# THE EFFECT OF SOIL AND CLIMATIC FACTORS ON ANTIMICROBIAL AND PHYTOCHEMICAL COMPOSITION OF STINGING NETTLE (*Urtica massaica)*

**BY**

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# DECLARATION

## DECLARATION BY THE CANDIDATE

I declare that this research thesis is my original piece of work presented to the Biological Sciences Department in the School of Science of the University of Eldoret and has not been presented elsewhere or in any other institution for an academic award or any other award.

**Kipruto Allan**

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**SC/PGB/028/14** **Date**

## DECLARATION BY THE SUPERVISORS

This research thesis has been submitted with our approval for examination as the university supervisors.

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## DEDICATION

I dedicate this research work to my family and friends who have all supported me throughout this important academic step in my life. Thank you all for inspiring me to attain this goal and above all I give thanks to The Almighty God for everything.

## ABSTRACT

Emerging multidrug resistance by many microorganisms callsfor exploration of new sources of drug alternatives. Plants are largely unexplored source of drug repository. Medicinal plants have great potential for providing novel drug leads with novel mechanism of action. Ecological and environmental factors have been reported to affect plant phytochemical contents. Soil and climate are some of these factors that are considered in the current study in the efficacy of *Urtica massaica* against some pathogenic bacteria.Therefore, the present study aimed at determining the antimicrobial activity and phytochemical profile of *Urtica massaica.*The study was conducted atthe Centre for Microbiology Research, Kenya Medical Research Institute. Plant samples were collected from Eldoret, Kericho, Kitale and Marigat;Kenya. The plants were dried in an oven to moisture content of 13%, and ground into fine powder, weighed and stored.Crude extracts from the stored fine powder were prepared using hot water and methanol.Reference (ATCC) strains and clinical isolates of *Staphylococcus aureus, Escherichia coli, Aspergillus flavus* and *Candida albicans* were used in bioassay testing of crude extracts at different concentrations.The antimicrobial activity of the extracts was determined by measuring the zones of inhibition using the disc diffusion method. The minimum inhibitory concentration for the extracts against each microorganism was determined and phytochemical screening was done on the crude extracts to determine the phytochemicals present. Analysis of variance was done to determine the difference in inhibition zones between methanolic and aqueous extracts of *U. massaica*. Significant differences ($p<0.05$) in inhibition zones between the crude extracts of *U. massaica* and positive control (Gentamycin and Ciprofloxacin) were reported against the selected group of microorganisms. However, non-significant differences ($P>0.05$) in inhibition zones between aqueous and methanolic crude extracts of *U. massaica* were reported among the four groups of microorganisms used in the study.Inhibition zones of aqueous crude extracts of *U. massaica* arraigned between 6.50 and 6.67mm while those of methanolic extracts were between 6.33 and 8.42mm.The aqueous crude extracts of *U.massaica* did not show any antimicrobial activity (6.00mm) against *E. coli.* There were no significant differences ($p>0.05$) in inhibition zones among the test plants from the four regions of the study. Inhibition zones of *U. massaica* crude extracts sampled from Kericho, Kitale, Marigat and Eldoret ranged between 13.08-14.75mm, 13.17-13.83mm, 13.17-14.00mm and 13.08-14.67mm respectively.The methanolic extract of the *U. massaica* from Eldoret showed presence of saponins, tarpenoids, steroids and flavonoids. Extracts from Kitale and Kericho recorded the presence of alkaloids, steroids and flavonoids, while those from Marigat reported the presence of saponins, tarpenoids, steroids and tannins. It was concluded that themethanolic extracts of *U. massaica* could bepotential source of drug formulation against the bacterial microorganisms (*S. aureus* and *E. coli*) and fungi (*C. albicans* and *A. flavus*).

# TABLE OF CONTENT

## LIST OF TABLES

# LIST OF PLATES

# LIST OF ABBREVIATIONS

AIDS – Acquired Immunodeficiency Virus

ASL – Above Sea Level

ATCC - American Type Culture Collection

CMR - Centre of Microbiology Research

CTMDR - Centre for Traditional medicine and Drug Research

CYP - Cytochromes P450

DMSO - Dimethyl sulphoxide

EAEC - Enteroaggregative *E. coli*

ETEC - Enterotoxigenic *E. coli*

FDA - Food and Drug Administration

GC - Gas Chromatography

GCMS - Gas chromatography Mass Spectrophotometry

GRAS - Generally Recognized as Safe

HIV - Human Immunodeficiency Virus

HP - Hewlett Packard

HPLC - High Performance Liquid Chromatography

IZD - Inhibition zone diameter

JICA - Japanese International Corporation Agency

KEMRI - Kenya Medical Research Institute

LPS - Lipopolysaccharide

MDR - Multi drug resistant

MHA - Müller-Hinton agar

MIC - Minimum inhibitory concentration

MRSA - methicillin-resistant *S. aureus*

MS - Mass Spectrophotometer

PBP - penicillin binding proteins

RIZD - Relative inhibition zone diameter

TLC - Thin Layer Chromatography

WHO - World Health Organization

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I would also like to thank my sister and grandfather for the support they gave to me. God bless you all.

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background information

Plants have shown considerable activity against various microbes and have been used for the development of new medicines. Some have different activities with less adverse effects than those produced by the drugs currently in use (Markova *et al.,* 2015; Wambugu *et al.,* 2009). Besides the domestication and the culture of food plants, all the civilizations of the world have developed the therapeutic research on the basis of the plant medicinal properties, which are contained in at least one of its parts (Bye, 1981; Bruneton, 1999).

The World Health Organization WHO (2003) and Kenya government have recognized the position of herbal folklore medicinal practice in primary health practices. This isbecause studies that found that 80% of the rural communities seek services from traditional healers (Marshal, 2001; Alphonse *et al*., 2010). Furthermore, the usage of plants, plants extracts or plant-derived pure chemicals to treat diseaseshas become a therapeutic modality that has stood the test of time (Ekeanyanwu, 2011; Njoroge *et al*., 2009).

In Kenya the ethnobotanical studies of several plants used by different communities has revealed that plants are important source of medicinal products that need further research to establish their efficacy, effectiveness and quality in health services (Amuka, 2014; Orwa, 2003; Barbour *et al.,* 2004; Figueiredo, 1996). According to Charimbu *et al.*, 2009, plant fungal diseases have been controlled by the use of *Urtica massaica* so as to reduce

use of synthetic fungicides, that has led to an increase agricultural costs and contaminate the environment (Njoroge, 2003).

*Urtica massaica* the plant in this study has been used traditionally to treat ear nose and throat (ENT) infections such as common cold, otitis media, and cough, tonsillitis, asthma, and chest pains in Rwanda (Alphonse *et al*., 2010). Locally, in Kenya, *U. massaica* has been used to treat diabetes, stomachache, kidneys, diarrhea, and wounds (Njoroge andBussmann, 2006). Occassionally, some fungal diseases of plants have been controlled by the use of *U. massaica* so as to reduce use of synthetic fungicides, that has led to an increase agricultural costs and contaminate the environment (Charimbu *et al.*, 2009).

Climatic and environmental factors have been known to affect plant development and health. Phytochemical contents mainly depend on the health of the plant, which is determined by the surrounding environment. Soil and climate are some of these factors that are considered in the current study that we think affect phytochemical production. These secondary metabolites have come to be known from research to maximize the potential yield of active constituents from herb and drug plants.However, new information about how resistance should stimulate further interest in the responses of these secondary metabolites to abiotic stress such as climate and soil of the area where they are found (Gershenzon, 1984). It remains to be seen whether a steady difference will be established, and to which extent the biotic and abiotic stresses, and factors such as soil biology, contribute to phytochemical contents in these medicinal plants (Zhao *et al.*, 2006).

The pathogens investigated in this study were; *Staphylococcus aureus* a Gram-positive and is non-motile small round shaped or non-motile cocci. It is found in grape-like (staphylo-) clusters (Bong *et al*., 2013). *Staphylococcus aureus* belongs to the family *Staphylococcaceae.* It affects all known mammalian species, including humans. Further due to its ability to affect a wide range of species, *S. aureus* can be readily transmitted from one species to another. This includes transmission between humans and animals (Morens *et al.,* 2004).

*Escherichia coli* is a Gram-negative gammaproteobacterium commonly found in the lower intestine of warm-blooded organisms. People and animals normally have some *E. coli* in their intestines, but some strains that cause infection enter the body mainly through; improper food handling (Dupont *et al.,* 2007). *Aspergillus flavus* is a saprotrophic and pathogenic fungus with an international distribution. It is known for its infection of cereal grains, legumes, and nuts (Ropars *et al.,* 2012). Many strains produce significant quantities of toxic compounds known as mycotoxins, which, when consumed, are lethal to mammals (Karkowska-Kuleta *et al.,* 2009).*Candida albicans* is a yeast normal part of the human gut flora. Humans have *C. albicans* in their gut, and usually it coexists without causing disease with the other bacteria and yeasts that are found in the intestines causing candidiasis (Sekirov *et al.,* 2010; Ishijimaand Abe, 2015).

**1.2 Statement of the problem**

With the recent upsurge in multidrug-resistant bacteria, there is interest in development of new drugs both synthetic and from natural sources, including plants. Medicinal plants are the major source of diverse therapeutic compounds that are potential source of

antibiotics (Aqil *et al*., 2006).Plants are the richest resource of drugs of traditional systems of medicine, therefore there is need to tap this vast natural resource to control both present and newly emerging diseases and antimicrobial resistance. *Urtica massaica* has been used traditionally to treat ENT diseases such common cold, Otitis media, and coughs, tonsillitis, asthma and chest pains; manage diabetes, stomachache, kidneys, diarrhea and wounds (Njoroge and Bussmann., 2006; Alphonse *et al*., 2010). However, only few studies (Hamill *et al.,* 2000) have scientifically tested its efficacy to control diseases and found it has activity against bacteria, fungi and viruses. With the availability of *U. massaica* locally, it is therefore providing a good option as an alternative in the approach of alleviating certain ailments. *Urtica massaica* is widely distributed in the country and is found in different geographic areas of varying climatic conditions and soil composition of which may affect the presence and/or quantities of secondary metabolites. This forms the basis of the current study that endeavored to determine whether these factors would affect the antimicrobial activity of the plant against selected pathogens (Figueiredo *et al.,* 2008).

**1.3 Justification**

Among many African communities, *U. massaica* is used to manage various ailments (Njoroge andBussmann, 2006; Kigen, 2016). Although antimicrobial bioassays to determine the antimicrobial activity have been conducted on this plant (Njoroge and Bussmann, 2006; Alphonse *et al*., 2010; Keter and Mutiso, 2012), none has reported on its efficacy on the basis of geographical location. Hence, the need to assess the plant parts for antimicrobial activity and phytochemicals against pathogenic bacteria on the basis of

geographical location. This plant is found in various geographical/ecological zones inKenya; hence the need to ascertain whether there would be any differences in its efficacy and phytochemical composition based on geographic location.

Phytochemicals naturally occur in medicinal plants are useful in the healing as well as curing of diseases. Therefore, the need to use plant extracts and plant parts, as practiced in our communities will be of great help in combating disease. There is need for a more natural control method of enteric bacteria. Very little attention has been paid to the changes in secondary metabolism that are occurring in the plants, affecting the levels of phytochemical contents (Gershenzon, 1984).

There has also been limited study of the biotic and abiotic factors that contribute to increased phytochemical content in organic crops (Zhao *et al*., 2006). With this gap, there is need for the study of the antimicrobial properties of *U. massaica* based on the ecological and soil factors.

## 1.4 Objectives

### 1.4.1 Broad objective

To evaluate the effect of climate and soil on the antimicrobial activity of *Urtica massaica* against *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus flavus*, and *Candida albicans.*

### 1.4.2 Specific objectives

1. To evaluate the antimicrobial and antifungal activity of the leaves of *U. Massaica* on *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus flavus*, and *Candida albicans* using different extraction methods.
2. To determinethe effects of climate and soil type on the antimicrobial effect of *U. massaica.*
3. To assess the effect of climate and soil type on phytochemical composition in *U. massaica.*

## 1.5 Hypothesis

### 1.5.1 Null hypothesis

1. Leaves of *U. massaica* from different climatic regionsdo not possess antimicrobial and antifungal activity against different microorganisms using different extraction methods.
2. Differences in climatic and soil conditionshave no effect of the antimicrobial efficacy of *U. massaica* on the selected microorganisms.
3. Differences in climatic and soil conditions have no effect on phytochemical composition of *U. massaica.*

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Overview

There is limited quantitative knowledge from different regions on patterns and ways of production and consumption of weedy plants, and on changing viewpoints towards the use of these plants (Marshall, 2001). In recent years, there has been an increase of interest in the pursuit of traditional health-care practices both in the western and developing world (Sen and Chakraborty, 2017).

Above and beyond the domestication and the culture of food plants, civilisations of the world came to develop therapeutic use because of medicinal plant quality. Plants are known to be medicinal, if at least one of its parts contains medicinal properties (Alphonse *et al.*, 2010). In recent years, there has been an increase in awareness of traditional health-care practices in both the western and developing world (Van Veen, 1997). The use of and search for drugs and dietary supplements obtained from plants has greately increased in recent years. Researchon plants with phytochemicals of medicinal importance and that can be further harnesed forthe treatment of various ailments and diseases has been on the rise. Indeginous healers have long utilized plants that prevent or cure contagious diseases (Cowan, 1999). However, some of these plants have not been tested scientifically.

Worldwide, over 80% of people have Acknowledgmentsmedicinal plant species to meet their day to day healthcare uses (Tadesse *et al*., 2005). Many plant species treat more than

one disease or sometimes can be used in combinations. This model of using medicinal plant species for variousconditions has been observed in many African indeginous communities (Kamatenesi *et al.,* 2011). Herbal drugs have been used for treatment for many centuries. Many drugs in modern medicine have herbal origin (Hossein *et al.*, 2007).

In Kenya, the use of plants as a source of herbal medicinal products has been well documented and scientific research on different plants which are believed to have medicinal value in different indeginous communities is ongoing so as to increase the awareness on the medicinal importance of indigenous plants (Rukangira, 2001). There is a relative upsurge in demand for herbal medicine both at home and overseas. Medicinal plants have played a major role in meeting health needs of rural communities in Kenya. About 75-90 % of the local community upcountry depends on medicinal plants (Ruth *et al.,* 2010).

The knowledge on,and about the use of plants as medicine is threatened to extinction, due to the fact that it is passed on orally from one generation to another, and also because of differences brought about by cultural changes due to development. The issue on habitat destruction has led to diminishing medicinal plants where communities exist and their surroundings, thus affecting their access to medicinal plants mostly affecting indigenous communities, as they depend on the plants for their main source of medicine (Wanjiku, 2010).

The recent upsurge in the numbers of multidrug-resistant bacteria has triggered great interest in new drugs or preparations from natural sources, including plants. Medicinal

plants are the new important source of various bioactive and therapeutic compounds that are to be considered as a new source of antibiotics (Aqil *et al*., 2006).

Plant parts such as roots are harvested for the medicinal properties they possess. This form of harvesting of plant roots however, threatens the growth of the plant. Plant species such as *Lantana camara, Urtica massaica* have had leaves, stems and roots being harvested. Harvesting of two or more plant parts can be more harmful to the plant, especially when the roots and barks/ stem are targeted. Therefore, from the conservation viewpoint, the high utilization of roots of plant species put these plant species at a risk because of the damages inflicted on the plant species (Kamatenesi *et al.,* 2011).

Stinging nettle, in different capacities has been used therapeutically to treat various ailments. This scented plant is primarily recommended in the treatment of rheumatic conditions, lower urinary tract infections, as a nutritional tonic by the indeginous medicinal healers (Prasad, 2005). In addition, the fresh, freeze-dried leaves arebeing used for the treatment of allergies. The root is used to manage benign prostate hyperplasia (Ochwang'i *et al*., 2014). Part of the application of the herb stinging nettle is owed to its presumed anti-inflammatory activity, which can be attributed to the clinical use of the plant to treating arthritic conditions and allergies (Upton *et al.,* 2013).

### 2.2 *Urtica massaica*

Plants that are growing in unwanted areas that are not sown or cultivated are known as weeds. Such plants may be found growing on cultivation fields meant for agricultural practices (Njoroge *et al*., 2004). Weeds are mainly considered to have adverse effects on

the plants they grow among bringing stress in competition for the available plant nutrients therefore bringing about low or even poor yields (Njoroge *et al*., 2004).

There is documentation of the use of wild and leafy plantsfor food and medicine in East Africa since as 1930. It is important to consider such plants otherwise considered as weeds as food and as medicine in combination with other plants, and are a good source of phytochemicals (Marshall, 2001).

*Urtica massaica* of the family Urticaceae, a dioecious upright plant thatdepending on the biotic and abiotic conditions can grow to a length of 2m vertically. It has aninflorescence of auxiliary groups of needle-like unbranched racemesand elliptical leaves. The plant mainly grows in abandoned cultivation fields and is greately used as a vegetable by people of Kenyan rural areas. Like other *Urtica* species, *U. massaica* has rough stinging hairs on the back and leaves. Each hair consists of a capillary tube with a little sac on the tip. The sac breaks off when it comes into contact with the skin, exposing a fine needle-like structure which subsequently penetrates the skin and in the process injects some fluid into the minute wound. Therefore, a stinging sensation is felt (Maitai *et al.,* 1980). Conventionally, some plant fungal diseases have been controlled by the use of *U.massaica* so as to reduce use of synthetic fungicides that has led to an increase agricultural costs and contaminate the environment (Charimbu *et al.,* 2009).

The commonest ENT diseases that traditional healers managed through the use of traditional methods are otitis media, cough, common cold, and tonsillitis, chest pains and asthma. *Urtica massaica* is one of the plants that areused in management of ENT infections in Central highlands of Kenya (Njoroge and Bussmann, 2006). The authors found that *U. massaica* was mentioned by at least four respondents during a survey and

they reported that the useful plant parts were collected when needed at that point in time and not prior to be stored for later use. The plant was prepared in concoctions and administered orally to treat ailments like blood glucose disorders (Keter and Mutiso, 2012).

It was found 19 diseases and symptoms treated by nine traditional healers by using 77 species of plants grouped into 71 genera and 39 families with 78 medicinal receipts. In total, 19 plants gathered in 12 families were listed as being consumed by the mountain gorillas and used in traditional medicine by the surrounding traditional healers featuring prominently was the family Urticaceae at 3.93% where *Urtica massaica* belongs. *Urtica massaica* was used to treat stomachache, kidneys, Diarrhea and wounds (Alphonse *et al*., 2010).

*Urtica massaica* boiled root preparations have been used in the management of abdominal pains in infants. A tincture made from its bark may also be used, it is peeled to the bark, crushed, a little water added and administered. The same preparation can also be used to treat chicken pox and infertility in men (Kigen, 2016).

### *2.3 Staphylococcus aureus*

*Staphylococcus aureus* is a bacterium that stains Gram-positive, non-motile,small round shaped or immobile cocci. It is found in grape-like (staphylo-) clusters. This is why it is called *Staphylococcus* (Bong *et al*., 2013). *Staphylococcus aureus* is a member the family *Staphylococcaceae.* It affects all known mammalian species, including humans. Further due to its ability to affect a wide range of species, *S. aureus* can be readily passed from

one species to another. This includes transission between humans and animals and vice versa (Morens *et al*., 2004).

*S. aureus* may be found freely suspended in the air we breathe and thus transmitted through the air we inhale. When an infected person coughs or sneezes, he or she releases large amounts minute particles of saliva that remain hanging in air (Hobday and Dancer, 2013). The bacteria can be transimitted through ingestion of contaminated foods or coming into contact with materials that have been infected with the microorganism. Quite a small fraction of the human population carries *S. aureus* in their nasopharynx cavity and on the epidermis as normal flora (Davis *et al.*, 2012).

*Staphylococcus* is among the five major causes of infections post traumatic injury or surgery. It affects almost half a million patients in American health facilities annually (Kerver *et al.*, 1988). It is shortened to "*S. aureus*" or "Staph aureus" in medical literary works pertaining to bacteriological studies. *S. aureus* was discovered in Aberdeen, Scotland in 1880 by the surgeon Sir Alexander Ogston in pus from surgical abscesses (Wilson, 1987).

This bacteriumcan be found present in someones nose, pharynx and on the skin without causing any harm. Almost 30% of individualsthat are healthy and with a well built immune system may present*S. aureus* colonization of the epidermis, gastrointestinal tract, or nasopharynx and may not exhibit any signs or disease (El Lakkis and Khardori, 2014). When *S. aureus* is isolated from an abscess or boil or other skin lesion, it is usually due to its secondary invasion of a wound rather than the primary cause of disease. *S. aureus* may

similarly be isolated from abscesses, breast abscesses or mastitis, dermatitis or skin infections and genital tract infections (Livingstone and Stringer, 1999).

The existence of *S. aureus* in culture is usually very minimal since this microorganism is abundant on the skin and nasopharynx of individuals and animals. The bacterium easily inhabits the nasopharynx or skin, or by culture of suspicious absecesses. On culture the bacterial colonies are usually distinguished by a shiny, opaque, cream appearance on blood agar (Hoffstadt and Youmans, 1932).

### *2.4 Escherichia coli*

*Escherichia coli* is a bacterium that is commonly found in the lower intestine of warm-blooded living organisms. A Gram-negative gammaproteobacterium,its derivatives i.e. two isolates, K-12 and B strains, are usually employed in molecular and genetic biology as both a microorganism and a model organism (Pelletier *et al.*, 2008).

*E. coli* belongs to the domain and kingdom Bacteria since members of this group are single celled microorganisms; phylum proteobacteria in that in this group members are Gram-negative bacterium with an outer membrane mainly made of lipopolysaccharides. *E. coli* is facultatively anaerobic and therefore considered to belong to the class Gamma Proteobacteria. *E. coli* belongs to the order Enterobacteriales because members of this group are rod-shaped facultatively anaerobic G- bacterium (Felis *et al*., 2009).

*E. coli* belongs to the family Enterobacteriaceae where members of this group are motile via peritrichous flagella that grows well at $37^{o}$C, is Oxidase negative, Catalase positive, and reduces nitrates, the Genus *Escherichia* according to Keter *et al*., 2012, (which is named for the person who discovered this genus, Theodor Escherich) for the members of

this group are mostly opportunistic flora that are enteric, and species recognized under the Genus *Escherichia* is *coli* (Shulman *et al*., 2007).

*E. coli* is unique in terms of its biochemical activities whereby it ferments lactose, has the enzyme lysine decarboxylase, is Vogus-Proskauer negative, produces indole, is inhibited by nitrate, and doesn't produce $H_2S$ gas (Solidônio *et al.*, 2013).

People and animals usually have some *E. coli* in their gut, although some strains are pathogenic and cause disease. The pathogenic microorganism that cause disease can gain access into your body through; ingestion of contaminated food, whether at home or in any other food handling facility. During the slaughtering process, poultry and meat products can be contaminated with bacteria from the animals' intestines,infected water containing the bacteria either from human or animal waste; or most commonly from person-to- person (Collins, 1997). Healthcare facilites, schools, and childcare institutions are particularly susceptible to the spread of the microorganism mainly through contact (Curtis *et al.*, 2003), animals and people who work with animals, especially cows, goats, and sheep, are at increased risk of infection. Anyone who handles animals or whoseentails handling of animals should wash their hands regularly and thoroughly to avoid transmission of the bacteria (Fairbrother and Nadeau, 2006).

Symptoms of *E. coli* infection include these signs: diarrhea, abdominal discomfort, and fever. Further high risk cases can lead to bloody diarrhea, dehydration, or even kidney failure. Immuno-compromised people with weakened immune systems, pregnant women, young children, and older adults are at increased hazard for developing these complications (Baldi *et al*., 2009). Proper food preparation and good hygiene is key to the reduction to the risk of developing agastrointestinal infection. Most cases of intestinal *E.*

*coli* infection can be treated at home. Signs of infectionusually clears out within a few days to a week (Bloomfield, 2006). *E coli* enteric infections require fluid replacement with solutions containing appropriate electrolytes as treatment and management. Antimicrobials are mostly considered to be useful in cases of traveler's diarrhea and include doxycycline, trimethoprim/sulfamethoxazole, fluoroquinolones, and rifaximin. They greatly reduce the duration of diarrhea by 24-36 hours. Antibiotics are not considereduseful in enterohemorrhagic *E coli* (EHEC) infection since they may make someone susceptible to the development of hemolytic uremic syndrome (HUS) (Dupont*et al.,* 2007).

### *2.5 Aspergillus flavus*

*Aspergillus flavus* is a saprotrophic and pathogenic fungus that is spread the world over. It is notorious for the infecting of cereals and legumes. *A. flavus* belongs to the kingdom: Fungi, division: Ascomycota, class: Eurotiomycetes, order: Eurotiales, family: Trichocomaceae, genus: *Aspergillus* and species: *flavus* (Ropars *et al.,* 2012).

*A. flavus* infections may occur even when the hosts are still in the field, but most do not show any signs until during storage. In addition to causing preharvest and postharvest contamination of grains, many strains produce significant quantities of toxic compounds known as mycotoxins that, when ingested, are harmful to both humans and animals. *A. flavus* is also an exploitative fungus that infects mammals, therefore leading to aspergillosis in immuno-compromised individuals (Pitt, 1994; Karkowska-Kuleta*et al.,* 2009). To protect grains and legumes against *A. flavus*, there are certain parameters that ought to be put in place before and after harvesting from the farm and plantations. Moisture levels should be kept below 11.5% and the temperature in storage should be as

low as possible since the fungi are not able to grow below temperatures of 5 °C (Jain, 2006). Fumigants are used to reduce the presence and the continued existence of pests, which enhances a rapid growth of the pathogen. Good and effective cleaning practice including, removal of aged and immature seeds, selection and differentiation of produce, and general tidiness will enhance mitigation and spread of the fungus (Litterick *et al.*, 2004).

Infection by *A. flavus* occurs mainly when the fungalspores gains access into the body through the nasal cavity and into the lungs. Also, other infections can occur on the body i.e. on the skin, nails and even at times the sinuses (Truitt and Tami, 1999).

Clinical manifestations of *A. flavus* are; fever, wheezing, coughs which may be severe with expectoration of large mucoid material and haemoptysis, general body malaise and severe headache (McCarthy and Pepys, 1971).

### *2.6 Candida albicans*

*Candida albicans* is a normal part of the human gut flora. Humans have *C. albicans* in their gut, and usually it coexists without causing disease with the other bacteria and yeasts that are found in the intestines. Combination of several factors can lead to the *C. albicans* population getting out of control. When out of control, it begins to affect your digestion, damage your intestinal wall, penetrating through into the bloodstream and releasing its toxic byproducts throughout your body and even weaken your immune system (Sekirov *et al.*, 2010).

*C. albicans* belongs to the Domain: Eukaryota, Phylum: Ascomycota, Class: Saccharomycetes, Order: Saccharomycetales, Family: Saccharomycetaceae, Genus: *C. albicans* and species: *albicans* (McManus and Coleman, 2014).

*C. albicans* is the most common type of oral, intestinal tract and vaginal yeast infections, and it may affect skin and other mucous membranes. This type of yeast infection is rarely serious if the body's immune system is working optimally. However, if immuno-compromised, the *C. albicans* infection can migrate to other areas of the body, including the blood and membranes around the heart and brain (Kim, 2011).

Candidiasis infections are mainlycaused by an overgrowth of *C. albicans* and can beavoided by watching a neat and healthy way of living. Maintaining a clean and healthy standard, and watching what one feeds on, and taking prescribed medicines can help alleviate candidiasis. Immunocompromised individuals can experience a relapse in infections or candidemia, but anti-fungal drugs can help boost their immunity (Tierno, 2004).

Candidiasis Symptoms are: Chronic Fatigue, Mood Disorders, Recurring Vaginal and Urinary Tract Infections, Oral Thrush, Sinus Infections, Intestinal Distress, Brain Fog, Skin and Nail Fungal Infections, Hormonal Imbalance (Ishijima and Abe, 2015).

In women, the most common of a vaginal yeast infection is irritation, intense itchiness of the vagina and the vulva coupled with a milky discharge. Occasionally, one feels pain during sexual intercourse or at times when passing urine one has a burning sensation when passing urine. Men mainly develop genital yeast infection after intercourse with a woman who has a vaginal yeast infection which is mainly the primary source of

infection. In men, candidiasis mainly manifests as an itchy rash, red skin, and swelling, irritation, itching around the head of the penis, and also pain when urinating and during intercourse, also it can be coupled with a milky discharge (Ahmed *et al*., 2014). In infants and adults, a *C. albicans* infection can appear in many different ways, oral candidiasis is called thrush. Thick, white lacy patches on top of a red base can form on the tongue, palate, or elsewhere inside the mouth.

Most cases of yeast infections can be managed with medications that can be self bought at any medicines outlet. Most of over the counter drugs are available like miconazole, tioconazole, butoconazole and clotrimazole (Morrell, 2014). For oral thrush, antifungal agent nystatin, Mycostatin, Nilstat around in the mouth is taken orally by swishing to maintain excellent oral hygiene (DeLuke *et al.,* 2015).

**2.7Climatic conditions and soil factors on phytochemical content in *Urticamassaica***

Biotic and abiotic stresses, such as high temperature, high light, soil and animal attack, are known to increase the release of volatile organic compounds from plants. Not much is known about the effect of multiple stress factors on plants. When the plant is subjected to two or more unfavourable conditions at times they become greately affected or may end up enhancing the growth and in turn improve in yield production greately (Holopainen and Gershenzon, 2010).

There is scanty or no information onsecondary metabolism that may occurs through abiotic factors, the information on secondary metabolites is mainly on how to get optimum produce due to utilization of active volatile compounds in herbs, spices and

medicinal plants. Another area of study on secondary metabolites is onthe reduction of harmful compounds in medicinal herbs (Gershenzon, 1984).

### 2.7.1 Soils

*Rosmanirus Officinalis* has shown high levels of most essential monoterpenes in plants growing on calcareous soils, while α-pinene, β-caryophyllene, and the total sesquiterpene content higher of higher levels in siliceous soils. Alloaromadendrene and δ-cadinene of *Cryptococcus albidus* showed higher concentrations on siliceous soils. Unlike *P. halepensis*, soil nutrients were not involved in terpene differences in calcareous and siliceous soils of these two herb species (Ormeño *et al.*, 2008).

Some soils in some areas have a high organic matter where evidence obtained showed that the soils supported and encouraged the presence of phytochemicals in the produce and yield though the climatic and soil stresses that encourage their presence can also be studied and documented. This will help to ascertain the level of influence they have on the plants (Zhao *et al.*, 2006).

### 2.7.2 Climate

Rainfall has had a notable negative effecton phytochemical composition between day time and night time (De Sousa Araújo *et al.*, 2015). Other factors that had influence on these compounds were found to be both temperature and relative humidity (Bisbis *et al*., 2018). Rainfall has been linked with an increase in the late afternoon contents of terpene and aldehyde volatiles with a known repellant effect on the codling moth, which is the major pest of apple fruit. In the summer of 2003, a season of low precipitation, some effects of drought on trees were tested by establishing relationships of volatile contents

with precipitaion. Wood by-products i.e. terpenes α-pinene, β-pinene and limonene were poorly affected by precipitaion. Another monoterpene, camphene, was only noted in this season but not in the previous years, and its release showed a very low relationship with precipitation. This showed that dry weather can impact the increase in formation of secondary metabolites. Two green leaf volatiles (*E*)-2-hexenal and (*Z*)-3-hexen-1-ol showed a negative correlation with precipitation, implying therefore that water deficit stress causes an upsurge in the production of lipoxygenase (Vallat*et al.*, 2005). The percentages of geraniol and its esters were highest during the cool winter season months of December and Januar. Isomenthone, 10-epi-gamma-eudesmol and other small terpenoid compounds did not show any significant seasonal trends (Kakaraparthi *et al.*, 2014). These favorable biotic and abiotic factors encouraged plant development and gave a high produce, essential oil production and optimum concentration of essential oil in sweet-scented geranium plants (Rao *et al.*, 1996).

Sunflower, castor bean, safflower, rape, and flax were grown at temperatures of 21, 26.5, 16, and 10°C. The content of oil of safflower, castor bean, and sun flower did not have any inteference by temperature. Optimum content of oil in flax and rape was reported at the most minimal temperature and continued reductionwas noted with elevation in temperature (Singer *et al.*, 2016). Fatty acid composition of thecastor oil and safflower bean oil was not interfered bytemperature alteration. In the other three species the amount of the more highly unsaturated fatty acids decreased as the temperature was increased. This decrease was accompanied by an increase in oleic acid (Canvin, 1965).

Oil content of some plants is not affected by temperature. In some plants, a very low temperature enhances high oil content and a high temperature impaired the oil content

production leading to decrease. Though temperature changes do not affect fatty acid composition. In the other species the amount of the more highly unsaturated fatty acids decreased as the temperature was increased. This reduction was coupled by an upsurge in oleic acid. Changesin temperaturedid not affect the levels of saturated fatty acids amongst all the species (Canvin, 1965).

### 2.7.3Plant genetic factors

Genetic factors are also known to have a directly impact all plant compounds. Physiologicaland environmental conditions may alter the ways in which the compounds express, though the major determining factor is the genetic background of the product. The phytochemical content in vegetables is dependent both quantitatively and qualitatively on the vegetable genetic composition (Kim *et al*., 2018). There are examples that show different phytochemical contents of various species ofsimilar genus and of varying cultivars of the same species in various conditions. In some cases, a reduction in water supply would enhance phytochemical content elevation. An example is the effect thatless irrigation caused broccoli glucosinolate levels to increaseand almost doubled (Valverde *et al.,* 2015). In plant metabolism, mineral nutrients are a key component. Increased sulphur application is attributed to the elevation of alliin amounts in onion and garlic due to the intensified development of sulphur-containing amino acids as from which alliin is derived (Shukla *et al.,* 2018). Elevation of sulphur levels have also led to an upsurge glucosinolate contents mainly because of glucoraphanin and glucoraphasatin in some plants. Reduction in nitrogen supply has enhanced the increase of amounts of glucosinolates, which can basically be attributed to the enhanced sulphur content which is non-protein, and therefore in an amplified presence of methionine. Contrary to improved

nitrogen application, the development of carotenoids and chlorophylls has been improved, where a decrease in phenolic conent may be evident (Schreiner, 2005).

## 2.8 Climate and soils of the study areas

### 2.8.1 Kitale

Kitale is located around the higher ground on the foot of Mount Elgon in Trans-Zoia County, Kenya. The slopes of Mount Elgon National Park are almost completely deforested due to need of wood as fuel and agriculturalland. The rainfall pattern has two peaks with the short rains from August to November and the long rains normally falling from April to July. Mt Elgon is the main water tower for the Turkwel River flowing into Lake Turkana in the north and also for the Nzoia River that wades its way into Lake Victoria in the south. The most preferred crops for cultivation in this area aresunflowers andmaize. The climate is classified as highland equatorial with an average annual temperature of 18°C and mean annual precipitation of around 1300 mm with the optimum between April and May and, also October and November. The soils by the mountain slopes are sandy, clay loams, a shade of red in color developed from ashes and basalt and contain a high content of organic matter. At the lower ends of the mountain the soils become dark brown withnitosols and osols. In the region bordering the Mt Elgon National Park, small-scale farms are located at altitudes ranging between 1800 and 2200 m above sea level (Horvath, 2006). The most commonly grown tree species in the area include *Cordia africana,Acacia spp, Persea americana, Sesbaniasesban, Passiflora edulis,Calliandraca lothyrsus, Markhamia lutea* and *Grevillea robusta* (Dharani, 2002; Gachene*et al.,* 2003; Maundu and Tengnäs, 2005).

### 2.8.2 Marigat

Marigat experirnces two rainfall seasons, the long rains between March and July and the short rains between September and November, and is reliable. The annual precipitation averagesbetween 300mm and 750 mm at the bottom of the Rift Valley close to Lake Bogoria to a high of 1200 mm in the elevated areas near Eldama Ravine. Temperature also is much more similar to the rainfall pattern. The mean temperature is about 28ºC almost all the year round. Lake Bogoria is hot, semi-arid to arid and is attributed to the rainfall and temperautre of the sorrounding. The average evaporation in a year is approximately 2,020 mm per annum andthe evaporation in this area ranges between a minimum of 1,800 mm to above 2,200 mm per year (JICA/MOARD, 1999).

Soil types and distribution in the area influenced by the topography, the steep slopes and rolling hills have soils developed from volcanic rocks, which are well drained varying from deep to shallow depths. Soils in these lowlands have developed on volcanic rocks and alluvial. In most areas the bedrock is basaltic and some pyroclastics. This has weathered over time under dry climatic conditions that gave rise to sandy loam soils in texture. They are well drained, moderately deep to very deep, brown to dark loams, sandy loams or clay loams and very erosive. Mogotio and Kisanana areas, soils are very shallow and at times extremely gravely to extremely stony. The soils vary in colour from mainly brownish to reddish brown depending on the mineral contents. Sandstones are common in Simotwet, Kamar and Koibos locations making them to be rich in sand. There are pockets of sedimentary rocks mainly shale and silt in depressions especially where streams enter swampy areas. Around Mugurin and MoloSirwe, soils have low water holding capacity, very shallow and extremely gravely clayloams. At the escarpments

soils have moderate water holding capacity, shallow depth, stony, gravely clay loams. Thick clay loam of alluvial origin is found around Marigat.

### 2.8.3 Uasin Gishu

This County lies between longitudes 34°50and 35°37 East and latitudes 0°30South and 0°55 North, some 300 km North North West of the capital Nairobi. UasinGishu County lies in UasinGishuplateau, which is one of Kenya's largest wheat producing areas, and most of it falls under the agroecological zone commonly known as wheat/barley zone (LH 3) (Republic of Kenya, 1997). Its terrain varies greatly with altitude, which ranges between 1500 m above sea level (ASL) at Kipkaren in the west to 2100 m ASL at Timboroa in the east. Eldoret (Kenya) town has an altitude of 2085 m above sea level and marks the boundary between the highest and the lowest altitudes of the County (Republic of Kenya, 1997). The general landscape is that of undulating plateau with no significant mountains or valleys. The land is higher in the east and declines in its western borders. The average rainfall is between 800 and 1000 mm, with temperatures of about 22°C. The soils are underlain by murram, are well-drained, moderately deep, dark red friable clay of petroplinthite. The soils are classified as RhodicFerralsols with pH 4.5–5.0 (Republic of Kenya, 1997; Nekesa *et al.,* 2011).

### 2.8.4 Kericho

Kericho experiences adequate rainfall of more than 100 mm/month throughout the year except in the month of January and December when it receives 78 and 80 mm respectively. The rainfall distribution pattern is described as unimodal, with April recording the highest (275 mm) and gradually decreasing to about 80 and 78 mm in

December and January respectively. The main annual monthly maximum and minimum temperatures are 22.2°C and 9.1°C respectively. The mean monthly averages show only slight variations between 14.8°C and 16.6°C. The mean annual temperature is therefore 15.7°C, designated as cool temperate. The Kericho soils are phonolites form what is called Losuguta type that is lavas that are typically porphyritic, usually vesicular and in some places, contains biotite identified with phenocrysts. To the north of Cheptabes River, the phonolites are remarkably uniform in composition. To the extreme south of the farm or along Itare River the phonolites are fine grained and in places glassy that are often seen when the rocks are broken or crushed. Due to rejuvenation, the rivers incised into the volcanic phonolites forming what is called interfluves, and the general tilt of the surface is in southwest direction. The general ground elevation is about 2120 m northeast, 1920 m to the west and about 1960 m to the south (Sombroek*et al*., 1982).

**2.9 Phytochemicals**

Plants are a rich source of a wide variety of phytochemicals that have been found to have antimicrobial properties (Cowan, 1999). The use and search for medicines and dietary supplements from plants have increased in recent years. Ethnopharmacologists, microbiologists, botanists and natural-products chemists are roaming the Earth for plants that have phytochemicals that could be developed for treatment of contagious diseases. Traditional healers have long used plants to prevent or cure infectious conditions. Western medicine practitioners are trying to follow their successes (Cowan, 1999).

Many medicinal plants contain numerous compounds with antibacterial activity (Marjorie, 1999). Chemotherapy is the main approach in the treatment of most infections

caused by bacteria but in clinical treatment antibiotics have faced a major issue of resistance that has led to treatment failure (Mckeegan*et al*., 2002).

Having fruits, spices and herbs as part of our diet do not include nutritional demand, growth and survival only, but they are also considered to be very rich sources of micronutrients, phytomedicines or neutraceuticals like alkaloids, carotenoids and polyphenols and so on (Aman*et al*., 2013).

Little attention has been accorded to the changes in secondary metabolism that may be occurring. Information on the effects of stress conditions on secondary metabolites has come mainly from research to make the most of the yield of active constituents from plants with medicinal properties and from attempts to reduce the levels of toxins in these plants. Advances in our understanding of the functional roles of secondary metabolites in plants should kindle additional interest in the responses of secondary metabolites (Gershenzon, 1984).

Attention has shifted to plant metabolites due to the increased incidences of drug-resistant pathogens of both clinical and agricultural importance. Medicinal plants have their inbuilt ability to resist pathogenic microorganisms that has led the researchers to investigate their mechanisms of action and isolate these phytochemicals. This has brought about exploitation of medicinal plants for the treatment of microbial infections of plants and humans by developing new antimicrobial agents. This novel search entails extensive research and it is therefore imperative to follow standard methods to authenticate claims of antimicrobial action (Das *et al.,* 2010).

Phytochemicals can also be found to be affected by environmental conditions as Ormeño *et al*., (2008) found out in the results obtained from tests on *R. officinalis* that showed high concentrations of numerous major monoterpenes in plants growing on calcareous soils, while α-pinene, β-caryophyllene, and the total sesquiterpene content were higher on siliceous soils. Finally, only alloaromadendrene and δ-cadinene of *C. albidus* showed higher concentrations on siliceous soils. Unlike *P. halepensis*, soil nutrients were not involved in terpene variation in calcareous and siliceous soils of these two shrub species (Ormeño *et al.*, 2008).

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Study area

The study was conductedinEldoret, Kericho, Kitale and Marigat, Kenya. These areas in Kenya are located in the Great Rift Valley. Eldoret and Kitale towns are geographically 330 km to the northwest of Kenya's capital, Nairobi. At an altitude of 2,085 meters above sea level, while Kericho town is to the southern part of the Rift Valley with an altitude of 1827 above the sea level (GoK, 2009). Kericho which is located in the central of the Rift Valley at an altitude of 2700 metres above the sea level, and finally Marigat that receives the lowest rainfall and has high temperatures is located on the Northern part of Rift Valley at an altitude of 900m above the sea level. (KFS, 2010; Wasonga *et al.,* 2011; Wandili *et al.,* 2013).

## 3.2 Collection and processing of plant samples

*Urtica massaica* leaf samples were collected from the field from the five different locations using a pair of scissors and packed in clear paper re-sealable zip-bags, and transported to KEMRI, CTMDR, Nairobi. At KEMRI, the plants were dried in an oven to a moisture content of 13%. The dried plant leaves was ground into fine powder, weighed and then stored in zip bags to be used in other subsequent procedures that followed. Plant identification followed Agnew and Agnew (1994) and voucher specimens were submitted to the University of Eldoret herbarium.

### 3.3 Preparation of plant extracts

Crude extracts from the stored fine powder were prepared using hot water and methanol. The crude extracts were later to be reconstituted to attain the desired concentration was constituted by dissolving 50 g of the ground samples with 100 ml of each of the respective solvents (Plate 3.1) (Usman and Osuji, 2008).

**Plate 3.1: Preparation of plant extracts**

### 3.4 Extraction

#### 3.4.1 Methanol extraction

Fifty grams of the ground plant parts were placed in a conical flask of 250 ml and 100 ml of 70% methanol added into the flask and shaken well. The mixed solution was left to settle for 24 hours. After which, they were filtered by the use of 6 mm filter paper (Whatman No. 1). The filtrate that was obtained was transferred into a flask with a round bottom. The flask was then pluggedto a rotary evaporator untilmethanol evaporated leaving a thick paste. The paste was transferred to a vial and left to dry in front of a fan until a solid was obtained indicating that all themethanol had evaporated from the paste.

The solid was then kept in a refrigerator at temperature of between 2°C - 4°C for other processes that followed.

### 3.4.2 Aqueous extraction

The damples were measured in 50g then dissolved in 100 ml of distilled water in 1litre conical flask then the mixture shaken until completely dissolved. The flask was then placed in a shaking water bath at a temperature of 70±1°C for one and a half hours. After incubation in a water bath, the mixture was then removed and filtered using surgical cotton wool in a glass funnel. The filtrate was left to cool and transferred into a 250 ml round bottom flask. The filtrate was put in a round-bottomed flask then inserted into a shallow tray containing acetone and dry ice to freeze dry, and coat on the flask. The sample was freeze-dried using Modulyo K4 freeze dryer (EDWARDS) so as to completely eliminate any water through vacuum until it was completely dry. The dried sample was removed from the flask, weighed into vials and refrigerated at 2 - 4 °C.

## 3.5 Evaluation of antimicrobial activity

ATCC strains and clinical isolates of *Escherichia coli, Staphylococcus aureus, C. albicans,* and *Aspergillus flavus* were used in bioassay testing using crude extracts at different concentrations. The tests were carried out to determine whether the plant extractsfrom plants collected from Eldoret, Kericho, Kitale and Marigatcontained antifungal or antibacterial capabilities. Controls, i.e. positive controls for both antibacterial and antifungal drugs included Ciprofloxacin Nystatin and Gentamicin. The test crude extractswere reconstituted using organic solvents (DMSO) and sterile distilled water necessary to ensure that the solvents that had been used for extraction and

dissolving the samples would not have inhibitory interference as all experiments were carried out in triplicates.

### 3.6 Antimicrobial assays

#### 3.6.1 Source of microorganisms

Bacteria of concern in the study were:for Gram positive; *Staphylococcus aureus* while Gram negative *Escherichia coli*. The fungi examined were *Aspergillus flavus* and *Candida albicans* (Miller *et al.,* 2006). The organisms and clinical isolates used for bioassay n the study were provided for from the KEMRI Centre for Microbiology Research, Nairobi (CMR). The Gram positive bacteria used were *Staphylococcus aureus* ATCC 2593 while Gram negative are *Escherichia coli* ATCC 27853. The fungi used were *Candida albicans* ATCC 24433 and *Aspergillus flavus* clinical isolates.

#### 3.6.2 Preparation of microorganisms

Preparation for bioassays was done by sub-culturing the fungi onto Sabouraud dextrose agar (SDA) then incubating at 35°C for 24 hours and 30°C at 72 hours respectively while the bacterial strains were then sub-cultured on Mueller Hinton agar at 37°C for 24 hours to obtain freshly growing strains (Rajakarunas and Towere, 2002).

#### 3.6.3 Preparation of McFarland standard

Inorder to standardize the inoculum density for a susceptibility testing, a $BaSO_4$ turbidity standard, and an equivalent to a 0.5 McFarland standard were used. Precisely, 0.5 McFarland standard was then prepared as described in National Committee for Clinical Laboratory Standards (NCCLS) (NCCLS, 1997). Approximately 1% V/V solution of

sulfuric acid was prepared by adding 1 ml of concentrated sulfuric acid to 99 ml of water and then mixed well. A 1.175% W/V solution of $BaCl_2$ was prepared by taking 2.35 g of $BaCl_2H_2O$ and dissolving in 200 ml of distilled water. To make the turbidity standard, 0.5 ml of the $BaCl_2H_2O$ solution was added to 1% 99.5 ml $H_2SO_4$ solution and mixed well. A small volume of the turbid solutions was transferred to a screw-capped tube of the same type as used for preparing the control inocula and stored in the dark at room temperature of 21°C (Andrews, 2001).

**3.6.4 Disc diffusion assay**

Determination of antimicrobial activity of the crude plant extracts was done by measuring the zones of inhibition by the use of disc diffusion method as described by (Bauer *et al.*, 1966). The bacteria were then sub-cultured and incubated for 24 hours at 37°C on nutrient agar. Fungi cultures were then incubated at 37°C for 96 hours and 37°Cfor 24 hours respectively in prescribed Potato dextrose agar supplemented with an antibiotic to obtain working cultures. Nutrient media for the growth of microorganisms were prepared as per the manufacturer's instructions. 3 to 4 colonies of cultured bacteria were then emulsified to make a suspension and then adjusted to match 0.5 McFarland's standard so as to produce inoculated agar with $1\times10^6$ colony forming units/ml. Then, aseptically the suspension was spread onto Mueller Hinton agar using a sterile cotton wool swab (Jorgensen and Turnidge, 2007).

Approximately 100 mg sample of an extract of each of the constituted samples was dissolved in 1ml of each solvent to produce 100 mg/ml. About 20 µl of each preparation was then measured and impregnated on to 6 mm filter paper discs prepared from Whatman No 1. These were sterilized in an autoclave purposely for disc diffusion assay

and let to air dry. The disks, after drying, were placed aseptically onto inoculated plates and then incubated for 24 hours at 37ºC. Fungal cultures were alsoproduced at 37°Cfor 72 hours and 35°C for 24 hours, respectively. After incubation, inhibition zone diameters were measured in millimetres and recorded against the corresponding concentrations as described by Badria and Elgayya, (2000). Positive controls were then prepared using standard antibiotics and the antifungal drugs alsowhile negative controls were prepared using disks impregnated with 20µlDMSO. Discs of Ciprofloxacin and Gentamycin (25µg) were used as standards for the positive control for bacteria while Nystatin discs (25µg) were positive controls for the fungal isolates. Discs containing 70 % DMSO were used as negative controls. Inhibition zone diameters was expressed as mean inhibition zones of the three assays. Classification of the antimicrobial activity was stipulated as ranging from little or no activity at ≤ 10 mm to very strong activity for inhibition zone diameters of ≥ 30 mm (Lee *et al*., 2004).

**3.6.5 Determination of minimum inhibitory concentration (MIC)**

Disk diffusion method was used to measure the minimum inhibitory concentration of the active crude extracts against the test microorganisms. This method is recommended by the Clinical Laboratory Standard Institute (CLSI) (NCCLS, 2002). The tests were carried out in well-micro-titer plates where the plant extracts were dissolved in the particular solvents then transferred into micro-titer plates to make serial dilutions ranging from $10^1$ - $10^{10}$. The volume in each well was 100 µl at the end, and then the wells were inoculated with 5µl of microbial suspension. The bactera and yeast were incubated at 37°Cfor 24 hours while fungi incubated at 37°C for 3-7 days. The MIC value was determined to be the lowest concentration of crude extract in broth medium that inhibited visible growth of

test microorganism as compared to the control where Dimethylsulphoxide (about 2 drops) dissolved in water (Motamedi, 2009). The MIC was recorded to be the lowest extract concentration with no visible growth as compared to the control broth turbidity (Michael *et al.,* 2003). Wells that had not been inoculated were used as controls for the experiment and the experiments were done in triplicates and the average results were recorded.

### 3.7 Evaluation of soil and climate

A desktop survey was done to get the various types of soil types and climatic conditions of the areas where the plant was collected from. A comprehensive study was done and we found abundant literature on the soil types and history of soil formation processes.

For climate, there is enough data on the regions where the plants were collected from, of which has been stored and recorded over time. There is also up to date data on climate that is updated regularly.

### 3.8 Phytochemical screening

Phytochemical analysis was done on the crude extracts on plants collected fromEldoret, Kericho, Kitale and Marigat. Silica gel plates were used in thin layer chromatography (TLC) plates were developed with Ethyl acetate: petroleum spirit (3:7) as the solvent system for methanol extracts while dichloromethane: methanol (9.5: 0.5) dilution system was utilized for aqueous extracts (Harborne, 1998). The silica gel plate was placed into a beaker containing the solvent system. The solvent moved up via capillary action and resulting bands were visualized in a UV chamber (Onwukaeme and Asonye, 2007; Bigoniya *et al.,* 2013).

### 3.8.1 Test for tannins

One gram of the dry plant powder was stirred in 30 ml distilled water then filtered using a filter paper. A few drops of ferric chloride reagent was added into the filtrate. The formation of a blue-black or green precipitate confirmed the presence of tannins (Trease and Evans, 2002).

### 3.8.2 Test for flavonoids

About, 0.2 g of the dry plant powder was dissolved on 4 ml ethyl acetate in a test tube vigorously shaken to ensure proper dissolution. The solution was decanted to remove plant particles and a few drops of ammonia solution were added into the filtrate. Since the liquids are immiscible the formation of an alkaline layer below the aqueous layer signifies the presence of flavonoids (Dharmedra *et al*., 2012).

### 3.8.3 Test for saponins

About 40 ml distilled water was boiled on a water bath for 10 min. Then 1 g of the dry plant powder was placed into a boiling tube and the warm water added into it and shaken for 10 minutes. The formation of thick persistent froth in the tube showed the presence of saponins (Ali *et al.,* 1990).

### 3.8.4 Test for terpenoids

One gram of dry plant powder was dissolved in 4 ml of chloroform in a test tube. Approximately 4 ml of concentrated $H_2SO_4$ was carefully added into the test tube. The formation of reddish- brown coloration on the interface indicated the presence of terpenoids (Sridevi *et al*., 2012; Siddiqui and Ali, 1997).

### 3.8.5 Test for glycoside

Few drops of ferric chloride and concentrated sulphuric acid were added to the solution of the extract in glacial acetic acid, and then observed for a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer confirmed the presence of Glycosides (Siddiqui and Ali, 1997).

### 3.8.6 Test for steroid

Four milligrams of extract were treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly, green bluish color was a positive result for steroids (Siddiqui and Ali, 1997).

### 3.8.7 Test for phenols

The sample was then dissolved in water, and a few drops of dilute ferric chloride solution were added to the solution. Formation of a blue, purple, red, or green coloration indicated the presence of phenols.

## 3.9 Data analysis

Anti-microbial activity for the extracts against the selected bacteria and fungi obtained using disk diffusion method where the zones diameter of inhibition was measured in millimeters, recorded in Excel spread sheet then exported to SPSS version 20 for analysis. Mean was calculated, and expressed as mean±standard deviation and data presented using tables. Significant variability between among solvents and regionswas determined using analysis of variance (ANOVA) at 5% significance level. Significant means were then further separated using Tukey’s test.

# CHAPTER FOUR

# RESULTS

## 4.1 Antimicrobial activity of *Urtica massaica*on different microorganisms using different extraction methods

Results obtained on the effect of methanolic and aqueous crude extracts of *U. massaica*on the selected groups of microorganisms are presented in Table 4.1and Plate 4.1. The significant differences ($p<0.05$) in inhibition zones between the crude extracts of *U. massaica*andthe positive control (Gentamycin and Ciprofloxacin) were reported against the selected group of microorganisms. However, significant differences ($P>0.05$) in inhibition zones between aqueous and methanolic crude extracts of *U. massaica* were reported among the four groups of microorganisms used in the study. The aqueous crude extracts of *U. massaica* showed lowest inhibition zone of 6.67 mm against *S. aureus*. However, this was not significantlydifferent from 8.42mm for the methanolic extract. Significant highest inhibition zones of 21.00mm and 20.00mm against *S. aureus* were recorded in plates with Gentamicin and Ciprofloxacin respectively.

**Table 4.1 Effect of solvents/controls on growth activity of selected group of microorganisms**

| Solvent/Controls | *S. aureus* | *E.coli* | *A. flavus* | *C. albicans* |
|---|---|---|---|---|
| Aqueous | 6.50±0.67a | 6.00±0.00a | 6.67±0.81a | 6.58±0.07a |
| Methanol | 8.42±2.25a | 6.33±0.49a | 7.58±3.94a | 6.92±0.90a |
| Gentamycin | 21.00±0.00c | 22.00±0.00c | 20.08±0.29b | 19.00±0.00b |
| Cipro | 20.00±0.00c | 21.00±0.00b | 21.00±0.00b | 20.00±0.00c |
| **F-Value** | **508.42** | **15550.00** | **178.31** | **2075.00** |
| **P-Value** | **0.00**** | **0.00**** | **0.00**** | **0.00**** |

*Means followed by different letters within a column are significantly different at P<0.05*
***denotes significance at p<0.05*

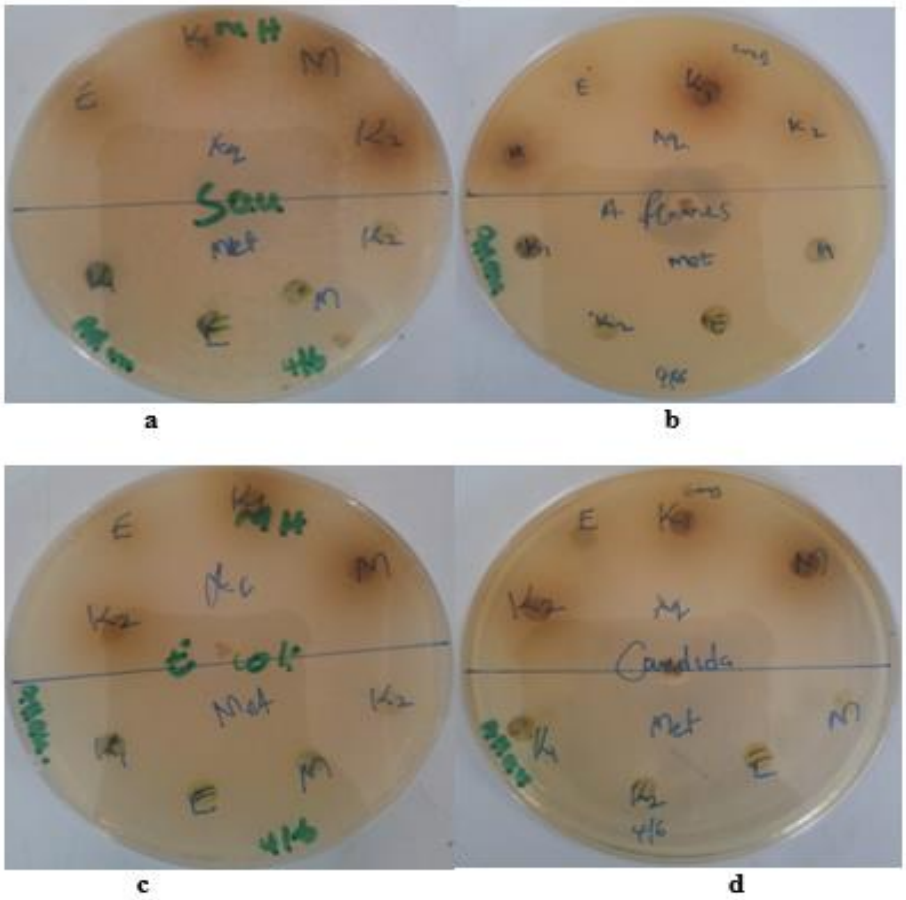


**Plate 4.1: Effect of methanolic and aqueous crude extracts on (a) *S. aureus*, (b) *A. flavus*, (c) *E. coli* and (d) *C. albicans***

The aqueous crude extracts of *U.massaica* did not show any antimicrobial activity (6.00mm) against *E. coli* while methanolic crude extracts recorded minimum inhibition zone of 6.33mm. Significant height inhibitions against *E. coli* were recorded in Gentamicin (21mm) and Ciprofloxacin (20mm). The growth inhibitions (mm) of aqueous and methanolic crude extracts against *A. flavus* were 6.67 and 7.58 respectively, which were significantly different from those of positive controls (Gentamicin 19.00mm and Ciprofloxacin 20mm). In addition, aqueous and methanolic crude extracts of *U.massaica*

were found to be not significantly different ($p>0.05$), with inhibition zones of 6.58mm and 6.92mm against *C. albicans* respectively. However, thesewere shown to be significantly lower from those Gentamicin (19mm) and Ciprofloxacin (20mm) as indicated in Table 4.1.

### 4.2. Minimum inhibition concentration

Table 4.2 shows Minimum inhibitory concentration in mg/ml of *U. massaica*extracts against the various pathogens. MICs were done on extracts that showed activity.Generally, *U. massaica* approved to be more active against *S. aureus* and *A. flavus*. The MIC results for *U. massaica* ranged from 0.1 mg/ml to 1000 mg/ml against the test isolates. The methanolic extracts were most active on different isolates with MIC value of 0.1 mg/ml to 100mg/ml against *S. aureus*.

For *A. flavus,* the MIC was higher with its aqueous extract from Kericho being the highest at 1000 mg/ml followed by its extract from Eldoret at 100mg/ml. The lowest was *U. massaica's* methanol extracts from Kericho at 0.1 mg/ml.

**Table 4.2Results of MIC of the plant and different solvents in mg/ml**

| Plant | Region | Test sample (solvent) | Test organism | MIC (mg/ml) |
|---|---|---|---|---|
| *U. massaica* | Marigat | Water | *S. aureus* | 100 |
| *U. massaica* | Kericho | Methanol | *S. aureus* | 0.1 |
| *U. massaica* | Marigat | Methanol | *S. aureus* | 10 |
| *U. massaica* | Eldoret | Methanol | *S. aureus* | 100 |
| *U. massaica* | Kericho | Water | *A. flavus* | 1000 |
| *U. massaica* | Eldoret | Water | *A.flavus* | 100 |

### 4.3 Effect of climatic and soil conditions on the antimicrobial activity of *Urtica massaica*

Findings on the effect of climatic and soil conditions on antimicrobial activity of *U. massaica* are shown in Table 4.2. Generally, inhibition zones among the four regions of the study were not significantly different($p>0.05$). Inhibition zones of 14.75mm,13.25mm, 14.00mm and 13.67mm against *S. aureus* were recorded in crude extracts of *U. massaica* from Kericho, Kitale, Marigat and Eldoret respectively. Crude extracts from Kericho, Kitale, Marigat and Eldoret showed inhibition zones of 13.83mm, 13.83mm, 13.92mm and 13.75mm against *E. coli* respectively. Similarly, inhibition zones among the four regions against *A. flavus* and *C. albicans* were not significantly different as indicated in Table 4.2

**Table 4.3 Effect of climatic and soil conditions on antimicrobial activity of U. massaica**

| **Region** | *S. aureus* | *E.coli* | *A. flavus* | *C. albicans* |
|---|---|---|---|---|
| Kericho | 14.75±6.43a | 13.83±8.02a | 13.67±7.17a | 13.08±6.73a |
| Kitale | 13.25±7.58a | 13.83±8.02a | 13.42±7.41a | 13.17±6.64a |
| Marigat | 14.00±6.81a | 13.92±7.94a | 13.58±7.25a | 13.17±6.66a |
| Eldoret | 13.67±7.16a | 13.75±810a | 14.67±7.31a | 13.08±6.73a |
| **F-Value** | **0.099** | **0.001** | **0.071** | **0.001** |
| **P-Value** | **0.960** | **0.99** | **0.975** | **0.99** |

*Means followed by different letters within a column are significantly different at P<0.05*

### 4.4Effect of climatic conditions and extraction methods on the antimicrobial activity of *Urtica massaica.*

Results in Table 4.3 showed a significant interaction ($p<0.05$) between the extraction solvent and climatic conditions on antimicrobial activity of *U. massaica* against *S.*

*aureus*. Significant highest inhibition zone of 11.67mm was recorded in methanolic crude extract of *U. massaica* from Kericho. Methanolic crude extract of *U. massaica* from Marigat and Eldoret showed inhibition zones of 7.67mm and 7.33mm respectively. On the other hand, inhibition zone of aqueous crude extract of *U. massaica* from Marigat was 7.33mm while those from Eldoret and Kericho was 6.33mm. Both methanolic and aqueous crude extracts of *U. massaica* fromKitale had no activity against *S. aureus.* Both positive controls recorded significant higher activity than crude extract of *U. massaica.*

**Table 4.4 Effect of climatic conditions and extraction methods on antimicrobial activity of U. massaica against S. aureus**

| | **Region** | | | |
|---|---|---|---|---|
| **Solvent** | Kericho | Kitale | Marigat | Eldoret |
| Aqueous | 6.33±0.58a | 6.00±0.00a | 7.33±0.58a | 6.33±0.58a |
| Methanol | 11.67±0.58b | 6.00±0.00a | 7.67±0.58a | 7.33±0.58b |
| Gentamycin | 21.00±0.00d | 21.00±0.00d | 21.00±0.00c | 21.00±0.00d |
| Cipro | 20.00±0.00c | 20.00±0.00c | 20.00±0.00b | 20.00±0.00c |
| **F-Value** | | **37.48** | | |
| **P-Value** | | **0.000**** | | |

*Means followed by different letters within a column are significantly different at P<0.05*
*** Denotes significance at p<0.05*

Results on the effect of climatic conditions and extraction methods on antimicrobial activity of *U. massaica* against *A. flavus* are presented in Table 4.4 showed an insignificant effect ($p>0.05$) of solvent by region interaction was reported. Positive control recorded significant activity which was higher ($p<0.05$) in inhibition zones than both aqueous and methanolic crude extracts of *U. massaica.* Methanolic crude extract of the test plant from Eldoret recorded highest inhibition zone of 11.00mm, against *A. flavus*

while lowest inhibition of 6.33mm was recorded in methanolic extract of *U. massaica* sampled from Kitale and Kericho as well as aqueous crude extract from Kitale.

**Table 4.5 Effect of climatic conditions and extraction methods on antimicrobial activity of U. massaica against A. flavus**

| | Region | | | |
|---|---|---|---|---|
| **Solvent/Control** | Kericho | Kitale | Marigat | Eldoret |
| Aqueous | 7.33±0.58b | 6.33±0.58a | 6.67±1.15a | 6.33±0.58a |
| Methanol | 6.33±0.58a | 6.33±0.58a | 6.67±0.58a | 11.00±7.81a |
| Gentamycin | 20.00±0.00b | 20.00±0.00b | 20.00±0.00b | 20.33±0.58b |
| Cipro | 21.00±0.00c | 21.00±0.00c | 21.00±0.00c | 21.00±0.00c |
| **F-Value** | | **1.043** | | |
| **P-Value** | | **0.434** | | |

*Means followed by different letters within a column are significantly different at P<0.05*

Two-way ANOVA results on the effect of solvent used in extraction and region on antimicrobial activity of *U. massaica* against *E. coli* are shown in Table 4.5 aqueous extract of *U. massaica* sampled from all the regions (Kericho, Kitale, Marigat and Eldoret) had no effect on *E. coli* (6mm).

**Table 4. 6 Effect of climatic conditions and extraction methods on antimicrobial activity of U. massaica against E. coli**

| | Region | | | |
|---|---|---|---|---|
| **Solvent** | Kericho | Kitale | Marigat | Eldoret |
| Aqueous | 6.00±0.00a | 6.00±0.00a | 6.00±0.00a | 6.00±0.00a |
| Methanol | 6.33±0.58b | 6.33±0.58b | 6.67±0.58b | 6.00±0.00a |
| Gentamycin | 22.00±0.00d | 22.00±0.00d | 22.00±0.00d | 22.00±0.00d |
| Cipro | 21.00±0.00c | 21.00±0.00c | 21.00±0.00c | 21.00±0.00c |
| **F-Value** | | **0.889** | | |
| **P-Value** | | **0.546** | | |

*Means followed by different letters within a column are significantly different at P<0.05*

However, methanolic extract of U. massaica plant samples from Marigat showed some activity (6.67mm), while those from Kitale and Kericho recorded inhibition zone of 6.33mm. Generally, Gentamicin and Ciprofloxacin drugs reported significant inhibition zones of 22 and 21mm respectively.

**Table 4.7 Effect of climatic conditions and extraction methods on antimicrobial activity of U. massaica against *C. albicans***

| | **Region** | | | |
|---|---|---|---|---|
| **Solvent** | Kericho | Kitale | Marigat | Eldoret |
| Aqueous | 6.33±0.58a | 6.67±0.58a | 6.33±0.58a | 7.00±0.99a |
| Methanol | 7.00±0.99a | 7.00±0.99a | 7.00±0.99a | 6.33±0.58a |
| Gentamycin | 19.00±0.00b | 19.00±0.00b | 19.00±0.00b | 19.00±0.00b |
| Cipro | 20.00±0.00c | 20.00±0.00c | 20.00±0.00c | 20.00±0.00c |
| **F-Value** | | **0.758** | | |
| **P-Value** | | **0.654** | | |

*Means followed by different letters within a column are significantly different at P<0.05*

Findings on climatic conditions on the effect of extraction methods on antimicrobial activity of *U. massaica* against *C. albicans* are presented in Table 4.6. The interaction effect of solvent and region was reported was reported to be not significant (p>0.05). Positive controls (Gentamicin and Ciprofloxacin) recorded were significantly higher (p<0.05) inhibition zones than both aqueous and methanolic extracts of the test plant. Methanolic crude extract of *U. massaica* sampled from Kericho, Kitale and Marigat reported inhibition zone of 7mm, while those sampled from Eldoret showed mean inhibition zone of 6.33mm aqueous crude extract of the test plant sampled from Eldoret recorded mean inhibition zone of 7mm, Kitale (6.67mm) while those from Kericho and Marigat showed mean inhibition zone of 6.33mm.

### 4.5 Phytochemical analysis of *U.massaica* from various geographical regions

**Table 4.8 Phytochemical analysis of U. massaica crude extracts**

| Phytochemical Screening | Eldoret | Kitale | Kericho | Marigat |
|---|---|---|---|---|
| Alkaloids | + | ++ | ++ | + |
| Glycosides | + | + | + | + |
| Saponins | ++ | + | ++ | ++ |
| Terpenoids | ++ | + | + | ++ |
| Phenols | + | + | + | + |
| Steroids | ++ | ++ | ++ | ++ |
| Tannins | + | + | + | ++ |
| Flavonoids | ++ | ++ | ++ | + |

(++) Conspicuously Present (+) Discreetly Present

Results on phytochemical analysis of *U. massaica* are shown in Table 4.7. A wide range of various phytochemicals; alkaloids, glycosides, saponins, tarpenoids, phenols, steroids, tannins and flavonoids were tested with their appropriate protocols and reagents. The *U. massaica* methanolic extracts showed presence of most of the phytochemicals tested.

The methanolic extract of the *U. massaica* from Eldoret showed the presence of saponins, tarpenoids, steroids and flavonoids in high levels as opposed to alkaloids, glycosides, Phenols and Tannins. Extracts from Kitale recorded high presence of alkaloids, steroids and flavonoids, as opposed to glycosides, saponins, terpenoids phenols and tannins. Extracts from Kericho recorded high presence of alkaloids, saponins, steroids and flavonoids, as opposed to glycosides, terpenoids phenols and tannins. Finally, extracts from Marigat recorded high presence of saponins, terpenoids, steroids and tannins, as opposed to alkaloids, glycosides, phenols and flavonoids. It is worth noting that *U. massaica* from all the regions exhibited highlevels of steroids as indicated in Table 4.7.

# CHAPTER FIVE

# DISCUSSION

## 5.1 Antimicrobial activity of *U. massaica* on different microorganisms using different extraction methods

Resultsindicate that inhibition zones among the solvents/ control varied significantly($p<0.05$). Methanolic crude extract of *U. massaica* showed mean inhibition zones that ranged from 6.33mm to 8.4mm, while those of aqueous extract ranged from 6mm to 6.67mm.

These results further showed that the methanolic crude extracts showed higher inhibition zones than the aqueous extract. This could be attributed to different polarity and extracting potential of methanol and water. Methanol can dissolve both polar and non-polar substances. A study by Cowan (1999) reported that most antimicrobial agents that have been identified from plants are soluble in organic solvents and this reveals the better efficiency of methanol as extracting solvent than water.

Similar findings were reported by Fasil, (2015) where high antibacterial activities observed in the methanolic extracts than aqueous extract of selected medicinal plants. Findings from the present study further indicated that aqueous *U. massaica* extract did not inhibit the growth of *E. coli.* these findings are contrary to those carried out by Ziarlarimi *et al*., (2011) on plants such as *Allium sativum* with medicinal value which showed antimicrobial activity against *Escherichia coli*. The variation could be attributed to the part of the part and moreover, the plants used could be of the different age. Generally, methanolic crude extract of *U. massaica* recorded antimicrobial activity against the test organisms; *S. aureus, E. coli, A. flavus* and *C. albicans.* The findings were

similar to those obtained in a study carried out on Gram negative and Gram positive bacteria as well as fungi by Kaithawas *et al.,* (2008) in India and Robson (1982) in England; on *Escherichia coli* and Agarry *et al.,* (2005) in Nigeria; on *Staphylococcus aureus* and *C. albicans* who found out that, extracts from a wide range of medicinal plants including *U. massaica* had antimicrobial activity against the test microorganisms. Moreover, findings from this study are in consonance with findings ofother researchers like Alamri and Moustafa (2012) who reported antimicrobial activity inmethanolic extracts of *Aloe vera* against *S. aureus* (18 mm) and *E. coli* (8.46 mm) at a using agar-well diffusion method.

The antimicrobial activities of *U. massaica* extracts could be due to the presence of bioactive ingredients. In addition, active ingredients not to be significant such as phenols have been reported to confer broad spectrum antibacterial activities (Alamri and Moustafa, 2012). These results, together with ethnobotanical studies made previously by other investigations (Tilahun and Mirutse, 2007; Yigezu *et al.,* 2014), suggest that *U. massaica* might have important compounds that can potentially be used for to inhibit pathogenic microorganisms.

**5.2 Effect of climatic and soil conditions on the antimicrobial activity of *U. massaica***

Findings indicate that crude extract of *U. massaica* sampled from various regions of the study showed varied antimicrobial activities. However, the variation was reported ($p>0.05$). These findings could be attributed to fact the*U. massaica* samples from all the regions recorded the presence of almost similar phytochemicals. Furthermore, it could be due to uniformity in age of the plant. The present findings are conflicting with those

reported by Sheen *et al.,* (1994), where geographical variation effected the level of medicinal active compounds of plants of the same species. Generally, most plants show a marked seasonal and climatic variation in their antimicrobial activity (Gaya, 2012).

Chemical diversity in nature is based on biological and geographical diversity, so researchers travel around the world obtaining samples to analyze and evaluate in drug discovery screens or bioassays (Newman and Cragg, 2007). Moreover, biochemical profiles of plants harvested at different times and locations may vary greatly (Quamina, 2003). In one of their studies on management of medicinal plant resources in Nyanza recommended that there should be studies to ascertain possible variations in medicinal plant active compounds in different geographical regions in order to prevent genetic erosion by planning their conservation (Ochieng *et al.,* 1995). A recent study by Kumar *et al.* (2017) recorded significant differences in antimicrobial activity of *Aloe vera spp.* from different agro-climatic conditions.

### 5.3 Phytochemical analysis of *U.massaica* from various geographical regions

Findings show that the extract from *U. massaica* from Kericho, Kitale, Marigat and Eldoret contained most of pharmacologically active components. The extract contained alkaloids, saponins, tarpenoids, phenols, steroids, tannins and flavonoids which may be responsible for the antimicrobial activity. Similar studies previously carried out have shown that some of the pharmacologically active components have antimicrobial activity. These findings concurred with those obtained in a previously carried out study by Mariita *et al.,* (2011) who after qualitative analysis of phytochemical components of *U. massaica* and *Aloe vera* extract used against *Escherichia coli, Salmonella typhi, Staphylococcus aureus* and *C. albicans* from plant collected along the Kenya lake region found it to

contained tannins, saponins, flavonoids, and alkaloids. Furthermore, similar findings were obtained in a study carried out in India by Arankumar and Methuselvan, (2009) who confirmed the presence of flavonoids, saponins, tannins and alkaloids in *Aloe* extract when used against *Staphylococcus aureus* and *Escherichia coli*. Qualitative analysis for the presence of phytochemicals in *Tagetes minuta* extract showed the presence of flavonoids, saponins, alkaloids and tannins. This pharmacologically active component might be responsible *Tagetes minuta* extract antimicrobial activity against the test microorganisms.

The current study found that alkaloids, saponins, tarpenoids and steroids were common phytochemicals found in plants collected from the four regions.These findingswere similar to those obtained in a study carried out in Pakistan by Tahir and Khan, (2012) and in Argentina by Tereschuk, (1997) who also confirmed the presence of phytochemicals flavonoids, saponins, tannins alkaloids, glycosides, phenols, steroids and tannins in *Tagetes minuta* extract used against Gram positive and Gram negative bacteria. The extract from *Bulbine frutescens* contained pharmacologically active compounds namely saponins, tannins, alkaloids, and saponins which could be responsible for antimicrobial activity. This study concurred with those of a previously done study in South Africa by Coopoosamy *et al.,* (2012) who found out that when the plant extract from *Bulbine frutescens* was used against *Staphylococcus aureus* and *Escherichia coli* and qualitative analysis of phytochemicals showed the presence of alkaloids, saponins, tannins, phenols, and flavonoids. However, the findings were contrary to those obtained in previously carried out a study in South Africa by Hutchings and Van Staden, (1994) who found out that, the extract from *Bulbine frutescens* did not contain any of the four phytochemicals.

This could be due to diverse plant metabolites associated with a geographical and ecological difference from where the plant was obtained and also the age of the plant used. The extracts from *Vernonia lasiopus* had active pharmacological compound; flavonoids, saponins, tannins, tarpenoids and alkaloids which could be responsible for the antimicrobial activity.

The findings of the current study were similar to those of a previously carried out study by Karer*uet al.*, (2008) who found out that, extracts from *Vernonia lasiopus* contained alkaloids, flavonoids, saponins, tapenoids and tannins when used against Gram positive and Gram negative bacteria (*Escherichia coli* and *Staphylococcus aureus*). Ayoola*et al.*, (2008) in Nigeria also found out that extract from the plant from the genus*Vernonia* contained flavonoids, alkaloids, tannins, terpenoids and saponins. However, the findings were contrary to those obtained in a study carried out in Southwestern region in Nigeria by Ibrahim *et al.*, (2012) who found out the extract from *Vernonia lasiopus* did not contain alkaloids. This difference could be associated with the geographical and soil factors of the area from which the plant was collected. Some soils in some areas have a high organic matter where evidence obtained seems in favor of enhancement of phytochemical content in organically grown produce, but there has been little methodical study of the factors that contribute to increased phytochemical content in plants. Consistent differences will be found, and the extent to which biotic and abiotic stresses, and other factors such as soil biology, contribute to those differences (Zhao *et al.*, 2006).

# CHAPTER SIX

# CONCLUSION AND RECOMMENDATIONS

## 6.1 Conclusion

1. Generally, the crude *U. massaica* extracts showed antimicrobial activity when used against *S. aureus, E.coli, A. flavus,* and *C. albicans*. Higher inhibition zone range of 6.33mm to 8.4mm was recorded in methanolic crude extract compared to 6-6.67mm of aqueous crude extract of the plant, concluding that methanol is a better solvent than water.
2. The crude extract of *U. massaica* sampled from Kericho, Kitale, Marigat and Eldoretrecorded varied antimicrobial activities. However, the variation was reported to be insignificant, concluding that geographical variation did not affect the level of medicinal active compounds in *U. massaica*. Insignificant interaction between region and extraction method on antimicrobial activity of *U. massaica* was observed.
3. There was presence of phytochemicals in the crude extracts of *U. massaica* from the four areas under study; alkaloids, saponins, tarpenoids phenols, steroids, tannins and flavonoids

## 6.2 Recommendations

- The methanolic extracts of *U. massaica* should be used in the formulation of a drug against the bacterial microorganisms (*S. aureus* and *E. coli*) and fungi (*C. albicans* and *A. flavus*) only after scientific validation of their safety.

- Since insignificant effect of geographical conditions on antimicrobial activity of *U. massaica* extract, therefore, the extract should be used regardless of the region sampled from.

- There is need to elucidate phytochemical components present in the extracts which might be responsible for the antimicrobial activity

### 6.2.1Suggestions for further studies

- There is a need to determine the possible mechanism of antimicrobial action of the extracts.
- Studies should be done to identify and quantitatively isolate individual phytochemical components in the extracts.

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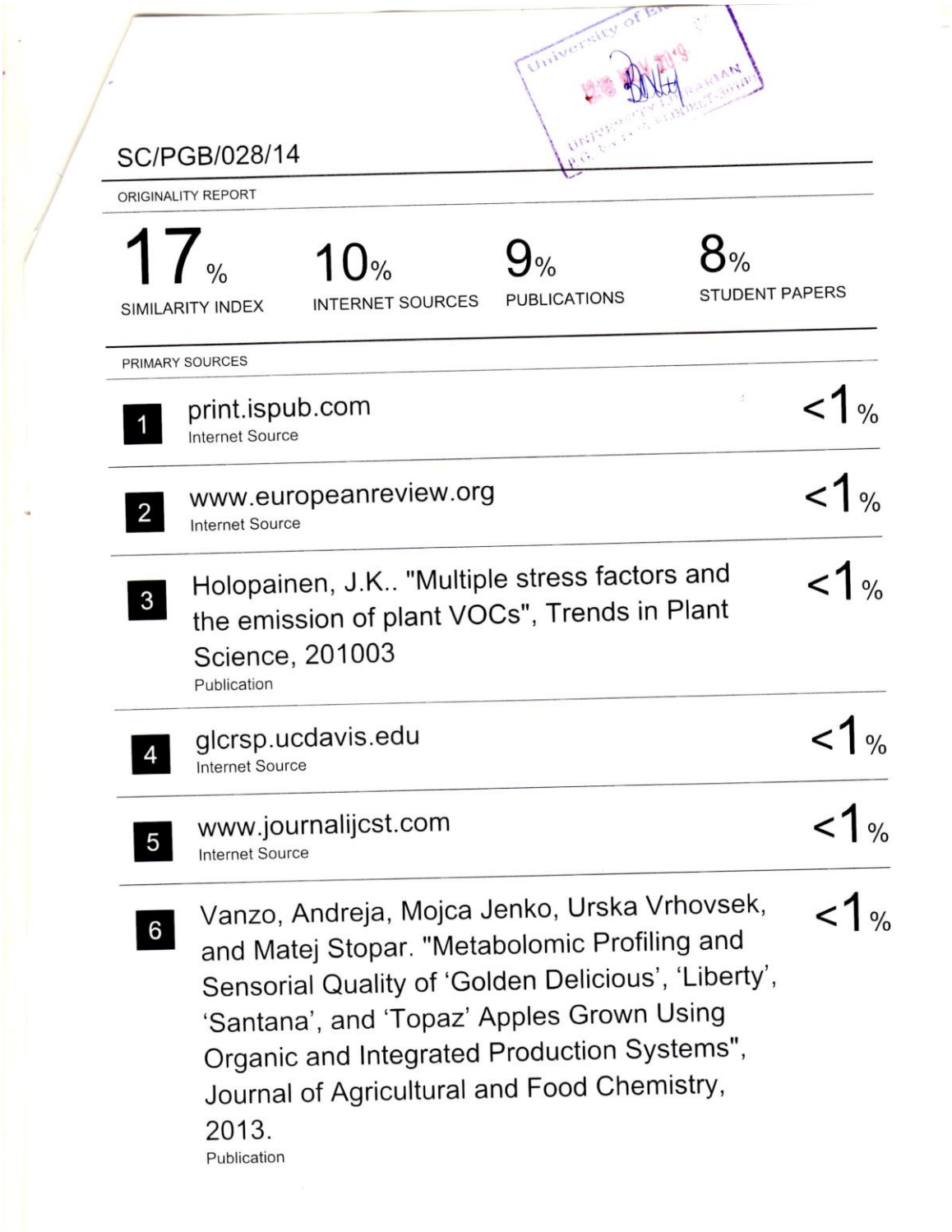
**Appendix 1:Similarity Report**



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