Antimicrobial Properties of Medicinal Plants used among the Kipsigis Community in Kenya

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DECLARATION

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

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DEDICATION

I dedicate this work to my wife Jacqueline Korir and my two sons Kiprotich and Kipruto. For you I will undertake all that is desirable within my reach.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ATCC	American type culture collection
CFU	Colony forming units
CLSI	Clinical Laboratory Standard Institute
D.N.A	Deoxyribonucleic acid
DCM	Dichloromethane
DMSO	Dimethylsulfoxide
FBS	Featal bovine serum
FDA	Food and drugs administration
HELF	Human embryonic lung fibroblast
HIV	Human immunodeficiency virus
LD	Lethal dose
MBC	Minimum bactericidal concentration
MEM	Minimum essential media
MIC	Minimum inhibition concentration
MRSA	Methicillin resistant *Staphylococcus aureaus*
NCCLS	National committee on clinical laboratory standards
PABA	Para aminobenzoic acid
PBP	Penicillin binding proteins
SPSS	Statistical package for social scientists
W.H.O	World Health Organization

ABSTRACT

The use of plants for medicinal purposes is an important part of the culture and tradition in Africa and the world at large. Up to 80% of the world's population depends directly on traditional medicine for health care. The main aim of this study was to determine antimicrobial properties of selected medicinal plants used among the Kipsigis community in Kenya. The plant materials were collected and taken to the KEMRI for processing. Four medicinal plants extracts were screened against selected bacteria and fungi. Inhibition zones diameters, minimum inhibitory concentrations, phytochemical profile and toxicity of the plants extracts determined. The most active plant extract against bacteria isolates was *C. polycephala* hexane extracts which had an MIC of 3.125 $x10^2$ μg/ml against methicillin resistant *Staphylococcus aureus*. Water extracts of *C. polycephala* was toxic with CV of 23.75% and 31.56% at 1000mg/ml and 5000mg/ml, respectively. Dichloromethane extracts of *S. didymobotrya* killed 80% of mice at a dose of 5000 mg/kg and 40% at 1000 mg/kg. The extracts had different chemical compounds that are responsible for the activities against the microbes. Significant variability within groups of plants solvents and organisms at 95% confidence interval (>p 0.05) were found to be significant. In both the cell and acute toxicity, it was observed that extracts at high concentration and at a high dose are toxic. The antimicrobial activity of the plants under investigation demonstrated support for the claimed antimicrobial uses of the plants in the traditional medicine and provides scientific proof for their medicinal uses.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 Background

It is estimated by W.H.O that 80% of the population, majority of this in developing countries, still rely on plant-based medicine for primary their health care (Mandal and Saunders, 1997). Herbal drugs are prepared from various parts of the plant such as leaves, stem, roots, seeds, tubers or exudates (Mukherjee, 2002). There are more than 1,340 plants known to be potential sources of antimicrobial compounds but few have been systematically studied scientifically (Wilkins and Board, 1989). Some plant like *Heteromorpha trifoliate* (wendl. Eckl and Zeyh) is used traditionally to treat stomach problems. It has been shown to have strong activity against several species of bacteria including *Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella gallinarum* and S*taphylococcus albus* (Desta, 1993; Baker *et al*., 2006). Although plants have been extensively used, specific evaluation on toxicity has not been done and could lead to serious complications (Kirby *et al.*, 1996).

However in 1953, during the outbreak of shigellosis in Japan, a strain of *Bacillus* causing dysentery was isolated which showed multiple drug resistance (WHO, 2004). Today, other microorganisms, such as M*ycobacterium tuberculosis* have developed multiple drug resistance (Ruddy *et al.*, 2004; Githui *et al*., 2008). This is one of the most prevalent and life threatening infectious disease in developing world today, as it is resistant to all first line drugs such as isoniazid and rifampicin (Viskum and Kok-jensen, 1997).

Indiscriminate exposure to antibiotics in patients hastens bacteria mutations that bring about drug resistance. This situation produces bacteria with greater ability to survive even the strongest antibiotics (WHO, 2002). More than 70% of the bacteria causing these infections are resistant to at least one of the drugs commonly used to treat them. Persons infected with drug resistant strains are more likely to have longer hospital stay and require treatment with second or third line drugs that may be effective but more toxic and expensive. Drug resistant bacteria have mainly been of nosocomial origin (Brun-Buisson *et al.*, 1987)

1.2 Microbial opportunistic infections

The antimicrobial resistance problem has been worsened by HIV pandemic and other immune suppressing conditions especially in individuals with cancer, organ transplant, stress and old age have lead to the emergence of opportunistic infection thereby leading to indiscriminate use of antibiotics (WHO, 2004). Human Immunodeficiency Virus (HIV)/ Acquired immune deficiency syndrome (AIDS) a disease of the human immune system (Rhodes, 1988; Sepkowitz and Sies, 2001). It is the most important public health problem in sub Saharan Africa today. The HIV virus progressively damages or reduces the effectiveness of the immune system and leaves individuals susceptible to opportunistic pathogens. Bacteria and fungi opportunistic infections accounts for up to 70% of HIV and AIDS defining illness (Sepkowitz and Sies, 2001; Shailaja, 2004). The opportunistic infections include bacterial pneumonia which is higher; Pneumocystis *pneumonia* (originally known as *Pneumocystis carinii* pneumonia or Pneumocystis pneumonia), Tuberculosis (TB) is unique among infections associated with HIV because it is transmissible to

immunocompetent people via the respiratory route (Shailaja, 2004). In early-stages of HIV infection (CD4 count >300 cells per µL), TB typically presents as a pulmonary disease. In advanced HIV infection, TB often presents atypically with extrapulmonary (systemic) clinical feature (Sepkowitz and Seis, 2001). Gastrointestinal infections which lead to unexplained chronic diarrhea in immune compromise persons is due to many possible causes, including common bacterial e.g. *Salmonellosis,* Pathogenic *E. coli* (Sadler, 1997).

Fungal infections including yeast and dermatosis are common cause of dermatological conditions in immune compromised individuals (Miller *et al.,* 2006; Vikin, 2006; Burns, 2006). While thousands of skin disorders have been described, only a small number account for most visits to the doctor (Burns, 2006). Fungal infection in immunocompromised patients is the leading cause of mortality and morbidity (Goldsmith and Lowel, 1983). The antimicrobial resistance to the available drugs is driving up healthcare costs, increasing the severity of disease, morbidity and mortality. The situation calls for urgent research on any potential source of new effective antimicrobials. Plants products are some of such sources, which have not been exhaustively utilized. In this study, selected medicinal plants used by the Kipsigis ethnic community of Kenya, were subjected to a systematic screening for antimicrobial activities.

1.3 Antimicrobial agents

An antimicrobial is a substance that kills (microbicidal) or inhibits (microbistatic) the growth of microbes such as bacteria, fungi, parasites or viruses (Davey and Warrell,

2000). Antimicrobial agents act in various ways; they either disrupt microbial processes or structures that differ from those of the host (Atlas, 1995). Damage to the pathogen may occur by inhibition of cell wall synthesis, inhibition of protein and nucleic acid synthesis, disrupting membrane structure and function or blocking metabolic pathways through inhibition of key enzymes. The drugs vary considerably in their range of effectiveness that is others have narrow spectrum while others have broad spectrum of action (Atlas, 1995).

The history of antimicrobials begins in 19th century with the observations of early scientist, who discovered that one type of bacteria could prevent the growth of another. They did not know at that time that the reason one bacterium failed to grow was that the other bacterium was producing an antibiotic (Atlas, 1995). Technically, antibiotics are only those substances that are produced by one microorganism that kill, or prevent the growth, of another microorganism. In today's common usage, the term antibiotic is used to refer to almost any drug that cures a bacterial infection. Antimicrobials include not only antibiotics, but also synthetic compounds (Seppala *et al.,* 1995). The era of antimicrobial chemotherapy began with the discovery of penicillin (Fleming's, 1929). This was followed by synthetic chemicals and sulfonamides, with broad antimicrobial activity (Domagk, 1935). These natural products were not only found to cure infections but also to possess incredibly low toxicity to animals (Benton *et al.,* 2003; Kirby *et al.,* 1996). This fact ushered in the age of antibiotic chemotherapy and an intense search for similar antimicrobial agents that were useful in the treatment of infectious diseases. With advances in medicinal chemistry, most antibiotics are now modified chemically from original compounds

found in nature, as is the case with beta-lactams (which include the penicillins, produced by fungi in the genus Penicillium, the Cephalosporin and the carbapenems). Some antibiotics are still produced and isolated from living organisms, such as the aminoglycosides; in addition, many more have been created through purely synthetic means, such as the quinolones (Finberg *et al*., 2004). The discovery of antimicrobials like penicillin and tetracycline paved the way for better health for millions around the world; no true cure for gonorrhea, strep throat, or pneumonia existed. Patients with infected wounds often had to have a wounded limb removed, or face death from infection. At present most of these infections can be easily cured with a short course of antimicrobials (Atlas, 1995).

However, the future effectiveness of antimicrobial therapy is somewhat in doubt. Microorganisms, especially bacteria, are becoming resistant to more and more antimicrobial agents. Bacteria found in hospitals appear to be especially resistant and are causes a lot of suffering to hospitalized patients. Bacterial resistance can only be combated by the discovery of new drugs. Microorganisms are becoming resistant more quickly than new drugs are being discovered. Thus, future research in antimicrobial therapy may focus on finding how to overcome resistance to antimicrobials, or how to treat infections with alternative means (Atlas, 1995). Herbal medicines are potential candidates for antimicrobial alternatives.

1.4 Antibiotic resistance

Antibacterial drugs (antibiotics) may target bacterial cell wall (penicillins, cephallosporins), cell membrane (polymixins,) and bacterial enzymes (quinolones

and sulfonamides which are bactericidal in nature). Those which target protein synthesis such as the aminoglycosides, macrolides and tetracyclines are usually bacteristatic (Finberg *et al.,* 2004). The emergence of antibiotic resistance is an evolutionary process that is based on selection for organisms that have enhanced ability to survive doses of antibiotics that would previously be lethal (Finberg, 2008). Antibiotics like penicillin and erythromycin which used to be one-time miracle cures are now less effective because bacteria have become more resistant (Pearson, 2007). Survival of bacteria often results from an inheritable resistance (Witte, 2004). Antibiotics themselves act as a selective pressure which allows the growth of resistant bacteria within a population and inhibits susceptible bacteria (Alekshun and Levy, 2007).

The underlying molecular mechanisms leading to antibiotic resistance can vary. Intrinsic resistance may naturally occur as a result of the bacteria's genetic makeup. The bacterial chromosome may fail to encode a protein which the antibiotic targets. Acquired resistance results from a mutation in the bacterial chromosome or the acquisition of extra-chromosomal DNA (Levy, 1994). The spread of antibiotic resistance between different bacteria may also be mediated by horizontal transfer of plasmids that carry genes which encode antibiotic resistance which may result in co-resistance to multiple antibiotics (Courvalin, 2001). These plasmids can carry different genes with diverse resistance mechanisms to unrelated antibiotics but because they are located on the same plasmid resistances to more than one antibiotic is transferred. Alternatively, cross-resistance to other antibiotics within the bacteria results when the same resistance mechanism is responsible for resistance to more

than one antibiotic (Baker *et al.,* 2006). Over use of broad-spectrum antibiotics, such as second- and third-generation cephalosporins, greatly hastens the development of methicillin resistance (Menhard *et al.,* 1993). Other factors contributing towards resistance include incorrect diagnosis, unnecessary prescriptions, improper use of antibiotics by patients, the impregnation of household items and children's toys with low levels of antibiotics, and the use of antibiotics as livestock food additives for growth promotion (Anaissie *et al.,* 1989; Seppala *et al.,* 1995; Benton *et al.,* 2003; Maubareck and Bourgiosi, 2003). These factors have lead to high incidence of resistance to commonly used antibiotics (White *et al.,* 1998). Antimicrobial resistance poses a major health problem and calls for search of alternative medicine (Anaissie and Bodey, 1993; Courvalin, 2001).

1.5 Antifungal agents

Antifungal drugs work by exploiting differences between mammalian and fungal cells to kill off the fungal organism without toxic effects to the host. Unlike bacteria, both fungi and humans are eukaryotes. Thus fungal and human cells are similar at the molecular level. This means it is more difficult to find a weakness in fungi to attack that does not also exist in human cells. However the fungal cell wall may be considered to be a prime target for selectively toxic to antifungal agents because of its chitin structure which is absent from human cells. No clinically available inhibitor of chitin synthesis analogous to the beta-lactams exists at present, even though much effort is being directed towards developing such agents. Other targets are currently being explored (Finberg *et al*., 2004). Consequently, these drugs require high doses resulting in unwanted side effects which can be life-threatening. There are relatively

few agents that can be used to treat fungal infections with relatively few side effects. Most of the antifungal drugs are known to be toxic to cells, liver and kidneys. Examples of antifungal drugs in use currently include the following: - Polyene antifungals, Imidazole and Triazole antifungals: Benzoic acid, Ciclopirox, Tolnaftate, Undecylenic acid – organic unsaturated fatty acids, Flucytosine, or 5-fluorocytosine, Haloprogin Griseofulvin, Allylamines and Echinocandins (Finberg, 2008). However, emerging drug resistant fungal strains are increasingly reported (White *et al.*, 2002; Buwa and Staden, 2006). Due to these shortcomings research on alternative antifungal drugs is needed.

1.6 Antimicrobial alternatives

Herbal medicine, also called botanical medicine or phytomedicine, refers to the use of any plant's material either ; seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Long practiced outside of conventional medicine, herbal remedies are becoming common as up-to-date analysis and research show their value in the treatment and prevention of disease. Herbs are plants or plant parts used for its scent, flavor or therapeutic properties. Many herbs have been used for a long time because of their claimed health benefits. They are sold as tablets, capsules, powders, teas, extracts and fresh or dried plants. While some may be effective, others may cause health problems, while some may not be effective or may interact with conventional drugs (Barrett and Lawrence, 1999). Plants have been used for medicinal purposes long before recorded history. For example, ancient Chinese and Egyptian papyrus writings describe medicinal plant uses. Indigenous cultures such as African and native American used herbs in their healing rituals, while others

developed traditional medical systems including Ayurveda and traditional Chinese medicine in which herbal therapies were used systematically. Scientific findings indicate that people in different parts of the globe tended to use the same or similar plants for the same purposes (Ang-Lee *et al.,* 2008). In the early 19th century, when methods of chemical analysis first became available, scientists began extracting and modifying the active ingredients from plants. Later chemists began making their own version of plant compounds, beginning the transition from raw herbs to synthetic pharmaceuticals. Over time, the use of herbal medicines declined in favor of pharmaceuticals (Blumenthal *et al.,* 2000).

Recently, the World Health Organization estimated that 80% of people worldwide rely on herbal medicines for some aspect of their primary healthcare (WHO, 2004). In the last twenty years in the United States of America, increasing public dissatisfaction with the cost of prescribed medications, combined with an interest in returning to natural or organic remedies, has led to an increase in the use of herbal medicines. In Germany, roughly 600 to 700 plant-based medicines are available and are prescribed by approximately 70% of German physicians (Barrett and Lawrence, 1999). Herbalists prefer using crude extracts rather than extracting single components from them. Whole plant extracts have many components. These components work together to produce therapeutic effects and also to lessen the chances of side effects from any one component. Several herbs are often used together to enhance effectiveness and synergistic actions and to reduce toxicity (Blumenthal *et al.,* 2000).

1.7 Fungal infections

1.7.1 Candidiasis

Candidiasis is a fungal infection of any of the *Candida* species, of which *Candida albicans* is the most common (Walsh and Dixon, 1996; Fidel, 2002). Candidiasis encompasses infections that range from superficial, such as oral thrush and vaginitis, to systemic and potentially life-threatening diseases. *Candida species* infections of the latter category are also referred to as candidemia and are usually confined to severely immuno-compromised persons, such as cancer, transplant and AIDS patients (Paus and Cotsareli, 1999). While clearly attributable to the presence of the opportunistic pathogens of the genus *Candida*, candidiasis describes a number of different disease syndromes that often differ in their causes and outcomes (Fidel, 2002). Commonly referred to as a yeast infection, it is also technically known as candidosis, moniliasis, and oidiomycosis (Saunders and Sinkuna, 2006).

1.7.2 *Cryptococcus neoformans*

Cryptococcus neoformans is an encapsulated yeast-like fungus and an important opportunistic pathogen in immunocompromised individuals. The fungus is very common in the environment mainly in soil, dirt and bird droppings. It enters into the body through inhalation into the lungs through to the blood, spinal column and the brain where it causes severe disease conditions (Eisenman, 2007). Infection with *C. neoformans* is termed cryptococcosis. Meningitis, especially as a secondary infection in AIDS patients, is often caused by *C. neoformans*. Cryptococcus can also infect the skin, lungs, or other parts of the body (Saag *et al.,* 2000; Martínez *et al.,* 2000; Loftus, 2005; Dadachova, 2007).

1.7.3 Dermatophytoses

Dermatophytoses is mainly caused by three genera of fungi namely *Trichophyton, Microsporum,* and *Epidermophyton* which causes infections in animals and humans. The three genera cause infections of the skin, hair and nails due to their ability to obtain nutrients from keratinized material. They are usually restricted to the nonliving cornified layer of the epidermis because of their inability to penetrate viable tissue of an immunocompetent host. Some of these infections are known as ringworm or tinea. The infectious agents can be acquired from contacts from infected individuals, animals, or contaminated soil (Van Rooji *et al.,* 2006).

1.8 Bacterial infections

1.8.1 *Escherichia coli*

The *E. coli* are normal flora in the guts of mammals and naturally do not cause infection. However there is pathogenic *E. coli* which causes different diseases syndromes like O157:H7 which is an enterohemorrhagic strain causing foodborne illness (Karch *et al.,* 2005). Infection often leads to bloody diarrhea, and occasionally to kidney failure, especially in young children and elderly people including HIV/AIDS patients due to their low immunity and may lead to haemolytic uremic syndrome (Remis *et al.,* 1983; Rendón, 2007).

1.8.2 *Staphylococcus aureus*

Staphylococcus aureus is extremely prevalent in atopic dermatitis patients, who are less resistant to it than other people (Kent *et al.,* 1997; Whitt *et al.,* 2002). It is difficult to treat patients with lowered immunity (Curran, 1980). Methicillin

reisistant *S. aureus* is by definition a strain of *Staphylococcus aureus* that is resistant to a large group of antibiotics called the beta-lactams which include penicillins and the cephalosporins (Raygada and Levine, 2009). The MRSA has evolved an ability to survive treatment with beta-lactam antibiotics and is very troublesome in hospital-associated (nosocomial) infections (Okuma *et al.,* 2002). In hospitals, patients with open wounds, invasive devices, and weakened immune systems are at greater risk for infection (Klein *et al.,* 2007).

1.8.3 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a common bacterium which can cause disease in animals and humans. It is found in soil, water, skin flora and most man-made environments throughout the world. It thrives not only in normal atmospheres, but also with little oxygen, and has thus colonized many natural and artificial environments (Walker *et al.,* 2004). An opportunistic pathogen of immunocompromised individuals, *P. aeruginosa* typically infects the pulmonary tract, urinary tract, burns, wounds and other blood infections (Ryan, 2004). It is the most common cause of infections of burn injuries and of the external ear (otitis externa) and is the most frequent colonizer of medical devices like catheters. *Pseudomonas species* can, in rare circumstances, cause community-acquired pneumonias (Anzai, 2000).

1.8.4 *Klebsiella pneumoniae*

Klebsiella pneumoniae is a normal flora of the mouth, skin, and intestines (Ryan, 2004). It is clinically the most important member of the *Klebsiella* genus of Enterobacteriaceae; it is closely related to *K. oxytoca* from which it is distinguished

by being indole-negative and by its ability to grow on both melezitose and 3-hydroxybutyrate (Podschun *et al.*, 1998). It naturally occurs in the soil and about 30% of strains can fix nitrogen in anaerobic condition (Postgate, 1998).

1.9 Plants under investigation

In Kenya about 400 plants species, which are used in traditional remedies has been documented by Kokwaro, (1993). Information obtained during the pilot study indicates that the use of traditional medicine among the Kipsigis community is very common due to cultural beliefs coupled with scarcity of resources to access conventional medicine. Plants with medicinal values exist in natural forest and are easily accessible.

Senna didymobotrya (Fresen) Irwin and Barneby (Caesalpinaceae) is a common plant in Bomet District. It is used traditionally by the Kipsigis community for the control of malaria as well as diarrhea. It is used to treat skin conditions in humans and widely used to treat livestock infections in Kenya as documented by Thoithi *et al.*, (2006). Products from *Senna spp* has not been evaluated by the Food and Drugs Administration (FDA) for safety, effectiveness, or purity. All potential risks and/or advantages of *S. didymobotrya* and other *Senna species* may not be known. Additionally, there are no regulated manufacturing standards in place for these compounds (WHO, 2004). Antimicrobial properties of this plant from Bomet district have not been documented. Plate 1.1 shows *Senna didymobotrya* branch with flowers and leaves.

Plate 1. 1: Branch of *Senna didymobotrya* with leaves and flowers

Clutia abyssinic Jaub. and Spach var. abyssinica "*Kurbanyat"* belong to the family Euphorbiaceae. It is a shrub of up to 2m high, sparsely branched with glabrous twigs. The leaves are lanceolate or elliptic lanceolate, tapering to obtuse apex at the base. It is distributed in dry grassland especially in Kenya, Tanzania, Zimbabwe, Ethiopia and South Africa. The plant extract is used traditionally in preparation of malaria remedy; leaves are used as an oral decoction for the treatment of hepatitis, externally as a local anti-inflammatory and as a medication for sores. The root extract is employed in the treatment of threatened abortion, convulsion, influenza, enlarged spleen and for the management of habitual miscarriage. Weak infusions of the roots are administered for stomachache and chest pain. Methanol extracts has been shown to exact weak relaxant activity on guinea pigs smooth muscles, uterine stimulant effect and no effect on skeletal muscles (Vlietinck *et al.,* 2000). Alcohol extract of *C. richardiana* exhibited activity against both Gram positive and Gram negative

bacteria (Hugo *et al.,* 2004). Extract of *Clutia spp* has been found to be devoid of *in-vitro* antimalarial or cytotoxicty activity (Maubareck, 2003). The antimicrobial properties of this plant have not been determined in our local environment despite the fact that it has been widely studied in Rwanda and Tanzania as documented by Hugo *et al.,* (2004) and Vlietinck *et al.,* (2000). Boiled leaves of *C. abyssinica* are used traditionally to treat malaria while boiled leaves and roots are used for headaches and stomach problems (Kokwaro, 2000). The leaves and bark are burned into ashes, mixed with margarine and used traditionally to treat oral thrash by the Kipsigis community in Kenya. Plate 1.2 shows *Clutia abyssinica* plant with branches and leaves.

Plate 1. 2: Leaves and branches of *Clutia abyssinica*

Cyathula polycephala auct. "Cheputpich" Belong to the family Amaranthaceae, (Judd, 2008). It is a genus of medicinal and ornamental plants and the species are distributed in Africa, Asia, Oceania and the Americas (Hugo *et al.,* 2004). The leaves are burned into ashes and are used to treat diabetes among the Mbeere and Ameru communities in Kenya (Kareru, 2007). Brine shrimp toxicity as well as

antiplasmodial properties of methanol extracts of *Cyathula polycephala* has been documented by Wanyoike *et al.*, (2003). The leaves are burned and the ashes are applied to skin infection while the roots are boiled and used to treat pneumonia by the Kipsigis community. The antimicrobial properties of this plant have not been documented. Plate 1.3 shows *Cyathula polycephala* shoots with leaves and flowers.

Plate1. 3: *Cyathula polycephala* shoots with flowers

Erythrina abyssinica "Chebisorwet" Lam belong to the family Leguminosae-Papilionoideae (Farjon, 1990). It is a flowering plant which is distributed in tropical and subtropical regions of the world. They grow up to 30 meters in height. There are about 130 species in the genus *Erythrina.* The seeds of at least one-third of the species contain potent erythrina alkaloids, and some of these are used for medicinal and other purposes by indigenous peoples. They are all toxic to some degree however, and the seeds of some can cause fatal poisoning. The main active

compounds in this genus generally seem to be alkaloids, such as scoulerine, erysodin and erysovin (namely in *E. flabelliformis*), and the putative anxiolytic erythravine (isolated from Mulungu *E. mulungu*) (Farjon, 1990). It is used traditionally by the Kipsigis people to treat skin conditions and trachoma. Antiplasmodial properties of this plant have been studied and reported to be potent by Abiy *et al.,* (2004). Antimicrobial properties of other species belonging to this genus have been studied like *E. subumbrans,* by Thitima *et al*., (2006). However, antimicrobial properties of *E. abyssinica* from Bomet District have not been determined. Plate 1.4 shows a mature *Erythrina abyssinica* tree with leaves and flowers.

Plate1. 4: Mature *Erythrina abyssinica* tree with flowers

1.10 Cell Toxicity

Cytotoxicity is the quality of being toxic to cells. Examples of toxic agents are a chemical substance, an immune cell or some types of venom (Riss and Moravec,

2004). Cytotoxicity assays are widely used by the pharmaceutical industry to screen for cytotoxicity in compound libraries. In research cytotoxic compounds are investigated, when one is interested in developing a therapeutic that targets rapidly dividing cancer cells, for instance; or one can screen "hits" from initial high-throughput drug screens for unwanted cytotoxic effects before investing in their development as a pharmaceutical (Decker, 1988). Cytotoxicity can also be monitored using the cell assays. Viable cells will reduce the reagent to a colored formazan product. A similar redox-based assay has also been developed using the fluorescent dye, resazurin. A label-free approach to follow the cytotoxic response of adherent animal cells in real-time is based on electric impedance measurements when the cells are grown on gold-film electrodes. This technology is referred to as electric cell-substrate impedance sensing. Label-free real-time techniques provide the kinetics of the cytotoxic response rather than just a snapshot like many colorimetric endpoint assays (Niles *et al.,* 2007). Some plants extracts like that of *Pittosporum viridiflorum* which is used traditionally in Kenya as antimalaria has been shown to exhibit the highest cell cytotoxicity on Vero E6 cells. Methanol and water extracts presented CC_{50} of 18.08 and 69.21g/mL, respectively, as reported by Muthaura *et al.,* (2007). The CC_{50} of this plant was below 50% thus it was reported to be very toxic to the cells. In East Africa a decoction or infusion of the stem bark of *P. viridiflorum* is used for malaria and fevers (Gakunju *et al.,* 1995; Irungu and Kigondu, 2007).

1.11 Acute toxicity test

Acute toxicity describes the adverse effects of a substance which result either from a single exposure or from multiple exposures in a short space of time (usually less than

24 hours). To be described as *acute* toxicity, the adverse effects should occur within 14 days of the administration of the substance (Meng *et al.,* 2000). Acute toxicity is distinguished from chronic toxicity, which describes the adverse health effects from repeated exposures, often at lower levels, to a substance over a longer time period (months or years). It is obviously unethical to test for acute (or chronic) toxicity in humans. However, some information can be gained from investigating accidental human exposures (e.g. factory accidents). Otherwise, most acute toxicity data comes from animal testing or, more recently, *in vitro* testing methods and inference from data on similar substances (Meng *et al.,* 2000).

1.12 Compounds founds in plants

Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms (Dewitt, 1996). They are produced by a large variety of organisms, including bacteria, fungi, plants and animals and are part of the group of natural products. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms. Alkaloids which are one of the largest groups of phytochemicals in plants have amazing effects on humans (Okuma *et al.,* 2002). Medications that contain significant levels of alkaloids have a direct toxic impact and hence when taken in excess they are bound to lead to symptoms like vomiting, diarrhea or affect the central nervous system (Fuller, 1986). Since plant alkaloids are natural, they often produce menacing effects of the remedies. However, many herbalists are of the opinion that the instant discomfort caused by the medications containing plant alkaloids are an important and necessary part of the stimulating action required for healing from any disorder (Cheeke, 1985).

Terpenes are a large and varied class of hydrocarbons, produced primarily by a wide variety of plants, particularly conifers (Ayafor, 1994). Examples of common terpenes are vitamin A, menthol, camphor (monoterpenes) farnesol and artemisin (sesquiternoids). Terpenes or ternoids are active against bacteria and fungi (Barrett and Lawrence, 1999; Ayafor, 1994) viruses and protozoa (Fujioka *et al.*, 1994; Ghosha, 1996). The mechanism of actions of terpenes is not fully understood but is assumed to involve membrane disruption by the lipophilic compounds (Ayafor, 1994).

Flavonoids are a class of secondary metabolites produced by plants. They can be classified into: - flavonoids, isoflavonoids and neoflavonoids derived from 4-phenylcoumarine (4-phenyl-1, 2-benzopyrone) structure. Flavonoids (specifically flavanoids such as the catechins) are "the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants" (Spencer, 2009). Flavonols, the original bioflavonoids such as quercetin, are also found ubiquitously, but in lesser quantities. They are widely distributed in plants fulfilling many functions including producing yellow or red/blue pigmentation in flowers and protection from attack by microbes and insects. They show anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity (Cushnie, 2005).

Phenolics acids are plant metabolites widely spread throughout the plant kingdom. Recent interest in phenolic acids stems from their potential protective role against oxidative damage diseases including protection against coronary heart disease, stroke, and cancers (Carmen, 2006). Phenolic compounds are found in fruits and

vegetable and are essential for the growth and reproduction of plants. They are produced as a response for defending injured plants against pathogens. The importance of antioxidant activities of phenolic compounds and their possible usage in processed foods as a natural antioxidant thus being highly utilized in recent years (Coll, 1972). Phenolic acid compounds seem to be universally distributed in plants. They have been the subjects of a great number of chemical, biological, agricultural, and medical studies (Carmen, 2006). The simplest of the class is phenol (C_6H_5OH). Some phenols are germicidal and are used in formulating disinfectants. Others possess estrogenic or endocrine disrupting activity (Coll, 1993). Other examples of phenols are orthophenyl phenol which are fungicide and used for waxing citrus fruits, polyphenol include flavonoids, tannins and thymol (2-Isopropyl-5-methyl phenol), an antiseptic that is used in mouthwashes (Carmen, 2006).

Anthraquinone (9, 10-dioxoanthracene) is an aromatic organic compound. It is a derivative of anthracene. It has the appearance of yellow or light gray to gray-green solid crystalline powder. Its other names are 9, 10-anthracenedione, anthradione, 9, 10-anthrachinon, anthracene-9, 10-quinone, 9, 10-dihydro-9, 10-dioxoanthracene, and trade names Hoelite, Morkit and Corbit (Macleod, 1988). Anthraquinones have a laxative effect on the body. They are generally not recommended for regular use due to concerns about the risk of habit-forming dependence and adverse side effects, including a higher risk for colorectal cancer (Moriarty *et al.*, 1988). Anthraquinones naturally occur in some plants, fungi, lichens, and insects, where they serve as a basic skeleton for their pigments. Natural anthraquinone derivatives tend to have laxative effects (Harbon, 1998; Moriarty *et al.*, 1988).

1.13 Statement of the problem

The past decade has witnessed a significant increase in the prevalence of microorganism's resistance to antibacterial and antifungal agents. Resistance to antimicrobial agents has important implications for morbidity, mortality and health care costs. Hence, substantial attention should be focused on understanding the mechanisms of antimicrobial resistance as well as developing new therapeutic options. Therefore medicinal plants present new therapeutic options for the treatment of infections caused by resistant microorganisms.

1.14 Justification

Plants have been and continue being a source of medicine since the existence of mankind. The plants are used among traditional communities in Kenya as herbal concoction and their activities and safety has not been established. Comprehensive compilation of the indigenous medical uses of Kenya herbs and authentification of the medicinal plants has not been thoroughly done. Documentation of such indigenous knowledge is crucial as it serves as a basis for further pharmacological and phytochemical studies for identification of compounds useful in drug development. It is widely acknowledged that many current drugs were discovered on the basis of their indigenous use. Such indigenous knowledge also needs to be documented before it is lost forever with the passing on of the older generation. Medicinal plants species warranting conservation may also be identified in the process of documentation of the indigenous medical knowledge and detailed pharmacological and phytochemical studies will enhance maximum exploitation of Kenyan medicinal plants species from a health as well as an economic perspective.

In Kenya many plants used in traditional therapy remain uninvestigated for microbial activities and toxicities. Therefore this study aims at providing scientific information on the antimicrobial activities and toxicities. This is essential for documentation and safeguarding the public from some plants with toxic properties.

1.15 Hypothesis

Alternative: Medicinal plants used among the Kipsigis community in Kenya have antimicrobial activities.

1.16 Objectives

1.16.1 General objective

To determine antimicrobial activities of the selected medicinal plants used among the Kipsigis community of Bomet District.

1.16. 2 Specific objectives

1. To determine antimicrobial activities of the selected plant extracts against both drug sensitive and drug resistance bacterial and fungal isolates.
2. To determine toxicities of the active plants extracts.
3. To perform phytochemical screening on the plant extracts with activity.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Preliminary experimental design

Test groups included selected organisms (ATCC strains and clinical isolates) and crude extracts at different concentrations. This was to determine whether the extracts were active either as antifungal or antibacterial agents. Positive controls; this consisted of both antifungal and antibacterial drugs (gentamycin and fluconazoles). These were to determine whether the test organisms used were sensitive to common drugs or were resistant. Negative controls; this consisted of sterile distilled water and dimethyl sulfoxide (DMSO), this was necessary to ensure that the solvents used for dissolution had no inhibitory actions on their own. The DMSO was used for dissolution as a universal solvent for organic solvents because it does not have antibacterial activity on its own. All experiments were carried out in triplicates in order to minimize experimental error.

2.2 Collection of the plants from the field

Four medicinal plants *Senna didymobotrya*, *Clutia abyssinica, Cyathula polycephala* and *Erythrina abyssinica* were collected from Bomet District. Selection of the plants was based on available ethnobotanical information from traditional health practitioners consulted as well as the available literature. The plants are used to treat both skin and diarrhea diseases. The herbalist advised on the samples to be collected as the plant part being used for treatment. The plants parts collected include *Clutia abyssinica* leaves, *Cyathula polycephala* shoots, *Senna didymobotrya* and *Erythrina*

abyssinica stem bark. The study plant materials were photographed and collected for authentification at the University of Nairobi Herbarium as shown in Table 2.1.

Table 2. 1: Plants collected for antimicrobial studies.

Botanical name/ Part collected/Herbarium No.	*Family Name*	*Vernacular Name*
Senna didymobotrya – Stem bark/KIFS 038	Caesalpinaceae	Senetwet
Clutia abyssinica – Leaves/ KIFS 009	Euphorbiaceae	Turmanyat
Cyathula polycephala- Shoots/ KIFS 092	Amaranthaceae	Cheputpich
Erythrina abyssinica – Stem bark/KIFS 023	Leguminosae	Kagarwet

The samples for extractions were collected in paper bags and taken to the Medicinal Chemistry Laboratory, Centre for Traditional Medicine and Drug Research KEMRI for processing and extractions.

2.3 Reagents

Analytical grade organic solvents; n-hexane, dichloromethane, chloroforms, ethyl acetate, methanol, acetone, and ammonia were sourced from Kobian, Nairobi, Kenya. Chloroform, methanol, vanillin, sulphuric acid and potassium hydroxide were also used.

2.4 ExtraCction of plant materials

Plants materials were dried at room temperature, grounded into fine powder using laboratory grinding mill and stored in a cool and dry place. Total extraction of each plant material was prepared by mixing with solvent in the ratio of 1: 10 (plant material/solvents). This procedure was done successively using hexane, dichloromethane and methanol where the plant materials were soaked in 1000ml conical flasks for 12 hours. The extracts were filtered using Whatman No. 1 filter paper and solvents removed through evaporation under reduced pressure at 45^{o}C using a rotary evaporator. The extracts were kept in stoppered sample vials at 4^{o} C until they were used (Kalmodin *et al.,* 2005; Mcloud, *et al*., 1998; Chhabra *et al.,* 1984). Total water extract of each plant material was done by soaking a weighed amount of the dry powder (27-50g) in distilled water and shaking it for two hours with an electric shaker at 65^{o}C. The suspension was filtered and the filtrate was kept in a deep freezer before it was evaporated to dryness by freeze drying. The lyophilized dry powder was collected in stoppered sample vials, weighed and kept in a desiccator, to avoid absorption of water, until they were used.

2.5 Microbial test organisms

Selection of microbial test organisms was based on the ethnobotanical information of the plants collected and the disease they are used for management and treatment. The herbalist advised on the plant part they use and the type of the disease they treat with. Using the herbalist information, when they use certain plant part say bark for diarrhea then the plant part extracts were tested against bacteria that cause the diarrhea. When they use for skin condition, both fungi and bacteria associated with

skin infections were tested. The bioassay procedures were conducted using methods recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2003). These methods are antimicrobial susceptibility test using disk diffusion and minimum inhibition concentration using broth dilution. The reference microbial organisms and clinical isolates were obtained from the Centre for Microbiology Research KEMRI, all preserved under -80^{o}C.

2.6 Test strains

The American Type Culture Collection (ATCC) organisms sometimes called reference organisms as well as clinical isolates used in this study include: Bacterial isolates; Gram positive:- *Staphylococcus aureus* ATCC 25923 and Methicillin resistant *Staphylococcus aureus* (MRSA) clinical isolate; Gram negative:- *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumonia*. (Clinical isolate): Fungi isolates: Yeast:-*Candida albicans* ATCC 90028, *Candida parapsilosis* ATCC 90029, *Candida krusei* ATCC 90026 and *Cryptococcus neoformans* ATCC 32602: Dermatophytes (Clinical isolates):- *Microsporum gypseum* and *Trychophyton mentagrophytes*.

2.7 Preparation of test organisms

Stocked bacterial strains were sub-cultured on Muller Hinton agar no. CM0337 (Oxoid Ltd, Basingstoke, Hampshire, England). Incubation was done at 37^{o}C for 12 – 18 hours to obtain freshly growing strains. Yeast and molds were subcultured onto Sabaraud Dextrose Agar no. CM 004 (Oxoid Ltd, Basingstoke, Hampshire, England). Each media was prepared according to the manufacturer's instructions.

Yeasts were incubated for 24 hours while molds were incubated for 72 hours at 30^{o}C to obtain freshly growing culture (Rajakaruna *et al*., 2002).

2.8 Preparation of McFarland standard

Exactly 0.5 McFarland equivalent turbidity standards was prepared by adding 0.6ml of 1% barium chloride solution ($BaCl_2.2H_2O$) to 99.4ml of 1% sulphuric acid solution (H_2SO_4) and mixed thoroughly. A small volume of the turbid solution was transferred to capped tube of the same type that was used to prepare the test and control innocula. It was then stored in the dark at room temperature (25^{o}C). Exactly 0.5 McFarland gives an equivalent approximate density of bacteria $1x10^{-6}$ colony forming units (CFU)/ ml (Kathirima *et al*., 2005).

2.9 Antimicrobial screening test

Five millimeters of sterile distilled water was used to make suspension. From an overnight growth of the test organism, 4-6 colonies were emulsified and the suspension was adjusted to match the 0.5 McFarland's standard. Respective plates were inoculated using a sterile cotton wool swab. Antimicrobial susceptibility test was done using disk diffusion methods. Briefly, 100 mg of each extract was dissolved in 1ml of the appropriate solvents and 10 µl of the mixture was impregnated onto 6mm sterile filter paper disk and air dried. The disk was placed aseptically onto the inoculated plates. The bacterial and yeast cultures were incubated at 37^{o}C for 12 to 18 hours while the molds were incubated at 25^{o}C for 72 hours. After incubation, inhibition zone diameter was measured in millimeters and recorded against the corresponding concentrations as described by Elgayyar and

Draugon, (2000). Positive controls were set against standard antibiotics and antifungal drugs while negative controls were set using disk impregnated with extraction solvents.

2.10 Determination of minimum inhibitory concentration

Broth micro dilution method was used to determine minimum inhibitory concentration for the active crude extracts against the test microorganisms. The method is recommended by the National Committee for Clinical Laboratory Standards now Clinical Laboratory Standard Institute (CLSI) (NCCLS, 2002). The tests were performed in 96 well-micro-titer plates. Serial doubling dilutions were done so that the concentration in the next well was half of the concentration in the former well. Serial dilutions ranging from10^{1}, 10^{-2}, 10^{-3}……..$10.^{-10}$ were done. The final volume in each well was 100 µl. The wells were inoculated with 5µl of microbial suspension. The yeast and bacteria were incubated at 37^{o}C for 24 hours while molds were incubated at 25^{o}C for 3-7 days in ambient air. The MIC was recorded as the lowest extract concentration demonstrating no visible growth as compared to the control broth turbidity (Benton *et al.,* 2003). Wells that was not inoculated was set to act as control. The MIC interpretation for the standard drugs gentamicin was adapted from NCCL's 2003 interpretive standards for dilution and disc diffusion susceptibility testing tables (Murray *et al.,* 1999). All the experiments were done in triplicates and average results were recorded after calculating the mean (average) using the three readings. The three results were added to get the sum and divided by three to get the average.

2.11 Cell toxicity

The extracts of the active plants were tested for *in vitro* cytotoxicity. This was done using human embryonic lung fibroblast (HELF) (Vero-199 cells line. They were prepared using 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (Mossman, 1983). The HELF cells were cultured and maintained in minimum essential medium (MEM) supplemented with 10% Featal calf bovin serum (FBS). The cells were cultured at 37^{o} C in 5% CO_2 and harvested by trypsinization, pooled in a 50ml vial. Approximately 100ul cell suspension (1×10^5 cells/ml) was added to each well in a 96-well micro-titer plate. Each sample was replicated 3 times and the cells incubated at 37^{o} in 5% CO_2 for 24 hrs for attachment. 150ul of the highest concentration (1000 mg/ml) of each of the test samples (a serial dilution, prepared in MEM) was added into the same row and a serial dilution done. The experimental plates were incubated further at 37^{o}C for 48hrs. The cells in media without drugs were used as controls. After 48hrs of incubation visualization dye was added into each well. The cells were incubated for 4 hrs or until a purple precipitate was clearly visible under a microscope. The medium together with the dye was aspirated off from the wells and dimethlysulfoxide (DMSO) (100µl) was added and the plates shaken for 5 min. This method was described by Kurokawa *et al.*, (2001). The absorbance for each well was measured at 562nm in a micro-titre plate reader (Mossman, 1983) and percentage cell viability (CV) calculated via an excel program with the formula;

$$\%CV = \frac{\text{Average abs of duplicate drug wells} - \text{Average abs of blank wells}}{\text{Average abs of control wells}} \times 100\%$$

Key: abs average absorbance

A dose–concentration graph was plotted which shows the % CV (of the Vero-199 cells) (Kigondu *et al.*, 2009).

2.12 Determination of acute toxicity

Fifteen Swiss albino mice were used in the study and were sourced from KEMRI animal house facility. Healthy mice (weight 20-22 g) were divided into two groups (control and treatment group) each cage with five mice. The mice were allowed to have access to water and food, except for a short fasting period of 12 hours before oral administration of the test sample. The active extracts suspension was administered orally at logarithmic dose of 1000.0mg/kg and 5000.0 mg/kg body weight. The general behavior of mice was observed continuously for 1 h after the treatment and then intermittently for 4 h, and thereafter over a period of 24 hours (Twaij *et al.*, 1983; Anaissie *et al.*, 1989). The mice were observed further for up to 14 days following treatment for any sign of restlessness and the latency of death and the lethal dose was determined (Rodney, 1985). During experiment all the dead mice were disposed according to KEMRI biosafety guidelines. After the experiment all the mice were sacrificed using chloroform and the carcasses incinerated.

2.13 Phytochemical screening

Phytochemical screening was done on the active extracts to identify the plants compounds present. Based on the initial bioassays the plant extracts exhibiting biological activity were screened for groups of chemical constituents. Thin layers chromatography (TLC) plates were developed with Chloroforms: Methanol (98:2) with five drops of glacial acetic acid before spraying with TLC visualization reagents

giving specific reactions, (Harborne, 1998). After plate development a purple colour is positive for terpenoids, orange to brown colour is positive for alkaloids, yellow to brown colour is positive for flavonoids, blue colour is positive for phenolics and yellow brown is positive for anthraquinones (red to violet or green purple under ultra violet light is also positive for anthraquinones). Table 2.2 shows the TLC visualizing reagents used in this study.

Table 2. 2: Thin layer chromatography visualization reagents

Compounds	Visualizing reagents
Terpenoids	Anisaldehyde
Alkaloids	Dragendorff reagents
Flavonoids	Ammonia fumes
Phenolics	Ethanolic potassium hydroxide
Anthraquinones	Methanolic potassium hydroxide (Kedde reagents)

2.14 Statistical data analysis

Statistical packages for social scientist SPSS Version 12.0 was utilized which enabled the analysis of variance by one way ANOVA to establish the significance variability between and within groups (Plants, Solvents and organisms) at 95% confidence interval ($p < 0.05$). The percentage cell viability was determined for the extracts and presented in a bar graph. *In-vivo* toxicity was checked for significant differences in lethal dose resulting to death of experimental and control groups of mice at the two doses. The data was presented inform of tables, plates and graphs.

CHAPTER THREE

3.0 RESULTS

3.1 Yields of extracted plant materials

Maximum and minimum yields were obtained from the water extract of *C. abyssinica* leaves (14.288 %.) and hexane extract of *S. didymobotrya* stem barks (0.166%), respectively. The methanol extract of *C. polycephala* shoots produced the highest yield (5.973%) followed by *E. abyssinica* stem bark (2.672 %) and *S. didymobotrya* stem bark (1.093 %) for organic solvents as shown in Table 3.1.

Table 3. 1: Percentage yields for sequential extractions

Plant species	Solvent	Part extracted	Initial wgts in grams	Wgts in grams	% yield
Clutia	Hexane	Leaves	80.0	0.41	0.513
abyssinica	DCM	Leaves	80.0	0.76	0.950
	Methanol	Leaves	80.0	1.829	2.286
	Water	Leaves	80.0	7.144	14.288
Senna	Hexane	Stem bark	80.0	0.1328	0.166
didymobotrya	DCM	Stem bark	80.0	1.0976	1.372
	Methanol	Stem bark	80.0	0.8744	1.093
	Water	Stem bark	80.0	2.2688	2.836
Cyathula	Hexane	Shoots	80.0	0.7896	0.987
polycephala	DCM	Shoots	80.0	0.2776	0.347
	Methanol	Shoots	80.0	4.7784	5.973
	Water	Shoots	80.0	5.6184	7.023
Erythrina	Hexane	Stem bark	80.0	0.4352	0.544
abyssinica	DCM	Stem bark	80.0	1.0608	1.326
	Methanol	Stem bark	80.0	2.1376	2.672
	Water	Stem bark	80.0	5.020	6.6936

*Percentage yields for sequential extractions (% yields) = extracted weights / initial weights x100). wgts – weights, DCM – Dimethylsulfoxide, % percentage

3.2 Biological activity evaluation

3.2.1 Inhibition zones diameters

Preliminary screening test to determine bioactivity was done using disk diffusion method and the results were captured using photographs denoted as plates as shown in plates 3.1.

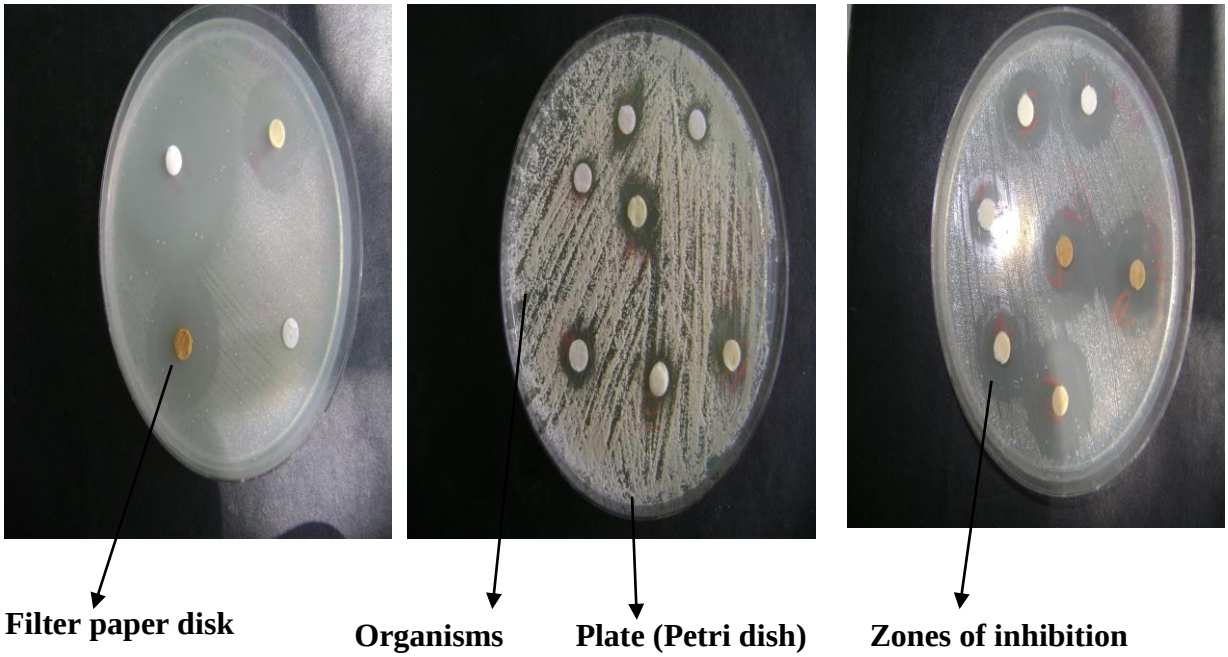


Plate 3. 1: Filter paper disks, microorganism and inhibition zone diameters

3.2.2 Antibacterial Activities

The results of the initial antimicrobial screening assay of the crude extracts of *C. abyssinica* on the selected microbial strains indicates that the dichloromethane (DCM) extract was the most active against *P. aeruginosa* with inhibition zone diameters of 15.0 mm and the methanol extract had the least activity against *P. aeruginosa* with inhibition zone diameters of 8.0 mm. The inhibition zone diameters are as shown in Table 3.2.

Table 3. 2: Antibacterial activities of different extracts of *Clutia abyssinica* against selected strains of bacteria

Plants	Test samples (extracts/drug)	Conc mg/ml	Inhibition zone diameters in millimeter for each Test organisms				
			S. aureus	*MRS. aureus*	*P. aeruginosa*	*E. coli*	*K. pneumoniae*
Clutia	Hexane	100.0	6.0	6.0	6.0	6.0	6.0
abyssinica	DCM	100.0	6.0	6.0	15.0	6.0	6.0
	Methanol	100.0	6.0	6.0	8.0	6.0	6.0
	Water	100.0	6.0	6.0	6.0	6.0	6.0
- Control	DMSO	100.0	6.0	6.0	6.0	6.0	6.0
+ Control	Gentamycin	100.0	19.0	9.0	21.0	19.0	17.0
- Control	Water	100.0	6.0	6.0	6.0	6.0	6.0

Key: DCM-Dichloromethane, DMSO- Dimethyl sulfoxide, MR- Methicilin Resistant.

The *S. didymobotrya* methanol and dichloromethane extracts against bacterial isolates had the highest activity as compared to water and hexane. Hexane extracts had no activity against *S. aureus, MR S. aureus, E. coli and K. pneumoniae* and the water extract had activity of 10.0 mm against *P. aeruginosa* and 11.0 mm against *K. Pneumoniae*, respectively. The results are as shown in Table 3.3.

Table 3. 3: Antibacterial activities of extracts of *Senna didymobotrya* against bacteria

Plant	Test samples (extracts/drug)	Conc mg/ml	Inhibition zone diameters in millimeter for each test organisms				
			S. aureus	*MRS aureus*	*P. aeruginosa*	*E. coli*	*K. pneumoniae*
Senna didymobotrya	Hexane	100.0	6.0	6.0	10.0	6.0	6.0
	DCM	100.0	9.0	10.0	16.0	9.0	12.0
	Methanol	100.0	9.0	11.0	8.0	9.0	12.0
	Water	100.0	6.0	6.0	10.0	6.0	11.0
- Control	DMSO	100.0	6.0	6.0	6.0	6.0	6.0
+ Control	Gentamycin	100.0	19.0	9.0	21.0	19.0	17.0
- Control	Water	100.0	6.0	6.0	6.0	6.0	6.0

Key: DCM-Dichloromethane, DMSO- Dimethyl sulfoxide, MR- Methicilin Resistant.

The methanol extract *C. polycephala* was active against *S. aureus*, MR *S. aureus*, and *P. aeruginosa* with inhibition zone diameters of 11.0, 11.0 and 10.0 mm respectively and no activity against the other bacterial isolates tested. The water extracts were active against *S. aureus, P. aeruginosa* M.R. *S. aureus. aureus and K. pneumoniae* with inhibition zone diameters of 10.0, 8.0, 9.0 and 9.0 mm, respectively. The results are as shown in Table 3.4.

Table 3. 4: Antibacterial activities of extracts of *Cyathula polycephala* against bacteria

Plant	Test samples (extracts/drug)	Conc mg/ml	Inhibition zone diameters in millimeter for each test organisms				
			S. aureus	*MRS aureus*	*P. aeruginosa*	*E. coli*	*K. pneumoniae*
Cyathula	Hexane	100.0	6.0	6.0	7.0	6.0	6.0
polycephala	DCM	100.0	6.0	6.0	7.0	6.0	6.0
	Methanol	100.0	11.0	11.0	10.0	6.0	6.0
	Water	100.0	10.0	9.0	8.0	6.0	9.0
- Control	DMSO	100.0	6.0	6.0	6.0	6.0	6.0
+ Control	Gentamycin	100.0	19.0	9.0	21.0	19.0	17.0
- Control	Water	100.0	6.0	6.0	6.0	6.0	6.0

Key: DCM-Dichloromethane, DMSO- Dimethyl sulfoxide, MR- Methicilin Resistant.

Hexane extracts of *E. abyssinica* were active against *P. aeruginosa* with inhibition zone diameters of 11.0 mm; it had no activity against other bacteria isolates tested. The dichloromethane extract was active against MR *S. aureus*, *P. aeruginosa* and *K. pneumonia.* Methanol extract was active against MR *S. aureus*, *P. aeruginosa* and *K. pneumoniae* with 9.0, 7.0 and 13.0 mm inhibition zones diameters, respectively. The inhibition zone diameters are as shown in Table 3.5.

Table 3. 5: Antibacterial activities of extracts of *Erythrina abyssinica* against bacteria

Plant	Test samples (extracts/drug)	Conc mg/ml	Inhibition zone diameters in millimeter of each test organisms				
			S. aureus	*MRS aureus*	*P. aeruginosa*	*E. coli*	*K. pneumoniae*
Erythrina	Hexane	100.0	6.0	6.0	11.0	6.0	6.0
abyssinica	DCM	100.0	6.0	10.0	12.0	6.0	11.0
	Methanol	100.0	10.0	9.0	7.0	6.0	13.0
	Water	100.0	6.0	6.0	6.0	6.0	6.0
- Control	DMSO	100.0	6.0	6.0	6.0	6.0	6.0
+ Control	Gentamycin	100.0	19.0	9.0	21.0	19.0	17.0
- Control	Water	100.0	6.0	6.0	6.0	6.0	6.0

Key: DCM-Dichloromethane, DMSO- Dimethyl sulfoxide, MR- Methicilin Resistant.

The hexane extracts of *S. didymobotrya* was active against *M. gypseum* with 16.0mm inhibition zone diameters and had no activity against the other fungal isolates tested. Dichloromethane extracts exhibited the following zone diameter 7.0, 7.5., 7.0, 18.0 and 15.0 mm against *C. albicans, C. parapsilosis, C. krusei*, *T. mentagrophyte* and *M. gypseum*, respectively. Methanol extracts had the following activities, 9.0, 9.0, 8.0, 7.0, 17.0 and 6.0 mm against *C. neoformans, C. albicans*, *C. parapsilosis, C. krusei* and *T. mentagrophyte*, respectively. Water extract was the least active with

8.0, 10.0 and 7.0 mm against *C. neoformans, C. albicans* and *C. parapsilosis,* respectively. The results are as shown in Table 3.6.

Table 3. 6: Antifungal activity of *Senna didymobotrya*

Plant	Test samples (extracts/drug)	Conc mg/ml	Inhibition zone diameters in millimeters of each tested isolate					
			C RPT	CA	CP	CK	TM	MG
Senna	Hexane	100.0	6.0	6.0	6.0	6.0	6.0	16.0
didymobotrya	DCM	100.0	6.0	7.0	7.5	7.0	18.0	15.0
	Methanol	100.0	9.0	9.0	8.0	7.0	17.0	6.0
	Water	100.0	8.0	10.0	7.0	6.0	6.0	6.0
- control	DMSO	100.0	6.0	6.0	6.0	6.0	6.0	6.0
+ control	Fluconazole	30 .0	23.0	27.0	24.0	26.0	20.0	20.0
- control	Water	100 .0	6.0	6.0	6.0	6.0	6.0	6.0

Key - CRPT – *Cryptococcus neoformans,* DCM – *Dichloromethane,* CA – *Candida albicans,* DMSO – *Dimethylsufoxide,* CP – *Candida parapsilosis,* CK – *Candida krusei,* TM – *Trichophyton mentagrophytes,* MG - *Microsporum gypseum*

The methanol extracts of *Cyathula polycephala* were active against *T. mentagrophyte* and *M. gypseum* with 14.0 and 15.0 inhibition diameters, respectively. Water extract showed less activity against *M. gypseum* with 7.0 mm zone of inhibition. The results are summarized in Table 3.7.

Table 3. 7: Antifungal activity of *Cyathula polycephala* extracts

Plant	Test samples (extracts/drug)	Conc mg/ml	Inhibition zone diameters in millimeter of each tested isolates					
			CRPT	C A	CP	CK	TM	MG
Cyathula	Hexane	100.0	6.0	6.0	6.0	6.0	6.0	6.0
polycephal	DCM	100.0	6.0	6.0	6.0	6.0	6.0	6.0
a	Methanol	100.0	6.0	6.0	6.0	6.0	14.0	15.0
	Water	100.0	6.0	6.0	6.0	6.0	6.0	7.0
- control	DMSO	100.0	6.0	6.0	6.0	6.0	6.0	6.0
+ control	Fluconazole	30.0	23.0	27.0	24.0	26.0	20.0	20.0
- control	Water	100.0	6.0	6.0	6.0	6.0	6.0	6.0

Key - CRPT – *Cryptococcus neoformans,* DCM – *Dichloromethane,* CA – *Candida albicans,* DMSO – *Dimethylsufoxide,* CP – *Candida parapsilosis,* CK – *Candida krusei,* TM – *Trichophyton mentagrophytes,* MG *Microsporum gypseum*

The hexane extracts of *E. abyssinica* had the highest activity with inhibition zone diameter of 23.0 mm against *M. gypseum* but had no activity against other fungi isolates tested. Dichloromethane extracts had different activities against the test isolates as follows, 8.0, 7.5, 14.0 and 20.0 mm zone sizes against *C. parapsilosis, C. krusei, T. mentagrophyte* and *M. gypseum,* respectively. The methanol extracts had the following activities 8.0, 10.0, 9.0, 8.5, 12.0 and 17.0 mm against *C. neoformans, C. albicans, C. parapsilosis, C. krusei, T. mentagrophyte* and *M. gypseum,* respectively. The water extracts had no activities against all the isolates tested. The results are recorded in Table 3.8.

Table 3. 8: Antifungal activity of *Erythrina abyssinica*

Plant	Test samples (extracts/drug)	Conc mg/ml	Inhibition zone diameters in millimeter					
			C RPT	CA	CP	CK	TM	MG
Erythrina	Hexane	100.0	6.0	6.0	6.0	6.0	6.0	23.0
abyssinica	DCM	100.0	6.0	6.0	8.0	7.5	14.0	20.0
	Methanol	100.0	8.0	10.0	9.0	8.5	12.0	17.0
	Water	100.0	6.0	6.0	6.0	6.0	6.0	6.0
- control	DMSO	100.0	6.0	6.0	6.0	6.0	6.0	6.0
+ control	Fluconazole	30.0	23.0	27.0	24.0	26.0	20.0	20.0
- control	Water	100.0	6.0	6.0	6.0	6.0	6.0	6.0

Key - CRPT – *Cryptococcus neoformans*, DCM – *Dichloromethane*, CA – *Candida albicans*, DMSO – *Dimethylsufoxide*, CP – *Candida parapsilosis*, CK – *Candida krusei*, TM – *Trichophyton mentagrophytes*, MG *Microsporum gypseum*, + control- Fluconazole

3.3 Minimum inhibition concentrations

3.3.1 Minimum inhibition concentrations plants extracts against bacteria

The MIC for the plant extracts which had inhibition diameters of 10 mm and above (significance activity) was determined. Microtitre plate showing MIC is shown in Appendix 1.

The *E. abyssinica* plant extracts recorded MIC ranging from 100.0 $x10^2$ µg/ml to 6.25 $x10^2$ µg/ml. Methanol extract of *E. abyssinica* recorded the lowest MIC of 6.25

$x10^2$ µg/ml against MRSA. Methanol extract demonstrated the lowest MIC. The results are indicated in Table 3.9.

Table 3. 9: Minimum inhibition concentration results of *Erythrina abyssinica*

Plants	Test samples (extracts/drug)	Test organism	MIC (µg/ml)
Erythrina abyssinica	Hexane	*P.aeruginosa*	50.0 $x10^2$ µg/ml
	DCM	*P. aeruginosa*	50.0 $x10^2$ µg/ml
		MRSA	50.0 $x10^2$ µg/ml
		K. pneumoniae	100.0 $x10^2$ µg/ml
	Methanol	*S. aureus*	25.0 $x10^2$ µg/ml
		P. aeruginosa	100.0 $x10^2$ µg/ml
		M RSA	6.25 $x10^2$ µg/ml
Standard	*Gentamycin–Control	All tested bacteria	0.5 µg/ml

Key: DCM Dimethylsulfoxide, MIC - Minimum inhibition concentration, MRSA-Methicilin Resistant *Staphylococcus aureaus*

The MIC of *Cyathula polycephala* plant extracts against different bacterial isolates ranged between 100.0 $x10^2$ µg/ml to 3.124 $x10^2$ µg/ml. Methanol extract of *C.*

polycephala recorded the lowest MIC of 3.124 $x10^2$ µg/ml against MRSA and 6.25 $x10^2$ µg/ml against *S. aureus.* The results are as shown in Table 3.10.

Table 3. 10: Minimum inhibition concentration of *Cyathula polycephala*

Plants	Test samples (extracts/drug)	Test organism	MIC in (µg/ml)
Cyathula polycephala	Hexane	*S. aureus*	100.0 $x10^2$ µg/ml
		P. aeruginosa	100.0 $x10^2$ µg/ml
	DCM	*P. aeruginosa*	100.0 $x10^2$ µg/ml
	Methanol	*S. aureus*	6.25 $x10^2$ µg/ml
		P. aeruginosa	25.0 $x10^2$ µg/ml
		MRSA aureus	3.125 $x10^2$ µg/ml
	Water	*S. aureus*	12.5 $x10^2$ µg/ml
		P. aeruginosa	25.0 $x10^2$ µg/ml
Standard	Gentamicin – control	All tested bacteria	0.5 µg/ml

Key: DCM Dimethylsulfoxide, MIC - Minimum inhibition concentration, MRSA Methicilin Resistant *Staphylococcus aureaus*

The Methanol extracts for *C. abyssinica* recorded an MIC of <0.1953 x10^2 µg/m against *P. aeruginosa*. The plants DCM extracts recorded an MIC of 50.0 x10^2 µg/ml against *Pseudomonas aeruginosa*. The results for *C. abyssinica* plant extracts MIC are as shown in Table 3.11.

Table 3. 11: Minimum inhibitory concentration results of *Clutia abyssinica*

Plants	Test samples (extracts/drug)	Test organism	MIC (mg/ml)
C. abyssinica	DCM	*P. aeruginosa*	50.0 x10^2 µg/ml
	Methanol	*P. aeruginosa*	<0.1953 x10^2 µg/ml
Standard	Gentamicin	All tested bacteria	0.5 µg/ml

Key: DCM Dimethylsulfoxide, MIC - Minimum inhibition concentration

Methanol extract recorded relatively a lower MIC, the low the MIC the best is the extracts. The readings were <0, 1953 µg/ml on *S. aureus,* 50 µg/ml on MRSA and *P. aeruginosa,* respectively. Hexane and methanol extracts *S. didymobotrya* had MICs of 100 x10^2 µg/ml against *P. aeruginosa* and *K. pneumonia*. This were the highest values for this plant extracts. The MIC for the conventional drug gentamicin was 0.5 µg/ml. The minimum inhibition concentration results are as shown in Table 3.12.

Table 3. 12: Minimum inhibitory concentration results for *Senna didymobotrya*

Plants	Test samples (extracts/drug)	Test organism	MIC (µg/ml)
S. didymobotrya	Hexane	*P. aeruginosa*	100 x10^2 µg/ml
	DCM	*S. aureus*	12.5 x10^2 µg/ml
	Methanol	*S. aureus*	<0.1953 x10^2 µg/ml
		P. aeruginosa	50 x10^2 µg/ml
		MRSA	50 x10^2 µg/ml
		K. pneumoniae	100 x10^2 µg/ml
Standard	Gentamicin	All tested bacteria	0.5 µg/ml

Key: DCM Dimethylsulfoxide, MRSA Methicilin Resistant *Staphylococcus aureaus*

3.3.2 The minimum inhibition concentrations plants extracts against fungi

The MIC results for *E. abyssinica* ranged from 100 x10^2 µg/ml to 6.25 x10^2 µg/ml against the test fungal isolates. The DCM extracts were generally active on different fungal isolates. The hexane extracts had the lowest MIC of 6.25 x10^2 µg/ml against *M. gypseum*. The MIC results are as shown in Table 3.13.

Table 3. 13 : The MIC values for *Erythrina abyssinica* extracts against fungal isolates

Plant	Test samples (extracts/drug)	Test organism	MIC (µg/ml)
Erithrina abyssinica	Dichloromethane	*C. neoformans*	25.0 x10^2 µg/ml
		T. mentagrophyte	25.0 x10^2 µg/ml
		M. gypseum	12.5 x10^2 µg/ml
Standard	Fluconazole	All tested fungi	1.0 and 0.5 µg/ml

Key: MIC - Minimum inhibition concentration

The MIC of *C. polycephala* plants extracts against dermatophytes was 25 x10^2 µg/ml and 50 x10^2 µg/ml for *T. mentagrophyte* and *M. gypseum*, respectively as shown in Table 3.14.

Table 3. 14: The minimum inhibition concentration for *C. polycephala* extracts against fungal isolates

Plant	Test samples (extracts/drug)	Test organism	MIC (µg/ml)
Cyathula polycephala	Methanol	*T. mentagrophyte*	25.0 x10^2 µg/ml
		M. gypseum	50.0 x10^2 µg/ml
Standard	Fluconazole	All tested fungi	1.0 µg/ml

Key: MIC - Minimum inhibition concentration

The *S. didymobotrya* plant extracts had different MIC range for different solvents against different fungal isolates. The MIC ranges from 100 $x10^2$ µg/ml to 12.5 $x10^2$ µg/ml. Methanol and hexane extracts recorded the lowest MIC of 25 $x10^2$ µg/ml and 12.5 $x10^2$ µg/ml against dermatophytes as compared to yeast isolates which recorded 100 $x10^2$ µg/ml. The results are as shown in Table 3.15.

Table 3. 15: The minimum inhibition concentration for *S. didymobotrya* extracts against fungal isolates

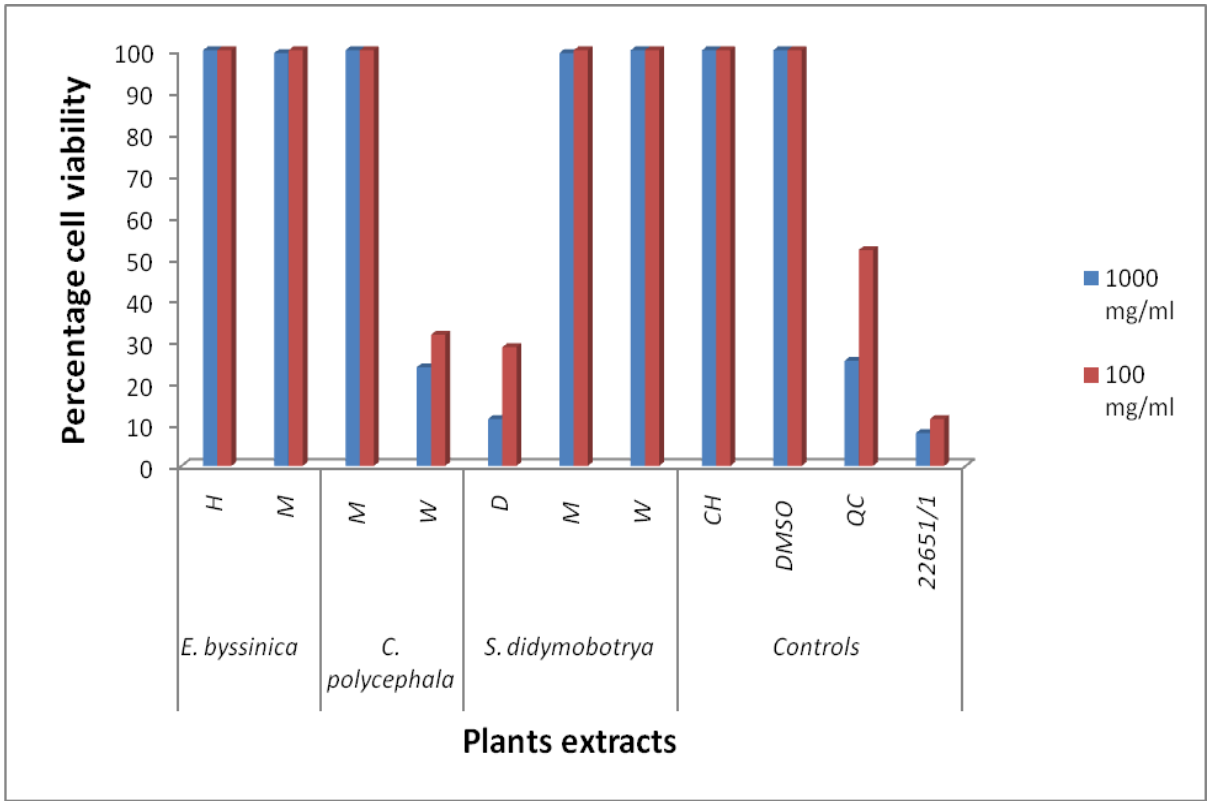
Plant	Test samples (extracts/drug)	Test organism	MIC (µg/ml)
S. didymobotrya	Hexane	*M. gypseum*	12.5 $x10^2$
	DCM	*C. albicans*	100.0 $x10^2$
		C. Tropicalis	100.0 $x10^2$
		C. Tropicalis	100.0 $x10^2$
		T. mentagrophyte	
		M. gypseum	25.0 $x10^2$
Standard	Fluconazole	All tested fungi	0.5 and 1.0

Key: MIC – Minimum inhibition concentration, DCM – Dimethylsulfoxide

3.4 *In vitro* toxicity of the plants extract.

Hexane extracts of *E. abyssinica* had a CV of 100% at both concentration 1000 mg/ml and100 mg/ml. The methanol extracts had a CV of 99.34% at concentration 1000 mg/ml and 100% at 100 mg/ml concentration. Methanol and water extract of *C. polycephala* had a CC_{50} of 100%. The water extracts recorded CV of 23.75% and

31.56% at 1000 mg/ml and 100 mg/ml concentration, respectively. The DCM extract of *S. didymobotrya* had a CV of 11.28% and 28.57% at 1000 mg/ml and 100 mg/ml, respectively. The methanol extracts recorded a CV of 99.34% and 100% at 1000 mg/ml and 100 mg/ml concentration, respectively. Dimethylsulfoxide and sterile distilled were used as negative control recorded a CV of 100 at both concentrations. Chloroquine (CQ) and TDR (22651/1 was used as positive controls. Chloroquine recorded CV of 25.28% and 51.94% at concentration 1000 mg/ml and 100 mg/ml, respectively. The other control TDR 22651/1 recorded a CV of 7.89% and 11.28% at concentration 1000 mg/ml and 100 mg/ml respectively. Cell toxicity was done on microtitre plates as shown in Appendix 2. The results are presented in Figure 3.1.



Key: H – Hexane, **M –** Methanol**, W** - Water extracts, **D** – Dichloromethane, **DMSO** - Dimethylsulfoxide (Negative control), **2265/1** and **QC** Chloroquine (Positive control)

Figure 3. 1: Extract concentration bar graph for cytotoxicity

3.5 Acute toxicity of *S. didymobotrya* and *C. polycephala* extracts

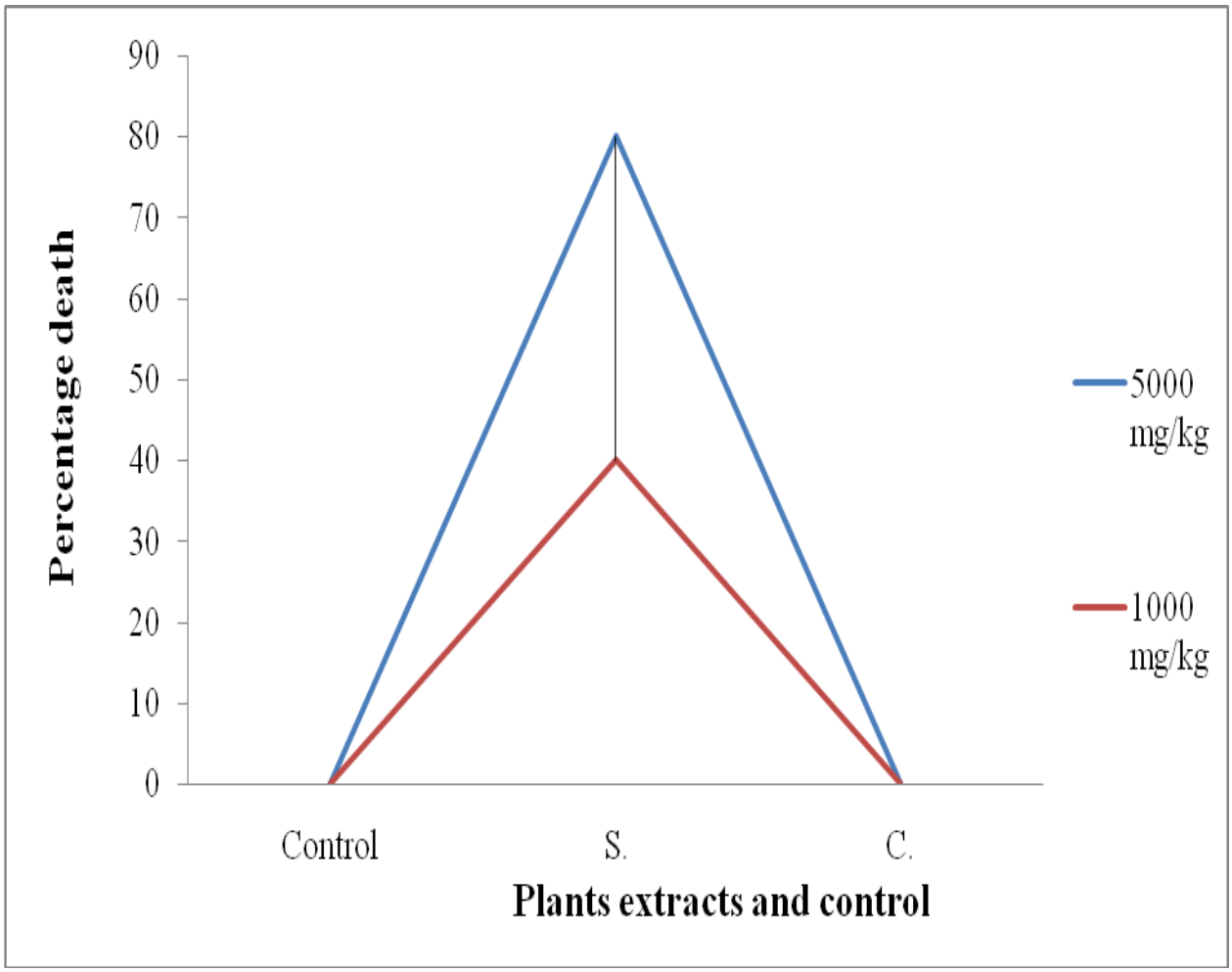
The lethal dose (LD) of *S. didymobotrya* (DCM) and *C. polycephala* (Water) extracts were determined at two doses, 5000 mg/kg and 1000 mg/kg respectively. The DCM extract of *S. didymobotrya* was very toxic at 5000 mg/kg and killed 80% of mice, while *C. polycephala* water extract at this dose did not kill any mice. At 1000 mg/kg dose the DCM extract of *S. didymobotrya* killed 40% of the mice. The results are summarized in Table 3.16.

Table 3. 16 : Acute toxicity of *Senna didymobotrya and Cyathula polycephala* extracts

Test Extract	Weight of Mice in Grams	Mortality within 24 hours			
		LD at 5000mg/kg		LD at 1000mg/kg	
		Deaths	% Deaths	Deaths	% Deaths
Senna didymobotrya (DCM)	17.8 ± 0.837g 21g	4/5	80%	2/5	40%
Cyathula polycephala (Water)	18.0 ± 0.707g 23.8± 1.304g	0/5	0%	0/5	0%
Control	18.8 ± 1.304g 24 ± 1.414g	0/5	0%	0/5	0/%

Key: DCM – Dimethylsulfoxide, **LD –** Lethal dose

Analysis of acute toxicity were done using excel and the dose response curve for *C. polycephala* (water) and *S.* didymobotrya (DCM) extracts is shown in Figure 3.2.



Key: S- *S. didymobotrya*, **DCM** extracts, **C-** *C. polycephala* water extracts, **Control-** Negative control

Figure 3. 2: Dose response curve

3.6 Data analysis

The results for data analysis showed that the plants extract were significant in inhibiting the growth of bacteria and fungi in the disk diffusion method. The inhibition zones were significant at 95% confidence interval (p- 0.05). The p values for all the variables are between <0.001 and 0.006. All this values are below 0.05 hence they are significant. Therefore the plants extracts under investigation can be used as antimicrobials (Table 3.17 and 3.18).

Table 3. 17: Between groups (plant, solvents and organisms) bacterial isolates

Tests of Between-Subjects Effects

Source of variation	Sum of square	df	Mean Square	F	P value
Plant	271.2	4	67.8	15.7	<0.001
Solvents	59.1	3	19.7	4.5	0.006
Organism	76.6	4	19.1	4.4	0.003
Error term	315.9	73	4.3		
Total	722.8	84			

Key: df-degree of freedom, F- f calculated, P- p value at 95% confidence interval

Table 3. 18: Between groups (plant, solvents and organisms) fungal isolates

Tests of Between-Subjects Effects

Source of variation	Sum of square	df	Mean Square	F	P value
Plant	714.6	4	178.6	21.2	<0.001
Solvents	87.0	3	29.0	3.4	0.020
Organism	183.4	5	36.7	4.3	0.001
Error term	751.1	89	8.4		
Total	1736.1	101			

R Squared = 0.567 (Adjusted R Squared = 0.509)

Key: df-degree of freedom, F- f calculated, P- p value at 95% confidence interval

Other tables for data analysis are shown in Appendix 4 and 5.

3.7 Thin layer Chromatography results

According to the results of the phytochemical screening study, all tested plant extracts were found to show a positive test for the presence of phenols. Methanol extracts of *E. abyssinica* and *S. didymobotrya* showed positive test for the presence of alkaloids and flavonoids, while *C. polycephala* methanol extract showed a negative result for the same test. Terpenoids and Anthraquinones were present on both *E. abyssinica* and *S. didymobotrya* dichloromethane extract and absent in the other plants extracts screened. Screening was done using thin layer chromatography (TLC) plates as shown in Appendix 3. The results are as shown in Table 3.19.

Table 3. 19: Phytochemical profile of the plants extracts

Plant species	Solvent	Plants compounds				
		Terpenoids	Alkaloids	Flavonoids	Phenolic	Anthraquinones
E. abyssinica	M	_	+	+	+	_
	D	+	+	+	+	+
C. polycephala	M	_	_	_	+	_
S. didymobotrya	M	_	+	+	+	_
	D	+	+	+	+	+

Key: **D** - Dichloromethane, **M**- Methanol, **+** -Present, **-** Absent

CHAPTER FOUR

4.0 DISCUSSION, CONCLUSION AND RECOMMENDATIONS

4.1 Discussion

The comparison of average percentage yields of all the solvents used showed that water extract had the highest yields in all the plants parts extracted. In general, the percentage yields for water extracts were more than the percentage yields for organic solvents. This is due to the fact that water is polar than organic solvents hence it is able to extract more compounds from a plant material. The leaves were noted to have a high percentage yields as compared to the stems bark in all the solvents used. Kigondu *et al*., (2009) also found similar results.

The results obtained in this study indicate a considerable difference in antimicrobial activity of different extracts from selected plant species. The most susceptible bacterial against all the extracts tested was *P. aeruginosa*. Similarly, *T. mentagrophyte* was found to be the most susceptible fungus against the extracts as compared to other fungal strains tested although extracts from *C. abyssinica* was found to have no activity against it. From all the fungal strains included in the test, *C. albicans* ATCC, *C. parapsilosis* , *C. krusei*, *C. neoformans* were found to have no activity against all the plant extracts except *E. abyssinica*. The least susceptible Fungus was *C. neoformans* probably because of the presence of a mucopolysaccharid capsule. The polysaccharide capsuler material in some of the pathogenic microorganism is responsible for virulence and antimicrobial resistance (Haloprogin, 2006). Some crude extracts recorded the highest activity on fungal strains for example inhibition zone diameters of 23.0 mm from hexane extract of *E. abyssinica*

against *T. mentagrophyte* and 16.0 mm from DCM extract of *S. didymobotrya*. The least activity was evidence with extracts having inhibition zone diameters between 7 and 10 mm. Those with 6 mm diameters had no activities since the filter paper disk was 6 mm in diameter. In terms of inhibition zone diameters most of the extracts displays antibacterial activity amounting to 10.0 mm this can be considered moderately active in consideration that the highest is at 23.0 mm and the lowest is 7.0 mm.

These findings are in line with the investigation of Bii *et al.,* (2008) which indicated that the lowest activity was at 7.0 mm and the highest was at 23.5 mm in diameters at the same concentrations. The solvents used for extraction were used as negative control while fluconazoles and gentamycin were used as positive controls. The conventional drugs gentamycin and fluconazole inhibition zone diameters was between 9.0 and 21.0 mm which compared well with that of the plant extracts although the extracts concentration was high as compared to the conventional drugs. A point to take home is that the drugs are pure compounds but the extracts are crude. When the antimicrobial activities of these plants extracts were compared to that of the positive controls, only *S. didymobotrya* dichloromethane extract at a concentration of 100 mg/ml was found to have almost comparable activity to the standard gentamycin against *P. aeruginosa*. Similarly, *S. didymobotrya, C. polycephala* and *E. abyssinica* at 100 mg/ml showed comparable activity against the fungi *T. menthagrophytes* and *M. gypseum* to that of fluconazole. However, in some cases, antimicrobial activities greater than the positive controls was observed, for example, in *E.* abyssinica against *M. gypseum*.

In general, among the tested microbial strains, bacteria were found to be more sensitive to many of the test extracts than fungi. The antibacterial activity was more pronounced on the gram-negative bacteria (*K. pneumonia*) than the gram-positive bacteria (*S. aureus and MRSA*). The reasons for variant activities between gram negative and gram positive bacteria could be due to their morphological difference. Gram negative bacteria have an outer phospholipid membrane carrying the structural lipopolysaccharide components. This makes the cell wall more permeable to lipophilic solutes, while porins constitute a selective barrier to the hydrophilic. Gram positive bacterial some times are more susceptible since they have only an outer pepetidoclycans layer which is not an effective permeability barrier as reported by Nostro *et al.,* (2003). But in this study found contradicting results. Some extracts are active on both Gram positive and Gram negative like *S. didymobotrya* (DCM) which was active against MRSA and *P. aeruginosa*, hence this can be considered as having broad spectrum of activity and present a potential for control of drug resistant strains. The *S. didmobotrya* is widely used among the Kipsigis for a wide range of conditions like malaria, diarrhea and skin infections hence this practices is supported by the broad spectrum of activity exhibited by its different extracts. The MIC plate is shown in appendix 1.

Water extracts of *S. didmobotrya* were generally active against both the tested bacteria and fungi therefore supporting the traditional methods of water extraction of most of the medicinal plants. Elsewhere studies have shown that *C. abyssinica* extracts have potent antimalarial activities (Irungu and Kigondu, 2007), this phenomenon could explain the low activity exhibited by this extracts against some

bacteria and fungi this means that extracts from this plant exhibit limited antibacterial properties. Generally extracts from organic solvents had were very active against bacteria and fungi as compared to water extracts, interestingly most medicinal plants are used as water extracts despite the low activities observed in this study (Kokwaro, 1993; Gachathi, 1989). These results agrees with the findings of Kigondu *et al.,* (2009) which indicated that methanol extracts were more active compared to water extracts.

The F values of plants, solvents and bacterial organisms was 15.7, 4.5 and 4.4, respectively, while the p value was <0.001, 0.006 and 0.003, respectively. The extracts had no activities against *E. coli* and leading to a significant difference between the extracts and gentamicin at 95% confidence interval. When the F value is greater than p value then it is considered significant, therefore the results were significant at 95% confidence interval where p value is 0.05. Variability for fungal *M. gypseu*m and *T. mentagrophye* were significant at 95% confidence interval, while those of *Candida specie* and *C. neorfomance* were less significant because their inhibition zones diameters were less than 10 mm. Analysis of variance between plants extracts and gentamicin recorded a p value less than 0.001 and a standard error of 1.04. Therefore the activities of the active plant extracts against bacteria and fungi in this study were significant at 95% confidence interval. There was no significance difference between plants extracts and fluconazole with a p value of less than 0.001. The results are also significant for variance of between solvents and test extracts. These results are in agreement with the findings of Muthaura *et al.,* (2007)

demonstrating the effects of solvents on activity in which the results were significant at $p>0.05$. The ANOVA graphs are shown in appendix 4.

The preliminary screening assays for antimicrobial activity can largely be considered as qualitative assays and are used for identifying the presence or absence of bioactive constituents in the extracts. The MIC of the crude extracts were 100 $x10^2$ µg/ml compared to the standard drugs which was 0.5 µg/ml for both yeast and bacterial and 1.0 µg/ml for dermatophytes. This is a clear indication that the active ingredient was present in low quantities, which necessitate the use of large amounts of crude extracts to gain the desired therapeutic concentrations. The differences in bioactivities could be attributed to the facts that plants differ phytochemically and the extraction procedures could also affect or alter their composition as seen from the work of Kigondu *et al.,* (2009). The tradition herbal practitioners are more often than not using plants in combination to attain therapeutic effects; the extracts could be acting synergistically this could have contributed to the absence of activities against *E. coli* in almost all the extracts tested in this study. Another reason is that the herbalists are using the extracts in large quantities hence having therapeutic activities since the concentration of the active ingredients increases with the increasing quantity of the crude extracts. The lowest MIC of the plant extracts range from 3.125 $x10^2$ µg/ml to 50.0 $x10^2$ µg/ml compared to 0.5 µg/ml and 1.0 µg/ml of gentamicin and fluconazole, respectively. Hexane extract of *E. abyssinica* was the most active against *M. gypseum* isolate with an MIC of 6.25 $x10^2$ µg/ml. Although this was significantly lower than that of fluconazole ($p.< 0.001$) it is a promising plant extract given that it was a crude extract compared to pure compound of fluconazole. Among

the Kipsigis community the plant is widely used for skin conditions which may be caused by dermatophytes and bacteria. This study therefore provides scientific evidence of antimicrobial activities of the active plant extracts against bacteria and fungi.

Toxicity studies are very important in screening medicinal plants. The extracts that had MIC of 50.0 $x10^2$ µg/ml and below were considered potent and their cell toxicity were determined. The CV for the plants extracts was tested on concentration 1000 mg/ml and 100 mg/ml to determine their toxic levels. Cell toxicity of the extracts were categorized into two; Toxic CV below 50% and safe CV above 50% as categorized by Zirihi *et al*., (2005). Hexane and methanol extracts of *E. byssinica* were considered to be safe because it recorded a CV which was above 50%. The two extracts *C. polycephala* water and *S. didymobotrya* DCM were very toxic as compared to the positive control Chloroquine which had a CV of 25.28% and 51.94% at concentration 1000 mg/ml and 100 mg/ml, respectively. The other control TDR 22651/1 had a CV of 7.89% and 11.28% at concentration 1000 mg/ml and 100 mg/ml respectively, this control was very toxic to cells since it recorded the lowest CV as compared to all the extracts tested and all the controls.

Except *C. polycephala* water extract, water extracts of the rest of the plants generally had lower cytotoxicities than both methanol and DCM. The results are in agreement with other work done by Muthaura *et al.,* (2007) on antimalaria properties of *Boscia angustifolia* water extracts which he found to have no cytotoxicities. Cepleanu *et al*., (1994) found out that the water stem/bark extracts of *B. angustifolia* had neither

cytotoxicity nor brine shrimp lethality. These results seem to confirm the validity of their traditional uses since traditionally herbs are boiled in water (Gessler *et al.,* 1995). Most of the extracts tested had low toxicity (CV above 50%) against HELF cells, suggesting that they may be safe as antimicrobials. This result agrees with other studies done by Kigondu *et al*., (2009) on cell toxicity of medicinal plants.

Acute toxicity was done on *S. didymobotrya* DCM extracts and *Cyathula polycephala* water extracts since they were found to be toxic to the cells. The extracts were considered safe when the death of mice was less than 50% which was observed at a lower dose of less than 5000 mg/kg (Zakeri, 2008; Gessler *et al.,* 1995). Between the two extracts tested *S. didymobotrya* DCM extract killed 80% of mice at a dose of 5000 mg/kg and 40% at 1000 mg/kg. Water extract of *C. polycephala* did not kill the mice at the two doses. This suggests that the *C. polycephala* extract can be safe as an antimicrobial agent at both doses. The extracts were tested on mice and the results cannot apply to human beings unless clinical trials are done. These results are in agreement with the findings of Muthaura *et al.,* (2007), who found that the water extract was less toxic than the DCM extract. The plant *S. didymobotrya* is widely used among the Kipsigis community in treating and managing skin and diarrhoea infections. However the method of preparation such as burning into ashes and then mixing with margarine for skin conditions as well as mixing with hot water and milk for stomach problems may be a way of reducing toxicity, otherwise the population may be continuously exposed to cytotoxic plants compounds.

Phytochemical screening is important in determining the type of chemical constituents found in a given herbal drug or extract. The plants were screened using thin layer chromatography visualization reagents. It revealed that the plants had the following compounds, *E. abysinica* methanol extracts had alkaloids, flavonoids and phenolics, while DCM extract had terpenoids, alkaloids, flavonoids, phenolics and anthraquinones. The *S. didymobotrya* extract DCM extracts had terpenoids, alkaloids, flavonoids, phenolics and anthraquinones while the water extract had alkaloids, flavonoids, phenolics. The presences of flavonoids, phenolics or terpenoids in plants have been reported to be responsible for antimicrobial activity in plants, (Nostro *et al.,* 2000). Flavonoids and flavonoid-derived plant natural products have long been known to function as antimicrobial defense compounds (Kazmi *et al.,* 1994). The different *in vitro* studies have also shown that they are effective antimicrobial substances against a wide spectrum of microorganisms (Schewe and Sies, 2003). For example the quercetin and naringenin (Meng *et al*., 2000), and the mixture of catechin compounds found in oolong and green teas (Rauha *et al.,* 2000) have been shown to exert antimicrobial activity against a wide range of microorganisms. Recent findings have also showed that ronane diterpenes, hugorosenone [3 ß-hydroxyrosa-1, 15-dien-2-one and 18-hydroxy hugoro senone among others] from *H. casteneifolia* have both larvicidal and antifungal activity (Baraza *et al.,* 2005). The antifungal and antibacterial activity of the plants studied was attributed to the presences of these secondary metabolites. In plants, their role is to protect plants against microorganisms and insects (Cowen, 2008).

4.2 Conclusion

Water extracts produced the highest yields of all the extraction solvents and therefore if one needs a lot of extracts then the best solvent for extraction is water. Extracts from methanol had good activities judged by the high inhibition zones of more than 10mm in diameters such as *E. abyssinica* stem bark therefore methanol is able to extracts a lot of the active ingredients in a given plant material while water extracts were not able to extracts active compounds.

The extracts of *E. abyssinica* and *S. didymobotrya* were very active against *P. aeruginosa* and *S. aureus* gram negative and gram positive bacteria thus it is considered to have broad spectrum. The plants extracts were also active against T*richophyton mentagrophtes* and *Cryptoccocus neorfomance* therefore they also have antifungal properties.

In both the cell and acute toxicity, it was observed that the DCM and water extracts of *S. didymobotrya* and *C. polycephala* were very toxic at high concentration and at high doses.

Plants compounds such as flavonoids, phenolics or terpenoids are responsible for antimicrobial activities in plants as reported elsewhere therefore this compounds in the plants extracts *E. abyssinica* methanol and *S. didymobotrya* DCM were responsible for antimicrobial activities.

The antifungal and antibacterial activity of the plants under investigation demonstrated support for the claimed ethnobotanical because they were active and the analysis should that they were significant at 95% confidence interval thus providing scientific prove for their medicinal uses.

4.3 Recommendations

Some extracts of *E. abyssinica* and *S. didymobotrya* plants have both antibacterial and antifungal analysis and isolation of the active compounds present as well as determination of their bioactivity should be done. Such an effort could lead to identification of a new range of compounds for management of fungi and bacterial infections.

Since this study was based on the plants part used by herbalist, it is worth recommending that other parts be studied especially the roots; the plant extracts be tested in combination to determine their synergistic activities. The medicinal plants should be conserved in their natural environment so as to conserve our heritage.

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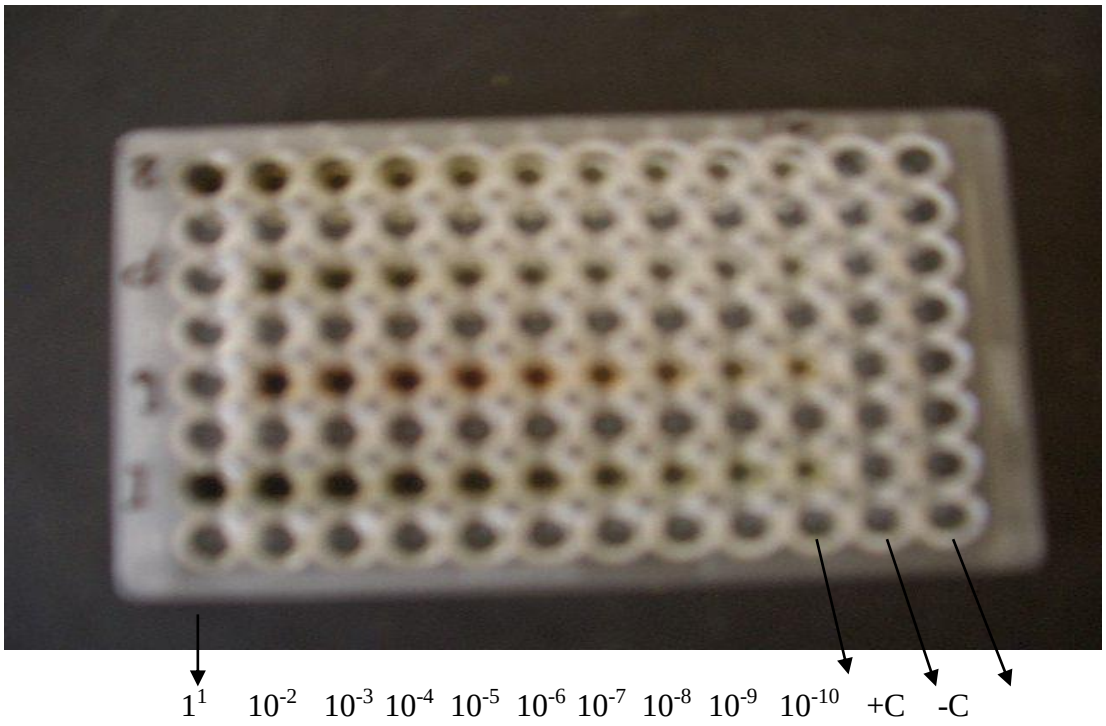
APPENDICES

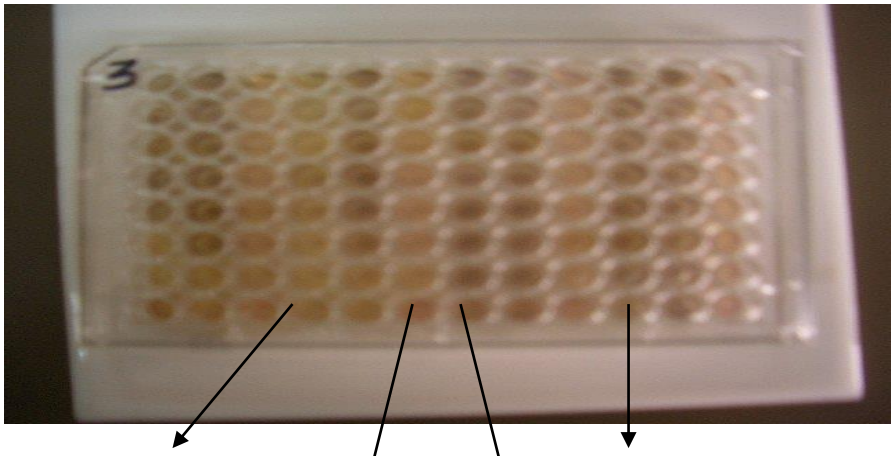
Appendix 1: Microtitre plate showing minimum inhibition concentrations

The plate shows four extracts at different concentration beginning from 1^{1} – 10^{-10} dilution factor. Gentamicin drug was used as a positive control for bacterial isolates while fluconazole was used as a positive control for fungal isolates. Sterile distilled water was used as a negative control for water extracts. Dimethylsulfoxide (DMSO) was used as a negative control for organic solvents. The photograph in appendix 1. shows the minimum inhibition concentrations. The fist well is the experiment while the second well was used a comparison (to compare the turbidity between the first well which was inoculated and the comparison well which was not inoculated).



Appendix 2: Cytotoxicity plates

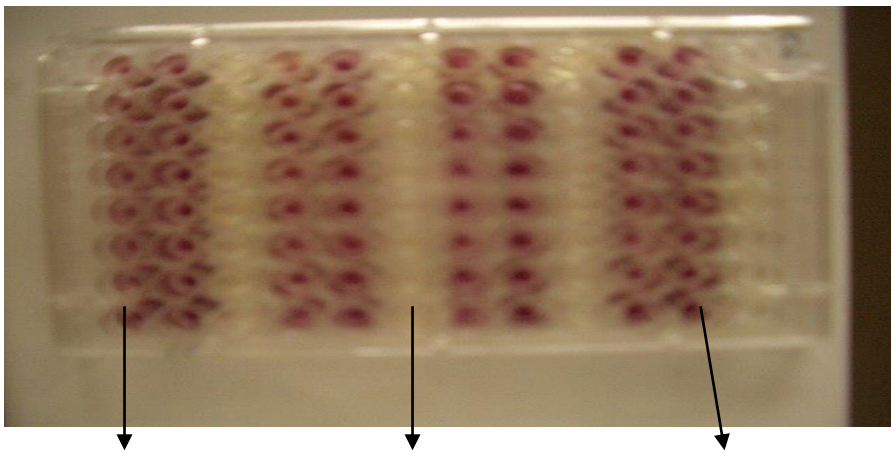
The following is a microtitre plate showing plant extract which was tested for cell toxicity. The extracts were tested at 1000mg/ml and 100mg/ml concentration. The clear wells indicate cell death while the dark wells indicate intact cells



Clear well (cells died) Intact cells (no cell death)

Positive control Negative control

The microtitre plate 2. gives the results of a plant extract which was not cytotoxic as seen from the wells with intact cells. The clear wells are positive control and the last two wells are negative controls.

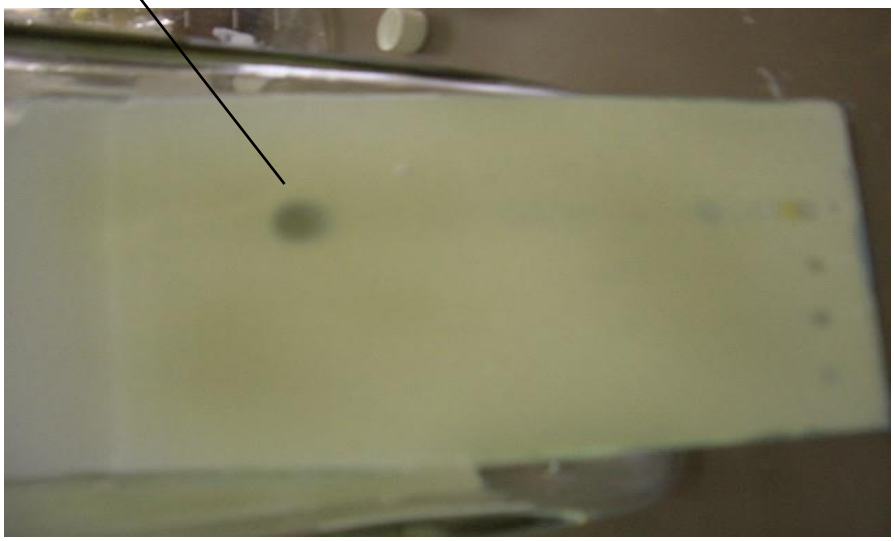


Plant extracts Positive control Negative control

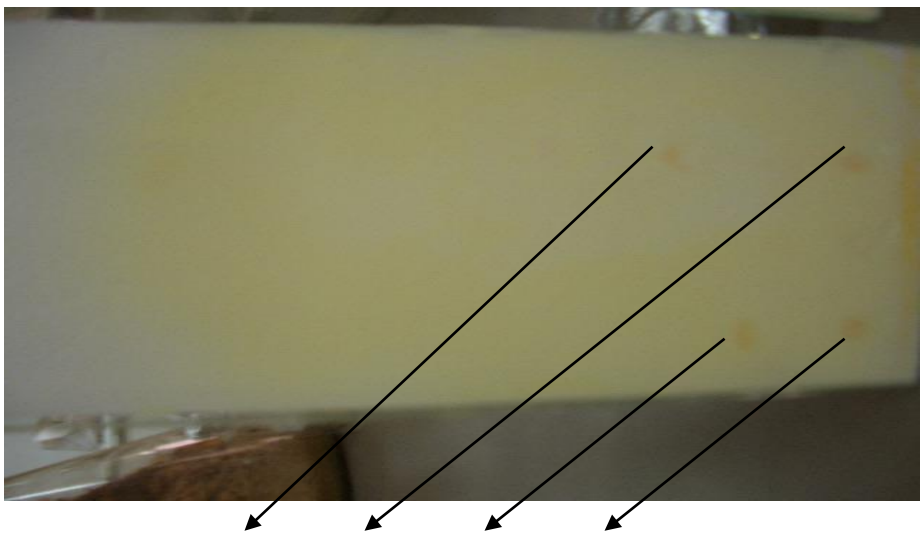
Appendix 3: Thin layer chromatography plates

Thin layer chromatography plate indicating a single compound as demonstrated by the presence of one dot

One dot indicating one compound



Thin layer chromatography plate indicating the presence of more than one compound as demonstrated by the presence of several dots



Four dots indicating four compounds (The dots are in different position because the compounds travel along the TLC plate at different wavelength)

Appendix 4: Statistical analysis – analysis of variance of bacterial data

Source of variation –In all the analysis the variable tested is significant P=0.05

Between plants

(I) Plant	(J) Plant	Mean Difference (I-J)	S. E	P value	95% Confidence Interval	
					Lower	Upper
E. abbyssinica	CONTROL	-6.85	1.04	<0.001	-9.31	-4.39
C. abbyssinica	CONTROL	-8.05	1.04	<0.001	-10.51	-5.59
C. polycephal	CONTROL	-7.20	1.04	<0.001	-9.66	-4.74
S. didymobotrya	CONTROL	-6.20	1.04	<0.001	-8.66	-3.74

Between test organism

(I) Organism	(J) Organism	Mean Difference (I-J)	S. E	P value	95% Confidence Interval	
					Lower	Upper
ECOLI	PAE	-2.82	0.71	0.001	-4.60	-1.04
KLEB	PAE	-0.82	0.71	0.603	-2.60	0.96
MRSA	PAE	-1.71	0.71	0.064	-3.49	0.08
SAU	PAE	-1.71	0.71	0.064	-3.49	0.08

Appendix 5: Statistical analysis – analysis of variance of fungal data

Between plants

(I) Plant	(J) Plant	Mean Difference (I-J)	S. E	P value	95% Confidence Interval	
					Lower	Upper
E. abbyssinica	Control	-8.67	1.33	<0.001	-11.80	-5.54
C. abbyssinica	Control	-11.58	1.33	<0.001	-14.71	-8.45
C. polycephal	Control	-10.92	1.33	<0.001	-14.05	-7.79
S. didymobotrya	Control	-9.08	1.33	<0.001	-12.21	-5.95

Between solvents

(I) Solvents	(J) Solvents	Mean Difference (I-J)	S. E	P value	95% Confidence Interval	
					Lower	Upper
HEXANE	Fluconazole	-10.54	1.33	<0.001	-13.67	-7.41-
DCM	Fluconazole	-9.54	1.33	<0.001	-12.67	6.41
METHANOL	Fluconazole	-8.83	1.33	<0.001	-11.96	-5.70
WATER	Fluconazole	-11.33	1.33	<0.001	-14.46	-8.20