emulsion, without shaking. The petroleum ether phase was passed through funnel with pad of glass wool and ten grams of sodium sulphate anhydrous was added into 50 milliliters volumetric flask. The extract was shaken and kept in a dark place covered the flask with aluminum foil. The absorbance of the carotenoid was read at the wavelength 450nm using the Spectrophotometer.

Concentration of total Carotenoid (μ/g) = $\frac{Absorbance * total\ volume * 10000}{weight\ of\ sample * 2592}$

3.5.3.2. Determination of vitamin C

The vitamin C or Ascorbic acid content was determined using the 2, 6_Dichlorophenol-Indophenol titration method (DCPIP) described in Association of Office Analytical Chemists (1996). The extraction involved the use of a mortar and a pestle to macerate 0.5 grams of each sample with 5 milliliters portions of 10% trichloro acetic acid (TCA) being added at a time. The extracts were quantitatively transferred into a clean 50 milliliters volumetric flask through glass wool to trap solid particles and made up to volume with TCA. The flask was shaken vigorously. The 2,6_DCPIP solution was standardized using 0.05 grams of industrial ascorbic acid into 100 milliliters of extracting solvent and pipetted ten milliliters into 100 milliliters volume flask, and diluted to mark with extracting solvent. Two milliliters of standard ascorbic acid were titrated into four conical flasks added five milliliters of extracting solvent, and titrated against DCPIP until a faint pink colour that persisted for one minute. The volume of DCPIP solution used was read from the burette and used to calculate the vitamin C content of the samples. The DCPIP was expressed as 0.0901 equivalents in mg/ml. The concentration of Vitamin C was expressed as mg of ascorbic acid equivalent/100g of sample.

Concentration of Vitamin C (mg/g) = $\frac{net\ titre\ value * Conc\ of\ DCPIP * total\ volume}{volume\ pipetted * sample\ weight}$ *100

3.5.4. Determination of total antioxidants

The total antioxidants were determined using 1,1-Diphenol-2-Picryl Hydrazyl radical method. About 0.5 to one gram of samples in duplicate was weighed into falcon tubes of approximately 15 milliliters capacity and ten milliliters of 80% methanol (extracting solvent) were added. The falcon tubes with their contents were suspended in an ultrasonic water bath and subjected to ultrasonic treatment for 20 minutes at room temperature. After 20 minutes of sonicating, the supernatant was collected into another falcon tube and kept it freezer and was centrifuged for ten minutes at 45000rpm. 2.9 milliliters of methanolic solution of DPPH were pipetted into test tubes and added 50µl of samples extracts. The test tubes were vortexed and allowed to stand for 20 minutes at room temperature. The absorbance was read at wavelength 517nm.

3.5.5. Minerals

3.5.5.1. Determination of Iron and Zinc

Quantification of Iron and Zinc was done by Atomic Absorption Spectrophotometric method using Flame and Furnace Technique (AOAC, 2006). Approximatively 1.0 g of well homogenized sample into a clean silica dish was weighted for each sample. 5mL of 20% sulphuric acid was added and mixed thoroughly with a glass stirring rod ensuring all sample material was wetted by the acid. The stirring rod was rinsed with water into silica dish, and then the contents of the dish were dried thoroughly in an oven set at 110°C. The dishes were then transferred to a furnace set at 250°C and the temperature was slowly and gradually (about 50°C/hr) rose to 500°C where the samples were ashed for about 6 hours. After that the dishes were removed from the furnace and were allowed to cool. The colour of the samples was monitored to ensure that the ashes were white or brownish red (essentially carbon free). For ash containing carbon particles, the down side of their dishes was washed with water combined with 2mL of HNO 3and well mixed. The dishes were then dried thoroughly on hot plate before being returned to furnace at 500°C and ashed for 30 minutes. Nitric acid treatment using 1mL increments of HNO_3 was repeated until white/brownish red, carbon free ash was obtained. When clean ash was obtained, the dishes were removed from furnace, cooled and 1mL HNO_3 and 10mL of water were added. The solution was heated on hot plate till the sample ash was dissolved. The contents of the dish were transferred to a 50mL volumetric flask and heated with 10mL of HCl (1+1). The solution was again transferred to the same volumetric flask to volume with water. Blank solution sample was prepared by following the same procedure as described for the samples. The determination of Zinc and Iron was done by flame AAS. The instrument was set and the absorbance of the samples and the blank were determined. The calculation of the heavy metal content was done from the standard curve.

3.5.5.2. Determination of Iodine

The iodine content of the leaves was determined using Titrimetric Method with Sodium Thiosulphate (Bosset and Giddey, 2002). About 50g of the test samples was taken and transferred into a 500 ml conical flask. 175ml of water were added and the flask was stirred to allow quick dissolution of the sample in water. The solution was then put into a 50ml conical flask. Four drops of methyl red and hydrochloric acid (0.1 mol/l) were added to the solution till the first colour change from yellow to orange. Then 1.5ml of bromine water was added immediately. The portions were allowed to stand for three minutes. Then some glass beads were added, heated and boiled for five minutes, with swirling and avoiding crystallization of the sample. After one minute standing, 1.0ml of formic acid was added till the whole inside surface of the conical flask was wetted and swirled. After 1 minute, the solution was allowed to cool to about 20 °C, 1.0 ml of phosphoric acid and 1.0 ml of potassium iodide solution were added. The conical flask was swirled, corked and allowed to stand in the dark for exactly five minutes. Titration was then done using a burette with a sodium thiosulphate standard volumetric solution 0.01mol/l. When the solution was nearly discolored, 1 ml of starch solution was added and the titration was resumed until the blue colour disappeared for at least 3 seconds.

The iodine content of the sample, W (I), is given by the formula:

$W(I) = 21.15 \times c\,(Na_2\,S_2\,O_3) \times [1000/m] \times (V_1 - V_0)$

- W(I) is the total iodine content, expressed as milligrams of iodine per kilogram of sample,
- m is the mass, in grams, of the test portion
- V_1 is the volume, in milliliters, of sodium thiosulphate used for the titration of the test solution
- V_0 is the volume, in milliliters, of sodium thiosulphate used for the titration of the blank solution

- $c(Na_2S_2O_3)$ is the molar concentration of the sodium thiosulphate standard volumetric solution

3.5.6. Anti-nutrients

3.5.6.1. Determination of total phytates

Phytic acid content was determined according to procedures developed by Harland and Oberleas, (1986) using a modified colorimetric (Wade reagent) method. About one gram of sample was weighed out and macerated in mortar & pestle to extract phytic acid using 100 ml of 2.4M HCl mixed with 0.1M sodium chloride. The clear supernatant of filtrate of the extract was further diluted 10 times into a 100 ml volumetric flask and made to the mark using distilled water. Thereafter, one milliliter of clear solution was diluted 10 times with distilled water and collected for colour development. Three milliliters of the diluted sample was mixed with one milliliter of Wade reagent (0.03% $FeCl3 \cdot 6H2O$ + 0.3% sulfosalicylic acid; 27308, Sigma-Aldrich Laborchemikalien GmbH) and thoroughly vortexed & centrifuge for 10 minutes. A series of calibration standards containing 0, 1.12, 2.24, 3.36, 5.6, 7.84 and 11.2 mg/ml was prepared from sodium salt M.W. 660.04g. Absorbance of color reaction products for both samples and standards was read at 500 nm on a spectrophotometer (Spectroquant Pharo 300M, EU). The amount of phytates in the samples was calculated as shown in equation from calibration curve.

3.5.6.2. Determination of oxalates

Oxalate composition in the leafy vegetables was determined by Day & Underwood, (1986) method. 2.5 g of the sample was mixed with 75 ml of 3 M H_2SO_4 and stirred for 1 h with a magnetic stirrer. The mixture was filtered and 25 ml of the filtrate was titrated while hot against 0.05 M $KMnO_4$ solution to a faint pink color that persisted for 30 s. The oxalate composition was calculated by assuming that 1 ml of 0.05 M KMnO4 is equivalent to 2.2 mg oxalate (AOAC, 1999).

3.6. Statistical analysis

Data was analyzed using the SPSS software program (Version 22). Frequencies were generated for survey data. Laboratory chemical analyses were done in triplicate and the mean value and standard deviations of each chemical parameter were computed. Analysis of Variance (ANOVA) was performed to calculate significant differences in means and least significant difference technique was used for separation of means at $p = 0.05$.

CHAPTER FOUR: RESULTS

4.1. Awareness, attitudes and practices of households regarding ILVs

4.1.1. Socio-demographic characteristics of households in Walungu/South Kivu

The socio-demographic characteristics of households interviewed in this study are presented in Table 1. Results indicated that up to 58.6% of the participants were female while 41.4% were male. Fewer household heads were below 20 years (10%) while the majority had an age between 21 and 50 years old. About 68.1% of household heads in this study population were married, 23.7% were widowed while the rest were either divorced (2.9%) or single but having a cohabiting partner (5.3%).

Table 1: Socio-demographic characteristics of households in Walungu

Socio-demographic characteristic		**Percentage**
Gender of the household head	Male	41.4
	Female	58.6
Age of the household head	Below 20 years	10
	21-30 years	26.2
	31-40 years	20.9
	41-50 years	20.6
	51-60 years	13.2
	Above 60 years	9.1
Marital status	Single with a cohabiting partner	5.3
	Married	68.1
	Divorced	2.9
	Widow	23.7
Level of Education	No education	40
	Primary school	33.5
	Secondary school	24.7
	University	1.5
	Other	0.3
Household size (members)	<5	29.9
	05-Oct	46.2
	>10	23.9

Majority (59.7%) of the household heads had an education degree (primary, secondary and university degree, 33.5%, 24.7% and 1.5 %, respectively). Only 40% of household heads had no education degree. However, 0.3% household heads had an apprenticeship trade. The majority of respondents were farmers at 61.8%. Other respondents were traders (19.4%), artisans (12%), civil servants (6.1%) and food service providers (0.8%). Most households had 5 to 10 members (46.2%) while others had less than 5 members (29.9%) whereas 23.9% of the households had more than 10 members.

4.1.2. Awareness of household heads on indigenous leafy vegetables

The indigenous leafy vegetables commonly known in Walungu/South Kivu are summarized in Table 2. Results indicated that 20 leafy vegetables are commonly known and consumed in South-Kivu, particularly in Walungu territory.

Table 2: Indigenous leafy vegetables commonly known and consumed in Walungu

Local name	English name	Scientific name
Bishoma/Bishogolo	Bean leaves	*Phaseolus vulgaris*
Bishusha	Pumpkin leaves	*Cucurbita ssp.*
Bisirusiru	Bracken, eagle fern	*Pteridium aquilinum*
Iragala	Gallant soldier, potato weed	*Galinsoga parviflora*
Kabalalalwage/Itunda	Woolflower	*Celosia trigyna*
Lengalenga	Amaranthes	*Amaranthus ssp*
Lujinji	Ehiopian Kale or dogmustard	*Erucastrum arabicum*
Lushenda	red pepper leaves	*Capsicum frutescens*
Madekere	Arrowleaf, elephant's ear	*Xanthosoma sagittifolium*
Mageru/Magara	Velvet bushwillow	*Hypoestes triflora*
Matembela/Ngozi	Sweet potato leaves	*Ipomoea batatas*
Muhashahasha/Mwengele	N/A	*Cyphostemma adenocaule*
Muhole	Spider plant	*Cleome gynandra*
Mulunda	Nightshade	*Solanum nigrum*

Mushaka	N/A	*Cleome Schimperi*
Mushinyungu	The calabash or bottle gour	*Lagenaria siceraria*
Nderema	Ceulon Spinach	*Basella alba*
Ngaingai/Mukeranshungu	Tropical hibiscus, rose mallow	*Hibiscus noldae*
Nyabudekere	Purlane	*Portulaca oleracea*
Sombe	Cassava leaves	*Mahinot esculenta* Syn.

For quick conceptualization of the 20 indigenous leafy vegetables indicated in table 4.2, photographs of the leaves (and flowers when available) are given in appendices (figures 2 - 11).

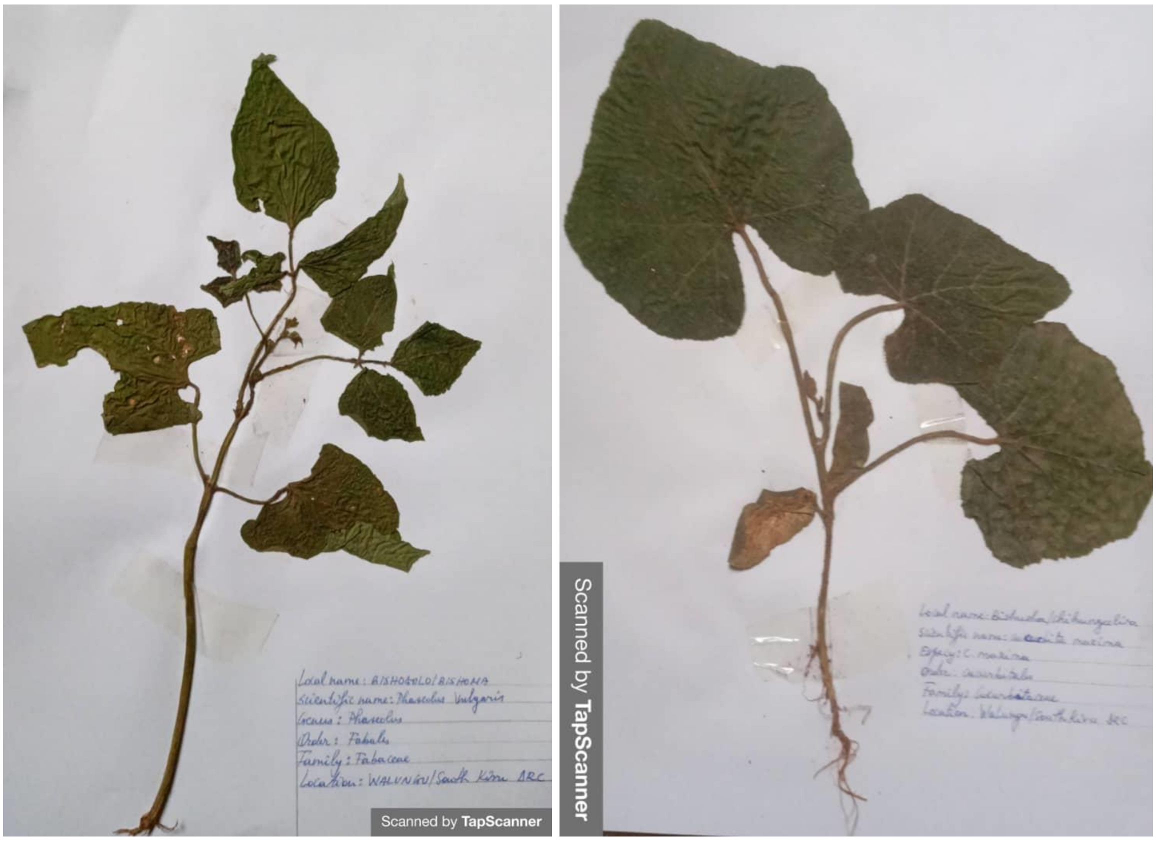

Figure 2: *Bishogolo or Phaseolus vulgaris* (L) and *Bishusha or Cucurbita spp* (R)

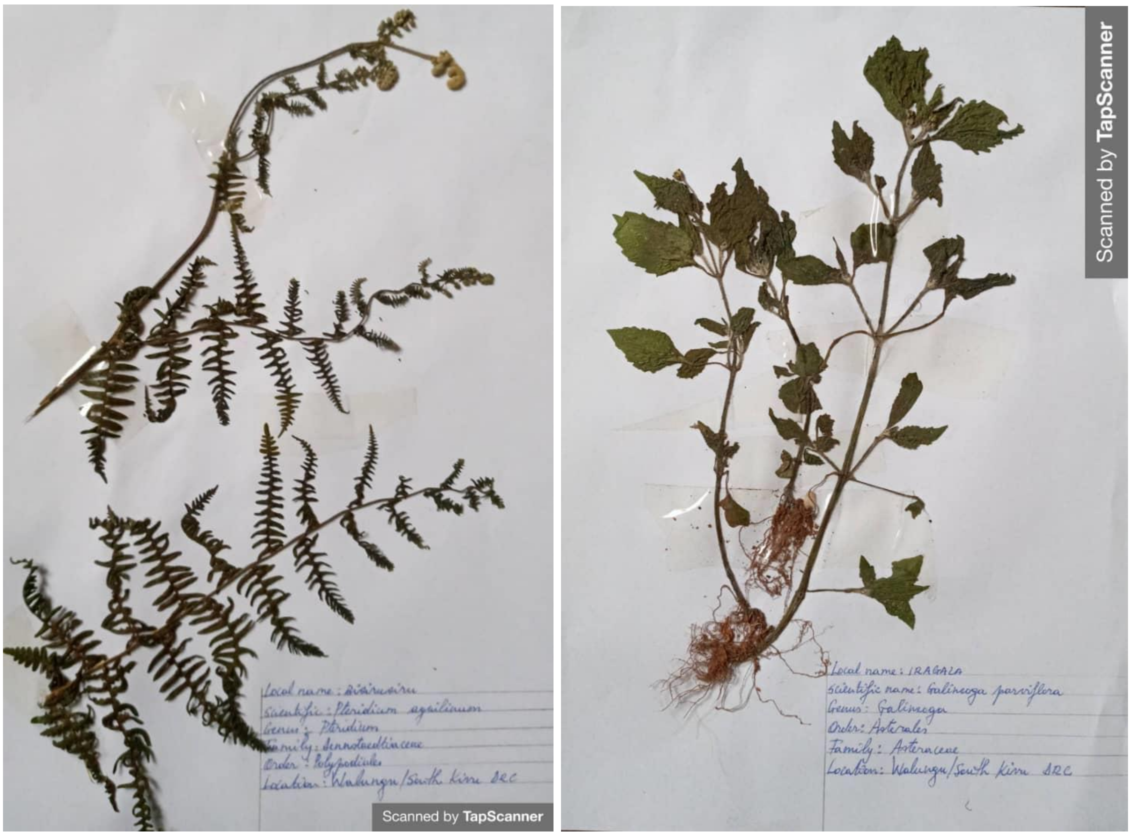

Figure 3: *Bisirusiru or Pteridium aquilinum* (L) and *Ragala or Galinsoga parviflora* (R)

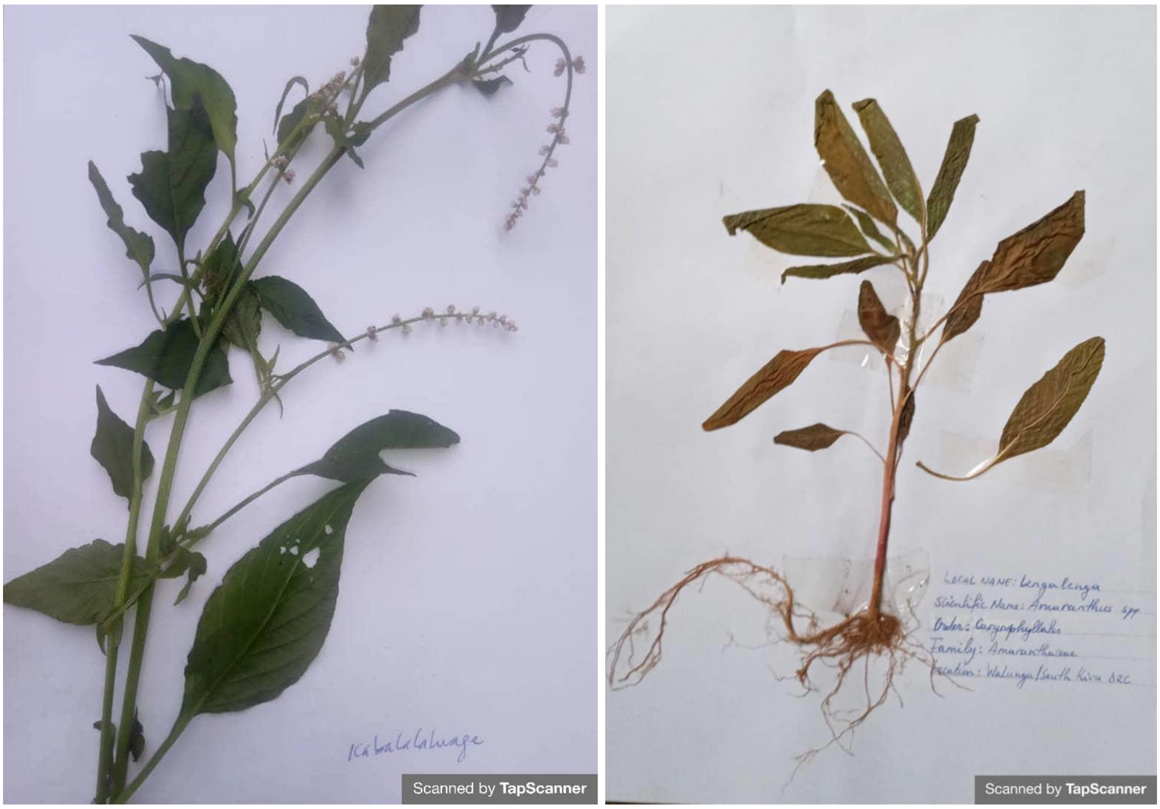

Figure 4: *Itunda or Celosia trigyna* (L) and *Lengalenga or Amaranthus ssp* (R)

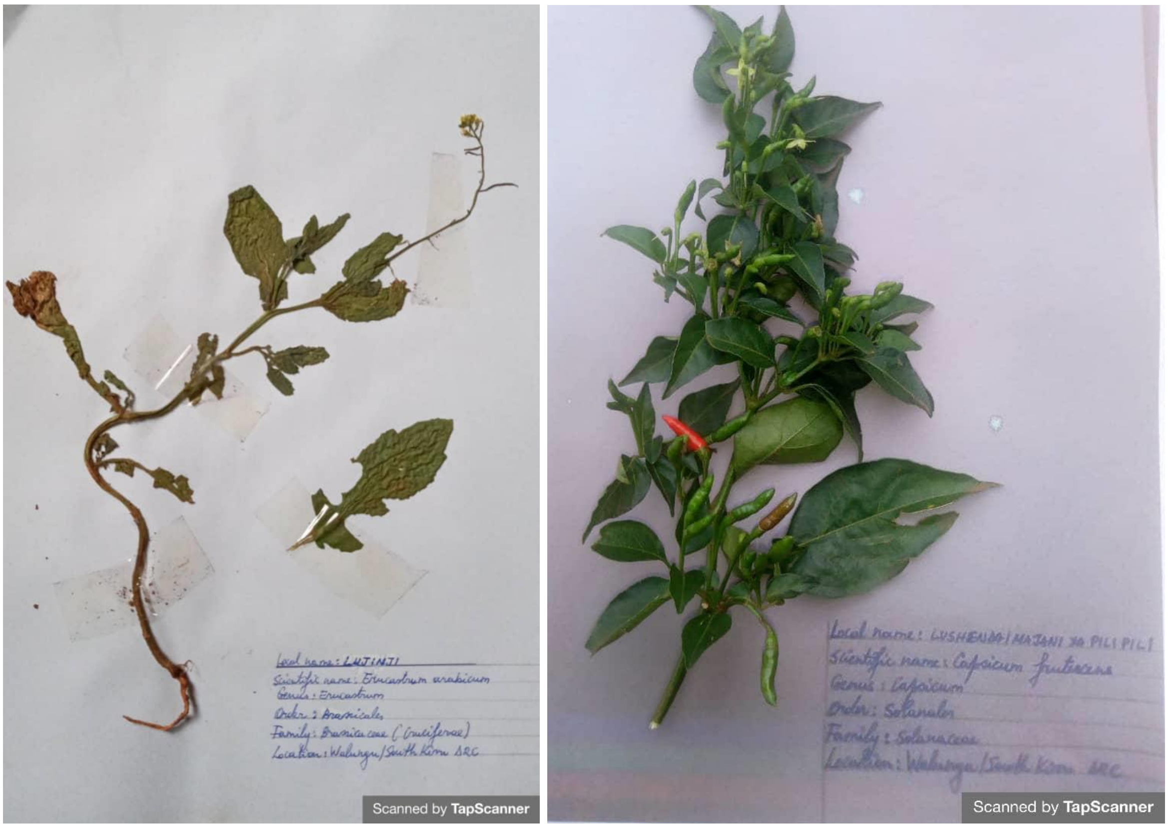

Figure 5: *Lujinji or Erucastrum arabicum* (L) and *Lushenda or Capsicum frutescens* (R)

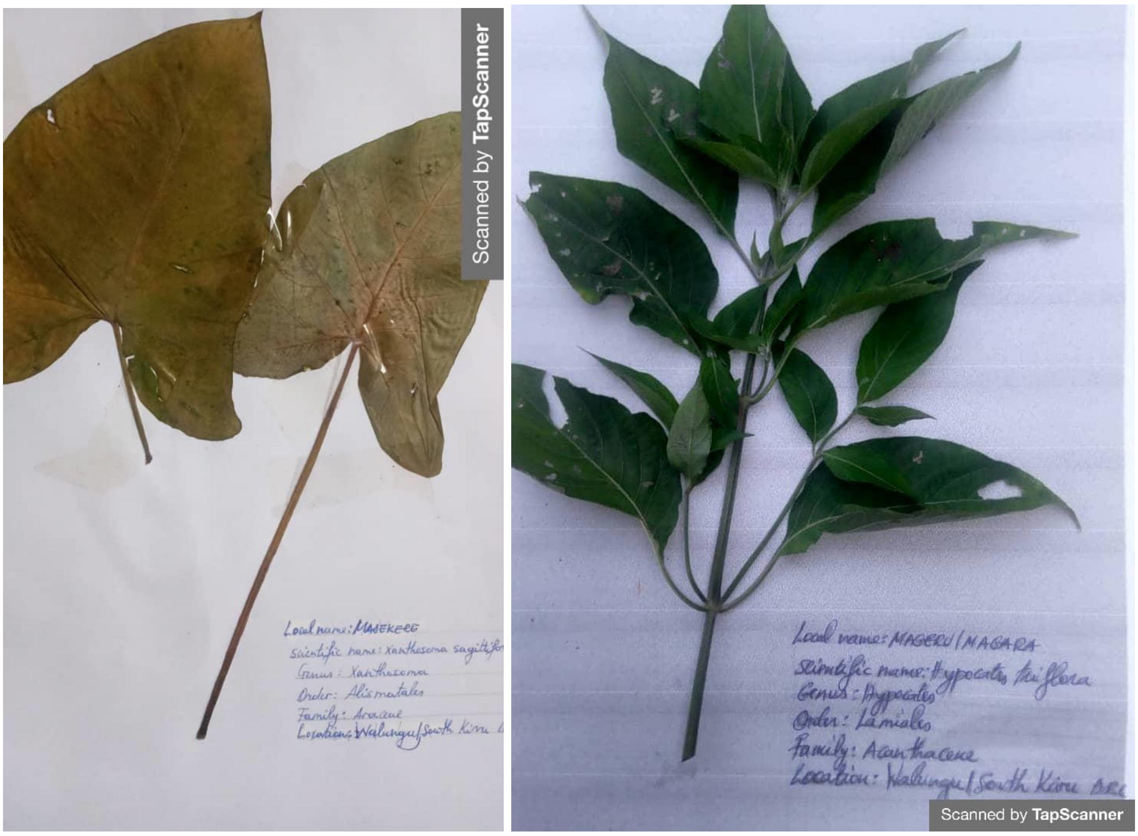

Figure 6: *Madekere or Xanthosoma sagittifolium* (L) and *Mageru or Hypoestes triflora* (R)

Figure 7: *Matembela or Ipomoea batatas* and *Muhashahasha or Cyphostemma adenocaule*

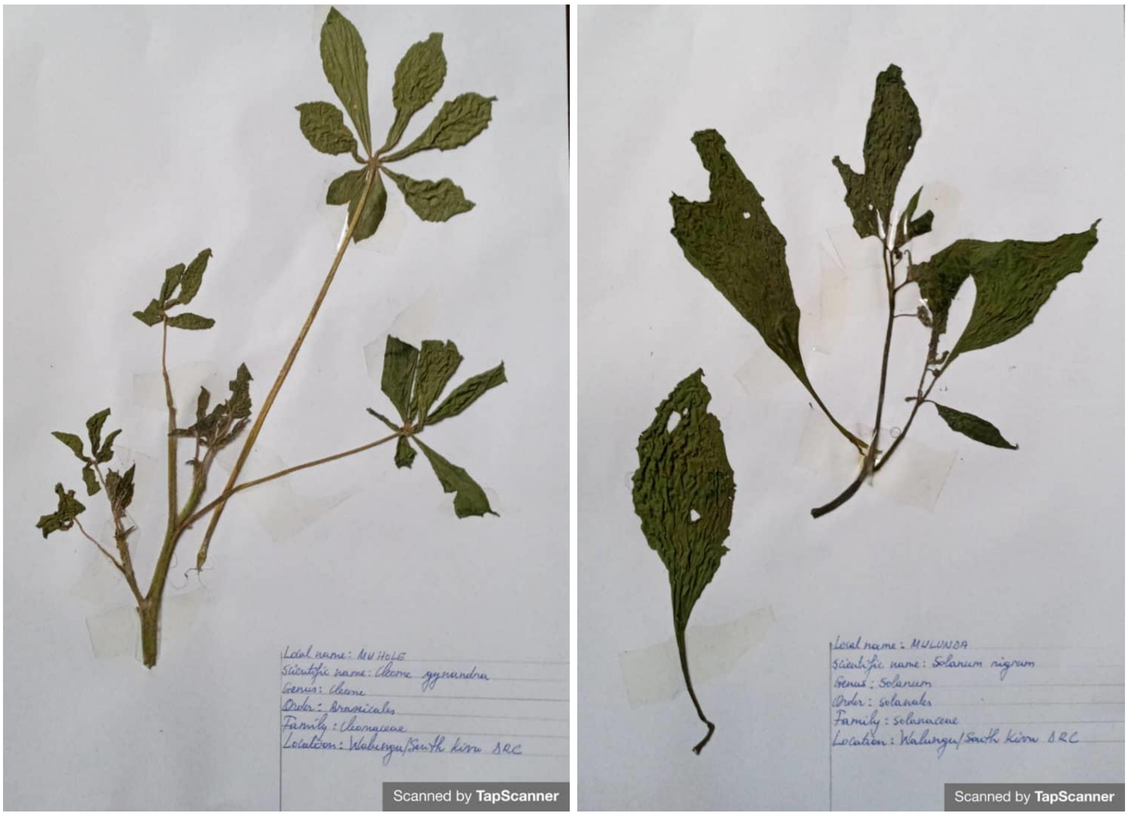

Figure 8: *Muhole or Cleome gynandra* (L) and *Mulunda or Solanum nigrum* (R)

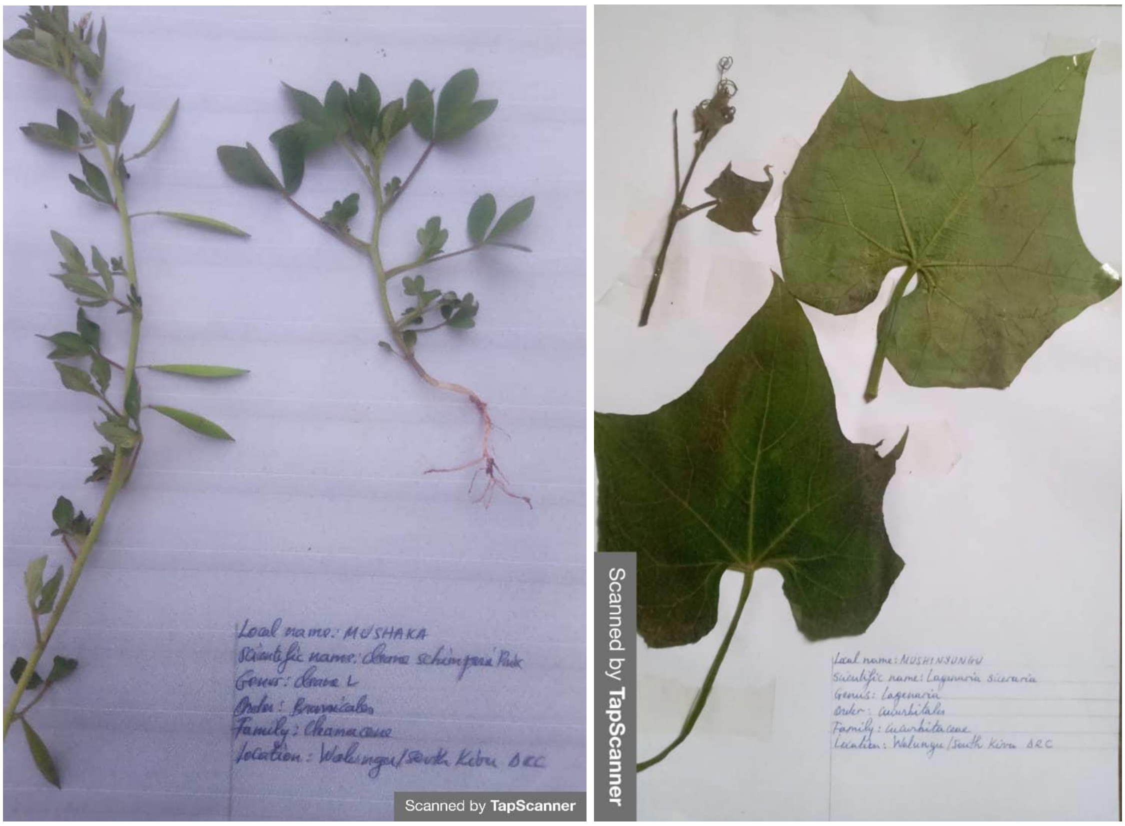

Figure 9: *Mushaka or Cleome Schimperi* (L) and *Mushinyungu or Lagenaria siceraria* (R)

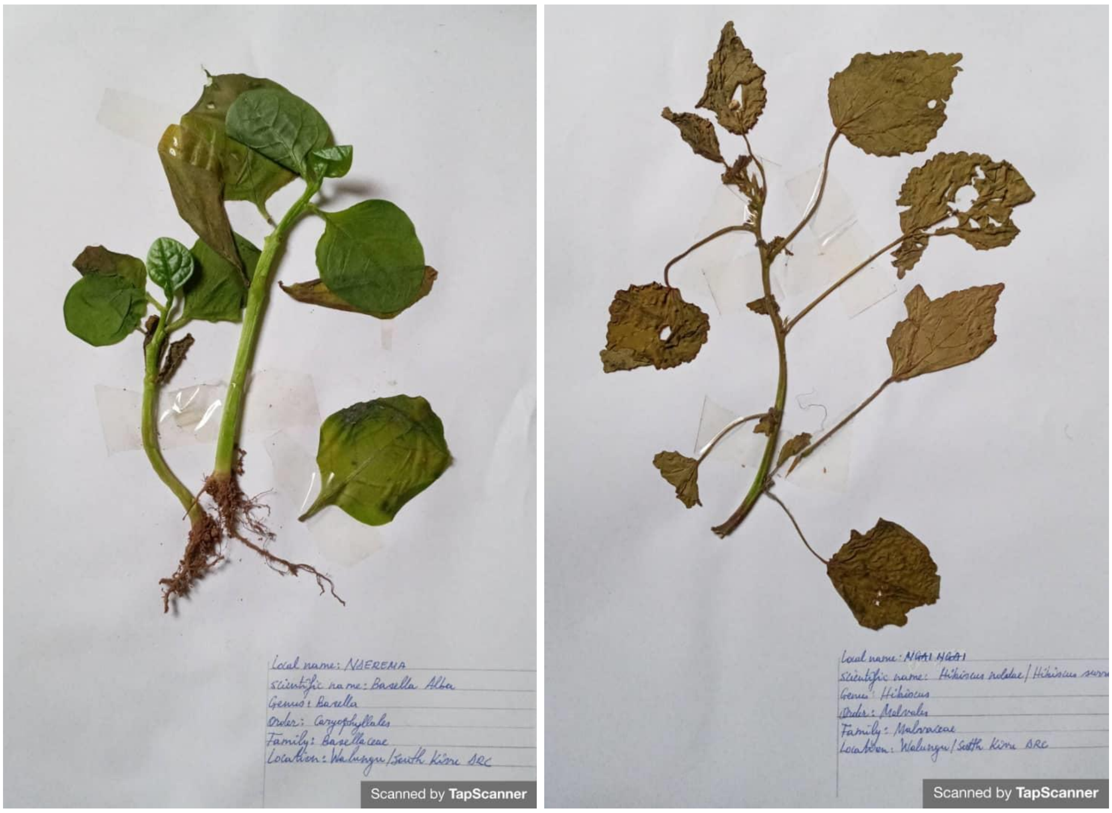

Figure 10: *Nderema or Basella alba* (L) and *Ngaingai or Hibiscus noldae* (R)

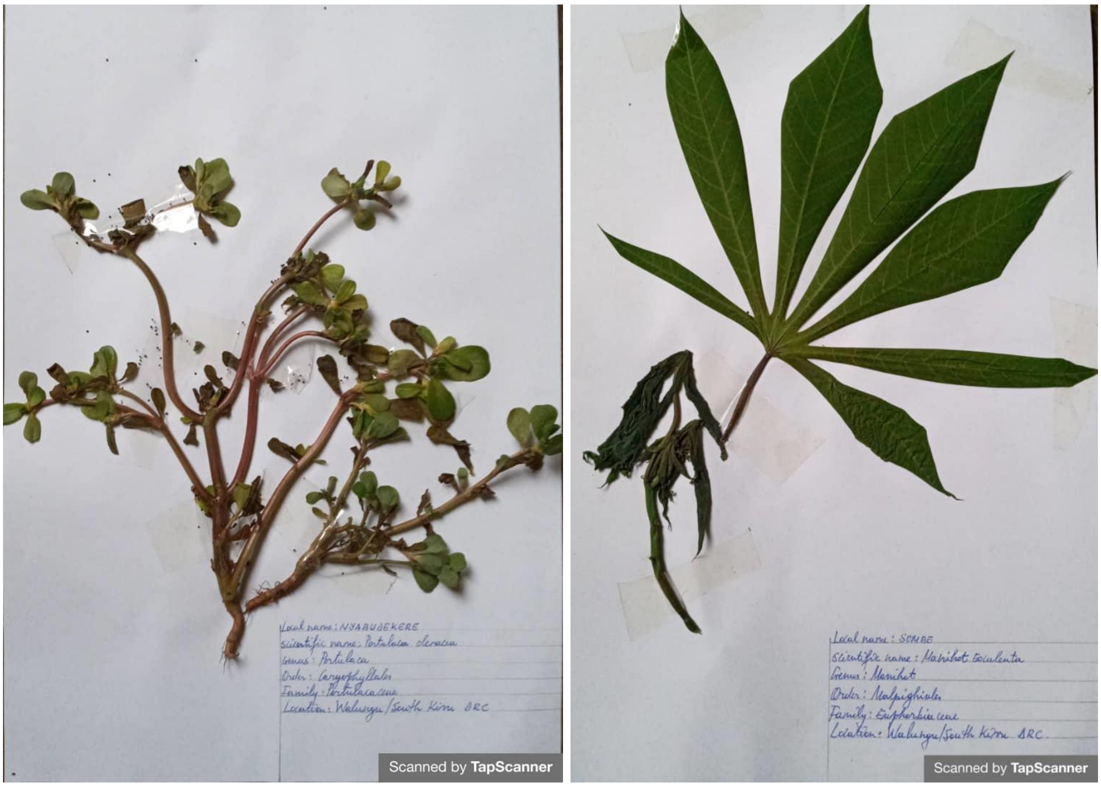

Figure 11: *Nyabudekere or Portulaca oleracea* (L) and *Sombe or Mahinot esculenta* (R)

4.1.3. 4.1.3 Reason for production of indigenous leafy vegetables for the households

The reasons for growing the indigenous leafy vegetables in Walungu are presented in Figure 12. Results indicate that, in all households, most of the indigenous leafy vegetables were grown mainly for food, secondly for income, and thirdly for medicinal purposes. As shown in Figure 3, *Sombe, Bishogolo* and *Lengalenga* were the most indigenous vegetables grown for food and income by over 80% and 35% respondents, respectively. *Bisirusiru* were mainly grown for medicinal purpose. Moreover, over 30% of respondents cultivated *Mulunda, Matamabazi* and *Lengalenga* for their medicinal benefits.

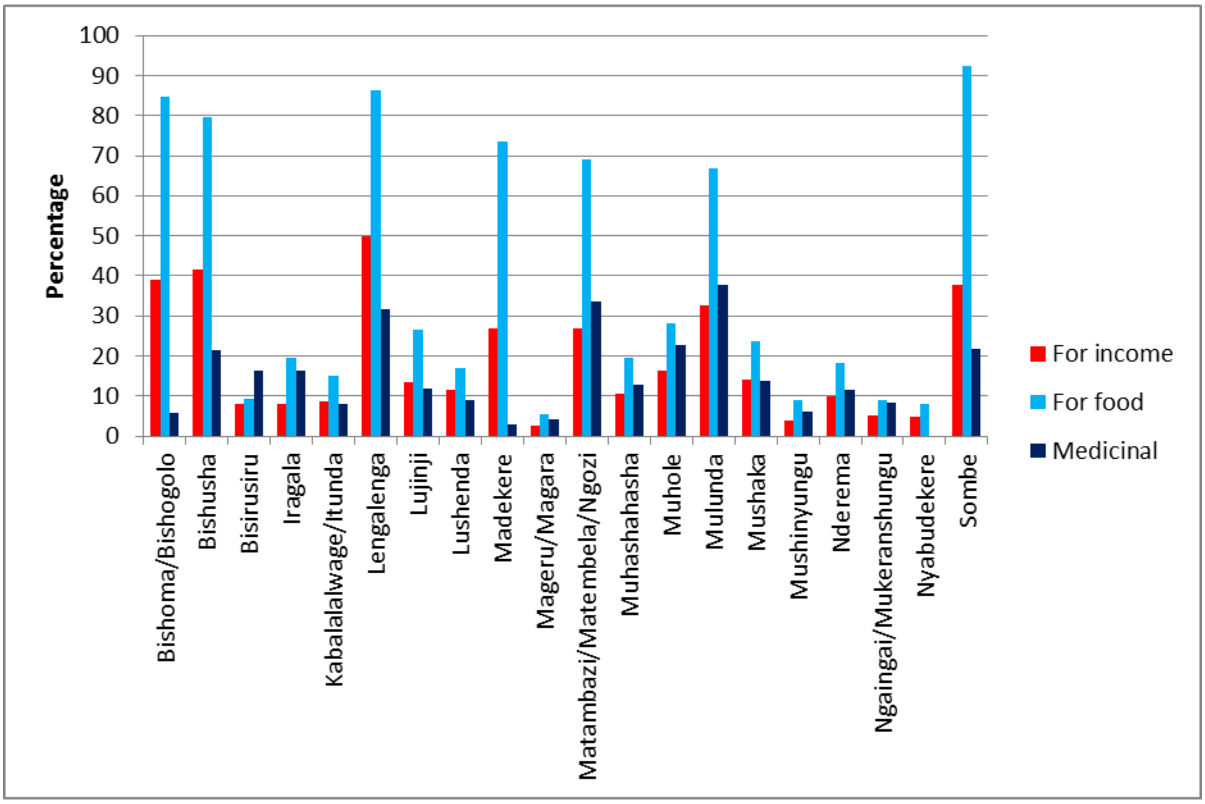

Figure 12: Reason for cultivating indigenous leafy vegetables in Walungu

4.1.4. Reason for indigenous leafy vegetables consumption

The reasons for the consumption of leafy vegetables in South-Kivu are summarized in Figure 13. Results showed that households in Walungu consume indigenous leafy vegetables mainly because they are available, tasty, and believed to be nutritious. The most available indigenous leafy vegetables were *Sombe, Bishogolo*, *Lengalenga*, *Bishusha* and *Madekere*. Respondents also believed that *Sombe* and *Lengalenga* are the most nutritious vegetables while *Bishogolo* were considered to be the tastiest among all the indigenous leafy vegetables consumed in Walungu.

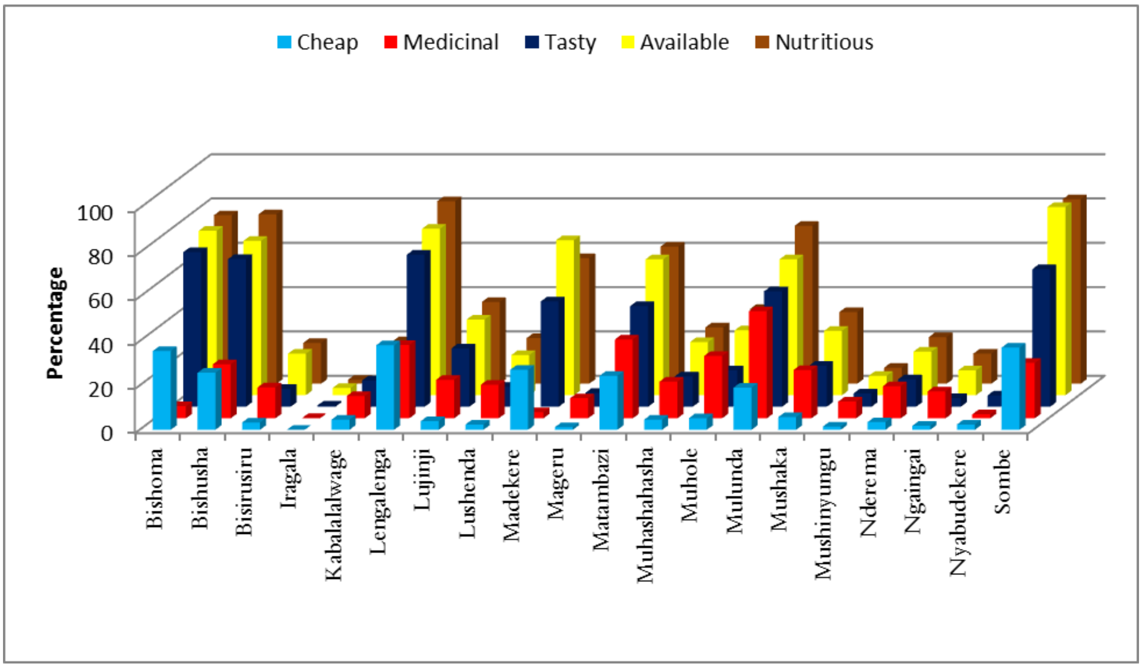

Figure 13: Distribution of raison for consumption Indigenous leafy vegetables by households in Walungu

4.1.5. Frequency of consumption of Indigenous leafy vegetables in Walungu

The regularity of consumption of indigenous leafy vegetables in Walungu is presented in Figure 14. Results showed regular daily consumption of indigenous leafy vegetables. Up to 55% of all households interviewed consumed ILVs two times per day in various quantities. About 27% of households interviewed consumed them three times per day while only 10% of households reported consumption once a day.

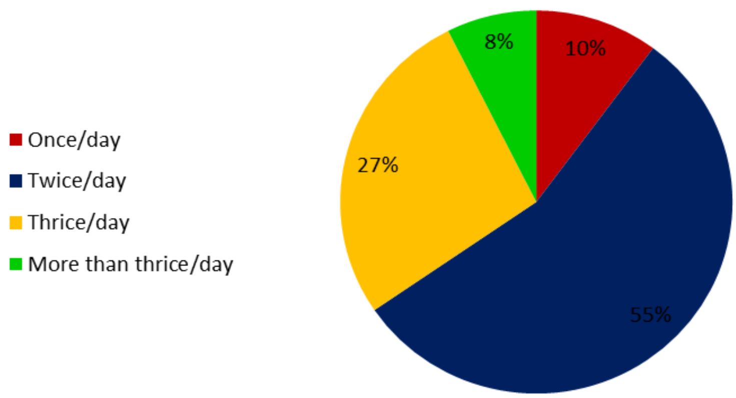

Figure 14: The frequency of the daily consumption of indigenous vegetables

4.1.6. Sources of indigenous leafy vegetables in Walungu

The sources of indigenous leafy vegetables consumed in Walungu are summarized in Figure 15. Majority 95.9% of households get indigenous leafy vegetables from family farms, 74.6% of households purchase them from the local market whereas 29.9% obtain the indigenous leafy vegetables from their backyard gardens. A few households (12.6%) reported harvesting indigenous vegetables from the bush or received as gifts.

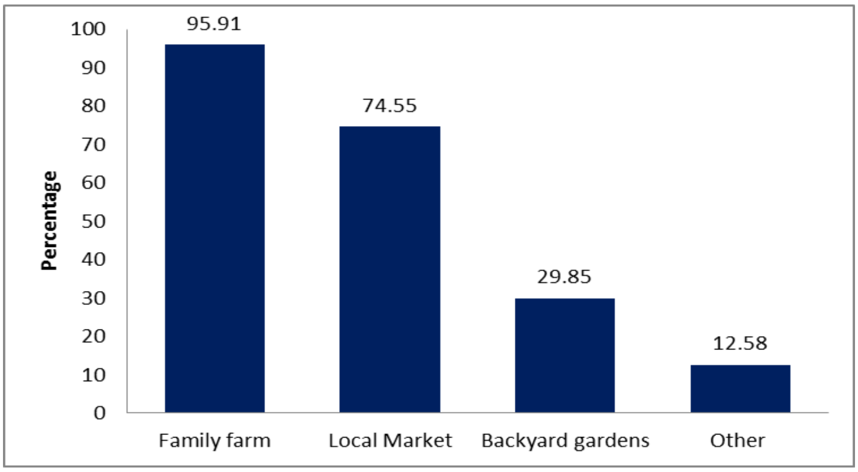

Figure 15: Sources of indigenous vegetables consumed and/or used

4.1.7. Preparation, Cooking and preservation of indigenous leafy vegetables

Preparation, cooking and preservation methods of indigenous leafy vegetables are summarized in figure 7-11. Results showed in Figure 16 that households in Walungu mainly wash their vegetables in cold water (89.1%) prior to cooking. Only a few households (9.7%) reported washing vegetables with warm water.

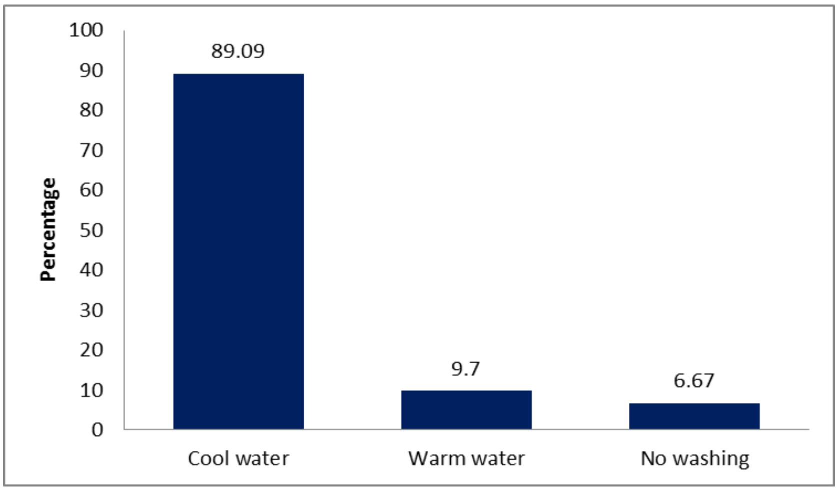

Figure 16: Washing patterns of ILVs before cooking

In Figure 17, Majority (92.3%) of households boiled their vegetables, 10.8% of households fried them while 6.4% steamed them before consumption. In other rare cases, indigenous leafy vegetables are consumed dried (0.76%) and/or raw. As result, boiling and steaming are the main preparation patterns of indigenous leafy vegetables in Walungu as indicated in Figure 4.18.

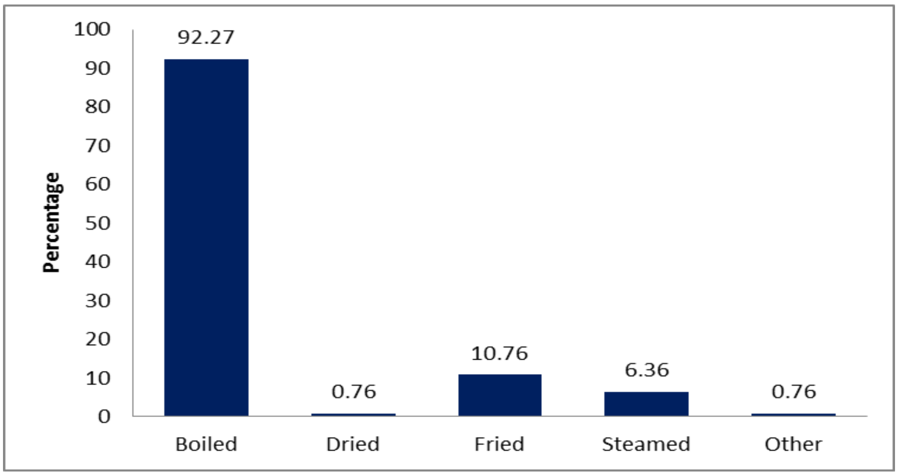

Figure 17: Use and/or consumption patterns of indigenous vegetables

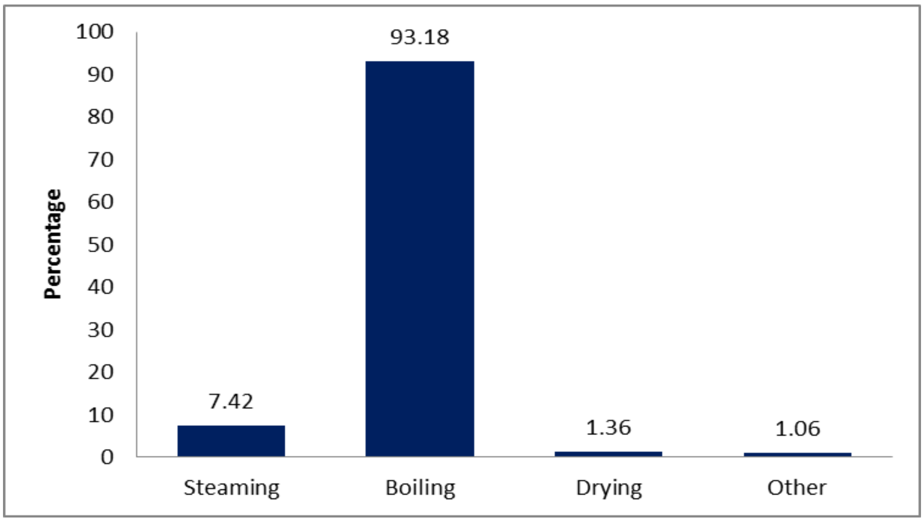

Figure 18: Preparation and/or cooking patterns

In Figure 19, More than half (58%) of all households interviewed cooked for ILVs one hour while some households (20% and 11.4%) reported cooking vegetables for 45 minutes and for 30 minutes, respectively. Only a few households' reported cooking vegetables for more than one hour (7.9%) and for 25 minutes (2.7%).

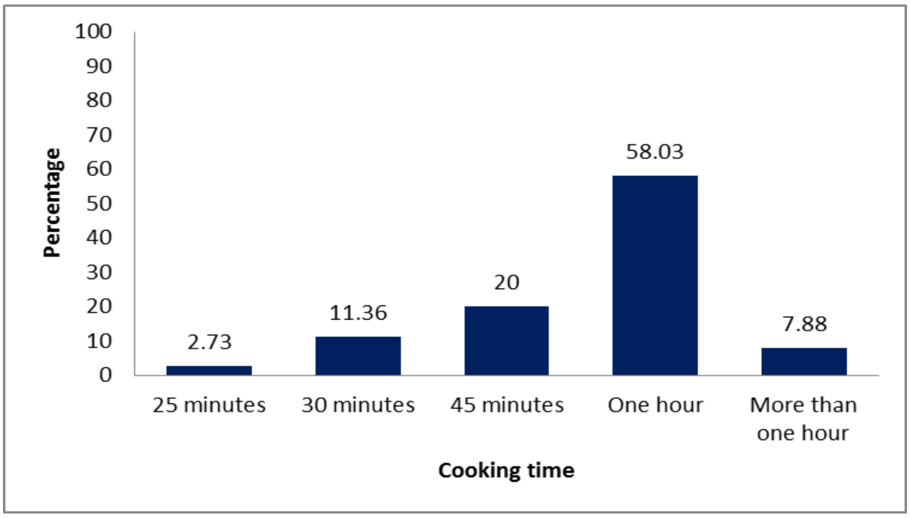

Figure 19: Duration (time) for cooking

Results in Figure 20 indicated that about a third (32.7%) of households reported drying the indigenous leafy vegetables via air drying while 26.5% of households used solar drying. About 40.2% of households reported not drying vegetables for preservation.

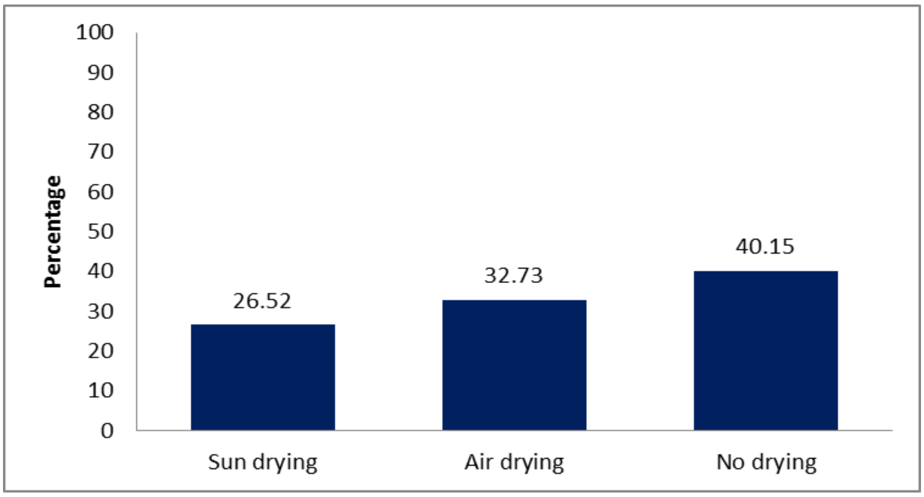

Figure 20: Drying patterns

4.2. Nutrient contents of Indigenous leafy vegetables

4.2.1. Proximate composition

The moisture content, carbohydrates concentration, crude protein and dietary fiber content of the Indigenous Leafy Vegetables commonly consumed in Walungu are summarized in Table 3. The moisture content of these vegetables varied from 78.6% in *Mushaka* to 94.1% in *Nderema*. All the vegetables with exception of *Mushaka* had a moisture content of more than 80%. Results also indicate variations in carbohydrates concentrations among Indigenous

Leafy Vegetables commonly consumed in Walungu. *Muhole* and *Lujinji* recorded the lowest carbohydrates content of 4.09 and 4.13 g/100g, respectively; whereas the highest carbohydrates content was recorded in *Mushaka* (8.9 g/100g). Significant differences ($p<0.05$) in protein content were observed between *Mushaka* (28.5%), *Lushenda* (8.3%), and the other leafy vegetables (average 4.1%). Results further showed variation in dietary fiber content of different leafy vegetables. Dietary fiber in major leafy vegetables consumed in Walungu ranged from 1.2% in *Nderema* to 38.6% in *Mushaka*. Significant differences in dietary fiber content were observed between vegetables. A higher dietary fiber content was recorded in *Bishogolo* (12.9%) and *Mushaka* (38.6%) than the rest of the varieties.

Table 3: Proximate composition of ILVs commonly consumed in Walungu

Leafy Vegetables	**Moisture content (%) on a wet basis**	**Carbohydrates (g/100g) on a dried basis**	**Crude protein (%) on a dried basis**	**Dietary fibre (%) on a dried basis**
Bishogolo (Phaseolus vulgaris)	84.4 ± 0.91^{bc}	5.43 ± 0.77^{a}	5.00 ± 0.37^{ab}	12.89 ± 1.74^{c}
Lujinji (Erucastrum arabicum)	86.9 ± 0.57^{c}	4.13 ± 0.73^{a}	5.22 ± 0.26^{b}	4.51 ± 0.36^{ab}
Lushenda (Capsicum frutescens)	81.9 ± 0.67^{ab}	4.84 ± 0.26^{a}	8.30 ± 0.12^{c}	8.23 ± 0.08^{bc}
Madekere (Xanthosoma sagittifolium)	84.3 ± 3.32^{bc}	5.24 ± 0.59^{a}	3.22 ± 0.07^{ab}	9.13 ± 0.85^{bc}
Muhole (Cleome gynandra)	81.8 ± 1.19^{ab}	4.09 ± 0.26^{a}	4.82 ± 0.26^{ab}	5.81 ± 0.36^{ab}
Mushaka (Cleome Schimperi)	80.7 ± 1.66^{a}	8.94 ± 0.25^{b}	28.46 ± 2.81^{d}	38.64 ± 5.49^{d}
Nderema (Basella alba)	94.1 ± 0.36^{d}	5.14 ± 1.34^{a}	2.77 ± 0.03^{a}	1.17 ± 0.11^{a}
Ngaingai (Hibiscus noldae)	84.22 ± 1.09^{bc}	5.67 ± 0.65^{a}	3.26 ± 0.02^{ab}	6.05 ± 0.71^{b}

Values are means ± SD. Values in a column with the same superscript are not significantly different ($p > 0.05$).

4.2.2. Mineral content of ILVs commonly consumed in Walungu

The results of minerals composition of Indigenous leafy vegetables commonly consumed in Walungu are presented in Table 4. The results show that Zinc and Iron were the dominant minerals in most of the vegetables, as compared to Iodine. In general, a high variation in the minerals was observed in all the selected leafy vegetables. There was a significant difference in minerals composition between the different Indigenous leafy vegetables. *Lujinji* (162.9 mg/Kg) and *Muhole* (14.3 mg/Kg) recorded the highest and lowest amount of Zinc, respectively. *Lushenda* (176.8 mg/Kg) and *Ngaingai* (113.3 mg/Kg) recorded the highest content of Iron. *Madekere* (2.4 µg/Kg), *Nderema* (1.8 µg/Kg) and *Ngaingai* (1.6 µg/Kg) recorded the highest mean content of Iodine.

Table 4: Mineral composition of ILVs commonly consumed in Walungu

Leafy Vegetables	Zinc in mg/Kg	Iron in mg/Kg	Iodine in µg/Kg
Bishogolo (Phaseolus vulgaris)	59.14 ± 0.18^{d}	83.77 ± 0.37^{d}	0.54 ± 0.01^{e}
Lujinji (Erucastrum arabicum)	162.96 ± 0.67^{a}	71.70 ± 0.20^{g}	0.79 ± 0.01^{d}
Lushenda (Capsicum frutescens)	87.36 ± 0.47^{c}	176.82 ± 0.77^{a}	1.05 ± 0.01^{c}
Madekere (Xanthosoma sagittifolium)	89.85 ± 0.06^{b}	69.52 ± 0.35^{h}	2.44 ± 0.05^{a}
Muhole (Cleome gynandra)	14.34 ± 0.12^{h}	81.39 ± 0.37^{e}	0.85 ± 0.05^{cd}
Mushaka (Cleome Schimperi)	43.45 ± 0.11^{f}	73.21 ± 0.37^{f}	0.87 ± 0.17^{cd}
Nderema (Basella alba)	35.14 ± 0.23^{g}	108.74 ± 0.29^{c}	1.77 ± 0.23^{b}
Ngaingai (Hibiscus noldae)	44.18 ± 0.15^{e}	113.28 ± 0.61^{b}	1.57 ± 0.24^{b}

Values are means ± SD. Values in a column with the same superscript are not significantly different ($p > 0.05$).

4.2.3. Vitamin and antioxidant content of ILVs commonly consumed in Walungu

The vitamin C, carotenoids and antioxidant in Indigenous leafy vegetables consumed in Walungu are shown in Table 5. Results indicate that antioxidant content varied from 63 to 80.3 mg/100g while vitamin C concentration varied from 27 to 56.8 mg/100g. Significant variations were recorded in carotenoids content of leafy vegetables consumed in Walungu.

Mushaka had the highest carotenoids content (136.1 µg/100g) as well as the highest concentration of vitamin C (56.8 mg/100g) and antioxidant content (80.3 mg/100g).

Table 5: Vitamin and antioxidant content of ILVs commonly consumed in Walungu

Leafy Vegetables	Carotenoids (µg/g)	Vitamin C (mg/100g)	Antioxidant (mg/100g)
Bishogolo (Phaseolus vulgaris)	28.58 ±5.64^{cd}	45.84 ±4.48^{b}	64.26 ±0.38^{c}
Lujinji (Erucastrum arabicum)	29.85 ±1.16^{cd}	33.51 ±2.95^{de}	63.24 ±0.20^{de}
Lushenda (Capsicum frutescens)	13.85 ±1.84^{e}	42.23 ±7.47^{bc}	63.85 ±0.24^{cd}
Madekere (Xanthosoma sagittifolium)	66.42 ±9.05^{b}	32.08 ±4.55^{e}	63.37 ±0.31^{de}
Muhole (Cleome gynandra)	25.41 ±8.26^{de}	33.67 ±6.73^{de}	63.00 ±0.21^{e}
Mushaka (Cleome Schimperi)	136.12 ±0.59^{a}	56.79 ±7.59^{a}	80.25 ±1.26^{a}
Nderema (Basella alba)	38.79 ±2.40^{c}	27.73 ±4.17^{e}	63.21 ±0.35^{de}
Ngaingai (Hibiscus noldae)	38.26 ±0.95^{c}	38.73 ±4.09^{cd}	68.39 ±0.71^{b}

Values are means ± SD. Values in a column with the same superscript are not significantly different ($p > 0.05$).

4.2.4. Total phenolic compounds, flavonoids and tannins content of ILVs commonly consumed in Walungu

The total phenolic compounds, flavonoids and tannins content in Indigenous leafy vegetables consumed in Walungu are summarized in Table 6. Results showed that total phenolic compounds content varied from 121.2 to 483.2 mg GAE/100g while flavonoids concentration ranged from 372.4 to 1127.9 mg/100g. Significant variations were also recorded in tannins content of leafy vegetables consumed in Walungu. *Mushaka* had the highest tannins content (63.7 mg/100g) as well as the highest concentration of total phenolic compounds (483.2 mg GAE/100g). *Ngaingai* had the highest flavonoids content (1127.9 mg/100g).

Table 6: TPC, flavonoids and tannins content of ILVs commonly consumed in Walungu

Leafy Vegetables	Total Phenolic Compounds (mg/g)	Flavonoids (mg/100g)	Tannins (mg/100g)
Bishogolo (Phaseolus vulgaris)	275.88 ±1.57^{d}	781.36 ±2.01^{bc}	9.39 ±1.85^{bc}
Lujinji (Erucastrum arabicum)	140.42 ±0.65^{b}	440.55 ±21.58^{a}	10.93 ±1.19^{cd}
Lushenda (Capsicum frutescens)	216.89 ±2.60^{c}	871.45 ±36.54^{c}	14.76 ±1.51^{d}
Madekere (Xanthosoma sagittifolium)	122.45 ±1.72^{a}	538.50 ±44.53^{ab}	4.16 ±0.11^{a}
Muhole (Cleome gynandra)	121.18 ±2.00^{a}	372.40 ±7.41^{a}	5.55 ±1.32^{ab}
Mushaka (Cleome Schimperi)	483.24 ±6.01^{f}	852.70 ±22.27^{c}	62.73 ±0.09^{e}
Nderema (Basella alba)	130.32 ±6.31^{ab}	420.42 ±57.02^{a}	7.34 ±1.14^{abc}
Ngaingai (Hibiscus noldae)	323.81 ±7.84^{e}	1127.85 ±79.48^{d}	8.30 ±2.72^{abc}

Values are means ± SD. Values in a column with the same superscript are not significantly different ($p > 0.05$).

4.2.5. Anti-nutrients components in ILVs commonly consumed in Walungu

Phytates and oxalates concentration in Indigenous leafy vegetables consumed in Walungu are shown in Table 7. Results indicated that phytates concentration varied from 7.2 to 9.3 mg/100g while oxalates content ranged from 19.9 to 23.5 mg/100g. *Muhole* had the least phytates content (19.9 mg/100g) while Nderema had the least oxalates concentration (19.9 mg/100g).

Table 7: Anti-nutrients components in ILVs commonly consumed in Walungu

Leafy Vegetables	Phytates (mg/100g)	Oxalates (mg/100g)
Bishogolo (Phaseolus vulgaris)	8.14 ±0.44[b]	21.95 ±1.44[ab]
Lujinji (Erucastrum arabicum)	7.82 ±0.50[ab]	23.17 ±0.52[b]
Lushenda (Capsicum frutescens)	7.79 ±0.39[ab]	20.40 ±1.23[ab]
Madekere (Xanthosoma sagittifolium)	8.00 ±0.18[b]	21.00 ±2.02[ab]
Muhole (Cleome gynandra)	7.23 ±0.36[a]	22.91 ±0.88[ab]
Mushaka (Cleome Schimperi)	7.72 ±0.27[ab]	23.47 ±0.34[ab]
Nderema (Basella alba)	7.44 ±0.27[ab]	19.91 ±2.69[a]
Ngaingai (Hibiscus noldae)	9.25 ±0.27[c]	21.79 ±1.09[ab]

Values are means ± SD. Values in a column with the same superscript are not significantly different ($p > 0.05$).

CHAPTER FIVE: DISCUSSION

5.1. Awareness, attitudes and practices of household in Walungu regarding the ILVs

Awareness, behavioral attitudes and practices of household are key factors when addressing food and nutrition security issues in a given community (Weerasekara, Withanachchi, Ginigaddara, and Ploeger, 2020). Household's food and nutrition-related awareness, attitudes and practices affect food security enhancement as well as nutritional status of household members. In this study, majority of household were represented by married women with at least primary education. This confirms the empirical data in Africa and particularly in DRC which have shown that most households in rural areas are headed by women (McKenna, Bartels, Pablo, and Walker, 2019; ODI, Weijs, Hilhorst, and Ferf, 2012). According to (Wulan, 2000), women play the main role in achieving a healthy food and nutrition status both in the household and the community as a whole. Therefore, women should take the lead position as key are important stakeholders and leaders in impacting nutrition of their family.

The study also showed that households in Walungu consume various ILVs and are aware of their related nutritional, health and income benefits. This corroborates the findings of other researchers (Ineke, Rensburg, Zijl, and Sonja, 2007; Kansiime et al., 2018; Voster et al., 2007) who established that the level of awareness and consumption of Indigenous Leafy Vegetables are correlated to their perceived nutritional and health benefits and income. According to Tabuti et al., 2004, the knowledge and consumption of indigenous plants depend on the health and nutritional issues faced by the population, food shortages (need for food and/or income) and the willingness of consumers to have supplements to major food crops.

Indigenous leafy vegetables in Walungu are consumed at least twice a day and mainly because of their availability, taste and their perceived nutritional value. This pattern can be attributed to the impact of food availability on its consumption. According to Bellisle, 2006, besides hunger, food choice is determined by socio-economic factors among which food availability and accessibility is a primary determinant. On the other hand, (Clark, 1998) established that there are strong evidences demonstrating the influence of taste on consumer food choice, but emphasized that taste was not as far up the list as one could think.

The majority of indigenous leafy vegetables in Walungu are sourced from family gardens and local market. This is in agreement with findings of (Sanya et al., 2018) who stated that majority of ILVs are produced locally by smallholder farmers and sold in local markets. According to (Malangen and Quartermain, 2018), local communities prefer to grow their own leafy vegetables for their home consumption and to sell part of their production due to local supply and demand factors.

ILVs in Walungu were washed with cold water before being boiled in water for one hour as the principal cooking method. This is similar to results by (Rensburg et al., 2004) who established that processing leafy vegetables in Africa involves mainly washing and cooking in water, with or without salt, for a few minutes to up to 2h, depending on species and culture. According to (Kansiime et al., 2018), better cooking methods of ILVs include steaming or boiling, as opposed to frying, which is the most common method for other food stuff.

The study further indicates that drying process was the principal method of preserving ILVs in Walungu. This could be attributed to the feasibility of the drying method, such as sun drying (directly in the sun) and solar drying (using solar dryers) which seems to be cheap and easy to conduct in remote rural areas. According to (Ineke et al., 2007; Rensburg et al., 2004), drying of leafy vegetables helps to address the food shortages during off-season period when fresh food must be bought.

5.2. Proximate composition of ILVs commonly known and consumed in Walungu

The moisture content of green vegetables affects both the nutritional content and the quality of the ready-to-eat product as well as the postharvest handling and storage (Garcia and Barrett, 2002). Vegetables with higher moisture content are less concentrated in nutrients and require special postharvest handling and storage since they rot fast (Munhuweyi, 2012). There were variations in moisture content among the 8 ILVs commonly consumed in Walungu. These variations can be attributed to differences in stages of plant maturity, variety of plant, species, and geographical location. Majority of the ILVs in this study recorded a moisture content (78-94 %) similar to that reported in other indigenous leafy vegetables (Uusiku et al., 2010).

Vegetables are an important component of daily diets as they are healthy source of carbohydrates. Diets without vegetables are often rich in carbohydrates promoting obesity associated health issues and other malnutrition disorders (Ineke et al., 2007). Leafy vegetables are generally low in carbohydrates. The carbohydrates content of the ILVs commonly consumed in Walungu ranged from 4.1 – 8.9 g/100g. Carbohydrates content in tropical leaves ranges from 4 – 16g/100g (FAO, 1990). According to Munhuweyi (2012), carbohydrates content of leafy vegetables is affected by variety, location, climatic conditions and fertilization. A significant difference was noticed in the carbohydrates content of leafy vegetables under this study. Since all the species were collected during the same season, the differences observed in carbohydrates content among the species can be attributed to the genetic make-up of each specie, the location and the soil fertility.

The protein content of the raw ILVs commonly known and consumed in Walungu ranged from 2.8 – 28.5%. A significant difference was noticed in the protein content of *Mushaka* (*Cleome schimperi*) (28%) when compared with the other ILVs (2.8-8.3%) found in Walungu. The large amount of protein in *Mushaka* could be attributed to genetic make-up of the *Cleome* genus along with their ability to absorb and stock nitrogenous compounds during the growing season (Abdullah, Elsayed, Abdelshafeek, Nazif, and Singab, 2016).

Leafy vegetables are good source of dietary fiber. The concentration of dietary fibre of ILVs in Walungu ranges from 1.2 to 38.6 g/100g. Similar results were obtained by (Uusiku et al., 2010). The total dietary fiber content of ILVs can vary due to differences in stages of plant maturity, seasonal variation, the use of fertilizers or chemicals, variety of plant, geographical location and the method used for analysis (Aletor et al., 2002; Punna and Paruchuri, 2004).

5.3. Nutritional characteristics and health benefits of ILVs commonly known and consumed in Walungu

Carotenoids comprise a group of chemical compounds responsible of fruits and leaves coloration in plant. Among this group, β-Carotene, also known as provitamin A, is a very important nutrient since vitamin A deficiency remains a major public health concern affecting billions of people around the world. The carotenoids of ILV species commonly eaten in Walungu ranged from 13.9 – 136.1 µg/100g. Variation in carotenoids content in ILVs consumed in Walungu could be attributed to species. Carotenoids were reported to be highly species dependent (Uusiku et al., 2010). This argument was already supported by (Rensburg et al., 2004) who even suggested genetic diversity approaches to enhance consumption of carotenoids rich food.

Leafy vegetables are the potential sources of vitamin C. The findings from this study have revealed that the leafy vegetables consumed in Walungu contains high vitamin C content ranging from 27.7 to 56.8 mg/100 g with *Mushaka* (*Cleome schimperi*) registering the highest value and *Ngaingai* (*Basella alba*) the lowest value, respectively. Vitamin C values for ILVs obtained in this study are in agreement with the findings of (Ineke et al., 2007; Lesten and

Kingsley, 2020; Uusiku et al., 2010b). However, these values were lower compared to the values of 64 mg and 94 mg/100 g, reported in other leafy vegetables such as Amaranths and Spinach leaves, respectively (Natesh, Abbey, and Asiedu, 2017).

Zinc is the least toxic metal among the three major trace metals found in plant. Zinc is an essential element in the human diet as it is required to maintain the proper functions of the immune system (Tautau, Martin, and Adebayo, 2014). Zinc content of Leafy Vegetables in Walungu ranged from 14.3 to 162.9 mg/Kg. According to (Nair and Balachandran, 1997), the acceptable limit for human consumption of Zn is 150 mg/Kg. However, the values obtained for Zn in this study are far above the maximum permissible limit of 3 mg/Kg for fruits and vegetables reported by World Health Organization (Wei Wong et al., 2019). Variation in Zinc content among ILVs under this present study can be attributed to species and location.

Iron is an essential element in production of red blood cells**.** From the results obtained, the iron concentration of ILVs consumed in Walungu varied from 71.1 to 176.8mg/Kg. This is in accordance with a previous study (Castro-Alba, Lazarte, Bergenståhl, and Granfeldt, 2019). According to Castro-Alba et al. (2019), leafy vegetables have a high mineral content but with a wide variation in iron. The variation in iron content in leafy vegetables depends on differences in species, plant maturity, and fertility of the soil.

Iodine is among the essential minerals supplied to human being by food and water. Its deficiency is the major cause of goiter and other iodine deficiency disorders (IDD) such as cretinism, endemic cognitive disorders and motor or intellectual sub-normality. This study revealed that iodine is present only in trace amounts in ILVs consumed in Walungu. This is in agreement with findings of (Gwarzo, 2012) who suggested that leafy vegetables should be taken as complement to iodine supplemented salt in order to prevent iodine deficiency disorders (IDD) in humans.

Phytochemicals can be generally categorized as nutritional (e.g., vitamins, minerals and phenolic compounds) and anti-nutritional (e.g., oxalates, tannins, phytates) chemical compounds. Leafy vegetables are good sources of natural antioxidants, such as vitamins, carotenoids, and phenolic compounds which include flavonoids, phenolic acids and tannins (Natesh N et al., 2017). Variation in phytochemicals content in the ILVs consumed in Walungu could be also attributed to the species. For instance, phenolic compounds are secondary plant metabolites that function beneficially to defend the plant against invading pathogens (Kammerer, Kramer, and Carle, 2012). The response of the plant depends on the combined effect of interactions between environment and plant species. On the other hand, plant can produce undesirable chemical substances referred to as anti nutrients (oxalates and phytates). These substances are abundant in both cultivated and wild plant species. Phytate content can affect significantly mineral bioavailability, which may in turn lead to mineral deficiencies (Castro-Alba et al., 2019). This study revealed variation in anti-nutrients (phytates and oxalates) content of ILVs consumed in Walungu. According to (Cooper-Driver, 1981; Natesh N et al., 2017), the content and the distribution of these anti-nutrients compounds vary with plant genera and species.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusion

The results of this study revealed that a wide range (20) of Indigenous Leafy Vegetables (ILVs) are commonly known in Walungu/DR Congo and that they are consumed because they available, tasty and believed to be nutritious.

Washing vegetables with clean water before boiling them for one hour was found to be the most common preparation and cooking method used while sun drying was the predominant preservation technology applied on vegetables in Walungu.

Mushaka (Cleome schimperi), Lushenda (Capsicum frutescens) and *Bishogolo (Phaseolus vulgaris)* leaves were the most nutritious due to their protein content (5-28%), dietary fiber, Iron content, carotenoids and vitamin C concentration.

6.2. Recommendations

Since a wide range of ILVs are known and consumed in Walungu, universities and researchers should focus on understanding their ontological cycle in order to improve the production and productivity of these vegetables.

There is need to popularize and promote these Indigenous Leafy Vegetables among restaurants and hotels as they can contribute at promoting good health using local and available resources.

Further studies should be performed to establish the effect of cooking methods on the quality of the final products (meals), their acceptability and shelf-life.

Further investigations should be undertaken to establish the impact of a daily consumption of these Leafy Vegetables on the health status of individuals from different age groups and gender.

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Appendices

Survey questionnaire

I. HOUSEHOLD/FARMER DEMOGRAPHICS

1. Gender of respondent

a) Male
b) Female

2. What is your role in this household?

a) Female head household
b) Wife of male head household
c) Male head household
d) Son/Daughter
e) Relative
f) Other and specify…………………………………………………………
…………………………………………………………………………………….

3. How old are you?

a) 20 years and below
b) 21-30 years
c) 31-40 years
d) 41-50 years
e) 51-60 years
f) Above 60 years

4. What is your current marital status?

a) Single
b) Single but having a cohabiting partner
c) Married
d) Divorced
e) Widowed

5. What is the highest level of education you have achieved?

a) No education
b) Primary school
c) Secondary school
d) Tertiary
e) Other and specify…………………………………………………………
……………………………………………………………………………………..
…………………………………………………………………………………….

6. What type of work do you do?

a) Farmer
b) Trader
c) Civil servant
d) Privately employed artisan
e) Food service
f) Other and specify…………………………………………………………
…………………………………………………………………………………….

…………………………………………………………………………………….

7. Number of people in the household

a) <5

b) 5-10

c) >10

II. INDIGENOUS VEGETABLES (Fruits and Leaves)

8. Which indigenous/traditional/wild vegetables do you know?

a) Lenga lenga (amaranths)

b) Sombe (Cassava leaves)

c) Bishusha (Pumpkin leaves)

d) Bishogolo (Bean leaves)

e) Mulunda

f) Madekerhe

g) Matembele (Sweet potato leaves)

h) Ndelama

i) Other and specify……………………………………………………………………….

……………………………………………………………………………………..

…………………………………………………………………………………….

…………………………………………………………………………………….

9. Which ones of those indigenous vegetables do you consume? (Those you grow/do not grow)

a) Lenga lenga (amaranths)

b) Sombe (Cassava leaves)

c) Bishusha (Pumpkin leaves)

d) Bishogolo (Bean leaves)

e) Mulunda

f) Madekerhe

g) Ndelama

h) Other and specify………………………………………………………………………

……………………………………………………………………………………..

……………………………………………………………………………………

……………………………………………………………………………………

10. Where do you usually get or buy the vegetables consumed?

a) Ground

b) Backyard gardens

c) Market

d) Other and specify……………………………………………………………………..

……………………………………………………………………………………

…………………………………………………………………………………….

11. How often do you eat/consume vegetables?

a) One time per day

b) Two times per day
c) Three times per day
d) More than three times per day

12. How do you use and/or consume those vegetables?

a) Boiled
b) Dried
c) Fried
d) Steamed
e) Other and specify…………………………………………………………………….
………………………………………………………………………………………
………………………………………………………………………………………
……………………………………………………………………………………..

13. How do you prepare those vegetables?

a) Steaming
b) Boiling
c) Drying
d) Other and specify…………………………………………………………………….
………………………………………………………………………………………
……………………………………………………………………………………..
………………………………………………………………………………………

14. Do you dry those vegetables? Yes or no?

a) If yes, how do you dry those vegetables?

i. Sun drying
ii. Air drying
iii. Other specify…………………………………………………………………

b) No

15. How many times do you take to cook those vegetables?

a) 25 minutes
b) 30 minutes
c) 45 minutes
d) 1 hours
e) More than one hour

16. Do you wash those vegetables before cooking? Yes or no?

a) If yes, how do you wash those vegetables before cooking?

i. Clean cool water
ii. Clean warm water
iii. Other and specify…………………………………………………………………
………………………………………………………………………………

b) No.

III. VEGETABLES GROWN

Vegetables grown/Local	For income (a)	For food (b)	Medicinal (c)	Other (d)

name				

IV. REASONS FOR CONSUMING VEGETABLES

Vegetable consumed/Local name	Cheap (a)	Expensive (b)	Medicinal (c)	Nutritious (d)	Tasty (e)	Available (f)	Other (g)