NUTRITIONAL, PHYTOCHEMICAL AND *IN VITRO* ANTIMICROBIAL SCREENING OF SOME INDIGENOUS LEAFY VEGETABLES

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Nutritional, Phytochemical and *In Vitro* Antimicrobial screening of some Indigenous Leafy Vegetables

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

To my parents, Mr. Henry Sitienei and Mrs. Esther Sitienei, my brothers and sisters, my beautiful wife Mrs. Susan Mibei and my lovely daughter Ms. Faith Chemutai. They not only shared every step of my study and research progress, but also are always the source of love, strength, support and encouragement. In addition, they have been an inspiration to me in carrying out this study. I love you all.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ACRONYMS AND ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometer
AIDS	Acquired Immune Deficiency Syndrome
ALVs	African Leafy Vegetables
AOAC	Association of Official Analytical Chemists
b.p	Boiling point
COMESA	Common Market for Eastern and Southern Africa
Conc	Concentration
CV	Coefficient of variation
DCIP	Dichlorophenol indophenol
DPPH	Diphenyl picryl hydrazyl
DWB	Dry weight basis
FAO	Food and Agriculture Organization
FST	Food Science and Technology
HCN	Hydrogen cyanide
HIV	Human Immunodeficiency Virus
ILVs	Indigenous Leafy Vegetables
IPGRI	International Plant Genetic Resources Institute
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KARI	Kenya Agricultural Research Institute
KEMRI	Kenya Medical Research Institute

LSD	Least significant difference
MIC	Minimum Inhibitory Concentration
ml	Milliliters
MoH	Ministry of Health
MUFA	Monounsaturated fatty acid
No.	Number
PROTA	Plant Resources of Tropical Africa
PUFA	Polyunsaturated fatty acid
R_f	Retention factor
SEM	Standard Error of the Mean
Sf	Solvent front
SFA	Saturated fatty acid
TLC	Thin Layer Chromatography
w/v	Weight to volume
WHO	World Health Organization

ABSTRACT

Indigenous leafy vegetables (ILVs) are traditional vegetables whose leaves, young shoots and flowers are consumed. Four commonly-used ILVs were investigated in this study, viz.: *Corchorus olitorius* L. (Jute mallow), *Crotalaria ochroleuca* G. (Slender leaf), *Solanum scabrum* Mill. (Black nightshade) and *Cleome gynandra* L. (Spider plant). These ILVs were planted at Jomo Kenyatta University of Agriculture and Technology (JKUAT) Experimental Farm and harvested between 1 to 2 months after planting. Fresh, cooked and dried (shade and solar drying) leaf samples were analyzed for nutritional and phytochemical composition. The samples were also investigated for relative antioxidant and antimicrobial activities.

Proximate composition results indicated that dry matter, protein, fiber, fat and vitamin C (16.8±0.17%, 3.7±0.62%, 2.1±0.50%, 1.4±0.27% and 153.68±7.75mg/100g DWB, respectively) was significantly higher in *C. olitorius* as compared to the other three ILVs ($P<0.05$). *Crotalaria ochroleuca* on the other hand exhibited higher contents of dry matter and ash (18.2±0.20% and 11.2±0.49%, respectively). Vitamin C and β-carotene contents of fresh leaf samples were significantly higher than those of dried and cooked samples ($P< 0.05$). *Cleome gynandra* exhibited significantly high β-carotene (8.73±0.16 mg/100g), while *S. scabrum* had the lowest amounts of both β-carotene and vitamin C (4.55±0.25 mg/100g and 62.61±4.57 mg/100g DWB, respectively). Besides, all cooked samples showed significantly lower β-carotene contents ($P < 0.05$) compared to fresh samples. The fatty acid profile indicated that the ILVs generally had a higher amount of

unsaturated fatty acids than saturated fats. *C. olitorius* exhibited predominant amounts of fatty acids, whereas *C. gynandra* reported significantly lower amount compared to the other ILVs.

The content of phenolic compounds and tannins in the leaf extracts was determined spectrophotometrically using Folin-Ciocalteu and Folin-Denis reagents and calculated as gallic acid and tannic acid equivalents, respectively. The total phenol contents varied from 0.40±0.03 to 4.45±0.10 g/100g and tannins from 0.70±0.03 to 7.25±0.05 g/100g DWB; *S. scabrum* gave relatively high tannins and phenolic compounds. Flavonoid and alkaloid contents were between 1.39±0.08 to 6.32±0.20 g/100g and 3.23±0.18 to 10.80±0.08 g/100g, respectively. The ability of the extracts to scavenge diphenyl picryl hydrazyl (DPPH) radicals was determined spectrophotometrically at 517nm. The four ILV extracts had significant radical scavenging effects and almost all reported a significantly higher percentage of DPPH inhibition than ascorbic acid ($P < 0.05$). The extracts of *C. olitorius* and *C. gynandra* were most effective since they had higher percentages of radical scavenging activity and lower IC_{50} values (concentration which scavenged 50% of the DPPH radicals).

The ILV extracts also displayed significant anti-microbial potency against micro-organisms such as *Escherichia coli*, *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa* and *Candida albicans*. The best activities against the micro-

organisms were observed in *C. gynandra* extracts with minimum inhibitory concentration (MIC) below 200 mg/ml.

The nutritional, phytochemical and antioxidant potential of the ILVs is of health or nutraceutical significance and should help encourage consumption of the ILVs. Though the processing and preparation methods such as solar drying and cooking reduced their final consumed amount, they should be dried in dry and dark place under low temperature and cooking should be done within a short time for maximum retention of nutrients. There is still much that begs for sustained research on ILVs, and this study forms the basis for future research, especially in regard to bio-prospecting and valorization (value addition) of the ILV biodiversity.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Plants have been major sources of medicines throughout human history (Hobbs, 1994) being used for traditional relief from numerous human diseases (Enzo, 2006). Their medicinal value lies in some chemical substances that produce definite physiological actions on the human body (Bilge and Ilkay, 2005). In developing countries, pharmaceutical drugs are not often affordable and approximately 60–80% of the world's population relies on traditional medicines as remedies for common illnesses (Owolabi *et al.,* 2007). A blend between traditional knowledge in medicinal plants, modern pharmacology and natural product chemistry has led to the discovery of very novel and efficacious forms of medication (Bhushan and Ashok, 2010).

According to the World Health Organization (WHO), medicinal plants are the best sources of a variety of drugs to combat various diseases. They advocate that countries should encourage traditional medicine with a view to identifying and exploiting safe and effective remedies for ailments of both microbial and non-microbial origins (WHO, 1978). They also recommend a daily intake of more than 400 g of vegetables and fruits per person to protect against diet related chronic diseases (WHO, 2003). Therefore ILV consumption could play an important role in the WHO global initiative on increased consumption of vegetables (Smith and Eyzaguirre, 2007).

Modernism often engenders contempt for traditional values, some of which may not necessarily be retrogressive. Much of contemporary environmental and socio-economic problems facing humanity are attributable to arbitrary adoption of modern ways at the expense of time-honored traditional practices, especially regarding farming systems and food habits.

In Kenya, both rural and urban families are increasingly consuming kales and other exotic vegetables at the expense of ILVs. This is in spite of the fact that some ILVs are nutritionally superior (FAO, 1988) and drought tolerant as compared to exotic vegetables. The ILVs are also naturally adapted to the agro-climatic conditions. Some of them even grow in the wild providing vegetative cover and organic replenishment of the soil. Apart from their use as food, some ILVs may also exhibit medicinal potency (Maundu *et al.*, 1999). In East Africa, literature that documents the use of ILVs as medicine and their analysis for active ingredients is limited. Modern trends in food technological research focuses on the food-medical interface (Sasson, 2005). The concern is to identify food sources and ingredients for the provision of both nutritional and pharmaceutical (jointly termed nutraceutical) benefits and ILVs may have an invaluable potential in this emerging area. In developing countries, the diets of the poor are predominantly cereal based, nutrient poor, with little vegetables and fruits (Michael, 1997).

1.2 Statement of problem

For centuries, ILVs have comprised an important segment of food security crops among Kenyan communities. However, seasonality of production, limited value-added products, loss of indigenous knowledge, a dearth of information on potential nutraceutical benefits, and changing food habits have progressively denigrated the dietary and economic importance of ILVs. This contributes to food insecurity and impoverishment of rural communities.

There is potential for developing new value-added products from ILV biodiversity, a process called valorization. This is afforded by the unique biochemical composition of ILVs. For example, identification and characterization of ILV bioactives may lead to discovery of novel anti-oxidants and anti-microbials of nutraceutical and pharmaceutical value. The need for such novel substances is necessitated by the emergence of microbial strains that are resistant to many commonly used pharmaceutical agents or drugs (Rekha *et al.*, 2009). This raises concerns that drug resistance is becoming so common and treatment failures for many diseases may prevail.

In view of the historical importance and value-added prospects of indigenous vegetables, it is necessary to screen some commonly consumed ILVs for their nutrient content, bioactive components, nutraceutical potential, and anti-microbial potency.

1.3 Justification

Man, more than ever before, needs a re-orientation on the sustainable use of his natural resources, particularly in this era of environmental degradation, loss of biodiversity and climate change. This, for example, is to source raw materials for medicine and harness the abundant rich flora for an improved primary health care delivery.

Due to the fact that traditional healers have long used plants to prevent or cure infections, many of these plants have currently been investigated scientifically for medicinal activity. Regrettably, the commonly consumed ILVs are often not addressed. These ILVs are popularly consumed in almost all the states of the Common Market for Eastern and Southern Africa (COMESA) with other parts of the world having similar gastronomic habits. For millennia, they have provided a delicate balance of food security and sustainable soil productivity. In East Africa, literature that documents their use as medicine and their analysis for active ingredients is limited. Therefore, research studies pertaining to their use as nutraceuticals should be emphasized. This will help exploit their potential for food security, nutrition and wealth creation for sustainable development to alleviate human suffering.

1.4 Hypothesis

Commonly used indigenous vegetables do not contain bioactive compounds of nutritional and medicinal importance.

1.5 Objectives of the study

1.5.1 Overall objective

To study the nutritional composition of selected indigenous leafy vegetables and screen for phytochemicals, antioxidant and *in vitro* antimicrobial activities.

1.5.2 Specific objectives

i. To evaluate the proximate composition of selected ILV leaves, viz.: *C. olitorius, C. ochroleuca, S. scabrum* and *C. gynandra;*

ii. To analyze phytochemical compounds found in fresh, solar dried and shade dried leaves of these vegetables;

iii. To evaluate the antioxidant and *in vitro* antimicrobial activities of the fresh, solar and shade dried vegetables.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Indigenous leafy vegetables

Indigenous Leafy Vegetables (ILVs) are traditional edible vegetables whose leaves, young shoots and flowers are consumed (Maundu *et al.,* 1999; Abukutsa-Onyango, 2003). Similarly, these vegetables have their secondary centre of origin as Africa and their natural habitat is in sub-Saharan Africa thus also referred to as "African Leafy Vegetables (ALVs)" (Schippers, 2002). The ILVs were introduced over a century ago and due to long use, have become part of the food culture in the sub-continent. They are increasingly recognized as possible contributors of both micronutrients and bioactive compounds to the diets of populations in Africa (Francisca and Eyzaguirre, 2007). Therefore, they form a significant part of the traditional diets of households and agricultural communities both in urban and the rural areas (Grubben and Denton, 2004). There has been a resurgence of interest in the ILVs during the past decade with several studies reporting on their regional availability and use (Odhav *et al.,* 2005). The species used and the wealth of indigenous knowledge vary with the culture, economic pursuits, species availability and level of influence by modernization.

2.2 ILVs in Africa

African environment is probably the least explored in terms of available untapped resources. It has many wild and weedy species of edible leafy vegetables which contribute significantly to the nutritional well-being of the rural population (Chweya and

Eyzanguire, 1999). Besides, these vegetables serve as a source of traditional medicine, therefore, herbal remedies is readily available in our floral biodiversity. While a few of these species have been domesticated or are semi-domesticated, most grow as weeds or are wild in virgin, undisturbed and/or cultivated areas in the rural areas (Kemei *et al.*, 1995). The vegetables are also important for their ecological, agronomic and cultural values (Geissler *et al.*, 2002) and are also adapted to many tropical conditions, pests and diseases. Therefore, they provide a gene pool for improvement of cultivated species to confer pests and diseases resistance. Despite these values, they have been neglected for many years by researchers, policy makers and funding agencies and are currently at risk of extinction in many countries (Adebooye and Opabod, 2004; Aphane *et al.*, 2002). If the situation is left unchecked it might result to loss of the rich ILV biodiversity and underutilization of these readily available resources.

2.3 Some Common ILVs

Worldwide there are about 13,000 species of plants used as food. The Plant Resources of Tropical Africa (PROTA) reported an estimated 6,376 useful indigenous African plants of which 397 are vegetables. It is however indicated that information is available on cultivation practices for 280 indigenous ILVs (PROTA, 2004). Examples of some ILVs found across Eastern Africa include African nightshade (*Solanum spp*), spider plant (*Cleome gynandra*), amaranth (*Amaranthus spp*), slenderleaf (*Crotalaria spp*), jute mallow (*Corchorus olitorius*), cowpea (*Vigna unguiculata* L.), pumpkin (*Curcurbita muschata*), African kale (*Brassica carinata* A.) *Basella alba* and *Commelina africana*

among many others (Abukutsa-Onyango *et al.,* 2006). This list is not exhaustive because Adebooye *et al.* (2003) reported an expanded list of ILVs that are consumed in Nigeria, Chweya (1997) in Kenya, Rubaihayo (1997) in Uganda, Seck *et al*. (1997) in Senegal and Okigbo (1977) for the entire tropical Africa.

2.4 ILVs in current use in Kenya

2.4.1 Corchorus species

Corchorus olitorius L. (Jute mallow - *mrenda*), is an erect woody herb growing up to 2.5 meters high. It belongs to the family *Tiliaceae* and is originally from Asia although Africa is currently the primary centre of its diversity. It is a popular vegetable in West Africa and usually recommended for pregnant women and nursing mothers because they are believed to be rich in iron (Oyedele *et al.,* 2006). *Corchorus olitorius* is the most frequently cultivated (Schippers, 2002) and is widely consumed by various communities in Kenya.

The leaves are believed to be rich in vitamins and micronutrients. The plant exhibits antioxidant activity with a significant α-tocopherol equivalent Vitamin E. The stems are harvested when the plant is in flower and are then retted (allowed to begin to rot) so that the fiber can be extracted. While perhaps better known as a fiber crop, jute is also a medicinal vegetable. Its bark and root have medicinal properties. In folk medicine, it is reported to be demulcent, deobstruent, diuretic, lactagogue, purgative, and tonic. It is a folk remedy for aches and pains, dysentery, enteritis, fever, dysentery, pectoral pains,

and tumors (Duke and Wain, 1981). Ayurvedics use the leaves for ascites, pain, piles, and tumors. Elsewhere the leaves are used for cystitis, dysuria, fever, and gonorrhea. The cold infusion is said to restore appetite and strength. However, they also contain hydrogen cyanide (HCN) and several cardiac glycosides that pose the risk of toxicity to humans

2.4.2 Crotalaria species

Crotalaria (slenderleaf - *mtoo*) is one ILV that has been grown and consumed in Kenya for a long time. It is a nitrogen-fixing legume and belongs to the family Fabaceae/Leguminaceae (Schippers, 2002; Abukutsa-Onyango, 2004). The genus *crotalaria* includes about 500 species of herbs and shrubs of which 400 species can be found in Africa (Schippers, 2002).

The two African species used as a vegetable are *Crotalaria ochroleuca* G. and *Crotalaria brevidens* Benth. *Crotalaria brevidens* has a wider agro-ecological range within Kenya, extending from Eastern Province to Lake Victoria and is distinguished by its wider leaves and longer, thinner pods. *Crotalaria ochroleuca* is mainly found in Nyanza and Western Provinces. It's a short-lived, erect perennial herb growing up to 1.5 meters in height. It has bright green leaves, pale yellow or creamish flowers and a mild taste (Schippers, 2002). It grows at elevations up to 2,000 m above the sea level and does best in warm areas. Its young leaves and shoots are consumed in combination with

other greens because it has bitter taste. *Crotalaria ochroleuca* also have medicinal properties that help reduce the effects of stomach ailments (Schippers, 2002).

2.4.3 Solanum species

Solanum (Black nightshade – managu) belongs to the family *Solanaceae* which also include a number of other important food crops namely; tomato, eggplant and potato. *Solanum nigrum* L. is native to North Africa, Europe and West Asia, and is renowned for its poisonous berries and leaves.

Solanum scabrum Mill. is broad leaved and cultivated widely in many regions in Africa and is non poisonous. It is recognized by its large dark purple fruit. According to Maundu *et al.* (1999), nightshade provides good levels of vitamins, minerals and trace elements at seven times the amounts derived from cabbage. The high levels of vitamins and micronutrients are especially important to people at risk of malnutrition and disease, particularly HIV/AIDS (Olembo *et al.,* 1995). The leaves are also used to treat a wide range of ailments in various parts of the world.

2.4.4 Cleome gynandra

Cleome gynandra L. */Gynandropsis gynandra* L. (Spider plant / cat's whiskers - *Isaka*) is an erect herbaceous annual herb with hairy, often purple stems and many branches. It belongs to the family *Capparaceae*. It grows as a weed in most tropical countries and can grow up to 1.5 m in height. In Kenya, it grows from sea level to 2400 metres. It is a fast-growing plant that is ready for harvest in as few as three weeks. It is believed to be a rich

source of nutrients, especially proteins, vitamins (A and C) and minerals (calcium and iron). Spider plant has mildly bitter taste which is derived from polyphenolics, and is assumed to constitute from 0.5% to 0.9% of the edible leaf (Chweya, 1997).

In many communities, they are used by women, pregnant and breastfeeding mothers (Schippers, 2002). It is believed to replenish or restore the blood supply. The boiled leaves are regarded as medicinal with anti-inflammatory properties and the leaf sap is used as an analgesic, particularly for headaches and earaches. A decoction or infusion of boiled leaves and/or roots is administered to facilitate childbirth, treat stomach ailments, constipation or thread-worm infection. The seeds and roots also have anthelmintic properties (Schippers, 2002).

2.5 Production of ILVs in Kenya

There is low adoption and production of ILVs in Kenya and this production is mainly on a subsistence basis (Nekesa and Meso, 1995). This could partly be attributed to their neglect as vegetable research efforts have mainly concentrated on exotics (Ndung'u *et al.,* 2004**).** However, several abiotic and biotic stresses do limit crop production in Kenya. These range from: drought to salinity; pests to diseases; poor quality seeds; lack of production and utilization packages; poor marketing and processing strategies and lack of knowledge of the correct choice of foods (Abukutsa-Onyango, 2003). In addition, changing food habits, loss of indigenous knowledge and ignorance of the nutritional and health benefits associated with ILV consumption have collectively

contributed to this apparent denigration. In spite of these, the report of a survey conducted by Abukutsa-Onyango (2003) showed that ILVs play a key role in income generation and offer a significant opportunity for the poor people especially women in western Kenya to earn a living. She reported that over 70% of the traded vegetables in rural markets were indigenous vegetables while in bigger towns was about 10% (Abukutsa-Onyango, 2003). Therefore, exploitation of ILVs adapted to the local environment will not only overcome these stresses, but also improve food security, nutrition, health and economy of the rural poor.

2.6 Patterns of consumption of ILVs

Indigenous leafy vegetables are relatively available and affordable particularly during the rainy seasons and represent one of the richest sources of biodiversity in African food systems (Chweya and Eyzanguire, 1999). They are a potentially rich source of nutrients but are found to be among the least produced or consumed foods (Maziya-Dixon *et al.*, 2004). Furthermore, many of these ILVs are mainly consumed for their nutritional values without much consideration for their medicinal importance. The decline in their production and consumption in many rural communities in Africa is believed to be due to the introduction of exotic vegetable varieties (Maundu *et al.,* 1999; FAO, 1988). In most cases, ILVs are considered out of fashion, associated with low-class people and also food and source of income for the poor and unemployed households in the urban and peri-urban slums (Mnzava, 1997). These misconceptions still linger in some places in Africa and will take time to change (Oiye *et al.,* 2009). Besides, they play an

important nutritional role and food security for these disadvantaged people (Onim and Mwaniki, 2008). Using this as the basis, there are a number of programs in Kenya that have attempted to increase the consumption of ILVs. Increasing ILV consumption and possibly substituting them for exotic vegetables in Kenya could lead to improved dietary supply of mineral, β-carotene and other vitamins. This may mainly curb the rise of non-communicable diseases related to the urban dietary practices (Oiye *et al.,* 2009).

2.7 Post harvest handling of ILVs

Post-harvest handling is the final stage in the process of producing high quality fresh produce. Ability to maintain a level of freshness from the field presents many challenges. Thus a grower who can meet these challenges is able to expand his or her marketing opportunities and better the ability to compete in the marketplace (Janet and Richard, 2000). Most leafy vegetables are highly perishable and have a very short shelf life lasting at best for only 3 days. They deteriorate very quickly in quality and flavor after harvesting and the extent of post harvest losses can be serious if the crop is handled poorly. This creates problems in the marketing chain with producers, traders or consumers (Schippers, 2002). Since quality cannot be improved after harvest but only maintained, it is important to harvest vegetables at the proper stage, size and at peak quality to avoid these post harvest constraints. Handling practices and techniques also need to be developed to minimize these problems.

2.8 Processing and Nutrient retention in ILVs

Processing is mainly the transformation of food from perishable produce into stable foods with long shelf lives. This is to maintain a supply of wholesome, nutritious food during the year and preservation for the time of scarcity (Habwe and Walingo, 2008). Nutrients are the building blocks of the human body and may be affected by food processing and cooking (Kirtan and Vaishali, 2005). Nutrient losses occur during harvesting and distribution, household and industrial handling, storage as well as catering (Somogyi, 1990).

While food processing still has the main objective of providing a safe nutritious diet in order to maintain health, other aspects, particularly the generation of wealth for the producer and seller, have become increasingly important. Most people in the rural areas still rely on traditional foods for their basic diet, while those in urban and cosmopolitan centers tend to purchase processed and packaged foods for convenience (Habwe and Walingo, 2008). However, vegetables are highly perishable food items thus require special processing treatments to prevent post harvest losses (Mepba *et al.,* 2007). Though drying has been used as a method of processing, it may increase the shelf life as well as alter the nutrient quality (Somado *et al.*, 2006). Owing to the lack of and/or inadequacy of processing methods, large quantities of perishable food spoil. Therefore, processing and preservation is one of the central problems facing developing countries. In Africa and Kenya in particular, this problem exists with many fruit and vegetable varieties (especially the indigenous ones) resulting in wastage during the in-season and

limited supply during the off-season. This is accompanied by high prices (Habwe and Walingo, 2008) because most locally available vegetables are seasonal and not available year-long (Abukutsa-Onyango *et al.,* 2006). Therefore, there is need for data on special processing treatments of locally consumed cooked preparations to prevent post harvest losses and promote nutrient retention (Mepba *et al.,* 2007).

2.9 Value and potential of ILVs

2.9.1 Micronutrient and health promotion

There is empirical evidence that ILVs have high nutritive value as well as several other advantages compared to the introduced exotic varieties. Quite a large number have long been known and reported to have health protecting properties and uses (Ayodele, 2005 and Okeno *et al.,* 2003) especially for households in poor economic settings. They are efficient sources of several important micronutrients, both with respect to unit cost of production and per unit of land area. This includes high micronutrient content, antioxidants and medicinal properties (IPGRI, 2006). They are also rich in vitamins, minerals, trace elements, dietary fiber and protein (Mulokozi *et al.,* 2004; Mathenge, 1997). Their consumption therefore, gives diversity to daily food intake, adding flavour and appetite to the diet (Asfaw, 1997). This is important for growing children and lactating mothers because they have a great role in boosting the immune system (Abukutsa-Onyango, 2003). Effectively, they are important in food security, during times of drought or poor harvest and are also vital for income generation. However, vegetable consumption is far below the level required to meet the micronutrient

requirements. Therefore, integrating micronutrient-rich foods such as vegetables into diets is the most practical and sustainable way to alleviate micronutrient deficiency (Ali and Tsou, 1997).

2.9.2 Medicinal and health benefits

From the literature, it is clear that in addition to serving as vegetables, some ILVs have potential nutraceutical value thus exhibit medicinal potency (Olembo *et al.,* 1995). Several of these have been used and continue to be used for prophylactic and therapeutic purposes by rural communities (Ayodele, 2005). According to a survey conducted by Kimiywe *et al.* (2007) in Nairobi, Kenya, the ILVs consumed by the respondents have a medicinal value attached to it. For example, many ILVs especially the sour or bitter ones like spiderplant, slenderleaf and African nightshades have been reported to heal stomach related ailments and malaria. Their bitter taste could be attributed to the presence of alkaloids and phenolic compounds (Abukutsa-Onyango, 2003). *Basella alba* L. on the other hand has been reported to relieve constipation in humans (Maundu *et al.*, 1999). The leaves of *Bidens pilosa* as well are used by local communities to treat wounds and boils; the juice to treat various eye and ear problems; a decoction for rheumatism, stomach disorders and intestinal helminthes and the roots are used for malaria. *Corchorus spp* is reported to be demulcent, diuretic; it is also a folk remedy for aches and pains, dysentery, pectoral pains, and has anti-tumor activity (Gupta *et al.*, 2003).

Some of the vegetables are also reported to cure more than one illness. A wide range of illnesses has been cited as being treated and/or managed by consumption of leafy vegetables (Olembo *et al.,* 1995). The most common illnesses cited are malaria, diarrhea, anemia, colds and coughs, skin infections, malnutrition, HIV/AIDS, diabetes and high blood pressure. However, there is need for further investigations to establish the basis for these perceptions (Kimiywe *et al.,* 2007). This is a significant aspect since modern trends in food technological research focuses on the food-medical interface. The concern therefore, is to identify food sources and ingredients for the provision of both nutritional and pharmaceutical (jointly termed nutraceutical) benefits. ILVs may have an invaluable potential in this emerging area.

2.9.3 Phytochemical composition

Phytochemicals are naturally occurring, biologically active chemical compound found in plants and have a beneficial effect on health. They are linked to protection against cardiovascular diseases, some forms of cancer and other degenerative diseases (Ayoola *et al.,* 2008). The most important action of these chemicals is that, they also function as antioxidants that react with the free oxygen molecules or free radicals in the body (Liu, 2003). More than 4000 of non-nutrient bioactive phytochemical compounds have been discovered from plants and it is expected that scientists will discover many more. Reports show that these compounds act as natural defense system for host plants and provide color, aroma and flavor.

Intake of phytochemicals should be from dietary sources rather than from supplements or pills. This is because these can only provide a few of the thousands of phytochemicals available and are thus less effective than a serving of fruits and vegetables. Therefore, since phytochemicals are found in all plant products, it is advised that a wide variety of fruits and vegetables should be consumed in order to gain maximum benefit from the nutrients and phytochemicals they contain. Additionally, phytochemicals work in synergy, thus the effect together is stronger (Liu, 2003).

2.9.4 Antioxidant and health benefits

Antioxidants are chemical compounds or molecules present in the biotic components and high in medicinal plants (Hae-Ryong *et al.,* 2006). These compounds are necessary for human health and nutrition (Kusum and Fazlu, 2002) as they protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite (David *et al.*, 2004). They play an important role in inhibiting and scavenging these free radicals and provide protection against infectious and degenerative diseases (Canadanovic *et al.,* 2005). High consumption of vegetables has been associated with a lowered incidence of degenerative diseases. These protective effects are attributed to the presence of antioxidants especially vitamins like ascorbic acid, tocopherol and β-carotene (Ganiyu, 2005). Antioxidants are also used to preserve food quality mainly because they arrest oxidative deterioration of lipids. Plant-based antioxidants are now preferred to the synthetic ones because of safety concerns.

2.10 Micronutrient deficiency in Kenya

Food insecurity and malnutrition is an issue of concern in Kenya and other countries in Sub-Saharan Africa. Over two billion people, mostly women of childbearing age and children under five years old, suffer from malnutrition. This is due to lack of micronutrient intake, which is a major cause of weakened immunity to diseases, leading to increased mortality in vulnerable groups. Micronutrient malnutrition, particularly vitamin A, iron, and iodine deficiency disorders are major public health concerns globally (ILSI/FAO, 1997)**.** The low level of fruit and vegetable consumption has devastating health effects (Lumpkin *et al.,* 2005) and poses a serious threat. It remains a major problem facing Kenya's poor and needy population and its impact in this population is worsened by the HIV/AIDS pandemic. If left unchecked, these deficiencies will set a vicious cycle effect that will take many generations to correct and this would translate into poor economic development.

Strategies to combat micronutrient deficiency in Kenya include food-based approach**.** The focus therefore, should be laid on programmes that intend to increase the study of micronutrient rich varieties of staple food crops. This is because most of the communities have traditional staple crops, which have been underutilized due to lack of information on their nutritional and medicinal value. These include the indigenous vegetables, fruits, tubers and roots, which if tapped or exploited are likely to be a more sustainable means as well as long-term solution to micronutrient deficiency elimination (MoH/ KEMRI, 1999).

2.11 Ethnobotanical research

There has been a renewed interest in ILVs by the policy makers and the international community due to the realization that vegetables have a potential that is yet to be exploited (Olembo *et al.,* 1995). Besides, ILVs have recently been attracting research attention not only in terms of their inherent nutrition quality, but also their healing power. Epidemiological and *in vitro* studies on medicinal plants and vegetables have strongly supported the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems (Wu *et al.,* 2004). However, literature that documents the use of ILVs as medicine and their analysis for bioactive ingredients is limited. This study was therefore carried out with the aim of studying the biochemical composition and *in vitro* antimicrobial activities of four selected ILVs.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection of plant materials

The four ILVs used were *Corchorus olitorius* L (Jute mallow – *mrenda*), *Crotalaria ochroleuca* G. (Slender leaf –*mitoo*), *Solanum scabrum* Mill. (Black nightshade – *managu*) and *Cleome gynandra* L. (Spider plant –*isaka*) (Appendix 1). Their seeds were collected from Kenya Agricultural Research Institute (KARI) as well as from the JKUAT Botanical Garden and planted at the JKUAT Experimental Farm.

3.1.1 Planting, Weeding and Irrigation

About ¼ acre piece of experimental plot was acquired at the JKUAT Research Farm for planting the ILVs. Tractor ploughing was done and farm workers engaged to manually prepare the land for planting. The seeds of the four ILVs were planted in blocks of 4m by 4m and spacing of 30cm by 10cm between vegetables. No artificial fertilizers were used but farm yard manure at 4 kg/m^2 applied at planting. Irrigation was undertaken regularly after every 3 days using overhead sprinklers and weeding was carried out biweekly.

3.1.2 Harvesting and processing of the plant

Mature leaves of each of the above vegetables were harvested between 1 – 2 months after planting. They were then washed properly with tap water, rinsed with sterile distilled water and subjected to post harvest processing. Some leaves were shade-dried

at room temperature to constant weight over a period of 5 - 9 days, while others were solar-dried using a solar drier at 55°C for four days. The dried leaves were then subsequently ground into fine powder. The prepared samples were stored at room temperature in airtight sterile containers protected from sunlight till further processing as described by Junaid *et al.* (2006).

3.2 Extraction of plant material

3.2.1 Cooking of ILV leaves

The fresh ILV leaf samples were sorted and weighed. Four hundred grams was washed properly with tap water then rinsed with distilled water and finally boiled in 1L distilled water for 15 minutes. The mixture was then macerated in a Warring blender for 10 minutes and then centrifuged at 4000*g* for 30 min. The supernatant was then filtered through Whatman No.1 filter paper and the extract was finally preserved aseptically in an airtight bottle at 5°C for later use.

3.2.2 Aqueous extraction of ILV leaves

Aqueous extraction was carried out for fresh leaves, where 40g of the ILV leaves was macerated in 100ml sterile distilled water in a Warring blender for 10 minutes. The macerate was first filtered through double layered muslin cloth and then centrifuged at 4000*g* for 30 min. The supernatant was then filtered through Whatman No.1 filter paper and sterilized at 120°C for 30 minutes. The extract was finally preserved aseptically in an airtight bottle at 5°C for later use (Babu *et al.*, 2007).

3.2.3 Solvent extraction

Initial methanol extraction was applied for the solar and shade dried samples. Fifty grams of the powdered plant material in a flask was covered with 500ml methanol and allowed to stand for 48 - 72 h. It was then filtered through Whatman filter paper No. 1 and distilled using rotary evaporator (Bibby Sterilin Ltd, RE 100B, UK) at 60°C until methanol free solid powder was obtained. The resulting extracts were then subsequently labeled as methanol extracts and preserved at 5°C in airtight bottles until further use (Alanis *et al.*, 2005).

3.3 Proximate analysis

Proximate composition of the ILV leaves was determined using the AOAC methods (2000) as described by Indrayan *et al.* (2005).

3.3.1 Moisture content

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

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

3.3.2 Ash content

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

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

3.3.3 Crude fat content

Fat extraction was determined using soxhlet method and hexane (b.p 65 - 70°C) was used. The extraction flask was heated to constant weight at 105°C for 1 h then cooled to room temperature in a desiccator. About 5g of sample was then weighed into the extraction thimble and stoppered with cotton wool. The thimble containing the sample was dried at 105°C for 1 h then placed in the extraction apparatus. Hexane was filled to two thirds of the extraction flask. The apparatus was set up and extraction started by heating the flask on the heated water bath for 4 h. The temperature was controlled so

that about 80 condensed droplets fell down on the thimble per minute. After extraction, hexane was allowed to drain down the flask then the thimble was quickly retrieved with forceps. The collected hexane was then evaporated on a water bath using rotary vacuum evaporator (Bibby Sterilin Ltd, RE 100B, UK). The flask containing the extracted fat was then dried at 105°C for 1 h, and then cooled to room temperature in a desiccator. The weight was finally taken % fat calculated as follows:

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

3.3.4 Crude protein

The crude protein was determined using micro Kjeldahl method described in AOAC (2000). About 1 g of the sample was weighed accurately and transferred to a digestion flask. Five grams of catalyst K_2SO_4 and 0.5g $CuSO_4$ was then added. Fifteen milliliters of concentrated H_2SO_4 was added and heated in a fume hood first with a small flame then increasing the temperature gradually. This converted any organic nitrogen to ammonium sulphate, $(NH_4)_2SO_4$, in solution. The contents were then heated until the liquid was colorless. After digesting, the contents of the flask were cooled to room temperature then transferred to a 100ml volumetric flask and filled up to the mark with distilled water. A 10ml aliquot of the digestion solution was taken into a distilling flask and 15ml of 40% NaOH was added to decompose $(NH_4)_2SO_4$ to alkaline. The solution was distilled using distillation apparatus. Twenty five milliliters of 4% boric acid

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

Nitrogen (%) = $(V_1 – V_2)$ x N x f x 0.014 x 100/v x 100/w

Where:
V_1 = Titre for sample (ml)
V_2 = Titre for blank (ml)
N = Normality of standard HCl solution (0.02)
F = factor of standard HCl solution
v = volume of diluted digest taken for distillation (10ml)
w = weight of sample taken.

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

3.3.5 Crude fiber

Fiber content was obtained from the loss in weight on ignition of dried residue remaining after digestion of fat-free samples under specified condition. This imitates the gastric and intestinal action in the process of digestion. Two grams of moisture and fat-

free material was refluxed for 30 minutes with 200ml of 1.25% H_2SO_4 in a reflux condenser. It is then removed and filtered using a Whatman filter paper No. 54. The insoluble matter is then washed with boiling water. After filtration and washing, the residue was treated with boiling 1.25% NaOH and again boiled under the reflux condenser for another 30 minutes. It was then filtered, washed with hot water and then 1% HCl and again with hot water and finally washed with diethyl ether. The residue was finally ignited in a muffle furnace and the ash weighed. The percentage crude fiber was obtained as follows (AOAC, 2000; Chopra and Kanwar, 1991):

$$\% \text{ crude fibre} = \frac{\text{Loss of weight of ignition}}{\text{Weight of sample used}} \times 100$$

3.3.6 Carbohydrate content

Carbohydrate was determined by subtracting the total ash content, crude fat, crude protein and crude fiber from the total dry matter content.

3.4 Chemical analysis

3.4.1 Determination of mineral composition

Minerals were determined after dry ashing according to the method described by the AOAC (2000). The total ash obtained after ashing was boiled with 10ml of 20% hydrochloric acid in a beaker and then filtered into a 100ml standard flask. It was then made up to the mark with deionized water. The minerals Na and K were determined from the resulting solution using emission flame photometer. The standard solutions of

0, 2, 4, 6, 8 and 10 ppm of Na and K were prepared from NaCl and KCl salt solutions and aspirated into the flame photometer. Absorbance was then recorded to prepare a standard curve.

The levels of Zn, Mn, Ca, Mg, and Fe were determined through atomic absorption spectrophotometer (AAS) using standard methods. Working standards of 0, 0.5, 1.0, 1.5, 2.0 and 2.5 ppm were prepared from the standard solution by serial dilution. Each standard was aspirated into AAS and its emission and absorption, respectively was recorded to prepare a standard curve. The same procedure was applied for the prepared sample solutions for each extract and results recorded. The mineral concentrations were calculated from the standard curve.

3.4.2 Fatty acid composition

The extracted fat was dissolved in 4ml hexane, transferred to a conical flask and evaporated on a hot plate. Four milliliters of 95% methanolic HCl solution was then added and heating was done under reflux for 1½ h. The digest was then cooled under tap water. Methyl esters were extracted by transferring the solution into a separating funnel and 4ml of hexane added. The funnel contents were placed on a shaker and vigorously shaken at room temperature and then let to stand for 10 minutes (Shaker Model KS 250 basic, Germany). The hexane layer was collected, the aqueous layer returned and extraction repeated one more time. The hexane fractions were combined and washed with 3 - 4 portions of distilled water to remove acid. Anhydrous sodium sulphate was

added in sufficient quantities to remove water. The filtrate was concentrated using nitrogen gas to about 0.5ml and the sample was injected into the gas chromatography. The standards were also injected and the procedure was repeated for all the samples (AOAC. 1998).

3.4.3 Vitamin C analysis

The amount of vitamin C in a sample was determined by redox titration method according to AOAC methods (1998) as described by Ranganna (2001). This involved the reaction between ascorbic acid in the sample and 2, 6-dichloroindophenol (DCIP). Trichloroacetic acid (TCA) reagent was prepared by dissolving 10g of TCA in 100ml of distilled water. Standard ascorbic acid (1 mg/ml) was then prepared. The DCIP solution was prepared by dissolving 0.25g of 2, 6-dichloroindophenol in about 500ml of distilled water. Sodium bicarbonate (0.21g) was then added and dissolved. The resulting solution was finally diluted to 1L with distilled water to make approximate concentration of 250mg DCIP/L. Five grams of the sample was ground in a mortar with acid washed sand using a suitable volume of 10% TCA. It was then transferred into a 100ml volumetric flask and made up to the mark with the TCA reagent, then immediately filtered through a fluted filter paper. Ten millimeters of the sample (0.05 mg/ml) was pipetted into a 100ml conical flask. A 50ml burette was filled with the DCIP solution which was then used to titrate the sample solution until a permanent light red or pink color appeared. The volume of DCIP needed to oxidize all of the ascorbic acid was recorded and the procedure was repeated. A blank determination was also carried out

with TCA. The ascorbic acid content was calculated using the dye factor. This was determined by the titration of the standard ascorbic acid solution with DCPIP dye using the balanced equation for the oxidation-reduction reaction between ascorbic acid and DCIP. Vitamin C content was then obtained as follows:

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

Where:

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

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

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

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

3.4.4 Beta carotene

Approximately 2g of ILV sample was weighed accurately. It was then placed in a mortar with about 10ml of acetone and ground thoroughly. The acetone extract was then transferred to a 100ml volumetric flask and the residue extracted again with 10ml of acetone and transferred to the volumetric flask. The extraction with acetone was repeated until the residue no longer gave color to acetone. The combined extract was then made to the 100ml mark. Twenty five millimeters of the extract was evaporated to dryness on a rotary vacuum evaporator (Bibby Sterilin Ltd, RE 100B, UK) and the residue dissolved in about 1ml petroleum ether. The solution was introduced into chromatographic column and eluted with petroleum ether. Beta-carotene went through

the column as a yellow pigment and was collected to 25ml volume in the volumetric flask with petroleum ether. Five solutions of standards with concentrations between 0.5µg/ml and 2.5µg/ml were prepared from a stock solution containing 2.5µg/ml pure β-carotene. The absorbance values of the solution were determined at 440 nm using UV-vis spectrophotometer (UV mini 1240 model, Shimadzu Corp., Kyoto, Japan) and plotted against their corresponding concentration to give a standard curve (Appendix II) (AOAC. 1998). The β-carotene content of the ILVs was then calculated per 100g of the material.

3.5 Phytochemical analysis

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

3.5.1 Determination of alkaloids

Two grams of the extract were extracted by warming it for 2 minutes with 20ml of 1% sulphuric acid in a 50ml conical flask on a water bath, with intermittent shaking. It was then centrifuged and the supernatant pipetted off into a small conical flask. One drop of Meyer's reagent was added to 0.1ml supernatant in a semi-micro tube. A cream precipitate indicated the presence of alkaloids. Quantification of alkaloids was done by

the alkaline precipitation gravimetric method described by Harborne, (1998). To 5g of the sample in 250ml beaker, 200ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 4 h at 28°C. It was later filtered via Whatman No 42 filter paper. The filtrate was then concentrated to one quarter of its original volume by evaporation. Concentrated ammonium hydroxide was added drop wise to the extract until the alkaloid precipitated. The alkaloid precipitated was received in a weighed filter paper, washed with 1% ammonia solution and dried in the oven at 80^0C. Alkaloid content was calculated and expressed as a percentage of the weight of sample analyzed.

3.5.2 Determination of flavonoids

Five milliliters of dilute ammonia solution was added to a portion of the aqueous filtrate of the extract followed by addition of concentrated H_2S0_4. A yellow coloration observed indicated the presence of flavonoids. The yellow coloration disappeared on standing. Quantity of flavonoids was then determined according to the method of Harborne (1998), where 5g of the sample was boiled in 50ml of 2 M HCl solution for 30 minutes under reflux. It was then allowed to cool and filtered through Whatman No 42 filter paper. A measured volume of the extract was then treated with equal volume of ethyl acetate starting with a drop. The flavonoid precipitated was recovered by filtration using weighed filter paper. The resulting weight difference gave the weight of flavonoid in the sample.

3.5.3 Determination of tannin

Tannin was determined by the Folin-Denis colorimetric method described by Kirk and Sawyer (1998). About 0.5 g of the dried powdered samples was boiled in 20ml of water in a test tube and then filtered through Whatman No. 42 filter paper. A few drops of 0.1% ferric chloride were added. A brownish green or a blue-black coloration indicated the presence of tannins. Tannin content was also determined by the same method (Kirk and Sawyer, 1998). Five grams of the sample was dispersed in 50ml of distilled water and shaken. The mixture was then allowed to stand for 30 min at 28°C before it was filtered through Whatman No. 42 filter paper. Two milliliters of the extract and standard tannin solution (tannic acid) 0, 0.1, 0.2, 0.3, 0.4 and 0.5 mg/ml was dispersed into a 50ml volumetric flask. Similarly 2ml of distilled water was put in separate volumetric flasks as a blank to calibrate the instrument to zero. Two milliliters of Folin-Denis reagent was added to each of the flasks followed by 2.5ml of saturated Na_2C0_3 solution. The content of each flask was made up to 50ml with distilled water and allowed to incubate at 28°C for 90 min. Their respective absorbance was measured in a UV-vis spectrophotometer (UV mini 1240 model, Shimadzu Corp., Kyoto, Japan) at 760nm.

3.5.4 Determination of phenolic compounds

Ferric chloride test were carried out where the extract were diluted to 5ml with distilled water. To this, a few drops of neutral 5% Ferric chloride solution were added. A dark green or a blue-black color indicated the presence of phenolic compounds. Total phenolic content in the vegetables was estimated spectrophotometrically using Folin-

Ciocalteu reagent, as described by Spanos and Wrolstad (1990) with slight modification, using gallic acid as a standard. An accurately weighed quantity (2-5 g) of the sample was homogenized or blended into a puree and passed through a cheese cloth to remove debris. It was then centrifuged at 4^{o}C and 12,000g for 20 minutes and the supernatant preserved. The sample was then passed through a 0.45 µL membrane filter. To 0.1ml of the sample extract, 5.0ml of 0.2 N Folin-Ciocalteu reagent and 4.0ml of saturated Na_2CO_3 solution were added. The standard curve was prepared using gallic acid (0, 0.1, 0.2, 0.3, 0.4 and 0.5 mg/ml). The mixture was allowed to stand for 90 min and absorbance measured at 765 nm using UV-vis spectrophotometer (UV mini 1240 model, Shimadzu Corp., Kyoto, Japan). The amount of total phenolics was expressed as mg gallic acid equivalents per 100 g sample.

3.5.5 Determination of steroids

Two ml of acetic anhydride were added to 0.5 g ethanolic extract of each sample with 2ml H_2S0_4. The color change from violet to blue or green in some samples indicated the presence of steroids (Harborne, 1998).

3.5.6 Determination of saponin

About 2 g of the powdered sample were boiled in 20ml of distilled water in a water bath and filtered. Ten milliliters of the filtrate was mixed with 5ml of distilled water and shaken vigorously to form a stable persistent froth. The froth was mixed with 3 drops of

olive oil and shaken vigorously, then observed for the formation of emulsion (Obadoni and Ochuko, 2001).

3.5.7 Test for terpenoids

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

3.5.8 Test for anthraquinones

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

3.6 Separation of phytochemicals by thin layer chromatography

For thin layer chromatographic studies of secondary metabolites, the extracts of all the ILVs were prepared and an aliquot (6 µl) of each solutions spotted on the precoated aluminum plates. The spots were placed 1.5 cm from the tip of the plate and 1 cm from each other. After complete solvent removal by drying, the spotted plate was loaded in the chromatographic tank or chamber saturated with the mobile phases. The chromatograms were developed and analyses were conducted after evaporating the

mobile phases. Bands were visualized at UV irradiation (254 nm) in a UV-viewing cabinet and retention factor (R_f) values calculated as follows:

$$\text{Retention factor } (R_f) = \frac{\text{Distance traveled by the spot}}{\text{Distance traveled by the solvent (solvent front)}}.$$

3.6.1 TLC study of alkaloids

The ILVs samples were wetted with half diluted NH_4OH and lixiviated with ethyl acetate for 24h at room temperature. The organic phase was separated from the acidified filtrate and basified with NH_4OH (pH 11-12). It was then extracted with chloroform (3X), condensed by evaporation and used for chromatography. The alkaloid spots were separated using the solvent mixture chloroform and methanol (15:1). The R_f values of the separated alkaloids were recorded after spraying with Dragendorff's reagent (Mallikharjuna *et al.*, 2007).

3.6.2 TLC study of flavonoids

One gram of the samples was extracted with 10ml methanol on water bath (60°C/ 5min). The filtrate was condensed by evaporation, added a mixture of water and ethyl acetate (10:1) and mixed thoroughly. The ethyl acetate phase thus retained was used for chromatography. The flavonoid spots were separated using chloroform and methanol (19:1) solvent mixture. The R_f values of these spots were recorded (Mallikharjuna *et al.*, 2007).

3.6.3 TLC study of phenols

The samples were lixiviated in methanol on rotary shaker (180 thaws/ min) for 24 h. The condensed filtrate was used for chromatography. The phenols were separated using chloroform and methanol (27:0.3) solvent mixture. The R_f values of these phenols were recorded under visible light after spraying the plates with Folin-Ciocalteu's reagent heating at 80°C/10 min (Harborne, 1998; Mallikharjuna *et al.,* 2007).

3.6.4 TLC study of saponins

Two grams of the sample were extracted with 10ml 70% ethanol by refluxing for 10 minutes. The filtrate was condensed, enriched with saturated *n*-BuOH, and thoroughly mixed. The butanol was retained, condensed and used for chromatography. The saponins were separated using chloroform, glacial acetic acid, methanol and water (64:34:12:8) solvent mixture. The R_f values of these spots were recorded by exposing chromatogram to the iodine vapours (Mallikharjuna *et al.,* 2007).

3.7 Determination of antioxidant activity

The radical-scavenging activity was determined using diphenyl picryl hydrazyl (DPPH) radical according to Ayoola *et al.* (2006). This provides information on the reactivity of the test compounds with a stable free radical and gives a strong absorption band at 517nm in the visible region. The following concentrations of the extracts were prepared, 0.05, 0.1, 0.5, 1.0, 2.0 and 5 mg/ml in methanol in cuvette placed in the spectrophotometer (Analar grade). Vitamin C was used as the antioxidant standard at the

same concentrations as the extract. One ml of the extract was placed in a test tube, and 3ml of methanol added followed by 0.5ml of 1 mM DPPH in methanol. The mixture was shaken vigorously and left to stand for 5 min. A blank solution was prepared containing the same amount of methanol and DPPH. The absorbance of the resulting solution was measured at 517 nm with a UV-vis spectrophotometer (UV mini 1240 model, Shimadzu Corp., Kyoto, Japan). All tests were run in triplicate and the radical scavenging activity was then calculated using the following formula:

% inhibition = {[Ab-Aa]/Ab} x 100

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

3.8 Microbial analysis

3.8.1 Collection and screening of test micro-organisms

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

3.8.2 Preparation of Paper Discs

The paper discs used for bioassay were prepared from Whatman No. 1 filter paper. Using a paper punch whose diameter was ascertained to be 6mm, the filter papers were

punched to produce 6 mm paper discs. These discs were then sterilized at 15 lbs pressure at 121°C for 15 minutes in a well sealed universal bottle.

3.8.3 Impregnation of extracts onto Paper Discs for Bioassay

Between 50-250 mg/ml of the crude extract was prepared and about 20 µL of each dissolved fraction was applied on to a sterile paper disc. The paper disc was left to dry completely at room temperature for about 2 h.

3.8.4 Microbial susceptibility testing

3.8.4.1 Antibacterial assay

The extracts were tested for antibacterial activity using agar disc diffusion assay according to Shahidi *et al.* (2004). The strains of bacteria obtained were inoculated in conical flask containing 100ml of nutrient broth or MacConkey and incubated at 37°C for 24 h. This is referred to as seeded broth. Media was prepared using Muller Hinton Agar (Himedia), poured on petri dishes and left to dry. The test organisms from the seeded broth was then placed on the dry agar with a loop and spread evenly on the plate using a sterile L glass rod and left to dry completely. Sterile extract impregnated discs were picked by forceps and very carefully placed on to the inoculated Muller Hinton Agar plates and incubated at 37°C for 24 h. Antibacterial activity was evaluated by measuring the inhibition zone formed around the discs using a ruler calibrated in millimeters. The assay was replicated thrice and the mean values calculated (Junaid *et al.*, 2006). Since the discs used were 6 mm in diameter, mean zone-sizes less than 6 mm

meant that the micro-organisms were resistant to the extracts, while greater than that were considered to be due to antimicrobial activity. Sterile distilled water and methanol served as negative control, whereas standard antimicrobial sensitivity discs containing eight antimicrobials, six being antibiotics and two antimycotics were used as positive controls. The antibiotics were augmentin (30µg/disc), nitrofuranicin (50µg/disc), norfloxacin (10µg/disc), ampicillin (25µg/disc), gentamicin (10µg/disc), nalidixic acid (30µg/disc).

3.8.4.2 Antifungal activity assay

The antifungal activity assay was carried out using the disc diffusion agar method (Ajaiyeoba, 2000). Saboraud dextrose agar (SDA) or potato dextrose agar (PDA) was poured in the sterile petri dishes and left to dry. About 100µl of the inoculum was introduced into the agar and spread evenly. Sterile extract impregnated discs were placed on fungal seeded plates and incubated at 28°C for 48 h. Discs impregnated with only methanol and water served as the negative controls. As a positive control, fluconazole (35µg/disc) and clotrimazole (50µg/disc) were used. Following an incubation period of 48 h, plates were removed from the incubator and antifungal activity was evaluated by measuring the zones of inhibition of fungal growth. Clear zones or mean zone-sizes less than 6 mm meant that the fungi were resistant to the extracts, while greater than 6mm meant that the extracts were active that were considered to be due to antimicrobial activity. The experiment was performed three times and antifungal activity test results were statistically analyzed.

3.8.5 Determination of minimum inhibitory concentration

The MIC of the extracts was determined by dilution to various concentrations according to the macro broth dilution technique (Akinyemi *et al.,* 2005). The ILV extracts were diluted to various concentrations (100, 150, 200, 250, 300 and 350 mg/ml). Equal volumes of the extracts and nutrient broth were mixed in the test tube. Specifically, 0.1ml of standardized inoculums was added to each tube. The tubes were incubated aerobically at 37℃ for 24 h for bacteria and at 28℃ for 48 h for fungi. Two control tubes were maintained for each test batch as follows: tube containing extracts and the growth medium without inoculums (antibiotic control) and the tube containing the growth medium, physiological saline and the inoculums (organism control). The MIC was then read as the concentration with the least inhibitory activity below which there was no further inhibition when compared with the control tubes (Akinyemi *et al.,* 2005).

3.9 Data management and analysis

Data was stored in both hard copy and electronically. MS access was used as the database. Descriptive statistics was used to present data on proximate, mineral and phytochemical composition. Differences in mean concentrations of the phytochemicals were determined using the Student's t- test (for two means) and analysis of variance (ANOVA) for more than two means. Within species and between species chemical composition differences were determined using ANOVA. SAS was used as the analysis software. The means were deemed significantly different when $P \leq 0.05$ and insignificant when $P > 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Cultivation ability of the ILVs

The vegetables germinated within 4 – 6 days after planting. They responded well with organic fertilizers and they had a short growth period with most of them being ready for harvesting within 3 – 4 weeks (Plate 1). Only *C. gynandra* had a short lifespan and started flowering between 8 – 10 weeks, whereas *C. olitorius* and *S. scabrum* took 12 – 16 weeks. *Crotalaria ochroleuca* thrived well and took 14 – 18 weeks.

Plate 1: One month old *Crotalaria ochrolueca* at JKUAT Experimental Farm.

4.2 Solvent extraction

The yields obtained by solvent extraction are summarized in Table 1. The solar dried ILVs gave significantly higher yield than the shade dried samples ($P < 0.05$). On the other hand, *C. gynandra* gave higher yield than the other ILVs, whereas *S. scabrum*

gave low content. The resulting extracts were then kept in airtight bottles (Plate 2) and preserved at 5°C.

Table 1: Percentage yield of the ILV methanolic extracts

Vegetable	**Treatment**	**Percent yield (g/100g)**
C. olitorius	Shade	3.3±0.75
	Solar	3.7±0.49
C. ochroleuca	Shade	2.4±0.53
	Solar	2.9±0.11
C. gynandra	Shade	3.6±0.36
	Solar	4.1±0.27
S. scabrum	Shade	2.1±0.39
	Solar	2.7±0.42

The results are the means of two replicates ± standard error of the mean.

Plate 2: Methanol crude extracts packed in airtight containers.

4.3 Proximate composition analysis

The nutrients in foods reside in the dry matter portion, which is the material remaining after removal of water. The ILV leaves demonstrated low dry matter values ranging between 12.8–18.2 % in all species (Table 2). *Solanum scabrum* had significantly ($P < 0.05$) lower dry matter content (12.8±0.14 %) as compared to *C. ochroleuca*, *C. olitorius* and *C. gynandra* with (15.1±1.38, 16.8±0.17 and 18.2±0.20 % DWB), respectively. *Corchorus olitorius* recorded significantly higher crude fiber content (2.1±0.50 % DWB) followed by *C. ochroleuca* with 1.2±0.37% DWB, whereas *S. scabrum and C. gynandra* reported low values below 1 %. Moreover, *C. olitorius* and *C. ochroleuca* had significantly higher ($P < 0.05$) composition of total fat, 1.4±0.27% DWB and 1.0±0.33% DWB, respectively, as compared to *C. gynandra* and *S. scabrum* with less than 1.0% of crude fat. Protein was significantly higher ($P < 0.05$) in *C. olitorius* (3.7±0.62% DWB) but there was no significant difference between the protein contents of *C. ochroleuca*, *C. gynandra* and *S. scabrum* ($P > 0.05$). Besides, the mean values indicated that, the ILV leaves had significantly low fat, carbohydrate and fiber contents as compared to the recommended dietary allowance. *Cleome gynandra* was also significantly ($P < 0.05$) rich in the amount of total ash (11.2±0.49% DWB) as compared to the other three vegetables.

Table 2: Proximate composition of the edible leaves of ILVs

	Percentage composition on DWB				
Parameter	*C. olitorius*	*C. ochroleuca*	*C. gynandra*	*S. scabrum*	*LSD*
Dry matter (WWB)	16.8^{a}±0.17	15.1^{b}±1.38	18.2^{a}±0.20	12.8^{c}±0.14	0.065
Ash	8.3^{b}±0.08	9.2^{b}±0.06	11.2^{a}±0.49	8.8^{b}±0.10	0.023
Protein	3.7^{a}±0.62	2.6^{b}±0.46	2.6^{b}±0.25	2.4^{b}±0.22	0.038
Fiber	2.1^{a}±0.50	1.2^{b}±0.37	0.8^{c}±0.28	0.6^{c}±0.36	0.035
Fat	1.4^{a}±0.27	1.0^{a}±0.33	0.8^{b}±0.09	0.7^{b}±0.07	0.020
Carbohydrate	1.3^{b}±0.01	1.1^{b}±0.02	2.8^{a}±0.93	0.3^{c}±0.00	0.010
Total organic matter	91.7^{a}±0.13	90.8^{a}±0.11	88.8^{b}±0.85	91.2^{a}±0.17	0.081

Values are given as means of three replicates ± SEM. Means with different superscript letters within a row are significantly different ($P < 0.05$). SEM= Standard error of the mean. LSD= Least significant difference.

4.4 Chemical Composition

4.4.1 Minerals

Elemental assays in all the ILVs revealed relatively high concentrations of calcium, magnesium and potassium (Table 3). The mineral contents of fresh ILVs did not vary significantly ($P > 0.05$) with that of the dried ILVs. As well, the mineral contents of fresh, shade and solar dried vegetables were significantly higher than that of cooked ILVs on DWB. Apparently, cooking of the vegetables led to an average loss of over 50% in some minerals. *Corchorus olitorius, C. gynandra* and *C. ochroleuca* on the other hand, had significantly higher iron contents (22.2±0.15, 15.4±1.51 and 13.6±0.52 mg/100g DWB, respectively) as compared to *S. scabrum* ($P < 0.05$). Other mineral elements detected in reasonable amounts were zinc, sodium and manganese. *Cleome gynandra* was found to be significantly rich in minerals as compared to the other ILVs.

Table 3. Mineral composition of ILVs

Vegetable	Treatment	Mineral concentration (mg/100g DWB)						
		Zn	Mn	Ca	Mg	Fe	K	Na
C. olitorius	Solar	3.9^{a}±0.01	23.5^{b}±2.08	34.0^{c}±3.51	53.9^{a}±3.89	20.0^{a}±0.34	13.9^{e}±0.14	11.2^{c}±0.36
	Shade	4.1^{a}±0.06	16.7^{c}±1.31	38.9^{c}±0.82	56.5^{a}±0.75	22.2^{a}±0.15	12.3^{e}±0.06	12.5^{c}±1.42
	Cooked	*BDL	11.1^{c}±1.04	26.2^{d}±2.48	23.4^{c}±1.61	11.2^{b}±0.20	7.0^{e}±0.11	4.8^{d}±0.21
	Fresh	5.0^{a}±0.17	23.5^{b}±2.01	40.3^{c}±2.13	67.2^{a}±2.03	19.9^{a}±1.03	13.8^{e}±0.19	13.2^{c}±0.97
C. ochroleuca	Solar	0.03^{d}±0.02	22.6^{b}±7.60	45.5^{c}±2.11	27.0^{c}±2.10	7.5^{c}±1.15	106.0^{b}±3.24	23.7^{b}±1.75
	Shade	0.05^{d}±0.01	24.1^{b}±1.15	74.8^{b}±6.98	26.6^{c}±3.77	13.6^{b}±0.52	121.7^{a}±2.60	21.4^{b}±1.78
	Cooked	*BDL	6.3^{d}±0.21	17.0^{d}±1.13	15.3^{d}±1.81	2.6^{d}±0.17	59.4^{d}±3.17	14.7^{c}±1.50
	Fresh	0.08^{d}±0.01	27.3^{b}±1.85	70.5^{b}±4.18	41.7^{b}±1.89	10.8^{b}±1.25	120.6^{a}±3.13	24.4^{b}±1.23
C. gynandra	Solar	*BDL	36.1^{a}±12.71	81.9^{a}±7.15	20.8^{d}±0.48	15.4^{b}±1.51	100.6^{b}±5.20	45.0^{a}±7.20
	Shade	*BDL	27.1^{b}±0.03	92.8^{a}±4.98	24.8^{c}±5.00	14.6^{b}±0.10	105.7^{b}±1.29	42.8^{a}±3.56
	Cooked	0.08^{c}±0.02	8.3^{d}±0.12	72.2^{b}±3.04	13.3^{d}±3.79	7.4^{c}±0.12	56.0^{d}±3.19	21.9^{b}±1.92
	Fresh	0.1^{c}±0.03	35.6^{a}±1.20	94.1^{a}±5.37	32.7^{c}±1.98	14.1^{b}±0.73	104.9^{b}±8.73	46.3^{a}±2.26
S. scabrum	Solar	0.7^{c}±0.02	3.6^{d}±1.63	89.9^{a}±8.30	44.2^{b}±4.20	4.1^{d}±1.01	86.9^{c}±2.62	4.1^{d}±1.80
	Shade	1.0^{b}±0.02	6.8^{d}±1.92	86.2^{a}±4.11	46.8^{b}±2.95	4.4^{d}±0.97	82.0^{c}±1.12	3.6^{d}±0.71
	Cooked	0.07^{d}±0.02	1.7^{e}±0.15	22.0^{d}±2.82	19.3^{c}±2.43	1.5^{d}±0.16	50.9^{d}±0.11	2.6^{d}±0.15
	Fresh	1.2^{b}±0.03	7.4^{d}±1.43	90.2^{a}±2.30	50.2^{b}±2.87	3.9^{d}±1.71	87.3^{c}±2.37	4.4^{d}±1.72

Values are given as means of two replicates ± standard deviation. Means with different small letters within a column are significantly different ($P < 0.05$).
*BDL= Below detectable level. DWB= Dry weight basis.

4.4.2 Fatty acid composition

The fatty acid profile of ILV leaves is shown in Table 4. Results indicated that, unsaturated fatty acids recorded higher amounts than the saturated fatty acids (SFA). This was attributed to the presence of linoleic acid ($C_{18:2\Delta9;12}$), α-linolenic ($C_{18:3\Delta9;12;15}$) and oleic ($C_{18:1\Delta9}$) acid. *Corchorus olitorius* exhibited significantly higher values of unsaturated fatty acids, linoleic acid ($C_{18:2\Delta9;12}$), α-linolenic ($C_{18:3\Delta9;12;15}$) and oleic ($C_{18:1;9}$) acids (300.6±15.12, 186.6±17.82 and 75.1±7.83 mg/100g DWB, respectively) ($P < 0.05$). The predominant SFA was palmitic acid ($C_{16:0}$) with 157.3±13.16 mg/100g and 169.8±9.31 mg/100g DWB in *C. ochroleuca* and *C. olitorius*, respectively. However, *C. olitorius* and *C. ochroleuca* was composed of high amounts of total fatty acids, whereas *C. gynandra* and *S. scabrum* had significantly lower ($P < 0.05$) amounts of the fatty acids.

Table 4: Composition of fatty acids in the ILVs

Fatty acid	**Fatty acid content of ILVs (mg/100g DWB)**			
	C. olitorius	***C. ochroleuca***	***C. gynandra***	***S. scabrum***
Caprylic(8:0)	23.6^{a}±5.81	10.01^{b}±1.23	5.8^{c}±0.24	9.2^{b}±0.07
Capric(10:0)	2.6^{b}±0.14	8.2^{a}±0.83	1.8^{b}±0.09	10.1^{a}±1.76
Lauric(12:0)	5.3^{a}±0.27	3.5^{b}±1.46	2.4^{b}±0.32	0.8^{c}±0.46
Myristic(14:0)	119.4^{a}±3.65	90.4^{b}±8.01	84.4^{b}±0.40	105.4^{a}±17.68
Palmitic(16:0)	169.8^{a}±9.31	157.3^{a}±13.16	108.0^{b}±7.03	113.1^{b}±2.08
Stearic(18:0)	35.2^{a}±0.41	37.7^{a}±5.93	18.4^{c}±0.08	29.5^{b}±1.82
Linoleic(18:2)	300.6^{a}±15.12	272.4^{a}±24.34	180.3^{b}±3.16	173.2^{b}±8.97
Linolenic(18:3)	186.6^{a}±17.82	130.2^{b}±2.39	67.3^{c}±10.03	129.9^{b}±2.86
Oleic(18:1)	75.1^{a}±7.83	38.5^{b}±6.97	27.3^{b}±0.18	32.3^{b}±0.08

Values are given as means of two replicates ± SEM. Means with different small letters within a row are significantly different ($P < 0.05$). SEM= Standard error of the mean

4.4.3 Vitamin C composition

Vitamin C content in fresh vegetables was significantly higher than that of dried samples (*P < 0.05*) (Table 5). However, solar drying led to significantly high loss in vitamin C content compared to shade drying or cooking. There was also a significant difference (*P < 0.05*) in the vitamin C content between the different ILVs. Fresh *C. olitorius* exhibited the highest amount of vitamin C content (153.7±7.75 mg/100g DWB), while *S. scabrum* had the least (62.6±4.57 mg/100g DWB). On the other hand, the vitamin C content of *C. ochroleuca* and *C. gynandra* were not significantly different. The loss in vitamin C on drying and cooking was between 79 – 97%.

Table 5: Vitamin C content of ILVs

Sample	**Vitamin C content mg/100g DWB**			
	Fresh	**Shade dried**	**Solar dried**	**Cooked**
C. olitorius	153.7^{aA}±7.75	16.7^{bA}±0.37	12.4^{cA}±0.23	26.3^{bA}±0.95
C. ochroleuca	92.8^{aB}±8.13	9.9^{bB}±0.91	4.0^{cB}±0.20	11.9^{bC}±1.13
C. gynandra	104.3^{aB}±6.68	14.7^{bA}±0.32	5.7^{cB}±0.40	16.2^{bB}±0.42
S. scabrum	62.6^{aC}±4.57	8.0^{bB}±0.17	2.4^{cC}±0.06	10.0^{bC}±1.73
LSD	0.63	0.15	0.12	0.21
CV%	12.2	24.2	20.4	31.2

Values are given as means of three replicates ± SEM. Means with different small letters within a row and capital letters within a column are significantly different (P < 0.05). SEM= Standard error of the mean. LSD= Least significant difference. CV= Coefficient of variation.

4.4.4 Beta-Carotene

Fresh ILVs had significantly higher β-carotene contents compared to the dried and cooked ILVs (*P < 0.05*). *Cleome gynandra* had the highest content of β-carotene (8.7±0.16 mg/100g DWB), while *C. olitorius* had 7.7±0.32 mg/100 g DWB and *C.*

ochroleuca, 6.2±0.19 mg/100g DWB. *Solanum scabrum* exhibited the lowest β-carotene content among the vegetables (4.6±0.25 mg/100g DWB) (Figure 1).

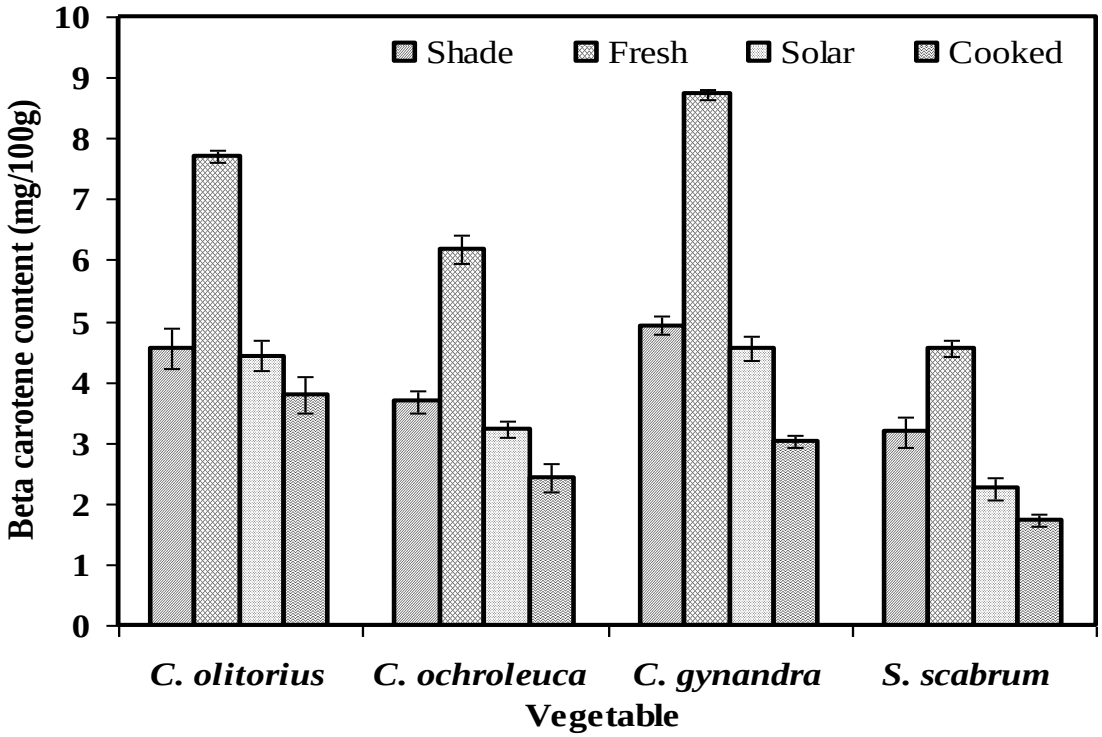


Figure 1: Beta-carotene content of ILV leaves. Values are given as means of three replicates ± standard error. Shade, fresh, solar and cooked in the key above includes the different treatments. Recommended Dietary Allowance (RDA) = 12 mg/100g. (Abukutsa-Onyango, 2003)

4.5 Phytochemical analysis

4.5.1 Qualitative analysis

The present study revealed the presence of a wide array of phytochemicals including alkaloids, flavonoids, tannins, saponins, steroids and phenols (Table 6). Flavonoids, alkaloids, saponins, phenols and amino acids were most common and present in almost all the ILVs, whereas terpenoids, steroids and anthraquinones were absent in most ILVs.

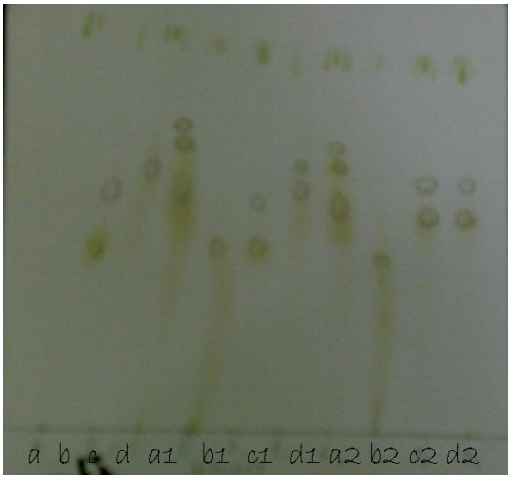
Table 6: Qualitative presentation of phytochemicals detected in the ILVs

Vegetable	Sample	Phytochemical compounds								
		Flav	**Alka**	**Sap**	**Tan**	**Phe**	**Anth**	**Ster**	**Terp**	**Aa**
C. olitorius	Fresh	+	+	+	+	+	+	+	-	+
	Shade	+	+	+	+	+	±	±	-	+
	Solar	+	+	+	+	+	-	-	-	+
	Cooked	+	+	+	+	+	-	-	-	+
C. ochroleuca	Fresh	+	+	±	+	+	+	+	-	+
	Shade	+	+	-	±	+	-	-	-	+
	Solar	+	+	±	+	+	-	-	-	+
	Cooked	+	+	-	±	+	-	-	-	+
C. gynandra	Fresh	+	+	+	±	+	-	+	+	+
	Shade	+	+	±	-	+	-	±	+	+
	Solar	+	+	±	-	+	-	-	+	+
	Cooked	+	+	±	-	+	-	-	+	+
S. scabrum	Fresh	+	+	+	+	±	+	+	-	+
	Shade	+	±	+	+	+	±	±	-	+
	Solar	+	-	+	+	+	-	-	-	+
	Cooked	-	+	+	+	-	-	±	-	+

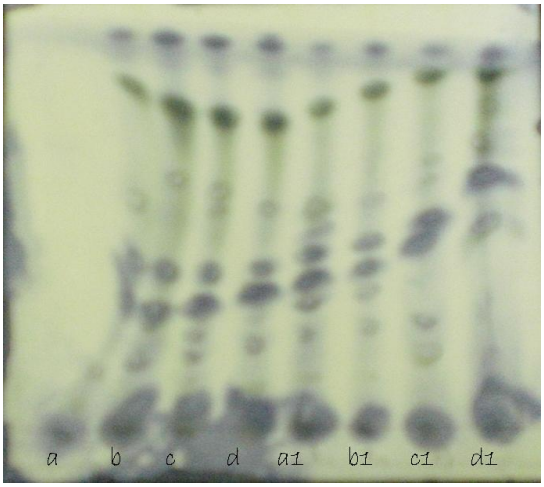
+ Present, – Absent, ± Doubtful.
Alka- alkaloid, Flav- flavonoid, Sap- saponin, Tan- tannin, Phe- phenol, Anth- anthraquinone, Ster- steroids, Terp- terpenoids, Aa- amino acids.

4.5.2 Thin layer chromatography

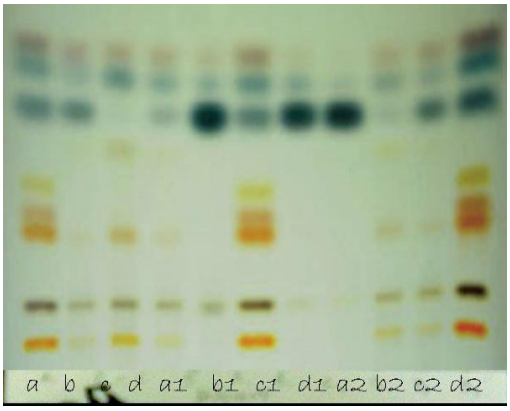
The TLC R_f values are tabulated in Tables 7, 8, 9 and 10 and presents the data for the qualitative separation of some phytochemicals from the ILV leaves. These results indicated that more alkaloid spots were found in samples of *C. ochroleuca* and *S. scabrum* (7 spots). Furthermore, fresh ILVs possessed more alkaloids as compared with the dried and cooked samples. One alkaloid spot was found to be common in all the ALV samples with R_f value 0.18 (Table 7).



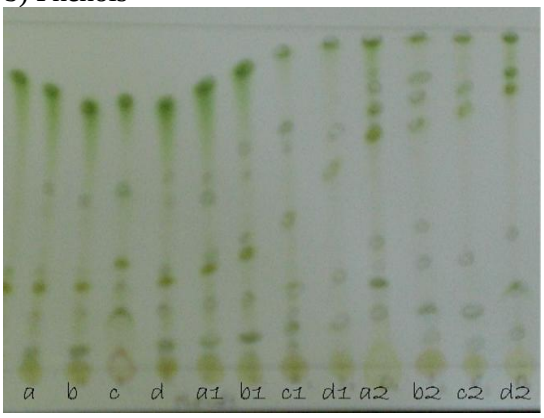
a) Saponins



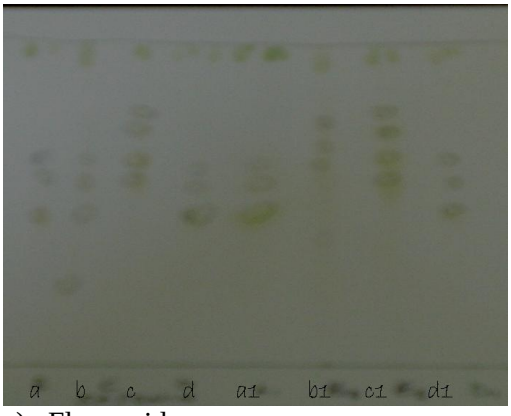
b) Phenols



c) Alkaloids



d) Total extracts



e) Flavonoids

Plate 3: TLC chromatograms of the ILV extracts. a, b, c, d; a_1, b_1, c_1, d_1; a_2, b_2, c_2, d_2 and a_3, b_3, c_3, d_3 represent fresh, shade, solar dried and cooked *C. olitorius, C. ochroleuca, C. gynandra, S. scabrum* respectively. The spots represent the different compounds which traveled according to their retention factors.

Table 7: Qualitative separation of alkaloids of ILVs by TLC (UV254nm)

Treatment	**R_f values of the vegetables (Sf = 9.8)**			
	C. olitorius	***C. ochroleuca***	***C. gynandra***	***S. scabrum***
Fresh	0.12±0.01	0.18^{a}±0.02*	0.14±0.02	0.08±0.00*
	0.18^{a}±0.03*	0.28±0.01	0.18^{a}±0.01*	0.19^{a}±0.02*
	0.24^{b}±0.02	0.31±0.05	0.24^{b}±0.02	0.21±0.01
	0.46±0.01	0.40±0.02	0.42±0.02	0.34±0.03*
	0.66^{c}±0.04	0.63±0.07	0.88^{d}±0.09*	0.52±0.02
	0.78±0.08*	0.88^{d}±0.14		0.68^{c}±0.03
	-	0.92±0.11	-	0.80±0.10*
Shade	0.10±0.01	0.18^{a}±0.02*	0.14±0.00	0.08±0.00*
	0.18^{a}±0.00*	0.24^{b}±0.01	0.18^{a}±0.02*	0.18^{a}±0.02*
	0.26^{b}±0.02	0.33±0.05	0.24^{b}±0.03	0.21±0.02
	0.42±0.03	0.40±0.04	0.89^{d}±0.11*	0.34±0.04*
	0.78±0.06*	0.85±0.09		0.52±0.09
	-	0.90±0.14	-	0.81±0.06*
Solar	0.17^{a}±0.02*	0.18^{a}±0.02*	0.18^{a}±0.01*	0.09±0.00*
	0.23^{b}±0.01	0.28±0.04	0.42±0.02	0.18^{a}±0.01*
	0.44±0.05	0.31±0.05	0.79±0.06	0.22±0.02
	0.78±0.09*	0.40±0.06	-	0.33±0.04*
	-	0.53±0.05	-	0.55±0.07
	-	0.92±0.15	-	0.80±0.09*
Cooked	0.14±0.01	0.16^{a}±0.00*	0.18^{a}±0.03*	0.08±0.00*
	0.18^{a}±0.04*	0.22^{b}±0.03	0.43±0.05	0.19^{a}±0.02*
	0.24^{b}±0.03	0.31±0.05	0.89±0.09*	0.34±0.04*
	0.44±0.05	0.42±0.02	-	0.82±0.15*
	0.78±0.12*	0.88^{d}±0.10	-	-

Values are Sf of the TLC spots given as means of three replicates ± SEM. SEM= Standard error of the mean. Sf – solvent front. R_f - Retention factor. Values with the same letters within a row indicate the spots with no significant difference in R_f values between the ILV samples. Asterisk (*) within a column indicates the spots common or retained in each ILV after the treatment.

Thin layer chromatography data for flavonoids is tabulated in the Table 8 below. Five flavonoid spots were reported in the *C. olitorius* and *C. gynandra* samples although with different R_f values, while, in the *C. ochroleuca* and *S. scabrum,* four and two spots were

observed, respectively. Two flavonoid spots were found to be common for all samples with R_f value ranging between 0.33- 0.36 and 0.40- 0.44.

Table 8: Qualitative separation of flavonoids of ILVs by TLC (UV254nm)

Treatment	**R_f values of the vegetables (Sf=9.6)**			
	C. olitorius	***C. ochroleuca***	***C. gynandra***	***S. scabrum***
Fresh	0.23±0.02*	0.20±0.01*	0.19±0.01*	0.34^{a}±0.04*
	0.36^{a}±0.01*	0.33^{a}±0.03*	0.36^{a}±0.04*	0.43^{b}±0.03*
	0.42^{b}±0.03*	0.42^{b}±0.02	0.43^{b}±0.03	0.76±0.04
	0.51±0.07	0.77±0.08	0.49±0.07	-
	0.83±0.09*	-	0.85±0.11*	-
Shade	0.22±0.04*	0.21±0.02*	0.19±0.02*	0.33^{a}±0.02*
	0.33^{a}±0.02*	0.40^{b}±0.07	0.33^{a}±0.04*	0.42^{b}±0.05*
	0.41^{b}±0.06*	0.75±0.08	0.43^{b}±0.06	0.75±0.04
	0.83±0.05*	-	0.85±0.13*	-
Solar	0.22±0.01*	0.21±0.01*	0.21±0.03*	0.34^{a}±0.02*
	0.33^{a}±0.04*	0.34^{a}±0.05*	0.36^{a}±0.05*	0.44^{b}±0.05*
	0.42^{b}±0.06*	0.75±0.08	0.49±0.02	-
	0.83±0.09*	-	0.85±0.12	-
Cooked	0.23±0.03*	0.33^{a}±0.04*	0.36^{a}±0.03*	0.33^{a}±0.04*
	0.36^{a}±0.07*	0.77±0.06	0.85±0.10*	0.41^{b}±0.07*
	0.83±0.14*	-	-	-

Values are Sf of the TLC spots given as means of three replicates ± SEM. SEM= Standard error of the mean. Sf – solvent front. R_f - Retention factor. Values with the same letters within a row indicate the spots with no significant difference in R_f values between the ILV samples. Asterisk (*) within a column indicates the spots common or retained in each ILV after the treatment.

Results on saponins (Table 9) revealed the presence of 4, 3, 3 and 1 spot in *S. scabrum, C. olitorius, C. ochroleuca* and *C. gynandra,* respectively, with one spot being common in all the ILVs (R_f = 0.69±0.01). Some spots were not observed after the treatments

Table 9: Qualitative separation of saponins of ILVs by TLC (UV254nm)

Treatment	**R_f values of the vegetables (Sf=9.8)**			
	C. olitorius	***C. ochroleuca***	***C. gynandra***	***S. scabrum***
Fresh	$0.69^{a}\pm0.05^{*}$	$0.69^{a}\pm0.07^{*}$	$0.69^{a}\pm0.04$	$0.68^{a}\pm0.06^{*}$
	$0.80\pm0.03^{*}$	$0.71^{a}\pm0.04$	-	$0.72^{a}\pm0.03$
	0.96 ± 0.14	$0.85^{b}\pm0.10^{*}$	-	$0.86^{b}\pm0.09^{*}$
	-	-	-	0.90 ± 0.17
Shade	$0.70^{a}\pm0.08^{*}$	0.66 ± 0.08	-	0.58 ± 0.07
	$0.79\pm0.11^{*}$	$0.86^{b}\pm0.10^{*}$	-	$0.70^{a}\pm0.03$
	-	-	-	$0.86^{b}\pm0.10^{*}$
	-	-	-	0.92 ± 0.16
Solar	$0.70^{a}\pm0.04^{*}$	$0.68^{a}\pm0.05^{*}$	-	0.58 ± 0.04
	$0.80\pm0.09^{*}$	$0.85^{b}\pm0.02^{*}$	-	$0.70^{a}\pm0.03^{*}$
	0.94 ± 0.12	-	-	0.90 ± 0.21
Cooked	$0.69^{a}\pm0.06^{*}$	$0.69^{a}\pm0.03^{*}$	-	0.55 ± 0.06
	$0.80\pm0.03^{*}$	-	-	$0.70^{a}\pm0.10^{*}$
	-	-	-	$0.86^{b}\pm0.15^{*}$

Values are Sf of the TLC spots given as means of three replicates ± SEM. SEM= Standard error of the mean. Sf – solvent front. R_f - Retention factor. Values with the same letters within a row indicate the spots with no significant difference in R_f values between the ILV samples. Asterisk (*) within a column indicates the spots common or retained in each ILV after the treatment.

The data of phenols is tabulated in Table 10 below. The highest number of phenols (4 spots) was reported in *C. olitorius* and *S. scabrum* with two similar spots with R_f values (0.39 and 0.49), while one spot (0.39±0.01) was common in all the ILVs.

Table 10: Qualitative separation of phenols of ILVs by TLC (UV254nm)

Treatment	**R_f values of the vegetables (Sf= 9.8)**			
	C. olitorius	***C. ochroleuca***	***C. gynandra***	***S. scabrum***
Fresh	0.16±0.01	0.17±0.02	0.39^{a}±0.04*	0.16±0.02
	0.39^{a}±0.03*	0.39^{a}±0.06*	0.58±0.07*	0.39^{a}±0.05*
	0.49^{b}±0.06	0.71±0.08	0.80±0.10	0.49^{b}±0.03*
	0.67±0.08	-	-	0.81±0.10
Shade	0.38^{a}±0.04*	0.39^{a}±0.04*	0.39^{a}±0.05*	0.38^{a}±0.03*
	0.66±0.03	0.70±0.05	0.57±0.03*	0.49^{b}±0.07*
Solar	0.39^{a}±0.03*	0.39^{a}±0.02*	0.38^{a}±0.04*	0.39^{a}±0.06*
	0.58±0.08	-	0.58±0.06*	0.52±0.09
Cooked	0.16±0.02	0.39^{a}±0.06*	0.59±0.08*	0.39^{a}±0.05*
	0.38^{a}±0.05*	0.71±0.09	-	0.49^{b}±0.02*
	0.58±0.05	-	-	-

Values are Sf of the TLC spots given as means of three replicates ± SEM. SEM= Standard error of the mean. Sf – solvent front. R_f - Retention factor. Values with the same letters within a row indicate the spots with no significant difference in R_f values between the ILV samples. Asterisk (*) within a column indicates the spots common or retained in each ILV after the treatment.

4.5.3 Quantitative analysis

The results for the quantification of phytochemicals are summarized in Table 11. There was no significant difference ($P > 0.05$) between the phytochemical contents of the solar and shade dried ILVs on DWB. Similarly, the phytochemical contents of fresh vegetables were significantly higher ($P < 0.05$) than those of the dried and cooked vegetables. *Solanum scabrum* contained the highest percentage of phenols and tannins than the rest, whereas *C. gynandra* was high in flavonoids. On the other hand, *C. ochroleuca*, *S. scabrum* and *C. olitorius* had significantly higher alkaloid contents.

Table 11: Phytochemical composition of ILV leaves

Plant species	Treatment	Composition expressed as g/100g DWB			
		Flavonoid	Alkaloid	Phenols	Tannins
C. olitorius	Fresh	4.4^{a}±0.07	9.4^{a}±0.08	2.2^{a}±0.08	3.9^{a}±0.06
	Shade	4.0^{b}±0.06	3.5^{c}±0.34	0.5^{c}±0.04	0.8^{c}±0.04
	Solar	3.1^{c}±0.59	4.7^{c}±0.29	0.7^{c}±0.08	1.2^{b}±0.05
	Cooked	3.5^{b}±0.25	6.6^{b}±0.18	1.3^{b}±0.06	1.7^{b}±0.05
C. ochroleuca	Fresh	2.9^{b}±0.11	10.4^{a}±0.19	2.9^{a}±0.09	2.5^{a}±0.05
	Shade	2.6^{b}±0.21	4.6^{c}±0.30	0.5^{b}±0.04	0.8^{c}±0.04
	Solar	3.3^{a}±0.20	4.5^{c}±0.15	0.4^{b}±0.03	0.7^{c}±0.03
	Cooked	1.4^{c}±0.08	7.4^{b}±0.27	0.7^{b}±0.07	1.1^{b}±0.04
C. gynandra	Fresh	6.3^{a}±0.20	6.4^{a}±0.04	2.3^{a}±0.05	3.7^{a}±0.04
	Shade	3.7^{c}±0.46	4.3^{b}±0.18	0.7^{c}±0.04	1.2^{c}±0.05
	Solar	2.9^{c}±0.09	3.2^{c}±0.18	0.7^{c}±0.13	1.2^{c}±0.03
	Cooked	4.9^{b}±0.13	4.2^{b}±0.19	1.0^{b}±0.11	1.8^{b}±0.05
S. scabrum	Fresh	3.4^{b}±0.12	10.8^{a}±0.08	4.5^{a}±0.10	7.3^{a}±0.05
	Shade	4.3^{a}±0.32	5.9^{b}±0.28	1.2^{c}±0.05	1.9^{c}±0.03
	Solar	3.4^{b}±0.21	4.7^{c}±0.16	1.4^{c}±0.23	2.0^{c}±0.08
	Cooked	1.4^{c}±0.08	4.0^{c}±0.13	2.7^{b}±0.06	2.8^{b}±0.06

Values are given as means of three replicates ± SEM. Means values appended by different small letters within a column are significantly different ($P < 0.05$). All the ILVs were analyzed on DWB. SEM= Standard error of the mean.

4.6 Antioxidant Activity

Free radical scavenging activity of methanolic extracts of ILVs was confirmed in this study. Figure 2 and 3 illustrates the absorbance versus concentration curve of methanol extracts of solar and shade dried ILVs, respectively. These results were compared with the absorbance versus concentration curves of ascorbic acid which is a standard antioxidant. The decrease in absorbance as the concentration increase was taken as a measure of the extent of radical scavenging activity (Kizhiyedathu *et al.,* 2005).

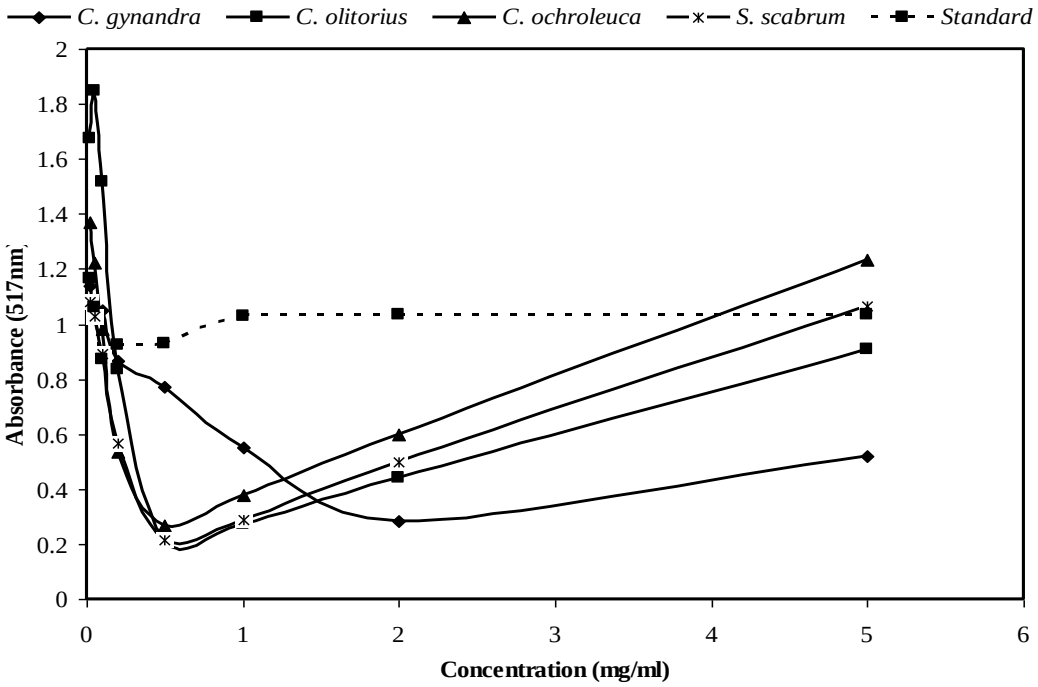


Figure 2: Changes in absorbance of methanolic extracts of solar dried ILVs with concentration (mg/ml).

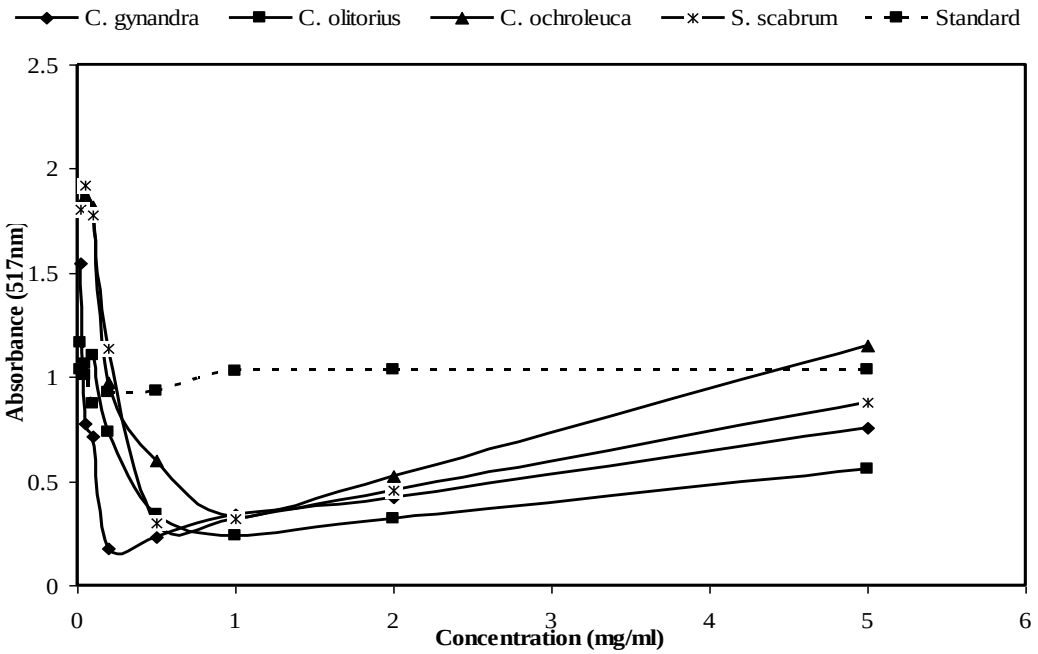


Figure 3: Changes in absorbance of methanolic extracts of shade dried ILVs with concentration (mg/ml).

Figure 4 and 5 shows the DPPH free radical scavenging activity (% inhibition) of solar and shade dried ILVs versus their concentrations, respectively. The scavenging effects of all extracts on DPPH radicals increased as the concentration increased in the range of 0.05 – 5.0 mg/ml and it was remarkable, especially in the case of *C. olitorius* and *C. gynandra*. However, the increase in activity was only marginal when the extract concentrations were higher than 1 mg/ml. Almost all the fractions showed significantly higher ($P < 0.05$) percentage of DPPH inhibition than ascorbic acid.

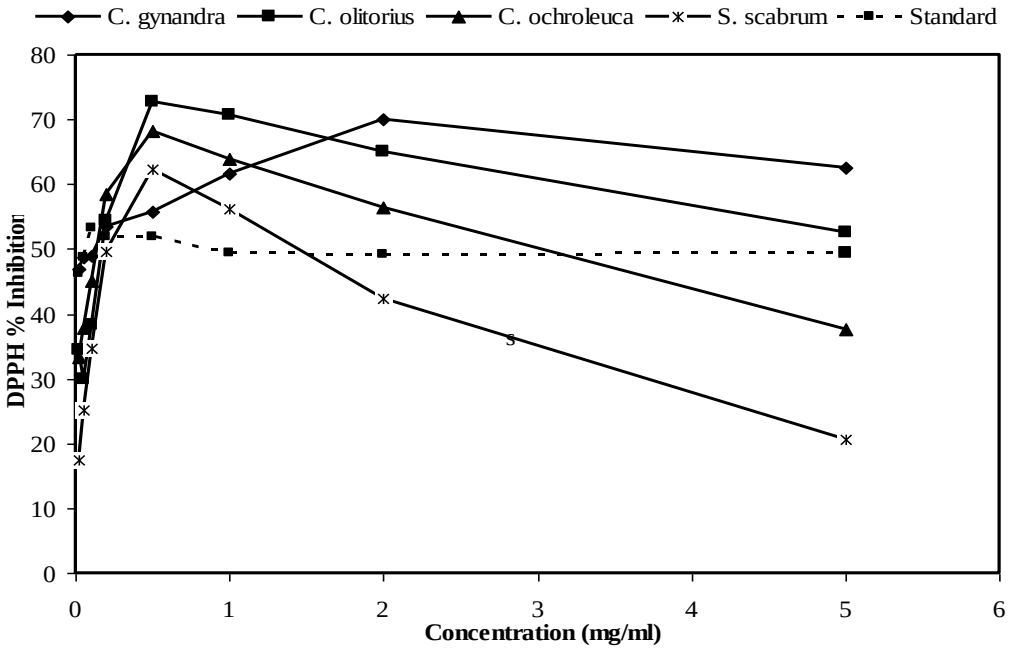


Figure 4: Arcsine transformed DPPH free radical scavenging activity (% inhibition) of solar methanolic ILV extracts

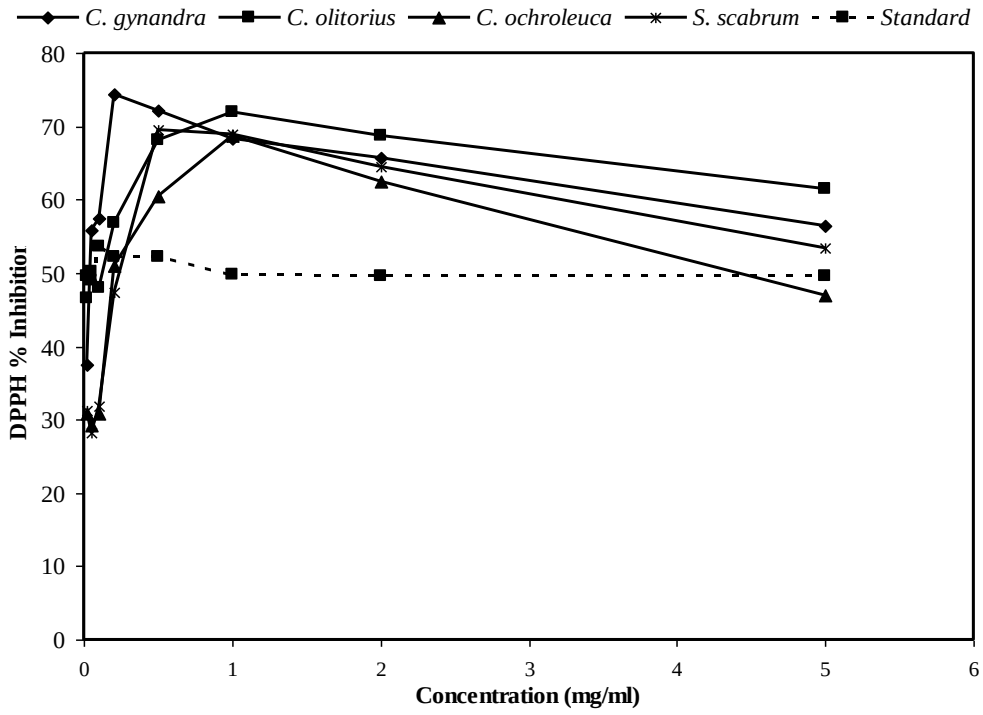


Figure 5: Arcsine transformed DPPH free radical scavenging activity (% inhibition) of shade methanolic ILV extracts

Table 12 presents the IC_{50} values (the concentration which scavenge 50% of the DPPH radicals) and the maximal extent of the radical scavenging activity. The IC_{50} values of the extracts ranged from 0.01±0.00 mg/ml to 0.19±0.07 mg/ml and the maximum percentage inhibition ranged between 64–93 % with solar and shade dried *C. gynandra, C. olitorius, C. ochroleuca* and *S. scabrum* reporting 88.3%, 91.2, 86.4, 78.4% and 92.8%, 90.4%, 86.9%, 87.8%, respectively. This was much higher than that of ascorbic acid, a standard antioxidant (64.2%). On the other hand, the IC_{50} value of ascorbic acid was low indicating strong antioxidation. The percentages of radical scavenging effect of

both solar and shade dried extracts were within close range therefore, demonstrated no significant difference (*P > 0.05*).

Table 12: IC_{50} and maximum percentage inhibition values for the ILV extracts

Plant species	Treatment	IC_{50} (mg/ml)	Maximum inhibition(%)	Concentration (mg/ml)
C. *olitorius*	Solar	0.14±0.03	91.2±3.54	0.5
	Shade	0.01±0.00	90.4±2.98	1.0
C. *ochroleuca*	Solar	0.10±0.01	86.4±1.74	0.5
	Shade	0.18±0.04	86.9±1.33	1.0
C. *gynandra*	Solar	0.01±0.00	88.3±3.32	2.0
	Shade	0.04±0.00	92.8±1.12	0.2
S. *scabrum*	Solar	0.19±0.07	78.4±1.21	0.5
	Shade	0.19±0.05	87.8±2.62	0.5
Ascorbic acid		0.01±0.00	64.2±1.03	0.1
		0.01±0.00	64.7±0.98	0.1

The results are the means of IC_{50} and maximum percentage inhibition values of two replicates ± SEM and their respective concentrations. SEM= Standard error of the mean. IC_{50} value - the concentration, which scavenged 50% of the DPPH radicals.

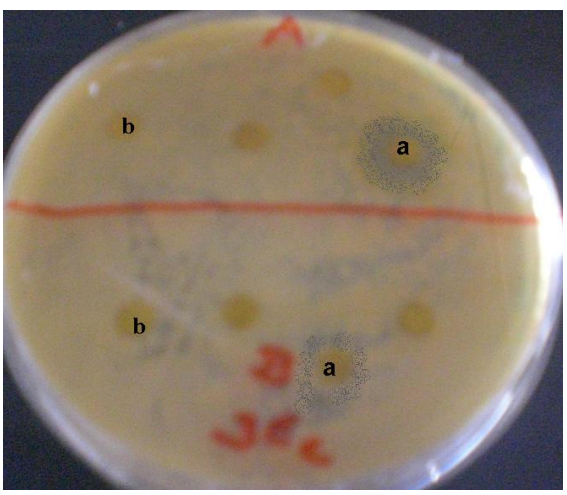
4.7 Antimicrobial susceptibility assay

4.7.1 Zone of inhibition

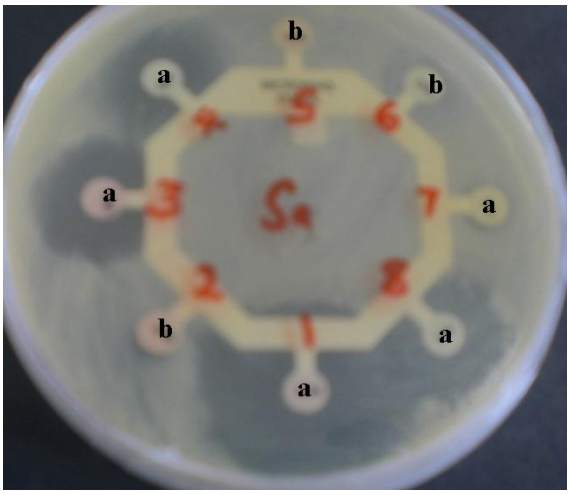
The inhibition zone of the extracts and the controls against microorganisms is shown in Plate 4. There was no inhibition with the negative controls (methanol and water), whereas some antimicrobials in the sensitivity discs and the ILV extracts exhibited inhibition against the organisms.

Tables 13, 14, 15 and 16 summarize the sensitivities of ILV extracts against *E. coli, S. aureus, B. subtilis, P. aeruginosa, C. albicans* and *A. niger*. From the results it was evident that the higher the concentration, the higher the zone of inhibition. On the other hand, almost all the ILV infusions were active against the test organisms. As well the

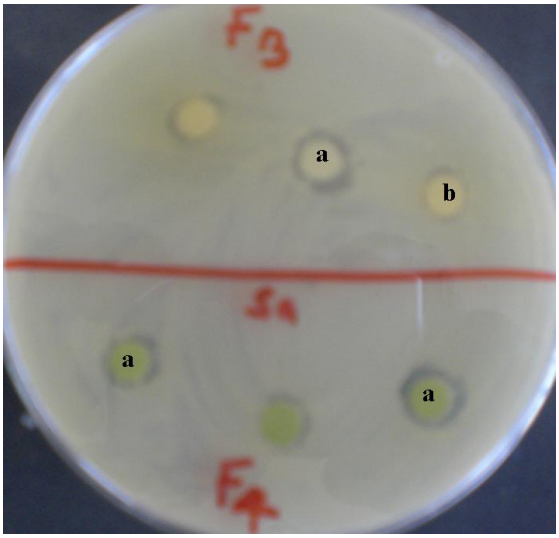
four bacterial strains *E. coli, S. aureus, B. subtilis* and *P. aeruginosa* were highly inhibited by all the ILV extracts and there was no significant difference between their zones of inhibition. *Cleome gynandra* extracts among the four ILVs exhibited the highest activity with most of the organisms especially *E. coli, S. aureus* and *B. subtilis*. This might be due to high concentration of flavonoids (Table 10). The two fungi and especially *A. niger* on the other hand, displayed resistance to the inhibitory effects of the ILV extracts since their zones of inhibition was low. In other words, the extracts appeared to be more bactericidal and less fungicidal. The solar and shade dried ILVs had higher inhibition zones as compared to the fresh and cooked ILVs on DWB.



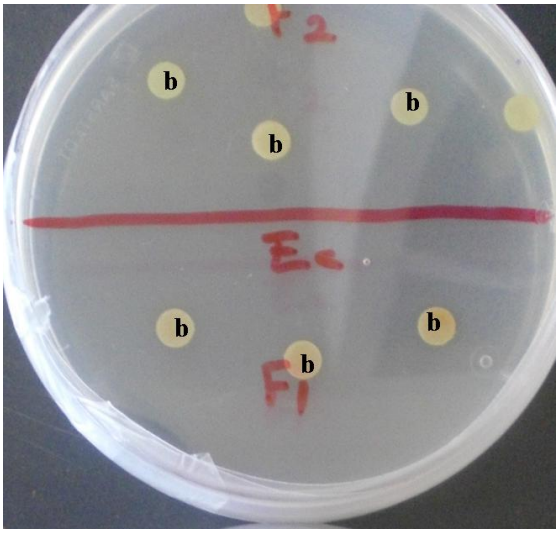
i) Solvent extracts (dried samples)



ii) Positive control (sensitivity discs)



iii) Aqueous extract (fresh/cooked samples)



iv) Negative control (methanol/water)

Plate 4: The inhibition zones of the extract and the controls. i) The solvent (methanol) extract of the dried ILV samples. ii) The positive control (antimicrobial sensitivity discs containing 8 antibiotics and 2 antimycotics). iii) The aqueous extracts of the fresh and cooked ILV samples. iv) The negative control (distilled water and methanol). The clear part around the discs indicates the zones of inhibition. The discs labelled (a) indicates the disc with inhibition, whereas (b) indicates the discs with no inhibition on the organisms.

Table 13: Bio-assay of the fresh ILV aqueous extract using disc diffusion method

Sample	Conc (mg/ml)	Zone of inhibition (mm)					
		E^c	S^a	B^s	P^a	C^a	A^n
Corchorus olitorius	100	2.3^{b}±2.3	4.7^{b}±2.3	2.3^{b}±2.3	0.0^{c}±0.0	0.0^{b}±0.0	0.0^{b}±0.0
	150	4.7^{b}±2.3	7.7^{ab}±0.3	7.0^{a}±0.0	4.7^{b}±2.3	4.7^{ab}±2.3	0.0^{b}±0.0
	200	7.7^{ab}±0.3	9.0^{a}±0.0	7.0^{a}±0.0	7.0^{ab}±0.0	4.7^{ab}±2.3	4.7^{a}±2.3
	250	11.0^{a}±0.6	10.7^{a}±0.3	9.0^{a}±0.6	9.7^{a}±0.7	8.3^{a}±0.3	7.3^{a}±0.3
LSD		5.49	3.88	3.92	3.96	5.41	3.84
Crotalaria ochroleuca	100	2.3^{b}±2.3	2.3^{b}±2.3	0.0^{c}±0.7	0.0^{c}±0.0	0.0^{b}±0.0	0.0^{b}±0.0
	150	8.3^{a}±0.3	2.3^{b}±2.3	0.0^{c}±0.0	7.7^{b}±0.3	0.0^{b}±0.0	0.0^{b}±0.0
	200	9.7^{a}±0.9	8.3^{a}±0.3	7.7^{b}±0.0	8.7^{a}±0.3	2.3^{b}±2.3	0.0^{b}±0.0
	250	10.7^{a}±0.7	11.0^{a}±0.0	9.3^{a}±0.3	9.3^{a}±0.3	8.0^{a}±0.0	4.7^{a}±2.3
LSD		4.25	5.41	1.22	0.94	3.80	3.80
Cleome gynandra	100	8.7^{b}±0.9	8.3^{b}±0.7	7.3^{b}±0.3	0.0^{c}±0.0	0.0^{b}±0.0	0.0^{c}±0.0
	150	9.7^{b}±0.3	9.3^{b}±0.9	7.7^{b}±0.3	5.0^{b}±2.5	5.0^{ab}±2.5	0.0^{c}±0.0
	200	11.3^{ab}±0.3	11.3^{a}±0.3	8.3^{b}±0.3	8.7^{ab}±0.3	7.3^{a}±0.3	7.3^{b}±0.3
	250	13.0^{a}±0.6	12.7^{a}±0.3	11.7^{a}±0.3	9.7^{a}±0.3	4.7^{ab}±2.3	9.7^{a}±0.7
LSD		1.88	1.96	1.09	4.17	5.62	1.22
Solanum scabrum	100	7.0^{b}±0.0	2.3^{b}±2.3	4.7^{b}±2.3	0.0^{b}±0.0	0.0^{b}±0.0	0.0^{b}±0.0
	150	8.0^{b}±0.6	8.7^{a}±0.3	4.7^{b}±2.3	4.7^{ab}±2.3	0.0^{b}±0.0	0.0^{b}±0.0
	200	10.0^{a}±0.7	11.3^{a}±0.3	8.0^{ab}±0.0	4.7^{ab}±2.3	0.0^{b}±0.0	2.3^{b}±2.3
	250	11.0^{a}±0.6	12.0^{a}±1.0	10.3^{a}±0.9	9.0^{a}±0.0	7.0^{a}±0.0	8.7^{a}±0.3
LSD		1.63	4.21	5.57	5.38	0.00	3.84

Results are given as mean of zone of inhibitions in mm ± SEM (n=3). Values < 6 mm show resistant while > 6 are sensitive. E^c= *E. coli*, S^a= *S. aureus*, B^s= *B. subtilis*, P^a= *P. aeruginosa*, C^a=*C. albicans*, A^n=*A.niger*. SEM= Standard error of the mean. LSD= Least significant difference.

Table 14: Bio-assay of the shade dried ILV methanolic extract using disc diffusion method

Sample	Conc (mg/ml)	Zone of inhibition (mm)					
		E^c	S^a	B^s	P^a	C^a	A^n
Corchorus olitorius	100	2.3^{b}±2.3	0.0^{b}±0.0	7.0^{c}±0.0	0.0^{d}±0.0	0.0^{d}±0.0	0.0^{b}±0.0
	150	2.3^{b}±2.3	2.3^{b}±2.3	8.3^{c}±0.7	5.0^{c}±2.5	4.7^{c}±2.3	2.3^{b}±2.3
	200	10.3^{a}±0.3	9.0^{a}±1.2	11.7^{b}±0.3	10.0^{b}±0.6	9.7^{b}±0.7	5.0^{b}±2.5
	250	15.7^{a}±0.3	10.7^{a}±0.7	16.7^{a}±0.9	17.3^{a}±0.3	14.3^{a}±0.3	13.7^{a}±0.3
LSD		5.44	4.38	1.88	4.25	3.99	5.62
Crotalaria ochroleuca	100	4.7^{c}±2.3	7.3^{c}±0.3	8.0^{c}±0.6	0.0^{b}±0.0	4.7^{c}±2.3	0.0^{b}±0.0
	150	8.0^{c}±0.6	7.3^{c}±0.3	9.1^{c}±0.0	4.7^{b}±2.3	8.0^{c}±0.0	0.0^{b}±0.0
	200	11.3^{b}±0.3	11.7^{b}±0.3	11.0^{b}±0.0	4.7^{b}±2.3	10.7^{ab}±0.3	0.0^{b}±0.0
	250	16.3^{a}±0.3	17.3^{a}±0.3	17.7^{a}±0.9	14.7^{a}±0.3	12.3^{a}±0.9	7.3^{a}±0.3
LSD		3.99	1.09	1.72	5.41	4.10	0.54
Cleome gynandra	100	4.7^{b}±2.3	0.0^{c}±0.0	8.7^{c}±0.3	4.7^{c}±2.3	5.0^{b}±2.5	0.0^{c}±0.0
	150	7.7^{b}±0.3	9.0^{b}±0.6	10.0^{c}±0.6	9.3^{b}±0.3	8.3^{b}±0.3	2.3^{c}±2.3
	200	13.7^{a}±0.4	12.3^{a}±0.3	13.3^{b}±0.3	12.7^{b}±0.3	13.7^{a}±0.7	9.3^{b}±0.9
	250	17.3^{a}±0.3	15.3^{a}±2.7	18.0^{a}±0.6	17.3^{a}±0.3	17.0^{a}±0.6	15.3^{a}±0.7
LSD		3.92	4.48	1.54	3.92	4.38	4.21
Solanum scabrum	100	7.3^{c}±0.3	7.3^{c}±0.3	7.3^{c}±0.3	2.3^{c}±2.3	0.0^{b}±0.0	0.0^{a}±0.0
	150	8.0^{c}±0.6	7.7^{c}±0.3	7.7^{c}±0.3	8.7^{b}±0.3	2.3^{b}±2.3	2.3^{a}±2.3
	200	10.0^{b}±0.0	11.0^{b}±0.6	12.7^{b}±0.7	9.3^{b}±0.3	4.7^{ab}±2.3	0.0^{a}±0.0
	250	16.7^{a}±0.7	17.3^{a}±0.3	17.3^{a}±0.3	17.0^{a}±0.0	8.3^{a}±0.7	0.0^{a}±0.0
LSD		1.54	1.33	1.44	3.88	5.49	3.80

Results are given as mean of zone of inhibitions in mm ± SEM (n=3). Values < 6 mm show resistant while > 6 are sensitive. E^c= *E. coli*, S^a= *S. aureus*, B^s= *B. subtilis*, P^a= *P. aeruginosa*, C^a=*C. albicans*, A^n=*A.niger*. SEM= Standard error of the mean. LSD= Least significant difference.

Table 15: Bio-assay of the solar dried ILV methanolic extract using disc diffusion method

Sample	Conc (mg/ml)	Zone of inhibition (mm)					
		***E*^{c}**	***S*^{a}**	***B*^{s}**	***P*^{a}**	***C*^{a}**	***A*^{n}**
Corchorus olitorius	100	$7.3^{c}\pm0.3$	$4.7^{c}\pm2.3$	$4.7^{b}\pm2.3$	$9.3^{c}\pm0.3$	$4.7^{b}\pm2.3$	$2.3^{a}\pm2.3$
	150	$9.3^{c}\pm1.5$	$12.7^{b}\pm1.2$	$5.3^{b}\pm2.7$	$10.3^{c}\pm0.9$	$4.7^{b}\pm2.3$	$4.7^{a}\pm2.3$
	200	$12.7^{ba}\pm0.3$	$12.7^{b}\pm0.3$	$12.7^{a}\pm0.3$	$14.3^{b}\pm0.3$	$5.0^{ab}\pm2.5$	$5.0^{a}\pm2.5$
	250	$17.0^{a}\pm0.6$	$19.3^{a}\pm0.9$	$16.7^{a}\pm0.3$	$17.7^{a}\pm0.3$	$11.7^{a}\pm0.3$	$8.7^{a}\pm0.3$
LSD		2.66	4.55	5.90	1.72	6.78	6.79
Crotalaria ochroleuca	100	$0.0^{c}\pm0.0$	$2.3^{c}\pm2.3$	$7.7^{c}\pm0.7$	$4.7^{b}\pm2.3$	$0.0^{c}\pm0.0$	$0.0^{c}\pm0.0$
	150	$5.0^{b}\pm2.5$	$9.3^{b}\pm1.2$	$8.0^{c}\pm0.6$	$4.7^{b}\pm2.3$	$2.3^{c}\pm2.3$	$0.0^{c}\pm0.0$
	200	$8.7^{ab}\pm0.3$	$10.7^{b}\pm0.3$	$12.7^{b}\pm0.3$	$13.3^{a}\pm0.7$	$8.7^{b}\pm0.3$	$4.7^{b}\pm2.3$
	250	$10.7^{a}\pm0.3$	$17.7^{a}\pm1.5$	$19.3^{a}\pm0.9$	$17.3^{a}\pm0.3$	$14.7^{a}\pm0.7$	$9.7^{a}\pm0.3$
LSD		4.18	4.92	2.11	5.52	3.99	3.84
Cleome gynandra	100	$7.7^{c}\pm0.3$	$8.0^{c}\pm0.6$	$8.7^{c}\pm0.9$	$4.7^{c}\pm2.3$	$5.0^{b}\pm2.5$	$0.0^{c}\pm0.0$
	150	$11.3^{bc}\pm1.2$	$9.7^{c}\pm0.9$	$10.0^{c}\pm0.6$	$8.3^{c}\pm0.9$	$8.0^{b}\pm0.6$	$5.0^{b}\pm2.5$
	200	$13.3^{b}\pm0.7$	$15.7^{b}\pm0.9$	$14.7^{b}\pm0.3$	$13.3^{b}\pm0.7$	$12.7^{a}\pm0.3$	$12.0^{a}\pm0.6$
	250	$19.7^{a}\pm1.8$	$22.0^{a}\pm1.5$	$16.7^{a}\pm0.3$	$18.3^{a}\pm0.9$	$17.0^{a}\pm0.6$	$14.7^{a}\pm0.7$
LSD		3.69	3.35	1.88	4.55	4.35	4.35
Solanum scabrum	100	$8.3^{c}\pm0.3$	$0.0^{d}\pm0.0$	$8.3^{c}\pm0.3$	$8.7^{c}\pm0.3$	$2.3^{a}\pm2.3$	$0.0^{a}\pm0.0$
	150	$10.3^{c}\pm1.8$	$10.0^{c}\pm0.6$	$8.7^{c}\pm0.3$	$9.3^{c}\pm0.3$	$4.7^{a}\pm2.3$	$0.0^{a}\pm0.0$
	200	$14.7^{b}\pm0.9$	$14.7^{b}\pm0.3$	$10.7^{b}\pm0.7$	$10.7^{b}\pm0.6$	$7.7^{ab}\pm0.3$	$0.0^{a}\pm0.0$
	250	$18.3^{a}\pm0.3$	$19.0^{a}\pm1.5$	$16.7^{a}\pm0.9$	$16.3^{a}\pm0.7$	$10.3^{a}\pm0.3$	$0.0^{a}\pm0.0$
LSD		3.31	2.72	1.96	1.72	5.44	0

Results are given as mean of zone of inhibitions in mm ± SEM (n=3). Values < 6 mm show resistant while > 6 are sensitive. *E*^{c}*= E. coli, S*^{a}*= S. aureus, B*^{s}*= B. subtilis, P*^{a}*= P. aeruginosa, C*^{a}*=C. albicans, A*^{n}*=A.niger.* SEM= Standard error of the mean. LSD= Least significant difference.

Table 16: Bio-assay of the cooked ILV aqueous extract using disc diffusion method

Sample	Conc (mg/ml)	Zone of inhibition (mm)					
		***E*^{c}**	***S*^{a}**	***B*^{s}**	***P*^{a}**	***C*^{a}**	***A*^{n}**
Corchorus olitorius	100	$2.3^{b}\pm2.3$	$0.0^{b}\pm0.0$	$2.3^{ab}\pm2.3$	$0.0^{b}\pm0.0$	$0.0^{b}\pm0.0$	$0.0^{b}\pm0.0$
	150	$2.3^{b}\pm2.3$	$2.3^{ab}\pm2.3$	$2.3^{ab}\pm2.3$	$4.7^{a}\pm2.3$	$0.0^{b}\pm0.0$	$0.0^{b}\pm0.0$
	200	$7.0^{ab}\pm0.0$	$2.3^{ab}\pm2.3$	$0.0^{b}\pm0.0$	$7.7^{a}\pm0.3$	$0.0^{b}\pm0.0$	$0.0^{b}\pm0.0$
	250	$9.0^{a}\pm0.6$	$7.7^{a}\pm0.7$	$7.7^{a}\pm0.3$	$8.0^{a}\pm0.6$	$7.3^{a}\pm0.3$	$4.7^{a}\pm2.3$
LSD		5.46	5.49	5.41	3.96	0.54	3.80
Crotalaria ochroleuca	100	$0.0^{b}\pm0.0$	$0.0^{b}\pm0.0$	$0.0^{b}\pm0.0$	$0.0^{b}\pm0.0$	$0.0^{a}\pm0.0$	$0.0^{a}\pm0.0$
	150	$0.0^{b}\pm0.0$	$4.7^{ab}\pm2.3$	$5.0^{a}\pm2.5$	$2.3^{ab}\pm2.3$	$0.0^{a}\pm0.0$	$0.0^{a}\pm0.0$
	200	$2.3^{b}\pm2.3$	$4.7^{ab}\pm2.3$	$7.7^{a}\pm0.3$	$8.0^{a}\pm0.0$	$0.0^{a}\pm0.0$	$0.0^{a}\pm0.0$
	250	$9.0^{a}\pm0.0$	$8.3^{a}\pm0.3$	$7.7^{a}\pm0.3$	$5.7^{ab}\pm2.8$	$2.3^{a}\pm2.3$	$0.0^{a}\pm0.0$
LSD		3.80	5.41	4.17	6.00	3.80	0.00
Cleome gynandra	100	$4.7^{b}\pm2.3$	$0.0^{b}\pm0.0$	$2.3^{b}\pm2.3$	$0.0^{b}\pm0.0$	$0.0^{b}\pm0.0$	$0.0^{b}\pm0.0$
	150	$7.3^{ab}\pm0.3$	$2.3^{b}\pm2.3$	$0.0^{a}\pm0.0$	$4.7^{a}\pm2.3$	$4.7^{a}\pm2.3$	$0.0^{b}\pm0.0$
	200	$8.0^{ab}\pm0.0$	$8.7^{a}\pm0.3$	$2.3^{b}\pm2.3$	$7.3^{a}\pm0.3$	$7.0^{a}\pm0.0$	$4.7^{a}\pm2.3$
	250	$9.3^{a}\pm0.9$	$9.7^{a}\pm0.3$	$9.3^{a}\pm1.2$	$8.0^{a}\pm0.6$	$7.7^{a}\pm0.3$	$8.0^{a}\pm0.6$
LSD		4.10	3.88	5.73	3.96	3.84	3.92
Solanum scabrum	100	$0.0^{b}\pm0.0$	$0.0^{b}\pm0.0$	$0.0^{b}\pm0.0$	$0.0^{b}\pm0.0$	$0.0^{a}\pm0.0$	$0.0^{a}\pm0.0$
	150	$0.0^{b}\pm0.0$	$0.0^{b}\pm0.0$	$0.0^{b}\pm0.0$	$2.3^{b}\pm2.3$	$0.0^{a}\pm0.0$	$0.0^{a}\pm0.0$
	200	$2.3^{b}\pm2.3$	$0.0^{b}\pm0.0$	$2.3^{b}\pm2.3$	$4.7^{ab}\pm2.3$	$0.0^{a}\pm0.0$	$0.0^{a}\pm0.0$
	250	$8.3^{a}\pm0.3$	$8.3^{a}\pm0.7$	$9.0^{a}\pm0.6$	$8.0^{a}\pm0.6$	$0.0^{a}\pm0.0$	$0.0^{a}\pm0.0$
LSD		3.84	1.09	3.92	5.46	0.00	0.00

Results are given as mean of zone of inhibitions in mm ± SEM (n=3). Values < 6 mm show resistant while > 6 are sensitive. *E*^{c}= *E. coli, S*^{a}= *S. aureus, B*^{s}= *B. subtilis, P*^{a}= *P. aeruginosa, C*^{a}=*C. albicans, A*^{n}=*A.niger.* SEM= Standard error of the mean. LSD= Least significant difference.

Table 17: Inhibition zone diameters of the controls

Control	Conc	Zone of inhibition (mm)					
	µg/disc	E^c	S^a	B^s	P^a	C^a	A^n
Augmentin	30	7.5±3.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Nitrofuranicin	50	9.0±2.0	9.5±0.5	7.5±0.5	7.5±0.5	0.0±0.0	0.0±0.0
Norfloxacin	10	29.0±1.0	23.5±3.5	20.1±2.0	17.5±0.5	0.0±0.0	0.0±0.0
Ampicillin	25	7.5±3.0	9.2±1.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Gentamicin	10	19.1±0.0	20.0±0.0	13.0±0.0	14.0±0.0	0.0±0.0	0.0±0.0
NalidixicAcid	30	16.0±2.0	16.0±4.0	21.5±0.5	18.5±0.5	19.5±5.5	0.0±0.0
Clotrimazole	50	9.0±0.5	0.0±0.0	0.0±0.0	0.0±0.0	31.5±1.5	0.0±0.0
Fluconazole	35	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	23.0±1.0	0.0±0.0
Methanol	-	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Water	-	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

Results are given as mean of zone of inhibitions in mm ± SEM (n=3). Values < 6 mm show resistant while > 6 are sensitive. E^c= *E. coli*, S^a= *S. aureus*, B^s= *B. subtilis*, P^a= *P. aeruginosa*, C^a=*C. albicans*, A^n=*A.niger*. SEM= Standard error of the mean.

Table 17 presents the data for the controls. Despite the fact that the antibiotics were not expired, augmentin exhibited low sensitivity (7.5±3.5mm) only in *E. coli* but was not active for *S. aureus*, *B. subtilis* and *P. aeruginosa*. Ampicillin on the other hand, exhibited sensitivity in *E. coli* and *S. aureus* (7.5±3.0 and 9.2±1.0mm, respectively) but not in *B. subtilis* and *P. aeruginosa*. This suggests that, these antibiotics might not be effective against the tested bacterial pathogens due to resistance by the bacteria. The two antimycotics; clotrimazole and fluconazole were effective on *C. albicans* but inactive on *A. niger*. The negative controls showed no inhibition on the bacterial and fungal isolates.

4.7.2 Minimum inhibitory concentration

The results of MICs are as shown in Table 18. This agrees with the results in section 4.5.1 as *C. gynandra* extracts exhibited best activities with an MIC of below 200 mg/ml

with most organisms. Similarly the four bacteria were highly inhibited by almost low concentrations of the ILV extracts, whereas the fungi were less inhibited. The MIC of all the ILV extracts against *C. albicans* and *A. niger* was greater than 200 mg/ml and 250 mg/ml, respectively. The MIC determination for the antimicrobials was not done because of the testing kits.

Table 18: Minimum inhibitory concentration of the ILVs against various micro-organisms

Vegetable	**Treatment**	**Minimum inhibitory concentration (mg/ml)**					
		***E*^{c}**	***S*^{a}**	***B*^{s}**	***P*^{a}**	***C*^{a}**	***A*^{n}**
Corchorus olitorius	Fresh	175±17	125±0	200±8	200±17	275±25	325±33
	Shade	175±25	150±8	100±0	175±8	225±0	300±17
	Solar	125±0	125±17	200±25	100±0	200±8	250±8
	Cooked	225±33	250±25	250±33	175±17	300±17	350±17
Crotalaria ochroleuca	Fresh	150±8	200±25	250±17	150±8	250±15	300±25
	Shade	125±17	150±0	100±8	200±25	225±17	275±33
	Solar	175±33	150±17	100±0	150±0	200±0	250±8
	Cooked	250±25	200±33	175±8	200±17	350±25	350±17
Cleome gynandra	Fresh	100±0	100±0	100±17	175±17	225±25	275±17
	Shade	125±8	150±17	100±8	125±0	200±17	275±8
	Solar	100±0	100±17	100±0	125±8	175±8	250±25
	Cooked	200±17	200±25	250±25	200±25	250±17	325±17
Solanum scabrum	Fresh	175±8	150±8	150±8	175±17	275±8	325±25
	Shade	100±17	150±0	125±17	125±0	225±17	325±33
	Solar	100±0	150±17	100±0	100±8	225±8	300±8
	Cooked	250±33	250±25	250±25	225±33	300±25	350±17

Results are given as the mean minimum inhibition concentrations (mg/ml) ± SEM (n=3). *E*^{c}*= E. coli, S*^{a}*= S. aureus, B*^{s}*= B. subtilis, P*^{a} *= P. aeruginosa, C*^{a}*=C. albicans, A*^{n}*=A.niger.* SEM= Standard error of the mean

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Nutrients

Nutritive quality was evaluated through determination of the amounts of dry matter, ash, crude protein, fiber, fat, carbohydrate, ascorbic acid and beta-carotene contained in the ILV leaves. The low dry matter reflects the high moisture content of the ILVs which renders them perishable. In that case, seasonal availability limits their utilization all round the year; therefore, there is need to preserve the nutrients through proper processing techniques for safe storage with efficient nutrient retention. *Cleome gynandra* exhibited high dry matter values among the other ILVs and this signifies its high nutritional properties. On the other hand, *C. olitorius* was found to be rich in fiber and this is beneficial nutritionally, since it has been reported that food fiber aids absorption of trace elements in the gut and reduces absorption of cholesterol. Besides, adequate intake of dietary fiber can lower the risk of coronary heart disease, hypertension, constipation, diabetes, colon and breast cancer (Ishida *et al*., 2000).

The mineral composition data indicated that all the ILVs are, on the average, good sources of minerals, except zinc. This agrees with the findings of Mnzava (1997). However, among the ILVs, *S. scabrum* exhibited relatively higher calcium content while *C. gynandra* had high manganese, calcium and sodium. On the other hand, *C. ochroleuca* apparently had superior amount of potassium, whereas *C. olitorius* exhibited

significantly higher zinc, iron, magnesium and lower potassium. Zinc was significantly low in the ILVs especially in *C. ochroleuca*, *S. scabrum* and *C. gynandra*. The mineral contents of fresh, shade and solar dried ILVs did not vary significantly ($P > 0.05$), whereas cooking led to an average loss of over 50 – 80%. This indicates that drying has no significant effect on the mineral composition of the ILVs while cooking leads to significant loss.

The high composition of minerals is advantageous since certain inorganic mineral elements intervene in therapeutic aspects such as aiding normal functioning of immune system (Anonymous, 1987). They play important roles in the maintenance of normal glucose tolerance and in the release of insulin from beta cells of islets of Langerhans (Choudhary and Bandyopadhyay, 1999). In addition, some minerals are important in connection with ischemic heart disease (Ishida *et al.*, 2000), bone formation and control of salt balance in human tissues. On the other hand, children, women of reproductive age and pregnant women need food with high iron content since they are most vulnerable to micronutrient deficiency and anemia. Iron is an essential trace element for haemoglobin formation, normal functioning of the central nervous system and in the oxidation of carbohydrates, protein and fats. Similarly, iron, zinc and manganese are antioxidant micronutrients and their presence could boost the immune system (Talwar *et al.*, 1989). In addition, some minerals such as calcium, iron, magnesium, manganese, copper and zinc are common cofactors in enzyme activity. Calcium is required as a component of the human diet, and it is essential for the full activity of many enzymes,

such as nitric oxide synthase, protein phosphatases, and adenylate kinase. Iron or manganese acts as a cofactor in catalase, an enzyme that catalyzes the conversion of hydrogen peroxide to water and oxygen. Magnesium as well is a co-factor of pyruvate dehydrogenase, an enzyme which transforms pyruvate into acetyl-CoA used in the citric acid cycle to carry out cellular respiration to release energy. With respect to this, the consumption of ILVs should be encouraged to acquire these important minerals.

The total unsaturated fatty acids were seen to be quite higher than the saturated fats. As well, the PUFA was predominantly higher than the MUFA and this was attributed to the presence of linoleic acid ($C_{18:2\Delta9;12}$) and α-linolenic acid ($C_{18:3\Delta9;12;15}$). These fatty acids are essential in the human diet since they cannot be synthesized by the body. They are also important to human immune system, as they help regulate blood pressure. This is an important component of membrane phospholipids, a precursor to arachidonic acid ($C_{20:4\Delta5;8;11;14}$) which is a critical fatty acid found in virtually all tissue membranes of humans (Glew *et al.*, 2004). These fatty acids are therefore important from the nutritional and stability point of view. Hence the intake of ILVs especially *Corchorus olitorius* and *C. ochroleuca* which contained highest amounts of the essential fatty acid would be beneficial.

Vitamin C is the most important vitamin in human nutrition that is supplied by fruits and vegetables. It is a powerful dietary antioxidant and highly sensitive to air, water, and temperature and is easily oxidized. In addition, it is water-soluble and thermo-labile. The method used here for the determination of vitamin C relies on the reaction between

ascorbic acid in the sample with 2, 6-dichlorophenol indophenol (DCIP) forming dehydro-ascorbic (DHAA) acid (Figure 6). It is instructive to note that both ascorbic acid and DHAA exhibit anti-scorbutic potency. Besides, any plant tissue contains the enzyme ascorbic acid oxidase which catalyzes the oxidation of ascorbic acid, the main biologically active form of vitamin C, to DHAA. This enzyme is sensitive to air and temperature and is denatured by high temperatures. During cooking, membranes break as temperatures increases, allowing ascorbic acid to leak into the water. However, ascorbic acid oxidase is also released as well and will continue to work on the ascorbic acid until it is denatured by higher temperature. Therefore, it implies that significant amount of vitamin C present in food may be lost by boiling and discarding the cooking water. Vitamin C content was appreciable in fresh leaves of *C. olitorius*, *C. gynandra*, and *C. ochroleuca*. Its thermo-labile nature was demonstrated by the huge loss and a significant destruction upon cooking and drying. In the case of *C. ochroleuca*, there was near total loss (about 87%) after cooking. This means that cooking in boiling water for 15 minutes, as was the case here, might be too severe if a significant residual amount is intended.

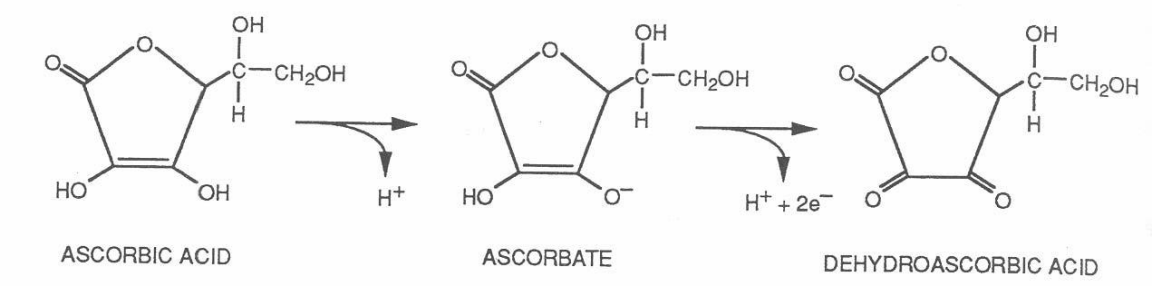


Figure 6: Degradation of ascorbic acid

Solar drying also reported a significantly higher loss of vitamin C content as compared to shade drying. This might be due to high temperatures as it has been reported for other vegetables (Mziray *et al.,* 2000). The results therefore, agree with the findings of Chaney and co-workers (Chaney *et al.,* 1979), which indicated that the most significant determinant of vitamin C content in foods is how the food is stored and prepared.

Vitamin C is important in the body and a small daily intake for an adult is required to avoid scurvy. Even in small amounts, it can protect indispensable molecules in the body, such as proteins, lipids (fats), carbohydrates, and nucleic acids from damage by free radicals and reactive oxygen species that can be generated during normal metabolism as well as through exposure to toxins and pollutants. However, there has been, and continues to be, vigorous debate on what the optimum daily intake of vitamin C should be. In an attempt to balance the competing claims, and ensure good health for the general population, the Federal Food and Drug Administration has adopted a recommended dietary allowance (RDA) of 60 mg/day for adults aged 15 or older, 15-45 mg/day for children, and 80-120mg/day for pregnant and lactating women (Brody, 1994).

The β-carotene content of the ILVs indicated that *C. gynandra* and *C. olitorius* exhibited superior β-carotene (pro-vitamin A) content than *C. ochroleuca* and *S. scabrum*. The results also indicated that fresh ILVs samples had a significantly higher β-carotene than the cooked and dried samples ($P < 0.05$). This therefore implies that cooking and drying of ILVs significantly reduces appreciable amount of β-carotene. However, the

destruction was relatively lower when initial drying temperature was low especially when dried in the shade. This agrees with the report that at high temperatures, the long chain polyunsaturated carbons undergo isomerization from the *trans*- to the *cis*- form, leading to loss of the vitamin activity (Tannenbaum, 1976). It has also been reported that light and oxidants catalyze the oxidation of β-carotene in stored dehydrated vegetables causing great losses (Gareth *et al.*, 1998). Therefore the loss of β-carotene in the dried ILVs was due to oxidation mainly by the oxygen retained in the environment and catalyzed by light.

The vitamin C contents of fresh *C. olitorius*, *C. ochroleuca*, and *C. gynandra* obtained in this study were all higher than literature values reported for cabbage, 42.3 mg/100 g fresh material, or spinach, 62.0 mg/100g fresh leaves (Lee and Kader, 2000). Similarly, the β-carotene content of the fresh ILVs were all higher than literature values reported for kales, 3.8 – 4.53 mg/100g fresh leaves (Lefsrud *et al.*, 2007). This underlines the superior nutritional quality of ILVs compared to the 'fashionable' exotic vegetables that have been embraced at the expense of ILVs.

5.1.2 Phytochemicals

Qualitative evaluation of phytochemicals was aimed simply to identify the presence of individual groups of phytochemical compounds. The ILVs was found to contain a wide array of phytochemicals. The TLC and quantitative results indicated that *S. scabrum* contained the highest percentage of phenols and tannins than the other ILVs, whereas *C. gynandra* was high in flavonoids. On the other hand, *C. ochroleuca*, *S. scabrum* and *C.*

olitorius had significantly higher alkaloid contents. On the other hand, the phytochemical components of fresh ILV samples were significantly higher ($P < 0.05$) than those of the dried and cooked samples. This might perhaps be due to the inter-conversion of these compounds into other derivatives, owing to the prolonged period of drying and cooking. However, there was no significant difference ($P > 0.05$) between the phytochemical compounds in solar and shade dried ILVs. The findings from this study are in agreement with those of work carried out in Nigeria which indicated that the presence of some of these anti-nutrients is reduced by various processing techniques (Elegbede, 1998). The risk of losing these plant chemicals as a result of cooking are therefore well founded and hence, the vegetables should be cooked in minimal heat and within a short time.

Phytochemicals act as antioxidants, suppressors of tumor growth, anti-mutagens, enzyme modulators, chemical inactivators and free radical scavengers. Flavonoids, tannins and saponins have been reported to have medicinal properties such as antimicrobial and antioxidant properties (Stephen *et al.,* 2009). Alkaloids on the other hand, are heterocyclic indole compounds which have proved to have pharmacological properties such as hypotensive activity, anticonvulsant activity, antiprotozoal, antimicrobial and antimalarial activities (Mallikharjuna *et al.,* 2007). They also have pronounced physiological effects particularly on the nervous system (Stephen *et al.,* 2009). Plants have the limitless ability to synthesize phenols or their derivatives. These phenolic compounds give the vegetable an astringent taste. They also bind proteins and

may lower protein digestibility and quality as well as reducing the risk of heart disease and certain types of cancers. The presence of phytochemicals in ILVs justifies their potential pharmacological and medicinal value. Therefore, their constant consumption is beneficial and should be encouraged.

5.1.3 Antioxidant activity

Biological molecules in the body are subject to free-radical attack causing damage (to DNA and membrane lipids) and cell impairment/death, eventually leading to disease conditions such as cancer, cardiovascular disease, rheumatoid arthritis, diabetes, and neurological disorders (Valko *et al.*, 2007). Antioxidants are substances that neutralize free radicals or their actions. They are mainly derived from dietary constituents (minerals, vitamins and phytochemicals); hence the need to know their composition in foods is imperative. The antioxidant activity was examined using DPPH by comparing the free radical scavenging activity of ILV methanolic extract with known antioxidants such as ascorbic acid. The various ILV extracts had significant scavenging effects and almost all reported a significantly higher percentage of DPPH inhibition than ascorbic acid ($P < 0.05$). It was demonstrated that the scavenging effects of all extracts on DPPH radicals increased as the concentration increased and it reached a maximum and then leveled off or reduced with further increase in the concentration of the extracts. This illustrated that the antioxidative activities of the ILV methanolic extracts are concentration dependent. However, the increase in activity was only marginal when the extract concentrations were higher than 1 mg/ml. This might probably be due to the

scavenging of all the DPPH radicals by higher concentrations of the extracts reducing their activity. The scavenging effect was highest in the case of *C. olitorius* and *C. gynandra* as compared to *C. ochroleuca* and *S. scabrum*. Subsequently, lower IC_{50} value reflects better protective action. Therefore, using the IC_{50} and the maximum DPPH percentage inhibition values, it was found out that the extracts of *C. gynandra* and *C. olitorius* were the most effective. This is because they exhibited low IC_{50} values and high percentage inhibition values compared to the other extracts. Thus indicates that the two ILVs have the strongest radical scavenging activity. On the other hand, there was no significant difference ($P > 0.05$) in the activity of the solar and shade dried ILV samples since their percentage radical scavenging effect was within close range. This suggests that using extracts from either solar or shade dried ILVs would relatively give the same effect. Therefore, the drying methods do not have any adverse effects on the radical scavengers.

5.1.4 Antimicrobial activity

Initial screening of plants for possible antimicrobial activity typically begins by crude extracts obtained using water or various organic solvents. Since nearly all of the components identified from plants that are active against microorganisms are aromatic or saturated organic compounds, they are most often obtained through initial ethanol or methanol extraction. In this study, methanol extraction was employed for the dried ILVs, whereas aqueous extraction was used for fresh samples. The highest antimicrobial activity was observed in *C. gynandra* extracts giving an MIC of below 200 mg/ml

against most of the organisms. *Bacillus subtilis* was highly inhibited by almost all the ILV extracts and this was comparable with the antibiotics. On the other hand, *A. niger* was highly resistant to the extract and antimycotics (fluconazole and clotrimazole). The results agree with other *in vitro* studies which indicate that *A. niger* is not sensitive to fluconazole even at higher concentrations (Usha *et al.,* 2006). Other results also indicate that *A. niger* is resistant to the commonly used drugs. As well, there were high inhibition zones on the solar and shade dried ILVs as compared to the fresh and cooked ILVs. This could be due to the concentration or accumulation of bioactive substances with inhibitory effects during drying. Similarly methanol is the best extraction solvent and might have extracted more inhibitory substances than water. Apparently, cooking of the ILVs affected the sensitivity of the extracts and this might be due to thermal degradation of the active ingredients. This is an area that requires further research to establish the exact route for loss of inhibitory potency as well as developing alternative processing techniques to preserve this medicinal benefit in ILV leaves.

5.2 Conclusions

Findings from this study indicate that the selected commonly consumed ILVs in Kenya are nutritious and can provide important nutrients needed for normal body function and maintenance. Apparently, *C. olitorius* is a relatively superior source of vitamin C, protein, fat and fiber; while *C. gynandra* popular with western Kenya communities, could be a good source of minerals especially manganese, calcium and sodium. It also exhibited over and above the normal recommended adult daily allowance of β-carotene

(pro-vitamin A) and vitamin C. *Crotalaria ochroleuca* and *S. scabrum* on the other hand, reported intermediate nutritional quantities. In addition, the ILVs are poor sources of fat making them good alternative food for obese people.

This study presents data that attests to the importance of ILVs in providing the much-needed dietary nutraceutical potential since they have different contents of phytochemicals and antioxidants. Generally, among the four ILVs, *C. gynandra* had significantly higher amount of flavonoid; *C. olitorius, C. ochroleuca* and *S. scabrum* exhibited relatively high alkaloid and *S. scabrum* contained high amount of phenols & tannins. The antioxidant activity of the ILV extracts was also remarkable especially in *C. gynandra* and *C. olitorius.*

Similarly, all the ILVs investigated were effective against all the bacterial strains tested and less effective on the fungal strains. *Cleome gynandra* and *C. olitorius* extracts had a significant antimicrobial activity, whereas *C. ochroleuca* and *S. scabrum* were not active especially to *A. niger*. Besides, the results were comparable to those of the standard antimicrobials. Thus, encouragement of people towards consumption of ILVs should be increased to enable full derivation of the nutrients.

Though the ILVs in particular contain relatively high levels of some nutrients, the processing and preparation methods prior to consumption reduce their final consumed amount. This is through leaching and oxidation due to cooking and drying. Therefore, it is essential to prepare vegetables in a manner that will retain maximum amount of

nutrients. This involves, drying the ILVs in dark and dry place under low temperature and also cooking should be within a short time for best retention of nutrients.

Finally, the data generated from this study provides an important insight on the value of the ILVs. It is therefore clear that, ILVs contain different bioactive compounds and nutritional qualities which when combined could be of tremendous benefit to the body. This provides the chemical basis of the ILVs which may be handy in probing their biochemistry. Because prevention is a more effective strategy than treatment, a constant supply, consumption and utilization of ILVs is essential to reduce the risk of diseases and malnutrition.

5.3 Recommendations

The recommendations that have been identified from the study include the need to:

1) Investigate, isolate and elucidate the compounds responsible for antioxidant and antimicrobial activity in ILVs using spectroscopic studies.
2) Carry out metabolomic fingerprinting of these ILVs to access the profiles of secondary metabolites in them.
3) Investigate better processing and preparation methods which will improve valorization strategies of ILVs.
4) Promote the production, utilization and conservation of ILVs through educational programs and market linkages to communities.
5) Promote diversification of ILVs among farmers by increasing their potential through improved seed production, breeding and selection.

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APPENDICES

Appendix I: Pictures of the target African Leafy Vegetables

a) Spider Plant *(Cleome gynandra)*

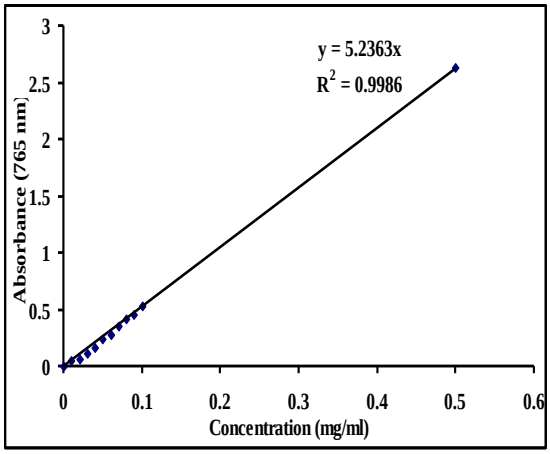
b) Jute mallow (*Corchorus olitorius)*

c) Slenderleaf *(Crotalaria ochroleuca)*

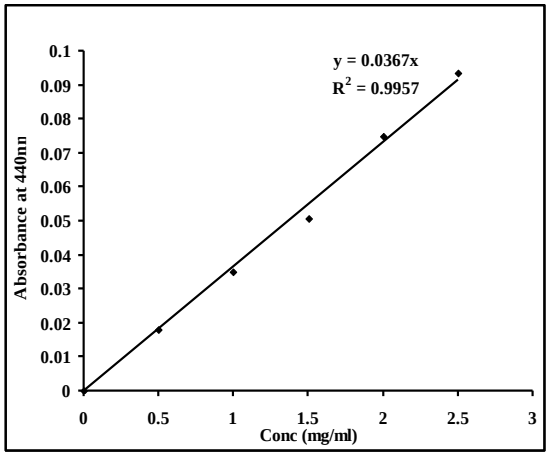
d) Nightshade (*Solanum scabrum)*

Appendix II: Standard curves

a) Gallic acid



b) Beta carotene



c) Tannic acid