ANTIMICROBIAL ACTIVITY OF SOME MEDICINAL PLANTS USED BY THE SAMBURU COMMUNITY OF NORTHEN KENYA AGAINST SELECTED BACTERIAL AND FUNGAL PATHOGENS

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DECLARATION

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

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DEDICATION

I dedicate this work to Mr and Mrs Meshack Ong'era for pivoting my life after my beloved parents Mr. and Mrs. Patrick Onyambu departed.

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ACRONYMS AND ABBREVIATIONS

ADD	Agar Disc Diffusion
AIDS	Acquired Immunodeficiency Syndrome
ANOVA	One-Way Analysis of Variance
ATCC	American Type Culture Collection
ATP	Adenosine-5'-triphosphate
APP	Acute-phase proteins
CI	Confidence Interval
DCM	Dichloromethane
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic acid
GIT	Gastrointestinal Tract
HIV	Human Immunodeficiency Virus
LC_{50}	Lethal Concentration Medium [at 50%]
MBC	Minimum Bactericidal Concentration
MDR	Multi-drug Resistant
MFC	Minimum Fungicidal Concentration
MHA	Muller Hinton Agar Media
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
PDA	Potato Dextrose Agar Media
SDA	Sabouraud Dextrose Agar Media
WHO	World Health Organization

ABSTRACT

Medicinal plants form an integral social and cultural component, and sometimes the only alternative available treatment for health problems. Infectious diseases concern the whole world because they cause an estimated 98% of deaths in both children and adults in developing countries. The aim of the study is to determine ethnobotanical uses and the bioactivity of some medicinal plants used by the Samburu community against selected bacterial and fungal pathogens. This study evaluated both antibacterial and antifungal activity of the selected twenty two medicinal plants used by the Samburu community. An ethnobotanical survey was conducted and collected information from herbalists on their use in the treatment of various infectious diseases. Plants identification was done with assistant of a taxonomist from Kenyatta University. Voucher specimens were prepared and deposited at the Department of Plant Sciences herbarium. Methanolic plant extracts were screened using Kirby-Bauer disk diffusion technique against eleven strains of bacteria and fungi obtained from Centre for Microbiology Research at Kenya Medical Research Institute, Nairobi Kenya. They were either American Type Culture Collection (ATCC) or clinical isolates; *Bacillus subtilis* (clinical isolate), *Staphylococcus aureus* (ATCC 25923), *Streptococcus pneumoniae* (ATCC 28910), *Escherichia coli* (ATCC 25922), *Vibrio cholerae* (ATCC 27622), *Shigella dysenteriae* (ATCC 26988), *Candida parapsilosis* (ATCC 18310), *Cryptococcus neoformans* (ATCC 19310), *Aspergillus flavus* (clinical isolate), *Microsporum gypseum* (clinical isolate), *Trichophyton mentagrophyte* (clinical isolate). Minimum inhibitory concentrations (MICs) were determined by measuring the diameter around the discs, while micro-dilution technique was used to determine the minimum bacteriocidal and fungicidal concentrations (MBCs/MFCs). Extracts of *Acacia nilotica, Thylachium africanum* and *Loranthus acaciae* produced high antibacterial and antifungal activity results of ≥9-15 mm and 9.375 - 18.75 mg/ml as showed by the MICs and MBCs/MFCs results. Screening for phytochemicals; tannins, saponins, flavonoids, cardiac glycosides, alkaloids, and terpenoids indicated presence at varying concentrations either high, moderate, low or absent. Significant difference of zones of inhibition means of all the strains was observed at $P \leq 0.05$. Clear indications from the data obtained were that these plants extracts serve as an enormous source of untapped antimicrobial agents. This study recommended that the Samburu community should continuously be sensitized on sustainable use of medicinal plants and that they should give priority to domesticating the medicinal plants. Further studies on the biological properties, isolation and identification of active components in the plant parts used in this research in order to test specific antimicrobial activity was also recommended.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Since ancient times, plants have formed the basis of traditional medical systems (Nadembega *et al.*, 2010). Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries. The World Health Organization (WHO) estimates that more than 80 percent of the world's population relies on folk medicine (Lawal *et al.*, 2010). It is also estimated that about 85% of the Samburu community medicare is from medicinal plants (Omwenga *et al.*, 2009). Even in the Western world, the use of herbal medicines is steadily growing with approximately 40 percent of the population reporting use of herbs to treat medical illnesses (Demma *et al.*, 2009). Dependence of such a large portion of the population on herbal medicine can be attributed to the fact that traditional medicines are easily accessible, affordable and extensive local knowledge and expertise amongst the local communities (Mariita *et al.*, 2010b). They are also generally more acceptable from a cultural and spiritual perspective, very popular and associated with little side effects (Rojas *et al.*, 2006).

Throughout the history of mankind, herbal remedies have been known to treat many infectious diseases. It has also been reported that natural products derivatives and analogs represent over 50 percent of all drugs in clinical use, in which natural products derived from higher plants represent about 25 percent of the total drugs in clinical use (Lawal *et al.*, 2010). The acceptance of natural plant medicine during the second half of

the 20th century as food preservative and also as agents for antimicrobial resistance led studies to investigate the antibacterial activity of several medicinal plants (Al-Bakri and Afifi, 2007; Lou *et al.*, 2010). These medicinal plants are known to produce a variety of secondary metabolites which constitute a major source of bioactive substances that have anti-bacterial and anti-fungal activities (Rangasamy *et al.*, 2007; Mbosso *et al.*, 2010). These secondary metabolites include the unsaturated long chain aldehydes, alkaloids, essential oils, flavones (flavonoids, flavonols, and quinones), lectins, polypeptides, phenolics, cardiac glycosides, polyphenols, saponins, terpenoids anthraquinones and steroids (Doughari *et al.*, 2008; Salama and Marraiki, 2010).

These metabolites have long been of interest to man and used either directly as precursors or as lead compound in the pharmaceutical industry (Mariita *et al.*, 2010a). The scientific research interest on medicinal plants has increased since it is expected that plant extracts target sites different from those used by antibiotics (Al-Bakri and Afifi, 2007; Mbosso *et al.*, 2010). This has led to pharmaceutical companies' spending considerable time and money in developing therapeutics based on natural products extracted from plants owing to their novel mechanism activities, coupled with the difficulty of microbes to develop resistance against them (Salama and Marraiki, 2010; Venugopal *et al.*, 2010). These compounds occur naturally in a wide range of plant parts in the seeds, stems, leaves, flowers, fruits, barks, roots or even within the whole plant (Turgis *et al.*, 2009; Meenakshi *et al.*, 2010). They have been extracted using four different solvents namely; acetone, hexane, dichloromethane (DCM) and methanol; of which the later, has quantitatively been the best extractant (Malabadi and Kumar, 2007).

The antimicrobial compounds exhibit various basic mechanisms of action. These may involve the formation of an encapsulated protein network that provides focal points for erythrocyte aggregation, inhibit spore germination of many spore producing pathogens, inhibit tumour growth, cause alterations in cell wall and membrane constitution in order to prevent defense peptides and proteins synthesis, disturb DNA synthesis, cause lethal breaks in the double-stranded DNA during DNA replication, inhibit enzyme functions, inhibit aflatoxin synthesis in fungal pathogens, among other mechanisms (El-Khallal, 2007; Khalil and Dababneh, 2007; Akkoc *et al.*, 2008; McCarrell *et al.*, 2008; Salama and Marraiki, 2010; Venugopal *et al.*, 2010). Therefore, the ability to develop novel powerful antimicrobial drugs is crucial in the fight against current and emerging biological threats (Russell *et al.*, 2010).

Infectious diseases have become a major health problem globally and very common in health care settings especially, in immunosuppressed individuals and vulnerable patients (Mariita *et al.*, 2010b). The increasing prevalence of multi-drug resistant bacterial and fungal strains with reduced susceptibility to antibiotics has raised the specter of 'untreatable' infections (Akkoc *et al.*, 2008). The growing disease burden worldwide from water, sanitation and hygiene has made the control of infectious diseases complicated even more with the manifestations of HIV/AIDS (Mariita *et al.*, 2010b). Surveys have revealed that almost no group of antibiotics has been introduced to which resistance had not been observed (Rangasamy *et al.*, 2007). This is indeed quite alarming considering that 98 percent of death in children in developing countries result mostly from infectious diseases (Rangasamy *et al.*, 2007).

Both Gram negative and Gram positive bacteria are known to cause infectious diseases by contaminating food, water, and the environment (Matasyoh *et al.*, 2008). *Escherichia coli,* is a Gram negative, rod-shaped bacterium, which is usually commensal in the gastrointestinal tracts of animals and humans (Karmali *et al.*, 2010). Serotype O157:H7 causes bloody diarrhoea and haemolytic uraemic syndrome due to food poisoning, also causes acute renal failure, thrombocytopenia, and microangiopathic haemolytic anaemia, accounting for 280 million episodes and more than 400,000 deaths annually (Chen *et al.*, 2009b). *Shigella dysenteriae* is a Gram-negative, rod-shaped, non-motile bacterium that causes shigellosis (Zafar *et al.*, 2009). It is estimated that about 160 million persons are affected annually and over one million die, most of which are children below the age of 10 years (Tiruneh, 2009).

Vibrio cholerae is a Gram-negative motile bacterium that causes cholera (Mohapatra *et al.*, 2009). Cholera is one of the leading causes of death in infants in developing countries and currently it accounts for an estimated three million deaths each year among children under the age of 5 years (Velazquez *et al.*, 2009). *Bacillus subtilis* is a Gram positive, rod-shaped, endospore forming bacterium commonly found in the soil and inhabits gastrointestinal tract of insects, animals and humans (Hong *et al.*, 2009). It produces subtilin a proteolytic enzyme that causes food spoilage and is responsible for the increase in outbreaks of food-borne diseases (Rahman and Kang, 2009). *Streptococcus pneumoniae* is a Gram-positive, alpha-hemolytic bacterium that causes acute exacerbations of chronic bronchitis and community-acquired pneumonia (Vila-Corcoles *et al.*, 2009). It is responsible for extensive morbidity and mortality among

infants and AIDS patients (Kone *et al.*, 2007). Commensal on the skin and in the nose is *Staphylococcus aureus* which is methicillin-resistant (MRSA) and mainly responsible for boils, post-operative wound infections, toxic shock syndrome, endocarditis, osteomyelitis, meningitis, and food poisoning (Rahman and Kang, 2009; Zampini *et al.*, 2009). This nomination reflects the high incidence of MRSA infections, substantial morbidity, and peculiar virulence factors circumventing usual antimicrobial therapy (Kudryavtsev *et al.*, 2009).

Antibiotics are widely used in human and veterinary medicine to control bacterial infections although resistance to old and newly produced drugs has been witnessed (Vaidya and Bhattarai, 2009; Tao *et al.*, 2010). Most antibiotics are poorly absorbed in human and animals gut with majority of them being excreted unchanged in faeces and urine which eventually find their way into the environment through the disposal of sewage, hospital wastewater and animal waste. This therefore, necessitates an urgent need to identify active chemo types from natural sources to facilitate the development of high, valuable and effective drugs (Saravanakumar *et al.*, 2009; Limsuwan *et al.*, 2009; Tao *et al.*, 2010).

Fungal infections range from superficial to systemic causing infectious diseases in immunocom promised patients (Lin, 2009). For example, *Candida parapsilosis* is a species of yeast that cause sepsis and infections of wound and tissue in immunocompromised and surgical patients (Hasan *et al.*, 2009). It is the second or third most common cause bloodstream infections (Dostal *et al.*, 2009) accounting for up to

50 percent morbidity and mortality of HIV/AIDS patients (Chen *et al.*, 2009a). *Cryptococcus neoformans* of the same class has a predilection for the central nervous system leading to severe, life-threatening pneumonia and meningoencephalitis in both immunocompetent and immunocompromised patients especially HIV-AIDS, cancer therapy and organ trasplantation (Bovers *et al.*, 2008; Ajesh and Sreejith, 2009; Masman *et al.*, 2009). It accounts for up to 30 percent of mortality and severe morbidity in AIDS patients and is responsible for over 95 percent of cryptococcosis cases worldwide (Lin, 2009).

Aflatoxin B1 is the most toxic form of mycotoxins produced by moulds like *Aspergillus flavus* for mammals. They present hepatotoxic, teratogenic and mutagenic properties, known to cause toxic hepatitis, hemorrhage, edema, immunosuppression and hepatic carcinoma (Brun *et al.*, 2009; Hell *et al.*, 2009; Reddy *et al.*, 2009). Majority of patients diagnosed with invasive aspergillosis die despite aggressive antifungal therapies (Lin, 2009). Dermatophytes on the other hand cause superficial infections characterized by hypopigmented or hyperpigmented macules and patches on the face, neck, chest and back in the cases of *Trichophyton mentagrophyte* mainly among immunocompromised patients (Derita *et al.*, 2009; Khosravi *et al.*, 2009). Up to 90 percent of the patients' infections become life-threatening fungal disease leading to high death rates in HIV/AIDS patients (Gauwerky *et al.*, 2009).

Microsporum gypseum on the other hand invades keratinized tissues, such as hair, skin or nails, of humans and other animals causing tinea corporis, tinea pedis and

onychomycosis (Koroishi *et al.*, 2008). The most affected patients are those with HIV infection and immunosuppression induced due to organ transplants or by cancer chemotherapy leading to high rate of mortality (Derita *et al.*, 2009; Khosravi *et al.*, 2009). Currently, a notable increase of multi-resistant fungal pathogens to current therapies from antifungal agents is disturbing (Ajesh and Sreejith, 2009). Therefore, the need for formulation of new antimicrobial agents and evaluation of the efficacy of natural plant products as a substitute for chemical antimicrobial agents is becoming increasingly obvious (Maregesi *et al.*, 2008; Zabka *et al.*, 2009). The appearance of multi-drug resistant pathogens has threatened antimicrobial chemotherapy since these microbes are capable of adopting and surviving unfavorable conditions. Some of these microbes multiply rapidly to double their numbers within every 20-30 min. Their individual cells also do not rely on their own genetic resources but rather on a large pool of itinerant genes which are capable of moving from one cell to another horizontally and/or vertically (Doughari *et al.*, 2008; Kummerer, 2009).

The problem of microbial resistance is as a result of years of widespread indiscriminate use, incessant misuse and abuse of antibiotics. In human medicine alone, the US Centre for Disease Control and Prevention estimates that approximately one-third of the 150 million prescriptions for antibiotics written each year were not needed (Rangasamy *et al.*, 2007). Hence, the increasing resistance of microorganisms against available antimicrobial agents is of major concern among scientists and clinicians worldwide (Orhan *et al.*, 2010). According to the World Health Report on infectious diseases, overcoming antibiotic resistance is one of the major issues of the WHO for the present

millennium. Hence the last decade witnessed an increase in the investigation of plants as a source of human disease management (Mbosso *et al.*, 2010).

New antimicrobial agents used to treat or control infectious diseases are being sought in order to decrease side effects, lower drug costs, and broaden the spectrum of activity against resistant microorganisms and opportunistic microbes affecting immunocompromised patients (Toledo *et al.*, 2011). The potential of higher plants as a source of new drugs is still largely unexplored yet plant derived drugs continue to serve as a prototype in developing more effective and less toxic medicines (Meenakshi *et al.*, 2010). Hence, researchers are increasingly turning their attention to natural products looking for new leads to develop better drugs against cancer, as well as viral and microbial infections (Philip *et al.*, 2009).

The rapid propagation in antibiotic resistance and the increasing interest in natural products, however, have placed medicinal plants back in the front lights as a reliable source for the discovery of active anti-microbial agents and possibly even novel classes of antibiotics (Rangasamy *et al.*, 2007). With such overwhelming evidence to date suggesting that plant antimicrobial compounds have therapeutic applications, the main aim of this study is to determine the main bioactive compounds of the medicinal plants growing in Samburu district of Samburu county, Kenya and to evaluate their antimicrobial activity against the selected bacteria and fungal strains mentioned above as the main focus in this study.

1.2 Statement Problem

The Samburu community occupies some of the northernmost parts of Kenya, a territory that has always been and still is one of the remotest accessible areas of Kenya. Due to remote location the Samburu, they received very little attention from the colonial administration as independence changed this only marginally (Bussmann, 2006). It is one of those communities in Kenya that are marginalized in terms of 'health care for all' as a basic human right and prerequisite to social-economic development (Omwenga *et al.*, 2009). This is due to the fact that despite the fact that the western style healthcare supplied by the government has been expanded in the last decades, but it is still often not readily available and many regions remain completely underserved. Subsequently, most communities still use herbal remedies as readily and cheaply available alternative (Bussmann, 2006). Despite the tremendous progress in human medicines, infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health (Arya *et al.*, 2010).

The problem is compounded by high poverty rate, poor sanitary conditions and inadequacy of clean water. For instance, pastoralism is a normal practice of the inhabitants' that leads to sharing of water with both domestic and wild animals which makes inhabitants ends up using water without proper treatment as it is scarce most of the year (Omwenga *et al.*, 2009). The dramatic and continued evolution of drug resistant strains has created an international crisis in health care. Some antibiotics have become almost obsolete. Consequently, new approaches for microbial control must be considered (Venugopal *et al.*, 2010).

Human infections particularly those involving microorganisms like; bacteria, fungi, viruses, nematodes cause serious damages in tropical and subtropical countries of the world. In recent years, multiple-drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of diseases (Umamaheswari *et al.*, 2008). There are high rates of occurrence of various ailments among the Samburu community. The diseases get magnified given the fact that they lack proper medication because of high poverty rates hence prefers the use of local treatment by use of medicinal plants. The community believes in medicinal plants first before the patient is hospitalized and in most cases hospitalization is as a result of intoxication due to overdoses (Omwenga *et al.*, 2009).

The "Witchcraft Act" of 1925 outlawed traditional medicine in Kenya. The practice however continued more concealed, until parts of the law were revoked with independence in 1963. Western style healthcare supplied by the government has been expanded in the last decades, but it is still often not readily available and many regions remain completely underserved. Subsequently, most communities still use herbal remedies as readily and cheaply available alternative. Most knowledge on therapeutic applications of medicinal plants is still transferred entirely orally in many communities from generation to generation with only very few written documents (Bussmann, 2006; Bussmann *et al.*, 2010). Hence research has been geared towards finding scientific evidence for the claims as to the therapeutic efficacy of African herbs by traditional healers (Lawal *et al.*, 2010).

1.3 Justification

Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind (Lawal *et al.*, 2010). They continue to provide the foundation for modern pharmaceuticals and drug leads (Siriwatanametanon *et al.*, 2010). It is estimated that over 60 percent of Africa's population consults traditional healers in addition to the modern medical services available to them (Matasyoh *et al.*, 2009). About 85 percent of the Samburu community use medicinal plants for their medicare (Omwenga *et al.*, 2009). Over the last centuries, intensive efforts have been made to discover clinically useful antimicrobial drugs (Umamaheswari *et al.*, 2008). Plants species have been employed as medicine, food, building and crafts material, forage, fuel and elements in spiritual activities (Zampini *et al.*, 2009). The use of natural products in disease prevention and control as well as in drug development has received increased attention in recent times (Amoo *et al.*, 2011). Hence, plants have been used to treat various diseases in East Africa especially Kenya (Mariita *et al.*, 2011). According to Amoo *et al.*, (2011), 11 percent of the 252 drugs considered as basic and essential by the WHO are solely of plant origin. It is estimated that over 90 percent of East Africa relying on folk medicine (Orwa *et al.*, 2008).

Over the years, the WHO has advocated traditional medicines as safe remedies for both microbial and non-microbial diseases. Several reports in the literature also indicate a wide spectrum of therapeutic activities for the crude extracts (Nenaah, 2010). These naturally occurring combinations of compounds are often synergistic, leading to a higher antimicrobial activity of crude extracts than the pure individual compounds (Lou

et al., 2010). Plant compounds have shown to have a wide range of biological activities, including antiallergic, antibacterial, antidiabetic, antiinflammatory, antiviral, antiproliferative, antimutagenic, antithrombotic, anticarcinogenic, hepatoprotective, oestrogenic, insecticidal, and antioxidant activitie. Therefore, the contribution of plant-derived natural products to modern pharmacotherapy is considerable (Orhan *et al.*, 2010).

This therefore, makes it credible to carry out efficacy studies on plant extracts obtained from the locally available plants because plant origin antimicrobials exhibit a very high potential as new therapeutic agents due to their novel mechanisms activity and the difficulty of microbes to develop resistance against them (Venugopal *et al.*, 2010). Therefore, evaluation of *in vitro* antimicrobial activity of medicinal plants is of great interest. It is estimated that two-thirds of the world population rely on traditional remedies due to the limited availability and high prices of most pharmaceutical products (Mbosso *et al.*, 2010). Antimicrobials of plant origin have enormous therapeutic potential, effective in the treatment of infectious diseases and simultaneously mitigate many of the side effects that are often associated with synthetic antimicrobials (Al-Bakri and Afifi, 2007).

Herbs with antimicrobial activity are being widely used at a commercial scale to prolong the shelf-life, improve the safety and to replace existing synthetic antimicrobials partly due to the mistrust of synthetic additives (Lou *et al.*, 2010). It has long been established that naturally occurring substances in plants have anti-bacterial

and anti-fungal activities (Rangasamy *et al.*, 2007). Therefore, in addition to plants' therapeutic use in herbal preparations, they can serve as important sources of new drugs, new drug leads and new chemical entities (Amoo *et al.*, 2011). In search for new plant-derived biologically active compounds against infectious diseases, ethnobotanical studies have been carried out on some medicinal plants used by the Samburu communities, the popularly cited were selected and antimicrobial assays performed to establish whether or not these plants contain activity against infectious disease causing microorganisms and to scientifically validate their use.

1.4 Research questions

i. Does the Samburu community have appropriate ethnobotanical information on medicinal plants they use in the treatment of selected bacterial and fungal pathogens?

ii. Do the selected medicinal plants from the Samburu community have antimicrobial activity against selected bacterial and fungal pathogens?

iii. What phytochemicals are present in the medicinal plants used by the Samburu community against selected bacterial and fungi pathogens?

1.5 Hypotheses

i. The Samburu community does not have appropriate information on the medicinal plants they use in the treatment of the selected bacterial and fungal pathogens.

ii. The medicinal plant extracts used by the Samburu community do not have antimicrobial active against the selected bacterial and fungal pathogens.

iii. Phytochemicals present in the medicinal plants used by the Samburu community are not known.

1.6 Objectives of the Study

1.6.1 General Objective

To determine ethnobotanical uses and the bioactivity of some medicinal plants used by the Samburu community against selected bacterial and fungal pathogens.

1.6.2 Specific Objectives

i. To investigate the ethnobotany of some medicinal plants used by the Samburu community in the treatment of infections caused by selected bacterial and fungal pathogens?

ii. To determine the antimicrobial activity of medicinal plants crude extracts used against selected bacterial and fungal pathogens.

iii. To determine the phytochemicals present in the selected medicinal plants.

CHAPTER TWO

LITERATURE REVIEW

2.1 Bacterial Infections

Infections due to a variety of bacterial etiologic agents are very common (Acharyya *et al.*, 2009). Examples include pathogenic *Escherichia coli, Shigella dysenteriae, Vibrio cholerae, Bacillus subtilis, Streptococcus pneumoniae* and *Staphylococcus aureus* which are of interest in this study. Morbidity and mortality due to these strains continue to be a major problem in many developing countries. There has been high prevalence of tropical infectious diseases occurring in immunosuppressed individuals in both endemic and non-endemic countries (Franco-Paredes *et al.*, 2010). The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Sharma *et al.*, 2009).

Antibiotic resistance in bacteria is the product of innate genetics and physiology, which are vertically transmitted through species, and the remarkable propensity of bacteria to exchange genetic material horizontally across species and genera. This combinatorial genetic strategy has resulted in the accumulation of multidrug-resistance (MDR) phenotypes in many species of bacteria thereby posing a serious threat to public health worldwide (Wright and Sutherland, 2007). Microorganisms' resistance to antibiotics has become a major global healthcare problem in the 21st century (Rangasamy *et al.*, 2007). Therefore, combating MDR requires: (i) a detailed understanding of the molecular basis, evolution and dissemination of resistance (ii) new chemicals with

antibiotic properties to fill the traditional antibiotic pipeline and (iii) innovative strategies that can extend the life of antibiotic molecules or that can uncover new approaches for controlling pathogen growth. Recent whole-genome sequence data for bacteria as well as exploration of the resistance burden in environmental bacteria suggest resistance is much more widespread than previously thought (Wright and Sutherland, 2007).

2.1.1 *Staphylococcus aureus*

Staphylococcus aureus is widely spread in nature as an indigenous microbiota of skin and mucosa of humans, animals and birds. The Gram positive cocci bacterium is carried asymptomatically in a number of body sites (Coutinho *et al.*, 2009). *S. aureus* is a biofilm producer and shows a better ability to attach itself to mucosal surfaces and cause infection than non-biofilm producer strains (Zampini *et al.*, 2009). Mainly, it is responsible for post-operative wound infections, toxic shock syndrome, endocarditis, osteomyelitis and food poisoning, skin infections such as boils, abscesses, carbuncles and wound sepses. Transmission from these sites causes both endemic and epidemic diseases (Rahman and Kang, 2009).

The ß-lactam antibiotics are the drugs of choice for the treatment of infections caused by *Staphylococcus aureus*. Resistance to ß-lactam compounds has been reported for methicillin, oxacillin, nafcillin, cloxacillin, and dicloxacillin. *S. aureus* has also been reported to have dramatic and continued evolution of drug resistant strains to other commonly used antimicrobial agents including aminoglycosides, macrolides,

chloramphenicol, tetracycline, and fluoroquinolones (Chomnawang *et al.*, 2010). With the emergence of methicillin resistance leading to treatment failure that was detected one year after its launch, has continued to pause a serious challenge in the management of infectious diseases (Dalhoff and Schubert, 2010). Resistance arises either passively as a result of pre-existing innate mechanisms or actively via the acquisition of new genetic material by means of mobile genetic elements such as plasmids or transposons (Wright and Sutherland, 2007).

The major mechanisms of antibiotic resistance include; enzymatic transformation, modification of the molecular target, sequestration of the drug, prevention of entry of the compound into the cell, active efflux from the cell interior and prevention of entry of the compound into the cell (Wright and Sutherland, 2007). Medicinal plants have been used as remedies for infectious diseases including the treatment of Methicillin-resistant *Staphylococcus aureus* (MRSA) infection because they have been known to decrease side effects, lower drug costs, and broaden the spectrum of activity against resistant microorganisms and opportunistic microbes affecting immunocompromised patients (Toledo *et al.*, 2011). Some of the plants that have been found to have high antimicrobial activity against *Staphylococcus aureus* include; *Polygonum aviculare* L., (Salama and Marraiki, 2010), *Elaeodendron schlechteranum*, *Ozoroa reticulate*, *Indigofera colutea*, *Combretum adenogonium*, *Harrisonia abyssinica* (Maregesi *et al.*, 2008), *Peganum harmala* (L) (Nenaah, 2010), *Entada abysinnica* Steudel ex A. Rich (Fabaceae) and *Lantana trifolia* (Mariita *et al.*, 2010b).

2.1.2 *Vibrio cholerae*

Cholera is an acute diarrhoeal disease caused by the non-invasive Gram negative bacillus *Vibrio cholerae* serogroup O1 or O139. The enterotoxin produced by this rod causes copious, painless, watery diarrhea leading to vomiting, severe dehydration and even death if treatment is not prompt (Pal *et al.*, 2010). It can spread as an endemic, epidemic, or pandemic disease affecting all age groups although most adult population have gained some degree of natural immunity because of prior illness or repeated asymptomatic infections. This therefore, makes it predominant among young children, who are exposed to the organism for the first time (Silva et al., 2008; Sharma *et al.*, 2010). It remains a serious public health problem for many developing countries and some developed countries of which epidemic proportions appear in Asia, Africa, and South America particularly in areas of inadequate sanitation and food hygiene practices. Clinical signs and symptoms in patients are sudden onset of belching abdominal pain within 5–6 h, with rice watery stool, vomiting, muscular cramping, and rapid progress of severe dehydration (Pal *et al.*, 2010).

Annual global figures (2009) reported to WHO had the majority of cases with 98 % reported from Africa. WHO estimates the actual global burden of disease as 3 to 5 million cholera cases and 100,000 to 130,000 deaths each year (Sharma *et al.*, 2009). Susceptibility to cholera depends on factors like; local intestinal immunity from previous exposure or vaccination, bacterial load and intrinsic host factors (such as stomach pH - gastric acid provides a barrier) and blood group where individuals with blood group O carrying the unmodified H antigen being more susceptible than those

with type AB being the most resistant. Blood-group antigens may also have the ability to confer protection against certain diseases, just as it has been recently observed for malaria and HIV infections (Holmner *et al.*, 2010).

Typically, antimicrobial agents are administered for 3-5 days; however, a single-dose therapy with tetracycline, doxycycline, furazolidone, or ciprofloxacin has been seen to be effective in reducing the duration and volume of diarrhoea (Sharma *et al.*, 2010). Other current antimicrobial agents used include; norfloxacin, ampicillin, streptomycin, neomycin, nalidixic acid, co-trimoxazole, gentamicin, azithromycin, sulfamethoxazole, trimethoprim, and chloramphenicol (Rajpara *et al.*, 2009). Despite the availability of these antimicrobials, a high percentage of resistance by *Vibrio cholerae* O1 to ciprofloxacin, norfloxacin, ampicillin, streptomycin, neomycin, nalidixic acid, furazolidone sulfamethoxazole, trimethoprim, chloramphenicol, streptomycin and co-trimoxazole has been observed (Pal *et al.*, 2010). The genes responsible often reside on mobile genetic elements for easy dissemination of drug resistance to other organisms. These include chromosomes or plasmids for the case of integrons and conjugative transposons, such as the SXT element (Rajpara *et al.*, 2009).

With the alarming incidence of antibiotic resistance in bacteria of medical importance, there is increasing interest in plants as a source of agents for the treatment of microbial diseases (Sharma *et al.*, 2010). Some of the plants that have been used against *V. cholerae* include; *Terminalia chebula* Retz. (Combretaceae), *Syzygium cumini* (L.)

Skeels (Myrtaceae), *Solanum nigrum* L. (Solanaceae), *Butea monosperma* (Lam.) Taub. (Papilionaceae) (Acharyya *et al.*, 2009).

2.1.3 *Escherichia coli*

Escherichia coli is a Gram negative, rod-shaped bacterium which is commensal in the gastrointestinal tracts of animals and humans (Karmali *et al.*, 2010), but serotype O157:H7 is enterotoxigenic and happens to be the most important pathogen of diarrhoea in infants, children, and adults (Chen *et al.*, 2009a). It causes fatal human illness whose clinical spectrum includes diarrhoea, haemorrhagic colitis, and the haemolytic uraemic syndrome (HUS), urinary tract infection, coleocystitis or septicaemia (Karmali *et al.*, 2010). Enterotoxigenic *E. coli* accounts for 280 million episodes and more than 400,000 deaths annually. It is also the most common pathogen of traveler's diarrhoea that affects 10 million travelers in developing countries (Chen *et al.*, 2009a).

Currently, the therapy used is loperamide which is administrated to patients with moderate or severe dehydration illness or bloody diarrhoea although not used for patients under 3 years (Chen *et al.*, 2009b). Other antibiotics widely used include; ampicillin, chloramphenicol, ciprofloxacin, levofloxacin, sulphamethoxazole/ trimethoprim (SXT), trimethoprim and tetracycline (Tao *et al.*, 2010). As much as these antibiotics kill bacteria, however, they are not capable of inhibiting the toxicity of bacterial toxin (Chen *et al.*, 2009a). *Escherichia coli* resistances to most antimicrobial agents have become a widespread medical problem evidenced in the permeability changes of the cell envelopes, chemical modifications of the antimicrobial agent,

enzymatic antibiotic degradation and the presence of membrane efflux systems which pump out the antimicrobial agents from the cytosol. Acquired resistance to antimicrobial agents results from generic cell changes and arises either by mutation or by the acquisition of genetic material (like plasmids) from another cell (Zampini *et al.*, 2009).

Today, the pharmaceutical arsenal available to control antibiotic-resistant bacteria *E. coli* is limited; hence, it is important to identify natural products with antimicrobial activity for the development of novel antibacterial therapies and adjunct treatments. Antibacterial activity has been reported for terpenoids from *Fabiana dens*a var. *ramulosa* and *Baccharis boliviensis* (Zampini *et al.*, 2009). Other herbal extracts, such as the fruit of *Chaenomeles speciosa,* the leaf of *Camellia japonica,* the gall of *Rhus chinensis*, exhibit anti-LT-induced diarrhoeal abilities via several mechanisms (Chen *et al.*, 2009b).

2.1.4 *Bacillus subtilis*

Although normally considered soil organisms, members of the Gram positive, rod-shaped, spore-forming genus *Bacillus* can inhabit the gastrointestinal tract (GIT) of insects, animals and humans (Hong *et al.*, 2009). *Bacillus subtilis* contaminates food by producing subtilin a proteolytic enzyme causing food spoilage and responsible for the increase in outbreaks of food-borne diseases (Rahman and Kang, 2009). The robustness of *Bacillus subtilis* spores enables them to survive transit through the stomach, after

which they germinate, proliferate and then re-sporulate before excretion in the faeces (Hong *et al.*, 2009).

Chloramphenicol, ciprofloxacin, ampicillin, trimethoprim and tetracycline are some of the antibiotic therapy used against *B. subtilis* (Salama and Marraiki, 2009; Nenaah, 2010). *B. subtilis* have genetic ability to transmit and acquire resistance to antibiotics particularly in endemic areas (Chen *et al.*, 2009b). This continous resistance has made many antibiotic therapies a non-viable solution, thereby becoming a major global healthcare problem in the 21st century. Plants continue to hold an important position in modern medicine because over 75% of the antibacterial drugs in clinical use are from natural origin. For example; plants of *Eruca* or *Diplotaxis* genera have been used against *Bacillus subtilis* in a number of times in promoting health or therapeutic properties, such as antiphlogistic, depurative, diuretic, digestive, aphrodisiac and rubefacient (Khoobchandani *et al.*, 2010). Other plants used against *B. subtilis* include; *Leucosidea sericea* (Aremu *et al.*, 2010), *Polygonum spectabile* Mart. (Polygonaceae) (Brandao *et al.*, 2010), *Stephania dielsiana* (Deng *et al.*, 2011).

2.1.5 *Shigella dysenteriae*

Shigella dysenteriae is a Gram-negative, rod-shaped, non-motile bacterium that causes shigellosis (Zafar *et al.*, 2009). The infective dose varies from 10 to 10^2 bacterial cells and the transmission is mainly fecal-oral, via the stool of the sick or convalescing persons or through asymptomatic carriers. *Shigella dysenteriae* type 1 strains were responsible for a dysentery epidemic in Ethiopia, Democratic Republic of Congo and

India (Antoine *et al.*, 2010). It is estimated that approximately 150 to 160 million persons are affected annually, of which 600,000 to 1 million die, most of which are children below the age of 10 years (Tiruneh, 2009). Further, a ninety-nine percent of shigellosis episodes occur in developing countries with majority of the cases and deaths occuring among children less than five years of age (Zafar *et al.*, 2009).

Shigellosis is characterized by acute dysenteric infectious diarrhoea. It is responsible for a high morbidity and mortality rate among persons in overpopulated and underprivileged areas where sanitary conditions are mediocre. The main virulence factor of *Shigella* is carried by the *inv* plasmid, a part of which harbours the *IpaA, IpaB, IpaC, IpaD* and *IpaH* genes implicated in the mechanism of characteristic cellular invasion. There also exists the *ial, set1 and set2* genes which play an important role in the virulence process and are involved in the passage from cell to cell (Antoine *et al.*, 2010). Currently, antibiotics administered for dysentery are ciprofloxacin, ticarcillin, amoxicillin, ampicillin, cotrimoxazole, chloramphenicol, tetracycline, clavulanic acid and sulfamide (Zafar *et al.*, 2009; Antoine *et al.*, 2010). In the recent past, the acquisition of new characteristics by *Shigella* has led to emergence of resistant strains to antibiotics. For example in Ivory Coast, Canada, Senegal and India species of the *Shigella* genus have been noted to have resistance to old and newly produced drugs (Antoine *et al.*, 2010).

Shigella is resistant to several antibiotics like ampicillin (90 %), tetracycline (90 %), sulfamide SFM (85 %), ticarcillin (77.5 %), amoxicillin + clavulanic acid (72.5 %),

cotrimoxazole (67.5 %) and Chloramphenicol (57.5 %) (Antoine *et al.*, 2010). Therefore, medicinal plants which represent a reservoir of effective chemotherapeutants and valuable sources of natural drugs have been used to inhibit different diseases (Vaidya and Bhattarai, 2009). Some of the plants that have been used against *Shigella* include; *Stephania dielsiana* (Deng *et al.*, 2010), *Ocimum canum* (Devi *et al.*, 2009), *Cassia occidentalis* L. (Yadav *et al.*, 2010), *Dactylorhiza hatagirea* (Ranpal, 2009), *Origanum minutiflorum* (Oke and Aslim, 2010).

2.1.6 *Streptococcus pneumoniae*

Streptococcus pneumoniae is a Gram-positive, alpha-hemolytic bacterium that causes acute exacerbations of chronic bronchitis and community-acquired pneumonia, meningitis, and acute otitis media (Campa *et al.*, 2009; Vila-Corcoles *et al.*, 2009). In many African countries including Côte d'Ivoire, it is responsible for extensive morbidity and mortality in infants and AIDS patients. Infections caused by *Streptoccocus pneumoniae* continue to be a growing public health concern (Kone *et al.*, 2007). Chloramphenicol, moxifloxacin, tetracycline, cotrimoxazole and fluoroquinolones like ciprofloxacin and levofloxacin, are being used in the treatment of pneumococcal patients (Campa *et al.*, 2009).

Pneumococcal resistance or reduced sensitivity to too many antibiotics including β-lactams, macrolides, tetracycline, fluoroquinolones and cotrimoxazole is acquired by point mutations, intraspecific recombination or interspecific recombination with the *S. mitis* group (Kone *et al.*, 2007; Campa *et al.*, 2009). Due to the occurrence of resistant

strains, there is a high demand to discover new antibiotics from medicinal plants to fight against resistant species (Limsuwan *et al.*, 2009). *Andira inermis*, *Combretum molle*, *Keetia hispida* and *Garcinia afzelii* are some traditional medicines that have effective antipneumococcal activity (Kone *et al.*, 2007).

2.2 Fungal infections

Fungal infections are one of the major threats in the field of medicine. Only a few successful drugs are currently available for the treatment of fungal infections especially for dermatomycoses (Ponnusamy *et al.*, 2010). Recently, there has been an increasing interest in the search for extracts and natural compounds from plants to replace existing synthetic antimicrobials partly due to the mistrust of synthetic additives (Lou *et al.*, 2010). This is coupled with the fact that plants are complex chemical storehouses of undiscovered biodynamic compounds with unrealized potential for use in modern medicine (Rangasamy *et al.*, 2007). The fungi strains of interest here are *Candida parapsilosis, Aspergillus flavus, Microsporum gypseum, Trichophyton mentagrophyte* and *Cryptococcus neoformans.*

2.2.1 *Candida parapsilosis*

Several *Candida* species are commensals and colonize the skin and mucosal surfaces of humans although their infections are commonly associated with biofilm formation that can occur both on mucosal surfaces and on plastic surfaces of in-dwelling devices. These biofilm consist of matrix-enclosed micro-colonies of yeast, hyphae and pseudohyphae, arranged in a complex structure. *Candida parapsilosis,* a yeast species

causes sepsis and infections of wound and tissue in immuno-compromised and surgical patients and is the most common fungal infection in AIDS patients (Hasan *et al.*, 2009). Candidiasis accounts for up to 50 % morbidity and mortality of HIV/AIDS patients (Chen *et al.*, 2009a).

In countries like India, where HAART (Highly Active Antiretroviral Therapy) is not universally available, oropharyngeal candidiasis is still common in HIV infected individuals. United States reports have also shown that *Candida* infections are higher among their patients (Hasan *et al.*, 2009). Currently used chemotherapy globally includes the administration of amphotericin B, fluconazole, voriconazole, ketoconazole and flucytosine (Chen *et al.*, 2009a; Hasan *et al.*, 2009). A notable resistance to antifungal agents is disturbing. For example *in vitro* susceptibility studies conducted in subtropical and tropical areas of Asia demonstrated a high frequency (Chen *et al.*, 2009b). The biofilms produced by the strains are inherently resistant to antifungal agents including amphotericin B and fluconazole (Hasan *et al.*, 2009). Despite great advances in modern medicine, fungal infections have continuously been treated using medicinal plants like; *Acalypha indica* L., *Cassia alata* L., *Lawsonia inermis* L., *Punica granatum* L., *Thespesia populnea* (L.) Sol., *Wrightia tinctoria* R. Br. (Ponnusamy *et al.*, 2010).

2.2.2 *Cryptococcus neoformans*

Cryptococcus neoformans is a saprophytic yeast with a predilection for the central nervous system and leads to severe, life-threatening pneumonia and

meningoencephalitis in both immunocompromised and immunocompetent patients especially HIV-AIDS, cancer therapy and organ transplantation (Masman *et al.*, 2009). It accounts for up to 30 % of mortality and severe morbidity in AIDS patients and responsible for over 95 % of cryptococcosis cases worldwide. Within the past three decades drastic rise in severe systemic fungal infections due to the increased immune-compromised population, mainly resulting from organ transplantation, cancer treatment, and HIV infection has complicated the treatment and management of infectious diseases. Cryptococcal meningitis alone kills about 624,000 people each year (Lin, 2009).

Treatment of cryptococcosis is achieved by administering miconazole, flucunazole, amphotericin B, and flucytosine. Currently, there is a problem of microbial drug resistance to available therapeutical measures and an increase of opportunistic infections (Maregesi *et al.*, 2008). Unfortunately too, only a few classes of antifungals are currently available, and they either lack potency or are toxic to human hosts. Thus the emergence of antifungal resistance adds more difficulties in treating fungal infections, which is exacerbated by the need for long-term usage of antifungals in high risk immunocompromised individuals (Lin, 2009). Despite emphasis being put in research of synthetic drugs, medicinal plants have been of interest due to the fact that a lot of synthetic drugs are potentially toxic and are not free of side effects on the host (Ponnusamy *et al.*, 2010). Plants like the *Elaeodendron schlechteranum* (leaves), *Rhynchosia sublobata* (root), *Acacia tortilis* (stem bark) and *Balanites aegyptiaca* (stem

bark) have been used in treating cyptococcal infections successfully for exhibiting high antifungal activity (Maregesi *et al.*, 2008).

2.2.3 *Aspergillus flavus*

Aspergillus flavus is a mould that produces aflatoxin B1 the most toxic form for mammals which presents hepatotoxic, teratogenic and mutagenic properties, causing damage such as toxic hepatitis, hemorrhage, edema/oedema, immunosuppression, brain abscesses and hepatic carcinoma (Brun *et al.*, 2009; Hell *et al.*, 2009; Reddy *et al.*, 2009). It is more virulent and the second leading cause of invasive and non-invasive aspergillosis resulting to majority of patients diagnosed with invasive aspergillosis dying despite aggressive antifungal therapies (Lin, 2009). The crude mortality from opportunistic fungal infections still exceeds 50% in most human studies and has been reported to be as high as 95% in bone marrow transplant recipients infected with *Aspergillus* sp (Mishra *et al.*, 2010).

About 5 billion people are exposed to aflatoxins in developing countries and aflatoxicosis is ranked 6th among the 10 most important health risks identified by WHO. Aflatoxins produced by toxigenic strains of *A. flavus*, have received significant attention throughout the world because of their hepatocarcinogenic, teratogenic, mutagenic and immunosuppressive properties (Prakash *et al.*, 2010). Currently most of the available effective antifungal agents are based on polyenes (amphotericin B), triazoles (fluconazole, itraconazole, voriconazole, posaconazole, metronidazole, ciprofloxacin) and β-lactam (ampicillin) (Brun *et al.*, 2009).

Despite a better understanding of the epidemiology of *Aspergillus* infections, important diagnostic limitations persist. Accordingly, the mortality for invasive aspergillosis remains very high simply because moulds have the acquired ability to resist chemical treatments and some preservatives, for example, some moulds can grow in the presence of potassium sorbate as others possess the ability to degrade sorbate (Dalie *et al.*, 2010). Plant compounds have been used to improve the shelf life, quality and nutritional value of stored foodstuffs because of their antifungal, antitoxigenic and antioxidant activities (Kumar *et al.*, 2010). Due to toxic and adverse effects, effective antifungal therapy from natural products are being sought to avoid the pain and side effects of drugs to patients (Murthy *et al.*, 2009).

2.2.4 *Trichophyton mentagrophyte*

Trichophyton mentagrophyte is a dermatophyte causing superficial infections characterized by hypopigmented or hyperpigmented macules and patches on the face, neck, chest and back mainly among immunocompromised patients (Derita *et al.*, 2009; Khosravi *et al.*, 2009). Up to 90 % of the patients' infections become life-threatening fungal disease leading to high death rates in HIV/AIDS patients (Gauwerky *et al.*, 2009). Tinea capitis caused by *Trichophyton* and *Microsporum* species account for the majority of fungal disease worldwide. Untreated infection or severe inflammation can result to the hair follicle being permanently damaged, leading to cicatricial alopecia (Mirmirani *et al.*, 2009). Since the early 1980s, fungal infections have emerged as major causes of morbi-mortality, mainly among immunocompromised patients (Derita *et al.*, 2009). Dermatomycoses cause great morbidity in patients receiving antineoplastic

chemotherapy, undergoing organ transplants or suffering from AIDS (Brandao *et al.*, 2010).

Conventional, antifungal agents such as chlorhexidine and imidazole derivatives, amphotericin B, fluconazole, itraconazole, voriconazole, posaconazole are used in the treatment of superficial infections (Bajpai *et al.*, 2009). Fungal infections have emerged as major causes of morbi-mortality. Polyenes cause serious host toxicity, whereas azoles are fungistatic and their prolonged use contributes to the development of drug resistance. Current drug treatments are effective, but the increased emergence of multi-resistant fungal pathogens is alarming (Khan *et al.*, 2009). Plant-derived antifungal compounds are attracting much interest as natural alternatives owing to their versatile applications, safety and effectiveness (Ajesh and Sreejith, 2009). Examples of some plants that have been used belong to the genera *Echinocaulon*, *Amblygonum*, *Persicaria*, *Tiniaria* and *Polygonum* (Derita *et al.*, 2009).

2.2.5 *Microsporum gypseum*

Microsporum gypseum is a dermatophyte that invades keratinized tissues, such as hair, skin or nail, of humans and other animals causing tinea corporis, tinea pedis and onychomycosis. The advent of HIV infection and immunosuppression induced for organ transplants or by cancer chemotherapy has lead to increased predisposition to fungal infections (Koroishi *et al.*, 2008). Fungal infections are one of the major threats in the field of medicine, especially ringworm infections or dermatophytosis widely affecting different body parts like skin, hair and nails (Ponnusamy *et al.*, 2010). Local

surveys in Kenya show that skin diseases take the third place in outpatient morbidity after malaria and respiratory diseases (Njoroge and Bussmann, 2007). Antifungal drugs of imidazoles, itraconazole, triazoles, griseofulvin, butenafine and terbinafine, have been used clinically for the topical treatment of dermathophytosis (Che *et al.*, 2009). Resistance to first-line antibiotic agents is a severe problem globally and their toxicity due to long term treatment also pose a real challenge to the clinical treatment of fungal infections caused by *Microsporum gypseum* (Ponnusamy *et al.*, 2010). These challenges of resistance have prompted scientists to find out new broad-spectrum antifungals for the effective management of dermatomycosis thereby shifting their interest to plants as natural sources for antimicrobial drugs (Koroishi *et al.*, 2008).

Considering the importance of fungal infections and the difficulties encountered in their treatment coupled with increased resistance to antifungals, many scientists have paid attention to extracts isolated from plant species used in herbal medicines inorder to identify new natural compounds that are not based on existing synthetic antimicrobial agents (Rojas *et al.*, 2006; Goncalves *et al.*, 2010). Some of the plants that are used against Microsporium species include; *Prunus africana* (Bii *et al.*, 2010), *Eryngium duriaei* subsp. *juresianum* (M. Laínz) M. Laínz (Cavaleiro *et al.*, 2010), *Thymus zygis* subsp. *sylvestris* (Goncalves *et al.*, 2010).

2.3 Medicinal Plants

In developing countries, medicinal plants are an important social and cultural component (Bussmann *et al.*, 2010). Often they are the only alternative available,

accessible, and affordable treatment for health problems (Toledo *et al.*, 2011). The use of plants for the treatment of various ailments is an old practice probably as old as mankind itself (Demma *et al.*, 2009). These uses are of tremendous importance in many societies, including most rural African communities (Bussmann, 2006). Apart from their therapeutical applications, plants are used as food for humans and livestock, nursing (Edeoga *et al.*, 2005). Due to this, they have attracted continued interest among organic and medicinal scientists over the years (Thomas *et al.*, 2010). In different laboratories of most countries, the antimicrobial effects of herbs have been searched since 1926 (Uslu *et al.*, 2009). This is attributed to the fact that herbal medicines are assumed to be of great importance in the primary healthcare of individuals and communities in many developing countries (Ayyanar and Ignacimuthu, 2005). Since, the traditional knowledge among the community members help scientists in targeting plants that may be medicinally useful instead of relying on trial and error, as in random screening procedures (Fennell *et al.*, 2004).

Ever since the knowledge of the distribution of pharmacologically active principles in higher plants, the importance of such plant-derived medicines in modern therapeutic practice has increased (Ponnusamy *et al.*, 2010). In a report recently published by the World Bank, it pointed out that preserving and enhancing the plant knowledge and use is equivalent to 'rescuing a global heritage'. Plant-based traditional knowledge has become a recognized tool in search for new sources of drugs and neutraceuticals (Ayyanar and Ignacimuthu, 2005). Plant species used for this purpose have been found to contain therapeutic substances which can be extracted and used in preparation of

drugs. The plant itself can also be used either directly or as an extract for medication, a practice that is particularly popular in developing countries (Demma *et al.*, 2009). Natural products, either as pure compounds or as standardized plant extracts, continue to provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity (Mariita *et al.*, 2010b).

Crude extracts, fractionated extracts and individual compounds contain a myriad of compounds that may be acting synergistically. Due to this, they have attracted continued interest among organic and medicinal scientists over the years (Thomas *et al.*, 2010). They have been screened for a wide range of applications as pharmaceutical agents like; antibacterial, antifungal, anti-HIV, anticonvulsant, anti-inflammatory, anti-allergic, antioxidant, anthelmintic, anti-amoebic, antibiotic, anticancer, antihypertensive, tyrokinase PDGF-RTK inhibition, antischistosomal and antimalarial, activity, as well as psychotropic, neurotropic and β-lactamase inhibition properties (Fennell *et al.*, 2004; Toledo *et al.*, 2011). About 20,000 plant species have been reported by WHO to have been used for medicinal purposes because they are well-known natural sources for the treatment of various diseases since antiquity (Maregesi *et al.*, 2008).

Therefore, drug discovery from these medicinal plants continue to provide new and important leads against various pharmacological targets including infectious diseases, malaria, and HIV/AIDS. This is why medicinal plants remain an important source of new drugs, drug leads, and chemical entities (Balunas and Kinghorn, 2005). In the past

few decades there have been intense pharmacological studies, brought about by the recognition of the value of medicinal plants as potential sources of new compounds for therapeutic use (Wagate *et al.*, 2010). The isolation in the early 19th century of early drugs such as morphine, cocaine, codeine, digitoxin, and quinine gives evidence of this claim (Balunas and Kinghorn, 2005).

According to WHO, the provision of safe and effective traditional medicines is a critical tool in increasing access to health care because plants continue to form an integral part of life in many indigenous African communities as a readily and cheaply available alternative to allopathic medicines (Wagate *et al.*, 2010). Knowledge of these plants is very important because not only is there the potential to discover new alternatives for the treatments of illnesses, but also from a conservation point of view. Due to either limited availability or affordability of pharmaceutical medicines about 80% of the rural population in Sub-Saharan Africa depends on traditional herbal remedies for primary health care and veterinary use (Thring and Weitz, 2006; Wagate *et al.*, 2010). Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with side effects and have an enormous therapeutic potential to heal many infectious diseases. For example, vincristine (an antitumor drug), digitalis (a heart regulator) and ephedrine (a bronchodilator used to decrease respiratory congestion) were all originally discovered through research on plants (Kumaraswamy *et al.*, 2008).

In particular, the antimicrobial activity of plant oils and extracts has formed the basis of many applications including raw and processed food preservation, pharmaceuticals,

alternative medicine and natural therapies (Sarac and Ugur, 2007). Despite the improvement on medicine and technology at the present day, the widespread consumption of natural products and the economical crisis has made herbs more effective and purposive in treating various infectious diseases (Uslu *et al.*, 2009). Natural products can be selected for biological screening based on ethnomedical use of plants, because many infectious diseases are known to have been treated with herbal remedies throughout the history of mankind. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries (Sarac and Ugur, 2007).

Every culture on earth today, through written or oral tradition, has relied on the vast variety of natural chemistries found in plants for their therapeutic properties because all drugs extracted from plants are substances with a particular therapeutic action (Seyydnejad *et al.*, 2010). The substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered candidates for developing new antimicrobial drugs (Sahgal *et al.*, 2009). Therefore, systematic screening of medicinal plants with the purpose of discovering new bioactive compounds is a routine activity in many laboratories devoted to biomedical research following the reports of World Health Organization stating that medicinal plants would be the best source to obtain a variety of drugs (Sahgal *et al.*, 2009). Hence, the integration of traditional and modern medicine is gaining increased recognition globally (Omoya and Akharaiyi, 2010).

The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, saponins, terpenoids, cardic glycoside and other phenolic compounds (Edeoga *et al.*, 2005; Mariita *et al.*, 2010a). Thus, phytochemical screening of some medicinal plants used by the Samburu community against selected multidrug resistant pathogens of bacteria and fungi were investigated using standard microbiological techniques to identify the constituents (Adegoke *et al.*, 2010).

Naturally occurring combinations of plant compounds are often synergistic, leading to a higher antimicrobial activity of crude extracts than the pure individual compounds (Lou *et al.*, 2010). The acceptance of natural plant medicine as an alternative for health care, food preservation and prevation of microbial resistance to antibiotics has led authors to investigate the antimicrobial activity of plants. Herbs with antimicrobial activity are being widely used at a commercial scale to prolong the shelf-life and improve the safety of foods. Natural components exhibited both antibacterial activity and antioxidant activity most times (Adegoke *et al.*, 2010; Lou *et al.*, 2010).

2.4 Phytochemicals in medicinal plants

The use of herbs and medicinal plants as the first medicines is a universal phenomenon (Seyydnejad *et al.*, 2010). Besides their importance in health care, plants have high socio-cultural and socio-economic values, providing off-farm income and employment opportunities to local people (Ranpal, 2009). Scientific interest in medicinal plants has

increased today with the search of new therapeutic agents against infectious diseases. The secondary metabolites produced by plants constitute a major source of bioactive substances (Mbosso *et al.*, 2010). The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, phenolic compounds and others (Sharma *et al.*, 2010; Mariita *et al.*, 2011).

2.4.1 Saponins

Saponins have been found in large amounts in the roots of intact plants reaching over 10% in some species. They have been exploited commercially for a variety of purposes including medicines, detergents, adjuvants and cosmetics (Eskander *et al.*, 2006). A large number of mono- and bi-desmosides in *Gypsophila* species have been isolated and characterized. The most common basic structures of their aglycones (sapogenins) are mainly gypsogenin but also in fewer amounts gypsogenic acid and quillaic acid. Some of the saponins are considered as the major bioactive components of the drugs, mainly used for their anti-inflammatory, spermicidal, hypocholesterolaemic, and antiviral activities. Some saponins from *Gypsophila* species have been reported to exert immunomodulant activities. Also, saponins with an aldehyde function at C-4 from *Gypsophila* old hamiana exhibited cytotoxic activity against different human cancer cell lines. *Gypsophila* saponins are of interest in terms of their applications in vaccines hence below is the Structure of Saponins contained in *Gypsophila gypsogenin* 3-O-glucuronide (Gevrenova *et al.*, 2010).

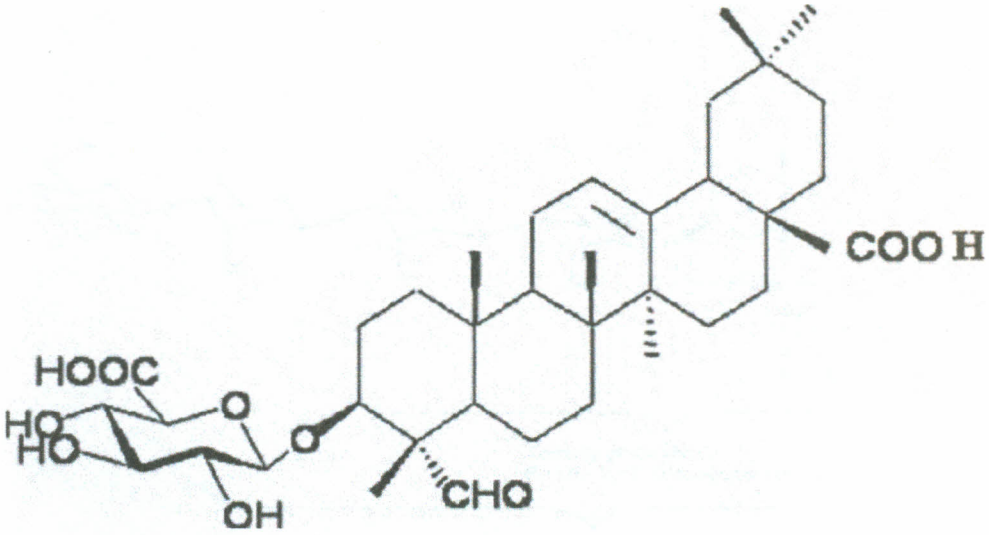


Figure 2. Structure of Saponins contained in *Gypsophila gypsogenin* (Gevrenova *et al.*, 2010).

2.4.2 Cardiac glycosides

Cardiac glycosides are compounds that are therapeutically relevant for the treatment of heart diseases, such as congestive heart failure and atrial fibrillation. The highest content is usually found in the leaves. Medicinal plants contain primary glycosides, such as lanatoside A, B and C. But once the plant is damaged it produces secondary metabolites namely; digitoxin, gitoxin and digoxin. Industrial source of digoxin, the active principle most frequently employed, is used for the semi-synthesis of β-methyldigoxin, which has the same pharmacological properties of its precursor, but a more rapid onset of action. *Digitalis lanata* Ehrh. (Scrophulariaceae) (yellow foxglove) is one such medicinal plant that contains cardiac glycosides (Pellati *et al.*, 2009).

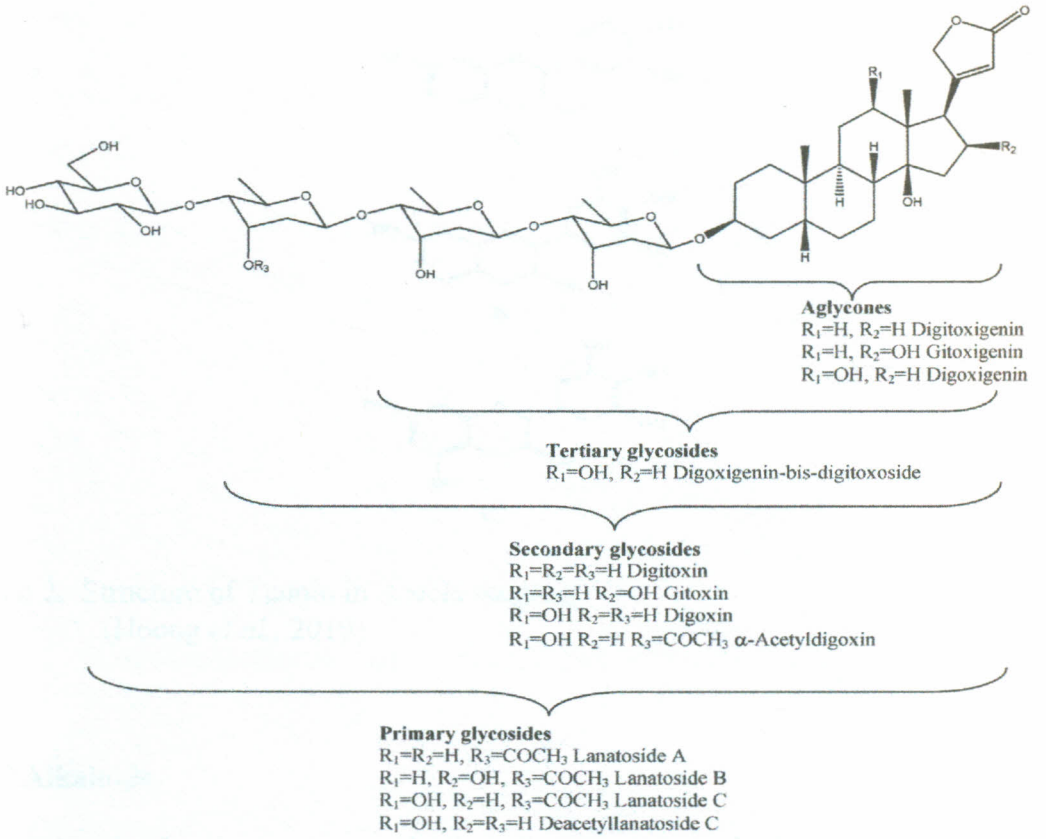


Figure 2. Chemical structure of *D. lanata* cardiac glycosides (Pellati *et al.*, 2009).

2.4.3 Tannins

Tannins are polyphenolic compounds with a molecular weight (Mw) ranging from 500 to 3000 Da. They can be found in the bark, stem, phloem, seeds, fruits, fruit pods, wood leaves and needles of dicotyledon plant. Some medicinal plants contain up to 18–25% tannin in their barks like *Acacia mangium.* Tannins protect plants from herbivores and invasive microbes because of their antibacterial and antifungal properties that they posses (Hoong *et al.*, 2010). Below is a a typical polymer structure of *Acacia mangium* tannin repeating unit: (A)-Profisetinidin, (B)-Prorobinetidin (C)-Prodelphinidin.

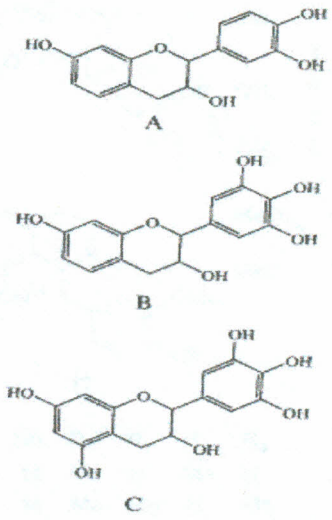


Figure 2. Structure of Tannin in *Acacia magium* (Hoong *et al.*, 2010)

2.4.4 Alkaloids

Alkaloids are chemical compounds that contain carbon, hydrogen and nitrogen. In addition, they may also contain oxygen, sulfur and more rarely other elements such as chlorine, bromine and phosphorus. They usually have heterocyclic structures and occur in approximately 10-20% of all plant species (Schiff, 2002). Alkaloids have both antifungal and antibacterial properties which enable them to commonly be used in the treatment of skin diseases, including various tinea, cutises itching, and skin ulcers (Rao *et al.*, 2009). Phytochemical studies on antifungal activity alkaloids from indigenous remedies resulted into the isolation of 22 alkaloids, including a new aporphine alkaloid fibrecisine (Rao *et al.*, 2009).

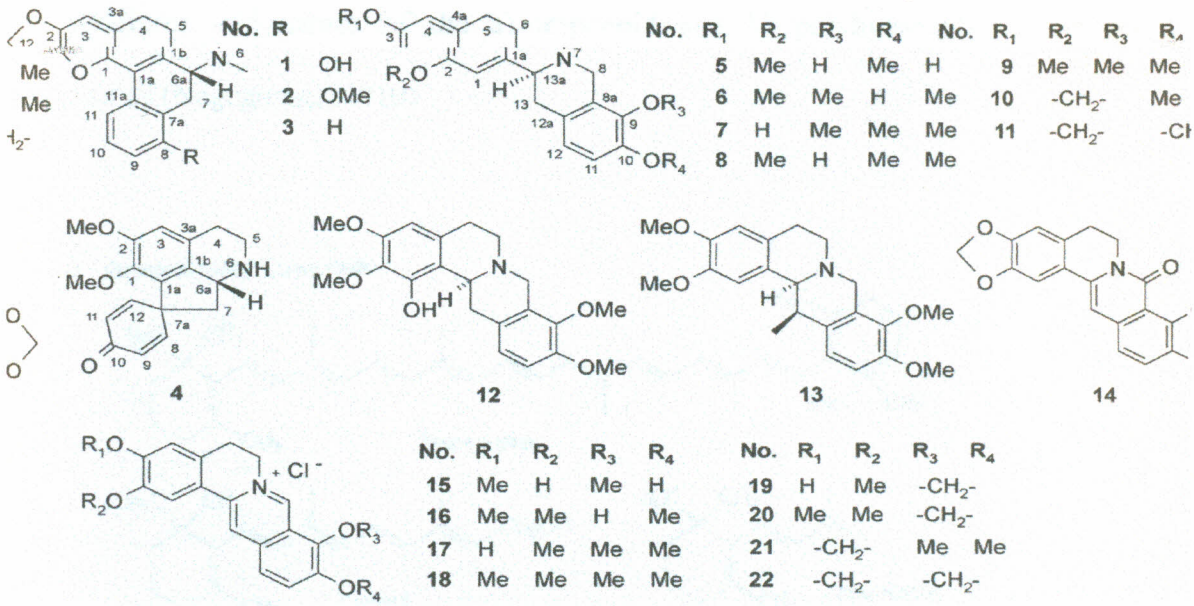


Figure 2. Structure of alkaloids in *Firaurea recisa* (Rao *et al.*, 2009).

2.4.5 Terpenoids

Volatile terpenoids represented by mainly isoprene (C5), monoterpenes (C10) and sesquiterpenes (C15) constitute the largest class of plant volatile compounds (Nagegowda, 2010). Terpenoids from *Spirostachys africana* Sond. (Tamboti tree), which belongs to the family Euphorbiaceae have been used in treating infant's body rashes, diarrhoea, stomach pains, and dysentery (Mathabe *et al.*, 2008). Apart from their importance in plant physiology and ecology, volatile terpenoids are also used as natural flavor and aroma compounds and have beneficial impact on humans as health promoting compounds (Nagegowda, 2010). More than 25,000 known compounds of terpenes and terpenoids form one of the largest and perhaps most structurally diverse groups of secondary metabolites. Triterpenoids of these plants have been isolated and reported to be cytotoxic and contain antimalarial activities (Mutai *et al.*, 2009). Below

are structures and names of the a) terpenoid and b) polyhydroxyphenol model compounds (Nagegowda, 2010).

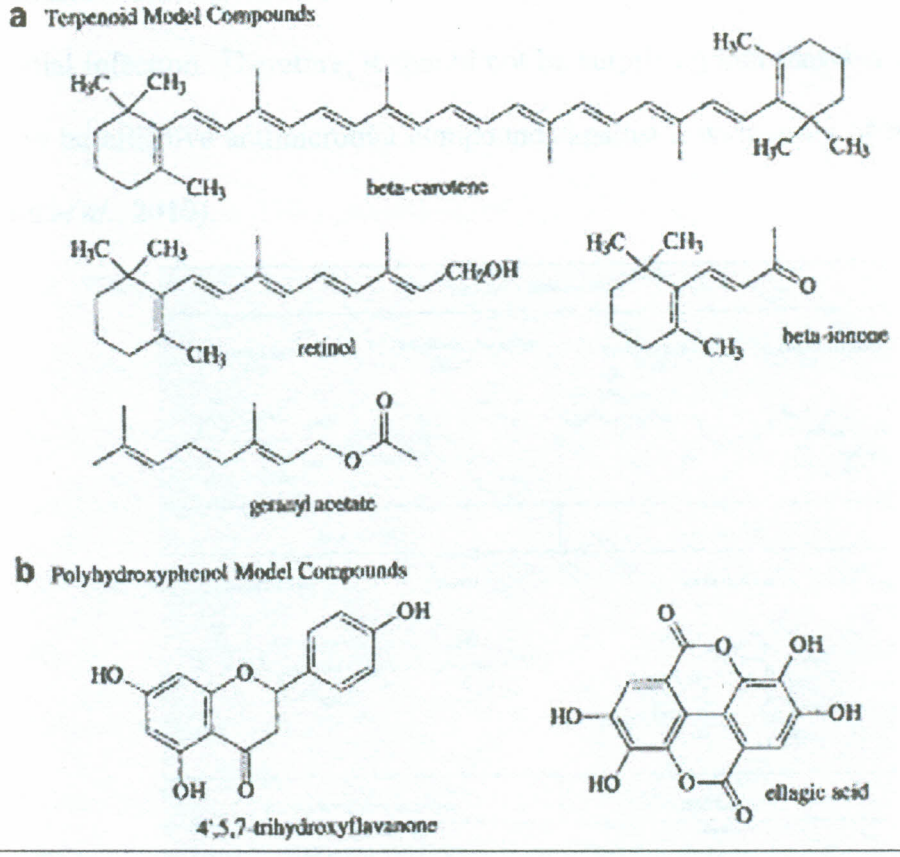


Figure 2. Structure of terpenoids (Nagegowda, 2010).

2.4.6 Flavonoids

Flavonoids have been proposed to exert beneficial effects in a multitude of disease states, including cancer, cardiovascular disease, and neurodegenerative disorders (Williams *et al.*, 2004). Plant-derived flavonoids are a large group of naturally occurring phenylchromones found in fruits, vegetables, tea, and wine. They have been

shown to have a wide range of biological activities like antiallergic, antibacterial, antidiabetic, antinflammatory, antiviral, anti- proliferative, antimutagenic, antithrombotic, anticarcinogenic, hepatoprotective, oestrogenic, insecticidal, and antioxidant. Some flavonoids are formed as antimicrobial barriers in plants response to microbial infection. Therefore, it should not be surprising that they have been found in vitro to be effective antimicrobial compounds against a wide array of microorganisms (Orhan *et al.*, 2010).

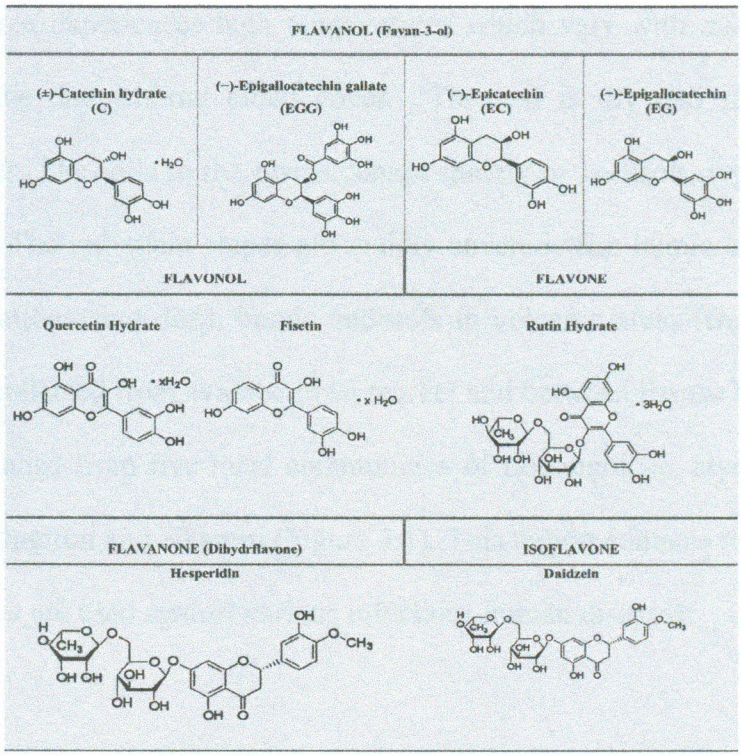


Figure 2. Structure of flavonoids
(Sousa *et al.*, 2009)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

The study was carried out at Wamba Division, Samburu sub-county, Samburu County, Northern Kenya whose main inhabitants is the Samburu community and is located 0.98°N and 37.34°E. This is an arid to semi-arid region with an annual rainfall of between 250 -500mm. The only semi-permanent river is the Ewaso Nyiro. For most of the year, the area experiences high temperatures which vary with altitude (24°C and 33°C) during the day without cloud cover. The soil is dry and sandy with poor vegetation cover. The soils in the plains consist mainly of vertisols, regosols, lithosols and cambisols. The mountain slopes are mainly covered with humic acrisols over the basement formations, and deep, humic andosols in volcanic areas (Bussmann, 2006). Samples were collected from Wamba town market and between Ewaso Nyiro River and the Mathews Range from five local communities of Lodungokwe, Nyamunyat, Ngilai West, Ngutuk Ongiron and Nkaroni (Figure 3.1). This region contains relatively diverse plant species that are used against various infectious human diseases.

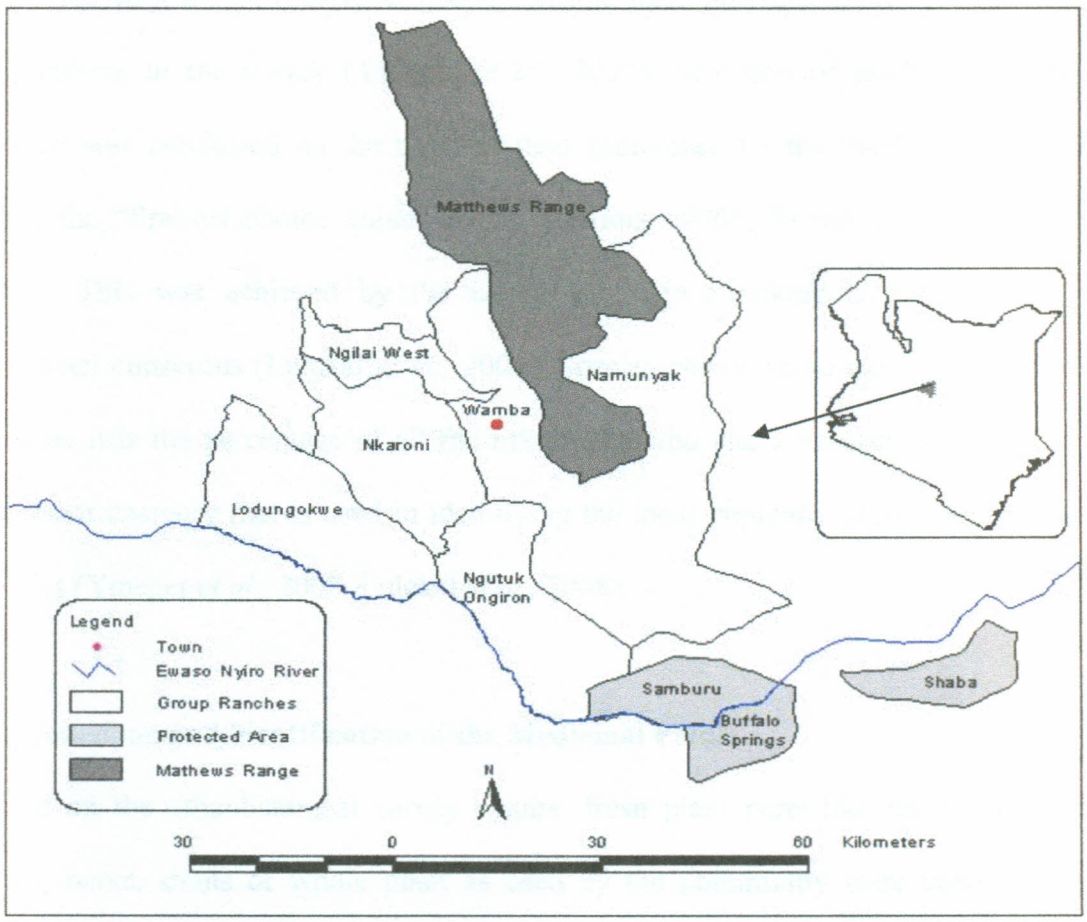


Figure 3. Map of Samburu County, Wamba Division, Northern Kenya (Mariita *et al.*, 2011).

3.2 Selection of medicinal plants

An ethnobotanical survey was carried out on the major medicinal plants used by the community for the treatment of common bacterial and fungal diseases. A questionnaire was administred, guided interviews conducted, focus group discussions with herbalists, market visits, checking various health websites and examining herbarium specimens were used. Informants who are knowledgeable practitioners (herbalists and locals) were

identified with the help of local people and the local administration then selected as respondents to the survey (Yineger *et al.*, 2007). Selection of medicinal plants of interest was conducted on the basis of their preference by the Samburu community using the "Species choice value" model (Gerique, 2006; Siriwatanametanon *et al.*, 2010). This was achieved by the use of preference-ranking technique based on informant consensus (Lulekal *et al.*, 2008). Species choice value model was preferred because it is the percentage of all the informants who cite a number of species for a particular category that is used in identifying the most popularly used medicinal plant species (Yineger *et al.*, 2007; Lulekal *et al.*, 2008).

3.3 Collection and identification of the Medicinal Plants

Based on the ethnobotanical survey results, fresh plant parts like the roots, leaves, seeds, barks, stems or whole plant as used by the community were collected from Wamba division, Samburu Sub-county in Northern Kenya. Plant identification was done by a plant taxonomist from the Department of Pharmacy and Contemporary Medicine, Kenyatta University, Nairobi, Kenya. Voucher specimens collected were prepared and deposited at the Kenyatta University herbarium for future reference (Thatoi *et al.*, 2008; Omwenga *et al.*, 2009; Aremu *et al.*, 2010; Mariita *et al.*, 2011).

3.4 Extraction of Plant Samples

All the twenty two medicinal plant samples collected were washed in tap water, chopped into small pieces and dried under the shade at room temperature for several days until they were completely dry (Sathiya *et al.*, 2008; Thatoi *et al.*, 2008). The dry

pieces were then ground using hammer type milling machine (Meecan, CM/L-1364548, India) at the Department of Pharmacy/Complimentary Alternative Medicine, Kenyatta University, Nairobi, Kenya. The powdered material was then transferred into the soxhlet extractor then extracted using methanol for 72 h (Mariita *et al.*, 2010b). A Whatmann filter paper No. 42 (125 mm) was used in filtering the extracts which were then concentrated using a rotary evaporator (Laborota 4000, SN 090816862, Germany) with the water bath set at 40 °C (Omwenga *et al.*, 2009; Mariita *et al.*, 2010a). The filtrate was dried in a dessicator over anhydrous copper sulphate, then the powdered residues transferred into vials and stored in a refrigerator at 4 °C in airtight vials until used for the biological testing and phytochemical screening (Braga *et al.*, 2007; Sathiya *et al.*, 2008; Aremu *et al.*, 2010; Mariita *et al.*, 2011).

3.5 Bio-Activity Study of the Selected Plant Extracts

The antimicrobial / inhibitory activities of the crude extracts from the selected medicinal plants were screened against a total of eleven microbes comprising of selected bacteria and fungal pathogens. The entire test cultures used in the study both the American Type Culture Collection (ATCC) and clinical isolates that had been tested and identified, were obtained from the Center for Microbiology Research (CMR), Kenya Medical Research Institute (KEMRI), Nairobi, Kenya. The Gram negative bacteria stains included; *Escherichia coli* (ATCC 25922), *Shigella dysenteriae* (ATCC 26988) and *Vibrio cholerae* (ATCC 27622), while the Gram positive bacteria strains included; *Bacillus subtilis* (clinical isolate), *Streptococcus pneumoniae* (ATCC 28910) and *Staphylococcus aureus* (ATCC 25923). The fungi strains were; yeasts of *Candida*

parapsilosis (ATCC 19310) and *Cryptococcus neoformans* (ATCC 18310); mold of *Aspergillus flavus* (clinical isolate) together with dermatophytes of *Microsporum gypseum* (clinical isolate) and *Trichophyton mentagrophyte* (clinical isolate). Common broth microdilution method in 96 multiwell microtiter plates in triplicates were used during the screening according to Al-Bayati and Al-Mola, (2008); Doughari *et al.*, (2008) and Sathiya *et al.*, (2008) models.

3.6 Maintenance of Microbial Stock Cultures

Stock cultures of *Escherichia coli, Shigella dysenteriae, Vibrio cholerae, Bacillus subtilis, Streptococcus pneumoniae* and *Staphylococcus aureus* were grown in Mueller-Hinton broth and incubated at 37 °C for 18-24 h (Nedorostova *et al.*, 2009). The yeasts of *Candida parapsilosis* and *Cryptococcus neoformans* were grown in Potato Dextrose Agar (PDA) and incubated at 37 °C for 24 h (Cruz *et al.*, 2007). *Aspergillus flavus* being a filamentous fungus was grown on PDA plates and incubated at 28 °C for 48 h in humid chambers (Costa *et al.*, 2010). The dermatophytes of *Microsporum gypseum* and *Trichophyton mentagrophyte* were grown on PDA and incubated at 37 °C for 72 h in humid chambers (Duraipandiyan and Ignacimuthu, 2009). Both the bacterial and fungal strains were maintained at 4 °C with periodic sub-culturing on Mueller-Hinton Agar (MHA) slants for bacteria (Bajracharya *et al.*, 2008) and PDA slants for fungi (Bajpai *et al.*, 2009).

3.7 Antimicrobial assays

3.7.1 Disc Diffusion technique (DD)

a) Antibacterial Assays

The disc diffusion technique (ADD) was carried out separately for the test microorganisms to determine the antibacterial activity of plant extracts against the selected microorganisms. Mueller Hinton agar was prepared using manufactures' instructions for purposes of culturing the bacteria. Normal saline solution was used to dilute a 24 h culture of the bacterial type culture or clinical isolate to attain a 0.5 McFarland standard. Spread plate method was used to culture 100 µl of the microbial suspension that was introduced into the petri dishes. Six dry sterile discs (6 mm diameter) were soaked in the plant extract (made by dissolving 300 mg of the extracts in 1000 µl of methanol), air dried and placed (distributed) evenly so that they are not closer than distances of 24 mm from center to center.

Discs impregnated with dimethyl sulfoxide (DMSO) and then air dried were used as negative controls while commercially available discs (HiMedia, Mumbai, India) of chloramphenicol (30 µg) were used as positive control. Incubations were done at 37 ^{O}C for 24 h in accordance with Clinical and Laboratory Standards Institute (CLSI) standards. The antibacterial activities were measured using a transparent ruler as the diameter (mm) of clear zone of growth inhibition around the discs. Three replicates of each test extract were examined and the mean values were recorded (Mutai *et al.*, 2009; Rajpara *et al.*, 2009; Mariita *et al.*, 2010b).

b) Antifungal Assays

To determine the antifungal activities of the plant extracts against the selected fungal strains, Potato Dextrose Agar (PDA) was prepared using manufactures' instructions for the purposes of culturing the fungi. Then the fungal test cultures were cultured by taking 0.1 ml from the broth and spreading on PDA and incubated at right conditions. The cork boarer was used to pick a section of the growing mycelium which had been placed at the center of the PDA plate. Dry disks impregnated with 0.1 ml of the plant extracts were placed (distributed) evenly so that they are not closer than distances of 24 mm from center to center around the section of the fungi (Hudzicki, 2009). The inocula were then incubated at respective conditions where the yeast cultures were incubated at 37 °C for 24 h (Cruz *et al.*, 2007), filamentous fungi at 28 °C for 48 h in humid chambers (Costa *et al.*, 2010) and dermatophytes at 27 °C for 72 h in humid chambers (Duraipandiyan and Ignacimuthu, 2009). Commercially available discs of miconazole were used as the positive control as discs treated with dimethyl sulfoxide served as negative control. All tests were carried out in triplicates and microbial growth inhibition determined by measuring the zones of inhibition using a transparent ruler (Mariita *et al.*, 2011).

3.7.2 Determination of Minimum Inhibitory Concentrations (MICs)

The minimum inhibitory concentrations (MICs) were done only where the plant extract showed high antibacterial activity of ≥9 mm by the disk diffusion method using the 96-well microplates (Mariita *et al.*, 2010a). The wells were filled with 50 µl of the Mueller Hinton broth for bacterial strains and potato dextrose broth for fungi. Then,

50 µl of the plant extract (prepared by mixing 300 mg of the plant extract with 1000 µl of dimethyl formamide (0.01% DMF) for complete dissolution) were dispensed into the first well before serial dilutions. The serial dilutions were achieved by transferring 50 µl of Mueller Hinton broth or potato dextrose broth respectively containing the extract from the first well through the second, third and fourth wells. A fifty microlitres of the test isolate were then dispensed into each well. One well was used as a negative control for microbe growth in the medium. Finally, 50 µl of the antibiotic (chloramphenicol / miconazole) were used as a positive control. Bacteria and yeasts were incubated at 37 °C for 24 h (Cruz *et al.*, 2007), filamentous fungi at 28 °C for 48 h in humid chambers (Costa *et al.*, 2010) and dermatophytes at 27 °C for 72 h in humid chambers (Duraipandiyan and Ignacimuthu, 2009). The concentration that showed no visible growth of the test organism were taken as the MIC (Doughari *et al.*, 2008; Matasyoh *et al.*, 2009; Rajpara *et al.*, 2009; Mariita *et al.*, 2010a).

3.7.3 Determination of Minimum Bactericidal/Fungicidal Concentrations (MBCs / MFCs).

All tests for determining MBCs and MFCs for the crude extracts were carried out in triplicates. The wells where there were no growths from MIC results, the bacteria were sub-cultured on Mueller Hinton agar and fungi on potato dextrose agar followed by incubations at respective right conditions for each microbe. Finally, the MBC or MFC were recorded at the lowest concentration of the plant extracts that did not yield any colony on the solid medium (Doughari *et al.*, 2008; Reuben, 2008; Mariita *et al.*, 2010b).

3.8 Phytochemical screening

The plant extracts were subjected to phytochemical tests using standard qualitative procedures to determine the groups of secondary metabolites present in the plant material.

3.8.1 Test for tannins

A 0.5 mg of the extract was boiled in 10 ml of water in a test tube and then filtered. Five drops of 0.1% ferric chloride were added and observed for brownish green or a blue-black colouration. High (+++), moderate (++) and low (+) levels of tannins were recorded as heavy, medium and slight precipitate (Yusha'u *et al.*, 2010).

3.8.2 Test for saponins

Using the frothing test method; 5 ml of distilled water was added into 0.5 mg of extract in a test tube. The solution was then shaken vigourously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigourously, after which it was observed for the formation of an emulsion (Ayoola *et al.*, 2008). Saponins concentration was recorded as low (+) with a froth height of 50 mm, moderate (++) with a froth height of 60 – 100 mm, and high level concentration (+++) if the height was greater than 100 mm.

3.8.3 Test for cardiac glycosides (Keller-Killani test)

Five ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicated a deoxysugar characteristic of

cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer (Edeoga *et al.*, 2005; Omwenga *et al.*, 2009). High (+++) concentrations of detectable cardiac glycosides were recorded when a deep green-blue colour was observed, a moderate (++) when medium green-blue colour was observed and a low (+) concentrations of detectable cardiac glycosides when a faint green-blue colour was observed (Mariita *et al.*, 2010b).

3.8.4 Test for alkaloids (Wagner's method)

Two hundred milligrams of plant extract was dissolved in 10 ml methanol and then filtered using Whatmann filter paper No. 42 (125 mm) filters. One mililitre of the filtrate was then mixed with 6 drops of Wagner's reagent. The presence of alkaloids was indicated by a creamish, brownish-red or an orange precipitate. An observation of a heavy yellowish-white precipitate, a high (+++) concentration was recorded, alight opalescence precipitate indicated a moderate (++) concentration while a faint turbidity indicated a low (+) concentration of alkaloids (Mariita *et al.*, 2010b).

3.8.5 Test for flavonoids

Five militres of dilute ammonia solution was added to a portion of the aqueous filtrate of each plant extract followed by 1ml of concentrated sulphuric acid. A yellow colouration that disappeared on standing indicated the presence of flavonoids. On the results' recording, a pale yellow colour indicated a low (+) concentration, a moderate yellow coloration indicated a moderate (++) while a high yellow coloration indicated a high concentration of flavonoids (Ayoola *et al.*, 2008).

3.8.6 Test for terpenoids (Salkowski test)

To 0.5 mg each of the extract was added 2 ml of chloroform and then 3 ml of concentrated sulphuric acid (H_2SO_4) was carefully added to form a layer. A reddish brown colour formation at the interface was noted as the presence of terpenoids. On the results, a faint reddish brown colour was recorded as low (+); medium reddish brown colour as moderate (++) concentration while deep reddish brown colour as high (+++) concentration (Manjamalai *et al.*, 2010).

3.9 Data Analysis

Minitab Statistical Software 13.20 © 2000 Minitab Inc. PA 16801-9928, USA was used in analyzing the data. The data analyzed were the average zones of inhibition values for each test cultures obtained from the antibacterial and antifungal assays expressed as means ± standard deviation means. One-way ANOVA at 5% significance level was used in testing the significance among the groups. A probability value of $P \leq 0.05$ was considered significant as Tukey's test was used in analyzing significant differences among group means (Rojas *et al.*, 2006; Mariita *et al.*, 2010a).

CHAPTER FOUR

RESULTS

4.1 Ethnobotanical Survey

The plant parts used by the community in treating various infectious diseases were harvested sustainably. Malaria, wounds, skin, respiratory and gastrointestinal problems were some of the human infections cited to be treated using these plant species. From Table 4.1, it could be noted that all the 22 plant species were distributed among 16 botanical families. Mimosaceae was the most represented with five plant species, followed by Euphorbiaceae with three while the remaining 14 families each had one plant species.

Table 4. Medicinal Plants used by the Samburu Community against selected bacterial and fungal ailments

Botanical Name	**Family Name**	**Local Name**	**Part used**	**Diseases Treated**	**Area collected from**
Aloe secundiflora (Engl.)	Aloaceae	Sukoroi	Leaves	Diarrhea, Polio, Malaria, Stomachach,Chest problems	Namunyak
Sericocomopsis hildebrandtii Schinz	Amaranthaceae	Lturkan	Roots	Malaria, Abdominal disorders.	Lodungokwe
Rhus ruspolii Engler.	Anacardiaceae	Lmugurusian	Seeds	Malaria, Diarrhoea.	Wamba
Acokanthera friesiorum Markgr.	Apocynaceae	Nchipilikwa	Roots	Malaria, Diarrhoea.	Namunyak
Cordia sinensis Lam.	Boraginaceae	Silipani	Stem Barks	Relief of dry coughing, Cold symptoms and joint aches, Stomach disorders, Malaria.	Nkaroni
Thylachium africanum Lour.	Capparaceae	Loimugi	Stem barks	Diarrhoea	Namunyak
Kedrostis pseudogijef (Gilg) C. Jeffrey	Cucurbitaceae	Sakurdumii	Stem (dried /fresh)	Yellow Fever, Diarrhoea.	Nkaroni
Croton megalocarpus Hutch.	Euphorbiaceae	Lmarguet	Root/Bark	Malaria, Fevers, Diarrhoea, Anaplasmosis, Wounds.	Namunyak
Croton macrostachysus Hochst. ex Ferret *et* Galinier	Euphorbiaceae	Ndoopa	Barks	Malaria.	Ngilai West
Euphorbia candelabrum var. erythraeae Berger	Euphorbiaceae	Mpopong'i	Stems Barks	Tuberculosis, Bronchitis, Asthma and Chest problems, URTI and GIT complications.	Namunyak
Albizia anthelmithica Brongn.	Leguminoceae	Lumurtana	Roots Barks	Parasite, Deworming.	Nkaroni

Loranthus acaciae Zucc.	Loranthaceae	Landenyai	Whole plant	Post-natal cleansing of the uterus after birth.	Nkaroni
Acacia tortilis (Forssk.) Hayne.	Mimosaceae	Ndapes	Barks	Diarrhoea, Stomachache	Namunyak
Acacia horrida (L.) Willd.	Mimosaceae	Lerai	Barks	Diarrhoea.	West gate
Acacia nilotica (L.) Del.	Mimosaceae	Lkiloriti	Roots Barks	Diarrhoea, Appetite, General fitness, Stomach problems, Chest Pain, TB, Abdominal pains,	Namunyak
Acacia senegal (L.) Willd.Var. *persica*	Mimosaceae	Lderekesi	Barks	Diarrhoea.	Nkaroni
Acacia ethaaica Schweinf.	Mimosaceae	Lcakwai	Stem Bark	Indigestion, Diarrhoea.	Namunyak
Plumbago dawei Rolfe	Plumbaginaceae	Lkirianthus	Roots	Malaria, Abdominal disorders.	Ngilai West
Cissus quadrangularis . L.	Rhamnaceae	Sukurtuti	Roots Stem	Diarrhoea, TB, Epilepsy, Pancreatitis, Malaria, Liver problems, Asthma, Wounds, Gastric ulcers, Neurosis, Schistosomiasis, Heumatism.	Nkaroni
Salvadora persica L. var. *persica*	Salvadoraceae	Sekotei	Roots	Measles, Preventing Miscarriages and Anti-plaque, Toothbrush.	Nkaroni
Clerodendrum myriacoides (Hochst.)Vatke subsp. *apperae* Verdc.	Verbenaceae	Lmakutukuti	Roots	Diarrhoea, Cold, Prostate Cancer, Malaria, Headache STDs, GIT, Arthritis / Joint Pain, Miscarriage Prevention, Lumbago, Polio, Typhoid.	West gate
Cissus rotundifolia Forsk. Vahl.	Vitaceae	Raraiti	Roots	Malaria,Polio, Diarrhoea.	Nkaroni

Table 4. Plants collected per family

Botanical Name	Family Name	Local Name	Part used	Diseases Treated	Area collected from
Aloe secundiflora (Engl.)	Aloaceae	Sukoroi	Leaves	Diarrhea, Polio, Malaria, Stomachache, Chest problems.	Namunyak
Sericocomopsis hildebrandtii Schinz	Amaranthaceae	Lturkan	Roots	Malaria, Abdominal disorders.	Lodungokwe
Rhus ruspolii Engler.	Anacardiaceae	Lmugurusian	Seeds	Malaria, Diarrhoea.	Wamba
Acokanthera friesiorum Markgr.	Apocynaceae	Nchipilikwa	Roots	Malaria, Diarrhoea.	Namunyak
Cordia sinensis Lam.	Boraginaceae	Silipani	Stem Barks	Relief of dry coughing, Cold symptoms and joint aches, Stomach disorders, Malaria.	Nkaroni
Thylachium africanum Lour.	Capparaceae	Loimugi	Stem barks	Diarrhoea	Namunyak
Kedrostis pseudogijef (Gilg) C. Jeffrey	Cucurbitaceae	Sakurdumii	Stem (dried /fresh)	Yellow Fever, Diarrhoea.	Nkaroni
Croton megalocarpus Hutch.	Euphorbiaceae	Lmarguet	Root/Bark	Malaria, Fevers, Diarrhoea, Anaplasmosis, Wounds.	Namunyak
Croton macrostachysus Hochst. ex Ferret *et* Galinier	Euphorbiaceae	Ndoopa	Barks	Malaria.	Ngilai West
Euphorbia candelabrum var. erythraeae Berger	Euphorbiaceae	Mpopong'i	Stems Barks	Tuberculosis, Bronchitis, Asthma and Chest problems, URTI and GIT complications.	Namunyak
Albizia anthelmithica Brongn.	Leguminoceae	Lumurtana	Roots Barks	Parasite, Deworming.	Nkaroni
Loranthus acaciae Zucc.	Loranthaceae	Landenyai	Whole plant	Post-natal cleansing of the uterus after birth.	Nkaroni

Acacia tortilis (Forssk.) Hayne	Mimosaceae	Ndapes	Barks	Diarrhoea, Stomachache	Namunyak
Acacia horrida (L.) Willd.	Mimosaceae	Lerai	Barks	Diarrhoea.	West gate
Acacia nilotica (L.) Del.	Mimosaceae	Lkiloriti	Roots Barks	Diarrhoea, Appetite, General fitness, Stomach problems, Chest Pain, TB, Abdominal pains.	Namunyak
Acacia senegal (L.) Willd.Var. *persica*	Mimosaceae	Lderekesi	Barks	Diarrhoea.	Nkaroni
Acacia ethaaica Schweinf.	Mimosaceae	Lcakwai	Stem Bark	Indigestion, Diarrhoea.	Namunyak
Plumbago dawei Rolfe	Plumbaginacea e	Lkirianthus	Roots	Malaria, Abdominal disorders.	Ngilai West
Cissus quadrangularis . L.	Rhamnaceae	Sukurtuti	Roots Stem	Diarrhoea, TB, Epilepsy, Asthma, Pancreatitis, Malaria, Liver problems, Wounds, Gastric ulcers, Neurosis, Schistosomiasis, Heumatism.	Nkaroni
Salvadora persica L. var. *persica*	Salvadoraceae	Sekotei	Roots	Measles, Preventing Miscarriages and Anti-plaque, Toothbrush.	Nkaroni
Clerodendrum myriacoides (Hochst.)Vatke subsp. *apperae* Verdc.	Verbenaceae	Lmakutukuti	Roots	Diarrhoea, Cold, Prostate Cancer, Malaria, Headache STDs, GIT, Arthritis / Joint Pain, Miscarriage Prevention, Lumbago, Polio, Typhoid, Aphrodisiac	West gate
Cissus rotundifolia Forsk. Vahl.	Vitaceae	Raraiti	Roots	Malaria,Polio, Diarrhoea.	Nkaroni

Plate 4. Some Samburu medicinal plants collected

(a) *Albizia anthelmithica*

(b) *Clerodendrum myriacoides*

(c) *Acacia nilotica*

(d) *Loranthus acacciae*

(e) *Salvadora persica*

(f) *Acokanthera friesiorum*

4.2 Antimicrobial results for bacteria

Based on bioassays conducted on the methanolic extracts of various medicinal plants, varied results against bacteria were obtained. Agar disc diffusion method was used in obtaining the zones of inhibition for all the twenty two medicinal plants. From the computed average values, antibacterial activity of ≥9–19 mm was recorded as high. From this study it was observed that; *Loranthus acaciae* exhibited a high antibacterial activity of 18.33 mm against *Vibrio cholerae*, and 18.00 mm against *Shigella dysenteriae*. *Rhus ruspoli* produced an antibacterial activity of 19.33 mm against *Streptococcus pneumoniae* while *Acacia nilotica* produced an activity of 13.66 mm against *Bacillus subtilis* and *Escherichia coli* (10.00 mm) (Table 4.3).

For *Clerodendrum myriacoides* a 17.66 mm antibacterial activity against *Streptococcus pneumoniae* was observed as *Thylachium africanum* produced an antibacterial activity of 16.00 mm against *Staphylococcus aureus* while *Acacia horrida* produced an activity of 13.33 mm against *Staphylococcus aureus*. Moderate antibacterial activity of ≥7–9 mm was also recorded on some extracts like that of *Cissus quadrangularis* and *Kedrostis pseudogijef* against *Bacillus subtilis* (8.66 mm). Most of the other extracts did not show any activity against the strains. For example, *Acacia tortilis* and *Acokanthera friesiorum* did not exhibit any visible activity (6.00 mm) against all the six bacterial strains (Table 4.3).

Table 4. Zones of Inhibition (mm) produced by the plant extracts against bacterial strains

Medicinal plants	*Escherichia coli*	*Vibrio cholerae*	*Staphylococcus aureus*	*Bacillus subtilis*	*Shigella dysenteriae*	*Streptococcus pneumoniae*
Cissus rotundifolia	6.00	6.00	6.00	7.33	6.00	6.00
Acacia tortilis	6.00	6.66	6.00	6.00	6.00	6.00
Acokanthera friesiorum	6.00	6.00	6.00	6.00	6.00	6.00
Acacia horrida	9.33	10.00	13.33	11.66	11.00	6.00
Acacia nilotica	10.00	9.00	11.00	13.66	10.33	6.00
Rhus ruspoli	6.00	6.00	6.00	6.33	6.00	19.33
Clerodendrum myriacoides	6.00	6.00	6.00	6.00	6.00	17.66
Albizia anthelmithica	6.33	10.66	8.00	6.66	6.00	6.00
Aloe secundiflora	6.00	6.66	6.00	6.00	7.33	8.66
Sericocomopsis hildebrandtii	6.00	7.00	6.00	6.00	6.00	6.00
Cissus quadrangularis	6.00	7.00	7.66	8.66	6.00	6.00
Thylachium africanum	7.00	11.66	16.00	13.00	12.66	6.66
Acacia senegal	6.00	6.66	6.00	10.66	6.00	6.00
Croton megalocarpus	6.00	6.00	7.00	8.00	7.00	7.00
Croton macrostachysus	6.00	6.00	6.00	6.00	6.00	6.00
Salvadora persica	7.00	6.00	6.00	6.00	6.33	6.00
Acacia ethaaica	9.33	7.00	12.00	11.33	10.33	6.00
Loranthus acaciae	10.00	18.33	18.00	17.33	16.66	6.00
Cordia sinensis	6.00	7.00	6.00	6.00	6.00	6.00
Euphorbia candelabrum	6.00	6.00	6.00	6.00	6.00	6.00
Kedrostis pseudogijef	6.33	6.00	6.33	8.66	6.00	6.00

Plumbago dawei	6.66	7.33	10.66	6.00	11.00	6.00
Positive control	19.00	21.33	24.66	25.33	17.00	33.66
Negative control	6.00	6.00	6.00	6.00	6.00	6.00

Controls: Negative control - Dimethyl sulfoxide (evaporated discs)

Positive control - Chloramphenicol (commercially produced discs)

In this study, most of the plant extracts did not showhigh antibacterial activity against *Escherichia coli* (ATCC 25922). *Acacia horrida* (9.33 mm), *Acacia nilotica* (10.00 mm) and *Loranthus accaciae* (10.00 mm) produced high zones of inhibition which were very high. *Salvadora persica* and *Thylachium africanum* produced moderate zones of inhibition of 7 mm while all the remaining plants extracts of *Cissus rotundifolia, Acacis tortilis, Acokanthera friesiorum, Aloe secundiflora, Sericocomopsis hildebrandtii* and *Cissus quadrangularis* were all completely inactive against the test organism. At $P \leq 0.05$, the means of the zones of inhibition showed a significant difference (Appendix II a.).

Based on the zones of inhibition against *Vibrio cholerae* (ATCC 27622) high antimicrobial activities were recorded on *Acacia horrida, Acacia nilotica, Albizia anthelmithica, Thylachium africanum* and *Loranthus acaciae.* Out of the five, *Loranthus acaciae* produced the highest zone of inhibition of 18.33 mm as *Acacia nilotica* produced the least of 9 mm in this group. A number of the remaining plants produced moderate zones of inhibition like *Sericocomopsis hildebrandtii, Cissus quadrangularis, Acacia ethaaica, Cordia sinensis* and *Plumbago dawei.* The remaining

plants extracts were all completely inactive against the test organism (Table 4.3). At $P \leq 0.05$, the means of the zones of inhibition showed a significant difference (Appendix II b).

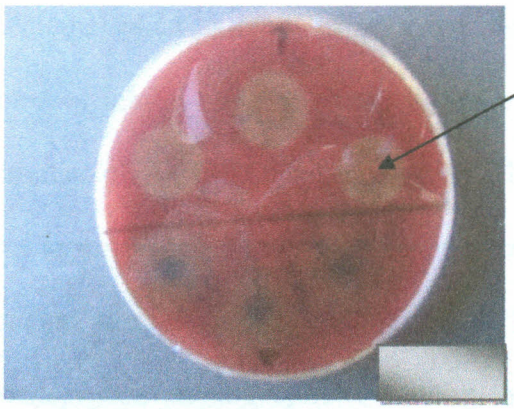
For the case of *Staphylococcus aureus* (ATCC 25923); *Acacia horrida, Acacia nilotica, Thylachium africanum, Acacia ethaaica, Loranthus acaciae* and *Plumbago dawei* produced high antibacterial activity of 13.33 mm, 11.00 mm, 16.00 mm, 12.00 mm, 10.66 mm, 18.00 mm and 16.00 mm respectively. It was noted that *Cissus quadrangularis* and *Croton megalocarpus* had low antibacterial activity while *Euphorbia candelabrum, Croton macrostachysus, Salvadora persica* among others proved to be completely inactive (6.00 mm) (Table 4.3). At $P \leq 0.05$, the means of the zones of inhibition showed a significant difference (Appendix II c).

Plant extracts with high antibacterial activity against *Bacillus subtilis* (Clinical isolate) included those of *Acacia horrida, Acacia nilotica, Thylachium africanum, Acacia senegal, Acacia ethaaica* and *Loranthus acaciae.* Worth mentioning is *Loranthus acaciae* which produced a zone of inhibition of 17.33 mm, closely followed by *Acacia nilotica* with 13.66 mm. *Cissus quadrangularis, Croton megalocarpus* and *Kedrostis pseudogijef* that produced moderate activity of about 8.00 mm. Most of the remaining plants like; *Acacia tortilis, Acokanthera friesiorum, Croton macrostachysus* among others showed complete inactivity against the pathogens (6.00 mm) (Table 4.3). At $P \leq 0.05$, the means of the zones of inhibition showed a significant difference (Appendix II d).

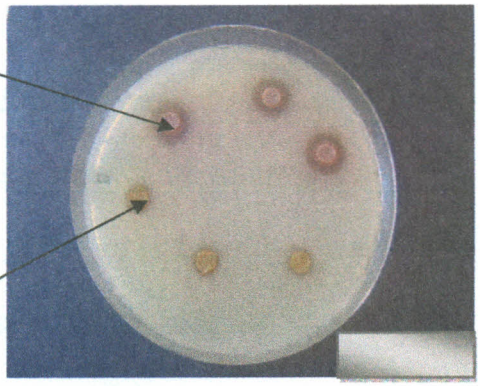
A total of six plant extracts had a high activity against *Shigella dysenteriae* (ATCC 26988). These include; *Plumbago dawei, Acacia ethaaica, Loranthus acaciae, Thylachium africanum, Acacia horrida* and *Acacia nilotica. Loranthus acaciae* still produced the highest inhibitory activity of 16.66 mm, while *Acacia ethaaica* and *Acacia nilotica* produced the least at 10.33 mm each within this group. Most of the remaining plants were inactive against this test organism (Table 4.3). At $P \leq 0.05$, the means of the zones of inhibition showed a significant difference (Appendix II e).

In the case of *Streptococcus pneumoniae* (ATCC 28910) only two plants extracts proved to be active which are *Rhus ruspoli* that produced a zone of inhibition of (19.33 mm) and *Clerodendrum myriacoides* (17.66 mm). On the other hand, *Aloe secundiflora* and *Croton megalocarpus* produced moderate activity of 8.66 mm and 7.00 mm respectively. All the remaining extracts were completely inactive (6.00 mm) (Table 4.3). At $P \leq 0.05$, the means of the zones of inhibition showed a significant difference (Appendix II f).

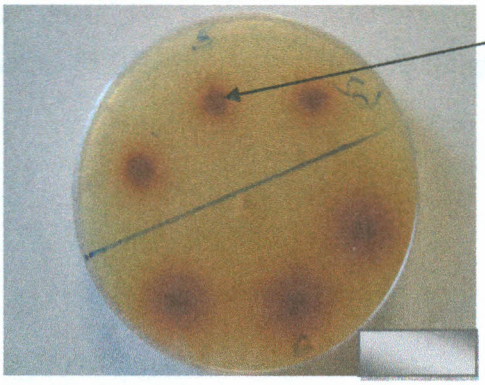
Plate 4. Some zones of inhibition produced by plant extracts against bacterial strains



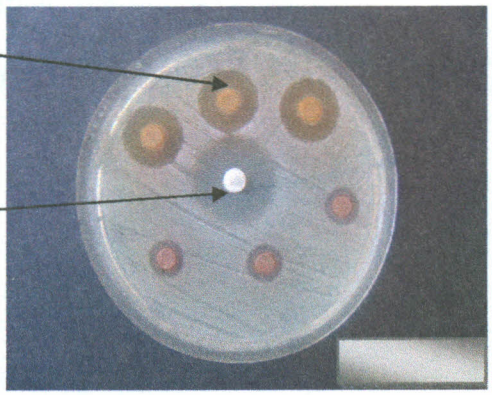
(a) Zones of inhibition of *Rhus ruspoli* (7) and *Clerodendrum myriacoides* (8) against *Streptococcus pneumoniae*



(b) Zones of inhibition of *Acacia nilotica* and negative control against *Escherichia coli*



(c) Zones of inhibitions of *Acokathera friesiorum* against *Shigella dysenteriae*



(d) Zones of inhibition of *Loranthus acaciae* (at the periphery) and that of the positive control (at the centre) *against Vibrio cholerae*

Key: The arrows are pointing at the area of the inhibition zones produced by plant extracts.

4.3 Antimicrobial results for fungi

Medicinal plant extracts screened against fungal strains produced quite varied results. Zones of inhibition were obtained by the use of disc diffusion method. High antifungal activities of ≥9–18 mm were recorded. The highest mean value of 18.66 mm was record on *Clerodendrum myriacoides* against *Microsporium gypseum,* followed by *Loranthus acaciae* producing a mean value of 14.66 mm both for *Aspergillus flavus* and *Trichophyton mentagrophyte.* Among the plant extracts most active against *Candida parapsilosis* are those of *Loranthus acaciae* which produced a zone of inhibition value of 14.33 mm. *Clerodendrum myriacoides* was the most active against *Cryptococcus neoformans* producing a mean value of 11.00 mm. A small portion of the remaining plants extracts showed moderate activities while a larger portion was completely inactive (6.00 mm) against the five fungal test cultures (Table 4.4).

Table 4. Zones of Inhibition (mm) produced by plant extracts against fungal strains

Medicinal plants	*Candida parapsilosis*	*Cryptococcus neoformans*	*Aspergillus flavus*	*Microsporium gypseum*	*Trichophyton mentagrophyte*
Cissus rotundifolia	6.00	6.00	6.00	6.00	6.00
Acacis torlitis	6.00	6.00	6.00	6.00	6.00
Acokanthera friesiorum	6.00	6.00	6.00	6.00	6.00
Acacia horrida	6.00	6.00	6.00	6.00	6.00
Acacia nilotica	12.66	6.00	11.66	13.33	11.66
Rhus ruspoli	6.00	6.00	6.00	8.33	6.00
Clerodendrum myriacoides	6.00	11.00	6.00	18.66	6.00
Albizia anthelmithica	6.00	6.00	7.00	6.00	6.00
Aloe secundiflora	6.00	6.33	6.00	6.00	6.00
Sericocomopsis hildebrandtii	6.00	6.33	6.66	7.66	6.00
Cissus quadrangularis	6.00	6.00	6.00	6.00	6.00
Thylachium africanum	10.66	9.00	10.00	10.66	10.66
Acacia senegal	6.00	6.00	6.00	6.00	6.00
Croton megalocarpus	6.00	6.00	6.00	7.00	6.00
Croton macrostachysus	6.00	6.00	6.00	6.00	6.00
Salvadora persica	6.00	6.00	6.00	6.00	6.00
Acacia ethaaica	6.00	9.66	6.00	8.66	6.00
Loranthus acaciae	14.33	6.00	14.66	14.00	14.66
Cordia sinensis	6.66	6.00	6.00	6.00	6.00
Euphorbia candelabrum	6.00	6.00	6.00	6.00	6.00
Kedrostis pseudogijef	6.00	6.00	6.00	9.33	6.00

Plumbago dawei	6.00	6.00	6.00	6.00	6.00
Positive control	20.66	21.00	19.00	20.30	22.00
Negative control	6.00	6.00	6.00	6.00	6.00

Controls: Negative control - Dimethyl sulfoxide (evaporated discs)

Positive control - Miconazole (commercially produced discs)

Acacia nilotica, Loranthus acaciae and *Thylachium africanum* produced high antifungal activity against *Candida parapsilosis (*ATCC 18310) producing mean values of 12.66 mm, 14.33 mm, and 10.66 mm respectively. All the remaining nineteen plant extracts were completely inactive (6 mm) (Table 4.4). At $P \leq 0.05$, the means of the zones of inhibition showed a significant difference (Appendix II g).

Three plant extracts showed high antifungal activities against *Cryptococcus neoformans* (ATCC 19310) which included; *Clerodendrum myriacoides* (11.00 mm), *Thylachium africanum* (9.00 mm), and *Acacia ethaaica* (9.33 mm). All the other species were completely inactive including *Loranthus acaciae* which was active against the other ten microbes (Table 4.4). The means of the zones of inhibition showed a significant difference at $P \leq 0.05$ (Appendix II h).

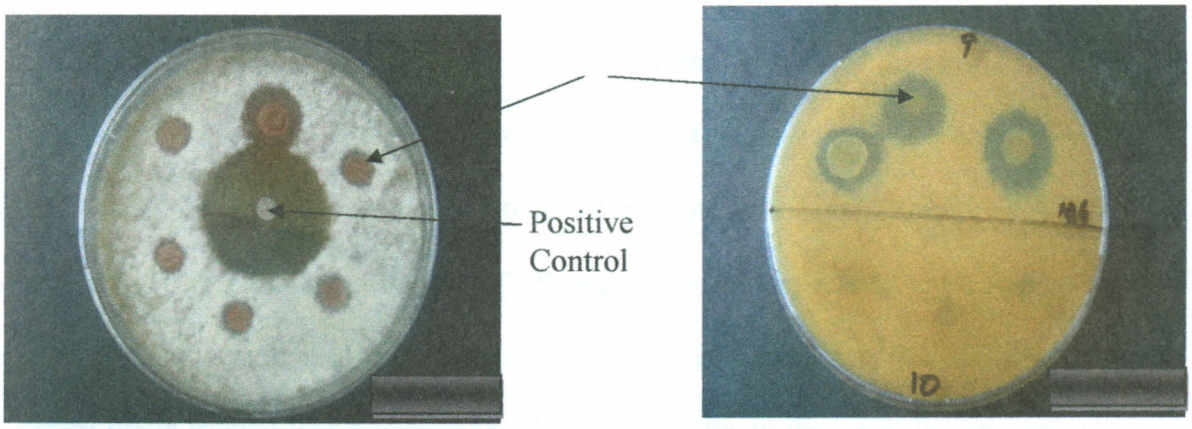
The zones of inhibition against a clinical isolate of *Aspergillus flavus* with high antifungal activities were recorded on *Acacia nilotica* (11.66 mm), *Thylachium africanum* (10.00 mm) and *Loranthus acaciae* (14.66 mm). *Albizia anthelmithica* produced a moderate antifungal activity of 7 mm while all the other remaining plant

extracts were completely inactive (6 mm) against this test organism (Table 4.4). At $P \leq 0.05$, the means of the zones of inhibition showed a significant difference (Appendix II i).

A number of plant extracts produced high antifungal activity against a clinical isolate of *Microsporum gypseum* with *Clerodendrum myriacoides* producing the highest zone of inhibition of 18.66 mm, then a mean of 14.00 mm produced by *Loranthus acaciae* followed by 13.33 mm recorded by *Acacia nilotica*. *Thylachium africanum* produced 10.66 mm and last in the group was *Kedrostis pseudogijef* with a mean zone of 9.33 mm. Moderate antifungal activities were observed among *Acacia ethaaica* (8.66 mm), *Croton megalocarpus* (7.00 mm), and *Rhus ruspoli* (8.33 mm). The remaining plant extracts of *Plumbago dawei, Cordia sinensis, Croton macrostachysus, Albizia anthelmithica, Aloe secundiflora, Acacia tortilis* and *Acokanthera friesiorum* among others were completely inactive producing a mean zone of 6.00 mm (Table 4.4). At $P \leq 0.05$, the means of the zones of inhibition there was no significant difference seen (Appendix II j).

Loranthus acaciae, Thylachium africanum and *Acacia nilotica* produced mean zones of inhibition of 14.66 mm, 10.66 mm and 11.66 mm respectively against *Trichophyton mentagrophyte* (Table 4.4). No plant extract exhibited a moderate antifungal activity but all the remaining extracts had no antimicrobial activity. Incidentally, there was no significant difference on the means of the zones of inhibition was showed at $P \leq 0.05$ (Appendix II k).

Plate 4. Some zones of inhibition produced by plant extracts against fungal strains



(a) Zones of inhibition of the positive control with that of *Thylachium africanum* against *Trichophyton mentagrophyte*

(b) Zones of inhibition of *Clerodendrum myriacoides* against *Microsporum gypseum*

Key: The arrows are pointing at the area of the inhibition zones.

4.4 The Minimum Inhibitory Concentrations (MICs) and the Minimum Bactericidal/Fungicidal Concentrations (MBCs/ MFCs).

Minimum Inhibitory Concentrations (MICs) was determined by the broth microdilution in 96-well microtiter plates and was considered as the lowest concentration of the sample that inhibits visible growth of a microbe. This was carried out only in cases where the plant extract showed high antibacterial activity by the disc diffusion method (≥9-15 mm) (Mariita *et al.*, 2010). The various results obtained are as presented in Table 4.5 for bacteria and Table 4.5 for fungi.

4.4.1 The Minimum Inhibitory Concentrations (MICs) and the Minimum Bactericidal Concentrations (MBCs) (mg/ml) for bacterial test cultures

Acacia horrida, Acacia nilotica, Acacia ethaaica and *Loranthus acaciae* are the only plant extracts that had their MICs and MBCs determined against *Escherichia coli* (ATCC 28922). This was decided upon and carried out based on their high antibacterial activities giving zones of inhibition of ≥9 mm. It was observed that there was no plant extract whose antimicrobial activity was equal to or more than that of the positive control while growth was observed in all concentrations in the tubes for the case of the negative control. It is only *Acacia ethaaica* that had a MIC not equal to MBC; it gave 37.5 mg/ml and 75 mg/ml respectively. On the other hand, *Acacia horrida, Acacia nilotica* and *Loranthus acaciae* all had the same MIC and MBC value of 37.5 mg/ml (Table 4.5).

For the case of *Vibrio cholerae* (ATCC 27622), *Loranthus acaciae* produced high MIC and MBC of 9.375 mg/ml very close to that of the positive control value of 4.687 mg/ml. *Acacia horrida* gave MIC value of 37.5 mg/ml and a MBC of 75 mg/ml. *Acacia nilotica, Thylachium africanum* and *Aloe secundiflora* all gave similar MICs and MBCs of 37.5 mg/ml. None of the plants gave a MIC similar to that of the positive control (Table 4.5).

Plant extracts screened for MICs and MBCs against *Staphyloccocus aureus* (ATCC 25923) showed high antimicrobial activity. For example, *Acacia horrida* gave results with similar MIC and MBC value of 18.75 mg/ml. *Acacia nilotica* produced MIC of

18.75 mg/ml and MBC of 37.5 mg/ml. The results of *Aloe secundiflora, Thylachium africanum* and *Loranthus acaciae* were very high too producing similar MICs and MBCs results of 9.375 mg/ml. No plant extract that gave similar results as those of the positive control (Table 4.5).

The determination of MICs and MBCs against *Bacillus subtilis* (Clinical isolate) proved to be equally high. *Acacia horrida* gave a similar activity for MIC and MBC of 37.5 mg/ml. *Acacia nilotica* and *Acacia ethaaica* gave similar values of MIC of 18.75 mg/ml and MBC of 37.5 mg/ml. *Thylachium africanum* showed MIC similar to MBC of 18.75 mg/ml. For this microbe, *Loranthus acaciae* had the highest activity giving a MIC of 9.375 mg/ml and MBC of 18.75 mg/ml (Table 4.5).

MICs and MBCs against *Shigella dysenteriae* (ATCC 26988) were equally determined. *Acacia horrida* produced high antimicrobial activity with MIC at 18.75 mg/ml and MBC as 37.5 mg/ml. Other extracts screened were those of *Acacia nilotica, Thylachium africanum, Acacia ethaaica* and *Loranthus acaciae* where *Acacia nilotica* and *Acacia ethaaica* produced similar MIC and MBC values of 37.5 mg/ml. *Thylachium africanum* produced MIC of 18.75 mg/ml and MBC of 37.5 mg/ml. *Loranthus acaciae* was the only plant extract that produced the highest antimicrobial activity against *Shigella dysenteriae* (ATCC 26988), producing similar MIC and MBC values of 18.75 mg/ml (Table 4.5).

Rhus ruspoli and *Clerodendrum myriacoides* were the only extracts that produced high antimicrobial activity against *Streptoccocus pneumoniae* (ATCC 28910). The point to note is that these plants were not active against the other bacterial microbes. *Rhus ruspoli* produced similar MIC and MBC of 18.75 mg/ml as *Clerodendrum myriacoides* produced MIC of 18.75 mg/ml and MBC of 37.5 mg/ml (Table 4.5).

Table 4. MICs (mg/ml) and MBCs (mg/ml) produced by plant extracts against bacterial strains

	E. coli		*V. cholerae*		*S. aureus*		*B. subtilis*		*S. dysenteriae*		*S. pneumoniae*	
Medicinal Plants	MIC (mg/ 50µl	MBC (mg/ 50µl)	MIC (mg/ 50µl	MBC (mg/ 50µl)	MIC (mg/ 50µl)	MBC (mg/ 50µl)	MIC (mg/ 50µl)	MBC (mg/ 50µl)	MIC (mg/ 50µl)	MBC (mg/ 50µl)	MIC (mg/ 50µl)	MBC (mg/ 50µl)
Acacia horrida	37.5	37.5	37.5	75	18.75	18.75	37.5	37.5	18.75	37.5	ND	ND
Acacia nilotica	37.5	37.5	37.5	37.5	18.75	37.5	18.75	37.5	37.5	37.5	ND	ND
Rhus ruspoli	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	18.75	18.75
Clerodendrum myriacoides	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	18.75	37.5
Thylachium africanum	ND	ND	37.5	37.5	9.375	9.375	18.75	18.75	18.75	37.5	ND	ND
Acacia ethaaica	37.5	75	ND	ND	ND	ND	18.75	37.5	37.5	37.5	ND	ND
Loranthus acaciae	37.5	37.5	9.375	9.375	9.375	9.375	9.375	18.75	18.75	18.75	ND	ND
Positive control	4.687	4.687	4.687	4.687	4.687	4.687	4.687	4.687	4.687	4.687	2.344	2.344
Negative control	Growth was observed in all concentrations in the tubes											

Controls: Negative controls - dimethyl sulfoxide (evaporated discs)

Positive control - Chloramphenicol (commercially produced discs)

Legend: ND - Not Done

4.4.2 The Minimum Inhibitory Concentrations (MICs) and the Minimum Fungicidal Concentration (MFCs) for fungal test cultures

Plant extracts with high antifungal activities with zones of inhibition of ≥9 mm were screened against selected test cultures for determining their MICs and MFCs that were tabulated as shown in Table 4.5. Extracts of *Acacia nilotica* and *Thylachium africanum* produced high antifungal activity against all fungal test cultures. At least three plant extracts were active against *Candida parapsilosis* (ATCC 19310). These were *Acacia nilotica, Thylachium africanum* and *Loranthus acaciae. Acacia nilotica* produced high MIC of 9.375 mg/ml and MFC of 18.75mg/ml. *Thylachium africanum* on the other hand showed a similar activity on MIC and MFC, of 18.75 mg/ml. *Loranthus acaciae* was also very good giving a high MIC of 9.375 mg/ml and MFC of 18.75 mg/ml (Table 4.6).

The plant extracts were screened for MICs and MFCs against *Cyptoccocus neoformans* (ATCC 18310) where only four extracts were proved to be active. Therefore, extracts of *Acacia nilotica, Albizia anthelmithica, Thylachium africanum* and *Acacia ethaaica* had their activity determined. Of the four, it's the extracts of *Thylachium africanum* that produced similar activity on MIC and MFC of 37.5 mg/ml. The remaining *Acacia nilotica, Albizia anthelmithica* and *Acacia ethaaica* extracts had similar MICs of 18.75 mg/ml and MFCs of 37.5 mg/ml (Table 4.6).

MICs and MFCs against *Aspergilus flavus* were also determined and its *Acacia nilotica* that produced similar activities for MICs and MFCs of 18.75 mg/ml. *Thylachium*

africanum produced different values for MIC and MFC of 18.75 mg/ml and 37.5 mg/ml respectively. Lastly for this microbe, the extracts of *Loranthus acaciae* produced very high activity producing MIC value of 9.375 mg/ml and MFC of 18.75 mg/ml. When *Acacia nilotica* and *Loranthus acaciae* were screened against *Microsporum gypseum*, they gave similar antimicrobial activity for MIC and MFC of 18.75 mg/ml. For *Albizia anthelmithica*, it produced very high activity both for MIC and MFC of 9.375 mg/ml. This was the highest activity compared to all the extracts that were active against the microbe. Similar antifungal activity for MIC and MFC were also observed when *Thylachium africanum* and *Kedrostis pseudogijef* were screened where both produced an activity of 37.5 mg/ml for both the MIC and MFC (Table 4.6).

Among all the plant extracts tested against *Trichophyton mentagrophyte*, only three were found to be active. *Acacia nilotica* produced similar MIC and MFC values of 18.75 mg/ml. *Thylachium africanum* showed high activity with MIC of 18.75 mg/ml and MFC of 37.5 mg/ml. *Loranthus acaciae* being one of the most active plant against the test cultures, it gave a very good MFC of 9.375 mg/ml and MIC of 18.75 mg/ml (Table 4.6).

Table 4. MICs (mg/ml) and MFCs (mg/ml) produced by plnat extracts against fungal strains

Test Cultures	*Candida parapsilosis*		*Cryptococcus neoformans*		*Aspergillus flavus*		*Microsporium gypseum*		*Trichophyton mentagrophyte*	
Plant	MIC (mg/ 50µl)	MFC (mg/ 50µl)	MIC (mg/ 50µl)	MFC (mg/ 50µl)	MIC (mg/ 50µl)	MFC (mg/ 50µl)	MIC (mg/ 50µl)	MFC (mg/ 50µl)	MIC (mg/ 50µl)	MFC (mg/ 50µl)
Acacia nilotica	9.375	18.75	18.75	37.5	18.75	18.75	18.75	18.75	18.75	18.75
Albizia anthelmithica	ND	ND	18.75	37.5	ND	ND	9.375	9.375	ND	ND
Thylachium africanum	18.75	18.75	37.5	37.5	18.75	37.5	37.5	37.5	18.75	37.5
Acacia ethaaica	ND	ND	18.75	37.5	ND	ND	ND	ND	ND	ND
Loranthus acaciae	9.375	18.75	ND	ND	9.375	18.75	18.75	18.75	9.375	18.75
Kedrostis pseudogijef	ND	ND	ND	ND	ND	ND	37.5	75	ND	ND
Positive control	4.687	4.687	4.687	4.687	4.687	4.687	4.687	4.687	4.687	4.687
Negative control	Growth was observed in all concentrations in the tubes									

Controls: Negative control - dimethyl sulfoxide

Positive control - miconazole

Legend: ND - Not Done

4.5 Preliminary phytochemical screening

All the twenty two medicinal plants methanolic extracts were screened for phytochemicals; Alkaloids, tannins, terpenoids, saponins, cardiac glycosides and flavonoids (Table 4.7).

4.5.1 Tannins

From the screening of phytochemicals presence, tannins were found to be present in all other plant extracts except in *Rhus ruspoli*. Most abundant concentrations of tannins were present in *Loranthus acaciae, Thylachium africanum, Acacia horrida,* and *Acacia nilotica* (Table 4.7). The blue-black colouration indicated the presence of tannins (Plate 4.4).

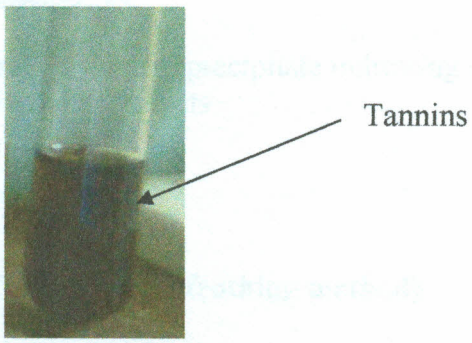


Plate 4. Blue-black colouration indicating the presence of tannins in *Acacia nilotica* extracts

4.5.2 Alkaloids (Wagner's test)

Alkaloids were tested using the Wagner's test. Only *Loranthus acaciae* was found to have high concentrations. *Salvadora persica, Thylachium africanum, Clerodendrum myriacoides, Acacia nilotica* and *Acacia horrida* had moderate amounts while low concentrations were witnessed in the extracts of *Kedrostis pseudogijef, Acacia ethaaica, Cissus quadrangularis, Sericocomopsis hildebrandtii, Albizia anthelmithica* and *Acacia tortilis*. Alkaloids were absent in plant extracts of *Plumbago dawei, Euphorbia candelabrum, Cordia sinensis, Acacia senegal, Croton megalocarpus, Croton macrostachysus, Aloe secundiflora, Rhus ruspoli, Acokanthera friesiorum* and *Cissus*

rotundifolia (Table 4.7). A creamish, brownish-red or orange precipitates indicated presence of alkaloids (Plate 4.5).

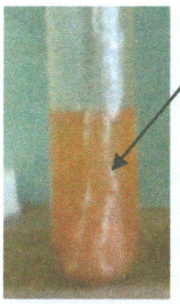


Plate 4. Orange precipitate indicating the presence of alkaloids in *Thyachium africanum* extracts

4.5.3 Saponins (frothing method)

Saponins' concentrations were high in the extracts of *Loranthus acaciae* and *Thylachium africanum.* In extracts of *Salvadora persica, Acacia ethaaica, Albizia anthelmithica* and *Acacia horrida* the saponins were moderate. The remaining plant extracts had low concentrations while these phytochemicals were completely absent in the extracts of *Acacia tortilis, Acokanthera friesiorum, Aloe secundiflora, Sericocomopsis hildebrandtii, Cordia sinensis* and *Plumbago dawei* (Table 4.7). A persistent froth indicated saponins' presence (Pate 4.6).

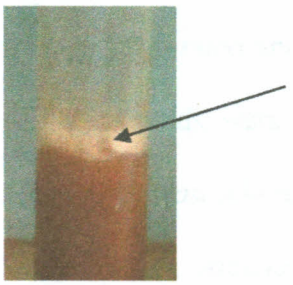


Plate 4. Persistent froth indicating the presence of saponins in *Loranthus acaciae* extracts

4.5.4 Cardiac glycosides

Most of the plant extracts were found to contain cardiac glycosides in moderate or low concentrations. A number of plant extracts too were found to contain no cardiac glycosides in them. These include *Cordia sinensis, Euphorbia candelabrum, Croton megalocarpus, Croton macrostachysus, Albizia anthelmithica, Aloe secundiflora, Sericocomopsis hildebrandtii* and *Cissus rotundifolia.* Only *Thylachium africanum* and *Acacia horrida* contained high concentrations (Table 4.7). The appearance of a green-blue colour indicated the presence of cardiac glycosides (Plate 4.7).

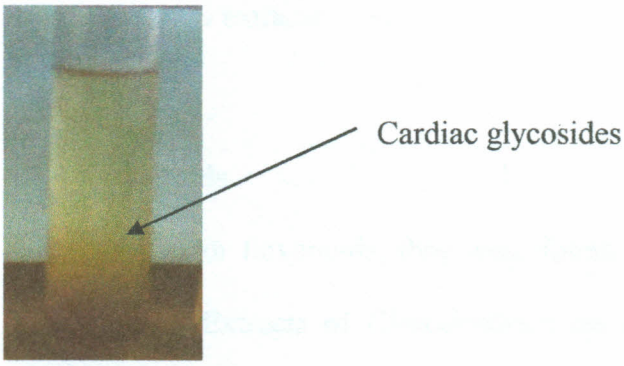


Plate 4. Green-blue colouration indicating the presence of cardiac glycosides in *Acacia horrida* extracts

4.5.5 Terpenoids

The terpenoids were mostly found in low concentrations in most extracts except in *Salvadora persica* and *Acacia ethaaica* where they were found in high concentrations. The terpenoids were found to be absent in *Plumbago dawei, Kedrostis pseudogijef, Croton macrostachysus, Croton megalocarpus, Thylachium africanum, Sericocomopsis hildebrandtii, Albizia anthelmithica, Acokanthera friesiorum* and *Cissus rotundifolia*

(Table 4.7). The terpenoids were indicated by the presence of reddish brown colouration at the interface (Plate 4.8).

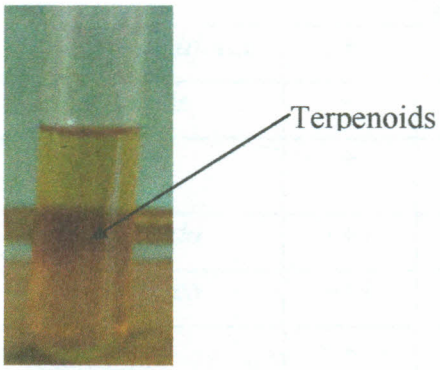


Plate 4. Reddish brown colouration at the interface indicates presence of terpenoids in *Acacia ethaacia* extracts

4.5.6 Flavonoids

On screening for flavonoids, they were found to be absent in *Cissus rotundifolia* and *Rhus ruspoli*. Extracts of *Clerodendrum myriacoides* and *Acacia nilotica* had high concentrations while the remaining extracts, the flavonoids were found mostly in low to moderate concentrations (Table 4.7). A yellow colouration that disappeared on standing indicated the presence of flavonoids (Plate 4.9).

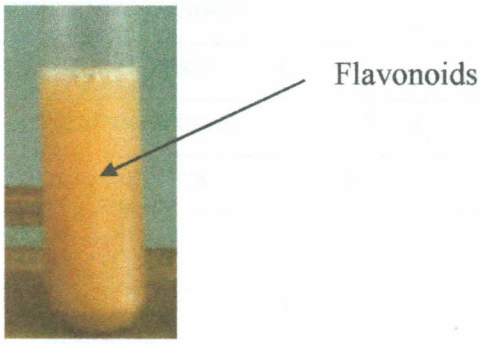


Plate 4. Yellow colouration indicates flavonoids presence in the *Clerodendrum myriacoides* extracts

Table 4. Phytochemicals screening results for the plant extracts

Botanical Name	Tannins	Alkaloids (Wagner's test)	Saponins	Cardiac glycosides	Terpenoids	Flavonoids
Cissus rotundifolia	+	-	+	-	-	-
Acacia tortilis	+	+	-	+	+	+
Acokanthera friesiorum	+	-	-	+	-	+
Acacia horrida	+++	++	++	+++	++	+
Acacia nilotica	+++	++	+	++	+	+++
Rhus ruspoli	-	-	+	+	+	-
Clerodendrum myriacoides	++	++	+	++	+	+++
Albizia anthelmithica	+	+	++	-	-	+
Aloe secundiflora	++	-	-	-	++	++
Sericocomopsis hildebrandtii	+	+	-	-	-	+
Cissus quadrangularis	++	+	+	+	++	+
Thylachium africanum	+++	++	+++	+++	-	++
Acacia senegal	+	-	+	+	++	+
Croton megalocarpus	++	-	+	-	-	+
Croton macrostachysus	+	-	+	-	-	+
Salvadora persica	++	++	++	+	+++	+
Acacia ethaaica	+	+	++	++	+++	+
Loranthus acaciae	+++	+++	+++	++	+	++
Cordia sinensis	+	-	-	-	+	+
Euphorbia candelabrum	+	-	+	-	+	+
Kedrostis pseudogijef	+	+	+	+	-	++
Plumbago dawei	+	-	-	+	-	++

Key: **+++ (High), ++ (Moderate), + (Low) and - (Not present)**

CHAPTER FIVE

DISCUSSION

5.1 Ethnobotanical Survey

From the ethnobotanical survey (Table 4.1), a total of twenty two medicinal plants were recored both in their local Samburu name, botanical name and family name. These plants were collected from the six conservancies within the district. Eight plants were collected, each from Namunyak and Nkaroni conservancies' of that these two conservancies have diverse plant species. Other plants were collected from Ngirai West and West Gate where two plants from each conservancy were collected and one plant each from Lodungokwe and Wamba conservancies. A total of sixteen different plant families were collected (Table 4.7) with Mimosaceae having five plants followed by Euphobiaceae with three plants. The rest of the families each had only one plant species collected. Most cited health problem was diarrhoea with others being malaria, fever, wounds, respiratory system illnesses and loss of appetite among others.

It was noted that the roots and stem barks were the most used plant parts in disease treatment while leaves, seeds and whole plants were cited to be used for few ailments. Since the locals and herbalists use different parts of the palnt like the seeds, barks, roots, leaves, or even the whole plant, this explains why most of the medicinal plants were becoming scarce due to non-sustainable harvesting of the bark which has been reported to accelerate the death of a tree. This means that the community should be encouraged to adopt sustainable harvesting methods and traditional healers domesticating some of

these plants, an idea that is becoming very popular in other regions of the world (Omwenga *et al.*, 2009).

5.2 Antimicrobial Activity

The positive controls for the disc diffusion assays was done by use of commonly used antibiotics commercially prepared discs of chloramphenicol for bacterial pathogens and miconazole for fungal pathogens while negative control was done by impregnating sterile discs with dymethyl sulphoxide (DMSO) which showed no inhibition. Average zone of inhibition was calculated for the the replicates that were carried out. A clear zone of ≥9 mm for both bacterial andfungal pathogens was considered as significant antibacterial activity (Marrita *et al.*, 2010a). The *in vitro* MIC results were classified with the MIC of 9.375 mg/ml considered to have very high antibacterial activity; 18.75 mg/ml, high antibacterial activity; 37.5 mg/ml, moderate activity; and 75mg/ml, low activity (Mathabe *et al.*, 2006).

Zones of inhibition results for both antibacterial and antifungal assays indicated that a number of plant extracts were very active although other extracts did not exhibit any antimicrobial activity. Plant extracts antimicrobial activity vary due to: their different chemical constituents that produce the activity; plant species; plant variety; plant part; conditions of growth (like soil, water and temperature); geographical regions; season; time of collection; different climatic conditions; and age of the plant (Arya *et al.*, 2010). Reports from previous studies indicate that medicinal plants have antiviral, antibacterial, antifungal, antihelminthic, anti inflammatory properties hence the reason

why these plants are active against the Gram-positive and Gram-negative bacteria, yeasts, moulds and dermatophytes (Bagyalakshmi, *et al.*, 2009).

5.2.1 Antibacterial Activity

Methanol extracts of the twenty two medicinal plants studied gave various results against the bacterial pathogens tested. These results from the study clearly indicate that the antimicrobial activity vary with the species the plants and plant material (Bagyalakshmi *et al.*, 2009). Considering that an antimicrobial activity of ≥9 mm is high (Mariita *et al.*, 2010b), its only the *Acacia horrida, Acacia nilotica, Rhus ruspoli, Clerodendrum myriacoides, Thylachium africanum, Acacia ethaaica* and *Loranthus acaciae* exhibited high antibacterial activity against the test cultures out of the twenty two screened plant extracts.

Acacia horrida exhibited high antibacterial activity against *Escherichia coli* (9.33 mm), *Vibrio cholerae* (10.00mm), *Staphylococcus aureus* (13.33 mm), *Bacillus subtilis* (11.66 mm), and *Shigella dysenteriae* (11.00 mm) but completely inactive against *Streptococcus pneumoniae* (6.00 mm) (Table 4.3). Phytochemical screening on *Acacia horrida* revealed the presence of tannins and cardiac glycosides in high concentrations, while alkaloids, saponins and terpenoids at moderate concentrations as flavonoids were present in low concentrations. The antibacterial activity against both the Gram-positive and Gram-negative bacteria could also be indicative of the presence of broad spectrum antibiotic compounds (Salama and Marraiki, 2008). From other studies, flavonoids and terpenoids have been reported in other studies to heal wounds, relief menstrual pain and treat female sterility (Al-Rehaily

et al., 2008). Some flavonoids are formed as antimicrobial barriers in plants response to microbial infection. They have also been found *in vitro* to be effective antimicrobial compounds against a wide array of microorganisms (Orhan *et al.*, 2010). Tannins are known for the treatment of bruises, boils and burns as well as unspecified wounds and skin disease (Adetutu *et al.*, 2009).

Acacia nilotica exhibited high antibacterial activity against *Escherichia coli, Vibrio cholerae, Staphylococcus aureus, Bacillus subtilis* and *Shigella dysenteriae* at producing zones of inhibition of 10.00 mm; 9.00 mm; 11.00 mm, 13.66 mm and 10.33 mm respectively (Table 4.3). Its high antibacterial activity against these test cultures was attributed to the psence of high concentrations of tannins and flavonoids, moderate concentrations of alkaloids and cardiac glycosides while terpenoids and saponins were present in low concentrations (Table 4.7). These phenolic compounds including tannins and flavonoids as well as saponins and alkaloids have been implicated in pharmacological activities such as anthelmintic, antimicrobial and anti-inflammatory activities (Aremu *et al.*, 2010). From previous studies, *Acacia nilotica* have been cited to contain antibacterial activities (Omwenga *et al.*, 2009). Although *Acacia nilotica* was completely inactive against *Streptococcus pneumoniae* (6.00 mm) probably because of the antagonistic effects by other compounds against the active principle(s) and not necessarily the inactivity of the plant extracts (Aremu *et al.*, 2010).

Rhus ruspolii and *Clerodendrum myriacoides* were the most active against *Streptococcus pneumoniae* producing zones of inhibition of 19.33 mm and 17.66 mm

respectively. Their activity provides proof of the claimed therapeutic value in respiratory conditions. Their activity on gram negative bacteria particularly drug resistant strain demonstrates their potential for control of drug resistance strains (Bii *et al.*, 2010). Regardless of their high antibacterial activity against *Streptococcus pneumoniae*, these two plants were completely inactive against other bacterial test cultures. This could be possible considering the fact that plant extracts often contain different chemicals with different pharmacological activities (Amoo *et al.*, 2011). Hence, the tannins, flavonoids and alkaloids that were found present in the roots of these plants could be having the medicinal value of the plant that inhibited *Streptococcus pneumoniae* (Bagyalakshmi *et al.*, 2009). Other studies reveal that flavonoids have been found to denature enzymes involved in cell wall biosynthesis in pathogens therefore, they are considered to be responsible for activity against *Streptococcus pneumoniae* because they were prent in these plants (Negi *et al.*, 2009). Tannins have the ability to act as hydrogen donors, reducing agents and singlet oxygen quenchers thereby attributing their antibacterial activity (Amoo *et al.*, 2011). Due to the high antimicrobial activity *in vitro*, *Rhus ruspoli* and *Clerodendrum myriacoides* it can be considered effective antimicrobial agents to treat infectious diseases caused by these pathogenic microbes (Duraipandiyan and Ignacimuthu, 2009). Hence, the trend of these plants to have higher activity against microbes is attributed to the various active, potentiate (synergism) or antagonistic compounds which possess antimicrobial properties and are present in various concentrations in the plant population (Marzouk *et al.*, 2010).

Thylachium africanum showed high antibacterial activity against *Vibrio cholerae* (11.66 mm), *Staphylococcus aureus* (16.00 mm), *Bacillus subtilis* (13.00 mm) and *Shigella dysenteriae* (12.66 mm) (Table 4.3). The phytochemical screened revealed that tannins, saponins and cardiac glycosides were present in high concentrations while alkaloids and flavonoids were moderately concentrated as terpenoids were absent. The presence of tannins could be responsible for antibacterial potential due to their basic character that allows them to react with proteins to form stable water soluble compounds there by killing the bacteria by directly damaging its cell membrane (Aliero *et al.*, 2008). Cardiac glycosides, flavonoids and tannins have bacterial activity, which validates the use of this plant in the control of *Staphylococcus aureus,* and *Shigella* species, and therefore implies a healing property for food poisoning, skin infections, ulcers, eczema, diarrhea and pneumonia (Cartaxo *et al.*, 2010). Saponins antimicrobial activity against bacterial strains is attributed to their anti-inflammatory, spermicidal, hypocholesterolaemic and antiviral activities (Gevrenova *et al.*, 2010).

Acacia ethaaica a exhibited high antibacterial activity against *Escherichia coli, Staphylococcus aureus, Bacillus subtilis* and *Shigella dysenteriae* at producing zones of inhibition of 9.33 mm; 12.00 mm; 11.33 mm, and 10.33 mm respectively (Table 4.3). The phytochemical screening results indicated that only the terpenoids were present at high concentrations while cardiac glycosides and saponins were present in moderate concentrations as tannins, alkaloids and flavonoids were present at low concentrations. The terpenoids represent a chemical defense against environmental stress and provide a repair mechanism for wounds and injuries (Salminen *et al.*, 2008). Terpenoids have also been found to have the ability to inhibit the functions of fatty acid synthase (Akor *et al.*,

2009). The presence of the other compounds in moderate concentrations may be responsible for the biological activities exhibited by the plant extracts therefore providing basic knowledge of the efficacy of the plant material (Aremu *et al.*, 2010). The plant extract was completely inactive against *Streptococcus pneumoniae* possibly because flavonoids were absent yet they are known to exhibit antioxidant activity and are effective scavengers of superoxide anions. Factors that can significantly affect the cell wall of *S. pneumoniae* which invariably may lead to the collapse of the cell wall and overall, and affecting the entire mechanism of the organism (Nwinyi *et al.*, 2009).

Loranthus acaciae had high antibacterial activity against other bacterial test cultures except inactive against *Streptococcus pneumoniae* (6 mm). Zones of inhibition of 10.00 mm, 18.33 mm, 18.00 mm, 17.33 mm and 16.66 mm against *Escherichia coli, Vibrio cholerae, Staphylococcus aureus, Bacillus subtilis,* and *Shigella dysenteriae* respectively were recorded. Phytochemical screening indicated that the plant extracts had high concentrations of tannins, alkaloids and saponins. Cardiac glycosides and flavonoids were moderately present, while the terpenoids were present in low concentrations. These chemicals have been used in healing wounds, sores, itching, cutaneous diseases, bone fracture, fever, ringworm, skin diseases, throat infection, diarrhoea among other symptoms (Omwenga *et al.*, 2009; Arya *et al.*, 2010). The reason why this plant is completely inactive against *Streptococcus pneumoniae* could be due to the presence of a prominent capsule composed of high molecular weight polysaccharides that differ in virulence, prevalence, and extent of drug resistance (Campa *et al.*, 2009). The antibacterial activity exhibited by its extract may be attributed to the presence of

alkaloids and flavonoids in high concentrations as observed in the phytochemical screening (Aliero *et al.*, 2008).

Despite *Plumbago dawei* containing the secondary metabolites in low concentrations and missing others, it showed high antimicrobial activity against *Staphyloccocus aureus* and *Shigella dysenteriae*. This could be attributed to the presence of flavonoids in moderate concentrations, low concentrations of tannins and cardiac glycosides. These compounds are powerful antioxidant, anti-inflammatory, anti-microbial and anti-cancer agents (Sousa *et al.*, 2009). The variation of antibacterial activity among the crude extracts might be due to distribution of varied antimicrobial substance (Devi *et al.*, 2009). The inhibitory activity of terpenoids on bacteria is used in the treatment of infants' body rashes, diarrhoea, dysentery and stomach pains (Mathabe *et al.*, 2008). Flavanones, hesperetin and naringenin have been reported to have inhibitory activity at a number of protein kinases (Williams *et al.*, 2004). This inhibition is mediated via the binding of the polyphenols to the Adenosine-5'-triphosphate (ATP) binding site, presumably causing three-dimensional structural changes in the kinase leading to its inactivity (Williams *et al.*, 2004). These therapeutic properties of alkaloids are used to treat conditions like; diarrhoea, hypertension, diabetes, dysentery and inflammations (Moyo *et al.*, 2010).

Albizia anthelmithica was the only plant that produced high antimicrobial activity of 10.66 mm against *Vibrio cholerae* probably because of the flavonoid compounds present that are known to inactivate cholera toxin thereby inhibiting *Vibrio cholerae* (Omwenga *et*

al., 2009). But *Albizia anthelmithica* was found to be completely inactive against all other bacterial test cultures. This could be due to the presence of saponins, whose main use is pegged on their anti-inflammatory, spermicidal and hypocholesterolaemic activities (Gevrenova *et al.*, 2010). Tannins, alkaloids and flavonoids that were present in low concentrations could also be of reference because they have been reported to have antibacterial activities. This plant had bactericidal activity against the test microorganisms (Acharyya *et al.*, 2009). Previous studies indicate that alkaloids in root and barks of *Albizia anthelmithica* have been used to treat fever, malaria, cholera, diarrhoea and rheumatism (Duraipandiyan and Ignacimuthu, 2009). This is why medicinal plants are important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also as stating materials for the syntheses of drugs or as models for pharmacologically active compounds (Devi *et al.*, 2009).

5.2.2 Antifungal Activity

General antifungal activity results revealed that extracts of *Acacia nilotica, Albizia anthelmithica, Thylachium africanum, Acacia ethaaica, Loranthus acaciae* and *Kedrostis pseudogijef* were the only active. All the remaining sixteen plant extracts were either moderately active or completely inactive. Most of these plant compounds screened; tannins, flavonoids, cardiac glycosides, terpenoids, alkaloids, saponins among others have high antifungal activity (Kategaonkar *et al.*, 2010).

Kedrostis pseudogijef Rolfe belongs to the family of Plumbaginaceae and was only active against *Microsporium gypseum* (9.33 mm) and completely inactive against all the other fungal test cultures. Phytochemical screening revealed that flavonoids were present in moderate concentrations while tannins, alkaloids, cardiac glycosides and saponins were present in low concentrations as terpenoids were absent. Flavonoids are known to be synthesized by plants in response to microbial infection. They have been found to be effective antimicrobial substances against a wide array of microorganisms' *in-vitro*. Their activity is probably due to their ability to complex with extracellular and soluble proteins in bacterial cell walls (Bii *et al.*, 2010).

Loranthus acaciae Zucc. belongs to the family of Loranthaceae and its extracts had high antifungal activity on other fingal test cultures except *Cryptococcus neoformans*. Phytochemical screening revealed that tannins, alkaloids and saponins were present in high concentrations, cardiac glycosides and flavonoids were present in moderate concentrations while terperpenoids were lowly concentrated. The reason why this plant was completely inactive against *Cryptococcus neoformans* could be due to the presence of a prominent capsule composed of high molecular weight polysaccharides that differ in virulence, prevalence, and extent of drug resistance (Masman *et al.*, 2009). Cardiac glycosides have been widely used in the treatment of heart failure for more than 200 years and they are one of the four categories of drugs that are recommended for routine use to treat heart failure by The American College of Cardiology/American Heart Association Joint Guidelines (Liu *et al.*, 2010). Tannins are known to make nutritional protein unavailable for microorganisms thereby preventing their development (Hoong *et*

al., 2010). In cases where one extract of the same plant depicted activity against most microorganisms, it would possibly mean that the compounds responsible for the antimicrobial activities were present in the extract at different concentrations (Kisangau *et al.*, 2007).

Acacia nilotica, Thylachium africanum and *Loranthus acaciae* were active against *Candida parapsilosis* with zones of inhibition of 12.66 mm, 10.66 mm and 14.33 mm respectively (Table 4.4). These plants have been cited to have several therapeutic properties like anti-inflammatory and hypolipidaemic, analgesic and sedative, anxiolytic, antimutagenic, antihypertensive, diuretic, antimicrobial, carminative, antispasmodic and relaxant (Begnami *et al.*, 2010). Tannins, alkaloids, terpenoids, saponins, cardiac glycosides and flavonoids were present in various concentrations except terpenoids that were not present in *Thylachium africanum*. Alkaloids have large spectrum of biological activities, including antiparasitic, antiviral, and cytotoxic. They are also potent inhibitors of cathepsin (v) also known as lysosomal cysteine peptidases, implicated in many pathological conditions (Severino *et al.*, 2011). Experiments with truncated cardiac glycosides provide evidence that the inhibitory effects on acute-phase proteins (APP) expression strictly correlate with blockade of the Na+/K+-ATPase, since cardiac glycosides without a C17 lactone or without a glycosidic part are only low Na+/K+-ATPase inhibitors (Kolkhof *et al.*, 2010).

Clerodendrum myriacoides (11.00 mm) and *Acacia ethaaica* (9.66 mm) were active against *Cryptococcus neoformans* (Table 4.4). The two plants had all the tested

phytochemicals in various concentrations which were able to act against the yeast. For example phytochemicals in high concentrations was flavonoids in *Clerodendrum myriacoides* extracts and terpenoids in *Acacia ethaaica* extracts. Saponins were found in moderate concentrations in both plant extracts while tannins, cardiac glycosides, and alkaloids were at either moderate or low concentrations. Their action may be attributed to the presence of flavonoids that have been known to disrupt microbial membranes (Bii *et al.*, 2010). Saponins have been proved to have ability to interfere with cellular membranes, inducting cytotoxicity even at low concentrations (Bottger and Melzig, 2010). Tannin consumption has shown to be beneficial for human and animal health through their bactericidal, fungicidal, antioxidant, nematocidal, and insecticidal properties (Fernandez-Salas *et al.*, 2011).

Screening of *Acacia nilotica, Thylachium africanum* and *Loranthus acaciae* extracts against *Aspergillus flavus* a filamentous fungus these plants were found to have high activity producing zones of inhibition of 11.66 mm, 10.00 mm and 14.66 mm respectively (Table 4.4). This may be due to the presence of flavonoids whose many biological actions have been attributed to their antioxidant properties, either through their reducing capacities per se or through their possible influences on intracellular redox status (Williams *et al.*, 2004). Saponins may also be a factor to consider since they have the ability to amplify the toxicity of type I ribosome-inactivating proteins (type I RIPs), lectins like saporin and agrostin in a synergistic manner (Bottger and Melzig, 2010). Tannins are known to exhibit a range of pharmacological activities which include; antimicrobial, anti-inflammatory, anti-diabetic and acetylcholinesterase

inhibitory effects (Moyo *et al.*, 2010). Terpenoids are known for their neuroprotective activity and have been reported to exhibit anti-microbial, insecticidal, anti-carcinogenic, anti-inflammatory, and many other kinds of activity (Chang *et al.*, 2007).

Acacia nilotica, Clerodendrum myriacoides, Thylachium africanum and *Loranthus acaciae* were active against *Microsporum gypseum* producing 13.33 mm, 18.66 mm, 10.66 mm, and 14.00 mm respectively (Table 4.4). They contain tannins, flavonoids, cardiac glycosides, saponins and alkaloids in high concentrations. Previously these same compounds have been reported to be active against fungi (Duraipandiyan and Ignacimuthu, 2009). The presence of tannins could be responsible for the antimicrobial activities because they have anti-inflammatory, antifungal, antioxidant and healing properties (Moyo *et al.*, 2010). Saponins cause inhibition of protein synthesis by removing adenine residues from the 28S ribosomal RNA (ribonucleic acid) during synthesis and prevent the penetration of the microbes through the cellular membrane (Bottger and Melzig, 2010). Flavonoids have been reported to be responsible for antioxidant activity. They act on enzymes and pathways involved in anti-inflammatory processes and also possess antifungal properties (Moyo *et al.*, 2010). Biological activities of alkaloids include antiamoebic, antibacterial, anti-inflammatory, and antifungal effects, as well as significant cytotoxic activity (Su *et al.*, 2008).

Screening against *Trichophyton mentagrophyte* resulted to *Acacia nilotica, Thylachium africanum* and *Loranthus acaciae* producing high antifungal activity with zones of inhibition of 11.66 mm, 10.66 mm and 14.66 mm (Table 4.4). Phytochemicals present

in high concentrations were tannins, alkaloids and saponins. With the high levels of phenolic compounds, these plants extracts also exhibited high antifungal activity (Moyo *et al.*, 2010).

5.2.3 Minimum Inhibitory Concentrations (MICs) and the Minimum Bactericidal / Fungicidal Concentrations (MBCs/MFCs)

The antimicrobial effects may be attributed, possibly in combination, to various phytochemicals detected during the extracts chemical screening and which are known to cause damage to cell membranes, causing leakage of cellular materials and ultimately the microorganism death (Marzouk *et al.*, 2010). *Acacia nilotica* (L.) Del. Belongs to the Mimosaceae family. The plant extract showed average activity against the bacterial test cultures with no big difference between the Gram positives and the Gram negatives except for *Streptococcus pneumoniae* which it was completely inactive against. In both, the average MICs and MBCs were almost the same with a concentration ranging from 18.75 mg/ml - 75mg/ml with only *Shigella dysenteriae* having a different MIC and MBC of 18.75 mg/ml and 37.5 mg/ml respectively. This concentration indicates that *Acacia nilotica* is possibly bactericidal to *Escherichia coli, Bacillus subtilis, Vibrio cholerae* and *Staphylococcus aureus* while bacteriostatic to *Shigella dysenteriae.* But the plant extract was completely inactive against *S. pneumoniae* hence no MIC/MBC was carried out. Thus the activity of the *Acacia nilotica* extract can be ascribed to the presence of flavonoids, terpenoids, cardiac glycosides and the alkaloids. Such phytochemicals had been earlier found to possess antibacterial activities (Hassan *et al.*, 2006).

Acacia horrida (L.) Willd. belongs to Mimosaceae family. The barks extract of *Acacia horrida* showed moderate to higher activity against the Gram positive and Gram negative bacteria. It was bactericidal given the fact that it produced same MICs and MBCs in *E.coli, V. cholerae* and *S. dysenteriae* of 37.5mg/ml respectively. The extract showed some difference between the MIC and MBC concentrations in *S. aureus,* and *B. subtilis* having an MIC and MBC of 18.75 mg/ml and 37.5mg/ml respectively. This indisputably demonstrates that the extract was possibly bacteriostatic against the two microbes. Therefore, because both concentrations are almost similar, the differences can be attributed to the synergistic or additive properties of the compounds present in the extract (Bottger and Melzig, 2010). For instance presence of all screened copounds of terpenoids, tannins, saponins, flavonoids, alkaloids and cardiac glycosides can have such properties together.

Rhus ruspolii Engler. belongs to Anacardiaceae family while *Clerodendrum myriacoides* (Hochst.)Vatke subsp. *apperae* Verdc. belongs to Verbenaceae family. These two plant extracts were the only extracts that had moderate to high activity against *Streptococcus pneumoniae. Rhus ruspolii* was highly active producing the same MIC and MBC of 18.75 mg/ml. this clearly indicates that it was bactericidal against *S. pneumoniae.* On the other hand, a difference in concentration was recored in *Clerodendrum myriacoides* with MIC of 18.75 mg/ml and MBC of 37.5mg/ml undoubtly indicating that the extract is bacteriostatic. Therefore, this indicates that the extracts did not act on the test cultures depending on their cell wall properties but most likely it is through the presence of flavonoids, alkaloids, cardiac glycosides, tannins, saponins and terpenoids plus other phytochemicals that may be present and were not screened for their presence. Such phytochemicals may possess some additive and/or synergistic properties that may be

responsible for its activity (Hassan *et al.*, 2006). Despite the fact that these two extracts exhibited complete inactivity against other test cultures and only active against *S. pneumoniae*, it means that the absence of significant activity could be due to factors such as dilution of active principle(s) and antagonistic effects by other compounds against the active principle(s) and not necessarily the inactivity of the plant extracts (Aremu *et al.*, 2010).

Belonging to Capparaceae family is *Thylachium africanum* Lour. which exhibited moderate to high antibacterial activity producing MIC and MBC concentrations ranging from 9.375 mg/ml to 37.5 mg/ml. The extract was bactericidal against *V. cholerae, S. aureus* and *B. subtilis* with same MIC and MBC concentrations of 37.5 mg/ml, 9.375 mg/ml, and 18.75 mg/ml respectively. This clearly indicates that the extract had high activity against *S. aureus* and *B. subtilis* and moderately active against *V. cholerae.* The extract was also bacteriostatic against *S. dysenteriae* with a difference concentration of 18.75 mg/ml (MIC) and 37.5 mg/ml (MBC). The phytochemical screening revealed the presence of tannins, saponins and cardiac glycosides in high concentrations while alkaloids and flavonoids were in moderate concentrations. The antimicrobial activities observed could be ascribed to the phytochemicals detected which can therefore be having a synergistic or additive activity to the antibacterial activity exhibited by the plant extract (Aremu *et al.*, 2010).

Loranthus acaciae Zucc. of Loranthaceae family exhibite high antibacterial activity against both the Gram positive and Gram negative bacteria except *S. pneumoniae.* In both, a range of MICs and MBCs of between 9.375 mg/ml to 37.5mg/ml were obtained. The extract was bactericidal against E. *coli, V. cholerae, S. aureus,* and *S. dysenteriae*

at concentrations of 37.5mg/ml, 9.375mg/ml, 9.375mg/ml and 18.75mg/ml respectively. The extract was equally bacteriostatic to *B. subtilis* producing different MICs and MBCs of 9.375mg/ml and 18.75mg/ml respectively. The compounds that were found to be present include; tannins, alkaloids and saponins in high concentrations, cardiac glycosides and flavonoids in moderate concentrations while terpenoids in low concentrations and such phytochemicals have been detected with antibacterial properties. This therefore, indicates that the activity of the extracts against the bacterial test organisms is based on the active compounds present in the extract that may have some additive or synergistic properties (Awoyinka *et al.*, 2007).

For the case of fungicidal, *Acacia nilotica* was fungicidal with both MIC and MFC of 18.75 mg/ml against *Aspergillus flavus, Microsporum gypseum* and *Trichophyton mentagrophyte*. *Albizia anthelmithica* was equally fungicidal with both MIC and MFC of 9.375 mg/ml against *Microsporum gypseum*. *Thylachium africanum* was fungicidal with both MIC and MFC of 18.75 mg/ml against *Candida parapsilosis*, 37.5 mg/ml against both *Cryptococcus neoformans* and *Microsporum gypseum*. *Loranthus acaciae* was only fungicidal with both MIC and MFC of 18.75 mg/ml against *Microsporum gypseum*. *Kedrostis pseudogijef* had both MIC and MFC of 37.5 mg/ml against *Microsporum gypseum*. The large spectrum of biological activities, including antibacterial, antifungal antiparasitic, antiviral, and cytotoxic (Severino *et al.*, 2011), depends on the concentration of phenolic compounds which have a dual bioactive role in plants, acting as both antioxidant and pro-oxidant agents at low and high concentrations, respectively (Moyo *et al.*, 2010). With all these wide spectrum

antibacterial and antifungal properties present in plants, they then can be good sources of the drugs to counter the multi-resistant microorganisms (Marzouk *et al.*, 2010).

5.2.4 Plants without antibacterial and antifungal activity

Out of the twenty two plants extracts that were screened, eleven of them listed below were completely inactive against all or some of the selected bacterial and fungal pathogens. Maybe they could be active because the naturally occurring combinations of compounds are often synergistic; leading to antimicrobial activity of crude extracts considering that traditionally the medicinal plants are usually boiled before use (Lou *et al.*, 2010). They include; *Cissus rotundifolia, Acacia tortilis, Acokanthera friesiorum, Aloe secundiflora, Sericocomopsis hildebrandtii, Cissus quadrangularis, Croton megalocarpus, Croton macrostachysus, Salvadora persica, Cordia sinensis,* and *Euphorbia candelabrum.* These extracts exhibited low pharmacological activity and therefore, this could be due to factors such as dilution of active principle(s) and antagonistic effects by other compounds against the active principle(s) and not necessarily the inactivity of the plant extracts (Aremu *et al.*, 2010b)

Although phytochemicals were present in these plant extracts, they could be acting in antagonism manner which made them to produce poor activity results (Kategaonkar *et al.*, 2010). Therefore, it is not surprising that there are differences in the antimicrobial effects of plants due to the phytochemical properties and differences among species. Some of these plant extracts may have contained antimicrobial constituents but not in

sufficient concentrations so as to be effective or it is also possible that compound may not be soluble in methanol (Bagyalakshmi *et al.*, 2009).

5.3 Phytochemical Screening of the plant extracts results

The phytochemicals screened for their presence included; tannins, saponins, flavonoids, terpenoids, cardiac glycosides and alkaloids. Results indicate that all the tested compounds were present and their activity is attributed to its broad spectrum of biological activities (Kategaonkar *et al.*, 2010).

5.3.1 Tannins

After phytochemical screening among the btwenty two plant extratcts, tannins were found to be present in twenty one extracts except in *Rhus ruspoli* extracts. High concentrations were witnenessed in extracts of *Acacia horrida, Acacia nilotica, Thylachium africanum* and *Loranthus acaciae* while moderate concentrations were recorded among extracts of *Clerodendrum myriacoides, Aloe secundiflora, Cissus quadrangularis, Croton megalocarpus,* and *Salvadora persica.* The remaining extracts had low concentrations of tannins (Table 4.7).

5.3.2 Alkaloids

The alkaloids were present in high concentrations among the extracts of *Loranthus acaciae* while moderate concetrations in *Thylachium africanum, Clerodendrum myriacoides, Acacia nilotica* and *Acacia horrida.* Notably was the absence of this compound in the extracts of *Cissus rotundifolia, Acokanthera friesiorum, Rhus ruspoli, Aloe secundiflora, Acacia senegal, Croton megalocarpus, Croton macrostachysus, Cordia sinensis* and *Plumbago dawei.* The other extracts had low conctrations of alkaloids (Table 4.7).

5.3.3 Saponins

The presence of saponins in the plant extracts was recorded in high concentrations among the *Thylachium africanum* and *Loranthus acaciae.* Moderate concentrations were present in extracts of *Acacia horrida, Albizia anthelmithica, Salvadora persica* and *Acacia ethaaica* while low conctrations were present in extracts of *Cissus rotundifolia, Acacia nilotica, Rhus ruspoli, Clerodendrum myriacoides, Cissus quadrangularis, Acacia senegal, Croton megalocarpus, Kedrostis pseudogijef, Euphorbia candelabrum* and *Croton macrostachysus.* Saponins were absent in all remaining plant extracts (Table 4.7).

5.3.4 Cardiac glycosides

Cardiac glycosides were found to be present in most extracts where they were in high concentrations in the extracts of *Acacia horrida* and *Thylachium africanum.* Moderate concentrations were present in *Acacia nilotica, Clerodendrum myriacoides, Acacia ethaaica* and *Loranthus acaciae.* Low concentrations of cardiac glycosides were present in extracts of *Acacia tortilis, Acokanthera friesiorum, Rhus ruspoli, Plumbago dawei, Acacia senegal, Salvadora persica, Kedrostis pseudogijef* and *Cissus quadrangularis.* Cardiac glycosides were absent in the remaining extracts (Table 4.7).

5.3.5 Terpenoids

Phytochemical screening for terpenoids revealed that the extracts of *Salvadora persica* and *Acacia ethaaica* contained the compound in high concentrations while *Acacia horrida, Aloe secundiflora, Acacia senegal,* and *Cissus quadrangularis.* Terpenoids were also found to be in low concetrations among the extracts of *Acacia tortilis, Acacia nilotica, Rhus ruspoli, Clerodendrum myriacoides, Cordia sinensis, Euphorbia candelabrum,* and *Loranthus acaciae.* The terpenoids were recorded as being absent in the remaining plant extracts (Table 4.7).

5.3.6 Flavonoids

The flavonoids were also screen for their presence and were found to be present in high concentrations in the extracts of *Acacia nilotica* and *Clerodendrum myriacoides.* Moderate concentrations were present in the extracts of *Aloe secundiflora, Thylachium africanum, Loranthus acaciae, Kedrostis pseudogijef* and *Plumbago dawei.* Flavonoids were equally absent in the extracts of *Cissus rotundifolia* and *Rhus ruspoli.* The remaining extracts had low conctrations of flavonoids (Table 4.7).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The main objective of this study was to determine the ethnobotanical uses and the bioactivity of some medicinal plants used by the Samburu community of Northern Kenya against selected bacterial and fungal pathogens.

Therefore, the conclusions made from this study includes:-

i. The ethnobotanical survey conducted revealed that it is true that the Samburu community uses medicinal plants to treat themselves against the infectious diseases caused by the bacterial and fungal pathogens bioassayed against in the study.

ii. Most of the plant extracts screened in deed had inhibitory properties ranging from high to moderate on the growth of the bacterial and fungal test cultures. On the other hand, some extracts did not exhibit any kind of inhibitory properties since they were completely inactive against the both the bacterial and fungal test cultures. Notable extracts with high inhibitory properties against bacterial pathogens include; *Acacia horrida, Acacia nilotica, Rhus ruspoli, Clerodendrum myriacoides, Thylachium africanum, Acacia ethaaica* and *Loranthus acaciae* while against fungal pathogens they include extracts of; *Acacia nilotica, Albizia anthelmithica, Thylachium africanum, Acacia ethaaica, Loranthus acaciae* and *Kedrostis pseudogijef.* MICs and MBCs/MFCs indicates moderate to high

antimicrobial activity with *Loranthus acaciae* being the highly bactericidal and fungicidal.

iii. Phytochemical screening revealed that all the six compounds screened for their presence were present at varying concentration in plant extracts; this could be the reason why the antimicrobial activities of the selected Samburu medicinal plants were screened. Among the six phytochemicals (tannins, alkaloids, saponins, cardiac glycosides, terpenoids and flavonoids) screened; tannins were found to be the most abundant found followed by flavonoids and saponins. Alkaloids were recorded to be absent in ten plant extracts a large number of extracts folled by terpenoids being absent in nine extracts as cardiac glycosides were absent in eight extracts. These phenolic compounds, these plants extract also exhibit high antifungal and antibacterial activity (Moyo *et al.*, 2010). Flavonoids are known to be synthesized by plants in response to microbial infection. They have been found to be effective antimicrobial substances against a wide array of microorganisms' *in-vitro*. Their activity is probably due to their ability to complex with extracellular and soluble proteins in bacterial cell walls. Flavonoids have also been known to disrupt microbial membranes (Bii *et al.*, 2010).

Therefore, this qualifies the research published by Wagate *et al.*, (2010), stating that plants form an integral part of life in many indigenous African communities as alternative to allopathic medicines. This is so because the Samburu herbalists' use these medicinal plants extracts in treating various ailments caused by bacterial and fungal

pathogens among the community members because they produce desired effects. These plant extracts provide evidence of broad spectrum antimicrobial activity based on the results which clearly demonstrate that the medicinal plants used by the Samburu community have a medicinal value. This is because most extracts were active against all the test organisms including *Streptococcus pneumoniae* and *Cryptococcus neoformans* that were most resistant. This therefore, ascertains the value of these medicinal plants used in the study.

6.2 Recommendations

The following were the recommendations made from this study;

i). The indigenous knowledge possessed by the Samburu community of Northern Kenya, about medicinal plants on their different use and application in health care should be recognized, promoted and protected because these medicinal plants in semi-arid areas exhibit antibacterial and antifungal activities against various infectious diseases.

ii). The Sambu community members together with their herablists' should continuously be sensitized on the sustainable use of medicinal plants and encouraged to give priority on the domesticating of the medicinal plants so as to reduce the chances of clearing them from the forests with indigenious plants. Some of these plants of importance to be domesticated include; *Acacia horrida, Acacia nilotica, Rhus ruspoli, Clerodendrum myriacoides, Thylachium africanum, Albizia anthelmithica* and *Loranthus acaciae.*This is because they exhibit high antifungal and antibacterial activities.

iii). The active plant extracts should be studied further to identify their antimicrobial mechanisms of action, evaluating possible synergism of antimicrobial activity among plant extracts used against bacteria and fungi pathogens.

iv). Further studies on *Acacia horrida, Clerodendrum myriacoides, Thylachium africanum, Albizia anthelmithica* and *Loranthus acaciae* should be done so as to isolate, purify and characterize the active components in them.

v). Further studies on *Cissus rotundifolia, Acacia tortilis, Acokanthera friesiorum, Aloe secundiflora, Sericocomopsis hildebrandtii, Croton macrostachysus, Salvadora persica, Cordia sinensis,* and *Euphorbia candelabrum* extracts should be done to confirm the activity because they indicated the presence of phytochemicals at varying concentrations although exhibited absence of pharmacological activity.

REFERENCES

Acharyya, S., Patra, A. and Bag, P. K. (2009). Evaluation of the Antimicrobial Activity of Some Medicinal Plants against Enteric Bacteria with Particular Reference to Multi-Drug Resistant *Vibrio cholerae*. *Tropical Journal of Pharmaceutical Research*, **8** (3): 231-237.

Adegoke, A. A., Iberi, P. A., Akinpelu, D. A., Aiyegoro, O. A., Mboto, C. I. (2010). Studies on phytochemical screening and antimicrobial potentials of *Phyllanthus amarus* against multiple antibiotic resistant bacteria. *International Journal of Applied Research in Natural Products*, **3** (3): 6-12.

Adetutu, A., Morgan, W. A., Corcoran, O. (2010). Antibacterial, antioxidant and fibroblast growth stimulation activity of crude extracts of *Bridelia ferruginea* leaf, a wound-healing plant of Nigeria. *Journal of Ethnopharmacology*, **133** (1): 116-9.

Ajesh, K. and Sreejith, K. (2009). Peptide antibiotics: An alternative and effective antimicrobial strategy to circumvent fungal infections. *Peptides*, **30**: 999–1006.

Akkoc, N., Akçelik, M., Haznedaroglu, I. C., Goker, H., Aksu, S., Kirazli, S., Firat, H. C. (2008). *In vitro* Anti-Bacterial Activities of Ankaferd Medicinal Plant Extract. *Ankaferd Drug Inc., University of Ankar, Istanbul/Turkey*, **2**: 471-485.

Akor, J. S. and Anjorin, T.S. (2009). Phytochemical and antimicrobial studies of *Commiphora africana* root extracts. *International Journal of Agriculture and Biology*, **11**: 795-797.

Al-Bakri, A. G. and Afifi, F. U. (2007). Evaluation of antimicrobial activity of selected plant extracts by rapid XTT colorimetry and bacterial enumeration. *Journal of Microbiological Methods*, **68**: 19–25.

Al-Bayati, F. A. and Al-Mola, H. F. (2008). Antibacterial and antifungal activities of different parts of *Tribulus terrestris* L. growing in Iraq. *Journal of Zhejiang University Science*, **9** (2): 154-159.

Aliero, A. A.; Aliero, B.L. and Buhari, U. (2008). Preliminary phytochemical and antibacterial screening of *Scadoxus multiflorus*. *International Journal of Pure and Applied Sciences*, **2** (4): 13-17.

Al-Rehaily, A. J., Albishi, O. A., El-Olemy, M. M., Mossa, J. S. (2008). Flavonoids and terpenoids from *Helichrysum forskahlii*. *Phytochemistry*, **69**: 1910–1914.

Amoo, S. O., Ndhlala, A. R., Finnie, J. F., Van Staden, J. (2011). Antifungal, acetylcholinesterase inhibition, antioxidant and phytochemical properties of three *Barleria* species. *South African Journal of Botany*, **10**: 101-116

Antoine, B., Adjehi, D., Nathalie, G., Valerie, G., Etienne, D. Marcellin, D. J. M., Mireille, D. (2010). Virulence Factors and Resistance Profile of *Shigella* Isolated During Infectious Diarrhoea in Abidjan, Côte D'Ivoire. *Journal of Applied Sciences Research,* **6** (6): 594-599.

Aremu, A. O., Fawole, O. A., Chukwujekwu, J. C., Light, M.E., Finnie, J. F., Van Staden, J. (2010). *In vitro* antimicrobial, anthelmintic and cyclooxygenase-inhibitory activities and phytochemical analysis of *Leucosidea sericea. Journal of Ethnopharmacology,* **131**: 22–27.

Arya, V., Yadav, S., Kumar, S., Yadav, J. P. (2010). Antimicrobial Activity of *Cassia occidentalis* L (Leaf) against various Human Pathogenic Microbes. *Life Sciences and Medicine Research,* **9**:1-11.

Awoyinka, O. A., Balogun, I. O., and Ogunnowo, A. A. (2007). Phytochemical screening and in vitro bioactivity of *Cnidoscolus aconitifolius* (Euphorbiaceae). *Journal of Medicinal Plants Research,* **1** (3): 063-065.

Ayoola, G. A., Coker, H. A. B., Adesegun, S. A., Adepoju-Bello, A. A., Obaweya, K., Ezennia, E. C., Atangbayila, T. O. (2008). Phytochemical Screening and Antioxidant Activities of Some Selected Medicinal Plants Used for Malaria Therapy in Southwestern Nigeria. *Tropical Journal of Pharmaceutical Research,* **7** (3): 1019-1024.

Ayyanar, M. and Ignacimuthu, S. (2005). Traditional knowledge of Kani tribals in Kouthalai of Tirunelveli hills, Tamil Nadu, India. *Journal of Ethnopharmacology,* **102**: 246–255.

Bagyalakshmi, B., Sridhar, D., Ponmurugan, P. (2009). Antimicrobial activity of important Indian medicinal plants against pyogenic infection. *Journal of Phytology,* **1** (6): 391–396.

Bajpai, V. K., Yoon, J. I., Kang, S. C. (2009). Antioxidant and antidermatophytic activities of essential oil and extracts of *Magnolia liliflora* Desr. *Food and Chemical Toxicology,* **47**: 2606–2612.

Bajracharya, A. M., Yami, K. D., Prasai, T., Basnyat, S. R. and Lekhak, B. (2008). Screening of Some Medicinal Plants used In Nepalese traditional Medicine against Enteric Bacteria. *Scientific World,* **6** (6): 107-110.

Balunas, M. J. and Kinghorn, A. D. (2005). Drug discovery from medicinal plants. *Life Scincies,* **78**: 431 – 441.

Becker, K., Hu, Y., Biller-Andorno, N. (2006). Infectious diseases – A global challenge. *International Journal of Medical Microbiology,* **296**: 179–185.

Begnami, A. F., Duarte, M. C. T., Furletti, C., Rehder, V. L. G. (2010). Antimicrobial potential of *Coriandrum sativum* L. against different *Candida* species *in vitro*. *Food Chemistry*, **118**: 74–77.

Bii, C., Korir, K. R., Rugutt, J. and Mutai, C. (2010). The potential use of *Prunus africana* for the control, treatment and management of common fungal and bacterial infections. *Journal of Medicinal Plants Research*, **4** (11): 995-998.

Bottger, S. and Melzig, M. F. (2010). Triterpenoid saponins of the Caryophyllaceae and Illecebraceae family. *Phytochemistry Letters*, **160**: 1–10.

Bovers, M., Hagen, F., Kuramae, E. E., Boekhout, T. (2008). Six monophyletic lineages identified within *Cryptococcus neoformans* and *Cryptococcus gattii* by multi-locus sequence typing. *Fungal Genetics and Biology*, **45**: 400–421.

Braga, F. G., Bouzada. M. L. M., Fabri, R. L., Matos, M. O., Moreira, F. O., Scio, E., Coimbra, E. S. (2007). Antileishmanial and antifungal activity of plants used in traditional medicine in Brazil. *Journal of Ethnopharmacology*, **111**: 396–402.

Brandao, G. C., Kroon, E. G., Duarte, M. G. R., Braga, F. C., Filho, J. G. S., Oliveira, A. B. (2010). Antimicrobial, antiviral and cytotoxic activity of extracts and constituents from *Polygonum spectabile* Mart. *Phytomedicine*, **17**: 926–929.

Brun, S., Fekkar, A., Busse, A., Seilhean, D., Lecs, M., Adler, D., Prodanovic, H., Mazier, D. and Datry, A. (2009). Case Report: *Aspergillus flavus* Brain Abscesses Associated with Hepatic Amebiasis in a Non-neutropenic Man in Senegal. *American Society of Tropical Medicine and Hygiene*, **81** (4): 583–586.

Bussmann, R. W. (2006). Ethnobotany of the Samburu of Mt. Nyiru, South Turkana, Kenya. *Journal of Ethnobiology and Ethnomedicine*, **2** (35): 1-10.

Bussmann, R. W., Malca-García, G., Glenn, A., Sharon, D., Chait, G., Díaz, D., Pourmand, K., Jonat, B., Somogy, S., Guardado, G., Aguirre, C., Chan, R., Meyer, K., Kuhlman, A., Townesmith, A., Effio-Carbajal, J., Frías-Fernandez, F., Benito, M. (2010). Minimum inhibitory concentrations of medicinal plants used in Northern Peru as antibacterial remedies. *Journal of Ethnopharmacology*, **132**: 101–108.

Campa, A. G., Ardanuy, C., Balsalobre, L., Perez-Trallero, E., Marimon, J. M., Fenoll, A. and Josefina Liñares, J. (2009). Changes in Fluoroquinolone-Resistant *Streptococcus pneumoniae* after 7-Valent Conjugate Vaccination, Spain. *Emerging Infectious Diseases*, **15** (6): 905-911.

Cartaxo, S, L., Souza, M. M. A., Albuquerque, U. P. (2010). Medicinal plants with bioprospecting potential used in semi-arid northeastern Brazil. *Journal of Ethnopharmacology*, **131**: 326–342.

Cavaleiro, C., Goncalves, M. J., Serra, D., Santoro, G., Tomi, F., Bighelli, A., Salgueiro, L. and Casanova, J. (2010). Composition of a volatile extract of *Eryngium duriaei* subsp. *juresianum* (M. Laínz) M. Laínz, signalised by the antifungal activity. *Journal of Pharmaceutical and Biomedical Analysis,* **9**: 039-050

Chang, H., Kim, H. J., Chun, H. S. (2007). Quantitative structure–activity relationship (QSAR) for neuroprotective activity of terpenoids. *Life Sciences,* **80**: 835–841.

Che, X., Sheng, C., Wang, W., Cao, Y., Xu, Y., Ji, H., Dong, G., Miao, Y., Yao, J., Zhang, W. (2009). New azoles with potent antifungal activity: Design, synthesis and molecular docking. *European Journal of Medicinal Chemistry,* **44**: 4218–4226.

Chen, K., Chen, Y., Lin, Y., Chou, H., Li, S. (2009a). The molecular epidemiology of serial *Candida tropicalis* isolates from ICU patients as revealed by multilocus sequence typing and pulsed-field gel electrophoresis. *Infection, Genetics and Evolution,* **9**: 912–920.

Chen, J., Ho, T., Chang, Y., Wu, S., Li, C., Hsiang, Y. (2009b). Identification of *Escherichia coli* enterotoxin inhibitors from traditional medicinal herbs by *in silico*, *in vitro*, and *in vivo* analyses. *Journal of Ethnopharmacology,* **12**: 372–378.

Chomnawang, M. T., Surassmo, S., Wongsariya, K., Bunyapraphatsara, N. (2009). Antibacterial Activity of Thai Medicinal Plants against Methicillin-resistant *Staphylococcus aureus*. *Fitoterapia,* **80**: 102–104.

Costa, R. M. P. B., Vaz, A. F. M., Oliva, M. L. V., Coelho, L. C. B. B., Correia, M. T. S., Carneiro-da-Cunha, M. G. (2010). A new mistletoe *Phthirusa pyrifolia* leaf lectin with antimicrobial properties. *Process Biochemistry,* **45**: 526–533.

Coutinho, H. D. M., Costa, J. G. M., Falcao-Silva, V. S., Siqueira-Junior, J. P., Lima, E. O. (2009). Effect of *Momordica charantia* L. in the resistance to aminoglycosides in methicilin-resistant *Staphylococcus aureus*. *Comparative Immunology, Microbiology and Infectious Diseases*, **717**: 1- 5

Cruz, M. C. S., Santos, P. O., Barbosa Jr. A. M., de M´elo, D. L. F. M., Alviano, C. S., Antoniolli, A. R., Alviano, D. S., Trindade, R.C. (2007). Antifungal activity of Brazilian medicinal plants involved in popular treatment of mycoses. *Journal of Ethnopharmacology,* **111**: 409–412.

Dalhoff, A. and Schubert, S. (2010). Dichotomous selection of high-level oxacillin resistance in *Staphylococcus aureus* by fluoroquinolones. *International Journal of Antimicrobial Agents,* **36**: 216–221.

Dalie, D. K. D., Deschamps, A. M., Richard-Forget, F. (2010). Lactic acid bacteria – Potential for control of mould growth and mycotoxins: A review. *Food Control*, **21**: 370–380.

Demma, J., Engidawork, E., Hellman, B. (2009). Potential genotoxicity of plant extracts used in Ethiopian traditional medicine. *Journal of Ethnopharmacology*, **122**: 136–142.

Deng, Y., Yu, Y., Luo, H., Zhang, M., Qin, X., Li, L. (2011). Antimicrobial activity of extract and two alkaloids from traditional Chinese medicinal plant *Stephania dielsiana*. *Food Chemistry*, **124**: 1556–1560.

Derita, M. G., Leiva, M. L., Zacchino, S. A. (2009). Influence of plant part, season of collection and content of the main active constituent, on the antifungal properties of *Polygonum acuminatum* Kunth. *Journal of Ethnopharmacology*, **124**: 377–383.

Devi, K., Devi, G. K., Thirumaran, G., Arumugam, R. and Anantharaman, P. (2009). Antibacterial Activity of Selected Medicinal Plants from Parangipettai Coastal Regions; Southeast Coast of India. *World Applied Sciences Journal*, **7** (9): 1212-1215.

Dostal, J., Brynda, J., Hruskova-Heidingsfeldova, O., Sieglova, I., Pichova, I., Rezacova, P. (2009). The crystal structure of the secreted aspartic protease 1 from *Candida parapsilosis* in complex with pepstatin A. *Journal of Structural Biology*, **167**: 145–152.

Doughari, J. H., El-mahmood, A. M. and Tyoyina, I. (2008). Antimicrobial activity of leaf extracts of *Senna obtusifolia* (L). *African Journal of Pharmacy and Pharmacology*, **2** (1): 007-013.

Duraipandiyan, V. and Ignacimuthu, S. (2009). Antibacterial and antifungal activity of Flindersine isolated from the traditional medicinal plant, *Toddalia asiatica* (L.) Lam. *Journal of Ethnopharmacology*, **123**: 494–498.

Edeoga, H. O., Okwu, D. E. and Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*, **4** (7): 685-688.

El-Khallal, S. M. (2007). Induction and Modulation of Resistance in Tomato Plants against *Fusarium* Wilt Disease by Bioagent Fungi (Arbuscular Mycorrhiza) and/or Hormonal Elicitors (Jasmonic Acid & Salicylic Acid): 2-Changes in the Antioxidant Enzymes, Phenolic Compounds and Pathogen Related- Proteins. *Australian Journal of Basic and Applied Sciences*, **1** (4): 717-732.

Eskander, J., Lavaud, C., Pouny, I., Soliman, H. S. M., Abdel-Khalik, S. M., Mahmoud. I. I. (2006). Saponins from the seeds of *Mimusops laurifolia*. *Phytochemistry*, **67**: 1793–1799.

Fennell, C. W., Lindsey, K. L., McGaw, L. J., Sparg, S. G., Stafford, G. I., Elgorashi, E. E., Grace, O. M., Staden, J. (2004). Assessing African medicinal plants for efficacy and safety: pharmacological screening and toxicology. *Journal of Ethnopharmacology,* **94**: 205–217.

Fernandez-Salas, A., Alonso-Díaz, M. A., Acosta-Rodríguez, R., Torres-Acosta, J. F. J., Sandoval-Castro, C. A., Rodríguez-Vivas, R. I. (2011). *In vitro* acaricidal effect of tannin-rich plants against the cattle tick *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae). *Veterinary Parasitology,* **175**: 113–118.

Franco-Paredes, C., Jacob, J. T., Hidron, A., Rodriguez-Morales, A. J., Kuhar, V., Caliendo, A. M. (2010). Transplantation and tropical infectious diseases. *International Journal of Infectious Diseases,* **14**: 189–196.

Gauwerky, K., Borelli, C. and Korting, H. C. (2009). Targeting virulence: A new paradigm for antifungals. *Drug Discovery Today,* **14** (3/4): 214-222.

Gerique, A. (2006). An Introduction to Ethnoecology and Ethnobotany Theory and Methods. *University of Giessen, Senckenbergstr.* **1**, 35390 Giessen, Germany.

Gevrenova, H., Stancheva, T., Voynikov, Y., Laurain-Mattar, D., Henry, M. (2010). Root *in vitro* cultures of six *Gypsophila* species and their saponin contents. *Enzyme and Microbial Technology,* **47**: 97–104.

Goncalves, M. J., Cruz, M. T., Cavaleiro, C., Lopes, M. C., Salgueiro, L. (2010). Chemical, antifungal and cytotoxic evaluation of the essential oil of *Thymus zygis* subsp. *sylvestris. Industrial Crops and Products*, **32**: 70–75.

Hamburg, M. A. (2008). Considerations for infectious disease research and practice. *Technology in Society,* **30**: 383– 387.

Hassan, S. W., Umar, R. A., Lawal, M., Bilbis, L. S., Muhammad, B. Y. and Dabai, Y. U. (2006). Evaluation of antibacterial activity and phytochemical analysis of root extracts of *Boscia angustifolia. African Journal of Biotechnology,* **5** (18): 1602- 1607.

Hasan, F., Xess, I., Wang, X., Jain, N., Fries, B. C. (2009). Biofilm formation in clinical *Candida* isolates and its association with virulence. *Microbes and Infection,* **11**: 753-761.

Hell, K., Gnonlonfin, B. G. J., Kodjogbe, G., Lamboni, Y., Abdourhamane, I. K. (2009). Mycoflora and occurrence of aflatoxin in dried vegetables in Benin, Mali and Togo, West Africa. *International Journal of Food Microbiology,* **135**: 99–104.

Holmner, A., Mackenzie, A., Krengel, U. (2010). Molecular basis of cholera blood-group dependence and implications for a world characterized by climate change. *FEBS Letters*, **584**: 2548–2555.

Hong, H. A., Khaneja, R., Tam, N. M. K., Cazzato, A., Tan, S., Urdaci, M., Brisson, A., Gasbarrini, A., Barnes, I., and Cutting, M. (2009). *Bacillus subtilis* isolated from the human gastrointestinal tract. *Research in Microbiology*, **160**: 134-143.

Hoong, Y. B., Pizzi, A., Tahir, P. M., Pasch, H. (2010). Characterization of *Acacia mangium* polyflavonoid tannins by MALDI-TOF mass spectrometry and CP-MAS 13C NMR. *European Polymer Journal*, **46**: 1268–1277.

Hudzicki, J. (2009): Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. *American Society of Microbiology*, **3**: 1-12.

Karmali, M. A., Gannon, V., Sargeant, J. M. (2010). Verocytotoxin-producing *Escherichia coli* (VTEC). *Veterinary Microbiology*, **140**: 360–370.

Kategaonkar, A. H., Pokalwar, R. U., Sonar, S. S., Gawali, V. U., Shingate, B. B., Shingare, M. S. (2010). Synthesis, *in vitro* antibacterial and antifungal evaluations of new ά-hydroxyphosphonate and new ά -acetoxyphosphonate derivatives of tetrazolo [1, 5-a] quinoline. *European Journal of Medicinal Chemistry*, **45**: 1128–1132.

Khalil, A. and Dababneh, B. F. (2007): Inhibition of Phytopathogenic Fungi by Extracts from Medicinal Plants in Jordan. *Journal of Biological Sciences*, **7** (3): 579-581.

Khan, R., Islam, B., Akram, M., Shakil, S., Ahmad, A., Ali, S. M., Siddiqui, M. and Khan, A. U. (2009). Antimicrobial Activity of Five Herbal Extracts Against Multi-Drug Resistant (MDR) Strains of Bacteria and Fungus of Clinical Origin. *Molecules*, **14**: 586-597.

Khoobchandani, M., Ojeswi, B. K., Ganesh, N., Srivastava, M. M., Gabbanini, N., Matera, R., Iori, R., Valgimigli, R. (2010). Antimicrobial properties and analytical profile of traditional *Eruca sativa* seed oil: Comparison with various aerial and root plant extracts. *Food Chemistry*, **120**: 217–224.

Khosravi, A. R., Shokri, H., Darabi, M. H., Kashani, A. Mansouri, P., Naser, A. (2009). Comparative study on the effects of a new antifungal lotion (*Artemisia sieberi* essential oil) and a clotrimazole lotion in the treatment of *Pityriasis versicolor*. *Journal de Mycologie Médicale*, **19**: 17-21.

Kisangau, D. P., Hosea, K. M., Joseph, C. C. and Lyaruu, H. V. M. (2007). *In vitro* antimicrobial assay of plants used in traditional medicine in Bukoba rural district, Tanzania. *African Journal of Traditional, Complementary, and Alternative*, **4** (4): 510 – 523.

Kolkhof, P., Geerts, A., Schafer, S., Torzewski, J. (2010). Cardiac glycosides potently inhibit C-reactive protein synthesis in human hepatocytes. *Biochemical and Biophysical Research Communications*, **394**: 233–239.

Kone, W. M., Atindehou, K. K., Kacou-N'Douba, A. and Dosso, M. (2007). Evaluation of 17 medicinal plants from Northern Côte D'ivoire for their *in vitro* activity against *Streptococcus pneumoniae*. *African Journal of Traditional, Complementary, and Alternative*, **4** (1): 17-22.

Koroishi, A. M., Foss, S. R., Cortez, D. A. G., Ueda-Nakamura, T., Nakamura, C. V., Filho, B. P. D. (2008). *In vitro* antifungal activity of extracts and neolignans from *Piper regnellii* against dermatophytes. *Journal of Ethnopharmacology*, **117**: 270–277.

Kudryavtsev, K. V., Bentley, M. L., McCafferty, D. G. (2009). Probing of the cis-5-phenylproline scaffold as a platform for the synthesis of mechanism-based inhibitors of the *Staphylococcus aureus* sortase SrtA isoform. *Bioorganic and Medicinal Chemistry*, **17**: 2886–2893.

Kumar, A., Shukla, R., Singh, P., Dubey, N. K. (2010). Chemical composition, antifungal and antiaflatoxigenic activities of *Ocimum sanctum* L. essential oil and its safety assessment as plant based antimicrobial. *Food and Chemical Toxicology*, **48**: 539–543.

Kumaraswamy, M. V., Kavitha, H. U. and Satish, S. (2008). Antibacterial Evaluation and Phytochemical Analysis of *Betula utilis* D. Don against Some Human Pathogenic Bacteria. World *Journal of Agricultural Sciences*, **4** (5): 661-664.

Kummerer, K., (2009). Antibiotics in the aquatic environment – A review – Part II. *Chemosphere*, **75**: 435–441.

Lawal, I. O., Uzokwe, N. E., Igboanugo, A. B. I., Adio, A. F., Awosan, E. A., Nwogwugwu, J. O., Faloye, B., Olatunji, B. O. and Adesoga, A. A. (2010). Ethno medicinal information on collation and identification of some medicinal plants in Research Institutes of South-west Nigeria. *African Journal of Pharmacy and Pharmacology*, **4** (1): 001-007.

Limsuwan, S., Trip, E. N., Kouwen, T. R. H. M., Piersma, S., Hiranrat, A., Mahabusarakam, W. Voravuthikunchai, S. P., Dijl J. M., Kayser, O. (2009). Rhodomyrtone: A new candidate as natural antibacterial drug from *Rhodomyrtus tomentosa*. *Phytomedicine*, **16**: 645–651.

Lin, X. (2009). *Cryptococcus neoformans:* Morphogenesis, infection, and evolution. *Infection, Genetics and Evolution*, **9**: 401–416.

Liu, T., Brown, D. A., O'Rourke, B. (2010). Role of mitochondrial dysfunction in cardiac glycoside toxicity. *Journal of Molecular and Cellular Cardiology,* **49**: 728–736.

Lou, Z., Wang, H., Lv, W., Ma, C., Wang, Z., Chen, S. (2010). Assessment of antibacterial activity of fractions from burdock leaf against food-related bacteria. *Food Control,* **21**: 1272–1278.

Lulekal, E., Kelbessa, E., Bekele, T. and Yineger, H. (2008). An ethnobotanical study of medicinal plants in Mana Angetu District, southeastern Ethiopia. *Journal of Ethnobiology and Ethnomedicine,* **4**:10.

Malabadi, R. B. and Kumur, V. (2007). Assessment of Antifungal Activity of Some Medicinal Plants. *International Journal of Pharmacology,* **3** (6): 499-504.

Manjamalai, A., Singh, R. S. S., Guruvayoorappan, C. and Grace, V. M. B. (2010). Analysis of Phytochemical Constituents and Anti-Microbial Activity of Some Medicinal Plants in Tamilnadu, India. *Global Journal of Biotechnology and Biochemistry,* **5** (2): 120-128.

Maregesi, S. M., Pieters, L., Ngassapa, O. D., Apers, S., Vingerhoets, R., Cos, P., Berghe, D. A. V., Vlietinck, A. J. (2008): Screening of some Tanzanian medicinal plants from Bunda district for antibacterial, antifungal and antiviral activities. *Journal of Ethnopharmacology,* **119**: 58–66.

Mariita, R. M., Ogol, C. K. P. O., Oguge, N. O., Okemo, P. O. (2010a). Antitubercular and Phytochemical Investigation of Methanol Extracts of Medicinal Plants Used by the Samburu Community in Kenya. *Tropical Journal of Pharmaceutical Research,* **9** (4): 379-385.

Mariita, R. M., Orodho, J. A. Okemo, P. O., Mbugua, P. K. (2010b). Antifungal, antibacterial and antimycobacterial activity of *Entada abysinnica* Steudel ex A. Rich (Fabaceae) methanol extract. *Pharmacognosy Research,* 2 (3): 163-168.

Mariita, R. M., Ogol, C. K. P. O., Oguge, N. O., Okemo, P. O. (2011). Methanol Extract of Three Medicinal Plants from Samburu in Northern Kenya Show Significant Antimycobacterial, Antibacterial and Antifungal Properties. *Research Journal of Medicinal Plant,* **5** (1): 54-64.

Marzouk, B., Marzouk, Z., Décor, R., Mhadhebi, L., Fenina, N., Aouni, M. (2010). Antibacterial and antifungal activities of several populations of Tunisian *Citrullus colocynthis* Schrad. immature fruits and seeds. *Journal de Mycologie Médicale,* **20**: 179—184.

Masman, M. F., Rodriguez, A. M., Raimondi, M., Zacchino, S. A., Luiten, P. G. M., Somlai, C., Kortvelyesi, T., Penke, B., Enriz, R. D. (2009). Penetratin and derivatives acting as antifungal agents. *European Journal of Medicinal Chemistry,* **44**: 212-228.

Matasyoh, J. C., Maiyo, Z. C., Ngure, R. M., Chepkorir, R. (2009). Chemical composition and antimicrobial activity of the essential oil of *Coriandrum sativum. Food Chemistry,* **113**: 526–529.

Mathabe, M.C., Nikolova, R.V., Lall, N. and Nyazema, N.Z. (2006). Antibacterial activities of medicinal plants used for the treatment of diarrhoea in Limpopo Province, South Africa. *Journal of Ethnopharmacology,* **105**: 286–293.

Mathabe, M. C., Hussein, A. A., Nikolova, R. V., Basson, A. E., Meyer, J. J. M., Lall, M. (2008). Antibacterial activities and cytotoxicity of terpenoids isolated from *Spirostachys africana. Journal of Ethnopharmacology,* **116**: 194–197.

Mbosso, E. J. T., Ngouela, S., Nguedia, J. C. A., Beng, V. P., Rohmer, M., Tsamo, E. (2010). *In vitro* antimicrobial activity of extracts and compounds of some selected medicinal plants from Cameroon. *Journal of Ethnopharmacology,* **128**: 476–481.

McCarrell, E. M., Gould, S. W. J., Fielder, M. D., Kelly, A. F., Sankary, W. E., and Naughton, D. P. (2008). Antimicrobial activities of pomegranate rind extracts: enhancement by addition of metal salts and vitamin C. *BMC Complementary and Alternative Medicine,* **8** (64): 1-7.

Meenakshi, B., Manish, M., Vijay, J., Anita, J. (2010). Phytochemical and Antimicrobial Studies of *Adhatoda zeylanica. International Journal of Research in Ayurveda and Pharmacy,* **1** (1): 90-97.

Mirmirani, P., Willey, A., Chamlin, S., Frieden, I. J. and Price, V. H. (2009). Tinea capitis mimicking cicatricial alopecia: What host and dermatophyte factors lead to this unusual clinical presentation? Case Reports. *Journal of the American Academy of Dermatology,* **60** (3). 490-495.

Mishra, B. B., Singh, D. D., Kishore, N., Tiwari, V. K., Tripathi, V. (2010). Antifungal constituents isolated from the seeds of Aegle marmelos. *Phytochemistry,* **71**: 230–234.

Mohapatra, S. S., Ramachandran, D., Mantri, C. K., Colwell, R. R., Singh, D. V. (2009). Determination of relationships among non-toxigenic *Vibrio cholerae* O1 biotype El Tor strains from housekeeping gene sequences and ribotype patterns. *Research in Microbiology,* **160**: 57-62.

Moyo, M., Ndhlala, A. R., Finnie, J. F., Staden, J. V. (2010). Phenolic composition, antioxidant and acetylcholinesterase inhibitory activities of *Sclerocarya birrea* and *Harpephyllum caffrum* (Anacardiaceae) extracts. *Food Chemistry,* **123**: 69–76.

Murthy, P. S., Ramalakshmi, K., Srinivas, P. (2009). Fungitoxic activity of Indian borage (*Plectranthus amboinicus*) volatiles. *Food Chemistry,* **114**: 1014–1018.

Mutai, C., Bii, C., Vagias, C., Abatis, D., Roussis, V. (2009). Antimicrobial activity of *Acacia mellifera* extracts and lupane triterpenes. *Journal of Ethnopharmacology,* **123**: 143–148.

Nadembega, P., Boussim, J. I., Nikiema, J. B., Poli, F., Antognoni, F. (2011): Medicinal plants in Baskoure, Kourittenga Province, Burkina Faso: An ethnobotanical study. *Journal of Ethnopharmacology,* **133** (2): 378-395.

Nagegowda, D. A. (2010). Plant volatile terpenoid metabolism: Biosynthetic genes, transcriptional regulation and subcellular compartmentation. *FEBS Letters,* **584**: 2965–2973.

Nedorostova, L., Kloucek, P., Kokoska, P., Stolcova, M., and Pulkrabek, J., (2009). Antimicrobial properties of selected essential oils in vapour phase against foodborne bacteria. *Food Control,* **20** (2): 157-160.

Negi, A. S., Kumar, J. K., Luqman, S., Saikia, D. and Khanuja, S. P. S. (2009). Antitubercular potential of plants: A brief account of some important molecules. *Medicinal Research Reviews,* **30** (4): 603-605.

Nenaah, G. (2010). Antibacterial and antifungal activities of (beta)-carboline alkaloids of *Peganum harmala* (L) seeds and their combination effects. *Fitoterapia,* 81: 779–782.

Njoroge, G. N. and Bussmann, R. W. (2007). Ethnotherapeautic management of skin diseases among the Kikuyus of Central Kenya. *Journal of Ethnopharmacology,* **111**: 303–307.

Nwinyi, O.C.; Chinedu, N.S.; Ajani, O.O.; Ikpo, C.O. and Ogunniran, K.O. (2009). Antibacterial effects of extracts of *Ocimum gratissimum* and *Piper guineense* on *Escherichia coli* and *Staphylococcus aureus*. *African Journal of Food Science,* **3** (3): 77-81.

Oke, F. and Aslim, B. (2010). Biological potentials and cytotoxicity of various extracts from endemic *Origanum minutiflorum* O. Schwarz & P.H. Davis. *Food Chemistry and Toxicology,* **48**: 1728–1733.

Omoya, F. O. and Akharaiyi, F. C. (2010). A Pasture Honey Trial for Antibacterial Potency on Some Selected Pathogenic Bacteria. *Journal of Natural Products,* **3**: 05-11.

Omwenga, E. O., Okemo, P. O., Mbugua, P. K. and Ogol, C. K. P. (2009). Ethnobotanical Survey and Antimicrobial Evaluation of Medicinal Plants used by the Samburu Community (Kenya) for treatment of Diarrhoea. *Pharmacognosy Magazine*, **4** (18): 169-176.

Orhan, D. D., Zc-elik, B., Zgen, S., Ergun, F. (2010). Antibacterial, antifungal, and antiviral activities of some flavonoids. *Microbiological Research*, **165**: 496-504.

Orwa, J. A., Jondiko, I. J. O., Minja, R. J. A., Bekunda, M. (2008): The use of *Toddalia asiatica* (L) Lam. (Rutaceae) in traditional medicine practice in East Africa. *Journal of Ethnopharmacology*, **115**: 257–262.

Pal, B. B., Khuntia, H. K., Samal, S. K., Kar, S. K., Patnaik, B. (2010). Epidemics of severe cholera caused by El Tor *Vibrio cholerae* O1 Ogawa possessing the ctxB gene of the classical biotype in Orissa, India. *International Journal of Infectious Diseases*, **14**: 384–389.

Pellati, F., Bruni, R., Bellardi, M. G., Bertaccini, A., Benvenuti, S. (2009). Optimization and validation of a high-performance liquid chromatography method for the analysis of cardiac glycosides in *Digitalis lanata*. *Journal of Chromatography A*, **1216**: 3260–3269.

Philip, K., Malek, S. N. A., Sani, W., Shin, S. K., Kumar, S., Lai, H. S., Serm, L. G. and Rahman, S. N. S. A. (2009). Antimicrobial Activity of Some Medicinal Plants from Malaysia. *American Journal of Applied Sciences*, **6** (8): 1613-1617.

Ponnusamy, K., Petchiammal, C., Mohankumar, R., Hopper, W. (2010). *In vitro* antifungal activity of indirubin isolated from a South Indian ethnomedicinal plant *Wrightia tinctoria* R. Br. *Journal of Ethnopharmacology*, **132**: 349–354.

Prakash, B., Shukla, R., Singh, P., Kumar, A., Mishra, P. K., Dubey, N. K. (2010). Efficacy of chemically characterized *Piper betle* L. essential oil against fungal and aflatoxin contamination of some edible commodities and its antioxidant activity. *International Journal of Food Microbiology*, **142**: 114–119.

Rahman, A. and Kang, S. C. (2009). *In vitro* control of food-borne and food spoilage bacteria by essential oil and ethanol extracts of *Lonicera japonica* Thunb. *Food Chemistry*, **116**: 670 - 675.

Rajpara, N., Patel, A., Tiwari,N., Bahuguna, J., Antony, A., Choudhury, I., Ghosh, A., Jain, R., Ghosh, A., Bhardwaj, A. K. (2009). Mechanism of drug resistance in a clinical isolate of *Vibrio fluvialis*: involvement of multiple plasmids and integrons. *International Journal of Antimicrobial Agents*, **34**: 220–225.

Rangasamy, O., Raoelison, G., Rakotoniriana, F. E., Cheuk, K., Urverg-Ratsimamanga, S., Quetin-Leclercq, J., Gurib-Fakim, A., Subratty, A. H. (2007). Screening for anti-infective properties of several medicinal plants of the Mauritians flora. *Journal of Ethnopharmacology,* **109**: 331–337.

Ranpal, S. (2009). An Assessment of Status and Antibacterial Properties of *Dactylorhiza hatagirea* in Annapurna Conservation Area (A case study of Paplekharka, Lete VDC, Mustang). *B. Sc. Forestry Research Thesis Submitted to Tribhuvan University, Institute of Forestry, Pokhara, Nepal.*

Rao, G. X., Zhang, S., Wang, H., Li, Z., Gao, S., Xu, G. (2009). Antifungal alkaloids from the fresh rattan stem of *Fibraurea recisa* Pierre. *Journal of Ethnopharmacology,* **123**: 001–005.

Reddy, K. R. N., Reddy, C. S., Muralidharan, K. (2009). Potential of botanicals and biocontrol agents on growth and aflatoxin production by *Aspergillus flavus* infecting rice grains. Food *Control*, **20**: 173–178.

Reuben, K. D. (2008). Phytochemical Screening and *in vitro* Antimicrobial Investigation of the Methanolic Extract of *Croton Zambesicus* Muell ARG. Stem Bark. *European Journal of Scientific Research,* **23** (1): 134-140.

Rojas, J. J., Ochoa, V. J., Ocampo, S. A., and Munoz, J. F. (2006). Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: A possible alternative in the treatment of non-nosocomial infections. *BMC Complementary and Alternative Medicine,* **6** (2): 1-6.

Russell, A. L., Kennedy, A. M., Spuches, A. M., Venugopal, D., Bhonsle, J. B., Hicks, R. P. (2010). Spectroscopic and thermodynamic evidence for antimicrobial peptide membrane selectivity. *Chemistry and Physics of Lipids,* **163**: 488–497.

Sahgal, G., Ramanathan, S., Sasidharan, S., Mordi, M. N., Ismail, S. and Mansor, S. M. (2009). Phytochemical and antimicrobial activity of *Swietenia mahagoni* crude methanolic seed extract. *Tropical Biomedicine,* **26** (3): 274–279.

Salama, H. M. H. and Marraiki, N. (2010). Antimicrobial activity and phytochemical analyses of *Polygonum aviculare* L. (Polygonaceae), naturally growing in Egypt. *Saudi Journal of Biological Sciences,* **17**: 57–63.

Salama, H.M.H. and Marraiki N. (2008). Antimicrobial activity and phytochemical analysis of *Polygonum aviculare* L. (Polygonaceae), naturally growing in Egypt. *Australian Journal of Basic and Applied Sciences,* **3** (3): 2008-2015.

Salminen, A., Lehtonen, M., Suuronen, T., Kaarniranta, K. and Huuskonen, J. (2008). Terpenoids: natural inhibitors of NF-kappa B signaling with anti-inflammatory and anticancer potential. *Cellular and Molecular Life Sciences,* **65** (19): 2979-2999.

Sarac, N. and Ugur, A. (2007). Antimicrobial activities and usage in folkloric medicine of some Lamiaceae species growing in Mugla, Turkey. *EurAsian Journal of BioSciences,* **4**: 28-37.

Saravanakumar, A., Venkateshwaran, K., Vanitha, J., Ganesh, M., Vasudevan, M. and Sivakumar, T. (2009). Evaluation of antibacterial activity, phenol and flavonoids contents of *Thespesia populnea* flower extracts. *Pakistan Journal of Pharmaceutical Sciences,* **22** (3): 282-286.

Sathiya, M., Parimala, P. and Muthuchelian, K. (2008). Preliminary Phytochemical Screening and Antibacterial Studies on the Ethanolic Leaf Extract of *Nyctanthes arbortristis* Linn. *Ethnobotanical Leaflets,* **12**: 337-342.

Schiff, P. L. (2002). Opium and Its Alkaloids. *American Journal of Pharmaceutical Education,* 66: 186-194.

Severino, R. P., Guido, R. V. C., Marques, E. F., Bromme, D., Silva, F. G. F., Fernandes, J. B., Andricopulo, A. D., Vieira, P. C. (2011). Acridone alkaloids as potent inhibitors of cathepsin V. *Bioorganic and Medicinal Chemistry,* **19** (4): 1477-481.

Seyydnejad, S. M., Niknejad, M., Darabpoor, I. and Motamedi, H. (2010). Antibacterial Activity of Hydroalcoholic Extract of *Callistemon citrinus* and *Albizia lebbeck. American Journal of Applied Sciences,* **7** (1): 13-16.

Sharma, A., Patel, V. K., Ramteke, P. (2009). Identification of vibriocidal compounds from medicinal plants using chromatographic fingerprinting. *World Journal of Microbiology and Biotechnology,* **25**: 19–25.

Sharma, A., Patel, V. K., Chaturvedi, A. N. (2010). Vibriocidal activity of certain medicinal plants used in Indian folklore medicine by tribals of Mahakoshal region of central India. *Indian Journal of Pharmacology,* **41** (3): 129-133.

Silva, A. P. G., Unks, D., Lyu, S., Ma, J., Zbozien-Pacamaj, R., Chen, X., Krensky, A. M., Clayberger, C. (2008): *In vitro* and *in vivo* antimicrobial activity of granulysin-derived peptides against *Vibrio cholerae. Journal of Antimicrobial Chemotherapy,* **61**: 1103–1109.

Siriwatanametanon, N., Fiebich, B., Efferth, T., Prieto, J. M., Heinrich, M. (2010). Traditionally used Thai medicinal plants: *In vitro* anti-inflammatory, anticancer and antioxidant activities. *Journal of Ethnopharmacology,* **130**: 196–207.

Sousa, F., Guebitz, G. M., Kokol, V. (2009). Antimicrobial and antioxidant properties of chitosan enzymatically functionalized with flavonoids. *Process Biochemistry,* **44**: 749–756.

Su, C., Damu, A. G., Chiang, P., Bastow, K. F., Morris-Natschke, S. L., Lee, K. and Wu, T. (2008). Total synthesis of phenanthroindolizidine alkaloids (±)-antofine, (±)-deoxypergularinine, and their dehydro congeners and evaluation of their cytotoxic activity. *Bioorganic and Medicinal Chemistry,* **16**: 6233–6241.

Tao, R., Ying, G., Su, H., Zhou, H., Sidhu, J. P. S. (2010). Detection of antibiotic resistance and tetracycline resistance genes in *Enterobacteriaceae* isolated from the Pearl Rivers in South China. *Environmental Pollution,* **158**: 2101-2109.

Thatoi, H. N., Panda, S. K., Rath, S. K., Dutta, S. K. (2008). Antimicrobial Activity and Ethnomedicinal Uses of Some Medicinal Plants from Similipal Biosphere Reserve, Orissa. *Asian Journal of Plant Sciences,* **7** (3): 260-267.

Thomas, K. D., Adhikari, A. V., Shetty, N. S. (2010). Design, synthesis and antimicrobial activities of some new quinoline derivatives carrying 1,2,3-triazole moiety. *European Journal of Medicinal Chemistry,* **45**: 3803-3810.

Thring, T. S. A. and Weitz, F. M. (2006). Medicinal plant use in the Bredasdorp/Elim region of the Southern Overberg in the Western Cape Province of South Africa. *Journal of Ethnopharmacology,* **103**: 261–275.

Tiruneh, M. (2009): Serodiversity and Antimicrobial Resistance of *Shigella* Isolates at Gondar University Teaching Hospital, Northwest Ethiopia. *Japanese Journal of Infectious Diseases,* **62**: 93-97.

Toledo, C. E. M., Britta, E. A., Ceole, L. F., Silva, E. R., Mello, J. C. P., Filho, B. P. D., Nakamura, C. V., Ueda-Nakamura, T. (2011). Antimicrobial and cytotoxic activities of medicinal plants of the Brazilian cerrado, using Brazilian cachaca as extractor liquid. *Journal of Ethnopharmacology,* **133** (2): 420-425.

Turgis, M., Han, J., Caillet, S., Lacroix, M. (2009). Antimicrobial activity of mustard essential oil against *Escherichia coli* O157:H7 and *Salmonella typhi*. *Food Control,* **20**: 1073–1079.

Umamaheswari, A., Shreevidya, R. and Nuni, A. (2008). *In vitro* Antibacterial Activity of *Bougainvillea spectabilis* Leaves Extracts. *Advances in Biological Research,* **2** (1-2): 01-05.

Uslu, H., Yoruk, O., Ayyıldız, A., Aktan, B. (2009). Antibacterial Spectrum of Umckaloabo (*Pelargonium sidoides*) On Upper Airway Infection Agents. *European Journal of General Medicine,* **6** (4): 245-248.

Vaidya, G. S. and Bhattarai, N. (2009). Antagonistic study of *Lantana camara* (linn) against pathogenic bacteria. *Scientific World,* **7** (7): 64-67.

Velazquez, C., Calzada, F., Esquivel, B., Barbosa, E., Calzada, S. (2009). Antisecretory activity from the flowers of *Chiranthodendron pentadactylon* and its flavonoids on intestinal fluid accumulation induced by *Vibrio cholerae* toxin in rats. *Journal of Ethnopharmacology,* **126**: 455–458.

Venugopal, D., Klapper, D., Srouji, A. H., Bhonsle, J. B., Borschel, R., Mueller, A., Russell, A. L., Williams, B. C., Hicks, R. P. (2010). Novel antimicrobial peptides that exhibit activity against select agents and other drug resistant bacteria. *Bioorganic and Medicinal Chemistry,* **18**: 5137–5147.

Vila-Corcoles, A., Bejarano-Romero, F., Salsench1, E., Ochoa-Gondar, I., Diego, C., Gomez Bertomeu, F., Raga-Luria, X., Cliville-Guasch, X. and Arija, V. (2009). Drug-resistance in *Streptococcus pneumoniae* isolates among Spanish middle aged and older adults with community-acquired pneumonia. *BMC Infectious Diseases,* **9** (36): 1-7.

Wagate, C. G., Mbaria, J. M., Gakuya, D. W., Nanyingi, M. O., Kareru, P. G., Njuguna, A., Gitahi, N., Macharia, J. K. and Njonge, F. K. (2010). Screening of some Kenyan Medicinal Plants for Antibacterial Activity. *Phytotherapy Research,* **24**: 150–153.

Williams, R. J., Spencer, J. P. E. and Evans, C. R. (2004). Flavonoids: Antioxidants or Signalling Molecules? *Free Radical Biology and Medicine,* **36** (7): 838 – 849.

Wright, G. N. and Sutherland, A. D. (2007). New strategies for combating multidrug-resistant bacteria. *Trends in Molecular Medicine,* **13** (6): 260-267.

Yadav, J. P., Arya, V., Yadav, S., Panghal, M., Kumar, S., Dhankhar, S. (2010). *Cassia occidentalis* L.: A review on its ethnobotany, phytochemical and pharmacological profile. *Fitoterapia*, **81**: 223–230.

Yineger, H., Kelbessa, E., Bekele, T., Lulekal, E. (2007). Ethnoveterinary medicinal plants at Bale Mountains National Park, Ethiopia. *Journal of Ethnopharmacology,* **112**: 55–70.

Yusha'u, M., Aliyu, B. S., and Olonitola, S. O. (2008). Preliminary Screening of *Acalypha* Extracts for *in vitro* Inhibitory Activity against Extended-Spectrum B-Lactamase Producing Enterobacteriaceae. *International Journal of Pure and Applied Sciences,* **2** (2): 1-5.

Zabka, M., Pavela, R., Slezakova, L. (2009). Antifungal effect of *Pimenta dioica* essential oil against dangerous pathogenic and toxinogenic fungi. *Industrial Crops and Products,* **30**: 250 -253.

Zafar, A., Hasan, R., Nizami, S. Q., Seidlein, L. V., Soofi, S., Ahsan, T., Chandio, S., Habib, A., Bhutto, N., Siddiqui, F J., Rizvi, A. Clemens, J. D., Bhutta, Z. A. (2009). Frequency of isolation of various subtypes and antimicrobial resistance of *Shigella* from urban slums of Karachi, Pakistan. *International Journal of Infectious Diseases,* **13**: 668 - 672.

Zampini, I. C., Cuello, S., Alberto, M. R., Ordonez, R. M., Almeida, R. D., Solorzano, E., Isla, M. I. (2009). Antimicrobial activity of selected plant species from "the Argentine Puna" against sensitive and multi-resistant bacteria. *Journal of Ethnopharmacology,* **124**: 499–505.

APPENDICES

Appendix I - Traditional Medical Practitioners (TMP) Questionnaire

Kenyatta University researchers in collaboration with Earthwatch are conducting research on the Use of medicinal plants to treat infectious diseases. We are soliciting information from you to determine the efficacy of some medicinal plants used against infectious diseases caused by some selected bacteria and fungi. We appreciate your cooperation and the information given will be treated with utmost confidentiality. Thank you in advance.

Improving the management of infectious diseases among the Samburu community

Recorder…… Date……

District…… Location……..

Sub-location…… Village……

A. Biodata

Name		Sex	
Age		Religion	
Main occupation		Other occupation	

B. Knowledge acquisition

1. Do you treat infectious diseases?

Yes	No

2. Which infectious diseases do you treat? *Please tick against what you treat*

Infectious Diseases	**Tick Appropriately**
Diarrhoea	
Wounds	
Ordinary cough	
Skin infections (patches on the neck, chest, face, back, hair, nails)	
Infections on hair and nails	
Cholera	
Blood diarrhoea	
Vomiting	
Abdominal pains	
Pneumonia	
Boils	
Carcinoma (turmor)	
Hemorrhage (excessive bleeding)	
Any other	

3. Please list the signs by which you recognize infectious diseases

_____

_____

_____

4. What are the local names given to the infectious diseases?

_____

_____

_____

5. What are the causes these infectious diseases?

_____

_____

_____

6. How did you learn how to treat infectious diseases? *Please tick against how you leanrt*

Learnt from parent	
From relatives other than parents	
From other healers	
Knowledge came through a dream	
Other source (s) *elaborate*	

C. Treatment practices and knowledge

1. Do you belong to a healers association?

Yes	No

2. How many patients do you treat per day?

_____

3. How many patients do you receive in a month?

_____

4. How many people work at your practice?

_____

5. How many people gather plants for you?

_____

6. What materials do you use in your treatments? *Please tick against the material used.*

Plant parts	Tick
Minerals (specify)	
Animal parts	
Other (specify)	

7. Are there any rituals involved?

Yes	No

8. Please fill in the table below to tell us about your way of treating infectious diseases

Infectious disease	Plant spp	Part used	Time of collection	Formula	Dose	Route administration	Preservation methods

Codes:

a. Infectious infections (1 Cholera; 2 Dysentery; 3 Food poisoning; 4 Skin infections; 5. Pneumonia; 6. Wounds;
7. Meningitis; 8. Bleeding; 9.Other)

b. Route of administration (1 drinking; 2 inhaling smoke from burnt plants; 3 steam bath; 4 lick ash……)

c. Time of collection (1 morning; 2 afternoon; 3 evening; 4 night time)

d. Part used (1 leaves; 2 stem bark; 3 root bark; 4 stem wood; 5 root wood; 6 flowers; 7 fruit).

e. Preservation method (1 powder; 2 liquid etc……..)

D. Patients

1. Are patients comfortable about seeking infectious diseases treatment from you?

Yes	No

2. Where do most of your customers come from?

Area	**Tick**
From this village	
From other districts	
From other countries	
Do not know	

3. Of the patients you treated in the last year, how many recovered?

_____

4. How did you assess that they had recovered?

_____

THANK YOU VERY MUCH

Appendix II - Tables representing degrees of significance of means of zones of inhibition in various test cultures.

a) *Escherichia coli*

Analysis of Variance for E. coli

Source	DF	SS	MS	F	P
Medicina	23	557.5417	24.2409	349.07	0.000
Error	48	3.3333	0.0694		
Total	71	560.8750			

Individual 95% CIs For Mean Based on Pooled StDev

Level	N	Mean	StDev	------+---------+---------+---------+
Acacia e	3	9.333	0.577	*)
Acacia h	3	9.333	0.577	*)
Acacia n	3	10.000	0.000	(*)
Acacia S	3	6.000	0.000	(*)
Acacis t	3	6.000	0.000	(*)
Acokanth	3	6.000	0.000	(*)
Albizia	3	6.000	0.000	(*)
Aloe sec	3	6.333	0.577	(*)
Cissus q	3	6.000	0.000	(*)
Cissus r	3	6.000	0.000	(*)
Cleroden	3	6.000	0.000	(*)
Cordia s	3	6.000	0.000	(*)
Croton m	3	6.000	0.000	(*)
Croton m	3	6.000	0.000	(*)
Euphorbi	3	6.000	0.000	(*)
Kedrosti	3	6.333	0.577	(*)
Loranthu	3	10.000	0.000	(*)
Plumbago	3	6.667	0.577	(*
Rhus rus	3	6.000	0.000	(*)
Salvador	3	7.000	0.000	*)
Sericoco	3	6.000	0.000	(*)
Thylachi	3	7.000	0.000	*)
Negative	3	6.000	0.000	(*)
Positive	3	19.000	0.000	(*

------+---------+---------+---------+

Pooled StDev = 0.264 8.0 12.0 16.0 20.0

Tukey's pairwise comparisons

Family error rate = 0.0500
Individual error rate = 0.000345
Critical value = 5.45

b) *Vibrio cholerae*

Analysis of Variance for V. chole

Source	DF	SS	MS	F	P
Medicina	23	1077.319	46.840	306.59	0.000
Error	48	7.333	0.153		
Total	71	1084.653			

Individual 95% CIs For Mean Based on Pooled StDev

Level	N	Mean	StDev	---------+---------+---------+-------
Acacia e	3	7.000	0.000	(*)
Acacia h	3	10.000	0.000	(*)
Acacia n	3	9.000	0.000	(*)
Acacia S	3	6.667	0.577	(*)
Acacis t	3	6.667	0.577	(*)
Acokanth	3	6.000	0.000	(*)
Albizia	3	6.000	0.000	(*)
Aloe sec	3	10.667	0.577	(*)
Cissus q	3	7.000	0.000	(*)
Cissus r	3	6.000	0.000	(*)
Cleroden	3	6.000	0.000	(*)
Cordia s	3	7.000	0.000	(*)
Croton m	3	6.000	0.000	(*)
Croton m	3	6.000	0.000	(*)
Euphorbi	3	6.000	0.000	(*)
Kedrosti	3	6.000	0.000	(*)
Loranthu	3	18.333	0.577	(*)
Plumbago	3	7.333	0.577	(*)
Rhus rus	3	6.667	0.577	(*)
Salvador	3	6.000	0.000	(*)
Sericoco	3	7.000	0.000	(*)
Thylachi	3	11.667	0.577	(*)
Negative	3	6.000	0.000	(*)
Positive	3	21.333	1.155	(*)

Pooled StDev = 0.391 10.0 15.0 20.0

Tukey's pairwise comparisons

Family error rate = 0.0500
Individual error rate = 0.000345

Critical value = 5.45

c) *Staphylococcus aureus*

Analysis of Variance for S. aureu

Source	DF	SS	MS	F	P
Medicina	23	1621.278	70.490	362.52	0.000
Error	48	9.333	0.194		
Total	71	1630.611			

Individual 95% CIs For Mean
Based on Pooled StDev

Level	N	Mean	StDev	-+---------+---------+---------+-----
Acacia e	3	12.000	0.000	(*)
Acacia h	3	13.333	0.577	(*)
Acacia n	3	11.000	1.000	(*)
Acacia S	3	6.000	0.000	(*)
Acacis t	3	6.000	0.000	(*)
Acokanth	3	6.000	0.000	(*)
Albizia	3	6.000	0.000	(*)
Aloe sec	3	8.000	0.000	(*)
Cissus q	3	7.667	0.577	(*)
Cissus r	3	6.000	0.000	(*)
Cleroden	3	6.000	0.000	(*)
Cordia s	3	6.000	0.000	(*)
Croton m	3	6.000	0.000	(*)
Croton m	3	7.000	0.000	(*)
Euphorbi	3	6.000	0.000	(*)
Kedrosti	3	6.333	0.577	(*
Loranthu	3	18.000	0.000	(*)
Plumbago	3	10.667	0.577	(*)
Rhus rus	3	6.000	0.000	(*)
Salvador	3	6.000	0.000	(*)
Sericoco	3	6.000	0.000	(*)
Thylachi	3	16.000	1.000	(*)
Negative	3	6.000	0.000	(*)
Positive	3	24.667	1.155	(*)

-+---------+---------+---------+-----
Pooled StDev = 0.441 6.0 12.0 18.0 24.0

Tukey's pairwise comparisons

Family error rate = 0.0500
Individual error rate = 0.000345

Critical value = 5.45

d) *Bacillus subtilis*

Analysis of Variance for B. subti

Source	DF	SS	MS	F	P
Medicina	23	1509.542	65.632	337.54	0.000
Error	48	9.333	0.194		
Total	71	1518.875			

Individual 95% CIs For Mean
Based on Pooled StDev

Level	N	Mean	StDev	-+---------+---------+---------+-----
Acacia e	3	11.333	0.577	(*)
Acacia h	3	11.667	0.577	*)
Acacia n	3	13.667	0.577	(*)
Acacia S	3	10.667	0.577	(*)
Acacis t	3	6.000	0.000	(*)
Acokanth	3	6.000	0.000	(*)
Albizia	3	6.000	0.000	(*)
Aloe sec	3	6.667	0.577	(*)
Cissus q	3	8.667	0.577	*)
Cissus r	3	7.333	0.577	(*)
Cleroden	3	6.667	0.577	(*)
Cordia s	3	6.000	0.000	(*)
Croton m	3	6.000	0.000	(*)
Croton m	3	8.000	0.000	(*)
Euphorbi	3	6.000	0.000	(*)
Kedrosti	3	8.667	0.577	*)
Loranthu	3	17.333	1.155	(*)
Plumbago	3	6.000	0.000	(*)
Rhus rus	3	6.000	0.000	(*)
Salvador	3	6.000	0.000	(*)
Sericoco	3	6.000	0.000	(*)
Thylachi	3	13.000	0.000	(*)
Negative	3	6.000	0.000	(*)
Positive	3	25.333	0.577	(*)

-+---------+---------+---------+-----
Pooled StDev = 0.441 6.0 12.0 18.0 24.0

Tukey's pairwise comparisons

Family error rate = 0.0500
Individual error rate = 0.000345

Critical value = 5.45

e) *Shigella dysenteriae*

Analysis of Variance for S. dysen

Source	DF	SS	MS	F	P
Medicina	23	805.500	35.022	210.13	0.000
Error	48	8.000	0.167		
Total	71	813.500			

Individual 95% CIs For Mean
Based on Pooled StDev

Level	N	Mean	StDev	-----+---------+---------+---------+-
Acacia e	3	10.667	1.155	(*-)
Acacia h	3	11.000	1.000	(*-)
Acacia n	3	10.333	0.577	(-*)
Acacia S	3	6.000	0.000	(*)
Acacis t	3	6.000	0.000	(*)
Acokanth	3	6.000	0.000	(*)
Albizia	3	6.000	0.000	(*)
Aloe sec	3	6.000	0.000	(*)
Cissus q	3	6.000	0.000	(*)
Cissus r	3	6.000	0.000	(*)
Cleroden	3	6.000	0.000	(*)
Cordia s	3	6.000	0.000	(*)
Croton m	3	6.000	0.000	(*)
Croton m	3	7.000	0.000	(*)
Euphorbi	3	6.000	0.000	(*)
Kedrosti	3	6.000	0.000	(*)
Loranthu	3	16.667	0.577	(-*)
Plumbago	3	11.000	0.000	(*-)
Rhus rus	3	7.333	0.577	(*)
Salvador	3	6.333	0.577	(*)
Sericoco	3	6.000	0.000	(*)
Thylachi	3	12.667	0.577	(*-)
Negative	3	6.000	0.000	(*)
Positive	3	17.000	0.000	(-*)

-----+---------+---------+---------+-

Pooled StDev = 0.408 7.0 10.5 14.0 17.5

Tukey's pairwise comparisons

Family error rate = 0.0500
Individual error rate = 0.000345

Critical value = 5.45

f) *Staphylococcus pneumoniae*

Analysis of Variance for S. pneum

Source	DF	SS	MS	F	P
Medicina	23	2857.542	124.241	1118.17	0.000
Error	48	5.333	0.111		
Total	71	2862.875			

Individual 95% CIs For Mean
Based on Pooled StDev

Level	N	Mean	StDev	---+---------+---------+---------+---
Acacia e	3	6.000	0.000	(*
Acacia h	3	6.000	0.000	(*
Acacia n	3	6.000	0.000	(*
Acacia S	3	6.000	0.000	(*
Acacis t	3	6.000	0.000	(*
Acokanth	3	6.000	0.000	(*
Albizia	3	17.667	0.577	*)
Aloe sec	3	6.000	0.000	(*
Cissus q	3	6.000	0.000	(*
Cissus r	3	6.000	0.000	(*
Cleroden	3	19.333	1.155	*)
Cordia s	3	6.000	0.000	(*
Croton m	3	6.000	0.000	(*
Croton m	3	7.000	0.000	(*
Euphorbi	3	6.000	0.000	(*
Kedrosti	3	6.000	0.000	(*
Loranthu	3	6.000	0.000	(*
Plumbago	3	6.000	0.000	(*
Rhus rus	3	8.667	0.577	(*
Salvador	3	6.000	0.000	(*
Sericoco	3	6.000	0.000	(*
Thylachi	3	6.667	0.577	*)
Negative	3	6.000	0.000	(*
Positive	3	33.667	0.577	*)

---+---------+---------+---------+---
Pooled StDev = 0.333 8.0 16.0 24.0 32.0

Tukey's pairwise comparisons

Family error rate = 0.0500
Individual error rate = 0.000345

Critical value = 5.45

g) *Candida parapsilosis*

Analysis of Variance for C. parap

Source	DF	SS	MS	F	P
Medicina	23	887.111	38.570	173.57	0.000
Error	48	10.667	0.222		
Total	71	897.778			

Individual 95% CIs For Mean Based on Pooled StDev

Level	N	Mean	StDev	----------+---------+---------+------
Acacia e	3	6.000	0.000	(*)
Acacia h	3	6.000	0.000	(*)
Acacia n	3	12.667	1.155	(*)
Acacia S	3	6.000	0.000	(*)
Acacis t	3	6.000	0.000	(*)
Acokanth	3	6.000	0.000	(*)
Albizia	3	6.000	0.000	(*)
Aloe sec	3	6.000	0.000	(*)
Cissus q	3	6.000	0.000	(*)
Cissus r	3	6.000	0.000	(*)
Cleroden	3	6.000	0.000	(*)
Cordia s	3	6.667	0.577	(*)
Croton m	3	6.000	0.000	(*)
Croton m	3	6.000	0.000	(*)
Euphorbi	3	6.000	0.000	(*)
Kedrosti	3	6.000	0.000	(*)
Loranthu	3	14.000	1.732	(*)
Plumbago	3	6.000	0.000	(*)
Rhus rus	3	6.000	0.000	(*)
Salvador	3	6.000	0.000	(*)
Sericoco	3	6.000	0.000	(*)
Thylachi	3	10.667	0.577	(*)
Negative	3	6.000	0.000	(*)
Positive	3	20.667	0.577	(*)

Pooled StDev = 0.471 10.0 15.0 20.0

Tukey's pairwise comparisons

Family error rate = 0.0500
Individual error rate = 0.000345

Critical value = 5.45

h) *Cryptococcus neoformans*

Analysis of Variance for C. neofo

Source	DF	SS	MS	F	P
Medicina	23	723.3194	31.4487	754.77	0.000
Error	48	2.0000	0.0417		
Total	71	725.3194			

Individual 95% CIs For Mean
Based on Pooled StDev

Level	N	Mean	StDev	---------+---------+---------+-------
Acacia e	3	9.667	0.577	*)
Acacia h	3	6.000	0.000	*
Acacia n	3	6.000	0.000	*
Acacia S	3	6.000	0.000	*
Acacis t	3	6.000	0.000	*
Acokanth	3	6.000	0.000	*
Albizia	3	11.000	0.000	*
Aloe sec	3	6.000	0.000	*
Cissus q	3	6.000	0.000	*
Cissus r	3	6.000	0.000	*
Cleroden	3	6.000	0.000	*
Cordia s	3	6.000	0.000	*
Croton m	3	6.000	0.000	*
Croton m	3	6.000	0.000	*
Euphorbi	3	6.000	0.000	*
Kedrosti	3	6.000	0.000	*
Loranthu	3	6.000	0.000	*
Plumbago	3	6.000	0.000	*
Rhus rus	3	6.667	0.577	*)
Salvador	3	6.000	0.000	*
Sericoco	3	6.333	0.577	(*
Thylachi	3	9.000	0.000	*
Negative	3	6.000	0.000	*
Positive	3	21.000	0.000	*

Pooled StDev = 0.204 10.0 15.0 20.0

Tukey's pairwise comparisons

Family error rate = 0.0500
Individual error rate = 0.000345

Critical value = 5.45

i) *Aspergillus flavus*

Analysis of Variance for A. flavu

Source	DF	SS	MS	F	P
Medicina	23	744.8750	32.3859	777.26	0.000
Error	48	2.0000	0.0417		
Total	71	746.8750			

Individual 95% CIs For Mean
Based on Pooled StDev

Level	N	Mean	StDev	------+---------+---------+---------+
Acacia e	3	6.000	0.000	(*)
Acacia h	3	6.000	0.000	(*)
Acacia n	3	11.667	0.577	*)
Acacia S	3	6.000	0.000	(*)
Acacis t	3	6.000	0.000	(*)
Acokanth	3	6.000	0.000	(*)
Albizia	3	6.000	0.000	(*)
Aloe sec	3	7.000	0.000	*)
Cissus q	3	6.000	0.000	(*)
Cissus r	3	6.000	0.000	(*)
Cleroden	3	6.000	0.000	(*)
Cordia s	3	6.000	0.000	(*)
Croton m	3	6.000	0.000	(*)
Croton m	3	6.000	0.000	(*)
Euphorbi	3	6.000	0.000	(*)
Kedrosti	3	6.000	0.000	(*)
Loranthu	3	14.667	0.577	(*
Plumbago	3	6.000	0.000	(*)
Rhus rus	3	6.000	0.000	(*)
Salvador	3	6.000	0.000	(*)
Sericoco	3	6.667	0.577	(*
Thylachi	3	10.000	0.000	(*)
Negative	3	6.000	0.000	(*)
Positive	3	19.000	0.000	(*

------+---------+---------+---------+

Pooled StDev = 0.204 8.0 12.0 16.0 20.0

Tukey's pairwise comparisons

Family error rate = 0.0500
Individual error rate = 0.000345

Critical value = 5.45

j) *Microsporium gypseum*

Analysis of Variance for M. gypse

Source	DF	SS	MS	F	P
Medicina	23	1188.444	51.671	218.84	0.000
Error	48	11.333	0.236		
Total	71	1199.778			

Individual 95% CIs For Mean Based on Pooled StDev

Level	N	Mean	StDev	----------+---------+---------+------
Acacia e	3	8.667	0.577	(*)
Acacia h	3	6.000	0.000	(*)
Acacia n	3	13.333	0.577	(*)
Acacia S	3	6.000	0.000	(*)
Acacis t	3	6.000	0.000	(*)
Acokanth	3	6.000	0.000	(*)
Albizia	3	18.667	0.577	(*)
Aloe sec	3	6.000	0.000	(*)
Cissus q	3	6.000	0.000	(*)
Cissus r	3	6.000	0.000	(*)
Cleroden	3	8.333	1.155	(*)
Cordia s	3	6.000	0.000	(*)
Croton m	3	6.000	0.000	(*)
Croton m	3	7.000	0.000	(*)
Euphorbi	3	6.000	0.000	(*)
Kedrosti	3	9.333	1.155	(*)
Loranthu	3	14.000	0.000	(*)
Plumbago	3	6.000	0.000	(*)
Rhus rus	3	6.000	0.000	(*)
Salvador	3	6.000	0.000	(*)
Sericoco	3	7.667	0.577	(*)
Thylachi	3	11.333	1.155	(*)
Negative	3	6.000	0.000	(*)
Positive	3	20.333	0.577	(*)

Pooled StDev = 0.486 10.0 15.0 20.0

Tukey's pairwise comparisons

Family error rate = 0.0500
Individual error rate = 0.000345

Critical value = 5.45

k) *Trichophyton mentagrophyte*

Analysis of Variance for T. menta

Source	DF	SS	MS	F	P
Medicina	23	1001.875	43.560	522.72	0.000
Error	48	4.000	0.083		
Total	71	1005.875			

Individual 95% CIs For Mean
Based on Pooled StDev

Level	N	Mean	StDev	---------+---------+---------+-------
Acacia e	3	6.000	0.000	(*)
Acacia h	3	6.000	0.000	(*)
Acacia n	3	11.667	0.577	*)
Acacia S	3	6.000	0.000	(*)
Acacis t	3	6.000	0.000	(*)
Acokanth	3	6.000	0.000	(*)
Albizia	3	6.000	0.000	(*)
Aloe sec	3	6.000	0.000	(*)
Cissus q	3	6.000	0.000	(*)
Cissus r	3	6.000	0.000	(*)
Cleroden	3	6.000	0.000	(*)
Cordia s	3	6.000	0.000	(*)
Croton m	3	6.000	0.000	(*)
Croton m	3	6.000	0.000	(*)
Euphorbi	3	6.000	0.000	(*)
Kedrosti	3	6.000	0.000	(*)
Loranthu	3	14.667	1.155	*)
Plumbago	3	6.000	0.000	(*)
Rhus rus	3	6.000	0.000	(*)
Salvador	3	6.000	0.000	(*)
Sericoco	3	6.000	0.000	(*)
Thylachi	3	10.667	0.577	*)
Negative	3	6.000	0.000	(*)
Positive	3	22.000	0.000	(*)

Pooled StDev = 0.289 10.0 15.0 20.0

Tukey's pairwise comparisons

Family error rate = 0.0500
Individual error rate = 0.000345

Critical value = 5.45