EFFICACY OF SOME MEDICINAL PLANTS USED IN VARIOUS PARTS OF KENYA IN TREATING SELECTED BACTERIAL AND FUNGAL PATHOGENS

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DECLARATION

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

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DEDICATION

To my late father, Noah Juma, my mother Phelesia Juma, my husband John Okanda and our children Blessing and Kinda for you I will undertake all that is desirable within my reach.

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ACRONYMS AND ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ANOVA	Analysis of Varience
ATCC	American Type Culture Collection
CI	Confidence Interval
CLSI	Clinical Laboratory Standard Institute
DCM	Dichloromethane
DMSO	Dimethyl Sulfoxide
GOI	Government of India
IRN	International Research Network
KEMRI	Kenya Medical Research Institute
MBC	Minimum Bactericidal Concentration
MFC	Minimum Fungicidal Concentration
MIC	Minimum Inhibitory Concentration
NCAPD	National Coordinating Agency for Population and Development
NCCLS	National Committee on Clinical Laboratory Standards
PKF	Pannell Kerr Forster
UNICEF	United Nations International Children's Emergency Fund
UNIDO	United Nations Industrial Development Organization

ABSTRACT

Medicinal plants have been used since time immemorial to treat and prevent human ailments. WHO indicates that up to 80% of the world's population uses traditional medicine. Infections caused by bacteria and fungi have become a major health problem globally accounting for over 50,000 deaths every day. It is estimated that more than 70% of the pathogenic bacteria are resistant to at least one of the antibiotics commonly used to treat them. Conventional drugs are expensive and have side effects. Many plants have been used by various communities in Kenya in the treatment of bacterial and fungal infections but they have not been validated. The main aim of this study was to determine the efficacy of some medicinal plants used by various communities in Kenya that treat the selected bacterial and the selected fungal diseases in man. An ethnobotanical survey was used to select and collect plants from Mwingi North, Kisii South and Rarieda Districts based on their use to treat infectious diseases such as skin infection, diarrhea and many others. Crude extracts from *Zanthoxylum chalybeum, Boscia angustifolia, Melia volkensii, Zanthoxylum gilletii, Fuerstia africana, Urtica dioica, Vernonia amygdalina, Ricinus communis, Commiphora africana, Psidia puntulata, Senna didymobotrya, Ormocarpum trichocarpum, Sesbania sesban, Balanites aegyptiaca, Albizia coriaria, Ficus sycomorus, Rhus natalensis* and *Tamarindus indica* believed to contain secondary metabolites were screened against ten microorganisms, including the bacteria:- *Salmonella typhi* ATCC 19430, *Escherichia coli* ATCC 25922, *Bacillus subtilis, Staphylococcus aureus* ATCC 25923 and Methicillin Resistant *Staphylococcus aureus.* The fungal strains that were used are; *Aspergillus niger*, *Candida albicans* ATCC 90028, *Microsporum gypseum, Crytococcus neoformans* ATCC 18310 and *Trichophyton mentagrophyte.* The plants were screened using Kirby Bauer disc diffusion method. Phytochemical screening was carried out to identify the presence or absence of classes of bioactive compounds. Data was analyzed using one way ANOVA, significant means were separated using Tukey's test. Generally, *Fuerstia africana, Zanthoxylum chalybeum, Balanites aegyptiaca*, *Ormocarpum trichocarpum, Senna didymobotrya* and *Tamarindus indica* gave strong antibacterial results of between 14.5 mm and 20 mm as *Albizia coriaria, Ficus sycomorus, Commiphora africana Rhus, natalensis Senna didymobotrya, Psidia puntulata,* and *Tamarindus indica* produced strong antifungal results of between 15.5 mm and 20.5 mm. The results of MICs and the MBCs/MFCs of the extracts of *Albizia coriaria, Ficus sycomorus, Senna didymobotrya, Psidia puntulata, Fuerstia africana, Balanites aegyptiaca* and *Tamarindus indica* showed a good activity of 0.9375 mg/ml in some test cultures. *Salmonella typhi* ATCC 19430 and *Escherichia coli* ATCC 25922 were the least sensitive bacteria while *Candida albicans* ATCC 90028 was the least sensitive fungus. The present study indicates that the majority of the plants tested are an important source of antibacterial agents especially on Gram positive bacteria (*Staphylococcus aureus, Bacillus subtilis* and Methicillin Resistant *Staphylococcus aureus*) and antifungal agents against the dermatophytes especially *Microsporum gypseum.* This study recommends that the plant extracts with good antimicrobial activity be subjected to both pharmacological and toxicological studies.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Infectious diseases are the leading cause of death worldwide (Bandow *et al.,* 2003; Parekh and Chanda 2007). The major infectious diseases cause over 11 million deaths annually (WHO, 2005). Half of all deaths in the tropical countries result from infectious diseases (Okigbo and Mmeka, 2008; Assob *et al.,* 2011). Infections caused by pathogenic bacteria and fungi remain an important public health concern particularly in developing countries because of factors such as: emergence of bacterial and fungal strains that are resistant to most useful antibiotics (Abad *et al.,* 2007; WHO, 2007) HIV/AIDS pandemic (Wagate *et al.,* 2008) and unavailability of vaccine (Assob *et al.,* 2011). Conventional drugs are expensive and the western health facilities are also inaccessible to rural people (Matu and Staden, 2003; Wagete *et al.,* 2008).

The problem of multiple drug resistance has come about due to the haphazard use of the antimicrobials and re-emergence of diseases caused by genetically versatile microbes (Olila *et al.,* 2001; Islam *et al.,* 2006; Wagete *et al.,* 2010). For instance, in many countries antimicrobials are bought directly from chemists with no prescriptions or advice from a health professional (WHO, 2005). About a third of the 150 million prescriptions for antibiotics each year in human medicine, are not needed according to the US centre for Disease Control and Prevention (Rangasamy *et al.,* 2007). Drug resistance made it necessary for researchers to search for alternative antimicrobial agents from other sources like plants (Pirbalouti *et al.,* 2010). In addition to that, the human body is much

better suited to treatment with herbal remedies than chemical compounds since humans have evolved with plants and our digestive system and physiology are geared to digesting and utilizing plant based foods with medicinal value (Farooq, 2005). Considering all these factors, the African Union in 2001, declared the years 2001 - 2010 as the decade for African traditional medicine thereby acknowledging therapeutic potential and endorsement, use and development of medicinal plants in developing countries (NCAPD, 2005).

Medicinal plants have been used since time immemorial to treat and prevent human ailments because they have components of therapeutic value (Hassan *et al.,* 2006; Gulluce *et al.,* 2006; Parekh and Chanda, 2007). Domesticated and non-domesticated animals in ordinary settings unconsciously treat themselves when sick by eating various parts of medicinal plants such as leaves, stems, barks and roots (Sindiga *et al.,* 1995). They may also treat their skin conditions by briskly rubbing themselves against suitable plants with curative properties (Sindiga *et al.,* 1995). Diets high in plant –based foods and beverages are associated with a lower risk of chronic diseases (Njoroge *et al*., 2012).

WHO estimates that up to 80% of the world`s population relies on plants for their primary health care needs (Doughari, 2006; Turker and Usta, 2008; Verma *et al.,* 2011). Such a large population depends on traditional medicine due to factors such as : Increase in resistance to the commonly used antibiotics, high cost and inaccessibility to antibiotics especially in rural areas. It is however noted that medicinal plants are readily available, they have little side effects and there is extensive local knowledge on herbal medicine

amongst the communities (Rojas *et al.,* 2006; Doughari *et al.,* 2008). There are about 20,000 plant species used for medicinal purposes (Gulluce *et al.,* 2006). From which at least 121 chemical substances are extracted (Olila *et al.,* 2007). Some of the known good sources of pharmacologically active compounds are natural products from fungi and higher plants (Olila *et al.,* 2001). Many of the effective drugs such as anti-malarial, anti-cancer, anti-diabetic and antibiotics such as atropine and ergometrine compounds have been purified from medicinal plants (Olila *et al.,* 2001; Samie *et al.,* 2005). Medicinal plants are also a source of many active ingredients in the pharmaceutical industries (Maundu and Tengnas, 2005). Besides that, when the deadly disease, AIDS came up, the Ministry of Health called on the herbalists to work together with them in search of a treatment for the disease (Sindiga *et al.,* 1995). Approximately two thirds of HIV/AIDS patients in many developing countries seek symptomatic relief and manage opportunistic infections through the use of traditional medicine (WHO, 2011). Therefore, it is sensible to study plants from the local flora which are used for treatment of such infections (Atindehou *et al.,* 2002).

The use of medicinal plants for treatment is the most preferred form of traditional medicine and is highly lucrative in the international market (WHO, 2008). The annual sales from herbal medicine and other plant products in Western Europe totalled to US dollar 5 billion in 2003-2004, US dollar 14 billion in China in 2005 and US dollar 160 million in Brazil in 2007 (WHO, 2008). There is also a large demand for crudely prepared medicinal plant parts mainly in Europe and America which usually are distributed as over the counter medications (Matu and Staden, 2003). In Africa parts of

medicinal plants are sold at every market in urban and peri-urban centres (Rukangira, 2001). For instance in East Africa, the dried leaves of *Securidaca longepedunculata* is used for treatment of wounds and sores, coughs, venereal diseases and snake bites. In Malawi, the same plant is used for wounds, coughs, bilharzias, venereal diseases, snake bites and headaches. While in Nigeria it is used for treatment of skin diseases (Rukangira, 2001).

The medicinal value of plants used for traditional medicine lies in the chemical substances they contain to treat chronic and common bacterial infections (Duraipandiyan *et al.,* 2006; Kareru *et al.,* 2008). These compounds represent secondary metabolites that serve as defense agents against invading micro-organisms and predators (Ghdeib and Shtayeh 1999; Nwodo *et al.,* 2010). They also help in regulation of growth in plants (Nwodo *et al.,* 2010). The secondary metabolites include the alkaloids, steroids, tannins, phenol compounds, flavonoids and fatty acids (Ashokkumar *et al.,* 2010). The numbers of flowering plants occurring on earth are conservatively estimated at 250,000. About 6% of these have been screened for biological activity and 15% are reported to have been evaluated phytochemically. These figures depict the need for a study to uncover a probable abundance of medicinal extracts in these plants (Turker and Usta, 2008).

The rate at which the incidence of new and re-emerging infectious diseases is increasing is alarming. The development of resistance to the antibiotics that are in current clinical use is also a problem (Parekh and Chanda, 2008). It is estimated that more than 70% of the pathogenic bacteria are resistant to at least one of the antibiotics commonly used to

treat them (Okemo *et al.,* 2011). This calls for urgent and continued need to find new antimicrobial compounds with varied chemical structures and new mechanisms of action (Parekh and Chanda, 2007) and has necessitated the need to search for new antimicrobial substances from other sources including plants (Doughari, 2006; Duraipandiyan *et al.,* 2006).

Many studies concerning the use of medicinal plants in Kenya have been carried out targeting different communities (Maundu and Tengnas, 2005; Kokwaro, 2009) other studies include: medicinal plants from Bomet district (Cheruiyot, 2009), ethnobotany of the Samburu and Turkana (Bussman, 2006; Omwenga *et al.,* 2009) and traditional medicine among the Embu and Mbeere people (Kareru *et al.,* 20 07). However, the ethnobotany in Kisii, Rarieda and Mwingi Districts is scarcely known. Many medicinal plants are used by the herbalists from Kisii, Rarieda and Mwingi Districts but their efficacy has not been established.

The ethnobotanical studies that are reported in Eastern province have focused on Embu, Mbeere, Makueni and Machakos districts, leaving out Mwingi district which is interior and likely to have more intact traditional knowledge (Njoroge *et al.,* 2010). Therefore, more studies should be done on plants from Mwingi district. The biodiversity of the plants found in Kisii district offer great possibilities in the search for natural products with antimicrobial compounds. The people from Rarieda district also have a strong cultural belief in traditional medicine (Osewe, 2011).

1.2 Problem statement and justification

Infectious diseases have become a major health problem globally, accounting for over 50,000 deaths everyday (Doughari and Manzara, 2008). This has been brought about by the increasing prevalence of multi-drug resistant bacterial and fungal strains to the available conventional medicine (Parekh and Chanda, 2007; Doughari and Manzara, 2008). It is estimated that more than 70% of the pathogenic bacteria are resistant to at least one of the antibiotics commonly used to treat them (Okemo *et al.*, 2011). The problem is major and unless planned action is taken in the whole world, we have the risk of getting back to pre- antibiotic period where many children died of infectious diseases and major surgery was not possible because of risk of infection (WHO, 2005). Traditional medicine has fewer side effects, better patient tolerance, acceptance due to long history of use, renewable in nature, the most affordable and available means of medical treatment to the rural people (WHO, 2002; Joshi *et al.*, 2011; Shagal *et al.*, 2013). Conventional medicine are expensive and have side effects (Okemo *et al.,* 2011) such as toxicity, allergic reactions and disruption of normal bacterial flora which could be life threatening (Saify *et al.,* 2000; Uzoigwe and Agwa, 2011). It was estimated that 2.22 million hospitalized patients had serious Adverse Drug Reactions (ADR) and 106,000 died in a single year in the U.S.A (Joshi *et al.*, 2011; Issazadeh *et al.*, 2012).

In recognition of the role of traditional medicine in primary health care, the Kenya government, in the 1979-1983 development plan, called for research to evaluate the functions and determine the extent of usefulness of traditional medicine (NCAPD, 2005). A key goal of Kenyans vision 2030 is to supply adequate health care for all. This can be

achieved faster by mainstreaming traditional health care practices into the national health care systems (Kaingu *et al.*, 2011). According to WHO (2008), there is lack of scientific evidence to evaluate the safety and efficacy of traditional medicine. Therefore, there is need to screen medicinal plants for better understanding of their properties, safety and efficacy (Doughari *et al.,* 2008) and also to validate their traditional uses and identify the active compounds (Demet *et al.,* 2008).

Many plants have been used by various communities in Kenya in the treatment of bacterial and fungal infections but they have not been validated (Korir *et al.,* 2012a). Attempts have been made to gather information on traditional medicine and the people who use them (Maundu and Tengnas, 2005; Kokwaro, 2009). However, only a small number of plants have been identified and the efforts have not covered all the communities in Kenya. Few studies have been carried out on the medicinal plants from Kisii and Mwingi Districts by Mariita *et al.* (2010a and 2011). However no research has been done on MRSA, *B. subtilis*, *C. neoformans*, filamentous fungi and dermatophytes. In studies done by Mariita *et al.*, (2010a and 2011) stem barks were used but in this research, leaves were used. In addition to that the solvent used for extraction was different. Mariita *et al.* (2010a and 2011) used methanol for extraction. However in the current study, the leaves were extracted using a mixture of dichloromethane and methanol in the ratio of 1:1 according to (Midiwo, 2010).

According to Kokwaro, (2009) these plants have been documented to be used by different communities without testing their efficacy. The efficacy of medicinal plants used by

herbalists from Rarieda District had not been established. Such indigenous knowledge also needs to be documented before it is lost with the passing on of the older generation (Kareru *et al.,* 2007; Vaghasiya *et al.,* 2011). Traditional healers by their nature do not keep records and most of the knowledge they have is passed on verbally from generation to generation (Kaingu *et al.*, 2011). There is an increased loss of therapeutic plants as a result of anthropogenic activities (Rukangira *et al.,* 2001).

Claims by the traditional healers about the efficacy of herbal preparations have little validation and documentation yet they treat many people. It is therefore imperative that chemical and bioassay analysis is carried out to find out the efficacy of the medicinal plants. We are likely to lose most of the information because of inadequate documentation and communication of the local health traditions through oral narratives. It is vital therefore that the efficacy is validated and documented. This supports the main objective of this study to investigate crude extracts of the selected plants using *in vitro* models of relevance to validate and document scientific antifungal and antibacterial activities of these plants.

1.3 Research questions

i. Do the communities from Mwingi North, Kisii South and Rarieda Districts have information on the medicinal plants used in the treatment of selected bacterial and fungal diseases?

ii. Are the candidate plants active against the selected bacterial pathogens?

iii. Are the candidate plants active against the selected fungal pathogens?

iv. Do the plant extracts have phytochemical constituents that are responsible for antimicrobial activity?

1.4 Hypotheses

i. The communities from Mwingi North, Kisii South and Rarieda Districts do not have information on the medicinal plants used in the treatment of common bacterial and fungal diseases.

ii. The selected medicinal plants do not have activity against the selected bacterial pathogens.

iii. The selected medicinal plants do not have activity against the selected fungal pathogens.

iv. The phytochemical constituents present in the medicinal plants are not known.

1.5 Objectives

1.5.1 General objective

To determine the efficacy of some medicinal plants used by various communities in Kenya that treats the selected bacterial and fungal pathogen.

1.5.2 Specific objectives

i. To investigate the ethnobotany of some medicinal plants used by the communities from Mwingi North, Kisii South and Rarieda Districts.

ii. To determine the antibacterial activity of the plant crude extracts against standard strains *S. typhi*, *E. coli*, MRSA, *S. aureus* and *B. subtilis.*

iii. To determine the antifungal activity of the plant crude extracts against standard strains of *C. albicans*, *A. niger, T. mentagrophyte*, *C. neoformans* and *M. gypseum.*

iv. To determine the MICs, MBCs and MFCs of the crude extracts showing antimicrobial activity against the selected bacterial and fungal pathogens.

v. To determine the phytochemicals present in the crude extracts.

CHAPTER TWO

LITERATURE REVIEW

2.1 Antimicrobial agents

An antimicrobial agent is a secondary metabolite produced by bacteria that has inhibitory properties against microorganisms which includes antibiotics and synthetic compounds but with minimal effects on mammalian cells (Elliott *et al.,* 2007). The drugs used in the treatment of infectious diseases are divided into two groups; chemical antimicrobials which are synthetic chemical compounds and antibiotics which are produced by bacteria and fungi (Elliott *et al.,* 2007). The main mechanisms involved in the action of antimicrobial drugs against microorganisms include; interference with cell wall synthesis, disruption of cell membrane, inhibition of nucleic acid synthesis, inhibition of protein synthesis, inhibition of enzymatic activity (Bhatia, 2008), inhibition of metabolic pathways (Tenover, 2006) and inhibition of folate fatty acid (Hancock, 2007).

Currently, multiple drug resistance has been developed due to increased misuse of antibiotics in the treatment of bacterial infections (Agyare *et al*., 2006; Khan *et al*., 2011). Antimicrobial resistance is one of the world`s most serious public health problem (WHO, 2010). With the increase in resistant bacterial and fungal strains, there is a corresponding rise in demand for natural antimicrobial treatments (Assob *et al*., 2011) and herbal medicine could be a reservoir of new antimicrobial substances waiting to be discovered (Assob *et al*., 2011).

2.2 Antibacterial drugs

Antibiotics are organic substances produced by microorganisms and are capable of inhibiting the growth of another microorganism at low concentration (Chinedu, 2005). The overuse of antibiotics in the treatment of bacterial infections has led to increased incidences of resistant pathogens to the available antibiotics (Agyare *et al.*, 2006). Bacterial resistance can only be combated by the discovery of new drugs and herbal medicines are potential candidates for antimicrobial alternatives.

2.2.1 Penicillins

These antibiotics are based on a structure called the beta-lactam ring which prevents cell wall synthesis by binding to an enzyme that makes peptidoglycan (Bhatia, 2008). Benzylpenicillin (penicillin G) exhibits activity against staphylococci, streptococci, neisseriae, spirochaetes and other organisms (Greenwood *et al.*, 2002). Resistance to penicillin was developed due to a beta – lactamase enzyme (penicillinase) which is able to break the beta - lactam ring and destroy the antibiotics (Bhatia, 2008) or changes in penicillin-binding protein as occurs in MRSA (Cheesbrough, 2000). Resistance to penicillin is widespread in staphylococci and increasingly in others such as *Streptococcus pneumoniae* and *Neisseria gonorrhea* (Bhatia, 2008).

2.2.2 Aminoglycosides

These are a group of antibiotics consisting of amino sugars and aminocyclitol ring held together by glycoside bonds. Common examples of aminoglycosides are streptomycin, neomycin, apramycin, kanamycin and gentamicin (Trene ry, 2011). Gentamicin is an

antibiotic widely used for the treatment of bacterial infections (Poormoosavi *et al.,* 2010). They inhibit formation of the ribosomal initiation complex and also cause misreading of messenger RNA (Greenwood *et al.*, 2002). Aminoglycosides are very poorly absorbed when taken orally and for the treatment of systemic infections, they must be given parenterally (Turnidge, 2003). Aminoglycosides are obligate nephrotoxins and renal impairment is eventually detected in all patients if treated for long (Trenery, 2011). They are mainly reserved for the treatment of severe sepsis caused by coliforms and other Gram negative aerobic bacilli (Cheesbrough, 2000; Elliot *et al.*, 2007). They have antimicrobial activity on *S. aureus,* Streptococci and many Gram-negative species such as neisseriae, *Haemophilus* and coliforms (Elliot *et al.*, 2007).

2.2.3 Tetracyclines

Tetracycline is a major member of a group of antibiotics with a broad spectrum of activity which is widely used in medicine and veterinary science to treat bacteria infections (Ali *et al.,* 2010). They have a broad spectrum of activity against many Gram-negative and Gram positive bacteria, chlamydiae, mycoplasmae and rickettsiae (Elliott *et al.*, 2007). The tetracycline antibiotics inhibit binding of tRNA to ribosomes (Ali *et al.,* 2010). Resistance to tetracycline is common in *Heamophilus influenzae, Streptococcus pneumoniae* and *Streptococcus pyogenes* (Cheesbrough, 2000). The most commonly used tetracyclines are doxycycline and minocycline (Greenwood *et al.*, 2002). The extensive use of tetracycline in the therapy of human and animal infections is due to their antimicrobial properties and lack of major adverse side effects (Chopra and Roberts, 2001).

2.2.4 Quinolones

These drugs are synthesized from nalidixic acid and inhibit the action of DNA gyrase, the enzyme responsible for super coiling of DNA (Bhatia, 2008). Examples are: Nalidixic acid, which has antimicrobial activity on enterobacteriaceae but with no activity on Gram-positive organisms. Fluoroquinolones such as ciprofloxacin, ofloxacin and norfloxacin with activity against coliforms and other Gram-negative bacilli (Elliott *et al.*, 2007). Ciprofloxacin is commonly used to treat urinary tract infections but it has been associated with tendonitis and has the tendency of altering the normal flora in the colon and also promotes the growth of bacteria, responsible for the development of inflammation of the colon (Uzoigwe and Agwa, 2011).

2.2.5 Macrolides

Macrolides cover a family of different antibiotics produced by fungi of the genus *Streptomyces* and some bacteria such as *Arthrobacter* spp. (Kwiatkowska and Maslinska, 2012). Its mode of action is to inhibit bacterial protein synthesis at ribosomes by binding to 50s ribosomal subunit (Kwiatkowska and Maslinska, 2012). They are mainly used for the treatment of staphylococcal infections, respiratory infections and urethritis (Cheesbrough 2000). They are used as alternatives to people with penicillin hypersensitivity (Bhatia, 2008; Kwiatkowska and Maslinska, 2012). Resistance may occur with *S. aureus, S. pyogenes* and *S. pneumoniae* (Cheesbrough, 2000). Examples of macrolides are erythromycin, clarithromycin and azithromycin (Elliott *et al.*, 2007).

2.2.6 Sulphonamides

Sulphonamides are a group of drugs containing sulfur dioxide and nitrogen directly linked to a benzene ring as a basic structure (Chantachaeng *et al.*, 2011). They are divided into two main groups according to chemical structure and therapeutic actions; sulfonamide antibiotics such as sulfamethoxazole, sulfamethoxypyridazine and sulfonamide non antibiotics such as furosemide, hydrochlorothiazide and glipizide (Chantachaeng *et al.*, 2011). They are broad-spectrum antibacterial agents which exhibit antimicrobial activity on *Streptococci, Neisseriae* and *H. influenzae* (Elliott *et al.*, 2007) but resistance is common and the group is also toxic (Greenwood *et al.*, 2002). Sulphonamides were introduced in 1935 and about 10 years later, 20 % of clinical isolates of *Neisseria gonorrhea* had become resistant to them (Chinedu, 2005). Due to the emergence of resistance to fluoroquinolones in 2007 (CDC, 2011) CDC now recommends dual therapy for gonorrhea with cephalosporin, ceftriaxone 250mg plus either azithromycin or doxycycline (CDC, 2011).

2.3 Antifungal agents

Treatment with antifungal agents depend on site, level of infection, species involved, the efficacy, safety profile and kinetics of the available drugs (Galgoczy *et al.*, 2008). It is unfortunate that at the moment, only a few classes of antifungals are available and they lack potency or are toxic to human hosts (Lin, 2009; Sai *et al.*, 2012). Modern antifungal agents have become expensive; have side effects and fungal strains have developed resistance to the already available fungal drugs (Koroishi *et al.*, 2008; Zuzarte *et al.*, 2011). This has led to the reduction of the number of available and effective antifungal

agents thus necessitating the development of new antifungal drugs (Hamza *et al.*, 2006). These have inspired an extended search for new classes of antifungals and compounds that inhibit resistant mechanisms because of the spread of multidrug resistant strains of fungi and the toxicity during prolonged treatment with several antifungal drugs (Garcia *et al.,* 2003; Yigit *et al.*, 2008). The above mentioned reasons have made it important to search for newer drugs from plants for the treatment of opportunistic fungal infections. One approach can be testing of plants which are used customarily for their antifungal actions as possible sources of drug development (Abad *et al.*, 2007).

Antifungals can be grouped into three classes based on their site of action: first, azole which prevents the synthesis of ergosterol, for example fluconazoles which are active against *Candida* species. Intraconazole is however active against *Aspergillus* species and dermatophytes (Elliot *et al.*, 2007; Ghannoum *et al.*, 1999). The second are polyenes which interact with fungal membrane and interfere with its sterol content for example, nystatin which is used for the treatment of local infections of the vagina and skin and amphotericin B which is used to treat histoplasmosis and blastomycosis. Lastly, flucytosine (5-fluorocytosine) which has antimicrobial activity against most yeasts such as *Candida* species (not *C. krusei)* and *C. neoformans* (Elliott *et al.,* 2007).

Most of the clinically used antifungals have various disadvantages in terms of toxicity, efficacy and cost. Their frequent use has led to the emergence of resistant strains (Abad *et al.*, 2007). Selective action against fungi without toxic side effects is not easy to get and

useful antifungal agents are few (Elliott *et al.*, 2007). The currently available polyenes are amphotericin B and nystatin, although differing safety profiles have reduced nystatin to topical use (Thompson *et al.*, 2009). In addition to that, there is limited scientific evidence on efficacy of medicinal plants in treatment of fungal infections (Hamza *et al.*, 2006). Due to these short comings, the efficacy of eighteen medicinal plants against five strains of fungi was determined.

2.4 Medicinal plants

Herbal medicines are defined as any preparations containing one or more active herbal substances or herbal extractives (Agyare *et al.*, 2006). The medicinal plants are the plants whose parts (leaves, seeds, stems, roots, fruits and leaves) extract infusions, decoctions and powders are used in the treatment of different diseases of humans, plants and animals (Jamil *et al.,* 2007). About 20,000 plant species used for medicinal purposes are reported by WHO (Gullece *et al.,* 2006; Khan *et al.,* 2011). Plants have been used since antiquity as a good source of anti-infective agents and during recent years, the search for plants with antimicrobial activities has continued to gain importance (Assob *et al.,* 2011). Antimicrobial agents can easily be obtained from medicinal plants that act as their source (Kubmarawa *et al.,* 2007; Rangasamy *et al.,* 2007). Therefore, there is need to screen plants for their antimicrobial properties.

Currently about 30 % of the modern pharmacological drugs are gotten directly or indirectly from plants (Doughari and Manzara, 2008). Plants have provided a rich source of new drugs and cure for diseases (Adesina, 2005). Many such medicines including

strychnine, asprin, vincristine and taxol which are made from plants (Agyare *et al.,* 2006). The antimicrobial drugs such as quinine and artemisine are either made from plants or had plant derived chemical structures used as templates (Muregi *et al.,* 2003). Purgative drugs are usually a mixture of anthraquinone derivatives from cassava or related species. Other plant derived drugs include anti-ulcers drugs, carbenoxole from *Glycyrrhiza glabra* roots and the antimalarial drug quinine from Cinchona bark, just to mention but a few (Adesina, 2005).

According to WHO, traditional medicine is one of the ways to achieve total health care coverage of the world`s population (Rukangira, 2001). Medicinal plants are used for primary healthcare by about 70 % - 90 % of populations in developing countries (WHO, 2011). For instance, *Nauclea latifolia* smith (Rubiaceae) is used to manage venereal diseases and constipation in Sierra Leone (Agyare *et al.,* 2006). A root infusion of *N. latifolia* is used for the treatment of gonorrhea in Sudan. The roots are used as chewing sticks for toothache and dental caries (Agyare *et al.,* 2006). In Ghana, the roots and leaves are used for stomach complaints and for treating sores while in Nigeria, the fruits are sometimes used for the treatment of piles and dysentery (Agyare *et al.,* 2006). Medicinal herbs can even cure deadly diseases such as cancer and AIDS that have resisted conventional drugs (Jamil *et al.,* 2007).

Skin diseases, diarrhoea, malaria, respiratory infection, bacterial and fungal infections are the most common health problem in rural areas (Pirzada *et al.,* 2010). In resource limited countries, a lot of medicinal plants are used traditionally for treatment against these

diseases (Pirzada *et al.,* 2010). The use of herbal and other alternative therapies are attractive in many developing countries because of poor health care management systems and the high cost of developing a new pharmaceutical drug that is estimated averagely over US $ 800 million (Muhammad and Awaisu, 2008).

For many generations, naturally occurring substances and locally made herbs for medication of wounds, including circumcision wounds and chronic skin ulcers have been known (Mboto *et al.,* 2009). Drug inventions from ethnopharmacology and natural products are still an important expectation in uplifting the deprived livelihoods of rural communities (Nanyingi *et al.,* 2008). The antimicrobial activity exhibited by the extracts of medicinal plants has been supported by several researchers (Rangasamy *et al.,* 2007; Doughari *et al.,* 2008).

2.4.1 Medicinal plants in the world

In Africa, Asia and other developing worlds, medicinal plants provide a rich source of raw materials for traditional medicine, particularly in the villages (Tsegaye, 2006). In as much as conventional medicine is easily accessible, many Asian countries; China, India, Japan and Korea still rely on traditional medicine (WHO 2002; Ramamorthy, 2010). . During the period of 2000, legislation on traditional medicines was developed by Canada, Nigeria, Australia, Madagascar and United States of America (WHO, 2002). This was formed since over 50 % of the population in the poorest parts of Africa and Asia do not have access to essential drugs (Muhammad and Awaisu, 2008) therefore making

medicinal plants the only other alternative. Most of the western medicinal preparations have one or more ingredients of plant origin (Nahak and Sahu, 2010).

About 25,000 effective plant base formulations are used in modern medicine and known to rural communities all over India and about 10,000 designed formulations are available in the local medicinal texts (GOI, 2000). The WHO estimates that not less than 25 % of all contemporary medicines are obtained from medicinal plants either directly or indirectly using modern technology (WHO, 2011). In India each plant is of use for man and animal (Nahak and Sahu, 2010) therefore, medicinal plants constitute the principal health care resources and making the consumption of modern medicine the lowest in the world (GOI, 2000).

There is tremendous increase in demand, production and sale of traditional medicinal products in the last decade due to expanded global market (WHO, 2011). There is a large demand of crudely prepared medicinal plants mainly in Europe and America which usually distributes them as over-the-counter medications (Matu and Staden, 2003). Medicinal plants make a valuable source of foreign exchange for a lot of developing nations and the universal market for herbs and medicinal plants run into several billion dollars per year. Countries such as Bulgaria, Germany, Poland and others in Africa and Asia are renowned as main exporters of plant based medicinal products and raw materials for overseas medicinal plant industries (Muhammad and Awaisu, 2008).

2.4.2 Medicinal plants in Africa

Herbal medicine has been used in Africa for hundreds of years even before colonization. Currently it is still being used and about 80 % of the population relies on it for basic health care services (Okigbo and Mmeka, 2008). Even though colonialists discouraged the use of herbal medicine in Africa, it is still used (Orwa *et al.*, 2008). Localized treatment for different diseases have been used since time immemorial in rural Africa and herbal medicines possessing antimicrobial activity have significant advantages in the societies that greatly used them (Parker *et al.*, 2007).

The proportions of traditional healers to conventional doctors in relation to the population of the whole of Africa are a testimony. A good example is Ghana where for every traditional healer there are 224 people as compared to one conventional doctor for about 21,000 people. This is also manifested in Swaziland where the ratio is 110 people per traditional healer and 10,000 people per conventional doctor (Rukangira, 2001). This is partially attributed to the fact that some conventional medicines are not effective or unable to treat some new diseases such as HIV/AIDS or have side effects (Kareru *et al.*, 2007). In addition, plants form an integral part of life in many indigenous African communities as readily and cheaply available alternative to allopathic medicine (Wagete *et al.*, 2010).

A variety of naturally occurring products in Africa are exported to pharmaceutical companies in the West yearly in exchange for quick cash and partly due to lack of machinery to develop them locally (Adesina, 2005). Most of the countries in Africa rely

on medicinal plants. Malaysia is cited as among the countries with commendable work in natural products use that should be copied by other countries (Muhammad and Awaisu, 2008). They have shown increased numbers of good quality herbal products in their market as a result of collaborative work with the government, drug industries, and other stakeholders that laid a strong base for the development of this sector (Muhammad and Awaisu, 2008).

In Ethiopia, medicinal plants have been used to treat different diseases by the local people since antiquity (Yinerger and Yewhalaw, 2007). Herbal medicine in South Africa is highly esteemed in value and over 70 % of the populace heavily relies on traditional health practitioners in matters of health care (Samie *et al.*, 2005).

Over 400 plant species are approximated to be applied to the management of regular diseases in East Africa (Mariita *et al.*, 2010a). In Uganda, the ratio of traditional medical practitioners is between 1:200 and 1:400 in comparison to the allopathic practitioners, whose ratio is 1:20,000 or less. The distribution of personnel is however not directly proportional, with a greater percentage in urban setups rendering them inaccessible to the rural populace (WHO, 2002). Treatment of measles has been achieved by using medicinal plants (Olila *et al.*, 2001).

Over 60 % of the rural population in Tanzania depends on traditional medicine in solving their health care problems (Moshi *et al.*, 2009). Those residing in urban setups where many modern health care facilities are available still go to traditional healers since the use

of herbal medicine still has a strong cultural influence (Moshi *et al.*, 2006). It is approximated that there is between 30,000 – 40,000 traditional practitioners compared to about 600 medical doctors in Tanzania. In Malawi the situation is worse with about 17,000 traditional practitioners to only 35 medical doctors (Rukangira, 2001).

2.4.3 Medicinal plants in Kenya

There are over 10,000 species of indigenous flora in Kenya, of which about 1,200 are of medicinal value (UNIDO, 2004). Studies and further proofs have demonstrated that there is overwhelming application of herbal medicine in Kenya since the conventional drugs are expensive (Orwa *et al.,* 2008; Kitonde *et al.,* 2013). This has been documented by ethnobotanical surveys (Kareru *et al.,* 2008; Nanyingi *et al.,* 2008, Njoroge *et al.,* 2010; Mariita *et al.,* 2010b, Okemo *et al.,* 2011). In Kenya, traditional medicine is used in the treatment of diseases caused by bacteria and fungal pathogens (Kokwaro, 2009). Not only is the usage extended to rural areas but also in urban regions as demonstrated by increased activities of traditional practitioners all over the country (Orwa *et al.,* 2008). The demand for herbal products in Nairobi has seen shelves of large supermarkets, drugs stores and health shops packed with herbal teas made from leaves of *Artemisia, Ajuga* and *Prunus* among others (Dharani and Yenesew, 2010). There are a lot of inequalities in health care service provision and those who live in urban areas enjoy easy access to health facilities. About 70 % of urban population has health facilities within 4 km, unlike their rural counterparts at 30 % of population (PKF and IRN, 2005).

Although the Government of Kenya has expanded conventional health care in the last 10 years, it is still not readily accessible and many regions are still totally underserved. This has made most communities to continue using traditional medicine which is affordable and available (Bussman, 2006). In rural Kenya, traditional healers are preferred over conventional trained doctors. Studies have shown that for one conventional doctor there are about 8,500 people while for a traditional herbalist, there are less than 1,000 people to each herbalist (Dharani and Yenesew, 2010). Data from the Ministry of Gender, Sports, Culture and Social Services reveals that the number of traditional medical practitioners registering their commercial enterprises/herbal clinics (usually in the urban areas) is on the increase. Further, the number of patients treated in these herbal clinics is also on the increase, sometimes reaching well over 500 patients per month (Njoroge, 2012). Kenya has a research programme in herbal medicine; The Traditional medicine and Drug Research Centre of Kenya Medical Research Institute, Nairobi (UNIDO, 2004). In addition to that, the department of Chemistry of the University of Nairobi isolates compounds from herbal medicine and supplies them to the pharmaceutical companies such as Merck (USA) and Jansen (Belgium) to test them for biological activity and process drugs from them.

In Kenya, many communities rely on traditional medicine, for instance in Eastern Province, Embu and Mbeere districts are equipped with a diversity of native herbal medicine. The plants are used in treating several diseases by the local herbalists (Kareru *et al.,* 2008). The Kenyan pastoralists of Samburu origin have also long kept their knowledge on the use of local plants for a diversity of purposes (Nanyingi *et al.,* 2008).

The Samburu still depend on a diet of milk, blood from animals and soups made from natural herbs, berries and other wild fruits. This culture has made herbal knowledge to remain widespread in the community (Bussmann, 2006). The Nandi community also relies on medicinal plants to treat various human and livestock ailments (Jeruto *et al.*, 2008). Further, the Kambas are well known as one of the tribes in Kenya that have greatly preserved their knowledge of the use of local plants for medicinal purposes (Njoroge *et al.,* 2010). Among the Luos, medicinal plants are effective in managing certain cultural problems which may not respond to conventional practices like Chiira- a condition manifesting as a curse due to broken taboos among the Luo community (Personal communication Osewe, 2011). The Kipsigis community in Kenya uses many plants preparations in managing various ailments (Korir *et al.,* 2012a).

2. 4. 4 Plants under investigation

2.4.4.1 *Ficus sycomorus* Linn. (Moraceae)

It is traditionally used for treating stomach pains (Samie *et al.*, 2010). It is reported to have activity against *S. aureus, S. typhi* and MRSA (Olusesan *et al.*, 2010). It possesses anti-diarheal activity (Ahmadu *et al.*, 2007).

2.4.4.2 *Balanites aegyptica* L. Drel (Balantiaceae)

This plant has a long history of traditional uses for many diseases (Chothani and Vaghasiya, 2011). It is one of the most common plants which are neglected (Chothani and Vaghasiya, 2011). The boiled roots are used to treat malaria and stomach pains (Yitebitu, 2004). Its stem bark has a high antifungal activity against *C. albicans* (Maregesi *et al.*, 2008).

2.4.4.3 *Sesbania sesban* Linn. (Papilionaceae)

The fresh roots and leaves are used to treat scorpion stings, boils and abscesses (Yitebitu, 2004). The leaves of this plant have been studied and moderately inhibited the growth of *E. coli* and *A. niger* (Hossain *et al.*, 2007).

2.4.4.4 *Tamarindus indica* L. (Fabaceae)

It is used in many cultures for a wide range of applications (Caluwe *et al.*, 2010). Its fruits were found to be active against *A. niger* and *C. albicans* (Caluwe *et al.*, 2010). While stem bark demonstrated activity on *E. coli, S. typhi*, *S. aureus* and *B. subtilis* (Doughari, 2006). *Tamarindus indica* L. from Rarieda District has not been tested against *E. coli, S. typhi*, *S. aureus*, *B. subtilis, A. niger* and *C. albicans.*

2.4.4.5 *Albizia coriaria* Welw. ex Oliv (Fabaceae)

This plant is highly regarded in the Luo community (Maundu and Tengnas, 2005). The leaves and stem bark are used for the treatment of cough (Olila *et al.*, 2007; Namukube *et al.*, 2011). From previous studies, it was found to be active against *B. subtilis* and *E. coli* but had no activity on *S. aureus* (Olila *et al.*, 2007).

2.4.4.6 *Ormocarpum trichocarpum* Taub. Engl. (Fabaceae)

The roots are used to treat stroke paralysis, bilharzias and for bone setting (Moshi *et al.*, 2006). This plant has been studied on *Giardia lamblia* (Johns *et al.*, 1995). However, the antimicrobial properties of this plant on the tested microorganism have not been done.

2.4.4.7 *Senna didymobotrya* Fresen. (Caesalpinaceae)

The leaves of the plant are used in the management of STDs and back pain in Central Kenya (Njoroge and Bussman, 2009) and as a remedy for ringworm infection (Tsegaye, 2006). The plant extracts are used in the management of hypertension, microbial infections, hemorrhoids, sickle cell aneamia, inflammation of the fallopian tubes and fibroids (Geisler *et al.*, 2002; Kamatenesi, 2004). This plant has been tested against *Plasmodium falciparum* (Ramalhete *et al.*, 2008) and *Entomoeba histolytica* (Raini, 2011). Despite well spelt ethnomedical application, very little bioassay guided studies have been conducted on the extractives from this plant.

2.4.4.8 *Commiphora africana* (A. Rich) Eng.Syn (Burseraceae)

The stems are used as toothbrushes by Rendile and Kamba communities (Maundu and Tengnas, 2005). From previous studies, the roots showed activities against *S. aureus*, *E. coli* and *C. albicans* (Akor and Anjorin, 2009). It exhibited activity as an antihelminthic (Gbolade, 2008).

2.4.4.9 *Psiadia punctualata* (DC) Vatke (Compositeae)

Is used for relief of colds and abdominal pains (Kokwaro, 2009). It showed a significant anti-leishmanial activity (Githinji *et al.,* 2009). It mildly inhibited the coffee berry disease fungus though it did not show any mosquito larvicidal activity (Midiwo *et al.*, 2002). Antimicrobial properties on the tested microorganism in this study have not been determined.

2.4.4.10 *Rhus natalensis* Krauss (Anacardiaceae)

Traditionally, it is used in the treatment of coughs, colds, headache and back pain (Gathirwa *et al.*, 2011). The root extract is employed in the treatment of diarrhea by the Luo community (Personal communication, Osewe, 2011). It has been found to possess antiviral activity (Parker *et al.*, 2007).

2.4.4.11 *Ricinus communis* Linn (Euphorbiceae)

It is traditionally used against headache, rheumatism, colic and acute diarrhea (Kota and Manthiri, 2011). The leaf extracts are active against *B. subtilis*, *E. coli* and *S. aureus* (Kota and Manthiri, 2011). The seed extract showed moderate activity on *S. aureus* and *E. coli.* (Jumbo and Enenebeako, 2007).

2.4.4.12 *Zanthoxylum chalybeum* Engl. (Rutaceae)

It is used in the treatment of malaria and other infectious diseases (Olila *et al.*, 2001). The stem barks have shown moderate antibacterial activity while the leaf extracts showed no antibacterial activity (Matu and Staden, 2003). Though this plant has been used as human and veterinary medicine, there still remains a lot that is unknown and therefore more research should be carried out (Olila *et al.*, 2001).

2.4.4.13 *Bosia angustifolia* A. Rich (Capparaceae)

It is used in the treatment of bacterial diseases (Hassan *et al.*, 2006). Its roots have been found to be active against *S. aureus, E. coli* but inactive against *S. typhi* (Hassan *et al.*, 2006). It has shown a strong antibacterial activity against *S. aureus* (Mariita *et al.*, 2011).

According to Omwenga *et al.* (2009) this plant showed weak activity against *S. aureus* and *B. subtilis.*

2.4.4.14 *Melia volkensii* Gurke (Meliaceae)

Its leaf extracts have shown weak activity against *Vibrio cholerae* but were active against *E. coli* (Akanga, 2008). Ethanolic extracts of its fruit have been found to be lethal against Southern green stink bug (Mitchell *et al.*, 2004).

2.4.4.15 *Zanthoxylum gilletii* De Wild (Rutaceae)

It has been found to be inactive against *S. typhi*, *S. aureus*, *E. coli* and *C. albicans.* (Mariita *et al.*, 2010a). This plant is used in the treatment of genitourinary and rheumatic troubles, severe coughs, colds, healing of the womb in women after giving birth, chest pains, mouth ulcers and sore throats (Kokwaro, 2009; Tarus *et al.*, 2006).

2.4.4.16 *Fuerstia africana* T.C.E. Fries (Lamiaceae)

Traditionally, the species has been used as a galactagogue, female fertility agent and a treatment for tapeworms and urinary problems (Koch *et al.*, 2006). It has been found to be inactive against *S. typhi*, *E. coli* and *C. albicans* but showed moderate activity against *S. aureus* (Mariita *et al.*, 2010a). However, not much has been done on the antimicrobial properties of this plant.

2.4.4.17 *Urtica dioica* Linn (Urticaceae)

It has a long history of use as medicine (Uzun *et al.*, 2004). It is used for treating anaemia, enlarged prostate and childlessness. Its leaves have been found to be very active against *S. aureus* but it showed moderate inhibitory activity against *E. coli* and was inactive against *C. albicans* (Demet *et al.*, 2008).

2.4.4.18 *Vernonia amygdalina* Del. (Asteraceae)

It is used in the treatment of nausea, diabetes, loss of apetite, dysentery and other gastrointestinal tract problems (Nwanjo, 2005). It has been found to be active against *S. aureus*, *E. coli* and *C. albicans* (Okigbo and Mmeke, 2008). This is contrary to Mariita *et al.* (2010a) and Cheruiyot *et al.* (2009) who reported that this plant did not have the ability to inhibit *E. coli* and *C. albicans*.

2.5 Bacterial infections

Bacterial infection is on the increase all over the world (Oyewole *et al.,* 2010) and HIV/AIDS pandemic, poor hygiene, overcrowding and resistance to conventional antimicrobials among other factors have led to increased prevalence of bacterial infections (Wagate *et al.,* 2008). Infectious diseases mostly diarrhoea threaten lives of millions of people around the world (Samie *et al.*, 2005).

2.5.1 *Escherichia coli*

Escherichia coli belong to the large group of Gram negative bacteria referred to as enterobacteria. It causes urinary tract infections, infections of wounds, sepsis, meningitis

and diarhoeal diseases (Cheesbrough, 2000). Although *E. coli* is a normal flora in the gut and is naturally harmless, it may cause gastro-intestinal diseases ranging from mild, self-limiting diarrhea to haemorrhagic colitis (Greenwood *et al.,* 2002). Serotype 0157:H7 is enterotoxigenic and is the most important pathogen that causes diarrhea in infants, children and adults (Chen *et al.,* 2009). It is estimated that 1.5 million children below 5 years die from diarrhoea each year. In addition to that more than 80% of child deaths in Africa and Asia is because of diarrhea (UNICEF/WHO, 2009). In Kenya 27,400 children die from diarrhea annually (UNICEF/WHO, 2009; Ndongwe, 2011). A study conducted on surveillance of antimicrobial resistance at a tertiary hospital in Tanzania revealed that *E. coli* had a high resistance to several antimicrobial agents such as ampicillin, trimethoprim-sulfamethoxazole, ceftazidine, tetracycline and sulfonamide (Blomberg *et al.*, 2004).

2.5.2 *Salmonella typhi*

Salmonella typhi is a Gram negative bacteria that causes enteric fever (typhoid and paratyphoid) and bacteraemia (Cheesbrough, 2000). The genus *Salmonella* has three species; *Salmonella typhi* and *Salmonella paratyphi* which cause enteric fever and *Salmonella enteritidis* which cause enteritis (Elliot *et al.*, 2007). Conventional antisalmonella drugs are becoming more and more unavailable to the common man in Africa due to increased cost (Gatsing and Adoga, 2007). Moreover, high morbidity and mortality caused by typhoid fever is increasing with the emergence and worldwide spread of *S. typhi* resistance to the most commonly used antibiotics (WHO, 2007). Typhoid fever is a public health problem in developing countries (Gatsing and Adoga, 2007). In 2000, it

is estimated that over 2.16 million episodes of typhoid occurred worldwide, resulting in 216,000 deaths (Bhuta *et al.,* 1999; Ochiai *et al.,* 2008). Typhoid is a common and serious disease amongst children and adults in Kenya (Mweu and English, 2008).

Antibiotic resistant strains of *S. typhi* are increasing rapidly, including multidrug resistant strains and those less sensitive to ciprofloxacin (Roeck, 2007) and are also resistant to chloramphenicol and cotrimoxazole, the antibiotics commonly prescribed for the treatment of typhoid fever (Oluyege *et al.,* 2009). The emergence and spread of drug resistance have challenged treatment options for typhoid in many countries (Chalya *et al.*, 2012).

2.5.3 *Staphylococcus aureus*

They are Gram positive bacteria which form clusters when grown on solid media. The most important is *S. aureus* which causes superficial infections and systemic infections such as endocarditis inflammation of bone or bone marrow (Parry *et a*l., 2004; Deurenberg and Stobberingh, 2008; Haque *et al.*, 2011). *S. aureus* is an opportunistic pathogen and a leading cause of hospital acquired infections (Klein, 2007; Haque *et al.*, 2011) and may also cause more serious infections such as ulcers, burns, pneumonia, osteomyelitis, septicaemia, mastitis, meningitis, food poisoning and toxic skin exfoliation (Cheesbrough, 2000). *S. aureus* is carried in the nose of 40% or more of healthy people, in feaces in about 20% of the people and skin carriage in 5-10% of the population (Cheesbrough, 2000; Elliot *et al.,* 2007).

Staphylococcus aureus has been successful in developing resistance to anti-microbial agents. Beta-lactamase producing strains of *S. aureus* emerged immediately after penicillin was introduced and now almost all isolates of *S. aureus* in hospitals are resistant to penicillin (Klein, 2007; Bhatia, 2008). A study done by Daini and Akano (2009) found out that 62% of *S. aureus* isolates had multiple resistance to the commonly used antimicrobial agents. It has a remarkable genetic diversity and can acquire new exogenous genes, which allow it to adapt to various changing environmental conditions to modulate its pathogenicity (Moellering, 2010). Infections caused by *S. aureus* are increasingly becoming difficult to control because the organism has acquired new antimicrobial resistance determinants (Jumbo and Enenebeaku, 2007; Oyetunji *et al.,* 2012). This necessitates a continuous search for alternative medicine, preferably from plants since plants are less toxic, readily available and cheap.

2.5.4 Methicillin resistant *Staphylococcus aureus*

These are strains of *S. aureus* which are resistant to the antibiotic methicillin. Many strains of MRSA are now resistant to multiple antibiotics (Elliot *et al.,* 2007; Oyetunji *et al.,* 2012) such as β-lactam agents, aminoglycosides and fluoroquinolones (Greenwood *et al.,* 2002). Infections caused by MRSA have become a major problem worldwide (Cuny *et al.,* 2010) and the risk of transmission and diseases caused by MRSA are greatest amongst hospital patients (Bhatia, 2008). MRSA have emerged as the predominant cause of infections in debilitated patients. For instance those in intensive care units, where the combinations of many antibiotics and the use of invasive devices contribute greatly to the risk of acquisition (Greenwood *et al.,* 2002). MRSA can cause sepsis ranging from world

infections to urinary tract infections and septicaemia (Elliot *et al.,* 2007). The measures taken to control MRSA in hospitals may be different depending on its prevalence and the particular clinical areas affected (Greenwood *et al.,* 2002). Vancomycin is however used for the management of MRSA infections (Cheesbrough, 2000).

2.5.5 *Bacillus subtilis*

The genus *Bacillus* are Gram positive, rod-shaped bacteria that form spores (Hong *et al.,* 2009) *Bacillus subtilis* and *Bacillus pumilis* have been implicated in causing food poisoning similar to that due to *Bacillus cereus* (Greenwood, 2007). They do not form toxins but some strains produce antibacterial peptides such as the antibiotic bacitracin, which could cause growth in the internal tract (Greenwood, 2007). Increase in out breaks of food-borne diseases has brought up concerns over pathogenic and spoilage micro-organisms in foods (Rahman and Kang, 2009). *B. subtilis* sometimes causes disease in immunocompromised humans such as meningitis, endocarditis, endophthalmitis, conjunctivitis or acute gastroenteritis (Bonjar and Farrokhi, 2004). *B. cereus* and *B. subtilis* may be found in wounds and tissues of immunocompromised or burned patients (Greenwood, 2007).

2.6 Fungal infections

Over the past few decades, fungal infections have increased rapidly (Yigit *et al.,* 2008) and this has been brought about by increased prevalence of immunocompromised populations such as organ transplant recipients, cancer and HIV/AIDS patients (Garcia *et al.,* 2003; Abad *et al.,* 2007). Fungal infection is one of the greatest killers of the

immunocompromised populations (Moyes and Naglik, 2011). Available literature indicates that up to 90% of all HIV patients suffer from fungal infections at some point during the course of the disease, of which 10-20% dies as a result of fungal infections (Runyoro *et al.,* 2006; Samie *et al.,* 2010).

Many plants have been used by herbalists and elders in the treatment of several fungal infections (Hamza *et al.*, 2010). The plants which are traditionally used should be tested for their fungal activities and drug developed from them since the fungal strains have developed resistance to antifungal drugs which are also expensive (Abad *et al.,* 2007; Koroishi *et al.,* 2008).

2.6.1 Dermatophytes

These are a group of related filamentous fungi, also referred to as ringworm fungi, which cause infection of the skin, hair and nails. They cause athlete`s foot (*Tinea pedis*), ringworm (*Tinea capitis)* and jock itch (*Tinea cruris)* (Greenwood *et al.,* 2002; Koroishi *et al.,* 2008). They are mainly caused by three genera of fungi namely T*richophyton*, *Microsporum* and *Epidermotophyton* (Greenwood *et al.*, 2002; Elliott *et al.*, 2007).

The dermatophytes infect the hair, skin and nails due to their ability to obtain nutrients from keratin for growth. Immunocompromised patients can be infected with more severe and extensive lesions (Galgoczy *et al.*, 2008). Dermatophytes cause a high morbidity in patients undergoing organ transplants or suffering from AIDS (Brandao *et al.*, 2010). Dermatophytosis is a trivial disease but has a lot of psychological effect and a costly

disease in terms of treatment (Sai *et al.*, 2012). Topical treatment with clotrimazole and terbinafine are commonly used for localized lesions. In widespread infections of *Tinea unguium* and scalp ringworm, complete antifungal treatment with oral drugs such as itraconazole, terbinafine and griseofulvin are necessary for systemic therapy (Galcoczy *et al.*, 2008, Koroishi *et al.*, 2008).

2.6.2 *Candida albicans*

It occurs in the mouths of up to 80% of healthy individuals and is mostly associated with normal oral flora (Williams *et al.,* 2011). *Candida* are the most common fungal micro-organism of humans and the causal agents of oral, gastrointestinal and vaginal candidiasis, causing severe morbidity in millions of persons worldwide (Lazzell *et al.,* 2009; Moyes and Naglik, 2011). Clinically significant risk factors for individuals to acquire *Candida* infections include: compromised immunity, over use of antibiotics, use of central venous catheters, prolonged hospital stay and tumors (Fleming *et al.,* 2011). For instance oral candidiasis mostly occurs after over using a large variety of antibiotics which facilitates later growth of *Candida* through lessening competitive pressure by lowering the oral bacterial population (Williams *et al.,* 2011).

Candida are the third leading cause of central venous catheters related infections with the second highest colonization to infection rate and the overall highest crude mortality (Lazzell *et al.,* 2009). *Candida vaginitis* is a common infection during pregnancy (Cheesbrough, 2000). Vaginal candidiasis alone affects about 75% of women at least once during their reproductive age which is about 30 million infection episodes annually (Moyes and Naglik, 2011). *Candida* infection are also the most common oral symptoms

of HIV infection, with half of HIV positive patients and 90% AIDS patients suffering from oral candidiasis (Moyes and Naglik, 2011). Oral candidiasis is the first and most frequent fungal infection in HIV patients (Hamza *et al.,* 2006; Runyoro *et al.,* 2006).

Candida albicans is already resistant to the limited toxic and expensive anticandida drugs available in the market (Runyoro *et al.,* 2006). Topical antifungal agents such as nystatin, miconazole, fluconazole, intraconazole and amphotericin B, are commonly used in treating oral candidiasis. However, a number of challenges such as a limited number of effective antifungal agents affect the management of *Candida* infections (Runyoro *et al.,* 2006).

2.6.3 *Cryptococcus neoformans*

Cryptococcus neoformans is responsible for about 1 million infections annually and over 600,000 deaths worldwide (Mansour *et al.,* 2011). The infection is transmitted by inhalation of the fungus from soil which is contaminated with bird droppings (Elliott *et al.,* 2007). It usually affects the lungs, brain and meninges and occasionally other parts of the body (Cheesbrough, 2000). Those at highest risk of developing cryptococcal infections are individuals with AIDS or lymphoma or recipients of chronic immunosuppressive medication (Barchiesi *et al.,* 2000; Mansour *et al.,* 2011).

Candida albicans and *C. neoformans* are the most common opportunistic infections in HIV/AIDS patients (Samie *et al.,* 2010). It causes meningoencephalitis mainly in immunocompromised host which is the most distressing symptom of cryptococcal

disease and is fatal unless treated (Florio *et al.,* 2011). In resource limited settings, cryptococcal meningitis can cause up to 30% mortality in AIDS patients (Lin, 2009). A report by CDC found cryptococcal meningitis to have killed about 624,000 people annually (Lin, 2009). Amphotericin B and triazoles are consecutively used to treat cryptococcal infections (Barchiesi *et al.,* 2000).

2.6.4 *Aspergillus niger*

Aspergillus spp. have been found to be the cause of food borne diseases or food spoilage (Pirbalouti *et al.,* 2010). One of the most important problems facing the food industries is food spoilage, infact developing and developed counties also find food-borne illnesses as a global problem (Rahman and Kang, 2009). Food –borne micro-organisms like *A. niger* exist freely in nature causing a lot of deaths and sickness in the population and can also be very dangerous agent to man as it can cause opportunistic infections (Sumathy *et al.,* 2010). For example in HIV-infected individuals, aspergillosis is caused by *A. flavus*, *A. niger* and *A. fumigates* (Runyoro *et al.,* 2006).

2.7 Phytochemicals in medicinal plants

Phytoconstituents are the natural bioactive compounds found in plants. They work with nutrients and fibres to form an integrated part of defense system against various diseases and stress conditions (Koche *et al.,* 2010). Phytochemicals are majorly divided into primary and secondary groups according to their activity in plants metabolism. Primary groups contain common sugar, amino acids, proteins and chlorophyll. While secondary groups are made of alkaloids, flavonoids, saponins, tannins, phenolic compounds and

many others (Khan *et al.*, 2011). Secondary metabolites of plants act as defense mechanisms against infestation by many microorganisms, insects and herbivores (Koche *et al.,* 2010). The medicinal value of plants depend on the bioactive phytochemical constituents which are found in parts of the plants. They give a definite physiological action on human body (Chletri *et al.*, 2008; Koche *et al.,* 2010; Sule *et al.*, 2011).

The most important of these bioactive compounds of plants are alkaloids, tannins and phenolic compounds (Chletri *et al.,* 2008). Phytochemical screening of plants has shown the presence of various chemicals such as alkaloids, tannins, flavonoids, steroids, glycosides and saponins (Koche *et al.,* 2010) which are well known for their laxative and pharmacological effects on humans and animals (Sule *et al.*, 2011).

2.7.1 Tannins

Tannins are heterogeneous groups of high molecular weight phenolic compounds with the capacity to form reversible and irreversible complexes with protein, polysaccharides (cellulose, hemicelluloses, pectin) alkaloids and nucleic acids (Frutos *et al.*, 2004). Tannins are generally more common in parts of the plant that are considered precious to it, for example new leaves and flowers which have high chances of being eaten by herbivores (Frutos *et al.*, 2004). Tannin content of plants is increased by high temperature, poor soil quality, water stress and extreme light intesities (Frutos *et al.*, 2004). Tannin is known for many physiological activities such as stimulation of phagocytic cells, host mediated tumor activity and various anti-infective actions (Cowan, 1999).

2.7.2 Saponins

Saponins are glycosides that are found commonly in plants. They are in large quantities in many foods eaten by animals and man (Soetan *et al.*, 2006). Saponin serve as lytic agent because it has detergent properties and are well known to show anti-inflammatory properties, while alkaloids and glycosides act as defense mechanism of the plant (Shital *et al.*, 2010). Saponins have been used commercially for various functions such as medicine, detergents, adjuvants and cosmetics (Eskander *et al.*, 2006). A lot of pharmacological activities such as antibiotic, antifungal, antiviral, hepatoprotective, anti-inflammatory and antiulcer have been reported on saponins (Soetan *et al.*, 2006).

2.7.3 Flavonoids

Flavonoids are a large family of polyphenolic components produced by plants. Studies have shown that flavonoids help reduce blood lipid and glucose and enhance human immunity (Ghasemzadeh *et al.,* 2011). They also have various biological activities such as antibacterial, antidiabetic, antiallergic, antiviral, antinflammatory, antimutagenic, anticarcinogenic and are insecticidal (Orhan *et al.,* 2010). Plants synthesise flavonoids as they respond to microbial infection (Cowan, 1999; Bii *et al.*, 2010).

2.7.4 Alkaloids

Alkaloid is derived from the Arabic word al-qali, which means the plant from which soda was first obtained (Kutchan, 1995). Alkaloids are one of the largest groups of phytochemicals in plants and are known to be toxic against cells of foreign organisms (Issazadeh *et al.*, 2012). Majority of natural products are physiologically active in mammals. Among the monoterpenoid indole alkaloid pharmaceuticals that are still

commercially isolated from plant material are the antimalarial drug quinine, the antineoplastic drug camptothecin, the rat poison and homeopathic drug strychnine and the antineoplastic chemotherapeutic agents, vincristine and vinblastine (Kutchan, 1995). Alkaloids have shown antimicrobial activity against *Giardia* and *entomoeba* species. They also possess antidiarrheal effect (Cowan, 1999).

2.7.5 Terpenoids

Terpenoids are synthesized from acetate units and they share their origin with fatty acids. Examples of common terpenoids are methanol and camphor (monoterpenes), farnesol and artemisin (sesquiterpenoids). They are known to be active against fungi, bacteria, protozoa and viruses (Cowan, 1999).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Ethnobotanical survey

A survey was carried out in Katse (Mwingi North) Iyabe and Kerina (Kisii South), Lweya and Ragengni Divisions in Rarieda Districts, Kenya (Figure 1) on the major medicinal plants used by communities inthe treatment of bacterial and fungal diseases. Selection of the plants was based on available ethnobotanical information from the herbalists consulted as well as available literature. In order for the survey to obtain indigenous knowledge on plants used, interviews were conducted using a semi-structured questionnaire (Appendix 2). Informants who are knowledgeable practitioners (herbalists) were identified with the help of local people and the local administration and selected as respondents to the survey (Yinerger and Yewhalaw, 2007).

Before carrying out the interview however, a written consent was sought from every informant (Kasolo *et al.*, 2010) appendix 1. Oral interviews were held in their homes. In the survey, the respondents were asked for the local names of the plants they used as medicine, parts used, target diseases, mode of preparation, administration of the resulting preparations and the plants availability.

Species choice value model was preferred because it is a 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 (Yinerger and Yewhalaw, 2007). Therefore; each prescription was considered authentic only after confirmation from three informants.

3.2 Collection and authentication of plant material

In this study, eighteen different medicinal plants which include; *Zanthoxylum chalybeum* Engl, *Boscia angustifolia* A. Rich, *Melia volkensii* Gurke, *Zanthoxylum gilletii* De Wild, *Fuerstia africana* T. C. E Fries (Figure 5), *Urtica dioica* Linn (Figure 6),, *Vernonia amygdalina* Del, *Ricinus communis* Linn (Figure 3), *Commiphora africana* (A. Rich) Eng. Syn, *Psidia puntulata* (DC) Vatke, *Senna didymobotrya* Fresen (Figure 7), *Ormocarpum trichocarpum* Taub. Engl, *Sesbania sesban* Linn (Figure 4), *Balanites aegyptiaca* L. Drel, *Albizia coriaria* Welw. ex Oliv, *Ficus sycomorus* Linn, *Rhus natalensis* Krauss and *Tamarindus indica* L. were collected from Kisii, Rarieda and Mwingi Counties. The plants collected were those for treatment of gastroenteritis, respiratory system illnesses as well as skin conditions. Fresh parts of the plant like the roots, leaves and barks were collected depending on the most commonly used by the traditional healers. Photographs of the selected plant species were taken. Plant identification was carried out by a plant taxonomist from Botany Department, Chiromo, University of Nairobi, Kenya. Voucher specimens collected were prepared and deposited at the University of Nairobi herbarium.

Figure 1: Map of Kenya showing Kisii, Bondo and Mwingi Districts.

3.3 Preparation of plant materials

The freshly collected parts of the plant were washed thoroughly with running tap water, chopped into smaller pieces and then dried under shade at room temperature for a period of two weeks until they were completely dry (Matu *et al.*, 2012). The small pieces were ground into powder using Wiley mill, (model no. 2, USA) at Botany Department, Chiromo University of Nairobi.

3.4 Extraction

The extraction was carried out at the Chemistry Department Chiromo, University of Nairobi. One hundred and fifty grams of each dry powder was mixed in a conical flask with 1000 ml of methanol and dichlomethane (1:1) mixture and left over night (Kitonde *et al.*, 2013; Midiwo, 2010). The extracts were then filtered through a whatmann filter paper No. 1.

3.5 Concentration into solid sample

Dry organic crude extracts were obtained after evaporating dichloromethane and methanol using a rotary evaporator (Buchii B-205 Switzerland) in a water bath set at a temperature of 40 °C (Omwenga *et al.*, 2009). The paste was collected into small vials which were then placed in an evaquated decicator filled with anhydrous copper sulphate to obtain dry powdered samples. All samples were stored in the refrigerator at a temperature of 4 °C if not bio-assayed immediately.

3.6 Microbial test organisms

A selection of microbial test organisms was based on the ethnobotanical information collected on the target disease, their significance as opportunistic pathogens and their resistance to conventional drugs. The chosen microorganisms also cause infectious diseases which are common to man and are easily transmitted. The standard reference microbial organisms and clinical isolates were obtained from the centre for Microbiology Research – KEMRI, Nairobi, Kenya. Standard reference microbial organisms, environmental microorganisms and clinical isolates used in this study were:

3.6.1 Bacterial isolates

Gram positive

- *Staphylococcus aureus* - ATCC 25923
- Methiciline resistant *Staphylococcus aureus* (MRSA) - Clinical isolates
- *Bacillus subtilis* – Environmental organism

Gram negative

Escherichia coli – ATCC 25922

Salmonella typhi -ATCC 19430

3.6.2 Fungal isolates

Yeast

Candida albicans – ATCC 90028

Cryptococcus neoformans – ATCC 18310

Dermatophytes

Microsporum gypseum – Clinical isolates

Trychophyton mentagrophytes – Clinical isolates

Filamentous fungi

Aspergillus niger – Environmental organism

3.7 Maintenance of microbial stock cultures

Stocked bacterial strains were subcultured on Muller Hinton agar no. CMO337. (Oxoid Ltd, Basingstoke, Hampshire, England). Incubation was carried out at a temperature of 37 °C for 24 h to obtain freshly growing strains (Omwenga *et al.*, 2009). Yeasts and molds were subculcultured onto Sabaraud Dextrose Agar No. CM 004 (Oxoid ltd Basingstoke, Hampshire, England). The Yeasts were incubated at a temperature of 37 °C for 24 h (Cruz *et al.,* 2007), filamentous fungus at 28 °C for 48 h in humid chamber (Costa *et al.*, 2010) and the dermatophytes at a temperature of 25 °C for 72 h (Korir *et al.*, 2012b). Both the bacterial and fungal strains were maintained at a temperature of 4 °C.

3.8 Antibacterial assays

The antibacterial bioassay was performed by Kirby Bauer disk diffusion method (Omori *et al.*, 2012). Muller Hinton agar no. CMO337 (Oxoid Ltd, Basingstoke, Hampshire, England) was prepared according to the manufacturers instructions for the purposes of culturing bacteria. Normal saline solution was used to dilute fresh 24 h culture of bacterial type cultures or clinical isolates to attain a 0.5 McFarland Standard which gives an equivalent approximate density of 1×10^8 of bacteria (Kitonde *et al.*, 2013). Spread

plate method was used to culture 100 µl of the bacterial suspension that was introduced into the petri dishes. Eighteen dry sterile discs (6 mm diameter) were soaked in 100 µl plant extract (made by dissolving 300 mg of each extract in 1000 µl (1ml) of DMSO). The discs were air dried and placed aseptically onto the inoculated plates at a distance of 3mm apart. Discs impregnated with DMSO and then air dried were used as negative controls while commercially available discs of chloramphenical were used as positive control for bacteria. Incubation was carried out at a temperature of 37 °C for 24 h. All tests were performed in triplicate. After incubation, bacterial growth inhibition was determined by measuring the diameter zones in millimeters using a transparent ruler and recorded against the corresponding plant extracts (Omwenga *et al.*, 2009; Mariita *et al.*, 2010a).

3.9 Antifungal assays

To determine the antifungal activities of the plant extracts against fungal strains, Sabourand Dextrose Agar no. CM OO4 (Oxoid Ltd, Basingstoke, Hampshire, England) was prepared using manufacturer's instructions for the purpose of culturing fungi. Normal saline solution was used to dilute fresh 24 h culture of fungal type cultures or clinical isolates to attain a 0.5 McFarland Standard. Spread plate method was used to culture 100 µl of the fungal suspension that was introduced into the petri dishes. Eighteen dry sterile discs (6 mm diameter) were soaked in 100 µl plant extract (made by dissolving 300 mg of each extract in 1000 µl (1 ml) of DMSO). The discs were air dried and placed aseptically into the inoculated plates at a distance of 3 mm apart. Discs impregnated with DMSO and then air dried were used as negative controls while

commercially available discs of miconazole were used as positive control (Bii *et al.*, 2010). The inocula were incubated at respective conditions where the yeast cultures were incubated at a temperature of 37 °C for 24 h (Cruz *et al.,* 2007) filamentous fungi at a temperature of 28 °C for 48 h in humid chambers (Costa *et al.*, 2010) and dermatophytes at a temperature of 25 °C for 72 h in humid chambers (Korir *et al.*, 2012b). All tests were performed in triplicate. After incubation, fungal growth inhibition was determined by measuring the diameter zones in millimeters using a transparent ruler and recorded against the corresponding plant extracts (Matu *et al* ., 2012).

3.10 Determination of minimum inhibitory concentration

Plant extracts were made by dissolving 300 mg of each crude extract in 1000 µl (1ml) of DMSO. Broth micro dilution method was used to determine minimum inhibitory concentration for the active crude extracts against the test microorganisms according to the National Committee for Clinical Laboratory Standards (NCCLS) now Clinical Standard Institute (CLSI) (Korir *et al.*, 2012b).

The tests were performed in 96 well micro-titer plates. Serial doubling dilutions were carried out so that the concentration in the next well was half of the concentration in the previous well. The MIC was determined only where the plant extract showed strong antimicrobial activity (≥ 9 mm) by the disc diffusion method (Mariita *et al.*, 2010a). The wells were filled with 50 µl of the Muller Hinton broth for bacterial strains and Sabourand Dextrose broth for fungi. Then, 50 µl of the plant extract (made by dissolving 300 mg of each extract in 1000 µl (1ml) of DMSO for complete dissolution) were

dispensed into the first well before serial dilutions. The serial dilutions were achieved by transferring 50 µl of Muller Hinton broth or Sabourand Dextrose broth containing the extract from the first well through the second, third and fourth wells. Then 50 µl of the test isolates were dispensed into each well. One row of wells was used as negative control of the growth of the microorganisms in the medium, whereas 50 µl of the antibiotic (Chloramphenical/miconazole) were used as a positive control. Micro titre plates were covered. Bacteria and yeasts were incubated at 37 °C for 24 h (Kitonde *et al.*, 2013) filamentous fungi at 28 °C for 48 h in humid chambers (Costa *et al.,* 2010) and dermatophytes at 25 °C for 72 h in humid chambers (Korir *et al.,* 2012b). Minimum inhibitory Concentrations (MIC) were determined by recording the lowest concentration of the active extracts that inhibited growth of micro-organisms as compared to the control broth turbidity (Wagete *et al.*, 2010; Kitonde *et al.*, 2013).

3.11 Determination of Minimum Bactericidal / Fungicidal Concentrations (MBCs/ MFCs)

The wells where there were no growth (not turbid) from MIC results, the bacteria were subcultured on Mueller Hinton Agar and fungi on Sabourand Dextrose Agar. The bacterial and yeast cultures were incubated at 37 °C for 24 h and the dermatophytes at a temperature of 25 °C for 72 h (Korir *et al.*, 2012b). The lowest concentration of the plant extracts that did not yield any colony on the solid medium after sub culturing and incubating for 24 h for bacteria was taken as the MBC and 72 h was taken as the MFC for dermatophytes and 24 h for yeasts. All tests were performed in triplicate (Samie *et al.*, 2010; Omori *et al.*, 2012).

3.12 Phytochemical screening

3.12.1 Test for alkaloids

Two hundred milligrams of plant extract were dissolved in 10 ml methanol and filtered, 1 ml of the filtrate was mixed with 6 drops of Wagner`s reagent (made by mixing 1.27 g iodine and 2 g potassium iodide in 100 ml of water). A creamish/ brownish-red/orange precipitate indicated the presence of alkaloids. A low (+) concentration was recorded if the addition of the reagent produced a faint turbidity, a moderate (++) concentration was recorded if a light opalescence precipitate was observed and a high (+++) concentration was recorded if a strong yellowish-white precipitate was observed (Omwenga *et al.*, 2009; Mariita *et al.,* 2011).

3.12.2 Cardiac glycosides

Five milligrams of the filtrate was treated with 2 ml of glacial acetic acid containing two drops of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. Green-blue colour indicated the presence of cardiac glycosides. A (+) reaction was recorded when a faint green-blue colour was observed (indicating low concentrations of detectable cardiac glycosides); A (++) reaction was recorded when a medium green-blue colour was observed (indicating moderate concentrations of detectable cardiac glycosides) and A (+++) reaction was recorded when a deep green-blue colour was observed indicating high concentrations of detectable cardiac glycosides (Edeoga *et al.*, 2005; Omwenga *et al.*, 2009; Mariita *et al.*, 2011).

3.12.3 Test for saponins

Zero point five milligrams of extract was added to a 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. Low (+) saponins concentration was recorded when the froth reached a height of 50 mm, medium (++) for the froth between 60-100 mm while high (+++) was represented by more than 100 mm.

3.12.4 Test for tannins

Zero point five milligrams of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for brownish green or a blue black colouration. Heavy precipitate indicated a high concentration of tannins (+++), medium precipitate (++) for medium concentration while a slight precipitate indicated a low concentration (+) (Mariita *et al.*, 2011).

3.12.5 Flavonoids

Five milliliters of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated sulphuric acid. The yellow colouration which disappeared steadily indicated the presence of flavanoids. By observing a strong yellow colouration a high (+++) concentration was recorded, moderate yellow indicated moderate (++) concentration while a pale yellow colour indicated a low (+) concentration of flavanoids (Edeoga *et al.*, 2005; Omwenga *et al.*, 2009; Mariita *et al.*, 2011).

3.12.6 Test for terpenoids

Zero point five milligrams each of the extract was added to 2 ml of chloroform and 3 ml of concentrated sulphuric acid was then added carefully to form a layer. The presence of terpenoids was indicated by the formation of a reddish brown colouration of the interface.

3.13 Statistical analysis

Minitab Statistical Software 13.20, 2000 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 mean standard deviation means. One way ANOVA at 95% CI was used in testing the significance among the groups. A probability value of ≤ 0.05 was considered significant as Tukey's test was used in analyzing significant differences among group means (Mariita *et al.*, 2010a).

CHAPTER FOUR

RESULTS

4.1 Ethnobotanical survey

The results of ethnobotanical survey are presented in Table 1, in which the plants are arranged in alphabetic synopsis according to families. The local names, parts used, drug preparation methods, diseases treated and the areas where the plants were collected from are also presented in table 1.

In this study a total of 18 species distributed in 15 families were identified. The family reported with the highest number of medicinal plant species was Fabaceae (3 species), this was followed by Rutaceae (2 species). Capparidaceae, Meliaceae, Lamiaceae, Urticaceae, Asteracea, Moraceae, Balantiaceae, Papilionaceae, Caesalpinaceae, Burseraceae, Compositae, Anacardiaceae and Euphobiaceae had one species each.

Eleven plants were collected from Lweya and Ragengni (Rarieda), 4 plants from Kerina and Iyabe (Kisii South) and 3 plants from Mwingi North. Various parts were harvested depending on the parts the communities prefer to use in the treatment of various ailments. The most frequently used preparations for administration were concoctions and decoctions. Only 2 plants were preferred by infusion.

The leaves (9 plants) were the most frequently used parts of the plant, followed by the bark (5 plants) then roots (4 plants). The diseases that were most frequently treated using herbal medicines are gastrointestinal (37%), respiratory (31%), other ailment (18%), skin infection (6%) genital urinary tract (4%) and wounds (4%) (Figure 2).

Table 1: Selected medicinal plants used by the communities from Mwingi North, Kisii South and Rarieda Counties in treating various bacterial and fungal ailments

Botanical Name	**Family name and Voucher number**	**Local name**	**Parts used**	**Methods**	**Diseases treated**	**Area collected from**
Rhus natalensis Krauss	Anacardiaceae 2011/IJ018	Sangla (Luo)	Roots	decoction	Coughs, colds, headache, diarrhea	Lweya (Rarieda)
Vernonia amygdalina Del.	Asteracea 2011/IJ015	Omosabakwa (Kisii)	leaves	concoction	Loss of appetite Gastrointestinal Problems, diarrhea	Kerina (Kisii South)
Balanites aegyptiaca L. Drel.	Balantiaceae 2011/IJ05	Othoo (Luo)	bark	decoction	Stomach pains, Dysentery	Ragengni (Rarieda)
Commiphora africana (A. Rich) Engl. Syn	Burseraceae 2011/IJ02	Arupiny (Luo)	bark	decoction	Diarrhea Fever	Lweya (Rarieda)
Senna didymobotrya Fresen	Caesalpinaceae 2011/IJ04	Owinu(Luo)	roots	decoction	Diarrhea Ringworm	Ragengni (Rarieda)

Boscia angusttifolia A. Rich	Capparidaceae 2011/IJ017	Mwenzenze (Kamba)	leaves	concoction	Chest pains Stomachache	Mwingi North
Psidia puntulata (DC) Vatke	Compositae 2011/IJ03	Atilili (Luo)	roots	decoction	Stomachache Colds, diarrhea	Lweya (Rarieda)
Ricinus communis Linn.	Euphorbiaceae 2011/IJ01	Odagwa (Luo)	leaves	concoction	Stomachache, diarrhea, pains	Lweya (Rarieda)
Tamarindus indica L.	Fabaceae 2011/IJ010	Chwa (Luo)	Bark	decoction	Diarrhea,cough Fever, tonsils	Ragengni (Rarieda)
Albizia coriaria Welw.ex Oliv.	Fabaceae 2011/IJ08	Ober (Luo)	Bark	decoction	Cough, diarrhea	Lweya (Rarieda)
Ormocarpum trichocarpum Taub. Engl.	Fabaceae 2011/IJ05	Det (Luo)	leaves	concoction	Diarrhea, typhoid	Lweya (Rarieda)
Fuerstia africana T.C.E Fries	Lamiaceae 2011/IJ011	Ekebunga Baiseke (Kisii)	leaves	concoction	Urinary problems, Tongue infections, diarrhea, Skin infections	Kerina (Kisii South)
Melia volkensii Gurke	Meliaceae 2011/IJ016	Mukau (Kamba)	Leaves	concoction	Pains in the body	Mwingi North
Ficus sycomorus	Moraceae	Ngowo(Luo)	Bark	decoction	Stomach pains,	Lweya

Linn.	2011/IJ09				Coughs, wounds	(Rarieda)
Sesbania sesban Linn.	Papilionaceae 2011/IJ06	Oyieko(Luo)	Roots	decoction	Diarrheal Diseases, boils	Ragengni (Rarieda)
Zanthoxylum chalybeum Engl.	Rutaceae 2011/IJ013	Mukenea (Kamba)	Leaves	concoction	Diarrhea, Sore throat, coughs, Chest pain	Mwingi North
Zanthoxylum gillettii De Wild	Rutaceae 2011/IJ014	Egekoma (Kisii)	Leaves	Infusion	Genitourinary, coughs, mouth ulcers, throat	Kerina (Kisii South)
Urtica dioica Linn.	Urticaceae 2011/IJ012	Rise (Kisii)	Leaves	concoction	Blood purification, Anaemia, boils	Iyabe (Kisii South)

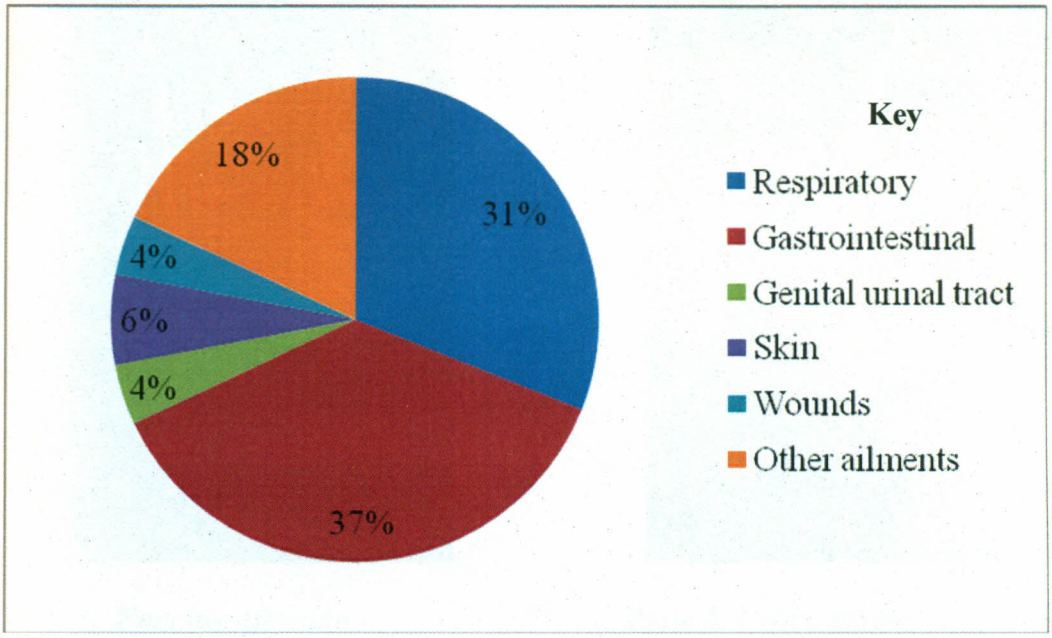

Figure 2: Percentage frequency of diseases treated using herbal drugs in Mwingi North, Kisii South and Rarieda Districts.

Plate 1. ***Ricinus communis***

Plate 2. ***Sesbania sesban***

Plate 3. *Fuerstia africana*

Plate 4. *Urtica dioica*

Plate 5. *Senna didymobotrya*

4.2 Antibacterial activity of the plant crude extracts against standard strains of bacteria

The results indicated that all the plant extracts tested had bacterial inhibitory effects. However, the inhibition of bacteria by the plant extracts varied with different plants. The zones of inhibition between 7 and 10 mm were reffered to as low/weak activity, between 11 and 14 moderate activity and between 15 and 21 high/ strong activity.

The plant extracts significantly inhibited the growth of *B. subtilis* and *S. aureus* ATCC 25922 compared to other strains of bacteria (Table 2). A total of five plant extracts had high activity against *B. subtilis*. These include; *Fuerstia africana* T. C. E Fries (17.0 mm), *Senna didymobotrya* Fresen (16.0 mm) (Plate 6), *Ormocarpum trichocarpum* Taub. Engl (15.5 mm), *Zanthoxylum chalybeum* Engl (15.0 mm) and *Tamarindus indica* L. (15.0 mm), while nine plant extracts had a moderate activity of between 11 and 13 against *B. subtilis, Ricinus communis* Linn (13.0 mm) (Plate 1), *Boscia angustifolia* A. Rich (13.0 mm), *Albizia coriaria* Welw. ex Oliv (12.0 mm), *Balanites aegyptiaca* L. Drel (12.00 mm), *Commiphora africana* (A. Rich) Engl. Syn (12.0 mm), *Rhus natalensis* Krauss (11.5 mm), *Sesbania sesban* Linn (11.00 mm), *Psidia puntulata* (DC) Vatke (11) and *Zanthoxylum chalybeum* Engl (11.0 mm). The remaining four plant extracts showed weak activity against *B. subtilis*. *Melia volkensii* Gurke, *Ficus sycomorus* Linn, *Urtica dioica* Linn and *Vernonia amygdalina* Del produced a weak antibacterial activity of 10.0 mm, 9.5 mm, 8.0 mm and 7.0 mm respectively. The zones of inhibition were significantly different ($P < 0.05$) (Table 2 and Apendix 7).

For the case of the *S. aureus* ATCC 25923, *Fuerstia africana* T. C. E Fries, *Senna didymobotrya* Fresen, *Tamarindus indica* L. (Plate 8), *Balanites aegyptiaca* L. Drel, *Psidia puntulata* (DC) Vatke, *Rhus natalensis* Krauss and *Ficus sycomorus* Linn gave antibacterial activity of 19.0 mm, 16.0 mm, 14.5 mm, 14.0 mm and 11.5 mm respectively (Table 2). On the other hand, six plants produced low activity against *S. aureus* ATCC 25923 with a zone of inhibition ranging betw een 7.5 -10mm. For example, *Albizia coriaria* Welw. ex Oliv (10.0 mm), *Commiphora africana (A. Rich) Engl. Syn* (9.5 mm), *Ormocarpum trichocarpum* Taub. Engl. (8.5 mm), *Sesbania sesban* Linn (8.0mm), *Zanthoxylum gilletii* De Wild (8.0 mm) and *Urtica dioica* Linn (7.5 mm). All the remaining plant extracts were completely inactive (6.0 mm). The zones of inhibition were significantly different ($P < 0.05$) (Table 2 and Appendix 4).

Fuerstia africana T. C. E Fries is the only plant that showed strong activity with a zone of inhibition of 20.0 mm against Methicillin resistant *Staphylococcus aureus* (Table 2 and Plate 7). *Albizia coriaria* Welw. ex Oliv, *Commiphora africana (A. Rich) Engl. Syn* and *Senna didymobotrya* Fresen produced moderate activity of 13.0 mm, 11.5 mm and 11.0 mm respectively while *Vernonia amygdalina* Del and *Zanthoxylum gilletii* De Wild gave a low antibacterial activity of 9.00 mm. All the remaining 11 plant extracts were completely inactive (6.0 mm) against this test organism. The zones of inhibition were significantly different ($P < 0.05$) (Table 2 and Appendix 3). In reference to Table 2, all of the plant extracts did not show any activity against *E. coli* ATCC 25922 and *S. typhi* ATCC 19430. (Appendix 5 and 6).

Table 2: Zones of inhibition produced by the plant extracts against the selected bacterial strains in mm

Medicinal plants	**MRSA**	***S. aureus***	***Salmonella***	***E. coli***	***B. subtilis***
Boscia angustifolia	6.0a	6.0a	6.0a	6.0a	13.0b
Fuerstia africana	20.0d	19.0c	6.0a	6.0a	17.0c
Melia volkensii	6.0a	6.0a	6.0a	6.0a	10.0b
Urtica dioica	6.0a	7.5a	6.0a	6.0a	8.0a
Vernonia amygdalina	9.0b	6.0a	6.0a	6.0a	7.0a
Zanthoxylum chalybeum	6.0a	6.0a	6.0a	6.0a	15.0c
Zanthoxylum gilletti	9.0b	8.0a	6.0a	6.0a	11.0b
Albizia coriaria	13.0c	10.0b	6.0a	6.0a	12.0b
Balanites aegyptiaca	6.0a	14.5c	6.0a	6.0a	12.0b
Commiphora africana	11.5b	9.5a	6.0a	6.0a	12.0b
Ficus sycomorus	8.5b	11.5c	6.0a	6.0a	9.5a
Ormocarpum trichocarpum	6.0a	8.5a	6.0a	6.0a	15.5c
Psidia puntulata	6.0a	14.0c	6.0a	6.0a	11.0b
Rhus natalensis	6.0a	14.0c	6.0a	6.0a	11.5b
Ricinus communis	6.0a	12.0b	6.0a	6.0a	13.0b
Senna didymobofrya	11.0b	16.0c	6.0a	6.0a	16.0c
Sesbania sesban	6.0a	8.0a	6.0a	6.0a	11.0b
Tamarindus indica	6.0a	16.0c	6.0a	6.0a	15.0c
+ve control	26.0d	24.0d	23.0b	25.5b	26.0d
-ve control	6.0a	6.0a	6.0a	6.0a	6.0a

Zones of inhibition in same column indicated by different letters are significantly different

4.2.1: Zone of inhibition plates

Plates 6 to 8 show zones of inhibition produced by plant extracts against bacterial strains.

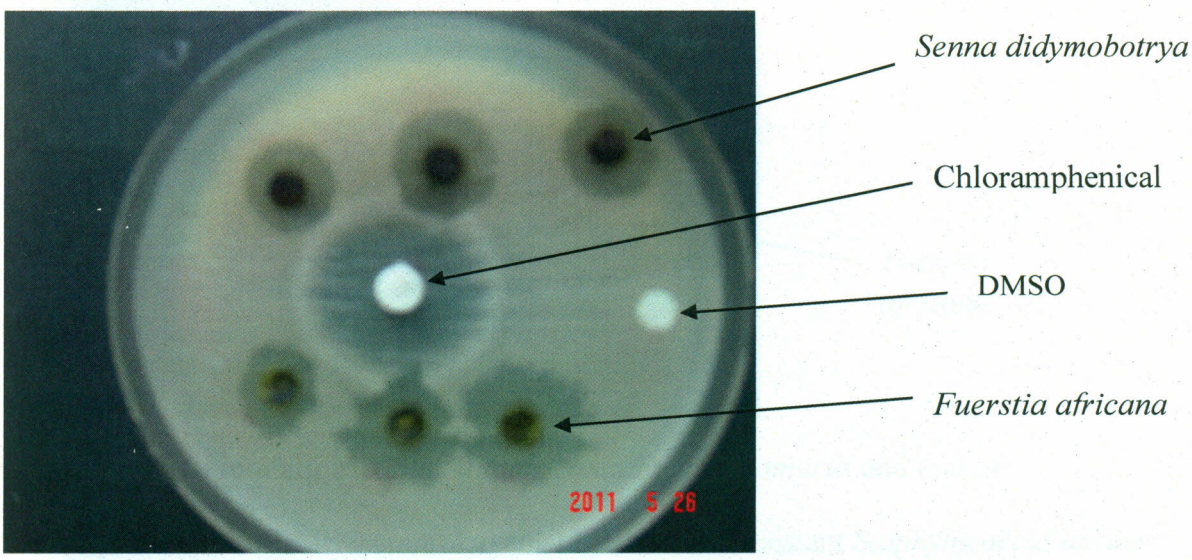

Plate 6: Zones of inhibition of *Fuerstia africana*, *Senna didymobotrya* and that of chloramphenical and DMSO control against *Bacillus subtilis*

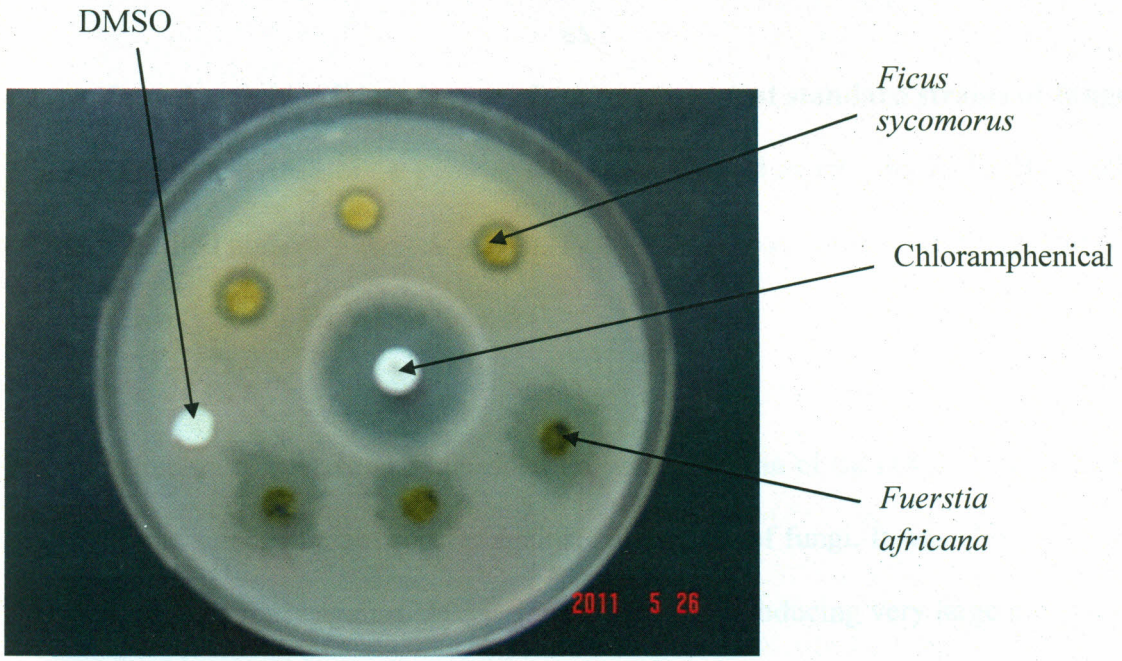

Plate 7: Zones of inhibition of *Fuerstia africana*, *Ficus sycomorus* and that of chloramphenical and DMSO control against Methicillin Resistant *Staphylococcus aureus*

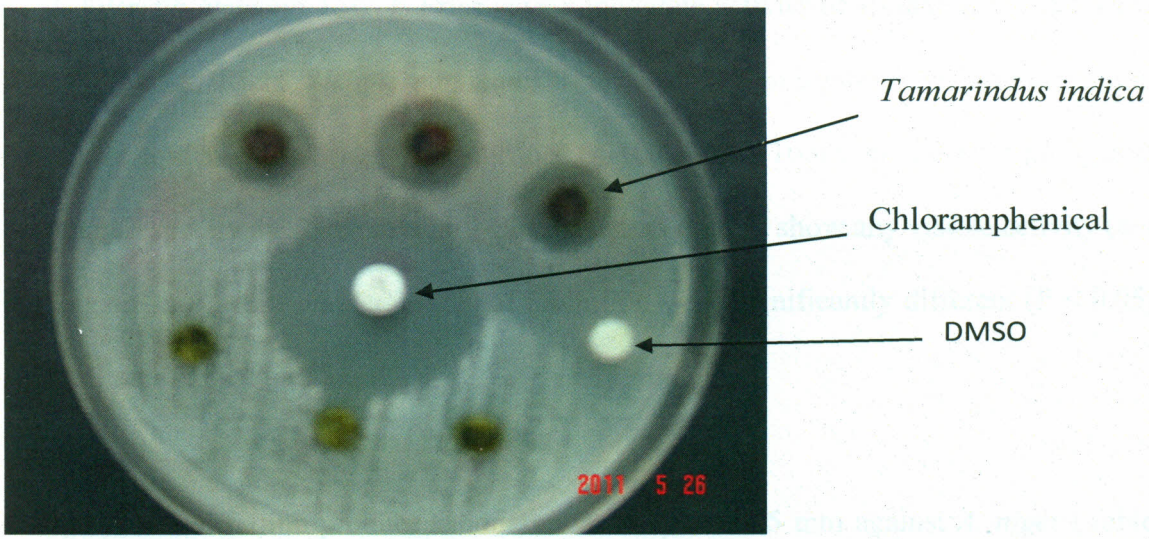

Plate 8: Zones of inhibition of *Tamarindus indica* and that of chloramphenical and DMSO control against *Staphylococcus aureus*

4.3 Antifungal activity of the plant crude extracts against standard strains of fungus

The antifungal activities of 18 plant species were assayed *in vitro* by Kirby Bauer disk diffusion method against 5 fungal species. From the findings a strong antifungal activity ranging between 9 -20.5 mm was recorded.

Table 3 summarizes the average microbial growth inhibition of the (DCM and methanol) extracts of the screened plant species against five strains of fungi. It was observed that 7 plants had great activity against *M. gypseum* with some producing very large mean zones of inhibition like *Psidia puntulata* (DC) Vatke (20.5 mm), *Commiphora africana* (A. Rich) Engl. Syn (17.5 mm) (Plate 9), *Senna didymobotrya* Fresen (17.0 mm), *Tamarindus indica* L. (16.0mm), *Albizia coriaria* Welw. ex Oliv (16.0 mm), *Ficus sycomorus* Linn (15.5 mm) and *Rhus natalensis* Krauss (15.5 mm) (Plate 10). On the hand, *Fuerstia africana* T. C. E Fries gave a moderate activity of 13.00mm while *Boscia angustifocia* A. Rich, *Ricinus communis* Linn and, *Ormocarpum trichocarpum* Taub. Engl gave low average zones of inhibitions of 10.00 mm, 10.0 mm and 8.5 mm against the strain respectively. The remaining plant extracts did not show any visible activity (6.0 mm) against *M. gypseum*. The zones of inhibition were significantly different ($P < 0.05$) (Table 3 and Appendix 12).

Ricinus communis Linn produced moderate activity of 11.5 mm against *A. niger* (Table 3) while *Fuerstia africana* T.C.E Fries, *Sesbania sesban* Linn and *Tamarindus indica* L. gave low activity of 8.0 mm, 8.0 mm and 7.5 mm respectively. The remaining 14 plant extract; *Zanthoxylum chalybeum* Engl., *Melia volkensii* Gurke, *Zanthoxylum gilletii* De

Wild, *Fuerstia africana* T.C.E Fries , *Urtica dioica* Linn, *Vernonia amygdalina* Del., *Commiphora africana (A. Rich) Engl. Syn, Psidia puntulata* (DC) Vatke, *Senna didymobotrya* Fresen, *Sesbania sesban* Linn, *Balanites aegyptiaca* L. Drel., *Albizia coriaria* Welw. ex Oliv, *Ficus sycomorus* Linn., *Rhus natalensis* Krauss and *Tamarindus indica* L. were inactive against *A. niger* (6.0 mm). The zones of inhibition were significantly different ($P < 0.05$) (Table 3 and Appendix 10).

Only *Fuerstia africana* T. C. E Fries showed weak activity of 9.0 mm against *C. neoformans* ATCC 18310 and moderate activity of 12.0 mm against *T. mentagrophyte* (Table 3, Appendix 9 and 10). All the remaining seventeen plant extracts were completely inactive producing a mean zone of 6.0 mm (Table 3). For the case of *C. albicans* ATCC 90028, all the eighteen plant extracts were inactive against this test organism. The zones of inhibition were not significantly different between the plant extracts but were significantly different between the plant extracts and the positive control ($P < 0.05$) (Table 3 and Appendix 8).

Table 3: Zones of inhibition produced by plants extracts against fungal strains in mm

Medicinal plants	*C.albicans*	*C. neoformans*	*A. niger*	*T. mentagrophyte*	*M. gypseum*
Boscia angustifolia	6.0a	6.0a	6.0a	6.0a	10.0b
Fuerstia Africana	6.0a	9.0b	8.0a	12.0b	13.0b
Melia volkensii	6.0a	6.0a	6.0a	6.0a	6.0a
Urtica dioica	6.0a	6.0a	6.0a	6.0a	6.0a
Vernonia amygdalina	6.0a	6.0a	6.0a	6.0a	6.0a
Zanthoxylum chalybeum	6.0a	6.0a	6.0a	6.0a	6.0a
Zanthoxylum gilletti	6.0a	6.0a	6.0a	6.0a	6.0a
Albizia coriaria	6.0a	6.0a	6.0a	6.0a	16c
Balanites aegyptiaca	6.0a	6.0a	6.0a	6.0a	6.00a
Commiphora africana	6.0a	6.0a	6.0a	6.0a	17.5c
Ficus sycomorus	6.0a	6.0a	6.0a	6.0a	15.5c
Ormocarpum trichocarpum	6.0a	6.0a	6.0a	6.0a	8.5b
Psidia puntulata	6.0a	6.0a	6.0a	6.0a	20.5d
Rhus natalensis	6.0a	6.0a	6.0a	6.0a	15.5c
Ricinus communis	6.0a	6.0a	11.5b	6.0a	10.0b
Senna didymobofrya	6.0a	6.0a	6.0a	6.0a	17.0c
Sesbania sesban	6.0a	6.0a	8.0a	6.0a	6.00a
Tamarindus indica	6.0a	6.0a	7.5a	6.0a	16.0c
+ve control	12.5b	13.0c	18,0c	21c	22d
-ve control	6.0a	6.0a	6.0a	6.0a	6.0a

Zones of inhibition in same column indicated by different letters are significantly different

4.3.1 Zone of inhibition plate against fungal strains

Plates 9 to 10 show zones of inhibition produced by plant extracts against fungal strains.

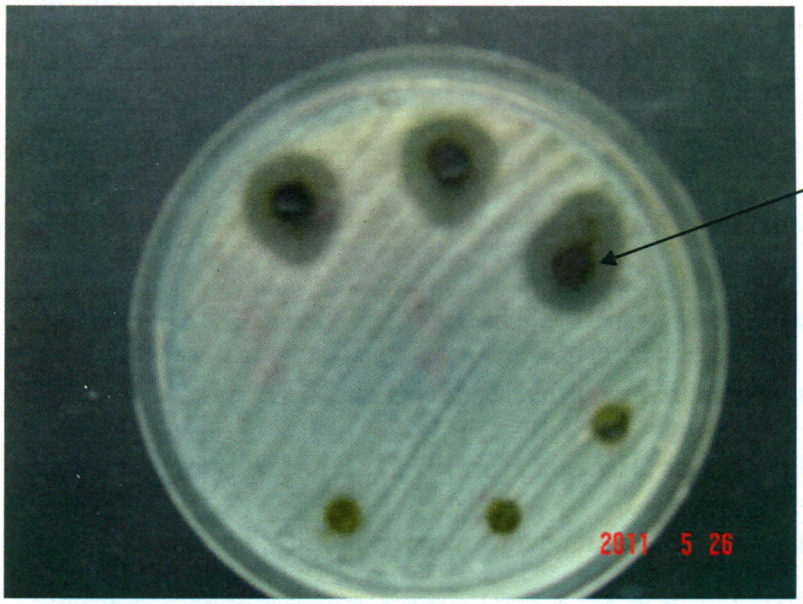

Plate 9: zones of inhibition of *Cammiphora africana* against *Microsporum gypseum*

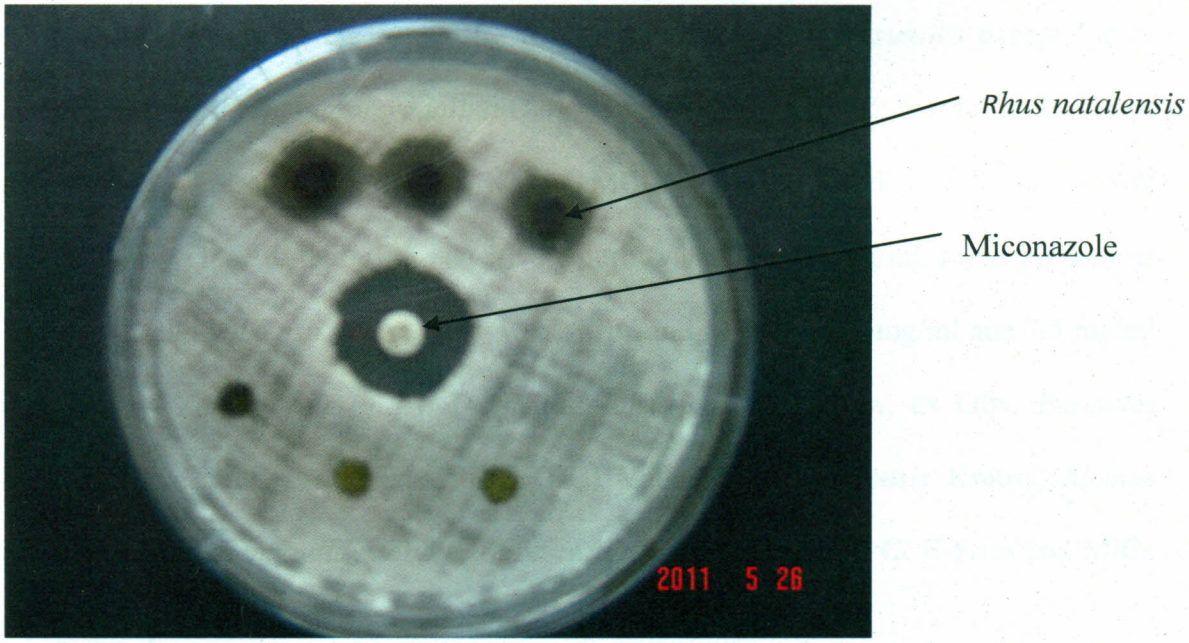

Plate 10: Zones of inhibition of *Rhus natalensis* and that of miconazole control against *Microsporum gypseum*

4.4 The Minimum Inhibitory Concentrations (MICs) and the Minimum Bactericidal Concentration (MBCS)

The minimum inhibitory concentration of the plant extracts which had inhibition diameters of 9 mm and above was determined by the broth micro dilution method in 96 well microtitre plates (plate 11) and the results are presented in table 4 and 5

Six plants had MIC and MBC against MRSA. *Albizia coriaria* Welw. ex Oliv and *Commiphora africana* (A. Rich) Eng. Syn gave MIC that were equal to MBC of 1.875 mg/ml. *Fuerstia africana* T. C. E Fries gave MIC and MBC of 0.9375 mg/ml and 1.875 mg/ml respectively while *Vernonia amygdalina* Del and *Zanthoxylum gilleti* De Wild gave MIC and MBC of 3.75 mg/ml and 7.5 mg/ml respectively while *Senna didymobotrya* Fresen gave MIC and MBC of 1.875 mg/ml and 3.75 mg/ml respectively. All the tested plants were screened for MIC and MBC against *B. subtilis* except *Urtica dioica* Linn and *Vernonia amygdalina* Del., *Fuerstia africana* T. C. E Fries and *Senna didymobotrya* Fresen gave a low MIC and MBC against *B. subtilis* of 0.9375 mg/ml which is very close to that of the positive control value 0.4688 mg/ml. *Ficus sycomorus* Linn and *Melia volkensii* Gurke had a weak MIC and MBC of 3.75mg/ml and 7.5 mg/ml respectively. *Zanthoxylum gilleti* De Wild *Albizia coriaria* Welw. ex Oliv, *Balanites aegyptiaca* L. Drel, *Psidia punctulata* (DC) Vatke, *Rhus natalensis* Krauss, *Ricinus communis* Linn, *Senna didymobotrya* Fresen, *Fuerstia africana* T. C. E Fries had MICs that were equal to MBC.

Balanites aegyptiaca L, *Psidia punctulata* (DC) Vatke, *Ricinus communis* Linn, *Senna didymobotrya,* Tamarindus indica L. and *Fuerstia africana* T. C. E Fries showed a low MIC and MBC of 0.9375 mg/ml against *S. aureus* ATCC 25923. Although the MIC and

MBC for positive control was lower, these are promising plant extracts given that they are crude extract compared to pure compounds of the positive control. *Zanthoxylum gilleti* De Wild showed a high MIC and MBC of 3.75 mg/ml and 7.5 mg/ml respectively and therefore exhibits the lowest activity.

Fungal strain especially *M. gypseum* gave the best MIC and MBC results (Table 5). These results were ranging between 0.9375 mg/ml to 3.75 mg/ml with 7 plants giving MIC of 0.9375 mg/ml. *M. gypseum* was therefore the most susceptible microbe amongst the tested strains. *Psidia punctulata* (DC) Vatke, *Albizia coriaria* Welw. ex Oliv, *Commiphora africana* (A. Rich) Engl. Syn, *Senna didymobotrya* Fresen and *Tamarindus indica* L. produced similar activity in MIC and MFC of 0.9375 mg/ml.

*Ficus sycomorus Linn.*and *Rhus natalensis* Krauss gave different values for MIC and MFC against *M. gypseum.* The MIC was 0.9375 mg/ml and MFC 1.875 mg/ml for both plants. *Boscia angustifolia* A. Rich and *Ricinus communis* Linn gave a low activity of 3.75 mg/ml for MIC and MFC against *M. gypseum.*

Among all the plant extracts tested against *C. neoformans* and *T. mentagrophyte* only *Fuerstia africana* T. C. E Fries was found to be active. *Fuerstia africana* T. C. E Fries produced a similar MIC and MFC of 3.75 mg/ml against. *T. mentagrophytes* and a weak MIC and MFC of 3.75 mg/ml and 7.5 mg/ml respectively against *C. neoformans. Ricinus communis* Linn was the only plant screened against *A. niger.* It gave a similar MIC and MFC of 3.75 mg/ml. No plant extracts were screened against *C. albicans* ATCC 90028 for MIC and MFC because all the plants were negative on it.

Table 4: The minimum inhibitory concentration and the minimum bactericidal concentration for bacterial test cultures in mg/ml

Plant	MRSA		S. aureus		B. subtilis	
	MIC	MBC	MIC	MBC	MIC	MBC
B. angustifolia	ND	ND	ND	ND	1.875	1.875
F. africana	0.9375	1.875	0.9375	0.9375	0.9375	0.9375
M. volkensii	ND	ND	ND	ND	3.75	7.5
U. dioica	ND	ND	ND	ND	ND	ND
V. amygdalina	3.75	7.5	ND	ND	ND	ND
Z. chalybeum	ND	ND	ND	ND	1.875	3.75
Z. gilletti	3.75	7.5	ND	ND	3.75	3.75
A. coriaria	1.875	1.875	1.875	3.75	1.875	1.875
B. aegyptiaca	ND	ND	0.9375	0.9375	1.875	1.875
C. africana	1.875	1.875	3.75	7.5	1.875	3.75
F. sycomorus	ND	ND	1.875	3.75	3.75	75.0
O. trichocarpum	ND	ND	ND	ND	0.9375	1.875
P. puntulata	ND	ND	0.9375	0.9375	3.75	3.75
R. natalensis	ND	ND	1.875	1.875	3.75	3.75
R. communis	ND	ND	1.875	3.75	1.875	1.875
S. didymobotrya	1.875	3.75	0.9375	0.9375	0.9375	0.9375
S. sesban	ND	ND	ND	ND	3.75	3.75
T. indica	ND	ND	0.9375	0.9375	0.9375	1.875
Positivecontrol	0.4688	0.4688	0.4688	0.4688	0.4688	0.4688
Negative control	Growth observed in all the tubes					

ND- Not done, MRSA- Methicillin Resistant *Staphylococcus aureus*, *B. subtilis- Bacillus subtilis*, MIC- minimum inhibitory concentration, MBC- minimum bactericidal concentration.

Table 5: The minimum inhibitory concentration and the minimum fungicidal concentration for fungal test cultures in mg/ml

Test Culture	***C. neoformans***		***T. mentagrophyte***		***M. gypseum***		***A. niger***	
Medicinal plants	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
B. angustifolia	ND	ND	ND	ND	3.75	3.75	ND	ND
F. africana	3.75	7.5	3.75	3.75	1.875	3.75	ND	ND
M. volkensii	ND	ND	ND	ND	ND	ND	ND	ND
U. dioica	ND	ND	ND	ND	ND	ND	ND	ND
V. amygdalina	ND	ND	ND	ND	ND	ND	ND	ND
Z. chalybeum	ND	ND	ND	ND	ND	ND	ND	ND
Z. gilletti	ND	ND	ND	ND	ND	ND	ND	ND
A. coriaria	ND	ND	ND	ND	0.9375	0.9375	ND	ND
B. aegyptiaca	ND	ND	ND	ND	ND	ND	ND	ND
C. africana	ND	ND	ND	ND	0.9375	0.9375	ND	ND
F. sycomorus	ND	ND	ND	ND	0.9375	1.875	ND	ND
O. trichocarpum	ND	ND	ND	ND	ND	ND	ND	ND
P. puntulata	ND	ND	ND	ND	0.9375	0.9375	ND	ND
R. natalensis	ND	ND	ND	ND	0.9375	1.875	ND	ND
R. communis	ND	ND	ND	ND	3.75	3.75	3.75	3.75
S. didymobotrya	ND	ND	ND	ND	0.9375	0.9375	ND	ND
S. sesban	ND	ND	ND	ND	ND	ND	ND	ND
T. indica	ND	ND	ND	ND	0.9375	0.9375	ND	ND
positive control	0.4688	0.4688	0.4688	0.4688	0.4688	0.4688	0.4688	0.4688
Negative control	Growth was observed in all the tubes							

MIC- Minimum Inhibitory concentration, MFC- Minimum fungicidal concentration, ND- Not done

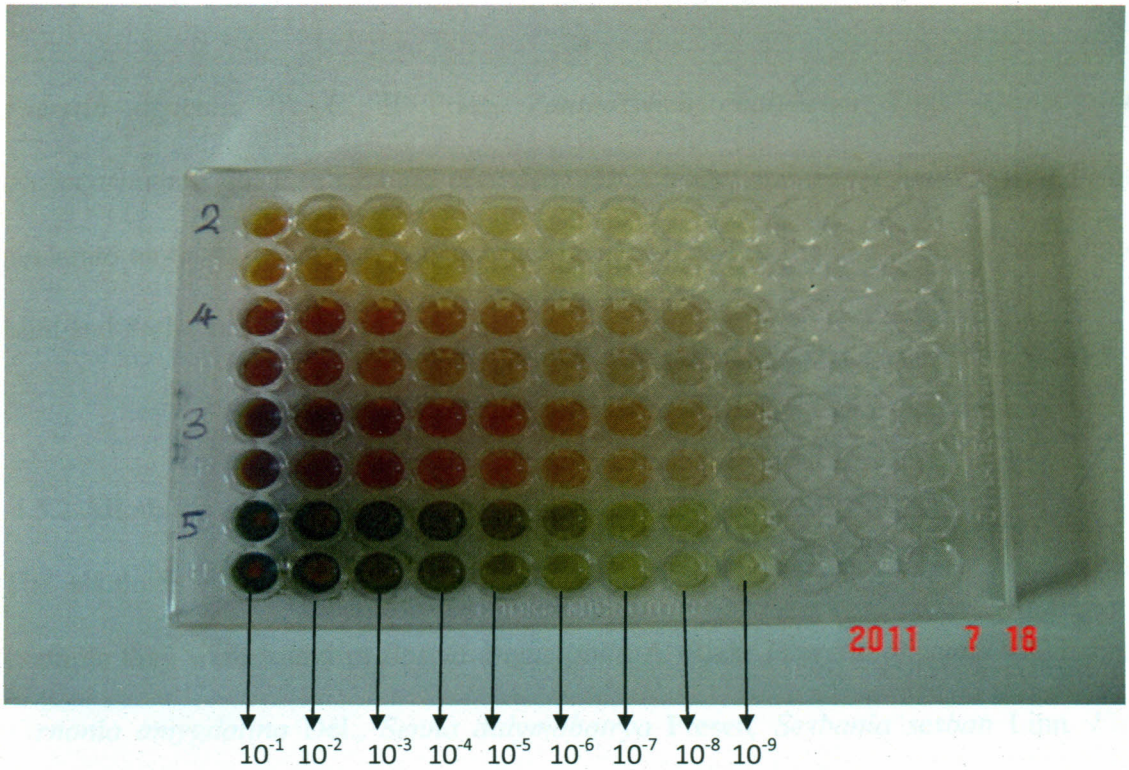

Plate 11: Microtitre plates showing Minimum inhibition concentration. The plate shows four extracts at different concentration beginning from 10^{-1} to 10^{-9} dilution factors

4.5 Phytochemical screening

All the eighteen medicinal plants extracts were screened for phytochemicals. Alkaloids, tannins, terpenoids, saponins, cardiac glycosides and flavonoids were the phytochemicals that had their presence determined and the results presented in table 6

4.5.1 Tannins

Tannins were found to be present in all plant extracts except in *Balanites aegyptiaca* L. Drel (Table 6). *Vernonia amygdalina* Del, *Zanthoxylum gilletti* De Wild, *Albizia coriaria* Welw.ex Oliv, *Commiphora africana* (A. Rich) Engl. Syn, *Ficus sycomorus* Linn, *Rhus natalensis* Krauss, *Ricinus communis* Linn, *Senna didymobotrya* Fresen and *Sesbania sesban* Linn were found to have high concentrations. *Boscia angustifolia* A. Rich,

Fuerstia africana T. C. E Fries, *Zanthoxylum chalybeum* Engl, *Ormocarpum trichocarpum* Taub. Engl, *Psidia puntulata* (DC) Vatke and *Tamarindus indica* L. had moderate amounts while low concentrations were noted in the extracts of *Urtica dioica* Linn and *Melia volkensii* Gurke.

4.5.2 Alkaloids

The alkaloids were mostly found in moderate concentrations in most extracts. For example they were found in *Boscia angustifolia* A. Rich, *Fuerstia africana* T.C.E Fries, *Vernonia amygdalina* Del., *Senna didymobotrya* Fresen, *Sesbania sesban* Linn, *Ficus sycomorus* Linn, *Albizia coriaria* Welw.ex Oliv and *Tamarindus indica* L. (Table 6) They were found in low concentrations in *Urtica dioica* Linn, *Zanthoxylum chalybeum* Engl., *Zanthoxylum gilletii* De Wild, *Balanites aegyptiaca* L. Drel., *Psidia punctulata* (DC) Vatke but were absent in *Ormocarpum trichocarpum* Taub. Engl, *Melia volkensii* Gurke, *Commiphora africana* (A. Rich) Engl. Syn, *Ricinus communis* Linn and *Rhus natalensis* Krauss.

4.5.3 Saponins

The saponins were completely absent in *Boscia angustifolia, Vernonia amygdalina* Del, *Urtica dioica* Linn, *Zanthoxylum chalybeum* Engl., *Zanthoxylum gilletii* De Wild, *Psidia punctulata* (DC) Vatke, *Senna didymobotrya* Fresen, *Ormocarpum trichocarpum* Taub. Engl and *Ficus sycomorus* Linn plant extracts (Table 6). In *Sesbania sesban* Linn, *Balanites aegyptiaca* L. Drel., *Albizia coriaria* Welw.ex Oliv, *Commiphora africana* (A.

Rich) Engl. Syn, *Rhus natalensis* Krauss and *Tamarindus indica* L. the saponins were moderate. *Fuerstia africana* T. C. E Fries, *Melia volkensii* Gurke and *Ricinus communis* Linn, had low concentrations of saponins in the plant extracts.

4.5.4 Cardiac glycosides

Most abundant cardiac glycosides were found to be present in *Albizia coriaria* Welw.ex Oliv, *Commiphora africana* (A. Rich) Engl. Syn , *Rhus natalensis* Krauss, *Fuerstia africana* T. C. E Fries, *Tamarindus indica* L. and *Ficus sycomorus* Linn plant extracts (Table). Cardiac glycosides were found in moderate concentration in *Melia volkensii* Gurke, *Balanites aegyptiaca* L. Drel, *Psidia punctulata* (DC) Vatke and *Ricinus communis* Linn while in low concentrations in *Vernonia amygdalina* Del., *Zanthoxylum chalybeum* Engl., *Ormocarpum trichocarpum* Taub. Engl and *Sesbania sesban* Linn. They were absent in plant extracts of *Boscia angustifolia* A. Rich, *Urtica dioica* Linn, *Zanthoxylum gilletii* De Wild and *Senna didymobotrya* Fresen.

4.5.5 Terpenoids

High concentrations of terpenoid were found in *Albizia coriaria* Welw.ex Oliv, *Commiphora africana* (A. Rich) Engl. Syn, *Rhus natalensis* Krauss, *Fuerstia africana* T. C. E Fries, *Ormocarpum trichocarpum* Taub. Engl, *Balanites aegyptiaca* L. Drel, *Senna didymobotrya* Fresen and *Ficus sycomorus* Linn plant extracts (Table 6). This was indicated by the formation of a reddish brown colouration of the interface (Plate 12). Extracts of *Melia volkensii* Gurke and *Psidia punctulata* (DC) Vatke and *Ricinus communis* Linn had low concentration. Terpenoids were found to be absent in *Boscia*

angustifolia, Urtica dioica Linn, *Vernonia amygdalina* Del., *Tamarindus indica* L. and *Sesbania sesban* Linn.

4.5.6 Flavonoids

Flavonoids were found in all other plant extracts except in *Melia volkensii* Gurke (Table 6). Flavonoids concentrations were high in the extracts of *Ormocarpum trichocarpum* Taub. Engl, *Balanites aegyptiaca* L. Drel, *Zanthoxylum gilletii* De Wild, *Psidia punctulata* (DC) Vatke and *Ricinus communis* Linn. In *Rhus natalensis* Krauss, *Fuerstia africana* T. C. E Fries, *Tamarindus indica* L., *Ficus sycomorus* Linn and *Zanthoxylum chalybeum* Engl the co ncentration of flavonoids in the plant extracts was moderate. *Boscia angustifolia* A. Rich, *Vernonia amygdalina* Del, *Urtica dioica* Linn, *Senna didymobotrya* Fresen, *Albizia coriaria* Welw.ex Oliv, *Commiphora africana* (A. Rich) Engl. Syn and *Sesbania sesban* Linn had low concentration of flavonoids.

Table 6: Phytochemical screening of the plants extracts

Medicinal plants	Tannins	Alkaloids	Saponin	Cardiac glycosides	Terpenoids	Flavanoids
Boscia angustifolia	++	++	–	–	–	+
Fuerstia africana	++	++	+	+++	+++	++
Melia volkensii	+	–	+	++	++	–
Urtica dioica	+	+	–	–	–	+
Vernonia amygdalina	+++	++	–	+	–	+
Zanthoxylum chalybeum	++	+	–	+	+	++
Zanthoxylum gilletti	+++	+	–	–	+	+++
Albizia coriaria	+++	++	++	+++	+++	+
Balanites aegyptiaca	–	+	++	++	+++	+++
Commiphora africana	+++	–	++	+++	+++	+
Ficus sycomorus	+++	++	–	+++	+++	++
Ormocarpum trichocarpum	++	–	–	+	+++	+++
Psidia puntulata	++	+	–	++	++	+++
Rhus natalensis	+++	–	++	+++	+++	++
Ricinus communis	+++	–	+	++	++	+++
Senna didymobofrya	+++	++	–	–	+++	+
Sesbania sesban	+++	++	++	+	–	+
Tamarindus indica	++	++	++	+++	–	++

+++ (High), ++ (Moderate), + (Low) and – (Not present).

Plate 12: Reddish brown colouration of the interface indicated the presence of terpenoid in *Fuerstia africana*

CHAPTER FIVE
DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

The present research provides information on 18 medicinal plants used in the treatment of diarrheal diseases, respiratory system illnesses, skin infection, fever, wounds among others. From this study, the most cited health problem was diarrhea (Figure 2). This may have been contributed by consumption of contaminated water (Jeruto *et al.*, 2008). The ethnobotanical survey results (Table 1) it indicated that traditional medicine still plays a significant role in meeting the basic healthcare needs of people from Mwingi North, Kisii South and Rarieda Districts in treating various diseases.

The 18 medicinal plants studied are distributed in 15 families. The most frequently used preparation for drug methods were concoctions and decoctions. It was observed that some of the plants were prepared by mixing more than one plant to make a concoction. This is supported by Nanyingi *et al.* (2008). The use of concoctions suggests that the compounds could only be active in combination, due to synergistic effects of several compounds (Omori *et al.*, 2012). On the plant parts used, it was noted that the leaves (9 plants) were the most frequently used parts of the plant followed by the bark (5 plants) then roots (4 plants) and this is in agreement with Nanyingi *et al.* (2008) findings. The leaves may be preferred because they are the main photosynthetic organs in plants and all the phytochemical compounds are made in them then later translocated to other parts like barks and roots (Jeruto *et al.*, 2008). The use of roots and barks is dangerous to the

existence of the harvested plant, since it contributes to the loss of plants obtained by such destructive harvesting methods (Jeruto *et al.,* 2011).

The results obtained in this study indicate a considerable difference in antibacterial activity of different extracts from selected plant species. Medicinal plants used by herbalists to treat non-fungal infections were also active against some strains of fungi tested. (Tables 2 and 3). This indicated that these extracts could be used for both bacterial and fungal infections. For example; *Tamarindus indica* L., *Senna didymobotrya* Fresen, *Rhus natalensis* Krauss, *Psidia puntulata* (DC) Vatke, *Ficus sycomorus* Linn, *Albizia coriaria* Welw. ex Oliv and *Fuerstia africana* T. C. E Fries exhibited strong activity against both bacterial and fungal strains. In general among the tested microbial strains, bacteria were found to be more sensitive to many of the tested agents compared to the fungi (Table 2 and Table 3). This is similar to Korir *et al.* (2012b) findings, who reported that the bacterial strains were more sensitive to most of the crude extracts than the fungal strains.

The antibacterial activity was more pronounced on the Gram positive bacteria than on the Gram negative bacteria, for example; *Tamarindus indica* L., *Senna didymobotrya* Fresen, *Rhus natalensis* Krauss, *Psidia puntulata* (DC) Vatke, *Albizia coriaria* Welw.ex Oliv, *Balanites aegyptiaca* L. Drel and *Fuerstia africana* T. C. E Fries produced high antibacterial activity against *S. aureus* ATCC 25923 and *B. subtilis but* no plant extract was active on *E. coli* ATCC 25922 and *S. typhi* ATCC 19430 (Table 2). This is in agreement with previous reports by some researchers (Duraipandiyan *et al.*, 2006;

Pirbalouti *et al.*, 2010). The probable reason for the variation in the activities between Gram negative and Gram positive bacteria could be due to their morphological difference (Maregesi *et al.*, 2008; Pirbalouti *et al.*, 2010). The Gram negative bacteria have an extra outer membrane in their cell wall which is richer in lipopolysaccharides and acts as a barrier to foreign substances including antibiotic molecules (Maregesi *et al.*, 2008; Pirbalouti *et al.*, 2010). It is also associated with the enzyme found in periplasmic space which can break down the molecules introduced from outside (Pirbalouti *et al.*, 2010).

B. subtilis and *S. aureus* ATCC 25923 were generally more sensitive to the plants extracts (Plates 6 and 8). This may be explained by the cell wall composition of the Gram positive bacteria (*S. aureus* and *B. subtilis*) which have a relatively thick layer of peptidoglycan sheets of interconnected glycan chains made up of polymer which is fully permeable to many substances and thus sensitive to most plant extracts (Kitonde *et al.*, 2013). This is supported by Samie *et al.* (2005) who reported that *Bacillus* spp and *S. aureus* were most sensitive whereas *E. coli* and *Salmonella anatum* were more resistant to crude extracts of plants.

Fuerstia africana T. C. E Fries was the most active plant extract. It is widely used among the Kisii for a wide range of conditions including: diarrhea, mouth infections, urinary tract problems and skin infections hence this practice is supported by the broad spectrum of activity displayed by the plant extract (Table 1). It gave zones of inhibition of 20.00 mm, 19.00 mm, 17.00 mm, 9.00 mm, 12.00 mm and 13.00 mm against MRSA, *S. aureus* ATCC 25923, *B. subtilis, C. neoformans* ATCC 18310, *T. mentagrophyte* and *M.*

gypseum respectively (Table 2 and 3). These results suggest that, the extract of *Fuerstia africana* T. C. E Fries could be used for management of bacterial diseases caused by *S. aureus* such as boils, sores, wounds and diarrhea. This result is in agreement with another study that found *Fuerstia africana* T. C. E Fries to be active against *S. aureus* and MRSA (Nge'ny *et al.*, 2011) but partly agrees with the findings of Mariita *et al.*, (2010a) who found that the plant showed a moderate activity on *S. aureus* but was inactive against *E. coli, S. typhi* and *C. albicans*. The difference may have been as a result of harvesting periods and solvents used in extraction of the plant (Samie *et al.*, 2005; Samie *et al.*, 2010; Asob *et al.*, 2011). Mariita *et al.* (2010a) used methanol as solvent for extraction while in this study DCM and methanol in the ratio of 1:1 was used.

The phytochemical analysis indicated that all the tested compounds were present hence its broad spectrum activity is attributed to the phytoconstituents it possesses (Doughari and Manzara, 2008). The tannins and alkaloids *Fuerstia africana* T. C. E Fries possessed are known to be cytotoxic to bacterial cells and could explain the broad spectrum activity (Omwenga *et al.,* 2009).

Amongst all the 18 plants tested, it's only *Fuerstia africana* T. C. E Fries that showed a low activity (9.00 mm) against *C. neoformans* ATCC 18310 (Table 3 and Appendix 9). This is supported by Korir *et al.* (2012a) who found that *C. neoformans* was resistant to all plant extracts. This may be because of the presence of polysaccharride capsule which is made of galactoxylomanna and glucuronoxylomannan (Susane *et al.*, 2009; Teresa and Alspaugh, 2012). The polysaccharide capsular material in *C. neoformans* is responsible

for virulence and antimicrobial resistance (Korir *et al.,* 2012a). For instance, the capsule enlargement has been associated with protection of the host fungus against host defense mechanism such as phagocytosis and oxidative burst (Susane *et al.*, 2009). In addition, capsular material also acts directly against the host. In macrophages, *C. neoformans* releases polysaccharide from its capsule into vesicles around the phagome and accumulation of these vesicles in the cytoplasm of the cell results in macrophages dysfunction and lysis (Hansang and Robin, 2009).

Senna didymobotrya Fresen showed activity of 16.00 mm against *S. aureus* ATCC 25923 and *B. subtilis* and 11.00 mm against MRSA. It was also very active against *M. gypseum* (17.00 mm) but it was completely inactive against all the other tested fungal and bacterial strains. This is contradictory to the previous findings in which *Senna didymobotrya* Fresen was active against *E. coli* and *C. albicans* (Korir *et al.,* 2012b). This may be attributed to the differences in locality of the plant species and time of collection (Matu and Staden, 2003). Plants collected during the rainy season will not have the same phytochemical concentrations as the ones collected during the dry season (Matu and Staden, 2003). The phytochemical screening indicated that tannins and terpenoids were present in high concentrations but the flavonoids were in low concentration. Tannins obtained from the bark of the stem of *Senna didymobotrya* Fresen are known to have antimicrobial activity (Chothani and Vaghasiya, 2011). Tannins act by reducing secretion and making the intestinal mucus resistant through the formation of protein tannate (Balogun *et al.*, 2011).

Albizia coriaria Welw. ex Oliv produced antibacterial activity with a zone of inhibition of 13.00 mm against MRSA, 12 mm against *B. subtilis* and 10.00 mm against *S. aureus* ATCC 25923 but was inactive against *S. typhi* and *E.coli* (Table 2). *Albizia coriaria* Welw. ex Oliv also exhibited a high antifungal activity with a zone of inhibition of 16.00mm against *M. gypseum* but was inactive against the remaining tested fungal strains. This is partly in agreement with the report by Olila *et al.* (2007) who found it to be active against *B. subtilis* and *E. coli* but had no activity against *S.aureus. C. africana* is also one of the most active plants that showed antimicrobial activity against four micro-organisms with a zone of inhibition of 11.50 mm, 9.5 mm, 12.00 mm and 17.5 mm against MRSA, *S. aureus* ATCC 25923, *B. subtilis* and *M. gypseum* respectively. This is partly in agreement with Akor and Anjorin (2009) who used the root extracts and found that it exhibited activity against *S. aureus, E .coli and C. albicans.* The difference in the results might be attributed to the parts of the plants used, physical factors (temperature, light and water) contamination by field microbes, substitution of plants and the locality (Okigbo and Mmeke, 2008).

The phytochemical analysis of *Albizia coriaria* Welw. ex Oliv and *Commiphora africana* (A. Rich) Engl. Syn indicated that tannins, terpenoids, saponin, cardiac glycosides, and flavonoids were present in various concentrations except alkaloid that were not present in *Commiphora africana* (A. Rich) Engl. Syn. Tannins possess antibacterial properties because of their ability to react with proteins to form stable water soluble compounds that kill bacteria by disrupting their cell membranes (Mariita *et al.*, 2011).

Ficus sycomorus Linn has been reported to posses anti-diarrheal activity (Ahmadu *et al.,* 2007). In this study, the bark extract was found to be active against *S. aureus* ATCC 25923 and *B. subtilis,* though it also exhibited a mild activity on MRSA with zones of inhibition of 11.50 mm, 9.5 mm and 8.5 mm (Table 2). It also exhibited a strong activity of 15.5 mm against *M. gypseum.* In previous research, crude ethanol extract of *Ficus sycomorus* Linn showed activity against *S. aureus,* MRSA *and S. typhii* (Kubmarawa *et al.,* 2007). This is partially in agreement with the present study. However in the current study, it was not active against *S. typhi* ATCC 19430. *Ficus sycomorus* Linn did not show any activity in the yeast species but was very active against *M. gypseum* and this is in agreement with *Samie et al.* (2010) who did not find any antifungal activity on the yeast. The difference might be attributed to differences in geographical location, type of solvents used in extraction, the type of tested microorganisms, parts used, storage conditions and methods of analysis (Wagete *et al.,* 2008; Olusesan *et al.,* 2010; Obeidat *et al.,* 2012).

The phytochemical analysis of *Ficus sycomorus* Linn revealed the presence of secondary metabolites such as: tannin, cardiac gl ycosides, terpenoids and flavonoids. The high concentration of terpenoids might have contributed to the antibacterial activity. Since this is supported by Chiruvella *et al.* (2007) who isolated terpenoids from *Soymida febrifuga* and reported that the antibacterial activity of terpenoids was due to membrane disruption by the terpenes. The presence of some of these compounds has been demonstrated in previous studies by other researchers. For example the presence of terpenes and tannins in the leaves has been demonstrated (Ahmadu *et al.*, 2007). The presence of glycosides,

tannins and flavonoids in the stem bark extract has been revealed (Kubmarawa *et al.,* 2007).

In this research, *Zanthoxylum giletii* De Wild was found active against *B. subtilis,* MRSA and showed a mild activity on *S. aureus* ATCC 25923 but was inactive against *E. coli* 25922 and *S. typhi* ATCC 19430. It also had no antifungal activity on the tested fungi. This is partially in agreement with Mariita *et al.* (2010a) who reported that the plant was inactive against *E. coli*, *S. typhi, S. aureus* and *C. albicans* but contradicts the findings of Agyare *et al.* (2006) who documented that the leaf extract of *Zanthoxylum gilletii* De Wild, was active against *E. coli, S. aureus, B. subtilis* and *C. albicans* but inactive against *A. niger*. The slight difference may be due to the solvent used for extraction and dosage (Okigbo and Mmke, 2008) climatic and environmental factors (Kubmarawa *et al.,* 2007; Okigbo and Mmke, 2008). Plants collected at different times and from different regions might have different activities against micro-organisms (Samie *et al.*, 2005). On testing for phytochemicals, it was found out that the plant possessed a high concentration of tannins and flavonoids, moderate concentration of terpenoids and alkaloids though it lacked saponins and cardiac glycosides. This finding partly agrees with the finding of Agyare *et al.,* (2006) who found out that the plant possessed alkaloids, tannins and saponins. The high concentration of flavonoids might have been responsible for the observed antimicrobial activity since they have been reported to exhibit strong antimicrobial activity (Olusesan *et al.*, 2010).

In the present study, *Balanites aegyptiaca* L. Drel was found to be active against *S. aureus* ATCC 25923 and *B. subtilis* but was inactive against MRSA, *S. typhi* ATCC 19430, *E. coli* ATCC 25922 and all the tested fungal strains. This is partially in agreement with a research carried out by Bidawat *et al.* (2011) who reported that the flavonoids fractions were active against *E. coli*, *B. subtilis* and *S. aureus.* On the contrary, *Balanites aegyptiaca* L. Drel stem bark exhibited a high antifungal activity against *C. albicans* (Maragesi *et al.*, 2008) and the leaves demonstrated a high antityphoid activity (Doughari *et al*,. 2007). Ethanol extracts of leaves were found to be more effective against *E. coli*, *B. subtilis, S. aureus* and *C. albicans* compared with the extract of stem bark (Gour and Kant, 2012). The difference could be as a result of extraction methods, geographical areas where the plants were collected, the part of plant material used and time of collection of the plant materials which in turn affected the amount of constituents of the plants (Ibrahim *et al.*, 2009; Okemo *et al.*, 2011) besides, the extraction procedures could also alter the phytochemical composition in plants (Korir *et al.,* 2012a).

The phytochemical analysis of *Balanites aegyptiaca* L. Drel revealed that the plant contained flavonoids, terpenoids, glycosides, saponins and traces of alkaloids but did not posses tannins. The antibacterial activity might have been due to the presence of flavonoids and terpenoids (Banson and Adeyemo, 2007). The high concentration of flavonoids could have contributed to the antimicrobial effect since flavonoids have been found to kill enzymes involved in cell wall biosynthesis in pathogens (Negi *et al.*, 2009). *Balanites aegyptiaca* L. Drel has a long history of traditional uses for a wide range of

diseases (Chothani and Vaghasiya, 2011). This could have been contributed by the presence of the secondary metabolites in various concentrations (Nwodo *et al.*, 2010).

Tamarindus indica L. is a multipurpose plant whose most parts find at least some use (Caluwe *et al.*, 2010). In the current research, the stem bark extract of *Tamarindus indica* L. demonstrated activity against *S. aureus* ATCC 25923, *B. subtilis and M. gypseum* but was inactive against MRSA, *E. coli* ATCC 25923, *S. typhi* ATCC 19430 and the other tested fungal strains. This is partially in agreement with Doughari (2006) whose report showed that *Tamarindus indica* L. showed activity against *E. coli, S. typhi, B. subtilis and S. aureus.* According to Daniyan and Muhammed (2008) fruit extract of *Tamarindus indica* L. was inactive against *S. typhi* but active against *E. coli and S. aureus.* This is supported by Nwodo *et al.* (2010) whose findings revealed that the plant was inactive against *S. typhi* but exhibited activity against *E. coli, B. subtilis* and *S. aureus*. The differences might have been caused by the solvents used for extraction, plant collection periods, age of the plant used, freshness of plant materials, adulteration and geographical difference (Okigbo and Mmeka, 2008). It has been documented that different solvents have diverse solubility capacities for different secondary metabolites (Doughari and Manzara, 2008). The phytochemical constituents such as: tannins, alkaloids, saponins, cardiac glycosides and flavonoids were present in the current study. The antimicrobial activity is therefore explained by the presence of the phytochemical constituents (Nwodo *et al.,* 2010). Flavonoids have been reported to possess antimicrobial activity (Olusesan, 2010) and are known to prevent gastric ulcers due to the astringent and antimicrobial

properties which appear responsible for gastric – protective activity (Njoroge *et al.*, 2012).

In this study, *Rhus natalensis* Krauss and *Psidia punctulata* (DC) Vatke demonstrated strong activity against *B. subtilis, S. aureus* ATCC 25923 and *M. gypseum. Rhus natalensis* Krauss gave zones of inhibition of 11.5 mm, 14.00 mm, and 15.5 mm respectively (Table 2). While *Psidia punctulata* (DC) Vatke gave 14.00 mm, 11.00 mm and 20.5 mm respectively. This signifies that, the extracts of could be used to produce antifungal and antibacterial compounds for the treatment of diseases caused by *M. gypseum, B. subtilis* and *S. aureus. Rhus natalensis* Krauss has been reported to exhibit antiplasmodial activity, (Gathirwa *et al.*, 2011). It has also been found to give protection from periodontopathic bacteria by preventing bacterial enzyme function (Parker *et al.*, 2007). *Psidia punctulata* (DC) Vatke has been reported to possess antileishmanial properties (Githinji *et al.,* 2009). It also demonstrated a mild activity against coffee berry disease fungus (Midiwo *et al.,* 2002).

The Phytochemical screening indicated that tannins, saponins, cardiac glycosides, terpenoids and flavonoids were present in *Rhus natalensis* Krauss, but alkaloids were absent. *Psidia punctulata* (DC) Vatke possessed tannins, alkaloids, cardiac glycosides, terpenoid and flavonoids but lacked saponins. The known active phytochemical constituents in *Psidia punctulata* (DC) Vatke are flavones and phenylpropenoids (Githinji *et al.*, 2010). The antibacterial activity of flavonoids is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls

(Banson and Mann, 2008). This could be attributed to the antimicrobial properties of the plant.

Sesbania sesban Linn and *Ormocarpum trichocarpum* Taub. Engl. showed moderate activity against *B. subtilis* by producing zones of inhibiting of 11.00 mm and 15.5 mm respectively. They also exhibited a mild activity against *S. aureus* ATCC 25923 of 8.00 mm and 8.50 mm respectively. They did not demonstrate any activity against MRSA, *E. coli* ATCC 25922, *S. typhi* ATCC 19430, the yeast and dematophytes though the leaf extracts of *Ormocarpum trichocarpum* Taub. Engl., were slightly active against *M. gypseum* (8.5 mm) and the bark extract of *Sesbania sesban* Linn also showed mild activity on *A. niger* of 8.00 mm. This result is partly in agreement with previous studies which revealed that the leaves of *Sesbania sesban* Linn showed activity against *B. cereus, E. coli* and *A. niger* (Hossain *et al.*, 2007). *Sesbania sesban* Linn did not show any activity on the yeast fungi but showed mild activity against *A. niger* (filamentous fungi) this may be due to differences in the structure of the cell wall of the organisms (Paiva *et al.*, 2010) the protein on *C. albicans*, function as selective transport system used to remove wastes and compounds that are dangerous to the cell (Nester *et al.*, 2004). This is of medical importance since it allows micro-organisms to oust antimicrobial medications that are used to destroy them and therefore make them resistant. This explanation could be one of the reasons why *C. albicans* ATCC 90028 was resistant to all crude extracts.

The phytochemical screening of *Ormocarpum trichocarpum* Taub. Engl indicated that terpenoid and flavonoids were present in high concentrations, while alkaloids and

saponins were absent. *Sesbania sesban* Linn had a high concentration of tannin, but alkaloids and saponins were moderate, cardiac glycosides and flavonoids were low and terpenoids were absent. Tannins, cardiac glycosides, flavonoids and saponins have been linked with antimicrobial activity (Nwodo *et al.*, 2010). For instance saponins act by breaking the cell membrane of bacteria (Omwenga *et al.*, 2009).

Ricinus communis Linn exhibited activity against *S. aureus* ATCC 25923, *B. subtilis, M. gypseum* and *A. niger.* The inhibition zones were 12.00 mm, 13.00 mm, 11.50 mm and 10.00 mm respectively. But the plant did not show any activity against the tested Gram negative bacteria and yeast. The activity of *Ricinus communis* Linn is in agreement with the findings of Verma *et al.* (2011) where the root extract was found to have activity against *S. aureus, B. subtilis,* and *A. niger* but contrasts with their findings that it was active against *E. coli*. From a study carried out by Jumbo and Enenebeako (2007) the fermented seed extract of *Ricinus communis* Linn was found to be active against *S. aureus* and *E. coli*. This also partly agrees with the findings of the current research. The difference could be attributed to the part of the plant used and geographical regions (Arya *et al.*, 2010).

The Phytochemical screening of *Ricinus communis* Linn indicates that tannins and flavonoids were present in high concentrations, cardiac glycosides and terpenoids in moderate concentration, saponins in low concentration but alkaloids were absent. The presence of tannins and flavonoids in high concentrations could be responsible for the antimicrobial activities. Tannins have been reported to have antimicrobial properties

since they can react with proteins forming stable water soluble compounds thus killing the bacteria by damaging the cell membrane directly (Okemo *et al.*, 2011). Flavonoids can also interact with mitochondria and interfere with pathways of intermediary metabolism (Williams *et al.*, 2004). Flavonoids and flavonoid – derived plant natural products have for a long time been known to function as antimicrobial defense compounds (Korir *et al.*, 2012b) The presence of tannins could be responsible for the antimicrobial activities because they have antifungal, anti-inflammatory and healing properties (Moyo *et al.*, 2010). This could explain the inhibition of *A. niger.*

In the present study, *Vernonia amygdalina* Del showed activity against MRSA but was slightly active against *B. subtilis.* It was inactive against *S. typhi* ATCC 19430, *S. aureus* ATCC 25923, *E. coli* ATCC 25923 and the tested dermatophytes, yeast and *A. niger.* These findings are in agreement to those of Mariita *et al.* (2010) and Cheruiyot *et al.* (2009) who reported that this plant did not have any ability to inhibit *E. coli, S. typhi* and *C. albicans.* This is also supported by Uzoigwe and Agwa (2011) who found that *E. coli* and *S. aureus* did not show susceptibility to both the leaf and stem extract of *Vernonia amygdalina* Del. This is contrary to reports by Okigbo and Mmeka (2008) and Ogundare (2011) in which *Vernonia amygdalina* Del was active against *E. coli, S. aureus* and *C. albicans.* The phytochemical analysis revealed that the plant had a high concentration of tannins, moderate concentration of alkaloids and traces of glycosides and flavonoids, though it did not have saponins and terpenoids. It is possible that the antibacterial activities of this plant may be due to the presence of bioactive components as noted by Ogundare (2011).

Findings from the current study shows that the leaf extracts of *Boscia angustifolia* A. Rich demonstrated activity against *B. subtilis* (13.00 mm) and *M. gypseum* (10.00 mm) only but was inactive against the rest of the microorganisms tested. This is partially in agreement with the findings by Omwenga *et al.* (2009) which revealed that the plant showed low activity against *S. aureus* and *B. subtilis* but was inactive against *S. typhi* and *E. coli.* This finding is contradicted by Hassan *et al.* (2006) who found that the root extracts were active against *E. coli.* The difference might have been attributed to different parts used since the concentration of the active phytochemicals can vary significantly between different parts of a given plant (Gathirwa *et al.,* 2011). The phytochemical screening revealed that the plant possesses tannins, alkaloids and flavonoids in various concentrations but lacked saponins, cardiac glycosides and terpenoids. Tannins have been found to form irreversible complexes with rich protein resulting in the inhibition of cell protein synthesis (Issazadeh *et al.*, 2012). Alkaloids are known to be toxic against cells of foreign organism (Issazadeh *et al.*, 2012).

The leaves extract of *Melia volkensii* Gurke and *Zanthoxylum chalybeum* Engl only inhibited *B. subtilis* with zone of inhibition of 10.00 mm and 15.00 mm respectively but they did not show any activity against the fungal strains (Tables 2 and 3). This is not in agreement with Akanga (2008) whose report on *Melia volkensii* Gurke showed activity against *E. coli.* The findings on *Zanthoxylum chalybeum* Engl is partially in accordance with Matu and Staden (2003) whose observations revealed that the stem bark had moderate antibacterial activity against *S. aureus*. *Zanthoxylum chalybeum* Engl has been

reported to have no antibacterial activity against *E. coli* and *S. aureus.* It also showed no antifungal activity against *C. albicans* (Olila *et al.*, 2001). The antidiarhoea activity could be as a result of their synergy with components from other plants and some metabolites (Doughari, 2006).

The preliminary phytochemical analysis of *Melia volkensii* Gurke revealed the presence of cardiac glycosides and terpenoids in moderate concentration and traces of saponin, while alkaloids and flavonoids were missing. This partially contradicts the phytochemical analysis results of Akanga (2008) which indicated that *Melia volkensii* Gurke possesses tannins, terpenoids, flavonoids, steroids, alkaloids, cardiac glycosides and saponin in varying amounts. The phytochemical test on *Zanthoxylum chalybeum* Engl indicated that the plant had tannin and flavonoids in moderate amounts, alkaloids, cardiac glycosides and terpenoids in low amounts but saponins were lacking. *Melia volkensii* Gurke and *Zanthoxylum chalybeum* Engl only inhibited the growth of *B. subtilis* and this might have been brought about by the fact that the plants may have contained antibacterial constituents. Besides this, the drying process may have caused conformational changes to occur in some of the chemical constituents found in *Melia volkensis* Gurke and *Zanthoxylum chalybeum* Engl (Parekh and Chanda, 2007).

Findings from the current study showed that *Urtica dioica* Linn exhibited mild activity on *S. aureus* ATCC 25923 with a zone of inhibition of (7.5mm) and *B. subtilis* (8.00 mm) but was inactive on all the tested fungal species. This is partially in agreement with Uzun *et al.* (2004) whose findings revealed that the plant was active against *S. aureus*,

moderately inhibited *E. coli* but was inactive against *C. albicans. Urtica dioica* Linn may not have any effect on the fungal strains tested but may participate in facilitating diffusion of active compounds into the blood, stabilizing the body temperature and reducing toxicity. This explains why herbal medicines are given in cocktail by herbalists (Adesina, 2005; Samie *et al.,* 2010). The low activity by *Urtica dioica* Linn against *B. subtilis* (8.00 mm) and *S. aureus* (7.5 mm) could suggest presence of resistance of these micro-organisms. The activity may be improved by increasing the concentration of the crude extract. This is supported by Kitonde *et al.* (2013) who reported that if drugs show less activity against the test organisms then that indicates the development of resistance by the micro-organisms. The antimicrobial activity would be increased by increasing the concentration of the extract (Kitonde *et al.,* 2013), since diluting crude extracts normally weaken their antimicrobial activity (Ramamoorthy *et al.,* 2010). The phytochemical screening of this plant revealed that the plant had traces of tannins, alkaloids and flavonoids but lacked saponins, cardiac glycosides and terpenoids.

The low concentration of the bioactive compounds in this plant might have been responsible for the poor antimicrobial activity (Nwodo *et al.*, 2010). Besides the absence of active ingredients, other reason that could explain the low antimicrobial activity include: the parts of the plant used, solvent used for extraction, the location where the plants were collected and the time when the plants were collected (Hamza *et al.,* 2006). The low activity by *Urtica dioica* Linn extract could also be due to low concentration of diffusible compounds (Ibrahim *et al.*, 2009). It is also possible that the drying process

could have caused disintergration reactions that lead to production of other non active chemicals (Omwenga *et al.*, 2009).

The absence of antimicrobial activity does not disapprove the ethnobotanical uses of the plants since the herbalists combine several plants. The herbalists use more than one plant to make a concoction for the management of a given disease because of the presence of different phytochemicals in different plants (Omwenga *et al.*, 2009). Synergy is a common concept in herbal medicine, suggesting that plant containing compounds potentiate each other's action (Koroishi *et al.*, 2008) while in this study, the bioassay test was done per plant. In addition to that the presence of some impurities has reduced the efficacy of the crude extracts (Ogundare, 2011).

Minimum inhibitory concentration is a quantitative assay and provides more information on the potency of the compounds present in the extracts (Korir *et al.,* 2012b). It was observed that there was no plant extract whose activity was equal to or more than that of the positive control while growth was observed in all concentrations in the tubes in the case of the negative control. The high MIC of crude extracts which was between 0.9375 mg/ml and 3.75 mg/ml compared to the standard drugs which was 0.4688 mg/ml is a clear indication that the active element in the crude extract was in low concentration which required the use of large amounts of crude extracts to increase the desired therapeutic effects (Korir *et al.*, 2012a). The lower the MIC the better the plant extract against the micro-organism tested (Korir *et al.*, 2012 b). *Albizia coriaria* Welw. ex Oliv and *Commiphora africana* (A. Rich) Eng. Syn gave MICs that were equal to MBCs of

1.875 mg/ml against MRSA. This clearly indicates that the concentration that inhibits the growth of MRSA is the same concentration of *Albizia coriaria* Welw. ex Oliv that kills the test organism. *Fuerstia africana* T. C. E Fries gave an MIC and MBC of 0.9375 mg/ml and 1.875 mg/ml respectively against MRSA. while *Vernonia amygdalina* Del, and *Zanthoxylum gilletii* De wild gave an MIC and MBC of 3.75 mg/ml and 7.5 mg/ml respectively while *Senna didymobotrya* Fresen gave an MIC and MBC of 1.875 mg/ml and 3.75 mg/ml respectively (Table 4). The crude extracts of medicinal plants at low concentrations shorten the length of treatment, reduce over dose and toxicity or side effects (Kitonde *et al*., 2013).

All the tested plants were screened for MIC and MBC against *B. subtilis* except *Urtica dioica* Linn and *Vernonia amygdalina* Del., *Fuerstia africana* T. C. E Fries and *Senna didymobotrya* Fresen gave a strong MIC and MBC against *B. subtilis* of 0.9375 mg/ml very close to that of the positive control value 0.4688 mg/ml. *Ficus sycomorus* Linn and *Melia volkensis* Gurke had a weak MIC and MBC of 3.75 mg/ml and 7.5 mg/ml respectively. *Zanthoxylum gilletii* De Wild *Albizia coriaria* Welw.ex Oliv, *Balanites aegyptiaca* L. Drel, *Psidia punctulata* (DC) Vatke, *Rhus natalensis* Krauss,, *Ricinus communis Linn, Senna didymobotrya* Fresen and *Fuerstia africana* T. C. E Fries had MICs that were equal to MBC. These 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).

Balanites aegyptiaca L. Drel., *Psidia punctulata* (DC) Vatke, *Ricinus communis* Linn, *Senna didymobotrya* Fresen, *Tamarindus indica* L. and *Fuerstia africana* T. C. E Fries showed a strong MIC and MBC of 0.9375 mg/ml against *S. aureus* ATCC 25923. Low MIC and MBC values are an indication of high efficacy against the organism in question (Doughari *et al.,* 2008). This study therefore provides scientific evidence of their efficacy against diarrhea caused by *S. aureus. Zanthoxylum gilletii* De Wild showed a high MIC and MBC of 3.75 mg/ml and 7.5 mg/ml therefore exhibits the lowest activity. Antimicrobial agents with low activity against a microbe have a high MIC while a very active antimicrobial agent has a low MIC (Banson and Adeyemo, 2007).

No plant extracts were screened against *E. coli* 25922 and *S. typhi* ATCC 19430 for MIC and MBC because all the plants were inactive on them. Lack of activity against *E. coli* could be attributed to the fact that *E. coli* are extended spectrum β-lactamase producers (ESBLS) and β-lactamase is one of the major causes of drug resistance (Mariita *et al.*, 2010a). *Escherichia coli* is known to quickly acquire drug resistance (Chandarana *et al.*, 2005). This could be the reason why all the plant extracts showed no efficacy against it.

Fungal strain especially *M. gypseum* gave the best MIC and MFC results. Ranging between 0.9375 mg/ml to 3.75 mg/ml with 7 plants giving MIC of 0.9375 mg/ml. *M. gypseum* was therefore the most susceptible microbe amongst the tested strains. *Psidia punctulata* (DC) Vatke, *Albizia coriara* Welw.ex Oliv, *Commiphora africana* (A. Rich) Engl. Syn *Senna didymobotrya* Fresen and *Tamarindus indica* L. produced similar

activity on MIC and MFC of 0.9375mg/ml against *M. gypseum*. These are very promising plants against *M. gypseum*, therefore supports the use of these plants for skin conditions.

Ficus sycomorus Linn and *Rhus natalensis* Krauss gave different values for MIC and MFC. The MIC was 0.9375 mg/ml and MFC 1.875 mg/ml for both plants against *M. gypseum*. *Boscia angustifolia* A. Rich and *Ricinus communis* Linn gave a low activity of 3.75 mg/ml for MIC and MFC for both plants. Among all the plant extracts tested against *C. neoformans* and *T. mentagrophytes* only *Fuerstia africana* T. C. E Fries was found to be active. It produced a similar MIC and MFC of 3.75 mg/ml against. *T. mentagrophytes* and a weak MIC and MFC of 3.75 mg/ml and 7.5 mg/ml respectively against *C. neoformans* 18310 (Table 5).This may be due to the high concentration of terpenoids which has cytotoxic activity against bacteria and fungi (Mamta and Jyoti, 2012). *Ricinus communis* Linn was the only plant screened against *A. niger* which had a similar MIC and MFC of 3.75 mg/ml. High values indicated that the micro-organism are less sensitive to the plant extracts (Obeidat *et al.*, 2012). No plant extracts were screened against *C. albicans* ATCC 90028 for MIC and MFC because all the plants were negative on it. This might have been brought about by the biofilms formed by *C. albicans* ATCC 90028 when single cells attach to a surface and grow into microcolonies which unite and form a complex 3 –D structure that is held together by hyphae and an exopolymer matrix (Lafleur, 2011).

The highest MIC and MBC/MFC values of the test microbes is an indication that either the plant extracts have low activity on the tested bacterial and fungal strains or that the

microorganisms have the potential of developing antibiotic resistance, while the low MIC and MBC/MFC values for the tested microbe is an indication that the plant extracts has the potential to treat any diseases associated with the pathogenic microbes effectively (Doughari and Manzara, 2008; Doughari, 2006). In this research all the active plant extracts were bacteriostatic/fungistatic since antibacterial substances are considered as bactericidal agents when the ratio MBC/MIC ≤ 4 and bacteriostatic agents when the ratio of MBC/MIC is ≥ 4 (Gatsing and Adoga, 2007).

5.2 CONCLUSIONS

The result of the study revealed that knowledge about the diseases treated by various plants is still maintained among the people of Kisii South, Rarieda and Mwingi North Districts. The survey shows that the community from Kisii South, Rarieda and Mwingi North Districts use medicinal plants to treat different ailments like gastrointestinal, respiratory problems, skin infections and other ailments. The leaves were the most frequently used parts of the plant and the most frequently used preparations for administration were concoctions and decoctions.

The present study indicates that the majority of the plants tested are important source of antibacterial agents especially on Gram positive bacteria and antifungal agents against the dermatophytes especially *M. gypseum*. Therefore supporting their uses by herbalists and locals for treatment of infectious diseases and giving scientific proof of their claimed efficacy against diarrhea.

The plant extracts showed activity against MRSA, *S. aureus* and *B. subtilis*. *Senna didymobotrya, Commiphora africana* (A. Rich) Engl. Syn, *Albizia coriaria* Welw. ex Oliv and *Fuerstia africana* T. C. E Fries exhi bited activity against the above three mentioned Gram positive bacteria. While *Ficus sycomorus* Linn., *Rhus natalensis* Krauss, *Psidia puntulata* (DC) Vatke, *Ricinus communis* Linn and *Tamarindus indica* L. also showed activity against *S. aureus* ATCC 25923 and *B. subtilis*. *Zanthoxylum chalybeum* Engl., *Melia volkensii* Gurke, *Sesbania sesban* Linn and *Ormocarpum trichocarpum* Taub. Engl. only inhibited *S. aureus* ATCC 25923. Therefore the plants can be used in treating diseases caused by MRSA, *S. aureus* ATCC 25923 and *B. subtilis* but not *E. coli* ATCC 25922 and *S. typhi* ATCC 19430.

The plant extracts were also active against *M. gypseum, T. mentagrophyte, C. neoformans* ATCC 18310 and *A. niger*. *Fuerstia africana* T. C. E Fries was the most effective in combating the pathogenic micro-organisms studied since it displayed antimicrobial activity against almost all the bacterial and fungal test cultures. *Fuerstia africana* T. C. E Fries showed a broad spectrum activity by inhibiting *M. gypseum, T. mentagrophyte, C. neoformans* ATCC 18310. The extracts of *Commiphora africana* (A. Rich) Engl. Syn, *Psidia puntulata* (DC) Vatke, *Senna didymobotrya* Fresen, *Albizia coriaria* Welw.ex Oliv, *Ficus sycomorus* Linn., *Rhus natalensis* Krauss, *Tamarindus indica* L. and *Ricinus communis* Linn showed activity against *M. gypseum*. No plant extract was found to be active against *C. albicans* ATCC 90028. Generally more extracts were found to be more active against bacteria than fungi.

The extracts of *Senna didymobotrya* Fresen and *Fuerstia africana* T. C. E Fries gave the strongest MIC and MBC of 0.9375 mg/ml against *B. subtitis* while *Psidia puntulata* (DC) Vatke, *Fuerstia africana* T. C. E Fries, *Senna didymobotrya* Fresen, *Tamarindus indica* L. and *Ricinus communis* Linn also gave the strongest MIC and MBC against *S. aureus* ATCC 25923. The plant extracts of *Commiphora africana* (A. Rich) Engl. Syn, *Psidia puntulata* (DC) Vatke, *Senna didymobotrya, Tamarindus indica* L. and *Albizia coriaria* Welw. ex Oliv produced similar activity on MIC and MBC of 0.9375 mg/ml against *M. gypseum.* Therefore the plants can be used for the treatment of diseases caused by *M. gypseum.*

The screening revealed the presence of various phytochemical compounds for example alkaloids, tannins, cardiac glycosides, terpenoids, saponins and flavonoids. All medicinal plants investigated were effective against *B. subtilis. Fuerstia africana* T. C. E Fries is widely used among the Kisii for a wide range of conditions. Hence this was supported by the broad spectrum of activity displayed by its crude extract making it the best amongst the crude extract used. *Melia volkensis* Gurke and *Urtica dioica* Linn were the least active plants.

5.3 RECOMMENDATIONS

i. Since this study has proved that the extracts of *Fuerstia africana* T. C. E Fries, *Albizia coriaria* Welw. ex Oliv, *Senna didymobotrya* Fresen, *Psidia puntulata* (DC) Vatke, *Tamarindus indica* L. and *Rhus natalensis Krauss* are readily

available sources of antifungal and antibacterial principles, their extracts should be subjected to both pharmacological and toxicological studies.

ii. The active principles responsible for antimicrobial activity in this study should be extracted and identified then screened against the selected bacterial and fungal pathogens used in their research.

iii. Bioassays of combinations of plant extracts that exhibited moderate and low activity for example *Boscia angustifolia* A. Rich, *Zanthoxylum gilletti* De Wild, *Ficus sycomorus* Linn, *Ormocarpum trichocarpum* Taub. Engl, *Rhus natalensis* Krauss, *Balanites aegyptiaca* L. Drel and *Ricinus communis* Linn should be carried out to establish any synergism between them.

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APPENDICES

Appendix 1: Consent form

Introduction

Hallo, I am India Jacqueline a student at Kenyatta University School of pure and applied sciences department of Microbiology. This information form seeks informed consent for your participation in a study that seeks to determine the efficacy of some medicinal plants used against infectious diseases caused by some selected bacteria and fungi.

Project title: Ef ficacy of some medicinal plants used in various parts of Kenya in treating selected bacterial and fungal pathogens.

Purpose: This is a study to determine the efficacy of some medicinal plants used in various parts of Kenya. The plants are used among the traditional communities in Kenya as herbal concoction but their efficacy has not been established. The results will provide scientific proof for their claimed medicinal use.

Procedure

If you consent to be in this study, I will ask you to answer survey questions in a private place at or near your home. The survey will take approximately 40 minutes. I will ask questions about medicinal plants and the diseases they treat. I will also ask how you acquire the knowledge on traditional medicinal. Example questions are: "which plants do you use routinely?", "How do you prepare these herbal drugs?", and "what diseases do they treat or prevent?". You do not have to answer any questions you do not want to answer.

Benefits of participation

You may not benefit directly from being in this study. However, the findings will provide useful information on the efficacy of the medicinal plants against the diseases caused by the selected bacterial and fungal pathogens.

Risks or discomforts

The risks of being in this study are low. Some of the questions are sensitive and you may feel uncomfortable giving all the information. You may also be inconvenienced because of the time involved.

Confidentiality

All the information in this study will be kept confidential. In order to protect your privacy, all hard copy data will be stored in designated lockers and access will be limited to the principle investigator. Using passwords only known to the principle will safeguard electronic data. Only people working directly on the study will be able to look at these records.

Voluntary participation

Being in this study is voluntary. You do not have to answer any question that you do not want to. You are free to stop taking part in this study at any time.

Who to contact

If you have questions about this research or feel you have been harmed by the research, please contact; India Jacqueline through 0722284276, the principle investigator of the study or Kenyatta University board of postgraduate studies.

Statement of consent

I have read and / or had this form explained to me. I understand the reason for the study. My questions have been answered. I agree to take part by my own choice.

_____ _____

_____

Participant`s name participant`s signature/ Thumb print date

_____ _____

_____

Witness`s name Witness`s signature date

(Applies for illiterate participants only)

I have explained purpose of this study to the participant, including the purpose, procedures, risks and benefits of the study. I have answered any questions she / he had.

_____ _____

_____

Investigator`s name Investigator`s signature Date

Appendix 2: Questionnaire

1. NAME ..... AGE (30-40) (40-50) (50-60) (70-ABOVE)
   COUNTY .....

   LOCATION .....

2. Which plants do you use routinely?
3. Name plants in your local language that you consider most medicinal.
4. How do you prepare these herbal drugs? Choose where applicable for each of the drugs you have mentioned. (Boiling, Soaking, burning, pounding, roasting).
5. What mode of drug application do you use? Chose where applicable for each of the plants you have chosen. (Inhaling, bathing, gurgling, swallowing, chewing, and other).
6. Do you get them locally or from elsewhere?
7. Where did you get these plants in the past and at present?
8. What diseases do they treat or prevent?
9. Pease list the signs by which you recognize the diseases?
10. What part is used?
11. Do you mix with other plants?
12. Where do you get your clients?
13. How many patients do you treat per day?
14. What is the availability of these plants?
15. Where did you get these plants in the past and at present?
16. How do you preserve these plants for future use?
17. How did you acquire the knowledge on traditional medicine?
18. Do you have any plans to pass this knowledge to your children and/or grandchildren etc?
19. How often do your customers come back after initial treatment?

Appendix 3 :One-way ANOVA, MRSA versus Plants

Analysis of Variance for MRSA

Source	DF	SS	MS	F	P
Plants	19	1633.500	85.974	*	*
Error	40	0.000	0.000		
Total	59	1633.500			

Individual 95% CIs For Mean

Based on Pooled StDev

Level	N	Mean	StDev	----------+---------+---------+------
Albizia	3	13.0000	0.0000	*
Balanite	3	6.0000	0.0000	*
Boscia a	3	6.0000	0.0000	*
Commipho	3	11.5000	0.0000	*
Ficus sy	3	8.5000	0.0000	*
Fuerstia	3	20.0000	0.0000	*
Melia vo	3	6.0000	0.0000	*
Neg. con	3	6.0000	0.0000	*
Ormocarp	3	6.0000	0.0000	*
post. co	3	26.0000	0.0000	*
Psidia p	3	6.0000	0.0000	*
Rhus nat	3	6.0000	0.0000	*
Ricinus	3	6.0000	0.0000	*
Senna di	3	11.0000	0.0000	*
Sesbania	3	6.0000	0.0000	*

Tamarind 3 6.0000 0.0000 *

Urtica d 3 6.0000 0.0000 *

Vernonia 3 9.0000 0.0000 *

Zanthoxy 3 6.0000 0.0000 *

Zanthoxy 3 9.0000 0.0000 *

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

Pooled StDev = 0.0000 12.0 18.0 24.0

Appendix 4: One-way ANOVA, *S. aureus* versus Plants

Analysis of Variance for *S. aureus*

Source	DF	SS	MS	F	P
Plants	19	1436.813	75.622	*	*
Error	40	0.000	0.000		
Total	59	1436.813			

Individual 95% CIs For Mean

Based on Pooled StDev

Level N Mean StDev --------+---------+---------+--------

Albizia 3 10.0000 0.0000 *

Balanite 3 14.5000 0.0000 *

Boscia a 3 6.0000 0.0000 *

Commipho 3 9.5000 0.0000 *

Ficus sy 3 11.5000 0.0000 *

Fuerstia 3 19.0000 0.0000 *

Melia vo 3 6.0000 0.0000 *

Neg. con 3 6.0000 0.0000 *

Ormocarp 3 8.5000 0.0000 *

post. co 3 24.0000 0.0000 *

Psidia p 3 14.0000 0.0000 *

Rhus nat 3 14.0000 0.0000 *

Ricinus 3 12.0000 0.0000 *

Senna di 3 16.0000 0.0000 *

Sesbania 3 8.0000 0.0000 *

Tamarind 3 16.0000 0.0000 *

Urtica d 3 7.5000 0.0000 *

Vernonia 3 6.0000 0.0000 *

Zanthoxy 3 6.0000 0.0000 *

Zanthoxy 3 8.0000 0.0000 *

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

Pooled StDev = 0.0000 10.0 15.0 20.0

Appendix 5: One-way ANOVA, *Salmonella* versus Plants

Analysis of Variance for *Salmonella*

Source	DF	SS	MS	F	P
Plants	19	823.6500	43.3500	*	*
Error	40	0.0000	0.0000		
Total	59	823.6500			

Individual 95% CIs For Mean

Based on Pooled StDev

Level	N	Mean	StDev	--------+---------+---------+--------
Albizia	3	6.0000	0.0000	*
Balanite	3	6.0000	0.0000	*
Boscia a	3	6.0000	0.0000	*
Commipho	3	6.0000	0.0000	*
Ficus sy	3	6.0000	0.0000	*
Fuerstia	3	6.0000	0.0000	*
Melia vo	3	6.0000	0.0000	*
Neg. con	3	6.0000	0.0000	*
Ormocarp	3	6.0000	0.0000	*
post. co	3	23.0000	0.0000	*
Psidia p	3	6.0000	0.0000	*
Rhus nat	3	6.0000	0.0000	*
Ricinus	3	6.0000	0.0000	*
Senna di	3	6.0000	0.0000	*
Sesbania	3	6.0000	0.0000	*
Tamarind	3	6.0000	0.0000	*
Urtica d	3	6.0000	0.0000	*
Vernonia	3	6.0000	0.0000	*
Zanthoxy	3	6.0000	0.0000	*
Zanthoxy	3	6.0000	0.0000	*

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

Pooled StDev = 0.0000 10.0 15.0 20.0

Appendix 6: One-way ANOVA, *E. coli* versus Plants

Analysis of Variance for *E. coli*

Source	DF	SS	MS	F	P
Plants	19	1083.713	57.038	1.0E+16	0.000
Error	40	0.000	0.000		
Total	59	1083.713			

Individual 95% CIs For Mean

Based on Pooled StDev

Level	N	Mean	StDev	-+---------+---------+---------+-----
Albizia	3	6.0000	0.0000	*
Balanite	3	6.0000	0.0000	*
Boscia a	3	6.0000	0.0000	*
Commipho	3	6.0000	0.0000	*
Ficus sy	3	6.0000	0.0000	*
Fuerstia	3	6.0000	0.0000	*
Melia vo	3	6.0000	0.0000	*
Neg. con	3	6.0000	0.0000	*
Ormocarp	3	6.0000	0.0000	*
post. co	3	25.5000	0.0000	(*
Psidia p	3	6.0000	0.0000	*
Rhus nat	3	6.0000	0.0000	*
Ricinus	3	6.0000	0.0000	*
Senna di	3	6.0000	0.0000	*
Sesbania	3	6.0000	0.0000	*

Tamarind 3 6.0000 0.0000 *

Urtica d 3 6.0000 0.0000 *

Vernonia 3 6.0000 0.0000 *

Zanthoxy 3 6.0000 0.0000 *

Zanthoxy 3 6.0000 0.0000 *

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

Pooled StDev = 0.0000 6.0 12.0 18.0 24.0

Appendix 7: One-way ANOVA, *B. subtilis* versus Plants

Analysis of Variance for *B. subtilis*

Source	DF	SS	MS	F	P
Plants	19	1059.413	55.759	9.8E+15	0.000
Error	40	0.000	0.000		
Total	59	1059.413			

Individual 95% CIs For Mean

Based on Pooled StDev

Level N Mean StDev -+---------+---------+---------+-----

Albizia 3 12.0000 0.0000 *

Balanite 3 12.0000 0.0000 *

Boscia a 3 13.0000 0.0000 *

Commipho 3 12.0000 0.0000 *

Ficus sy 3 9.5000 0.0000 *

Fuerstia 3 17.0000 0.0000 *

Melia vo 3 10.0000 0.0000 *

Neg. con 3 6.0000 0.0000 *

Ormocarp 3 15.5000 0.0000 *

post. co 3 26.0000 0.0000 *

Psidia p 3 11.0000 0.0000 *

Rhus nat 3 11.5000 0.0000 *

Ricinus 3 13.0000 0.0000 *

Senna di 3 16.0000 0.0000 *

Sesbania 3 11.0000 0.0000 *

Tamarind 3 15.0000 0.0000 *

Urtica d 3 8.0000 0.0000 *

Vernonia 3 7.0000 0.0000 *

Zanthoxy 3 15.0000 0.0000 *

Zanthoxy 3 11.0000 0.0000 *

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

Pooled StDev = 0.0000 6.0 12.0 18.0 24.0

Appendix 8: One-way ANOVA, *Candida* versus Plants

Analysis of Variance for *Candida*

Source	DF	SS	MS	F	P
Plants	19	228.1500	12.0079	8.4E+15	0.000
Error	40	0.0000	0.0000		
Total	59	228.1500			

Individual 95% CIs For Mean

Based on Pooled StDev

Level	N	Mean	StDev	-+---------+---------+---------+-----
Albizia	3	6.0000	0.0000	*
Balanite	3	6.0000	0.0000	*
Boscia a	3	6.0000	0.0000	*
Commipho	3	6.0000	0.0000	*
Ficus sy	3	6.0000	0.0000	*
Fuerstia	3	6.0000	0.0000	*
Melia vo	3	6.0000	0.0000	*
Neg. con	3	6.0000	0.0000	*
Ormocarp	3	6.0000	0.0000	*
post. co	3	12.5000	0.0000	(*
Psidia p	3	6.0000	0.0000	*
Rhus nat	3	6.0000	0.0000	*
Ricinus	3	6.0000	0.0000	*
Senna di	3	6.0000	0.0000	*
Sesbania	3	12.5000	0.0000	(*
Tamarind	3	6.0000	0.0000	*
Urtica d	3	6.0000	0.0000	*
Vernonia	3	6.0000	0.0000	*
Zanthoxy	3	6.0000	0.0000	*
Zanthoxy	3	6.0000	0.0000	*

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

Pooled StDev = 0.0000 6.0 8.0 10.0 12.0

Appendix 9: One-way ANOVA, *C. neoformans* versus Plants

Analysis of Variance for *C. neoformans*

Source	DF	SS	MS	F	P
Plants	19	159.0000	8.3684	*	*
Error	40	0.0000	0.0000		
Total	59	159.0000			

Individual 95% CIs For Mean

Based on Pooled StDev

Level	N	Mean	StDev	----------+---------+---------+------
Albizia	3	6.0000	0.0000	*
Balanite	3	6.0000	0.0000	*
Boscia a	3	6.0000	0.0000	*
Commipho	3	6.0000	0.0000	*
Ficus sy	3	6.0000	0.0000	*
Fuerstia	3	9.0000	0.0000	*
Melia vo	3	6.0000	0.0000	*
Neg. con	3	6.0000	0.0000	*
Ormocarp	3	6.0000	0.0000	*
post. co	3	13.0000	0.0000	*
Psidia p	3	6.0000	0.0000	*
Rhus nat	3	6.0000	0.0000	*
Ricinus	3	6.0000	0.0000	*
Senna di	3	6.0000	0.0000	*
Sesbania	3	6.0000	0.0000	*

Tamarind 3 6.0000 0.0000 *

Urtica d 3 6.0000 0.0000 *

Vernonia 3 6.0000 0.0000 *

Zanthoxy 3 6.0000 0.0000 *

Zanthoxy 3 6.0000 0.0000 *

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

Pooled StDev = 0.0000 8.0 10.0 12.0

Appendix 10: One-way ANOVA, *A. niger* versus Plants

Analysis of Variance for *A. niger*

Source	DF	SS	MS	F	P
Plants	19	474.1500	24.9553	1.8E+16	0.000
Error	40	0.0000	0.0000		
Total	59	474.1500			

Individual 95% CIs For Mean

Based on Pooled StDev

Level N Mean StDev ---+---+---+---+---

Albizia 3 6.0000 0.0000 *

Balanite 3 6.0000 0.0000 *

Boscia a 3 6.0000 0.0000 *

Commipho 3 6.0000 0.0000 *

Ficus sy 3 6.0000 0.0000 *

Fuerstia 3 8.0000 0.0000 *

Melia vo 3 6.0000 0.0000 *

Neg. con 3 6.0000 0.0000 *

Ormocarp 3 6.0000 0.0000 *

post. co 3 18.0000 0.0000 *

Psidia p 3 6.0000 0.0000 *

Rhus nat 3 6.0000 0.0000 *

Ricinus 3 11.5000 0.0000 *

Senna di 3 6.0000 0.0000 *

Sesbania 3 8.0000 0.0000 *

Tamarind 3 7.5000 0.0000 *

Urtica d 3 6.0000 0.0000 *

Vernonia 3 6.0000 0.0000 *

Zanthoxy 3 6.0000 0.0000 *

Zanthoxy 3 6.0000 0.0000 *

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

Pooled StDev = 0.0000 7.0 10.5 14.0 17.5

Appendix 11: One-way ANOVA, *T. mentagrophyte* versus Plants

Analysis of Variance for *T. mentagrophyte*

Source	DF	SS	MS	F	P
Plants	19	716.8500	37.7289	6.6E+15	0.000
Error	40	0.0000	0.0000		
Total	59	716.8500			

Individual 95% CIs For Mean

Based on Pooled StDev

Level	N	Mean	StDev	---------+---------+---------+-------
Albizia	3	6.0000	0.0000	*
Balanite	3	6.0000	0.0000	*
Boscia a	3	6.0000	0.0000	*
Commipho	3	6.0000	0.0000	*
Ficus sy	3	6.0000	0.0000	*
Fuerstia	3	12.0000	0.0000	*
Melia vo	3	6.0000	0.0000	*
Neg. con	3	6.0000	0.0000	*
Ormocarp	3	6.0000	0.0000	*
post. co	3	21.0000	0.0000	*
Psidia p	3	6.0000	0.0000	*
Rhus nat	3	6.0000	0.0000	*
Ricinus	3	6.0000	0.0000	*
Senna di	3	6.0000	0.0000	*
Sesbania	3	6.0000	0.0000	*
Tamarind	3	6.0000	0.0000	*
Urtica d	3	6.0000	0.0000	*
Vernonia	3	6.0000	0.0000	*
Zanthoxy	3	6.0000	0.0000	*
Zanthoxy	3	6.0000	0.0000	*

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

Pooled StDev = 0.0000 10.0 15.0 20.0

Appendix 12: One-way ANOVA, *M. gypseum* versus Plants

Analysis of Variance for *M. gypseum*

Source	DF	SS	MS	F	P
Plants	19	1763.212	92.801	4.1E+15	0.000
Error	40	0.000	0.000		
Total	59	1763.213			

Individual 95% CIs For Mean

Based on Pooled StDev

Level	N	Mean	StDev	---------+---------+---------+-------
Albizia	3	16.0000	0.0000	*
Balanite	3	6.0000	0.0000	*
Boscia a	3	10.0000	0.0000	*
Commipho	3	17.5000	0.0000	*
Ficus sy	3	15.5000	0.0000	*
Fuerstia	3	13.0000	0.0000	*
Melia vo	3	6.0000	0.0000	*
Neg. con	3	6.0000	0.0000	*
Ormocarp	3	8.5000	0.0000	*
post. co	3	22.0000	0.0000	*
Psidia p	3	20.5000	0.0000	*
Rhus nat	3	15.5000	0.0000	*
Ricinus	3	10.0000	0.0000	*
Senna di	3	17.0000	0.0000	*
Sesbania	3	6.0000	0.0000	*

Tamarind 3 16.0000 0.0000 *

Urtica d 3 6.0000 0.0000 *

Vernonia 3 6.0000 0.0000 *

Zanthoxy 3 6.0000 0.0000 *

Zanthoxy 3 6.0000 0.0000 *

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

Pooled StDev = 0.0000 10.0 15.0 20.0