ETHNOBOTANICAL SURVEY, ANTIMICROBIAL EFFICACY AND PRELIMINARY PHYTOCHEMICAL SCREENING OF SELECTED ANTI-DIARRHOEAL MEDICINAL PLANTS USED BY THE SAMBURU COMMUNITY, WAMBA, SAMBURU DISTRICT, KENYA

OMORI ERIC OMWENGA (BSC)

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Declaration

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

NAME: OMORI ERIC OMWENGA

DEPARTMENT OF PLANT AND MICROBIAL SCIENCES

SIGNATURE…………………………………. DATE……………………..

We confirm that the work reported in this thesis was carried out by the candidate under our supervision.

1. PROF. OKEMO O. PAUL
   KENYATTA UNIVERSITY
   DEPARTMENT OF PLANT AND MICROBIAL SCIENCES

   SIGNATURE……………………………………DATE………………………....

2. DR. MBUGUA PAUL K.
   KENYATTA UNIVERSITY
   DEPARTMENT OF PLANT AND MICROBIAL SCIENCES

   SIGNATURE…………………………………………DATE……………………

Dedication

This work is dedicated to the Samburu people especially the elders, other local informants and the local herbalists who volunteered the information about the usage of the antidiarrhoeal plants.

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ACRONYMS AND ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
ATCC	American Type Culture Collection
CDC	Centre for Disease Control
CFU	Colony Forming Units
DMF	Dimethyl formanide
DNA	Deoxyribonucleic Acid
HIV	Human immunodeficiency virus
HMR	High-level mupirocin-resistant
LMR	Low-level mupirocin-resistant
MBC	Minimum bactericidal concentration
MIC	Minimum inhibitory concentration
MRSA	Methicillin Resistant *Staphylococcus aureas*
ORT	Oral rehydration therapy
PDA	Potato Dextrose Agar
PPM	Parts per million
QR	Quinolone-resistant
RNA	Ribonucleic Acid
STD	Standard
TLC	Thin Layer Chromatography
WHO	World Health Organization

ABSTRACT

Despite advances in understanding of the causes, treatment and prevention of diarrhoeal diseases an estimated 4.6 million people die from diarhoreal diseases every year. Diarrhoea can either be acute or chronic, with acute diarhoea being the most common form. The main agents of diarrhoreal diseases include viruses like rotavirus, bacteria such as *Enterobacteria* e.t.c and parasitic agents like *Cryptosporidium.* The disease has been found to attack mostly the children who are below the age of 5years old and the elderly. It is also a major opportunistic infection amongst the HIV/AIDS patients. Oral rehydration therapy (ORT), antibiotics and medicinal plants have been used as remedials. But antibiotics have been found to posssess antimicrobial resistence hence need for search of new drugs. Plants have been found to have active compounds (phytochemicals) which have antidiarrhoreal activity. Therefore this study evaluated the efficacy of the antidiarrhoeal plants used by the Samburu community. By use of simple questionnares a survey was carried out that was targeting the Samburu informants and herbalists on the common antidiarrhoreal plants they use. Thirty three antidiarrhoeal plants were collected, dried, chopped, ground by the laboratory grinding miller. Voucher specimens were prepared and deposited at the Kenyatta University herbarium. Extraction of the selected medicinal plants was done by use of methanol (solvent) and then screened by use of the disk diffusion method against the selected microorganisms that were collected from Kenyatta National Hospital. There was significant difference of the means of the zones of inhibition of the *Staplylococcus aureas, Salmonella typhi* and *Pseudomonas aeruginosa* at $P \leq 0.01$ except in *Escherichia coli*, *Bacillus subtilis* and *Candida albicans* that showed no significant difference even at $P \leq 0.05$. No activity was observed on *A. flavus, Aspergillus niger, Penicillium spp,* and *Fusarium lateritium*. *Pseudomonas aeruginosa* was affected by most plant extracts in terms of zones of inhibition. The The MICs and the MBCs of the extracts were determined by use of the microtitre plate method with *Acacia ethaaica, Acacia nilotica, Acacia tortilis, Albizia anthelmitica, Clerodendrum myriacoides, Plumbago dawei & Thylachium africanum* extracts showing good MICS and MBCS results of 9.38- 18.75 (mg/ml) in most test cultures except *C. albicans* that seemed to be insensitive to any extract. The extracts produced high MICs and MBCs againsts *P. aeruginosa* and *S. aureas.* Preliminary phytochemical screening of tannins, terpenoids, cardiac glycosides, alkaloids, and saponins was also carried out. Tannins were the most abundant phythochemicals present that were found to be present in most plant extracts. Generally the Gram negative isolates were more affected than the Gram positive isolates hence the activeity of the extracts can not be ascribed to the cell wall properties but to other factors like the sensitivity of the test organism to the extracts, the diffusion rate of the extract e.t.c. For the fungi isolates the activity of the extracts was very low and thus it can be ascribed to the cell wall properties that may have made the extracts not to diffuse into the test micro-organisms. Also presence of different pythochemicals may explain the activity of the extracts whereby they can be offering some synergism/additive or antagonism activity against the test isolates. More work needs to be done on the highly active extracts like *Acacia nilotica* and *Plumbago dawei*. Thus the results obtained clearly back the use of the medicinal plants selected from the community as they showed good activity against the test cultures.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

The Samburu community is one of those communities in the sub-Saharan Africa that practices nomadic pastoralism due to the kind of climatic conditions in the region that is semi-arid with the rainfall distribution of about 250mm per year. In Kenya it is one of the marginalized areas leave alone in the sub-Saharan Africa mainly in terms of 'HEALTH FOR ALL' as a basic human right and prerequisite to social-economic development. Pastoralism as a normal practice of the inhabitants leads to sharing of water with both domestic and wild animals hence inhabitants end up using water without proper treatment since it is scarce in most times of the year and this has led to an increase in diarrhoreal diseases. Also lack of good sanitary conditions in the region like lack of pit latrines to a large extent has led to the increase of the diarrhoreal diseases in the region. The diseases get magnified given the fact that they lack proper medication because the community is poor and tends to prefer the use of local treatment by use of medicinal plants instead of hospitalization. It is estimated that about 85% of the Samburu community medicare is from medicinal plants as the community believes in medicinal plants first before the patient is hospitalized like in most communities in developing nations (Bussmann, 2006). Thus there is need for the scientific evaluation of the medicinal plants the Samburu community uses to treat diarhoreal diseases.

The Samburu people like the rest of the world contribute to the estimated 4.6 million people, including 2.5 million children, die from diarrhoreal diseases every year (Kosek *et* *al.,* 2003; Thapar and Sanderson, 2004), particularly in developing countries. The WHO defines diarrhoea as three or more loose or watery stools in a period of 24h (Allen *et al.*, 2003), although changes in the consistency of the stools are important indicators of the disease. Diarrhoea can be classified as acute or chronic, with acute diarrhoea being the most common form. Acute diarrhoea has an abrupt onset, resolves within about 14 days and is usually caused by infectious agents, although drugs, poisons (including bacterial toxins) or acute inflammatory reactions can contribute (Thapar and Sanderson, 2004). Worldwide, rotavirus is the major cause of infectious diarrhoea, particularly among young children, however, other viral (adenovirus, enterovirus and norovirus), bacterial (enterobacteria amongst many*)* and parasitic (*Cryptosporidium and Giardia)* agents are important pathogens (Palombo, 2006).

Fungi can also cause diarrhoea, for instance *Candida* species form an ubiquitous genus of yeast present throughout the environment. They are part of the normal flora in the alimentary canal and on mucocutaneous membranes. *C. albicans* is the most common yeast species isolated from human faeces, being identified in 65% of stool samples from healthy adults (Forbes *et al*., 2008). Nevertheless, several reports have suggested that it may cause diarrhoea as they have identified *Candida*, but not other enteric pathogens, in the stools of patients with diarrhoea and have reported symptom resolution following treatment (Forbes *et al*., 2008).

Oral rehydration therapy (ORT) remains the major treatment for diarrhoea, although it does not reduce the volume or duration of diarrhoea (Subbotina *et al.,* 2003). Other treatment options include antibiotics and gut motility suppressing agents, which reverse dehydration, shorten the length of the illness and reduce the period of time an individual is infectious (Allen *et al*., 2003; Guerrant, 2001). Treatment with pharmacological agents that are pathogen specific or that suppress severe symptoms would be of benefit to patients suffering from prolonged diarrhoea (Takahashi *et al.*, 2001; Oi *et al.*, 2002).

Irrespective of the fact that antibiotics are used widely in management of diarrhoreal diseases, microbial resistance to antibiotics in use nowadays is a major concern. It is actually a global problem as most pathogens have been found to be resistant to the mostly used antibiotics and provides the need for the search for new compounds with potential effects against pathogenic bacteria (Larhsini *et al.*, 2001). For instance over the last three decades, methicillin resistant *Staphylococcus aureus* caused major problems in hospitals throughout the world (Chambers, 1997).

Antibiotics have also been found to cause side effects like nausea, diarrhoea, constipation, memory loss, cardiotoxicity, hepatotoxicity, nephrotoxicity amongst many with most causing depression of the immune system (Gralla *et al.*, 2005). Thus there is need for search of antimicrobials that offer less of these toxities. On the other hand medicinal plants, because of their often multiple targets, minor side-effects, low potentials to cause resistance and low costs, are increasingly being projected as suitable alternative sources of antimicrobial agents (Jassim and Naji, 2003).

Traditional plants have also been used in treatment of diarrhoreal diseases since time immemorial (Tanaka *et al.*, 2002). Indigenous plants such as *Andrographis paniculata-* (Burm.f.) Nees, *Asparagus racemosus* Willd., *Butea monosperma* (Lam.) Taub, *Cassia auriculata* (Linn.) and others are widely used for the treatment of diarrhoea (Kumar *et* *al.,* 2001). These amongst many that have been scientifically evaluated against most microorganisms show indeed plants can be alternatives. In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents (Krishnaraju *et al.,* 2005). Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections (Jigna and Sumitra, 2007).

However in the traditional systems of medicine, there is lack of proper diagnosis and the underlying disease could be quite difficult to interprete. This means that medicinal plant administration is based mainly on the patient's symptoms and is therefore important to test the extracts against the pathogens to establish whether the activity exists or not. One plant may be used to treat more than one disease while a single disease may be treated by several plant species. Thus it is difficult to ascertain which plant is actually effective for a particular complaint unless we carry out scientific tests to find out the sensitivity of particular pathogens to the extracts (Gathu, 2006). Palombo (2006) found out that the cases of intoxication due to overdosing of the medicinal plants or inefficiency due to underdosing are also arising. Thus the scientific evaluation of such extracts in terms of quality, efficacy and safety of such preparations should be well assessed.

The advantage of the use of medicinal plants is their ability to deliver high local concentrations of antibiotic irrespective of vascular supply. Further benefits include the absence of adverse systemic effects, and a low incidence of resistance (Spann *et al.,* 2003).

1.2 Problem statement and justification

Irrespective of the fact that ORT, antibiotics and medicinal plants are used in the management of the diarrhoreal diseases still the condition has not been fully eradicated. Most of the antibiotics used in management of diarrhoea have been found to have a lot of side effects like nausea, cardiotoxity, memory loss e.t.c. (Gralla *et al.,* 2005). On the hand most of the targeted microorganisms by such antibiotics have developed some resistance to them thus there is need for such of new drugs (Larhsini *et al.*, 2001). Intoxication and inefficiency cases have been also on an increase on the usage of the medicinal plants on the other hand. This can be due overdosing or underdosing of the medicinal plants used thus there is need for scientific evaluation of such medicinal plants to ascertain their activity (Palombo, 2006). Lastly administration of such medicinal plants by most communities is mainly based on the patient's symptoms but not on proper diagnosis of the disease thus the underlying disease could be difficult to interprete. On the other hand one plant can be used to treat different diseases or many plants can be used to treat one disease. Therefore it is important to evaluate the activity of such plants against the pathogens to ascertain there activity (Gathu, 2006).

1.3 HYPOTHESIS

HO-There is no significant efficacy of the selected medicinal plants used by the Samburu community in the treatment of the diarrhoreal diseases.

1.4 OBJECTIVES OF THE STUDY

1.4.1 General objective

To determine the efficacy of selected medicinal plants used by the Samburu community against diarrhoeal diseases.

1.4.2 Specific objectives

a. To carry out an Ethnobotanical survey on the antidiarrhoeal plants used by the Samburu community.

b. To screen selected plant extracts *in-vitro* against diarrhoea causing bacteria and fungi by use of bioassay methods.

c. To determine the MICs and MBCs of the medicinal plants against diarrhoeal agents.

d. To determine the phytochemicals present in the extracts.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

The word diarrhoea is derived from the Greek words for "flowing through". For most persons, diarrhoea means “the frequent passage of loose stools” (Talley *et al.,* 1994). This definition includes two major components: loose stool consistency (pourable stools) and increased stool frequency (more than two bowel movements daily). Physicians often include a third component: increased stool weight (> 200 g/24 h), but patients are poor estimators of stool output.

Diarrhoea is a universal human experience. Most persons have had acute infectious diarrhoea at some time during their lives. The incidence of acute diarrhoea is roughly 5% to 7% annually (Dupont, 1997). Infectious diarrhoea is associated with contaminated food and water and is typically spread via fecal-oral transmission. Chronic diarrhoea (lasting > 4 weeks) is also common, with a prevalence of approximately 5% in the United States (Fine and Schiller, 1999). It is less likely to be caused by infection and is more likely to be a symptom of other disorders.

Diarrhoea results from excess water in the stool (Wenzl *et al.,* 1995). To understand the pathophysiology of diarrhoea, it is necessary to understand how water is transported across the mucosa of the gastrointestinal tract. Water moves in response to osmotic gradients established by the absorption of salts (mainly sodium chloride, but also potassium and bicarbonate salts) and nutrients (e.g., monosaccharides, amino acids, and fatty acids). Salts and nutrients move both passively, in response to electrochemical gradients across the mucosa, and actively, in response to molecular pumps located in the enterocyte membranes (Sellin, 2002).

Diarrhoea develops if the overall efficiency of absorption declines by as little as 1%. This can occur under the following circumstances: when the rate of intestinal nutrient and salt absorption decreases; net electrolyte secretion develops (an unusual circumstance except with severe diarrhoea such as the diarrhoea associated with cholera, in which stool output can exceed 10 L/day); transit through the intestine speeds up, thereby limiting the time available for absorption; or poorly absorbable substances are ingested and increase intraluminal osmotic activity, causing the retention of water within the intestine (osmotic diarrhoea) (Schiller, 2000).

Common problems that primarily cause a reduction in the rate of intestinal nutrient and salt absorption include mucosal diseases, such as celiac disease; inflammatory diseases that disrupt the integrity of the intestinal mucosa (e.g., Crohn disease); and infections with pathogens that produce toxins that affect enterocyte function like bacteria (such as *Campylobacter jejuni, E. coli, Salmonella spp., Vibrio cholerae, Yersinia enterocolitica, Aeromonas spp., Staphylococcus aureas* amongst many), enteroparasites (*Giardia spp., Cryptosporidium spp.*, and *Entamoeba histolytica*), and viruses (adenovirus, Norwalk virus, and rotavirus) (Vargas *et al.*, 2004)**.**

2.2 Diarrhoea epidemiology

Diarrhoeal illness is common world-wide, causing a wide spectrum of signs and symptoms ranging from minor discomfort to dehydration which may result in death. Episodes of diarrhoea can also lead to subsequent ill health (Cumberland *et al.*, 2003;

Helms *et al.,* 2003) particularly in more vulnerable groups such as children, older people, or those with an underlying disease. There are also substantial social and economic costs associated with the high frequency of diarrhoeal illness (Hellard *et al.,* 2003; Roberts *et al.,* 2003). Many of the cases are infectious, and are caused by a variety of viruses, bacteria, or protozoa.

Some information on the frequency of diarrhoeal illness is available from laboratory-based communicable disease reporting, outbreak surveillance, and other routine public health surveillance activities. However, these sources underestimate the burden of diarrhoeal illness as they usually only represent persons coming into contact with health services. For this reason, population-based studies have been conducted in several countries to obtain more comprehensive estimates of the prevalence of diarrhoeal illness in the community (Herikstad *et al.,* 2002; Imhoff *et al.,* 2004). While these studies provide important information on the epidemiology of diarrhoeal illness, the use of different study designs and varying case definitions has made international comparisons difficult.

Nevertheless acute diarrhoeal disease among children younger than 5 years remains a major cause of morbidity and mortality worldwide (Olesen *et al.,* 2005). An Expert Committee of the World Health Organization recently estimated that diarrhoea causes 18% of the 11 million deaths among children younger than the age of 5 years in the world, nearly the same mortality as pneumonia (19%) which is the leading cause of infant mortality (Bryce, *et al.,*2005). Disease and death caused by diarrhoea is a global problem, but is especially prevalent in developing countries (Kosek *et al.,* 2003). Adults are also affected and need special attention in treatment and management, especially in acute- and long-term care residents, because of their multiple comorbidities, immunosenescence, frailty, and poor nutritional status (Trinh and Prabhakar, 2007). The incidence of diarrhoeal illness in adults is estimated to be 1.4 episodes/adult/year (Altekruse *et al.,* 1995). On the other hand it is identified as a major opportunistic disease among HIV/AIDS patients (Kone *et al.*, 2004). *Blastocystis hominis* for instance has been known to cause diarrhoea in immunocompromised hosts (Gassama *et al*., 2001).

International programs are encouraging the use of oral rehydration therapy (ORT) which has greatly reduced infant mortality in recent years by treating dehydration (Victoria *et al.,* 2000; Bhan, 2000). In addition antibiotics are recommended for treating diarrhoea to shorten the duration of illness, decrease morbidity and mortality, and reduce duration of bacterial shedding (Guerrant *et al.,* 2001). However, diarrhoeal diseases remain a serious public health problem and continue to be a leading cause of disease and death in children worldwide (Guerrant *et al.*, 2002).

Severe infectious diarrhoea occurs most frequently under circumstances of poor environmental sanitation and hygiene, inadequate water supplies, and poverty. This is not to say that the problem occurs only in developing countries because such conditions are common in underdeveloped countries as well. Diarrhoea in developed countries is prevalent (at least 1 to 3 episodes per child per year) and has large economic costs (Ethelberg *et al.,* 2006). In developed countries, childhood diarrhoea has been linked with daycare attendance and lack of breast-feeding. Contact with symptomatic persons in the home or elsewhere, and exposure to contaminated food and water is common also in the children and the elderly (Kenrad, 2006).

Despite considerable advances in the understanding and management of diarrhoeal disorders in childhood, globally these still account for a large proportion (20%) of childhood deaths, with an estimated 2.2 million deaths (Black *et al.*,2003). In a global estimate of the burden of diarrhoeal disorders in 1980 the World Health Organization calculated that there were over 700 million episodes of diarrhoea annually in children under 5 years of age in developing countries (excluding China), with approximately 4.6 million deaths (Zulfiqar, 2006). More recent reviews of studies published in the last 10 years indicate that although global mortality may have been reduced, the overall incidence remains unchanged at about 3.2 episodes per child per year (Yusufzai and Bhutta, 2000).

2.3 Virulence factors of diarrhoea causing microorganisms

Most of the pathogens that cause diarhoea have such virulent traits or presumed virulent traits being distinct for each of the groups and are divided into several broad groups: adherence factors that allow pathogens to attach to the intestinal mucosa and begin to proliferate; toxins that interrupt normal intestinal cell secretion and absorption (secretory toxins); and toxins that damage the intestinal cell (cytotoxins) and produce diarrhoeal disease (Wanke, 2001). Others like *S. typhi* are dependent on their ability to invade cells, possession of a complete lipopolysaccharide coat, the presence of the Vi antigen, and the production and excretion of a protein known as invasin. Invasin allows non-phagocytic cells to take up the bacterium, where it is able to live and replicate intracellularly (Huang and DuPont, 2005).

2.4 Chemotherapy

2.4.1. Historical background

The discovery of antimicrobial agents ranks as one of man's greatest achievements. The sulphonamides were introduced into medical practices in the 1930's, penicillin and streptomycin in the 1940's, the broad spectrum bacteriostatic antibiotics during the 1950's and the broad spectrum bactericidal ones in the 1960's. Other important synthetic chemicals and highly specific narrow spectrum antibiotics were introduced during these years. These were all outstanding achievements which revolutionalized the treatment of bacterial diseases (Baldry, 1976; McKane and Kandel, 1996). However, bacteria have developed methods of resisting the action of these substances so that some have been rendered useless (Ocharo, 2005).

2.4.2. Groups of the drugs used for treatment of diarrhoeal diseases

A lot of drugs have been used to treat such diarrhoeal infections and they are mainly targeted at the pathogen's structure. Such antimicrobials can either be bactericidal/fungicidal (they kill microbes directly) or bacteriostatic (they prevent microbes from growing). For instance some antibiotics inhibit the cell wall synthesis e.g. penicillin (Fig. 1) and cephalosporin. Because they tend to target the synthesis process of the cell wall, only the actively growing cells are affected by these antibiotics. Also, since human cells do not have the peptidoglycan cell walls, such antibiotics have less toxicity to the host cells.

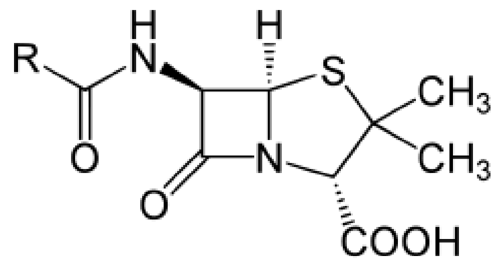


Fig. 1: Structure of Penicillin.

Others interfere with the structure and function of the nucleic acid e.g. sulfonamides. They interfere with the processes of DNA replication and transcription in microorganisms. Some drugs with this mode of action have extremely limited usefulness because they interfere with mammalian DNA and RNA (Tortora *et al.,* 2002).

Others interfere with protein synthesis process of the bacteria since it is the common feature of all cells. Chloramphenicol (Fig. 3), erythromycin, streptomycin and tetracycline (Fig. 2) are among the antibiotics that interfere with the protein synthesis. Binding to 50s portion of the 70s prokaryotic ribosome, chloramphenicol inhibits the formation of peptide bonds in the growing polypeptide chain. Erythromycin also reacts with the 50s portion of the 70s prokaryotic ribosome. Most drugs that inhibit protein synthesis have a broad spectrum activity; erythromycin is an exception as it does not penetrate the Gram negative cell wall, hence mostly affects the Gram positive bacteria. Tetracycline interferes with the attachment of the tRNA carrying the amino acids to the ribosome, preventing the addition of the amino acids to the growing polypeptide chain. Aminoglycosides like streptomycin and gentamicin, interfere with the initial steps of protein synthesis by changing the shape of the 30s portion of the 70s prokaryotic ribosome. This interference causes the genetic code on the mRNA to be read incorrectly (Tortora *et al.,* 2004).

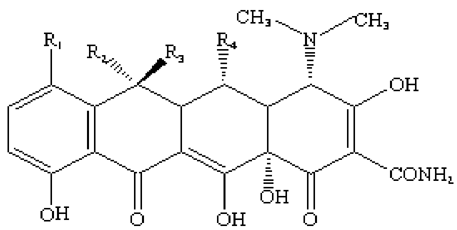


Fig 2: Chemical structure of a tetracycline

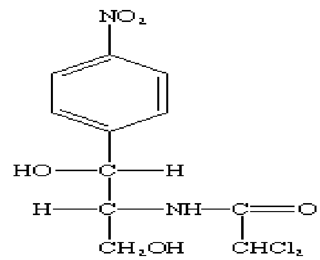


Fig 3: Chemical structure of chloramphenicol.

Some damage the cell membrane by distorting the cell surface especially polypeptide antibiotics. They usually interfere with the permeability of the plasma membrane; these changes result in the loss of important metabolites from the microbial cell. For example, polymyxin B causes disruption of the cell membrane by attaching to the phospholipids of the membrane (Talaro, 2005).

The more recent triazole derivatives, such as fluconazole, itraconazole, and voriconazole (a triazole in development), owe their antifungal activity at least in part to inhibition of cytochrome P-450-dependent 14α-sterol demethylase. Although more recent azole antifungals are 14α-demethylase inhibitors, there exists heterogeneity of action among these antifungals **(**Sanati *et al.*, 1997). The earlier imidazole derivatives (such as miconazole, econazole, and ketoconazole) have a complex mode of action, inhibiting several membrane-bound enzymes as well as membrane lipid biosynthesis. For larger polyenes, such as amphotericin B, it has been proposed that the interaction of the antifungal with membrane sterol results in the production of aqueous pores consisting of an annulus of eight amphotericin B molecules linked hydrophobically to the membrane sterols. This configuration gives rise to a pore in which the polyene hydroxyl residues face inward, leading to altered permeability, leakage of vital cytoplasmic components, and death of the organism (Mahmoud *et al.*, 1999).

2.5. Resistance of micro-organisms to drugs

Antibiotic resistance is a global public health problem. Although all countries are affected, the extent of the problem in the developing nations is unknown. With increasing travel and patient movement throughout the world, transmission of drug-resistant organisms from one country to another became a possibility (Richet *et al.*, 2001). Drug resistance is more frequently encountered in hospital-acquired pathogens; however the incidence of antibiotic-resistant pathogens in community-acquired infections has been also on the rise in recent years (Hooton and Levy, 2001).

Bacteria have continued to react to human attempts to control them by evading the mechanism of antibiotics action. Both Gram-negative and Gram-positive organisms have demonstrated excellent capability to undermine the effectiveness of one or more antimicrobial agents. More worrisome is the emergence of multidrug resistance shown by certain strains of Gram-negative bacteria such as *Pseudomonas, Klebsiella*, *Enterobacter, Acinetobacter, Salmonella* species and Gram-positive organisms such as *Staphylococcus*, *Enterococcus* and *Streptococcus* species. In recent years there has been a steady increase in frequency of methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus* species and extended spectrum beta-lactamase productinging *Klebsiella pneumoniae* and *Escherichia coli* (Ang *et al.*, 2004).

A retrospective review in Chicago in 1998 showed an increase in the prevalence of community-acquired methicillin-resistant *Staphylococcus aureus* in children, without identified predisposing risk, from 10 per 100,000 admissions in 1990-1998 to 259 per 100,000 admissions in 1993-1995 (Herold *et al.*, 1998). At the same time Methicillin resistant *S. aureus* (MRSA) had caused major problems in hospitals throughout the world. The first outbreak caused by MRSA occurred in European hospitals in the early 1960’s (Chambers, 1997). Recent reports indicate that MRSA strains account for 10 to 40% of *S. aureus* isolated from some European hospitals (Zhao *et al.*, 2001; Akinyemi *et al.*, 2005). Also two mupirocin-resistant phenotypes, low-level (LMR) and high-level resistance (HMR), have been identified. LMR is thought to be the result of a mutational change in the chromosomally encoded *ileS-2* (*mupA*) gene, and has been shown to develop in *S. aureus* isolates exposed *in vitro* to progressively higher concentrations of mupirocin. The proposed genetic basis for HMR is the acquisition of a transferable plasmid containing the *ileS-2* gene encoding an additional IRS enzyme **(**Upton *et al.*, 2003).

A lot of resistance has also been reported in other drugs like quinolone (fluoroquinolones). Resistance in *E. coli* is becoming a clinical problem in some areas, several reports have appeared (Goettsch *et al.*, 2000). Quinolone-resistant (QR) *E. coli* may have lower invasive capacity than does quinolone-susceptible *E. coli* (Velasco *et al*., 2001). A number of mechanisms for this resistance are known, including mutations in the topoisomerase II and IV genes and decreased intrabacterial accumulation of quinolone, mostly secondary to the over expression of efflux pumps or decreases in cell wall permeability (Vila *et al.*,1999). It was also found that *S. typhi* isolates were resistant to ciprofloxacin (MIC range 7–125 mg/L) before the routine prescription of this drug in clinical practice and the resistance was found to be chromosomally based (Tikoo *et al.*, 2000). These amongst many shows that the resistance incidences are on the increase and therefore there is need for search of new drugs.

Although antibiotic resistance is a natural expression of evolution and microbial genetics, certain factors are thought to contribute immensely to enhance the expression and spreading of this bacterial inherent potential. Overuse of antibiotics and consequent antibiotic selective pressure is thought to be the most important factor contributing to the appearance of different kinds of resistant bacteria (CDC, 1999). The more often a drug is used, the more likely bacteria are to develop resistance to it. In fact one recent study indicated that as much as 60% of the hospital prescriptions for vancomycin are not in accordance with guidelines (CDC, 1999). It is therefore not a surprise that vancomycin-intermediate and vancomycin-resistant strains of *S. aureus* have been described recently (CDC, 2002a; CDC, 2002b).

Other factors are poor implementation of infection control measures, admission to intensive care unit, severity of underlying illness, total number of days in the hospital, previous colonization with multi-drug resistant organisms and invasive procedures such as urinary catheterization and dialysis (Ang *et al.,* 2004).

2.6. Medicinal plants

2.6.1. Historical background of the medicinal plants

Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world and can be alternatives since their reputed efficacies have been experienced and passed on from one generation to another (Akinyemi *et al.,* 2005; Jachak and Saklani, 2007). In rural areas of developing countries, they continue to be used as the primary source of medicine (Chitme *et al.*, 2003). About 80% of the people in developing countries use traditional medicines for their health care (Kim, 2005).

It is estimated that there are 250,000 to 500,000 species of plants on earth (Borris, 1996). Relatively small percentages (1 to 10%) of these are used as foods by both humans and other animals. It is possible that even more are used for medicinal purposes (Moerman, 1996). Hippocrates (in the late fifth century B.C.) mentioned 300 to 400 medicinal plants (Schultes, 1978). In the first century A.D., Dioscorides wrote *De Materia Medica*, a medicinal plant catalogue which became the prototype for modern pharmacopoeias. The Bible offers descriptions of approximately 30 healing plants (Cowan, 1999). Indeed, frankincense and myrrh probably enjoyed their status of great worth due to their medicinal properties. They were reported to have antiseptic properties and were even employed as mouthwashes. The fall of ancient civilizations forestalled Western advances in the understanding of medicinal plants, with much of the documentation of plant pharmaceuticals being destroyed or lost (Cowan, 1999). During the Dark Ages, the Arab world continued to excavate their own older works and to build upon them. Of course, Asian cultures were also busy compiling their own pharmacopoeia. In the West, the Renaissance years saw a revival of ancient medicine, which was built largely on plant medicinals.

Thus the mainstream medicine is increasingly receptive to the use of antimicrobial and other drugs derived from plants, as traditional antibiotics (products of microorganisms or their synthesized derivatives) become ineffective and as new, particularly viral, diseases remain intractable to this type of drug. Another driving factor for the renewed interest in plant antimicrobials in the past 20 years has been the rapid rate of (plant) species extinction (Lewis and Elvin-Lewis**,** 1995). There is a feeling among natural-products chemists and microbiologists that the multitude of potentially useful phytochemical structures which could be synthesized chemically is at risk of being lost irretrievably (Borris, 1996). The ethno botanists/ethno pharmacologists, have started to utilize the impressive array of knowledge assembled by indigenous peoples about the plant and animal products they have used to maintain health (Silva *et al.*,1996). Lastly, the ascendancy of the human immunodeficiency virus (HIV) has spurred intensive investigation into the plant derivatives which may be effective, especially for use in underdeveloped nations with little access to expensive Western medicines (De Clercq, 1995).

2.6.2. The phytochemicals in medicinal plants

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives (Lahlou, 2004). Most of the deriveretives are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total (Schultes, 1978). In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores. Some of them, such as terpenoids, give plants their odors; others (quinones and tannins) are responsible for plant pigments. Many compounds are responsible for plant flavor (the terpenoid capsaicin from chili peppers), and some of the same herbs and spices used by humans to season food yield useful medicinal compounds. Such secondary metabolites like tannins, terpenoids, flavonoids, alkaloids, saponins, reducing sugars, sterols and triterpenes have been found to have antidiarrhoeal or antimicrobial activity (Yu *et al.*, 2000; Al-Rehaily *et al.*, 2001).

2.6.2.1 Tannins

Tannin is a general descriptive name for a group of polymeric phenolic substances capable of tanning leather or precipitating gelatin from solution, a property known as astringency (Cowan, 1999). They are found in almost every plant part: bark, wood, leaves, fruits, and roots (Scalbert, 1991). They have been found to have antidiarrhoeal effect and these substances may precipitate proteins of the enterocytes, reduce peristaltic movement and intestinal secretion (Al-Rehaily *et al.*, 2001). Tannins also can be toxic to filamentous fungi, yeasts, and bacteria. Condensed tannins have been determined to bind cell walls of ruminal bacteria, preventing growth and protease activity (Jones *et al.*, 1994). At least two studies have shown tannins to be inhibitory to viral reverse transcriptases (Cowan, 1999).

2.6.2.2 Flavones, flavonoids and flavonols

Flavones are phenolic structures containing one carbonyl group shown in Figure 4. Since they are known to be synthesized by plants in response to microbial infection (Dixon *et al.*, 1983), it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. More lipophilic flavonoids may also disrupt microbial membranes (Tsuchiya *et al.*, 1996).

Catechins, as shown in Figure 5 is the most reduced form of the C_3 unit in flavonoid compounds and has been found to inhibit *Vibrio cholerae* O1 *in vitro* (Borris, 1996), *Streptococcus mutans* (Batista *et al.*, 1994; Sakanaka *et al.*, 1992; Tsuchiya *et al.*, 1994), *Shigella* (Vijaya *et al.*, 1995), and other bacteria (Sakanaka *et al.*, 1992). The catechins inactivated cholera toxin in *Vibrio* (Borris, 1996) and inhibited isolated bacterial glucosyltransferases in *S. mutans* (Nakahara *et al.*, 1993), possibly due to complexing activities. This latter activity was borne out in *in vivo* tests of conventional rats. When the rats were fed a diet containing 0.1% tea catechins, fissure caries (caused by *S. mutans*) was reduced by 40% (Ooshima *et al.*, 1993). *In vitro* and *in vivo* experiments have shown that flavonoids are able to inhibit the intestinal secretory response induced by prostaglandin E_2 (Su *et al*., 2000).

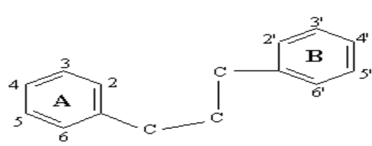


Fig 4: The general structure of the flavonoid.

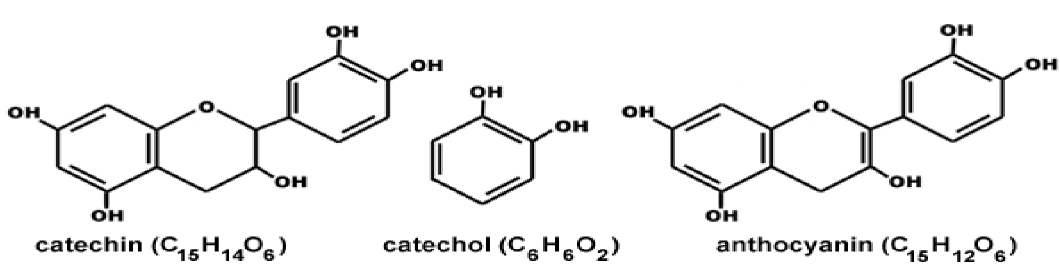


Fig 5: The structures of the catechins, catechol, and anthocyanin

2.6.2.3 Terpenoids and essential oils

Terpenoids as shown in Figure 6 are synthesized from acetate units, and as such they share their origins with fatty acids. They differ from fatty acids in that they contain extensive branching and are cyclized. Examples of common terpenoids are methanol and camphor (monoterpenes) and farnesol and artemisin (sesquiterpenoids). Artemisin and its derivative α-arteether, also known by the name qinghaosu, find current use as antimalarials (Vishwakarma, 1990).

Terpenenes or terpenoids are active against bacteria (Amaral *et al.*, 1998; Mendoza *et al.*, 1997), fungi (Suresh *et al.*, 1997; Taylor *et al.*, 1996), viruses (Sun *et al.*, 1996; Xu *et al.*, 1996), and protozoa (Ghoshal *et al.*, 1996). In 1977, it was reported that 60% of essential oil derivatives examined to date were inhibitory to fungi while 30% inhibited bacteria (Chaurasia and Vyas, 1977). The mechanism of action of terpenes is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds. Accordingly, Mendoza *et al*., (1997) found that increasing the hyrophilicity of kaurene diterpenoids by addition of a methyl group drastically reduced their antimicrobial activity. Food scientists have found the terpenoids present in essential oils of plants to be useful in the control of *Listeria monocytogenes* (Aureli *et al*., 1992). Oil of basil, a commercially available herb, was found to be as effective as 125 ppm chlorine in disinfecting lettuce leaves (McIntosh *et al.*, 2000, 2003).

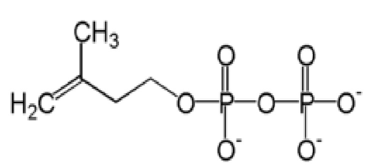


Fig 6: Chemical structure of the terpenoid isopentenyl pyrophosphate.

2.6.2.4 Alkaloids

Heterocyclic nitrogen compounds are called alkaloids as shown in Figures 7 and 8 overleaf. The first medically useful example of an alkaloid was morphine, isolated in 1805 from the opium poppy *Papaver somniferum* L. (Cowan, 1999). Codeine and heroin are both derivatives of morphine. Diterpenoid alkaloids, commonly isolated from the plants of the Ranunculaceae, or buttercup family (Atta-ur-Rahman and Choudhary, 1995), are commonly found to have antimicrobial properties (Omulokoli *et al.,* 1997). Solamargine, a glycoalkaloid from the berries of *Solanum khasianum* Clarke., and other alkaloids may be useful against HIV infection (McMahon *et al.,* 1995) as well as intestinal infections associated with AIDS. While alkaloids have been found to have microbiocidal effects [including against *Giardia* and *Entamoeba* species (Ghoshal *et al.,* 1996), the major antidiarrhoeal effect is probably due to their effects on transit time in the small intestine. While many alkaloids are poisonous, some are used medicinally as analgesics (pain relievers) or anaesthetics, particularly morphine and codeine, and for other uses.

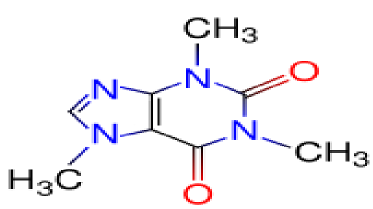


Fig 7: Chemical structure of caffeine

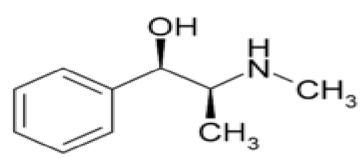


Fig 8: Chemical structure of ephedrine- alkaloid

2.6.2.5 Quinones

Quinones are aromatic rings with two ketone substitutions. They are ubiquitous in nature and are characteristically highly reactive (Stern *et al.,* 1996). These compounds, being coloured, are responsible for the browning reaction in cut or injured fruits and vegetables and are an intermediate in the melanin synthesis pathway in human skin (Cowan, 1999). The switch between diphenol (or hydroquinone) and diketone (or quinone) occurs easily through oxidation and reduction reactions. The individual redox potential of the particular quinone-hydroquinone pair is very important in many biological systems. Vitamin K is a complex naphthoquinone. Its antihemorrhagic activity may be related to its ease of oxidation in body tissues.

In addition to providing a source of stable free radicals, quinones are known to complex irreversibly with nucleophilic amino acids in proteins (Stern *et al.*, 1996), often leading to inactivation of the protein and loss of function. For that reason, the potential range of quinone antimicrobial effects is great. Probable targets in the microbial cell are surface-exposed adhesins, cell wall polypeptides, and membrane-bound enzymes. Quinones may also render substrates unavailable to the microorganism. As with all plant-derived antimicrobials, the possible toxic effects of quinones must be thoroughly examined.

Kazmi *et al.*, (1994) described an anthraquinone a type of quinones from *Cassia italica* (Mill.) Lam., a Pakistani tree, which was bacteriostatic for *Bacillus anthracis*, *Corynebacterium pseudodiphthericum*, and *Pseudomonas aeruginosa* and bactericidal for *Pseudomonas pseudomalliae*.

2.6.2.6 Lectins and polypeptides

Peptides which are inhibitory to microorganisms were first reported in 1942 (Balls *et al.*, 1942). They are often positively charged and contain disulfide bonds (Zhang and Lewis, 1997). Their mechanism of action may be the formation of ion channels in the microbial membrane (Terras *et al.*, 1993; Zhang and Lewis, 1997) or competitive inhibition of adhesion of microbial proteins to host polysaccharide receptors. Recent interest has been focused mostly on studying anti-HIV peptides and lectins, but the inhibition of bacteria and fungi by these macromolecules, such as that from the herbaceous *Amaranthus spp.*, has long been known (De Bolle *et al.*, 1996).

Thionins are peptides commonly found in barley and wheat and consist of 47 amino acid residues (Colilla *et al.*, 1990). They are toxic to yeasts and Gram-negative and Gram-positive bacteria (Cowan, 1999). Thionins AX1 and AX2 from sugar beet are active against fungi but not bacteria (Kragh *et al.*, 1995). Fabatin, a newly identified 47-residue peptide from fava beans, appears to be structurally related to γ-thionins from grains and inhibits *E. coli*, *P. aeruginosa*, and *Enterococcus hirae* but not *Candida* or *Saccharomyces* (Zhang and Lewis, 1997).

The larger lectin molecules, which include mannose-specific lectins from several plants (Balzarini *et al.*, 1991), MAP30 from bitter melon (Lee-Huang *et al.*, 1995), GAP31 from *Gelonium multiflorum* A. Juss. (Bourinbaiar and Lee Huang, 1996), and jacalin (Favero *et al.*, 1993), are inhibitory to viral proliferation (HIV, cytomegalovirus), probably by inhibiting viral interaction with critical host cell components.

2.6.2.7 Other Compounds

Many phytochemicals not mentioned above have been found to exert antimicrobial properties. This review has attempted to focus on reports of chemicals which are found in multiple instances to be active. It should be mentioned, however, that there are reports of antimicrobial properties associated with polyamines (in particular spermidine), isothiocyanates (Iwu *et al.*, 1991), thiosulfinates and glucosides (Rucker *et al.*, 1992).

2.6.3. The medicinal plants use

2.6.3.1 Some anti-bacterial medicinal plants

Several studies have evaluated the effectiveness of such medicinal plants used in treatment of bacteria known to cause diarrhoea, in all different continents (Rani *et al*., 1999). Like the *Ocimum gratissimum* L. has been reported to have medicinal properties. The leaf extracts are popularly used for the treatment of diarrhoea while the cold leaf infusions are used for the relief of stomach upset and haemorrhoids (Akinyemi *et al.*, 2005). The thymol-rich leaf has been reported to have antimicrobial properties. The bark extracts of *Phylanthus discoideus* (Bail.) M ll.Arg. are used to treat stomachache and lumbago (Akinyemi *et al.*, 2005). The extracts of *Annona senegalensis* Pers., *Steganotaenia araliacea* Hochst. var. *araliacea*. and *Securidaca longipendiculata* Fresen. were also found to have an activity against *S. aureus* (Lino and Deogracious, 2006). Voravuthikunchai *et al.* (2004) reported good antibacterial activity in *Peltophorum pterocarpum* (DC.) K. Heyne, and *Punica granatum*- L., against *E.coli* using aqueous and methanol extracts. Also soluble extracts of onions and ginger have been found to have antibacterial properties (Azu and Onyeagba, 2007). When the extracts were tested in one study on *E. coli, S. typhi* and *B. subtilis*, it was found that the widest zone of inhibition was obtained with *S. typhi* followed by *E. coli* (Azu and Onyeagba, 2007) hence can offer alternative medicare.

In Central America, *Aristolochia trilobata* L. extracts showed an interesting anti-bacterial activity against *S. aureus* with a MIC = 0.31 mg/ml for the hexane extract of the leaves and a MIC = 0.625 mg/ml for the hexane extract of the bark (Camporese *et al.,* 2003). This plant species is one of the most popular herbal remedies of Belize and it is used in traditional medicine for hangovers, flu, colds, amoebiasis, colitis and to clean the urinary tract (Camporese *et al.,* 2003). It seems that neither phytochemical nor biological studies have been carried out on this *Aristolochia* species: only its topical anti-inflammatory activity has been recently described (Sosa *et al*., 2002). However, plants belonging to the genus *Aristolochia* were reported to contain aristolochic acids, which are known to be both mutagenic (Bianucci *et al*., 1993; Pistelli *et al*., 1993) and carcinogenic (Camporese *et al.,* 2003). The mutagenicity of these compounds could explain the mechanism of their anti-microbial action. In fact, these compounds were also isolated from *Aristolochia longa* L. (Hinou *et al.,* 1990) and they inhibited *E. coli*, *P. aeruginosa*, *S. faecalis*, *S. aureus* and *S. epidermidis*.

Chiranthodendron pentadactylon Larreategui. on the other hand was found to posses several compounds including flavonoids, steroids, hydrocarbons, sugars and gallic acid, which showed moderate activity against bacteria (Lara and M´arquez, 1996; Alanis *et al*., 2003). Alkaloids have been isolated from *Bocconia frutescens* L. and the methanolic and chlorophorm extracts showed antibacterial activity against *V, cholerae*, a causal agent of diarrhoea (Perez, 2000; Caballero *et al*., 2002, 2003). The antibacterial activity seen in the most active extracts of this study together with the antisecretory effect are evidence that support the use of *Chiranthodendron pentadactylon* Larreategui., *Geranium mexicanum* var. *resimum* and *Bocconia frutescens* L. in Mexican traditional medicine to treat diarrhoea (Vel´azquez *et al.,* 2006).

In another ethnopharmacological survey, extracts of the six East African medicinal plants *Entada abyssinica* Steud. ex A. Rich (stem bark), *Terminalia spinosa* Engl. (young branches), *Harrisonia abyssinica* Oliv. (roots), *Ximenia caffra* Sond. (roots), *Azadirachta indica* L. (stem bark and leaves), and *Spilanthes mauritiana* DC. (roots and flowers) were tested against 105 strains of bacteria from seven genera (*Staphylococcus*, *Enterococcus*, *Pseudomonas*, *Escherichia*, *Klebsiella*, *Salmonella*, *Mycobacterium*) (Fabry *et al.,* 1998). The minimum inhibitory concentration reached by 50% (MIC50%) and 90% (MIC90%) of the strains for the extracts of *E. abyssinica* Steud. ex A. Rich, *T. spinosa* Engl., *X. caffra* Sond. , and *A. indica* L. (stem bark) ranged from 0.13–8 mg/ml and from 0.5 to 8 mg/ml, respectively. Their minimum bactericidal concentration by 50% (MBC50%) and MBC90% were all between 0.5 and 8 mg/ml. *H. abyssinica* Oliv., *A. indica* L. (leaves), and *S. mauritiana* DC. (roots and flowers) had MIC and MBC values of 8 mg/ml. Mycobacteria were not inhibited at extract concentrations of 0.5–2 mg/ml. lt is concluded that plant extracts with low MIC and MBC values may serve as sources for compounds with therapeutic potency (Fabry *et al.,* 1998). These amongst many plants clearly show that the medicinal plants can be alternatives against most of these bacteria that are causing a lot of diseases to man.

2.6.3.2 Some anti-fungal medicinal plants

A review of the literature on the evaluation of medicinal plant extracts shows that many studies into their antifungal activities have been carried out in recent years (Abad *et al.,* 2007). For instance nineteen plant species from fourteen families used in traditional North American Indian medicine were tested for their fungicidal (*Cladosporium cucumerinum* and *Candida albicans*) activity. Of the species investigated, nine were active against *Cladosporium cucumerinum* and nine against *Candida albicans*. A programme was designed for the pharmacological screening of species used by the Mayan people in the highlands of Chiapas in southern Mexico to treat gastrointestinal and respiratory diseases. It demonstrated that 63% of the botanical species showed antifungal properties against *Candida albicans* (Abad *et al.*, 2007).

In Ethiopia, it was also demonstrated that the plant extracts had an activity against the fungi. The antimicrobial activity profile of all species of plants (except *Phytolacca dodecandra*) against the tested strains indicated an activity against *Trichophyton mentagrophytes* which was found to be the most sensitive fungus although some species such as *Calpurnia aurea*, *Kalanchoe petitiana*, *Phytolacca dodecandra* and *Verbascum sinaiticum* were found to be inactive against it. Of all the fungal strains included in the test, *Aspergillus niger* was found to be virtually insensitive to all plant extracts and *Candida albicans*, which is an isolate, was found to be the least inhibited fungus (Tadeg *et al.*, 2005). Also in Tanazania the methanol extracts of *Cineraria grandiflora*, *Marattia fraxinea*, and *Pavonia urens*, which to a varying extent inhibited the growth of the fungi (*Aspergillus fumigatus*, *Fusarium culmorum*), however *Candida albicans* was the only organism basically insensitive to any of the extract treatments (Boer *et al*., 2005).

Some Indian plants *Beta vulgaris*, *Sapindus* sp., *Casuarina equistifolia*, *Nelumbo nucifera*, *Portulaca quadrifolia*, *Vitis vinifera*, *Cordia dichotoma* and *Nyctanthes arbortristis* were reported to have some antifungal activity against *C. albicans* (Ahmad and Beg, 2001). These examples amongst many clearly indicates that plants can still offer alternative medicare against fungal ailments that have caused many problems to man.

CHAPTER THREE

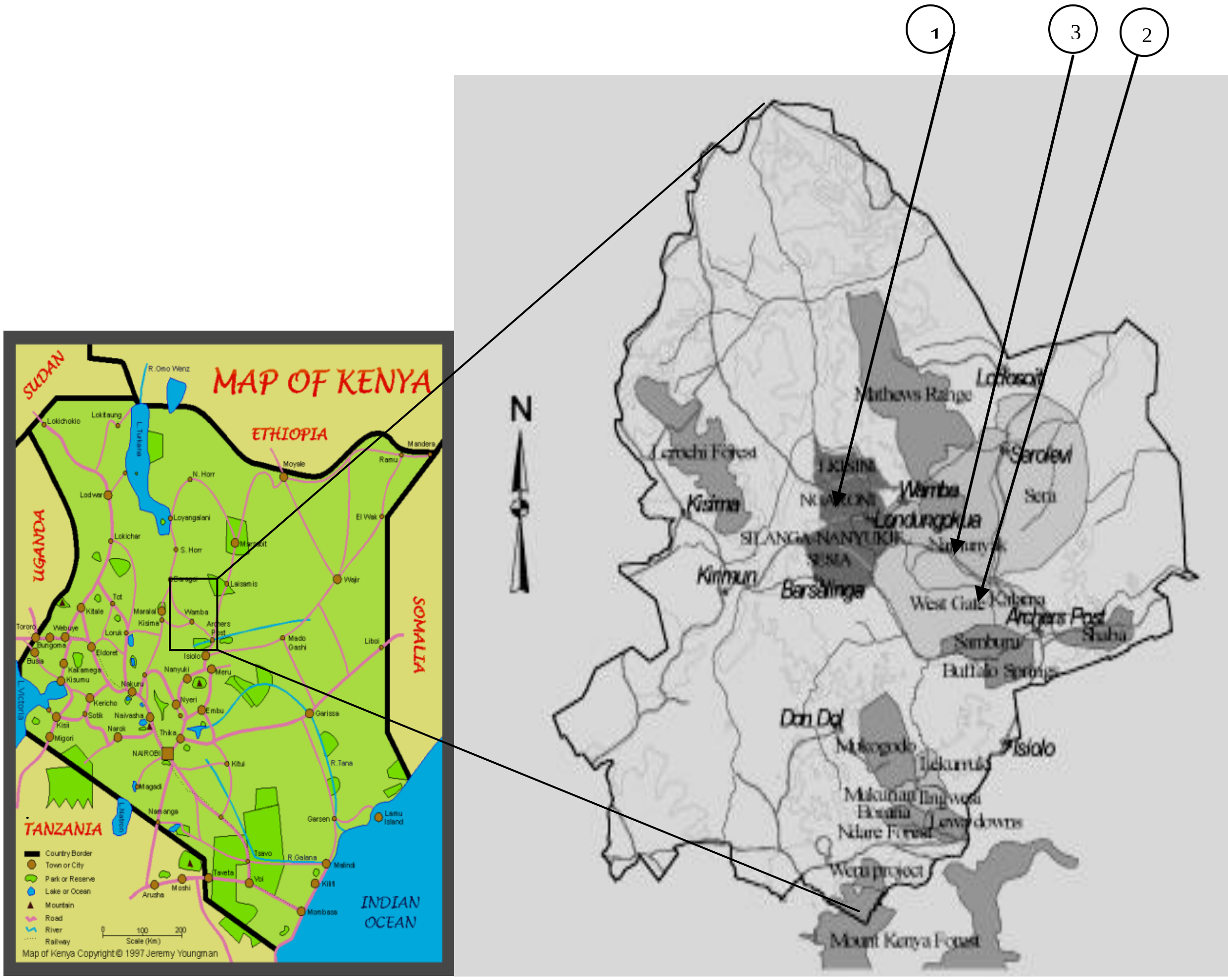
3.0 MATERIALS AND METHODS

3.1 Study site.

This study was carried out at Wamba Division, Samburu District, Rift Valley Province that is 0.98°N and 37.34°E. The major inhabitants of Wamba Division are the Samburu community. The area is arid to semi-arid with annual rainfall of between 250 – 500mm. The only permanent river is the Ewaso Nyiro, but other occasional water sources include ephemeral laggas, man-made reservoirs and natural ponds which only contain water during the wet season. For most of the year, the area experiences high temperatures which vary with altitude and are generally between 24ºC- 33 ºC during the day with no cloud cover. The soil is dry and sandy with a poor vegetation cover.

Samburu District is situated in the northern half of the Rift Valley. It is bordered by five Districts in the Rift Valley and Eastern Provinces. To the north west is Turkana District while to the south west is Baringo District. Marsabit District is to the north east, Isiolo District to the east and Laikipia District to the south. The District lies within the semi-arid areas of the country and covers approximately 21,126.5 square kilometers (including 3,250 square kilometers of gazetted forest). The District is divided into six divisions.

Traditionally, conservation of these medicinal plants was strictly adhered to as no one was allowed by the culture to harvest from one plant severally before it completely recovered, while total uprooting was a taboo (Bussmann, 2006). But due to the increased of the population medicinal plants are experiencing a danger as some species are disappearing due to over-harvesting. Loss of traditional knowledge and biodiversity are a major challenge in the sustaining of traditional medical systems in the community.The laboratory work for this research was carried out at Wamba Catholic Hospital laboratory.



Key: 1-Nkaroni conservancy; 2. West gate conservancy 3. Namunyak conservancy

Fig 9.Map of the study site area

3.2 Ethnobotanical survey and Identification of the plants

Plant samples were collected from the various conservancies around Wamba (Samburu) - Namunyak, Nkaroni and West Gate where questionnaires were used to get data of the plants most used by the herbalists and the community in treatment of diarhoreal diseases (Appendix 1). The stem bark, roots, root bark and leaves were sampled as they were the major parts the community used. Voucher specimens were also collected for identification and deposited at the Kenyatta University herbarium.

A literature survey on the plants mentioned by the herbalists and the local informants of the community was conducted to eliminate plants that had been previously screened for diarhoreal diseases from this study to avoid duplication of the data. This literature survey was made possible through Natural Product Alert (NAPRALERT) database of University of Illinois, USA.

Preliminary identification of the plants was done by the taxonomist at the Kenyatta University herbarium. Herbarium specimens were prepared to aid confirmation of the plants identity. Voucher specimens are deposited at the herbarium of the Plant and Microbial Sciences Department, Kenyatta University.

3.3 Solvent extraction of the plant material

Plant extracts were prepared by cold percolation method (Alanis *et al.*, 2005). The plant materials collected were air-dried under shade and then chopped into small pieces before being ground using the laboratory grinding mill (Edeoga *et al.,* 2007). Some 50g of dried powder were soaked in 300 ml 80% methanol (MW-32.04)) for 12-48h with intermittent shaking. The plant extracts were filtered by use of Whatman No. 1 filter paper. The filtrates were then evaporated until a constant dry weight of each extract was obtained by using the rotary evaporator (VV 2000 Heidolph, Germany) set at 40-50°C. The crude extracts were then used for bioassays.

3.4 Test cultures

The type culture isolates were obtained from Kenyatta National Hospital in Nairobi which included *Staphylococcus aureus* (Gram +ve cocci) - ATCC 20591, *Bacillus subtillis* (Gram +ve spore forming *bacilli*) - Local isolate, *Salmonella typhi* (Gram –ve rod)- ATCC 2202, *Escherichia coli* (Gram-ve rod) - STD. 25922 and *Pseudomonas aeruginosa* (Gram-ve rod) - ATCC 25852. They were selected on the basis of their cell wall properties. The fungal test cultures included *Candida albicans* ATCC EK138, *Aspergillus niger* ATCC 16404, *Aspergillus flavus*-Local isolate, *Fusarium lateritium*-Local isolate, and *Penicillium spp.*-Local isolates.

The test strains of the bacteria were kept refrigerated on Muller-Hinton (Merck, Germany) agar slants during the experimental period and were subcultured and incubated for 24h at 37ºC then tested using standard biochemical methods for purity before use. Fungal strains were maintained on Potato Dextrose Agar (PDA) at 4 ºC and were also subcultured in PDA broth before testing for purity using biochemical and morphological characteristics.

3.5 Susceptibility testing (disc diffusion)

The agar disc diffusion method (NCCLS, 2002; Paulo, 2007) was used to evaluate the antimicrobial activity by measuring the inhibition zone in mm against the test microorganisms by use of a transparent ruler. Plates were prepared with a base layer of Mueller Hinton agar and Potato Dextrose Agar (PDA) for purposes of culturing the bacteria and fungi respectively. It was prepared by weighing the quantities recommended by the manufacturer and dissolving in recommended quantities of distilled water. The media was then allowed to settle for 10min then swirled before autoclaving at 121˚C, at

15psi for 15min. The media was allowed to cool and then dispensed on plates in the clean work bench before it solidified ready for use.

A 12-24h culture of the bacterial type culture isolate was diluted with physiological saline solution and the turbidity corrected by adding sterile physiological saline until a MacFarland turbidity standard of 0.5 (10^6 CFU/ml) was obtained. Afterwards, a top layer of Mueller Hinton agar was inoculated with 0.1mls of the microbial suspension that was poured over the Petri dishes then spread by spread plate method (Paulo, 2007). The dry sterile discs (6mm diameter) impregnated with 0.1ml of the plant extracts that were made by dissolving 300mg of the extracts in 1ml of methanol were mounted on the spread microorganisms. Discs impregnated with methanol and left to dry overnight in an oven set at 40ºC were used as negative control. Amoxicillin (250 mg) and tetracycline (300 mg) were also dissolved using ethanol before they were impregnated onto discs by dispensing 0.1ml of the dissolved drug into the sterile discs (6mm diameter) then left to dry. They were used as positive controls. The plates were then incubated at 35ºC for 12-48h.

For the fungal isolates they were cultured by taking 0.1mls from the broth and spreading it by spread plate method and incubated at 25˚C for 48-72h. Thereafter by use of the cork boarer, a section with the young mycelium was picked and placed on a PDA plate and dry disc (6mm) treated with 0.1ml of the plant extracts that were made by dissolving 300mg of the extracts in 1ml of methanol were placed at a distance around the section of the mycelium extracted and they were incubated at 25˚C for 48-72h. Fluconazole (300 mg) were also dissolved using ethanol before they were impregnated onto discs by dispensing 0.1ml of the dissolved drug into the sterile discs (6mm diameter) then left to dry before they were also mounted onto the PDA with the extracted mycelium. Dry discs treated with methanol were also used as the negative control.

Microbial growth inhibition was determined by measuring the zones of inhibition from the end of growth to the disc at one end to the beginning of growth at the other end. All tests were performed in triplicate for purposes of statistical analysis.

3.6 Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC).

Minimum Inhibitory Concentration (MIC) is the least amount of antimicrobial that will inhibit visible growth of an organism after an overnight incubation. Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) is the amount of the antimicrobial that prevents growth after subculture of the organism to antibiotic free medium.

The Minimum Inhibitory Concentration (MIC) values of the methanol extracts were determined by using the micro dilution broth method (Andrews, 2001) in 96–well micro plates. 300mg (0.3g) of the plant extract were taken and mixed with 1ml (1000µl) nutrient broth containing 0.01% Dimethyl formanide (DMF) to enable complete dissolving of the extract.

The wells were then filled with 50µl each of the Nutrient broth for bacterial strains and PDA broth for fungal strains that was made by following the manufacturer's procedures before they were autoclaved at 121˚C, at 15psi for 15min and left to cool before they were used.

Then 50µl (15mg) of the plant extract in nutrient broth containing 0.01% DMF was dispensed into the well before serial dilutions were done four times. One well was used as negative control of the growth of the microorganisms in the medium. 50µl of the antibiotic (Cefrodoxima/ fluconazole) were used as positive controls that were also serial diluted to reduce the concentration as in the plant extracts. The innoculum were adjusted for each test organism to yield a cell concentration of 10^6 CFU/mL that is equivalent to MacFarland turbidity standard of 0.5 $\times 10^6$CFU/ml. Then 50µl of the test innoculum was dispensed to each well before the micro titre plates were incubated at 37ºC for 12-24h. The MIC values were determined as the lowest concentrations of the extract capable of inhibiting microorganisms' growth. The wells where no growth was observed were then subcultured. The lowest concentration of the plant extracts that did not yield any colony growth on the solid medium (PDA or Nutrient agar) after sub culturing and incubating for 48-72h for fungi and 12-24h for bacterial strains was taken as the MFC/MBC. All tests were performed in triplicate (Evans *et al.*, 2002; Ruttoh, 2009).

3.7 Determination of the phytochemicals

3.7.1 Phytochemical screening

Qualitative phytochemical analysis of the crude extracts of the plants collected was determined by the methods used by Edeoga *et al.*, (2005); Jigna and Sumitra, (2007).

3.7.1.1 Test for Tannins

Tannins presence was determined by dissolving 200 mg plant material in 10 ml distilled water, and then filtered. Some 2 ml of the filtrate was taken and 2 ml Iron (III) chloride solution was added. Blue-black precipitate indicated the presence of tannins. A (+) reaction was recorded when a slight precipitate was observed; a (++) reaction was recorded when a medium precipitate was observed; and a (+++) reaction was recorded when a heavy precipitate was observed. These were used to indicate the presence of different concentrations of detectable tannins with (+) representing low, (++), moderate and (+++) high levels of tannins (Edeoga *et al.*, 2005).

3.7.1.2 Test for the alkaloids

Alkaloids presence was determined by dissolving 200 mg plant extract in 10 ml methanol, and was filtered. 1 ml of the filtrate was then mixed with 6 drops of Wagner's reagent (1.27g iodine + 2g potassium iodide in 100ml of water). Creamish precipitate/brownish-red precipitate/orange precipitate indicated the presence of alkaloids. A low (+) reaction was recorded if the addition of the reagent produced a faint turbidity; a moderate (++) reaction was recorded if alight opalescence precipitate was observed; and a high (+++) reaction was recorded if a heavy yellowish-white precipitate was observed. The development of turbidity indicated the presence of alkaloids (Obdoni and Ochuko, 2001).

3.7.1.3 Test for Saponins

Saponins presence was determined by the frothing test method where 0.5 ml of the filtrate was mixed with 5 ml distilled water. Frothing persistence indicated the presence of saponins (Jigna and Sumitra, 2007). A (+) sign was recorded when the froth reached a height of 0.5cm; a (++) sign with the height of 0.6-1cm; and a (+++) sign with a height of more than 1cm to indicate low, moderate or high concentration of saponins respectively in the plant extract.

3.7.1.4 Test for Cardiac glycosides

Cardiac glycosides presence was determined by Keller-Kiliani test where 2 ml of the filtrate was mixed with 1 ml glacial acetic acid, few drops of Iron (III) chloride and conc. sulphuric acid. Green-blue colour indicated the presence of cardiac glycosides. A (+) reaction was recorded when a faint green-blue colour was observed; a (++) reaction was recorded when a medium green-blue colour was observed; and a (+++) reaction was recorded when a deep green-blue colour was observed. These was used to indicate the presence at different concentrations of detectable cardiac glycosides with (+) representing low, (++), moderate and (+++), high levels of cardiac glycosides (Obdoni and Ochuko, 2001).

3.7.1.5 Test for Terpenoids

Terpenoids presence was determined by taking 5mls of the plant extract that was mixed with 2mls of chloroform, and 3mls of conc. sulphuric acid that is carefully added to form a layer. A reddish brown coloration of the interface formed indicated the presence of terpenoids. A (+) reaction was recorded when a faint reddish brown coloration was observed; a (++) reaction was recorded when a medium reddish brown coloration was observed; and a (+++) reaction was recorded when a deep reddish brown coloration was observed. These were used to indicate the presence at different concentrations of detectable terpenoids with (+) representing low, (++), moderate and (+++) high levels of terpenoids (Edeoga *et al.,* 2005).

3.7.1.6 Test for flavonoids

Flavonoids presence was determined by taking 5 ml of dilute ammonia solution that was added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated sulphuric acid. A yellow coloration observed that disappeared on standing in each extract indicated the presence of flavonoids. A (+) reaction was reported in pale yellow colour; (++) in moderate yellow and (+++) in strong yellow coloration (Ahmad and Beg, 2000; Okwu, 2001).

3.8 Statistical analysis

The average zones of inhibition values were expressed as means ± standard error. The mean zones of inhibition for each test cultures were analyzed by one-way analysis of variance (ANOVA) to get the differences among group means. P value < 0.05 was considered as significant. The significant means were separated by the Tukey's test. The software STATISTICA® was employed for the statistical analysis. Simple descriptive statistics was also used to present the data of the average zones of inhibition.

CHAPTER FOUR

4.0: RESULTS

4.1 Ethnobotanical survey

A total of 33 medicinal plants used by the Samburu community were collected and studied after carrying out simple interviews. The plants were collected from the various conservancies around the study area (Wamba- Samburu). Sixteen medicinal plants were collected at Namunyak conservancy, twelve from Nkaroni conservancy and five from West gate conservancy. The species belonged to different families as shown in Table 1. The family of Mimosaceae had the highest number of the medicinal plants (six), Vetaceae, Boraginaceae, Capparaceae, and Fabaceae families had two species each and the rest of the families had one medicinal species each.

Various parts were harvested depending on the parts the community used in the treatment of the various diseases. The bark, roots and the leaves were the ones that were harvested, but the part that is used most was found to be the bark of the stem mainly followed by the roots and then the leaves.

Most of the medicinal plants collected were used to treat diarrhoeal diseases but others treat yellow fever, mumps, malaria, sexually transmitted infections like gonorrhoea, pneumonia, eye problems, wounds, dewormers, oral thrush, whooping cough, and ear infections amongst many other uses as per the findings of the interviews.

Table 1: Summary of the medicinal plants used by the Samburu community.

BOTANICAL NAME	FAMILY NAME	SAMBURU NAME	PART USED	DISEASES TREATED	AREA FOUND
Acacia ethaaica Schweinf.	Mimosaceae	Lchakwai	Bark	Stomach ache	Namunyak
Acacia horrida (L.) Willd.	Mimosaceae	Lerai	Bark	Diarrhoea	West gate
Acacia nilotica (L.) Del.	Mimosaceae	Lkiloriti	Bark/ roots	Stomach ache	Namunyak
Acacia nubica Benth.	Mimosaceae	Ldepe	Bark	Diarrhoea	Nkaroni
Acacia senegal (L.) Willd.Var.	Mimosaceae	Lderekesi	Bark	Stomach ache	Nkaroni
Acacia tortilis (Forssk.) Hayne.	Mimosaceae	Ndapes	Roots	Stomach ache	Namunyak
Acokanthera friesiorum Markgr.	Apocynaceae	Nchipilikwa	Roots/leaves	Diarrhoea	Namunyak
Albizia anthelmitica Brongn.	Leguminosae	Lumurtana	Roots/bark	Deworming/ Diarrhoea	Nkaroni
Aloe secundiflora Engl.	Aloaceae	Sukuroi	Whole	Stomach ache	Namunyak
Balanites aegyptiaca (L.) Del.	Balanitaceae	Sirai	Roots	Stomach ache	West gate
Boscia angustifolia Guill. and Perr	Capparaceae	Lororoi	Bark	Diarhoea, gonorrhea	Nkaroni
Cissus rotundifolia Forsk. Vahl.	Vitaceae	Raraiti	Root	Stomach ache	Nkaroni
Cissus quadrangularis L.	Vitaceae	Sukurtut	Stem	Diarrhoea	Nkaroni
Clerodendrum myriacoides (Hochst.) Vatke subsp. *napperae* Verdc	Verbenaceae	Makutukuti	Roots	Diarrhoea, malaria, polio & STIs	West gate
Commiphora africana (A. Rich) Engl. Var.	Burseraceae.	Lcheni-ngiro	Bark	Diarrhoea, eye problem	Nkaroni
Cordia monoica Roxb.	Boraginaceae	Seki	Roots	Diarrhoea	West gate
Cordia purpurea (Picc.) Aiton	Boraginaceae	Lgiriai	Roots bark	Diarhoea	Namunyak
Cordia sinensis Lam.	Boraginaceae	Silapani	Bark	Diarhoea	Nkaroni
Croton macrostachysu (A. Rich). Benths	Euphorbiaceae	Ndoopa	Roots	Stomach ache, ear infection	Namunyak
Euclea divinoram Hiern.	Ebenaceae	Nchingei	Roots	Diarrhoea	Namunyak
Euphorbia scarlatica (L) O. Ktze	Euphorbiaceae	Mpopongi	Leaves/whole plant	Diarrhoea, Common cold	West gate
Gomphocarpus fruticosus (L) W.T. Aiton	Apocyneacea	Lekule	Whole plant	Diarrhoea	Namunyak
Grewia simi K. Schum.	Tiliaceae	Ngaliyoi	Roots	Stomach ache	Nkaroni
Jasminum abyssinicum Hochst. ex DC.	Oleaceae	Laresoro	Bark	Diarrhoea, Oral thrush	Namunyak
Kedrostis pseudogijef (Gilg) C. Jeffrey	Cucurbitaceae	Sakurdumii	Stem	Diarrhoea, Yellow fever	Nkaroni
Loranthus acaciae Zucc.	Loranthaceae	Lardenyei	Whole plant	Stomach ache	Nkaroni
Ocimum suave Willd.	Lamiaceae	Lemuran	Bark	Diarrhoea	Namunyak
Ormocarpum trachycarpum (Taub) Harms	Leguminosae	Lekweita	Bark	Diarrhoea, whooping cough	Namunyak
Plumbago dawei Rolfe.	Plumbaginaceae	Lkiriantus	Bark	Diarrhoea, Malaria	Namunyak
Salvodora persica L. var. persica	Salvadoraceae	Sokotei	Roots/ branches	Stomach ache, pneumonia	Nkaroni
Solanum incanum L.	Solanaceae	Ltulelei	Roots	Diarrhoea, malaria	Namunyak
Teclea simplicifolia (Engl.) Verdc.	Rutaceae	Lgilai	Bark	Diarrhoea, malaria	Namunyak
Thylachium africanum Lour.	Capparaceae	Loimugi	Bark	Diarrhoea	Namunyak

The plates below represent some of the medicinal plants collected.

Plate 1: *Loranthus acaciae*

Plate 2: *Grewia simi*

Plate 3: *Cissus quadrangularis*

Plate 4: *Euphorbia scarlatica*

Plate 5: *Clerodendrum myriacoides*

Plate 6: *Balanites aegyptiaca*

Plate 7: *Salvodora persica*

Plate 8: *Acokanthera friesiorum*

Plate 9: *Acacia nilotica*

Plate 10: *Albizia anthelmitica*

Plate 11:*Cordia sinensis*

Plate 12: *Kedrostis pseudogijef*

4.2 Antibacterial results

The raw data of the results got for the disc diffusion method is attached (Appendix II). The extracts produced promising results as most of them inhibited the growth of the test cultures. Some of the zones of inhibition produced by the extracts are shown in the plates 13-18 with the arrows pointing to the area of the inhibition zones.

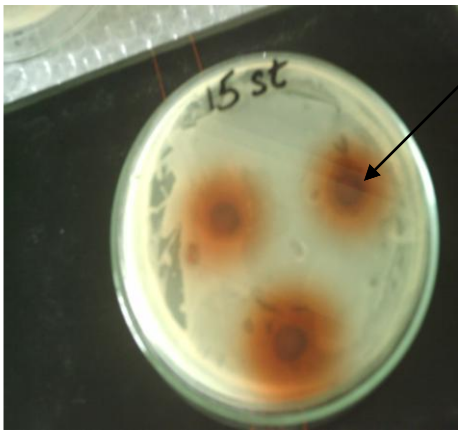


Plate 13: *Commiphora africana*. Zone of inhibition against *S. typhi*

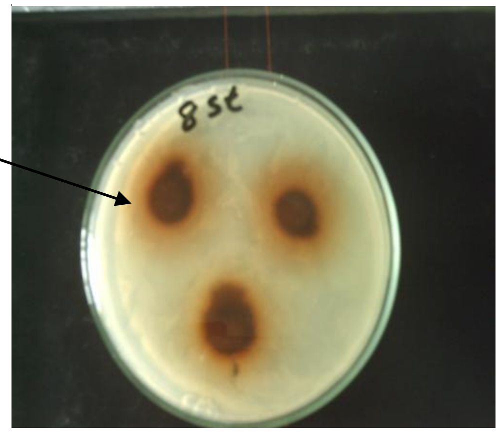


Plate 14: *Clerodendrum myriacoides* zone of inhibition against *S. typhi*.

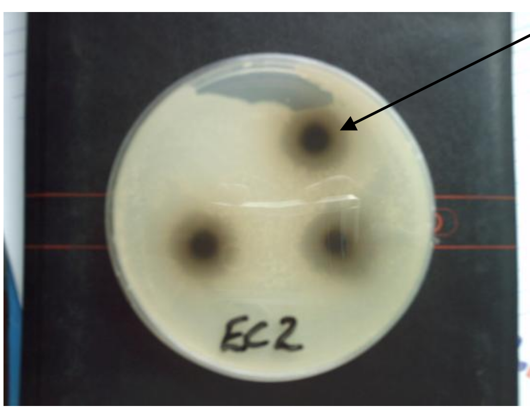


Plate 15: *Plumbago dawei* zone of inhibition against the *E.coli*

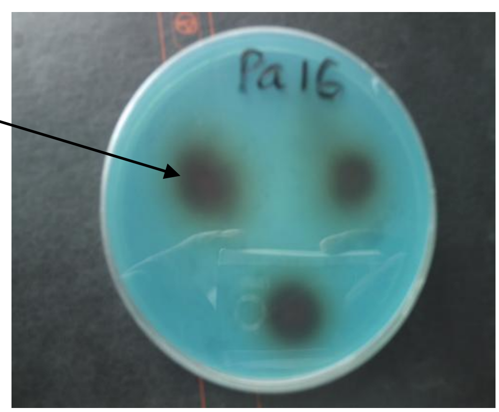


Plate 16: *Acacia ethaaica* zone of inhibition against *P. aeruginosa*.

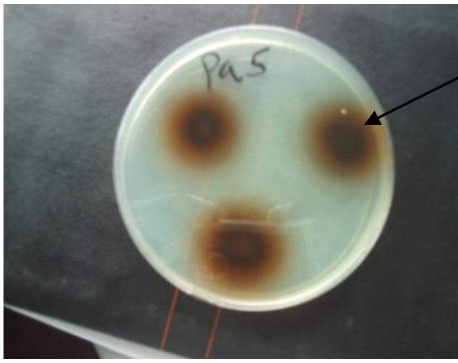


Plate 17: *Acacia nilotica* zone of inhibition against *P. aeruginosa*

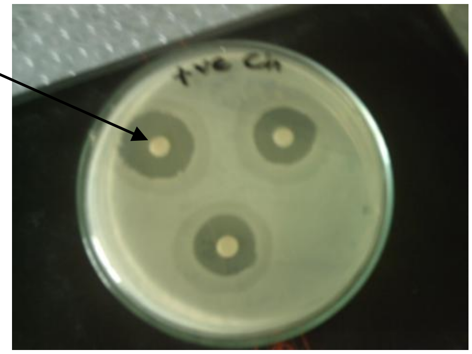


Plate 18: Zone of inhibition of chrolophenical against the *S. aureas* (+ve control)

4.2.1 Antibacterial results for *S. aureas*- ATCC 20591

4.2.1.1 Disc diffusion screening test

The performance of most of the extracts were quite promising with most having an average zone of inhibition as shown in the graph below (Fig. 10) and in the raw data on Appendix II. There was a significant difference of the means of the zones of inhibition at P≤ 0.01 as shown in Appendix V (a). None of the extracts showed no activity against *S. aureas*. *Salvodora persica, Plumbago dawei* and *Croton macrostachysu* showed higher zones of inhibition of 22mm, 26mm, and 24mm respectively. On the other hand *Boscia angustifolia, Gomphocarpus fruticosus,* and *Ormocarpum trachycarupm* gave narrow zones of inhibition of 07mm, 08mm, and 08mm respectively.

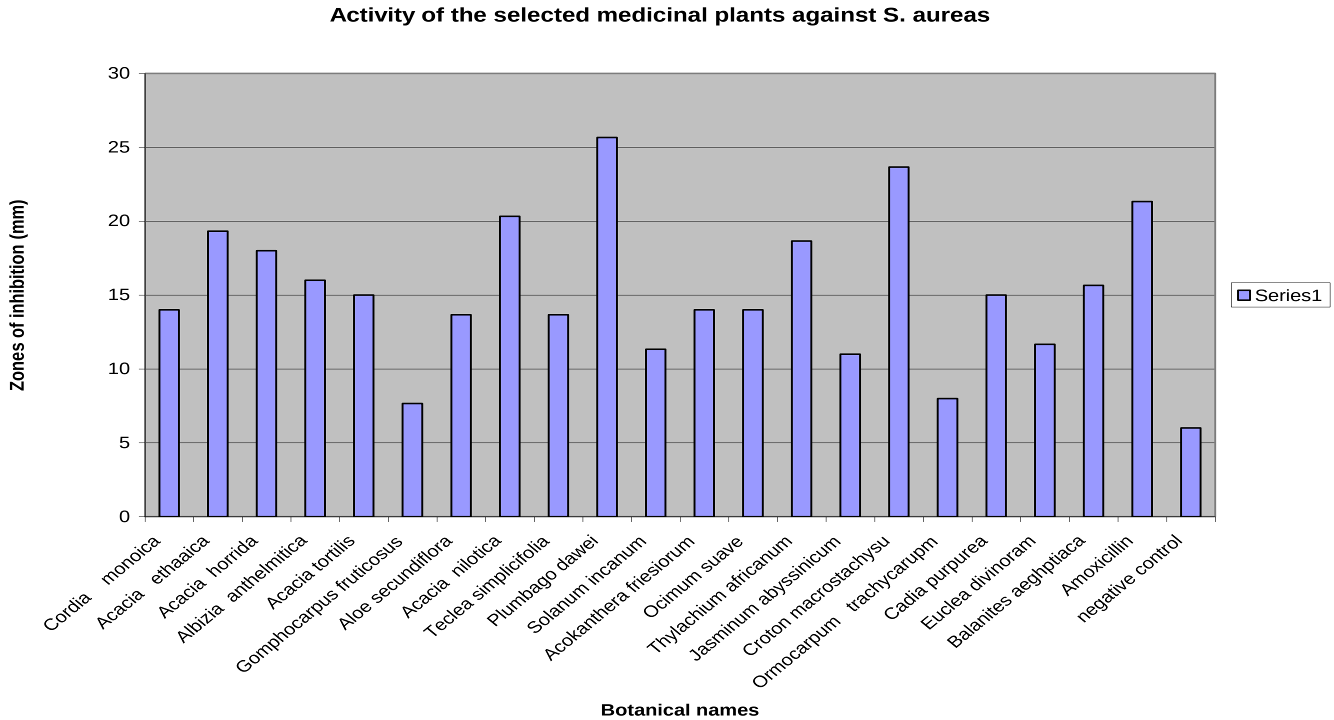


Fig 10: The graph representing the zones of inhibition of some extracts against *S. aureas* **-**ATCC 20591.

4.2.1.2 Minimum inhibitory concentrations (MICs) and the Minimum bactericidal concentration (MBCs) of the medicinal plants against *S. aureas* ATCC 20591.

The extracts also showed some good activity on the test culture *S. aureas* ATCC 20591. *Acacia ethaaica* and *Plumbago dawei* showed the very high MIC and MBC value which had the same concentration of 9.38 mg/ml- Fig 11 and the raw data on appendix IV. This clearly shows that the concentration that inhibits the growth of *S. aureas* is the same concentration of the two extracts that kills this test organism. This was different from what was observed from *Clerodendrum myriacoides, Acacia tortilis, Acacia nilotica, Cordia purpurea* and *Thylachium africanum* that showed also a high MICs and MBCs of 18.75 mg/ml. Other medicinal plants showed MICs and MBCs of 18.75 mg/ml and 37.5

mg/ml respectively. This clearly demonstrates that the concentration of these extracts that inhibits the growth of the *S. aureas* can not kill that test organism. Fourteen (14) of the extracts tested gave MICs and MBCs of 37.5 mg/ml. Other 5 extracts produced MICs and MBCs of 37.5 mg/ml and 75 mg/ml respectively. *Salvodora persica, Solanum incanum* and *Ormocarpum trachycarpum* gave the weakest results. The extracts produced MICs and MBCs of 75 mg/ml. Cefrodoxima antibiotic was used as a positive control and it produced an MIC and MBC of 18.75 mg/ml respectively.

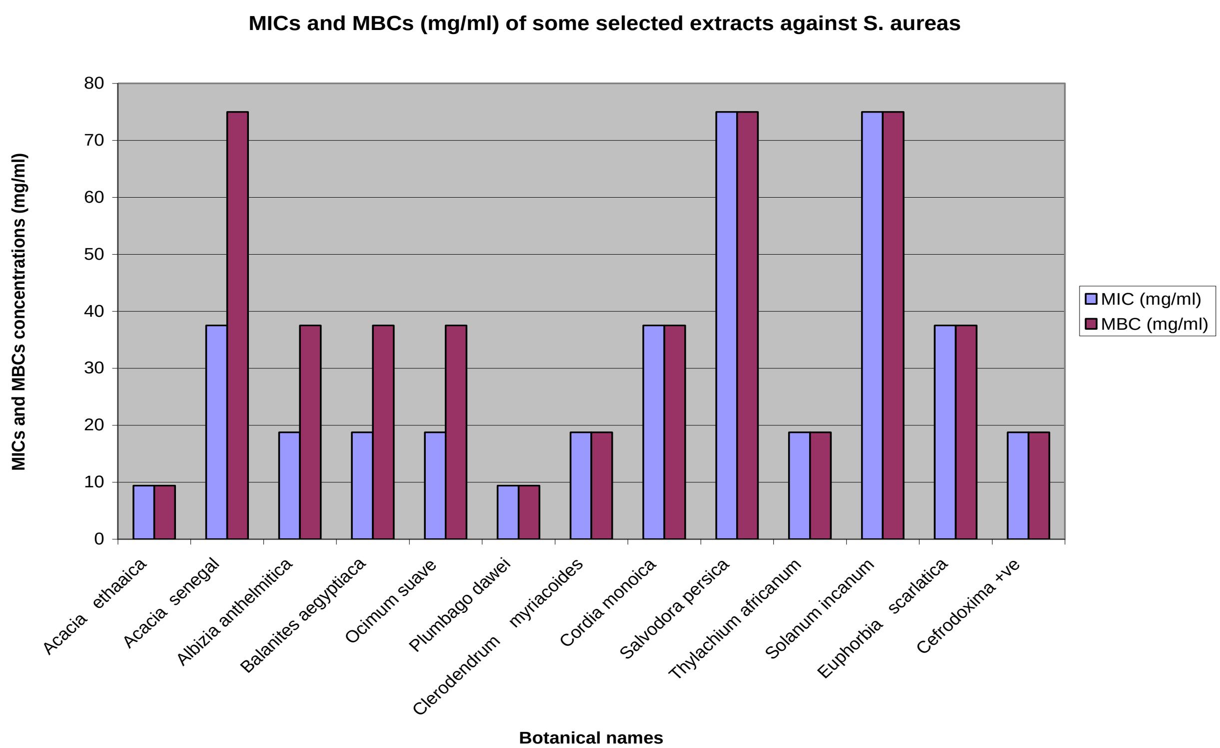


Fig 11: Graph representing the MICs and MBCs (mg/ml) produced by the selected Samburu medicinal plants against *S. aureas*-ATCC 20591.

4.2.2 Antibacterial results for *B. subtillis*- Local isolate.

4.2.2.1 Disc diffusion screening test.

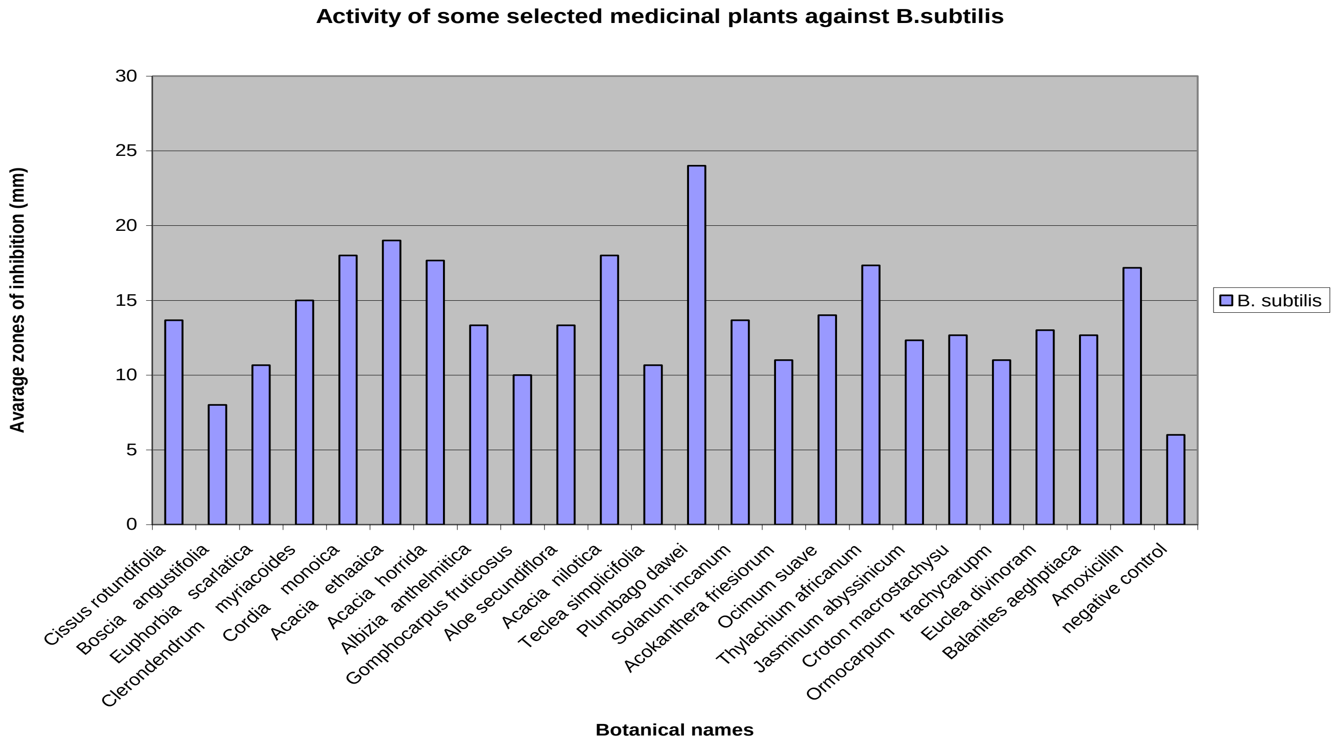


Fig 12: The graph representing the zones of inhibition of some selected extracts against *B. subtilis* –Local isolate.

Most extracts performed quite well against the *B. subtilis* local isolate with most having a zone of inhibition of more than 10mm. Each extract had an activity against the spore forming organism as they inhibited the growth of the organism by producing different zones of inhibition. *Plumbago dawei* again produced the widest average zone of inhibition with *B. subtilis* of 24mm. The extract was followed by *Acacia ethaaica* that had a zone of inhibition of 19mm. *Acacia horrida, Acacia nilotica* and *Cordia monoica* which had average zones of inhibition of 18mm respectively. Most of the extracts had the zones of inhibition of between 10mm-17mm as shown in Fig. 12 and the raw data on Appendix II. Incidentally there was no significant difference of the means of the zones of inhibition at P≤ 0.05 (Appendix V-b). *Boscia angustifolia* is the only extract that showed a zone of inhibition of <10mm i.e. 08mm against this test organism.

4.2.2.2 Minimum inhibitory concentrations (MICs) and the Minimum bactericidal concentration (MBCs) of the medicinal plants against *B. subtillis*

The extracts also showed good MICs and MBCs values against this spore forming micro-organism. For instance *Acacia ethaaica* and *Plumbago dawei* showed MICs of 9.38mg/ml and the MBCs of 18.75 mg/ml –Fig. 13 and the raw data on appendix IV. These are the plants that showed the highest bacteriostatic and bactericidal activities against this test organism. *Clerodendrum myriacoides* also gave high MIC and MBC of 18.75 mg/ml. *Cordia sinensis*, *Commiphora africana*, *Euphorbia scarlatica*, *Acacia tortilis*, *Acacia nilotica*, *Ocimum suave*, *Euclea divinoram* and *Balanites aegyptiaca* showed high MICs and MBCs values of 18.75 mg/ml and 37.5 mg/ml respectively. *Acacia nubica*, *Albizia anthelmitica*, and *Thylachium africanum* showed same MICs and MBCs values of 37.5 mg/ml against *B. subtilis*. Nineteen (19) of the extracts screened further showed the same MICs and MBCs of 37.5 mg/ml and 75 mg/ml. Also Cefrodoxima antibiotic was used as a control and produced an MIC and MBC of 18.75 mg/ml respectively.

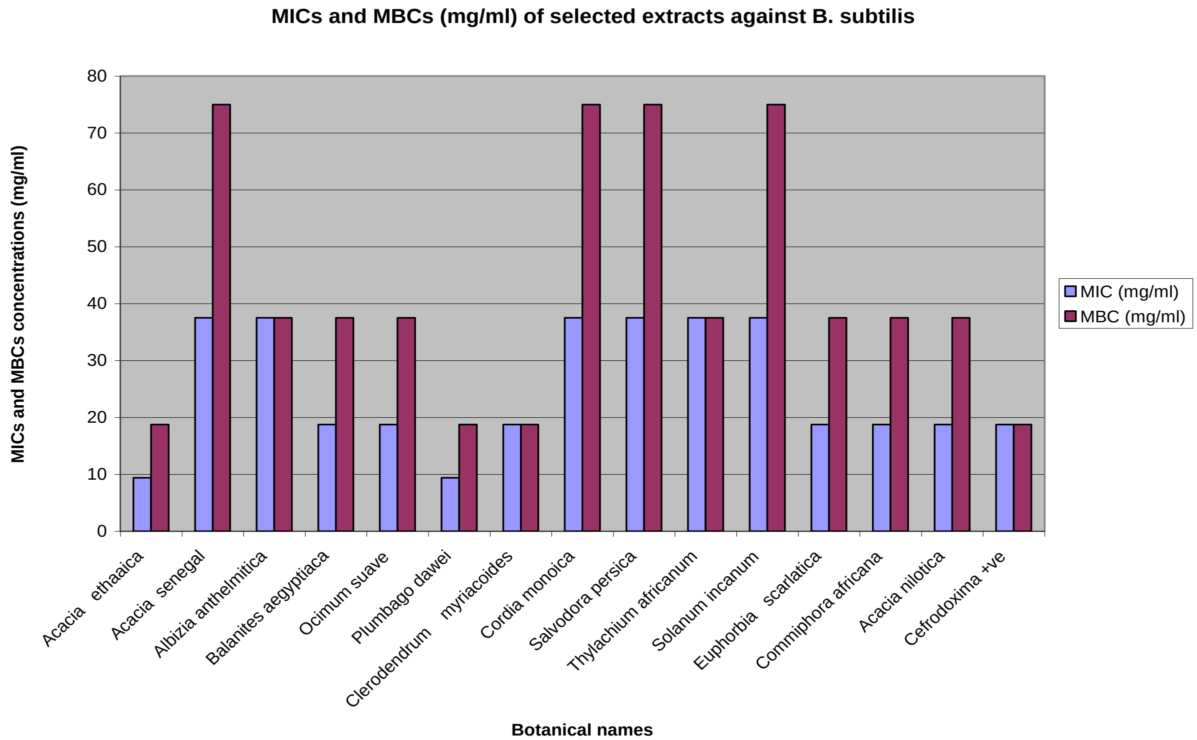


Fig 13: Graph representing the MICs and MBCs (mg/ml) produced by the selected Samburu medicinal plants against *B. subtillis*-Local isolate.

4.2.3 Antibacterial results for *S. typhi*- ATCC 2202

4.2.3.1 Disc diffusion screening test.

The performance of most of the extracts against the *S. typhi* ATCC 2202 was quite promising. No extract was completely inactive against this test organism. *Acacia nilotica* extracts produced the widest zone of inhibition (21mm) against this test organism. The *Acacia horrida* extract also produced a substantial zone of inhibition of 19mm. *Acacia ethaaica, Ocimum suave,* and *Thylachium africanum* produced an average zone of inhibition against the test organism of 16.66mm. With *Plumbago dawei* and *Commiphora africana* producing an average zone of inhibition of 16mm each against *S. typhi*. The rest of the extracts produced a zone of inhibition as is represented in Fig. 14

and the raw data on Appendix II. The means of the zones of inhibition showed a significant difference at P ≤ 0.01 as shown in Appendix V (c).

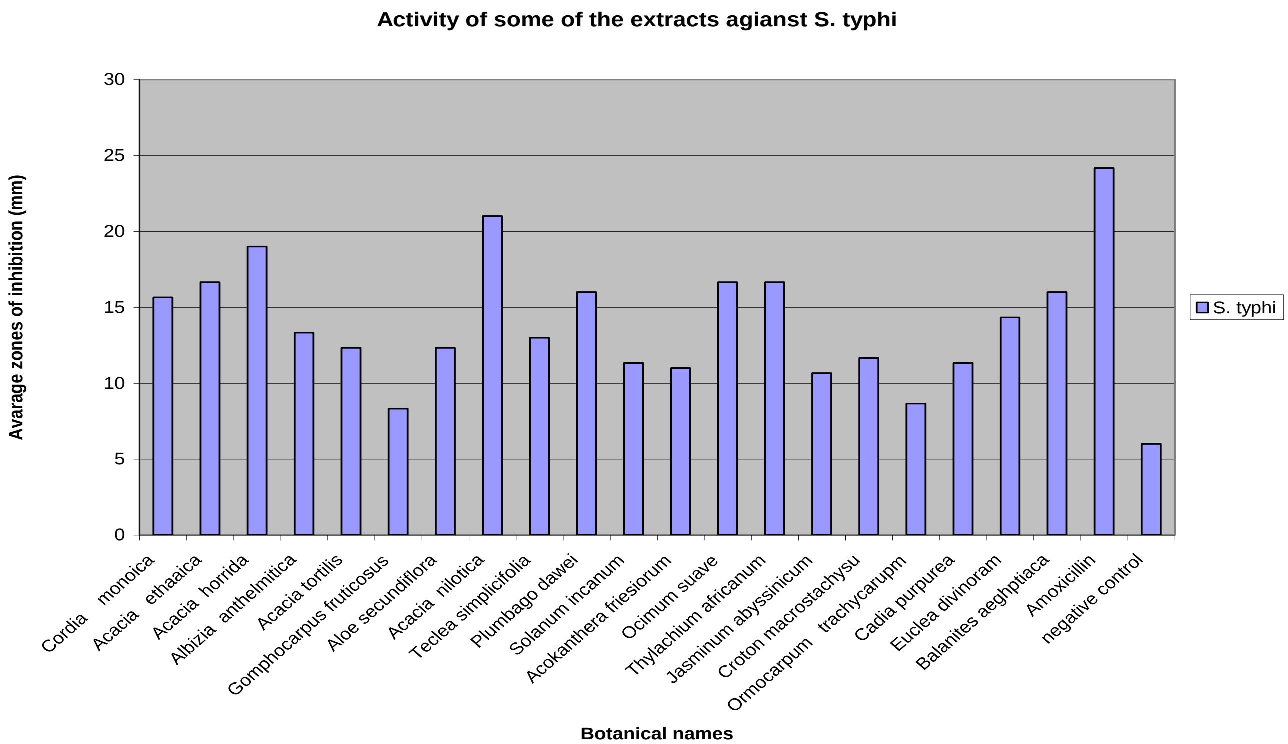


Fig 14: The graph representing the zones of inhibition of some selected extracts against *S. typhi* -ATCC 2202.

4.2.3.2 Minimum inhibitory concentrations (MICs) and the Minimum bactericidal concentration (MBCs) of the medicinal plants against *S. typhi*-ATCC 2202.

In all the extracts tested, most produced very promising results- Fig 15 and the raw data on appendix IV. Some of the extracts had the same MICs and MBCs 0f 18.75mg/ml respectively which was similar to that of the positive control against *S. typhi* ATCC 2202. Such extracts include *Cordia sinensis, Commiphora africana, Euphorbia scarlatica, Clerodendrum myriacoides, Acacia ethaaica, Acacia nilotica, Thylachium africanum, Euclea divinoram* and *Balanites aegyptiaca.* Only *Plumbago dawei* produced an MIC and MBC that is greater than that of the positive control i.e. 9.38mg/ml. Eight (8) extracts produced MICs and MBCs of 37.5mg/ml respectively. Such plants include *Cissus quadrangularis*, *Acacia senegal*, *Acacia horrida*, *Albizia anthelmitica* amongst many as shown in table below. Other extracts (13) produced MICs and MBCs of 37.5 mg/ml and 75 mg/ml respectively. Such extracts includes *Kedrostis pseudogijef*, *Salvodora persica*, *Acacia nubica*, *Ormocarpum trachycarupm*, *Grewia simi* amongst many as represented in the Fig. 15 and the raw data on appendix IV.

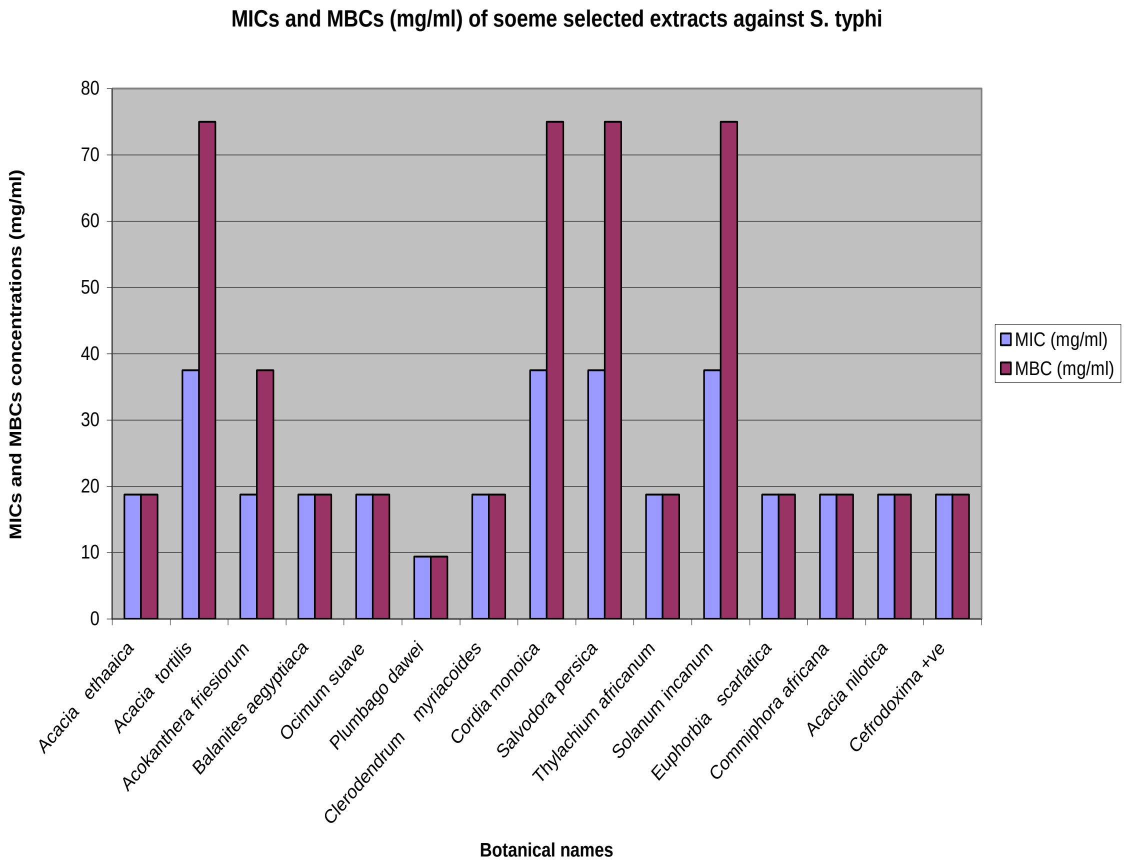


Fig 15: Graph representing the MICs (mg/ml) and MBCs (mg/ml) produced by the selected Samburu medicinal plants against *S. typhi* ATCC 2202.

4.2.4 Antibacterial results for *E.coli*- STD. 25922

4.2.4.1 Disc diffusion screening test.

The performance of most of the extracts was quite promising as none of the extracts were found not to posses any inhibitory activity against the STD *E.coli* 25922. All the extracts produced zones of inhibition of more than 10mm with *Acacia nilotica* producing the widest average zone of inhibition of 21.66mm among the screened extracts. Average zones of inhibition of 18.66mm, 18mm, 18mm, and 17.66mm were produced by *Acacia horrida, Ocimum suave, Balanites aegyptiaca,* and *Acacia ethaaica* respectively. Other extracts produced low zones of inhibition of between 10mm-16.33mm. Some of such extracts include *Acokanthera friesiorum, Euclea divinoram, Aloe secundiflora, Clerodendrum myriacoides, Teclea simplicifolia, Gomphocarpus fruticosus* e.t.c. as presented in Fig. 16 and the raw data on Appendix II. The extracts also produced no significant differences in the means for the Zones of inhibition at $P \leq 0.05$ as is shown in Appendix V (d).

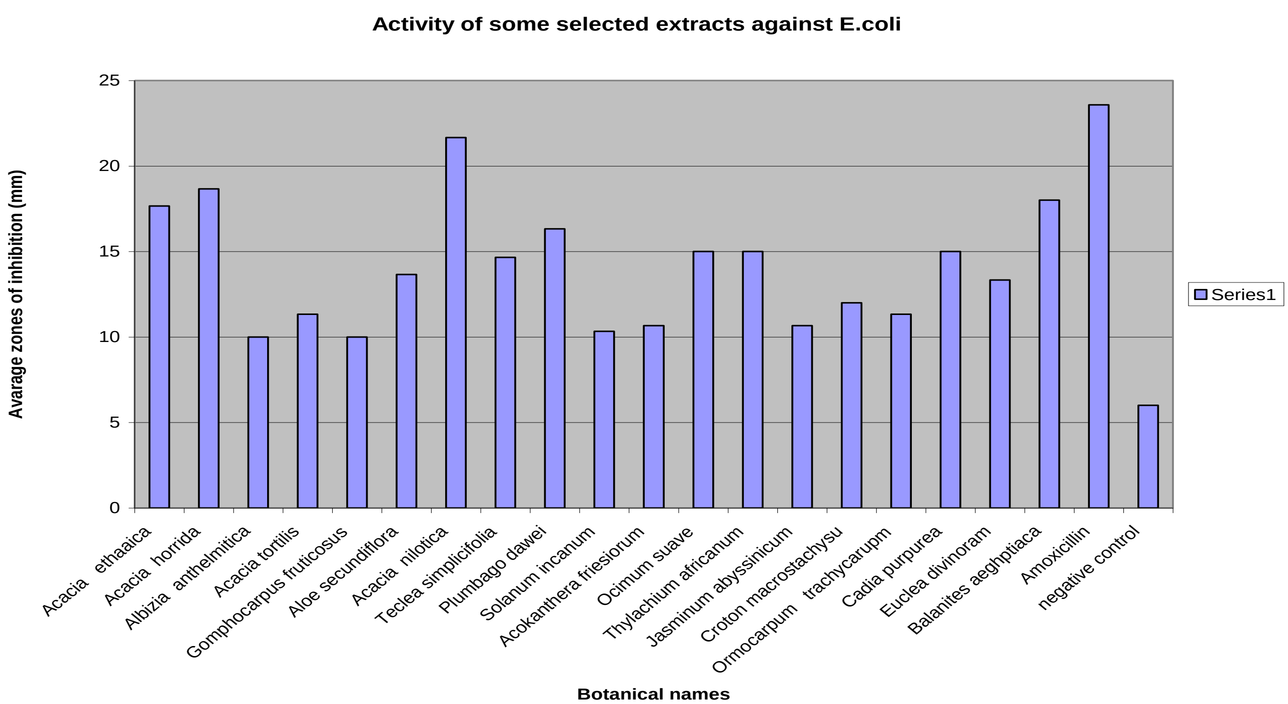


Fig 16: The graph representing the zones of inhibition of various extracts against *E. coli*-ATCC 25922.

4.2.4.2 Minimum inhibitory concentrations (MICs) and the Minimum bactericidal concentration (MBCs) of the medicinal plants against *E.coli* STD. 25922.

The extracts produced good MICs and MBCs against *E.coli* STD. 25922. For instance *Plumbago dawei* and *Acacia ethaaica* extracts produced MICs and MBCs of 9.38 mg/ml respectively, a concentration that is lower than that of the Cefrodoxima antibiotic. The *Commiphora africana, Acacia senegal, Clerodendrum myriacoides, Acacia tortilis, Acacia nilotica, Ocimum suave, Acokanthera friesiorum, Cordia purpurea,* and *Euclea divinoram* produced MICs and MBCs of 18.75 mg/ml respectively, a concentration that is equal to the concentration of the Cefrodoxima antibiotic. Several extracts on the other hand produced MICs and MBCs of 37.5 mg/ml respectively. Such extracts include *Cissus quadrangularis, Kedrostis pseudogijef, Acacia horrida, Albizia anthelmitica, Gomphocarpus fruticosus, Aloe secundiflora* amongst many as presented in Fig. 17 and the raw data on appendix IV. *Teclea simplicifolia* and *Cordia monoica* extracts produced MICs and MBCs of 37.5 mg/ml and 75 mg/ml respectively against the test culture.

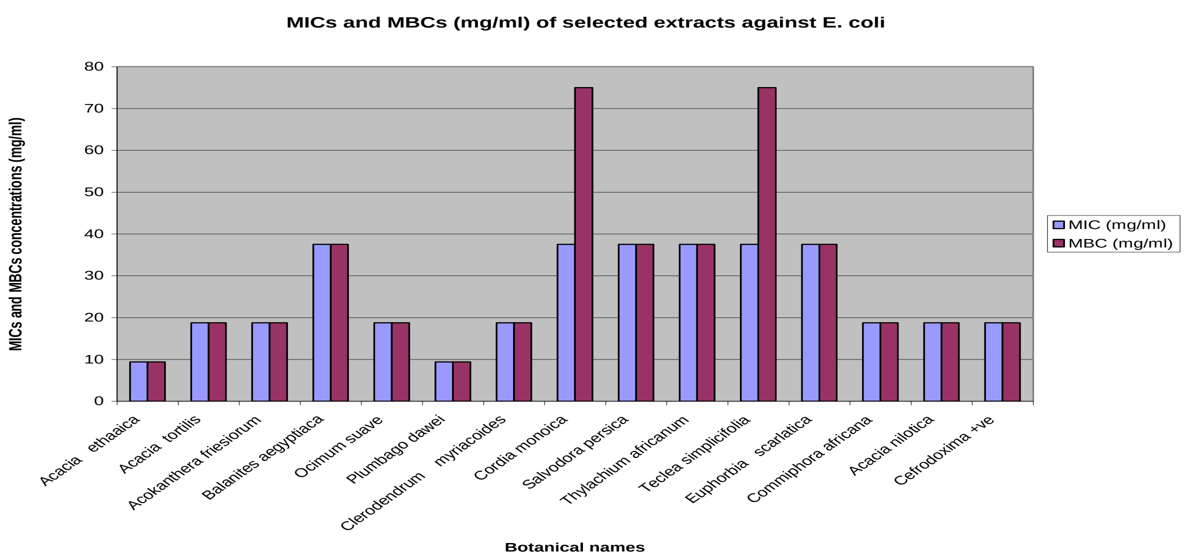


Fig 17: Graph showing the MICs (mg/ml) and MBCs (mg/ml) produced by the selected Samburu medicinal plants against *E. coli* STD. 25922.

4.2.5 Antibacterial results for *P. aeruginosa* ATCC 25852

4.2.5.1 Disc diffusion screening test

The plant extracts screened against *P. aeruginosa* ATCC 25852 produced substantial results. None of the extracts screened produced an average zone of inhibition of less than 12mm and most of the extracts produced higher zones of inhibition of more than 20mm with *Cordia monoica* producing the widest average zone of inhibition of 36.33mm. Among the plants that produced average zones of inhibition of more than 20mm are *Cissus quadrangularis* (23mm), *Acacia horrida* (28.66mm), *Albizia anthelmitica* (28.66mm), *Acacia tortilis* (21.33mm), *Ormocarpum trachycarpum* (25.33mm), *Cordia purpurea* (23.66mm), *Aloe secundiflora* (27mm), *Solanum incanum* (20mm), *Thylachium africanum* (23.33mm) amongst many as presented in Fig. 18 and the raw data on Appendix II. Some of the extracts produced average zones of inhibition of less than 20mm but more than 10mm and such extracts include *Kedrostis pseudogijef* (18mm), *Grewia simi*(14mm), *Acacia nubica* (15.33mm), *Acacia senegal* (18.33mm), *Cissus rotundifolia* (17.66mm), *Euphorbia scarlatica* (13mm), *Acacia ethaaica* (19.33mm), *Gomphocarpus fruticosus* (12.66mm), *Teclea simplicifolia*(18.33mm), *Acokanthera friesiorum* (19mm), *Croton macrostachysu* (18mm), *Euclea divinoram* (19.33mm) and *Balanites aegyptiaca* (17mm). The means of the zones of inhibition had a significant difference at $P \leq 0.01$ as shown in Appendix V (e).

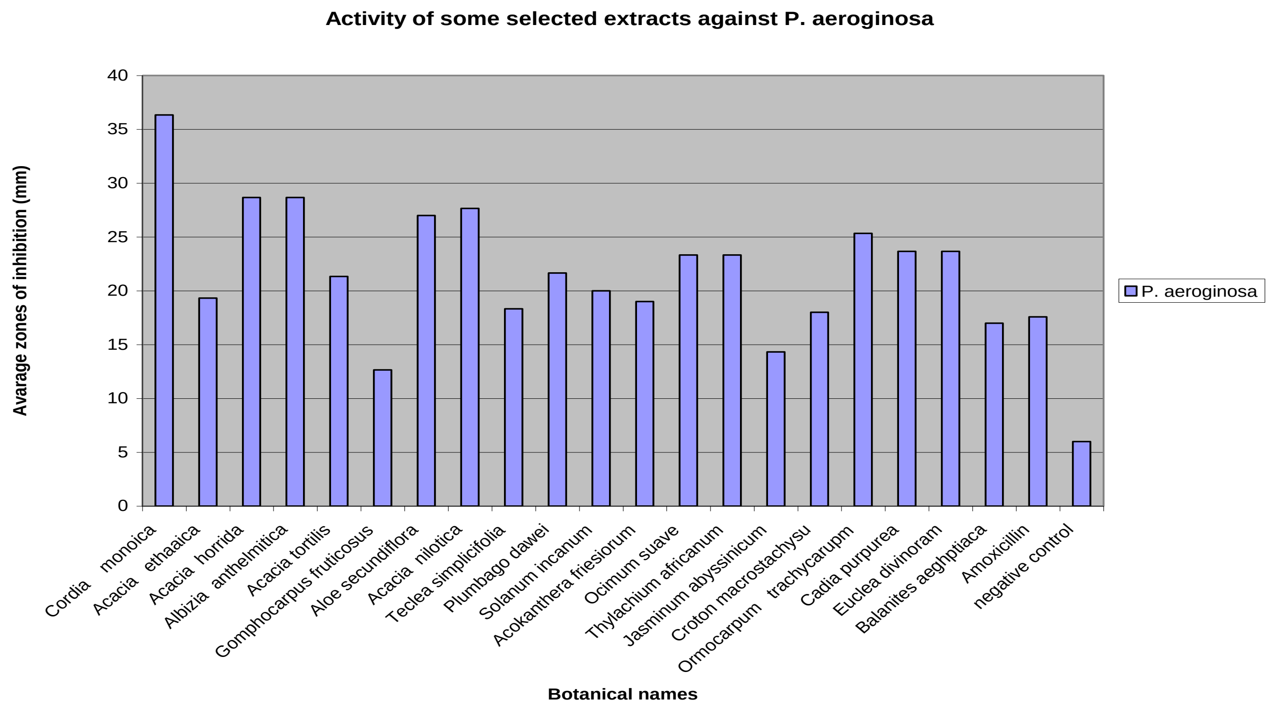


Fig 18: The graph representing the zones of inhibition of various extracts against *P. aeruginosa***-**ATCC 25852.

4.2.5.2 Minimum inhibitory concentrations (MICs) and the Minimum bactericidal concentration (MBCs) of the medicinal plants against *P. aeruginosa* ATCC 25852

Most extracts produced very good MICs and MBCs with *P. aeruginosa* ATCC 25852 than in most of the other test cultures screened. Three extracts produced MICs and MBCs that are same as those produced by the positive control antibiotic (Cefrodoxima). They include *Acacia ethaaica*, *Acacia nilotica* and *Plumbago dawei* and they produced MICs and MBCs of 9.38 mg/ml respectively. *Commiphora africana, Cissus rotundifolia, Clerodendrum myriacoides, Acacia horrida, Albizia anthelmitica, Teclea simplicifolia, Thylachium africanum* and *Croton macrostachysu* produced good MICs and MBCs of 18.75 mg/ml respectively against *P. aeruginosa* ATCC 25852. Significantly good activity was also observed in other extracts. Some of these extracts produced MICs of 18.75 mg/ml and MBCs of 37.5 mg/ml include *Kedrostis pseudogijef*, *Acacia nubica*, *Grewia simi*, *Cissus rotundifolia*, *Euphorbia scarlatica*, *Cordia monoica*, *Acacia tortilis*, *Acokanthera friesiorum*, *Cordia purpurea*, *Euclea divinoram and Balanites aegyptiaca* as shown in Fig. 19 and the raw data on Appendix IV. Five extracts produced MICs and MBCs of 37.5 mg/ml respectively. Such extracts includes those from *Cissus quadrangularis*, *Salvodora persica*, *Cordia sinensis*, *Loranthus acaciae*, *Acacia senegal*, and *Ocimum suave*. Others produced MICs and MBCs of 37.5 mg/ml and 75 mg/ml respectively against the test culture e.g *Boscia angustifolia* and *Aloe secundiflora.*

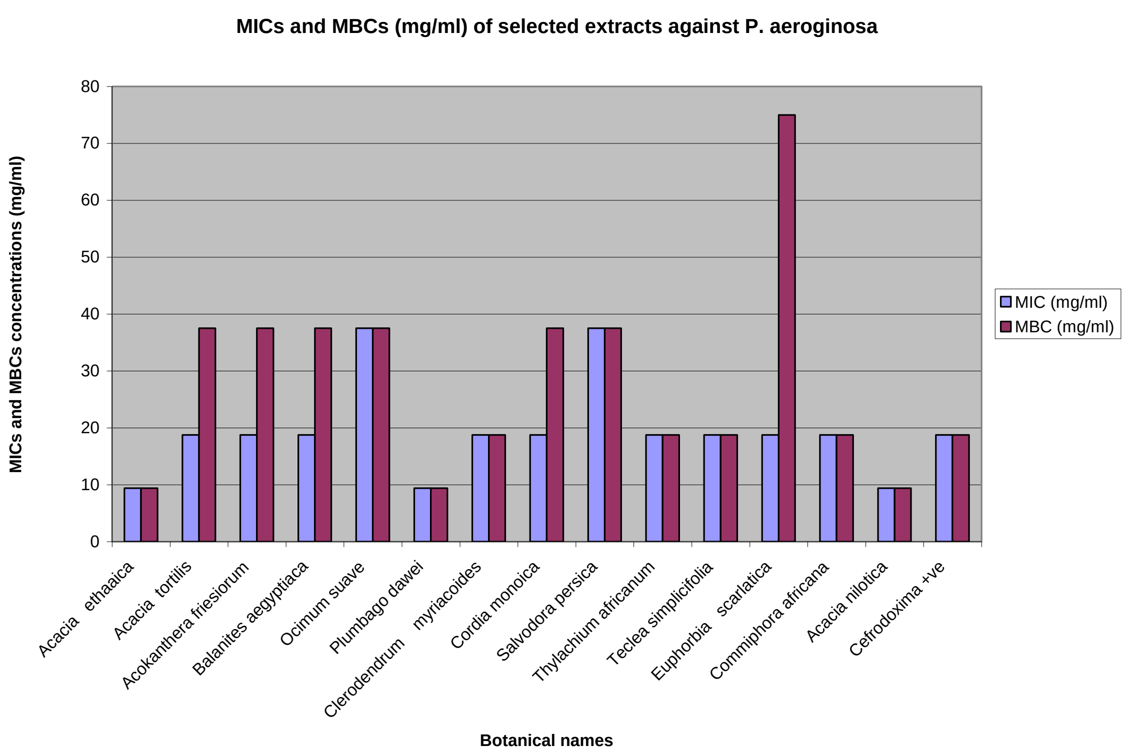


Fig 19: Graph showing the MICs (mg/ml) and MBCs (mg/ml) produced by the selected Samburu medicinal plants against *P. aeruginosa* ATCC 25852.

4.3 Antifungal disc diffusion results

Sixteen (16) medicinal plants were screened against the five (5) fungal cultures. The extracts showed no activity against the fungal test cultures except with the *C. albicans* where four extracts showed slight activity that was almost same as that of the positive control (fluconazole). *Albizia anthelmitica* produced a higher zone of inhibition against the fungus than the other extracts as shown in Fig. 20 and the raw data on Appendix III. There was no significant difference of the means of the zones of inhibition. Thus these findings did not necessitate the carrying out of the MICs and the MFCs of the extracts against the fungal test cultures.

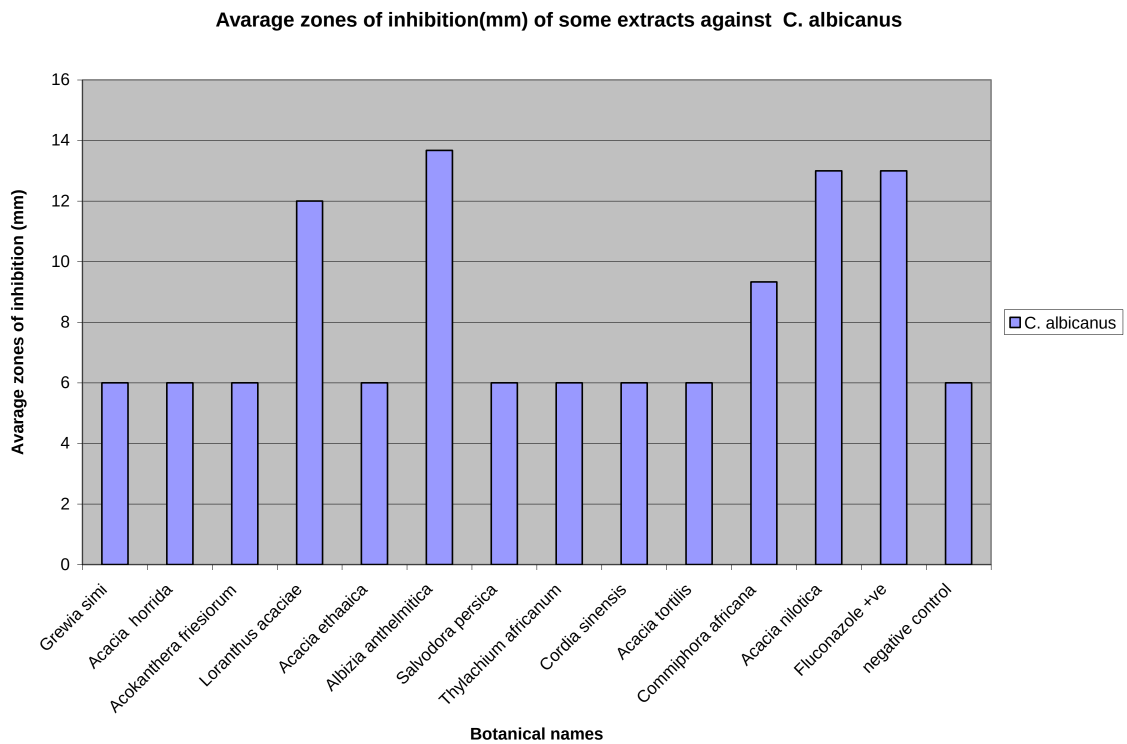


Fig. 20. The graph representing the zones of inhibition of various extracts against *C. albicans* ATCC EK138.

4.4 Phytochemical screening of the plant extracts results.

Various phytochemicals were screened for their presence i.e. tannins, saponins, flavonoids, terpenoids, cardiac glycosides and alkaloids.

4.4.1 Tannins

Among the phytochemicals screened tannins were found to be the most abundant as they were found to be present in most extracts. It was actually found to be in higher amounts in such extracts as C*ordia sinensis*, *Grewia simi*, *Loranthus acaciae, Clerodendrum myriacoides, Acacia nilotica, Teclea simplicifolia, Solanum incanum, Ocimum suave* and *Thylachium africanum.* Others also had the tannins as shown in the Table 2.

4.4.2 Saponins

Saponins were also present in some extracts and were more abundant in extracts of *Grewia simi, Cissus rotundifolia* and *Thylachium africanum.* The phytochemical was not found in some extracts like *Kedrostis pseudogijef*, *Salvodora persica, Acacia nubica, Boscia angustifolia* e.t.c as shown in Table 2.

4.4.3 Flavonoids

Flavonoids were found to be present as presented in Table 2. The phytochemical was found to be abundant in *Clerodendrum myriacoides,* and *Acacia nilotica.* The phytochemical was not found in such extracts like *Commiphora africana, Boscia angustifolia, Cordia monoica,* e.t.c.

4.4.4 Terpenoids

Terpenoids presence was also screened for there presence- Table 2. The phytochemical was found to be present in most extracts in substantial amounts with it being abundant in such extracts as *Cissus quadrangularis, Euphorbia scarlatica*, *Acacia ethaaica,* *Jasminum abyssinicum* and *Euclea divinoram.* The phytochemical was not present in some extracts like *Grewia simi, Gomphocarpus fruticosus, Albizia anthelmitica* and *Croton macrostachysu*.

4.4.5 Cardiac glycosides

Cardiac glycoside was also found to be present in most extracts. The phytochemical was abundant in *Commiphora africana, Clerodendrum myriacoides, Gomphocarpus fruticosus,* and *Thylachium africanum.* Some extracts also possessed the phytochemical but in small proportions as presented in the Table 2. Others did not possess it completely. Such extracts included *Cordia sinensis, Boscia angustifolia, Aloe secundiflora, Jasminum abyssinicum, Euclea divinoram* e.t.c as presented in the Table 2.

4.4.6 Alkaloids

The presence of alkaloids was determined by use of the Wagner's test method. The phytochemical was found to be present in most extracts but was more abundant in a few of the extracts like *Commiphora africana, Acacia horrida, Cordia purpurea* and *Thylachium africanum.* The phytochemical was also in small proportions in such plant extracts as *Solanum incanum, Cissus quadrangularis, Acacia tortilis,* e.t.c as presented in the Table 2. *Kedrostis pseudogijef, Plumbago dawei, Acokanthera friesiorum, Ormocarpum trachycarpum, Croton macrostachysu* e.t.c. did not possess this phytochemical.

Table 2: The plant extracts phytochemical screening test results

Species name	**Tannins**	**Saponins**	**Flavonoids**	**Terpenoids**	**Cardiac glycosides**	**Alkaloids (wagner's test)**
Cissus quadrangularis	++	+	+	+++	+	+
Kedrostis pseudogijef	-	-	+	+	++	-
Salvodora persica	-	-	+	+	+	+
Acacia nubica	-	-	+	++	++	-
Cordia sinensis	+++	+	+	+	-	-
Grewia simi	+++	+++	+	-	+	-
Loranthus acaciae	+++	+	+	-	+	+
Commiphora africana	++	+	-	-	+++	+++
Acacia Senegal	+	++	+	++	+	-
Cissus rotundifolia	++	+++	-	-	-	++
Boscia angustifolia	+	-	-	-	-	+
Euphorbia scarlatica	+	-	++	+++	++	++
Clerodendrum myriacoides	+++	+	+++	++	+++	++
Cordia monoica	++	+	-	+	-	-
Acacia ethaaica	+	++	++	+++	++	+
Acacia horrida	++	++	-	++	++	+++
Albizia anthelmitica	+	++	+	-	-	++
Acacia tortilis	++	+	+	+	++	++
Gomphocarpus fruticosus	+	-	+	-	+++	-
Aloe secundiflora	+	-	++	++	-	-
Acacia nilotica	+++	++	+++	++	+	++
Teclea Simplicifolia	+++	+	+	++	+	+

Species name	**Tannins**	**Saponins**	**Flavonoids**	**Terpenoids**	**Cardiac glycosides**	**Alkaloids (wagner's test)**
Plumbago dawei	++	-	++	-	+	-
Solanum incanum	+++	++	+	-	++	+
Acokanthera friesiorum	++	-	++	-	+	-
Ocimum suave	+++	-	+	+	+	++
Thylachium africanum	+++	+++	++	-	+++	+++
Jasminum abyssinicum	-	++	-	+++	-	-
Croton macrostachysu	-	+	+	-	-	-
Ormocarpum trachycarpum	++	+	-	+	++	-
Cordia Purpurea	+++	++	+	-	-	+++
Euclea divinoram	++	+	+	+++	-	+
Balanites aegyptiaca	+++	+	++	+	-	+

Key: +++ Most abundant ++ Abundant + Less abundant

\- Not present

The following plates represent the presence of the above tested phytochemicals as they were observed during the preliminary tests for their presence in some of the extracts.

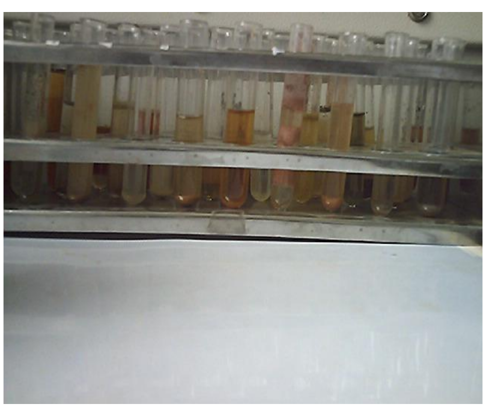


Plate 19: The tubes show the various phytochemicals screened for there presence.

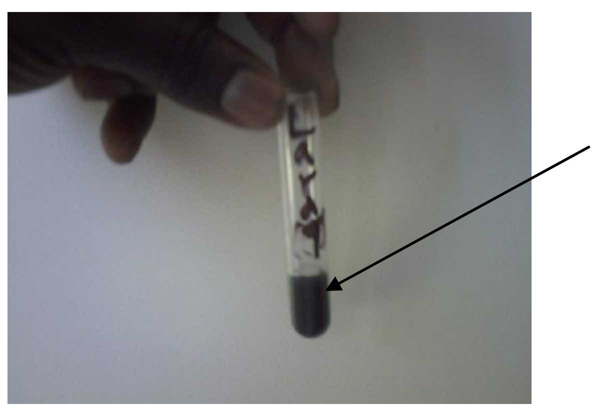


Plate 20:*Loranthus acaciae* extracts showing presence of tannins

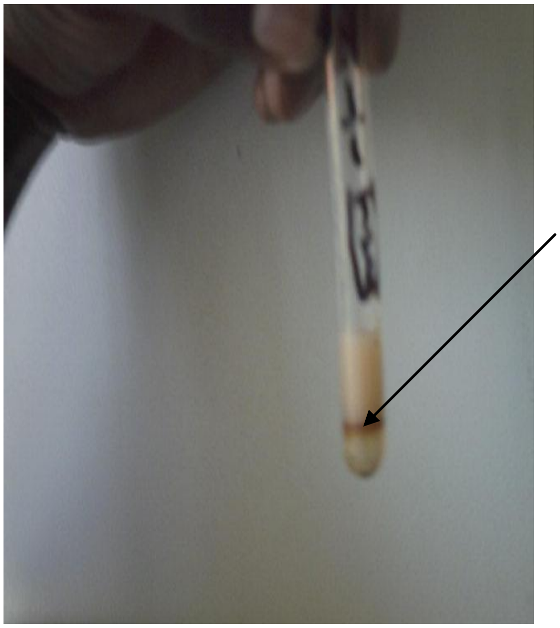


Plate 21:*Cissus quadrangularis* extracts showing presence of terpenoids.

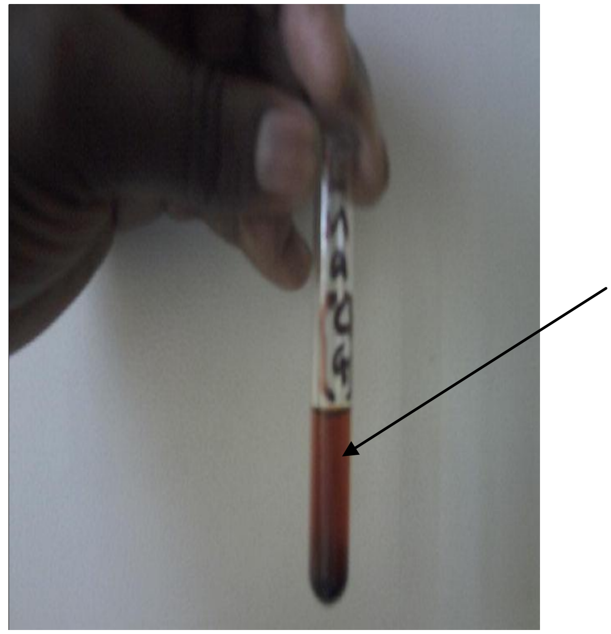


Plate 22:*Commiphora africana* extracts showing presence of alkaloids.

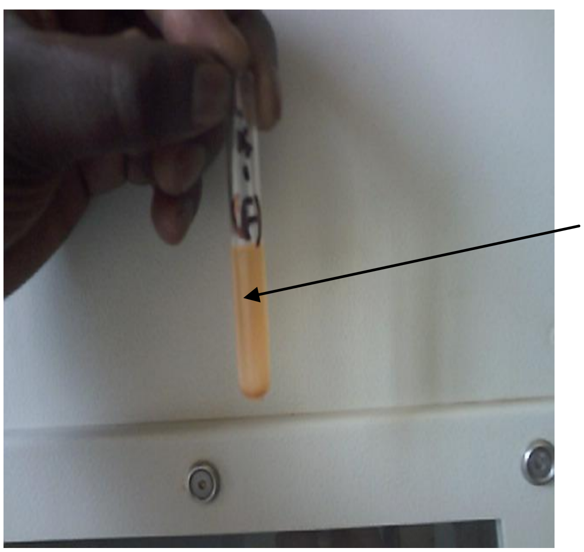


Plate 23: *Acacia ethaaica* extracts showing presence of flavonoids

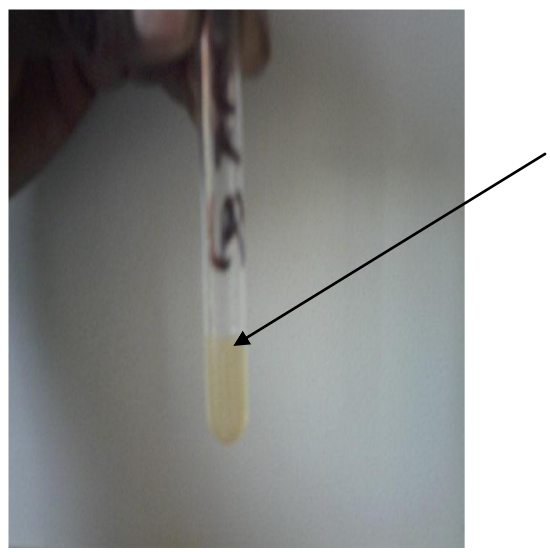


Plate 24: *Albizia anthelmitica* extracts showing presence of alkaloids

CHAPTER FIVE

5.0 DISCUSSIONS

5.1 Disc diffusion and MIC ratings of the extracts

The negative controls of the disc diffusion testing was done by use of methanol that showed no inhibition, while positive control was done by use of commonly used antibiotics such Amoxicillin and chloramphenicol that were impregnated in the sterile discs using the same procedure as that of the extracts impregnation. The average zone of inhibition was calculated for the 3 replicates. A clearing zone of 8mm for Gram positive and Gram negative bacteria was used for designating significant antibacterial activity (Ruttoh, 2009). The *in vitro* MIC results were classified with the MIC of 9.38 mg/ml considered to have very high antibacterial activity; 18.75 mg/ml, high antibacterial activity; 37.5 mg/ml, moderate activity; and 75mg/ml, low activity (Mathabe *et al.*, 2006).

5.2 *Cissus quadrangularis* L.

The *Cissus quadrangularis* belongs to the Vitaceae family. On the basis of the current investigation, it is possible to point out that the broad antibacterial activity of *Cissus quadrangularis* L. methanol extracts towards bacterial strains used may be ascribed apparently to the presence of tannins, saponins, flavonoids, terpenoids, cardiac glycosides and the alkaloids. These phytochemicals have been reported to have antimicrobial activity (Ibrahim *et al.*, 2005) and they might be responsible for the antimicrobial activity of the *Cissus quadrangularis* L. since the possibility of having synergistic or additive antibacterial activities might be present.

Murthy *et al*., (2003) reported that *Cissus quadrangularis* L. is highly potent to the Gram positive bacteria (*B. subtilis*, *B. cereus*, *Staphylococcus aureus*, and *Streptococcus* species), whereas Gram-negative bacteria (*P. aeruginosa* and *E. coli*) exhibited high resistance to the plant extract. This is exactly the opposite of what was observed where, Gram negative bacteria were found to be more susceptible to the extract than the Gram positives. The extract had higher zones of inhibition on *P. aeruginosa, S. typhi and E. coli*. Further the bacteriostatic concentration of the extract that inhibited the growth of the test cultures also differed with the Gram positives concentration being higher than that of the Gram negatives. These differences might also be attributed to the changes in environmental conditions like changes in the amount of rainfall distribution and temperatures.

The extract was also screened against the fungal isolates but it did not show any activity regardless of the presence of the phytochemicals. This can be attributed to the possibility of antagonistic activity of the phytochemicals present in the extracts since they have been known to have some antifungal activity (Ruttoh, 2009).

5.3 *Kedrostis pseudogijef* (Gilg) C. Jeffrey.

The *Kedrostis pseudogijef* (Gilg) C. Jeffrey belongs to the family Cucurbitaceae. The medicinal plant extract produced almost the same average zones of inhibition with both the Gram positives and the Gram negative bacteria test cultures that were between 12mm and 13mm. It’s only in *P. aeruginosa* that the extract produced a zone of inhibition of 18mm. The same observation was made when the extract was screened for the MICs and MBCs. The extract produced MICs and MBCs ranging from 37.5 mg/ml to 75mg/ml. The extract only produced a different pattern for the *P. aeruginosa* with the MIC of 18.75mg/ml and MBC of 37.5mg/ml. Since the level of action is almost similar the mechanism of activity can not be attributed to cell wall properties but most likely can be due to the presence of flavonoids, terpenoids and cardiac glycosides. This is generally because the presence of the phospholipids and other forms of lipids tends to be impermeable to materials crossing the cell wall hence Gram negative bacteria cell wall tends to inhibit entry of most substrates passing through it. Since therefore the activity was similar in both groups of bacteria, it can be due to synergistic or additive antibacterial activities which are almost similar in both the Gram positives and the Gram negatives bacteria (Ruttoh, 2009).

5.4 *Salvodora persica* L. var. *persica*

On the basis of the current investigation, it is possible to point out that the broad antibacterial activity of *Salvodora persica* methanol extracts towards bacterial strains used may be ascribed apparently to the presence of flavonoids, terpenoids, cardiac glycosides and the alkaloids. This is because such phytochemicals like flavonoids have been associated with antibacterial activities before (Krishnaraju *et al.,* 2005). The antimicrobial activities of the phytochemicals detected can therefore be having a synergistic or additive activity to the antibacterial activity exhibited by the plant extract.

P. aeruginosa and *E. coli* were the sensitive strains among the ones tested with the MICs and MBCs of 37.5 mg/ml respectively. This shows that the extract has a moderate activity against the Gram negatives than the Gram positives. For instance the extract produced an MIC and MBC of 75 mg/ml with the *S. aureas.* This is in agreement with other studies that clearly demonstrate that *Salvodora persica* has a low activity against the S. *aureas* (Almas, 2001).

The extract was also screened for the antifungal activity but did not show any antifungal activity. Thus it is possible to point out that the absence of the antifungal activity of *Salvodora persica* methanol extracts towards the fungal strains to be attributed to the antagonistic activity of the phytochemicals that were found to be present (Jigna and Sumitra, 2007).

5.5 *Acacia nubica* Benth.

The plant specimen belongs to the Mimosaceae family. The extract showed some average activity against the test cultures with no big difference between the Gram positives and the Gram negatives. In both, the average MICs and MBCs were almost the same with a concentration ranging from 37.5mg/ml- 75mg/ml with only *P. aeruginosa* having a different MIC and MBC of 18.75 mg/ml and 37.5 mg/ml respectively. This difference in the concentrations indicates that *Acacia nubica* is possibly bacteriostatic to some of the test cultures except against the *E.coli*, *B. subtilis*, and *S. typhi.*

Thus the activity of the *Acacia nubica* extract can be ascribed to the presence of flavonoids, terpenoids, cardiac glycosides and the alkaloids. Such phytochemicals had been earlier found to possess antibacterial activities (Hassan *et al.*, 2006). They could be having synergistic or additive activities among them and among the other phytochemicals which may be present but were not tested for their presence. Since the *Acacia nubica* is commonly used as antidiarrhoeal medicinal plant by the Samburu community. The antibacterial properties therefore can suggest its potential usefulness in traditional medicine for the treatment of diarrhoea. But further studies need to be carried out to determine it's mode of action.

5.6 *Cordia sinensis* Lam.

The plant extract belongs to Boraginaceae family (Hess, 2005).The *Cordia sinensis* extract produced high to moderate activities against both the Gram positives and the Gram negatives. For instance the extract produced a high MIC and MBC with the Gram negative *S. typhi* of 18.75mg/ml respectively. Almost similar results were got when the extract was bioassayed against the Gram positive spore forming *B. subtilis* (MIC-18.75mg/ml and MBC-37.5mg/ml). Thus partly one can conclude that the difference can be ascribed to differences in the cell wall properties. Also the extract can be bactericidal in nature since in some test cultures the MIC and the MBC concentrations are the same, for instance the extract produced an MIC and MBC of 37.5mg/ml against the *S. aureas, E. coli* and *P. aeruginosa.* Also the same was observed with *S. typhi*.

Presence of tannins, saponins, flavonoids and terpenoids could be the ones that are responsible for the activity of the extract. This is because such phytochemicals have been found to possess activity on the test organisms before (Cowan, 1999). Synergism or additive activity of such compounds present and those that may be present but were not detected can be a possibility for such an extract action.

5.7 *Grewia simi* K. Schum.

The plant belongs to Tiliaceae family. There was moderate activity of *Grewia simi* extracts against the Gram positive and Gram negative bacteria. The extract showed some difference between the MIC and MBC concentrations in *S.typhi, B. subtilis* and *P. aeruginosa* with the first two having an MIC and MBC of 37.5mg/ml and 75mg/ml respectively and the last one having the MIC of 18.75mg/ml and MBC of 37.5mg/ml. This clearly demonstrates that the extract is possibly bacteriostatic. On the other hand the extract is bactericidal given the fact that it produced same MICs and MBCs in *S. aureas* and *E.coli* of 37.5mg/ml respectively.

Since both concentrations are almost similar, the differences can not be ascribed to the cell wall properties but to the synergistic or additive properties of the compounds present in the extract. For instance presence of tannins, saponins, flavonoids, and cardiac glycosides among those that were tested for their presence can have such properties together with those that were not screened for their presence and might be present.

5.8 *Loranthus acaciae* Zucc.

The plant belongs to the Loranthaceae family. There was moderate activity of the *Loranthus acaciae* extracts against both the Gram positive and Gram negative bacteria. In both, a range of MICs and MBCs of between 37.5mg/ml to 75mg/ml was obtained. The extract was bacteriostatic to *B. subtilis* and *S. typhi* thus producing similar MICs and MBCs of 37.5mg/ml and 75mg/ml respectively. On the other hand the extract was bactericidal to *P. aeruginosa, S. aureas*, and *E. coli* with both MIC and MBCs being similar at 37.5mg/ml respectively in all the organisms.

Thus this clearly demonstrates that the activity of the extracts against the bacterial test organisms is not based on the cell wall properties but rather on the active compounds present in the extract that may have some additive or synergistic properties. For instance tannins, saponins, flavonoids, cardiac glycosides, and the alkaloids were found to be present and such phytochemicals have been detected with antibacterial properties before (Awoyinka *et al.*, 2007).

The extract further showed some slight activity against the *C. albicans.* The moderate activity of the extract is difficult to be associated with any of the class(es) of the compounds present due to the type of activity presented. Nevertheless, it is reasonable to speculate that the activity could be related to the synergism or additive activity of two or more of the compounds present.

5.9 *Commiphora africana* (A. Rich) Engl. Var. *persica*

The *Commiphora africana* plant belongs to the Burseraceae family. The extracts of *Commiphora africana* showed a high activity with the Gram negative bacteria and moderate activity with the Gram positive bacteria. For instance the extract proved to be bactericidal to the Gram negative test cultures by producing similar MICs and MBCs of 18.75mg/ml respectively against *S. typhi*, *E. coli* and *P. aeruginosa.* This is one of the best MICs and MBCs concentrations got from the plants screened for their efficacy. The extract was bactericidial to *S. aureas* (MIC and MBC of 37.5mg/ml) and bacteriostatic to *B. subtilis* (MIC-18.75mg/ml and MBC of 37.5mg/ml).

The differences in the activity of the extract against the test cultures can not be ascribed to the differences in the cell wall properties but may be ascribed to the presence of tannins, saponins, cardiac glycosides and the alkaloids. These are the phytochemicals that have been found to possess the antibacterial properties before, thus their synergistic or additive properties can be the ones responsible for the activity of the extract. Thus the antibacterial properties suggest the potential usefulness of the bark of this plant in traditional medicine for the treatment of diarrhoea. Further studies need to be done to determine the mode of action of the extract.

5.10 *Acacia senegal* (L.) Willd.Var. *persica*

The *Acacia senegal* plant belongs to the Mimosaceae family. Its gum is used for soothing mucous membranes of the intestine and to treat inflammed skin. It is also reportedly used for its astringent properties, to treat bleeding, bronchitis, diarrhoea, gonorrhea, leprosy, typhoid fever and upper respiratory tract infections (Van Wyk and Ben-Erik, 2005).

The extract showed a moderate activity in both the Gram negative and Gram positive bacteria tested. The extracts were bacteriostatic with *S. aureas* and *B. subtilis* with an MIC and MBC of 37.5mg/ml and 75mg/ml respectively. The extract also on the other hand was bactericidal in nature against *S. typhi* and *P. aeruginosa* with the MIC and MBC of 37.5mg/ml. Further bactericidal activity of the extract was observed in *E. coli* which had a high activity with the MIC and MBC of 18.75mg/ml.

These differences in activity can not be ascribed to the differences in cell wall properties since the activity of the extract is almost the same except in *E. coli.* Thus the differences can be greatly ascribed to the presence of the tannins, saponins, flavonoids, terpenoids, and cardiac glycosides which tend to possess synergism or additive activity as it has been found before (Edeoga *et al.,* 2005).

The *Acacia senegal* bark extract is used in the treatment of stomach ailments. The antimicrobial findings of this plant support its usefulness in traditional medicine for treatment of the gastroenteritis common pathogens. Further studies need to be carried out to find out the mode of activity of the plant.

5.11. *Cissus rotundifolia* Forsk. Vahl.

The *Cissus rotundifolia* plant belongs to the family of Vitaceae. The *Cissus rotundifolia* extracts showed moderate activity against the Gram positive and Gram negative test cultures. The extract was bacteriostatic in most of the test cultures with it producing an MIC of 18.75mg/ml and MBC of 37.5mg/ml with *S. aureas* and *P. aeruginosa*. This presented the high activity in both the Gram positive and negative bacteria respectively. Further bacteriostatic activity was observed with *B. subtilis* and *S. typhi* with the MIC of 37.5mg/ml and MBC of 75mg/ml. The extract was bactericidal to *E. coli* with the MIC of 37.5mg/ml and MBC of 37.5mg/ml.

Thus the activity of the extract can not be ascribed to the cell wall properties since the activity is similar in both the Gram positive and negative test bacteria. The activity of the extract is suspected to be due to the presence of tannins, saponins and alkaloids which have been found to be active against most of the bacteria (Edeoga *et al.,* 2005). From the survey *Cissus rotundifolia* root extracts are used by the community for treatment of stomach ache. The antibacterial properties suggest its potential usefulness in traditional medicine for treatment of stomach ailments caused by Gram negative bacteria. However some investigations on its mode of action need to be carried out.

5.12 *Boscia angustifolia* Guill. and Perr.

Boscia angustifolia plant belongs to the Capparaceae family (Haston *et al.*, 2007). The *Boscia angustifolia* bark extracts showed moderate activity against Gram positive and Gram negative test cultures. The extract showed bactericidal activity against *S. aureas* and *E. coli* (MIC of 37.5mg/ml and MBC of 37.5mg/ml). The extract was bacteriostatic against *B. subtilis, S. typhi and P. aeruginosa* with the MIC of 37.5mg/ml and MBC of 75mg/ml.

This clearly demonstrates that the extract did not act on the test cultures depending on their cell wall properties but most likely it is through the presence of the tannins and the alkaloids plus other phytochemicals that may be present and were not screened for their presence. Such phytochemicals may possess some additive and/or synergistic properties that may be responsible for its activity. Hassan *et al.*, (2006) showed that *B. angustifolia* possess significant ($P < 0.05$) inhibitory activities against all the tested bacterial isolates (*S. aureus, P. aeruginosa, E. coli* and *S. pneumoniae*) with the exception of *S. typhi* at 30-120 and 10-120 mg/ml, respectively. This partly agrees with this study and partly not as it showed activity in most of the test organisms including *S. typhi*.

From the survey the *Boscia angustifolia* bark is used by the Samburu community to treat diarrhoeal diseases. The antibacterial properties suggest its potential usefulness in traditional medicine for the treatment of diarhoreal diseases. Further investigations need to be done on the mode of activity of the plant.

5.13 *Euphorbia scarlatica*. (L.) O. Ktze.

The *Euphorbia scarlatica* plant belongs to the Euphorbiaceae family. The *Euphorbia scarlatica* plant extract showed a moderate to high activity against the Gram positive and Gram negative bacteria. For instance the extract was bactericidal with *S. aureas* (MIC of 37.5mg/ml and MBC of 37.5mg/ml), *S. typhi* (MIC of 18.75mg/ml and MBC of 18.75mg/ml) and *E.coli* (MIC of 37.5mg/ml and MBC of 37.5mg/ml). This clearly shows that the extract was more active against *S. typhi*.

On the other hand the extract has a possibility of being bacteriostatic, for instance the extract had an MIC of 18.75mg/ml and MBC of 37.5mg/ml with the *B. subtillis*. Also the extract produced MIC of 18.75mg/ml and MBC of 75mg/ml with the *P. aeruginosa.* The mode of action can be ascribed to cell wall properties because in general the extract was more active against the Gram negatives than the Gram positives. At the same time the cell wall properties can be supported by the presence of tannins, flavonoids, terpenoids, cardiac glycosides, and alkaloids which can offer some synergistic or additive properties. This clearly demonstrates that the medicinal plant has the potential of treating diarhoreal diseases. Further activity needs to be done to find out the mode of action of the extract against the test bacteria.

5.14 *Clerodendrum myriacoides* (Hochst.)Vatke subsp. *napperae* Verdc)

The *Clerodendrum myriacoides* belongs to the Verbenaceae family. The *Clerodendrum myriacoides* root extracts showed a high activity against the Gram negative and Gram positive bacteria. The extract further showed bactericidal properties against the two groups of the test bacteria. For instance the extract produced MICs and MBCs of 18.75mg/ml respectively in all the test cultures, a feature that was not observed in other extracts.

These activities of the *Clerodendrum myriacoides* root extracts can not be ascribed to the cell wall properties since the activity is similar in both the Gram positive and the Gram negative. This is because the presence of the phospholipids and lipopolysaccharides in the cell wall of the Gram negative are supposed to offer more resistance to permeability of the extract's phytochemicals. But the extract had high activity against the Gram negative and Gram positive bacteria thus presence of tannins, saponins, flavonoids, terpenoids, cardiac glycosides, and alkaloids can be the most possible factor for its activity given the fact that they have been found to possess some antibacterial activity before (Yu, *et al.,* 2000). For instance the antidiarrhoeal activity of flavonoids has been connected to their ability to inhibit intestinal motility and hydro-electrolytic secretion, (Rao, *et al*., 1997). Tannins on the other hand can evoke an antidiarrhoeal effect and these substances may precipitate proteins of the enterocytes, reduce peristaltic movement and intestinal secretion (Al-Rehaily, *et al.,* 2001).

The *Clerodendrum myriacoides* root extracts should be further investigated because it is clearly showing high activity in both the Gram positive and Gram negative bacteria. May be this is the reason why the Samburu community uses it to treat various diseases like

STIs, malaria, polio amongst many. Thus the findings of this research suggest its potential usefulness in traditional medicine for the various treatments it is used for. Further studies on its mode of action need to be done.

5.15 *Cordia monoica* Roxb.

The *Cordia monoica* plant belongs to the Boraginaceae family. The *Cordia monoica* root extract produced moderate activity in both the Gram negative and Gram positive test cultures. From the results obtained, the extract is possibly more of bacteriostatic to both the Gram positive and Gram negative bacteria. The extract actually produced an MIC of 37.5mg/ml and MBC of 75mg/ml in most of the test cultures other than the *P. aeruginosa* where it produced a MIC of 18.75mg/ml and MBC of 37.5mg/ml.

The results obtained can not be ascribed to the cell wall properties differences since the extract can inhibit the growth of the test cultures at the same concentrations. Therefore most likely the activity of the extract can easily be ascribed to the presence of tannins, saponins, and terpenoids among the phytochemicals that were screened for. These together with other phytochemicals that were not screened for might possibly be having some synergistic or additive activities against the test organisms. Despite the moderate activity of the extract, this still supports the traditional use of the plant for treatments but further studies need to be done to deduce it's mode of action.

5.16 *Acacia ethaaica* Schweinf.

The *Acacia ethaaica* plant belongs to the Mimosaceae family. The *Acacia ethaaica* bark extract produced very high activity against the Gram positive and the Gram negative test cultures. The activity of the extract was highest in *S. aureas, E.coli* and *P. aeruginosa* with an MIC of 9.38mg/ml and MBC of 9.38mg/ml respectively. The extract was also active on *B. subtillis* and *S. typhi* (MIC of 9.38mg/ml and MBC of 18.75mg/ml respectively). This clearly demonstrates the possibility of the extract to be bactericidal in nature since the MIC and MBC concentrations are equal.

The above activity can not be ascribed to the cell wall properties but possibly to the synergistic or additive activity of such compounds like tannins, saponins, flavonoids, terpenoids, cardiac glycosides, and alkaloids that were found to be present and have been found to possess antibacterial activity before (Ocharo, 2005). The *Acacia ethaaica* bark is used to treat stomach-ache by the Samburu community. The antibacterial properties suggest its usefulness in traditional medicine for the treatment of stomach-ache that can be caused by both the Gram positive and Gram negative bacteria. From the study, the *Acacia ethaaica* posses a rich source of antibacterial agents. Further studies for the isolation of the active principle(s) can provide biologically active constituents that may be used as drugs or to develop more safe and potent antibacterial life saving drugs.

5.17 *Acacia horrida* (L.) Willd.

The *Acacia horrida* plant belongs to the Fabaceae family. The *Acacia horrida* bark extract was more active against *P. aeruginosa* producing a MIC of 18.75mg/ml and MBC of 18.75mg/ml. In other Gram negatives the extract produced a moderate activity with the MIC of 37.5mg/ml and MBC of 37.5mg/ml. This clearly demonstrates that the extract could be having bactericidal effects against Gram negative bacteria. On the other hand the extract was also bactericidal in nature with the *S. aureas* (MIC of 37.5mg/ml and

MBC of 37.5mg/ml). Further activity was also observed in *B. subtilis* where a moderate bacteriostatic effect was observed (MIC of 37.5mg/ml and MBC of 75mg/ml).

Since the activity of the extract is almost the same except with the *P. aeruginosa* and *B. subtilis,* it can not however be ascribed to cell wall properties of the test organisms. This can be ascribed to the presence of the tannins, saponins, terpenoids, cardiac glycosides, and alkaloids which have been found to have the antibacterial properties (Ruttoh, 2009).

5.18 *Albizia anthelmitica* Brongn.

The *Albizia anthelmitica* plant extracts belongs to the Leguminosae family. There was moderate activity of *Albizia anthelmitica* plant extracts against both the Gram positive and Gram negative test cultures. For the Gram positive bacteria, the extract showed high activity against the *S. aureas* than the *B. subtilis* (MIC of 18.75mg/ml and MBC of 37.5mg/ml and MIC of 37.5mg/ml and MBC of 37.5mg/ml respectively). This can be ascribed to the fact that *B. subtilis* is spore forming and such spores can offer some resistance to the extract activity (Nakano and Zuber, 1998). On the other hand almost same activity was observed in the Gram negative test cultures with more activity of the extract observed with the *P. aeruginosa* (MIC of 18.75mg/ml and MBC of 18.75mg/ml).

The extract was more of bactericidal than bacteriostatic since it only had a different MIC and MBC with *S. aureas* (MIC of 18.75mg/ml and MBC of 37.5mg/ml). The activity of the extract can not be fully ascribed to the cell wall properties of the test cultures but can be due to the synergistic or additive properties that may be present due to the presence of the tannins, saponins, flavonoids, and alkaloids in this extract. This average activity of the extract against the test cultures screened might be the reason for the Samburu community not using the plant for the treatment of diarrhoeal diseases but just as a dewormer. Thus there is need for further studies on the plant to find out its effect on the worms.

The crude extracts at least showed activity against the yeast fungus *C. albicans*. The extract produced slightly higher zones of inhibition than that of fluconazole. This can be attributed to the synergistic or additive properties that may be present from the tannins, saponins, flavonoids, and alkaloids. On the other hand flavonoids have been associated with the antifungal properties before (Ruttoh, 2009). In the current upsurge of fungal infections due to HIV/AIDS scorch, there is need to focus on plants that have antifungal activity. This is with a view to contributing towards the possibility of discovery of new biologically active compounds with decreased or no toxicity and the side effects present in the current antifungal drugs.

5.19 *Acacia tortilis* (Forssk.) Hayne.

The *Acacia tortilis* plant belongs to the Mimosaceae family. The *Acacia tortilis* root extracts showed high activity against the Gram positive and the Gram negative test cultures. For the Gram positive test cultures the extract produced almost similar results. From the results got, it clearly demonstrates that the extract has a possibility of having both the bacteriostatic and bactericidal effect on the test cultures. For instance the extract produced an MIC of 18.75mg/ml and MBC of 18.75mg/ml with *S. aureas* and *E. coli.* Also the extract produced MIC of 18.75mg/ml and MBC of 37.5mg/ml with *B. subtillis* and *P. aeruginosa.*

This activity can not be ascribed to cell wall properties but to the presence of phytochemicals such as tannins, saponins, flavonoids, terpenoids, cardiac glycosides, and alkaloids (Vidari *et al.*, 2003). The presence of these compounds is in conformity with the findings of Kubmarawa *et al.,* (2007) who also got the same compounds in the same extracts. From studies done, flavonoids inhibit intestinal transit, secretion and motility also it modulates arachidonic metabolism (Rao *et al*., 1997). Incidentally the extract was not active against the fungal isolates.

These results also are in agreement with those of Kubmarawa *et al.,* (2007) that clearly demonstrated that the *Acacia tortilis* is highly active against *B. subtilis*, (NCTC8236) *E. coli*, (ATCC 9637) *S. aureus* (ATCC 13709) *P. aeruginosa*(ATCC 27853). In the same study the *Acacia tortilis* was found to have such compounds as saponins, glycosides, tannins, alkaloids, volatile oils and flavonoids. This also agrees with the findings of this study.

5.20 *Gomphocarpus fruticosus* (L.) W.T. Aiton.

The *Gomphocarpus fruticosus* plant belongs to the Apocynaceae family. The extracts of *Gomphocarpus fruticosus* showed moderate activity against both the Gram positive and Gram negative test cultures. There was no big difference between the MIC and MBC in most test cultures like the *S. aureas, S. typhi, E.coli and P.aeruginosa.* This clearly indicates that *Gomphocarpus fruticosus* is possibly bactericidal given the fact that both the MICs and the MBCs are at the same level (37.5mg/ml). It is only on *B. subtilis* that *Gomphocarpus fruticosus* produced a bacteriostatic activity with an MIC of 37.5mg/ml and MBC of 75mg/ml.

Thus from the results we can ascribe the activity of the *Gomphocarpus fruticosus* to the presence of tannins, flavonoids and cardiac glycosides together with some of the phytochemicals that might be present but whose presence was not investigated. Such phytochemicals may be having the synergistic or additive activity that could be responsible for the activity of the extract.

5.21 *Aloe secundiflora* Engl.

The *Aloe secundiflora* plant extracts also belongs to the Aloeaceae family. The extract produced moderate activity against the Gram positive and Gram negative test cultures. The extract was more bactericidal since most of the test cultures had both the MIC and MBC at the same level (MIC of 37.5mg/ml and MBC of 37.5mg/ml). It's only in *B. subtilis* and *P. aeruginosa* that the extract proved to be bacteriostatic with the MIC of 37.5mg/ml and MBC of 75mg/ml. The activity of the extract can not therefore be ascribed to the cell wall properties of the test cultures since the extract has almost the same activity to the test cultures. A possibility of additive or synergism activity might be present in the extract as it was found to have tannins, flavonoids, and terpenoids which have been found to possess antibacterial activities (Edeoga *et al.*, 2005). The extracts did not show any activity against the fungal test cultures.

The findings of this research therefore can back the traditional use of the medicinal plant in the treatment of stomach ache problems. However, studies should be carried out to determine its toxicity levels and the mode of action of the extract against enteric pathogens.

5.22 *Acacia nilotica* (L.) Del. Subsp. Adansonii (Guill. & Perr.)

The *Acacia nilotica* plant belongs to the Mimosaceae family. The *Acacia nilotica* roots/bark extracts showed a very high activity against the Gram positive and Gram negative test cultures. The extract is more bactericidal in nature because it actually had both MICs and MBCs of most test cultures at the same level. For instance *S. aureas, S. typhi* and *E.coli* had an MIC of 18.75mg/ml and MBC of 18.75mg/ml each. At the same time *P. aeruginosa* had the highest MIC of 9.38mg/ml and MBC of 9.38mg/ml. On the other hand the extract proved to be bacteriostatic in nature with the *B. subtillis* by producing a MIC of 18.75mg/ml and MBC of 37.5mg/ml.

Since there is no difference of the activity of the extract against the Gram positive and Gram negative bacteria then the activity can not be ascribed to the cell wall properties. The extract was found to possess tannins, saponins, flavonoids, terpenoids, cardiac glycosides, and the alkaloids among the tested phytochemicals. These were among others that could be present and were not tested for their presence like the anthraquinones and comaurins might possess the synergistic or additive activity against the test cultures. These antimicrobial studies suggest that *Acacia nilotica* is a rich source of antibacterial agents. The antibacterial properties suggest its potential usefulness in traditional medicine for treatment of diarhoreal diseases. Further studies for the isolation of the active principle(s) can provide biologically active constituents that may be useful drugs or to develop more safe and potent antibacterial life saving drugs.

This study on the other hand is in accord with others that have been done. For instance Rani and Khullar, (2004) found that *Acacia nilotica* has activity against *S. typhi*. Also the findings back its use by the Samburu community in the treatment of diarrhoreal diseases.

On the other hand the crude extract of *Acacia nilotica* showed some substantial activity against the yeast fungi *C. albicans* only. This can be ascribed to the additive or synergistic activity of the phytochemicals present as they have been known to possess such a property (Bohs, 2001).

5.23 *Teclea simplicifolia* (Engl.) Verdc.

The *Teclea simplicifolia* plant belongs to the Rutaceae family. The bark extract of *Teclea simplicifolia* showed moderate to high activity against the test cultures. There was no significant difference between the Gram positive and the Gram negative test cultures. The extract proved to be more bactericidal in most of the test cultures like the *S. typhi* and *S. aureas* with the MIC of 37.5mg/ml and MBC of 37.5mg/ml each. The extract was also bactericidal to *P. aeruginosa* with it having a higher activity than in other test cultures (MIC of 18.75mg/ml and MBC of 18.75mg/ml). The extract was also bacteriostatic in two of the test cultures i.e. *B. subtilis* and *E.coli* (MIC of 37.5mg/ml and MBC of 75mg/ml).

Since the activity is almost the same in both the Gram negative and Gram positive bacteria, it can not be attributed to the cell wall properties. But presence of such active compounds like tannins, saponins, flavonoids, terpenoids, cardiac glycosides and alkaloids can be a contributing factor since such compounds have been found to be active against bacteria (Jigna and Sumitra, 2007). However medicinal plant did not act so well with the test cultures and it might be the reason why the Samburu community uses it for malaria treatment. Thus there is need for further investigation against its antimalarials properties.

5.24 *Plumbago dawei* Rolfe.

Plumbago dawei Rolfe belongs to Plumbaginaceae family. The bark extracts of *Plumbago dawei* showed a tremendous effect on the test cultures in both the Gram negatives and the Gram positives. It is one of the medicinal plants that produced excellent results. The extract was more of bactericidal in all the test cultures except with the *B. subtilis* (MIC of 9.38mg/ml and MBC of 18.75mg/ml). It actually produced MICs of 9.38mg/ml and MBCs of 9.38mg/ml against *S. aureas, S. typhi, E. coli*, and *P. aeruginosa.*

The activity of the extract also can not be ascribed to the cell wall properties since the activity is similar in both the Gram positive and the Gram negative bacteria. The activity can be due to the presence of such phytochemicals as tannins, flavonoids, and cardiac glycosides that were present but in moderate amounts. These together with the other phytochemicals that were not screened for their presence can be a possible ascription for the activity of the extract.

This antimicrobial study suggests that *Plumbago dawei* is a rich source of antibacterial agents. Further studies for the isolation of the active principle(s) can provide biologically active constituents that may be useful drugs or to develop more safe and potent antibacterial life saving drugs. Also the cytoxicity screening and antimalarials efficacy needs to be done on the extract. The findings support the traditional usage of the plant for treatment of diarrhea since it has proved to inhibit the growth of the bacterial isolates.

5.25 *Solanum incanum* L.

The *Solanum incanum* plant belongs to Solanaceae family. The extract showed poor activity against the test cultures other than the *P. aeruginosa* though it proved to be more of bacteriostatic in most test cultures than bactericidal. For instance the extract was bacteriostatic with *B. subtilis, S. typhi* and *E. coli* (MIC of 37.5mg/ml and MBC of 75mg/ml in each). Poor bactericidal activity of the extract was observed in *S. aureas*-MIC of 75mg/ml and MBC of 75mg/ml. On the other hand the extract showed high bactericidal activity against *P. aeruginosa* with MIC of 18.75mg/ml and MBC of 18.75mg/ml.

The activity of the extract can be ascribed partly to the differences in the cell wall properties but also the presence of tannins, saponins, flavonoids, cardiac glycosides while alkaloids compounds can play a good role towards its activity. This study agrees with others as the extracts possess alkaloids which are known to be diverse in the *Solanum* genus (Bohs, 2001).

The activity of the extract so far is the poorest when it is compared with other test cultures. The findings can agree with the Samburu community who use the plant for treatment of the malaria. Thus more studies need to be carried out to determine the efficacy of the plant against the *Plasmodium spp* that is known to cause malaria.

5.26 *Acokanthera friesiorum* Markgr.

The *Acokanthera friesiorum* plant belongs to the Apocynacea family. There was moderate to high activity of the extract against the Gram positive and Gram negative test cultures respectively. The extract possessed both bacteriostatic and bactericidal effects on the test cultures. For instance the extract was bactericidal with *S. aureas* (MIC of 37.5mg/ml and MBC of 37.5mg/ml) and *E. coli* (MIC of 18.75mg/ml and MBC of 18.75mg/ml). On the other hand the extract was bacteriostatic against *B. subtilis* (MIC of 37.5mg/ml and MBC of 75mg/ml), *S. typhi* (MIC of 18.75mg/ml and MBC of 37.5mg/ml) and *P. auroginosa* (MIC of 18.75mg/ml and MBC of 37.5mg/ml).

These findings clearly demonstrate that the differences in the activity of the extract may be not due to the cell wall differences since the activity of the extract was higher on the Gram negative than the Gram positive test cultures. The reason for the different sensitivity could be ascribed to the cell wall morphological differences between these micro organisms (Ruttoh, 2009). Also the activity of the extract can also be ascribed to the presence of the tannins, flavonoids, and cardiac glycosides which could be having some synergistic or additive activity against the test cultures.

The findings of the study can therefore support the usage of the plant for the treatment of the diarhoreal diseases but further studies need to be done to deduce the cytotoxicity effects and mode of action of this medicinal plant.

5.27 *Ocimum suave* Willd.

The *Ocimum suave* plant belongs to the Lamiaceae family. There was a high activity of the extract against the Gram positive and Gram negative test cultures. The extract showed both bacteriostatic and bactericidal properties on the test cultures. For instance the extract had a bacteriostatic activity against *S. aureas* and *B. subtilis* with MIC of 18.75mg/ml and MBC of 37.5mg/ml in both. On the other hand the extracts showed bactericidal activity with *S. typhi* (MIC of 18.75mg/ml and MBC of 18.75mg/ml), *E.coli* (MIC of 18.75mg/ml and MBC of 18.75mg/ml) and *P. auroginosa* (MIC of 37.5mg/ml and MBC of 37.5mg/ml).

The activity of the extract can not be ascribed to the cell wall morphology differences since the results are almost similar. Thus the results can be ascribed to the presence of tannins, flavonoids, terpenoids, cardiac glycosides and the alkaloids which can possess the additive and synergistic activities to the test cultures. Such phytochemicals have been found to possess some antibacterial activities (Mallikharjuna *et al.,* 2007). The findings therefore can back the traditional usage of the medicinal plant in the treatment of diarhoreal diseases. However studies should be carried out to determine its toxicity levels and the mode of action against the enteric pathogens.

5.28 *Thylachium africanum* Lour.

Thylachium africanum belongs to Capparaceae family. There was a high activity of the extract among the Gram positive and Gram negative test cultures. According to the findings the extract proved to be bactericidal in nature since both the MICs and MBCs are at the same level. The high activity was observed in *S. aureas, S. typhi,* and *P. aeruginosa* with all having MICs of 18.75mg/ml and MBCs of 18.75mg/ml. *E. coli* and

B. subtilis had also same MIC of 37.5mg/ml and MBC of 37.5mg/ml. These differences can be possibly be due to the spore forming nature of the *B. subtilis* which can offer some resistance to this extract.

At the same time the activity of the extract can not be ascribed to the cell wall properties as the extract showed the same activity against the Gram positive and the Gram negative test cultures. Thus most probably the presence of such compounds like tannins, saponins, flavonoids, cardiac glycosides, and alkaloids can produce a synergistic activity.

The extract also produced some activity against *C. albicans* a yeast fungus. The moderate activity of the extract is difficult to be associated to any of the class (es) of the compounds present due to the type of activity presented. Nevertheless, it is reasonable to speculate that the activity could be related to the synergism or additive activity of two or more of the compounds present e.g. the tannins, saponins, flavonoids e.t.c. Therefore the findings of the study can support the usage of the *Thylachium africanum* for the treatment of the diarhoreal diseases but further studies need to be done to deduce the cytotoxicity effects and mode of action of the medicinal plant.

5.29 *Jasminum abyssinicum* Hochst. ex DC.

The *Jasminum abyssinicum* belongs to Oleaceae family. The *Jasminum abyssinicum* bark extracts showed a moderate activity against the Gram positive and Gram negative test cultures. The extract showed both the bacteriostatic and bactericidal activity. For instance the extract was bactericidal against *S. aureas*, *E.coli* and *P. aeruginosa* each having an

MIC of 37.5mg/ml and MBC of 37.5mg/ml. On the other hand the extract produced a MIC of 37.5mg/ml and MBC of 75mg/ml against the *B. subtilis* and *S. typhi*.

This clearly demonstrates that the activity of the extract is not based on the cell wall properties but can be ascribed to the presence of saponins and the terpenoids that have the antimicrobial properties (Toda *et al.*, 1989). This is because the extract acted in the similar manner in both Gram positive and Gram negative test cultures.

5.30 *Croton macrostachysu* (A. Rich). Benths

Croton macrostachysu is a plant that belongs to the Euphorbiaceae family. There was poor activity of the extract against the test cultures except for the Gram negative *P.aeruginosa*. The extract proved to have bacteriostatic and bactericidal activity against both the Gram positive and Gram negative test cultures. For instance the extract produced the same MIC of 37.5mg/ml and MBC of 75mg/ml against the S. *aureas*, and *B. subtilis.* The extract was only bactericidal against the Gram negative test cultures with it producing high activity against *P.aeruginosa* (MIC of 18.75mg/ml and MBC of 18.75mg/ml). Thus the extract has can be used to control the growth of the *P.aeruginosa* that is known to offer a lot resistance to most antibiotics (Omwenga *et al.*, 2009).

Thus there was low activity on the Gram positive bacteria than the Gram negative bacteria and can partly be ascribed to differences in the cell wall properties. Secondly the presence of the saponins and flavonoids can also have some additive effect against the test cultures.

5.31 *Ormocarpum trachycarpum*. (Taub) Harms

The extract belongs to Legumiminosae family. The *Ormocarpum trachycarpum* extract showed some poor activity against most of the test cultures. The extract proved to have both the bacteriostatic and bactericidal activities. For instance bacteriostatic activities were observed when the extract acted against *P. aeruginosa*, *S. typhi* and *B. subtilis* all having equal MICs and MBCs of 37.5mg/ml and 75mg/ml respectively. Bactericidal activities were observed in *S. aureas* and *E.coli*.

Thus the activity of the extract can not be ascribed to the cell wall properties as in *Acokanthera friesiorum* since the activity is almost the same. But the activity can be ascribed to the presence of tannins, saponins, terpenoids, and cardiac glycosides which could be offering the synergistic activities to the test cultures. For instance the tannins have been found to have an ability to inactivate microbial adhesins, enzymes, cell envelope transport proteins, etc. There is also evidence for direct inactivation of microorganisms (Cowan, 1999).

5.32 *Cordia purpurea* (Picc.) Ait. Aiton

Cordia purpurea belongs to the Boraginaceae family. There was a moderate to high activity of the *Cordia purpurea* extract against both the Gram positive and the Gram negative test cultures. The extract exhibited both the bacteriostatic and bactericidal activities. There was a big difference in terms of activity against the two Gram positive test cultures and may be ascribed to the fact that the *B. subtillis* is a spore forming pathogen and such spores may offer some resistance to the extract action.

Bacteriostatic activities were observed when the extract acted against the *B. subtilis* (MIC of 37.5mg/ml and MBC of 75mg/ml), *S. typhi* (MIC of 37.5mg/ml and MBC of 75mg/ml), *and P. aeruginosa* (MIC of 18.75mg/ml and MBC of 37.5mg/ml). On the other hand the extract had a high bactericidal activity against *S. aureas* (MIC of 18.75mg/ml and MBC of 18.75mg/ml) and *E.coli* (MIC of 18.75mg/ml and MBC of 18.75mg/ml). The activity of the extracts may be due to the presence of tannins, saponins, flavonoids, and alkaloids that could be having some synergistic activities. Such compounds have been found before to be having antidiarrhoeal effect (Palombo, 2006). Thus the findings of this study can back the traditional use of the medicinal plant as it has been found to posses some antidiarrhoeal activity. However, further studies need to be carried out to deduce its cytotoxicity levels and mode of action.

5.33 *Euclea divinoram* Hiern.

The *Euclea divinoram* plant belongs to the Ebenaceae family. There was a moderate to high activity of the extract against Gram positive and Gram negative test cultures. The extract was found to exhibit both the bactericidal and bacteriostatic activities. The extract proved to have higher activity against the Gram negatives than the Gram positives. Bactericidal effects of the extracts were observed in S. *aureas* (MIC of 37.5mg/ml and MBC of 37.5mg/ml), *S. typhi* (MIC of 18.75mg/ml and MBC of 18.75mg/ml), and *E. coli* (MIC of 18.75mg/ml and MBC of 18.75mg/ml). Bacteriostatic activities were observed in *B. subtilis* and *P. aeruginosa* both with MIC of 18.75mg/ml and MBC of 37.5mg/ml.

Thus the activity of the extract can not be due to the cell wall properties thus different morphological differences that may vary the action of the extract (Ruttoh, 2009). Also the presence of tannins, saponins, flavonoids, terpenoids and alkaloids can offer some additive activity against the test cultures differently. Such phytochemicals have been found to possess some antibacterial activity before (Martins *et al.*, 2001).

These antimicrobial studies suggest that *Euclea divinoram* is a rich source of antibacterial agents. Further studies for isolation of the active principle(s) can provide biologically active constituents that may be used as drugs or develop more safe and potent antibacterial life saving drugs. It also supports the use of this plant in traditional medicine.

5.34 *Balanites aegyptiaca* (L.) Del.

The *Balanites aegyptiaca* belongs to the Balanitaceae family. The root extracts of *Balanites aegyptiaca* showed moderate to higher activity against the test organisms. The results obtained in this study indicate considerable differences in antibacterial activity of the *Balanites aegyptiaca* extracts against Gram positive and Gram negative bacteria. This activity was more pronounced against Gram positive than the Gram negative bacteria. The reason for the different sensitivity could be ascribed to the morphological differences between these microorganisms, Gram negative bacteria having an outer phospholipids membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to lipophilic solutes, while the porins constitute a selective barrier to the hydrophilic solutes with an exclusion limit of about 600 Da. On the other hand the Gram positive bacteria are susceptible because of having only an outer peptidoglycan layer which is not an effective permeability barrier (Ruttoh, 2009).

The extract also exhibited both the bactericidal and bacteriostatic activity against the test cultures. For instance the extract was bactericidal against *S. typhi* (MIC of 18.75mg/ml and MBC of 18.75mg/ml) and *E. coli* (MIC of 37.5mg/ml and MBC of 37.5mg/ml). On the other hand the extract was bacteriostatic against *S. aureas* (MIC of 18.75mg/ml and MBC of 37.5mg/ml), *B. subtilis* (MIC of 18.75mg/ml and MBC of 37.5mg/ml) and *P. aeruginosa* (MIC of 18.75mg/ml and MBC of 37.5mg/ml). These findings also agree with those got by Doughari *et al.*, (2007) that the extracts of *Balanites aegyptiaca* are active against such Gram negative bacteria like the *S. typhi*. *B. aegyptiaca* produced a higher zone of inhibition (16 mm zone of inhibition) than that of ciprofloxacin (10 mg/ml, 10 mm zone of inhibition) and the rest of the antibiotics. This is an indication that the plant extracts when purified will be very potent antityphoid agents. The results also agree with results from other studies; for instance Liu and Nakanishi, (1982) found that the extract is active against *B. subtilis.*

This activity can be ascribed to the presence of tannins, saponins, flavonoids, terpenoids and alkaloids since they have been known to have antibacterial properties. Such phytochemicals have also been found to be present in the same plant by another study reported to account for the exertion of antimicrobial activity by plants (Pretorius and Watt, 2001; Doughari *et al*., 2007).

The antibacterial properties got from the study suggest its potential usefulness in traditional medicine. However, further studies needs to be carried out on the extract to deduce its mode of action and cytotoxicity levels.

5.35 Plants with no activity.

The plants that had no antimicrobial activity especially against the fungal test cultures could be having other medicinal value including immune boosting or nutritional properties. There is also a possibility of synergism as some of them are in combination and boiled before use. Studies based on these possibilities should be carried out on plants such as *Salvadora persica, Cordia sinensis, Grewia simi, Acacia horrida, Acacia ethaaica*, *Aloe secundiflora*, *Cissus quadrangularis* e.t.c

5.36 Standard antibiotics.

The bacteria used in the study showed susceptibility to the common antibiotics. The NCCLS (2002) recommendations for the drugs used to be considered as resistant are amoxicillin ≤ 13mm, chloramphenicol ≤ 12mm, tetracycline ≤14mm and fluconazole ≤10mm. The standard antibiotics used as quality control in the determination of MICs showed activity in conformity with the patterns determined by NCCLS (2002). Amoxicillin produced zones of inhibition as follows: *S. aureas* (21.33mm), *S. typhi* (24.17mm), *B. subtillis* (17.17mm), *E. coli* (23.58mm) and *P. aeruginosa* (17.58mm). Thus no test culture proved to be resistant to amoxicillin.

Cefrodoxima was used as positive control for activity in performing the MIC assay. The results showed MICs against *S. aureas* (18.75 mg/ml), *S. typhi* (18.75 mg/ml), *B. subtillis* (18.75 mg/ml), *E. coli* (18.75 mg/ml) and *P. aeruginosa* (9.38 mg/ml). The negative controls did not show any sign of inhibiting bacterial growth.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The aim of the project was to find out whether the medicinal plants used by the Samburu community are active against the diarhoea causing pathogens. Among the conclusions that were observed from the study includes:-

- Ethnobotanical survey revealed that in deed the Samburu community uses medicinal plants to treat themselves against the diarrhoreal diseases.
- Most of the extracts screened actually had substantial inhibitory properties on the growth of the bacterial test cultures. However it was noted that the extracts had no substantial inhibitory properties against the fungal test cultures except for the *C. albicans* that was slightly sensitive to four extracts (*Acacia nilotica, Thylachium africanum, Albizia anthelmitica & Loranthus acaciae*). More inhibition for growth was observed among Gram negative bacterial cultures than Gram positive bacterial cultures. Among the Gram positive test cultures *S. aureas* was more sensitive to the extracts than the *B. subtilis* while *P. aeruginosa* was the more sensitive Gram negative bacteria to the extracts and the most affected by the extracts in general.
- Most extracts also produced moderate to high MICs and MBCs against the bacterial isolates with most showing both bactericidial and bacteriostatic properties in the different varieties of bacterial test cultures. *Acacia ethaaica* and *Plumbago dawei* and Acacia *nilotica* were among the extracts that showed very high activity on the bacterial test cultures. However all the plants except *Teclea*

Simplicifolia and *Cordia monoica* extracts showed bacteriocidial property against *E. coli*. *P.aeruginosa* was the most sensitive to the extracts than all other bacterial test cultures.

- The presence of a variety of the phytochemicals that were screened can be the reason for the activity of the Samburu medicinal plants screened. Among the phytochemicals screened tannins were found to be the most abundant followed by flavonoids and saponins. Such phytochemicals have been found to possess some synergistic/additive or antagonistic properties that may be responsible for their activity. For instance flavonoids have also been found to possess some activity and their activity is probably due to their ability to complex with extra cellular and soluble proteins and also complex with bacterial cell walls. More lipophilic flavonoids may also disrupt microbial membranes (Tsuchiya *et al.,* 1996).

Thus herbal remedies may consist of a single plant species though in some cases it may be made of two or more different plant remedies. Since the herbalists use single or more plant materials, these plants can therefore be considered to have the desired effects. Several extracts are promising due to their broad spectra of activity. Thus this study ascertains the value of these plants.

Also the results clearly demonstrate that the medicinal plants used by the Samburu community have a medicinal value. This is because most extracts were active against most of the test organisms including *P. aeruginosa* which is a difficult micro organism to control. The results thus demonstrate that medicinal plants can be used as alternatives in treatment of diarrhoreal diseases. Therefore there is need for establishing a good working relationship in Kenya between traditional healers and scientific institutions to rapidly identify and evaluate these plants before they are extinted by the forces of nature.

6.2 Recommendations

Following the results of this study the following recommendations are proposed:-

I. Indigenous knowledge of Samburu community on use of these medicinal plants should be recognized, protected and promoted since they are found to have the antidiarrhoeal activity.

II. The community should be encouraged to conserve and cultivate the important medicinal plants like *Acacia ethaaica, Albizia anthelmitica, Acacia nilotica, Euclea divinoram,* and *Plumbago dawei* amongst many which grow in the wild since they have been found to possess high antimicrobial activity against the tested isolates. This is to reduce chances of being cleared from the indigenous forests.

III. More work on *Acacia ethaaica, Albizia anthelmitica, Acacia nilotica, Euclea divinoram,* and *Plumbago dawei* amongst the other active plants as per this study are recommended in order to isolate, purify and characterize the active component(s) in them.

IV. Fairly large populations of Kenyans use herbal preparations as an alternative to modern medicines and their preparations need to be evaluated for safety, efficacy and quality.

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APPENDICES

Appendix I - QUESTIONNAIRE

Medicinal plants Questionnaire

Recorder: ……………………………………….SEX………………………………
Date:…………………………..……………… District:………………………….
Division:……………………………….. Conservancy:…………………….

1. Have you visited a traditional healer this year?

Yes ……………………… No………………………

2 .Have other members of your family also visited a traditional healer this year?

Yes ………………………. No………………………

3. How do you travel to the traditional healer?

4. If you walk, how long does it take you?

Less than 1 hour	
1hr to 1.5hrs	
Over 2 hrs	

5. How many times did you visit a clinic or doctor last year? (Not a traditional healer).

None	
Once	
Two to three times	
More than six times	

6. How do you travel to the clinic?

7. If you walk, how long does it take you?

8. If you use a matatu how much does it cost you (return fare)?

9. If you get treatment at the clinic is it more expensive or cheaper than the traditional treatment?

Cheaper	
Same	
More expensive	

10. If a clinic was near to your house, would you use traditional medicines more or less often?

Less often	
More often	
It depends on what treatment i need	

11. Do you prefer traditional medicine for the treatment of stomach illness?

Yes…………………………… … No…………………………………

12. Why do you prefer traditional medicine to hospital medicine?

TM is cheap	
TM is readily available	
Hospitals are far away	

13. Which plants are used for treating people with stomachache?

14. Which parts of these plants (above) that are used?

15. How do you prepare these plants (above) before they are administered to the patient?

APPENDIX II. Average zones of inhibition (mm) of the plant extract against the bacterial test cultures

BOTANICAL NAME	*S.aureas*	*B. subtilis*	*S. typhi*	*E. coli*	*P. aeruginosa*
1) *Acacia ethaaica*	19.33	19	16.66	17.66	19.33
2) *Acacia horrida*	18	17.66	19	18.66	28.66
3) *Acacia nilotica*	20.33	18	21	21.66	27.66
4) *Acacia nubica*	17.33	15.66	13.33	13.33	15.33
5) *Acacia senegal*	11.33	15	11	12.66	18.33
6) *Acacia tortilis*	15	13	12.33	11.33	21.33
7) *Acokanthera friesiorum*	14	11	11	10.66	19
8) *Albizia anthelmitica*	16	13.33	13.33	10	28.66
9) *Aloe secundiflora*	13.66	13.33	12.33	13.66	27
10) *Balanites aegyptiaca*	15.66	12.66	16	18	17
11) *Boscia angustifolia*	7	8	10.33	11.33	20
12) *Cissus rotundifolia*	14.33	13.66	9.66	11.33	17.66
13) *Cissus quadrangularis*	10.33	12.66	12.66	13.66	23
14) *Clerodendrum myriacoides*	14.33	15	14	16	25.33
15) *Commiphora africana*	13	14.33	16	16.33	21.66
16) *Cordia monoica*	14	18	15.66	16.33	36.33
17) *Cordia purpurea*	15	13	11.33	15	23.66
18) *Cordia sinensis*	14.33	13.33	12.33	16.33	19.66
19) *Croton macrostachysu*	23.66	12.66	11.66	12	18
20) *Euclea divinoram*	11.66	13	14.33	13.33	19.33
21) *Euphorbia scarlatica*	11.33	10.66	10.66	11.33	13
22) *Gomphocarpus fruticosus*	7.66	10	8.33	10	12.66
23) *Grewia simi*	14.33	13.66	12.66	12.66	14
24) *Jasminum abyssinicum*	11	12.33	10.66	10.66	14.33
25) *Kedrostis pseudogijef*	13.33	12.66	11.66	12	18
26) *Loranthus acaciae*	16.66	15	11.66	16.33	25
27) *Ocimum suave*	14	14	16.66	18	21
28) *Ormocarpum trachycarpum*	8	11	8.66	11.33	25.33
29) *Plumbago dawei*	25.66	24	16	16.33	21.66
30) *Salvodora persica*	21.66	16.33	12.33	14.33	20
31) *Solanum incanum*	11.33	13.66	11.33	10.33	20
32) *Teclea simplicifolia*	13.66	10.66	13	14.66	18.33
33. *Thylachium africanum*	18.66	17.33	16.66	15	23.33

TABLE CONT…

BOTANICAL NAME	***S. aureas***	***B. subtilis***	***S. typhi***	***E. coli***	***P. aeroginosa***
34. Amoxicillin	21.33	17.17	24.17	23.58	1758
35) -ve control	6	6	6	6	6

Appendix III: Average zones of inhibition (mm) of the plant extract against the fungal test cultures

PLA NT NAME	*C. albicans*	*A. niger*	*A. flavus*	*F. lateritium*	*Penicillium spp*
***Cissus quadrangularis* L.**	06	06	06	06	06
Salvodora persica L. var. persica	06	06	06	06	06
***Cordia sinensis* Lam.**	06	06	06	06	06
Grewia simi	06	06	06	06	06
***Loranthus acaciae* Zucc.**	12	06	06	06	06
***Commiphora africana* (A. Rich) Engl. Var.**	9.33	06	06	06	06
Euphorbia scarlatica	06	06	06	06	06
***Acacia ethaaica* Schweinf.**	06	06	06	06	06
***Acacia horrida* (L.) Willd.**	06	06	06	06	06
***Albizia anthelmitica* Brongn.**	13.67	06	06	06	06
***Acacia tortilis* (Forssk.) Hayne.**	06	06	06	06	06
***Aloe secundiflora* Engl.**	06	06	06	06	06
***Acacia nilotica* (L.) Del.**	13	06	06	06	06
***Acokanthera friesiorum* Markgr.**	06	06	06	06	06
***Thylachium africanum* Lour.**	06	06	06	06	06
Fluconazole	13	12	14	13	11
Negative control	06	06	06	06	06

Appendix IV: The MICs and MBCs (mg/ml) produced by the selected Samburu medicinal plants against the bacterial test cultures.

	S. aureas		***B. subtilis***		***S. typhi***		***E. coli***		***P. aeroginosa***	
BOTANICAL PLANT NAME	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Acacia ethaaica Schweinf.	9.38	9.38	9.38	18.75	18.75	18.75	9.38	9.38	9.38	9.38
Acacia horrida (L.) Willd.	37.5	37.5	37.5	75	37.5	37.5	37.5	37.5	18.75	18.75
Acacia nilotica (L.) Del.	18.75	18.75	18.75	37.5	18.75	18.75	18.75	18.75	9.38	9.38
Acacia nubica Benth.	37.5	75	37.5	37.5	37.5	75	37.5	37.5	18.75	37.5
Acacia senegal (L.) Willd.Var. *Persica*	37.5	75	37.5	75	37.5	37.5	18.75	18.75	37.5	37.5
Acacia tortilis (Forssk.) Hayne.	18.75	18.75	18.75	37.5	37.5	75	18.75	18.75	18.75	37.5
Acokanthera friesiorum Markgr.	37.5	37.5	37.5	75	18.75	37.5	18.75	18.75	18.75	37.5
Albizia anthelmitica Brongn.	18.75	37.5	37.5	37.5	37.5	37.5	37.5	37.5	18.75	18.75
Aloe secundiflora Engl.	37.5	37.5	37.5	75	37.5	37.5	37.5	37.5	37.5	75
Balanites aegyptiaca (L.)Del.	18.75	37.5	18.75	37.5	18.75	18.75	37.5	37.5	18.75	37.5
Boscia angustifolia Guill. and Perr.	37.5	37.5	37.5	75	37.5	75	37.5	37.5	37.5	75
Cissus rotundifolia Forsk. Vahl.	18.75	37.5	37.5	75	37.5	75	37.5	37.5	18.75	37.5
Cissus quadrangularis L.	37.5	75	37.5	75	37.5	37.5	37.5	37.5	37.5	37.5
Clerodendrum myriacoides (Hochst.) Vatke subsp. *napperae* Verdc	18.75	18.75	18.75	18.75	18.75	18.75	18.75	18.75	18.75	18.75
Commiphora africana (A. Rich) Engl. Var. *persica*	37.5	37.5	18.75	37.5	18.75	18.75	18.75	18.75	18.75	18.75
Cordia monoica Roxb.	37.5	37.5	37.5	75	37.5	75	37.5	75	18.75	37.5
Cordia purpurea (Picc.) Aiton	18.75	18.75	37.5	75	37.5	75	18.75	18.75	18.75	37.5
Cordia sinensis Lam.	37.5	37.5	18.75	37.5	18.75	18.75	37.5	37.5	37.5	37.5

TABLE CONT….

	S. aureas		***B. subtilis***		***S. typhi***		***E. coli***		***P. aeroginosa***	
BOTANICAL PLANT NAME	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Croton macrostachysu (A. Rich). Benths	37.5	75	37.5	75	37.5	37.5	37.5	37.5	18.75	18.75
Euclea divinoram Hiern.	37.5	37.5	18.75	37.5	18.75	18.75	18.75	18.75	18.75	37.5
Euphorbia scarlatica S. Carter	37.5	37.5	18.75	37.5	18.75	18.75	37.5	37.5	18.75	75
Gomphocarpus fruticosus (L) W.T. Aiton	37.5	37.5	37.5	75	37.5	37.5	37.5	37.5	37.5	37.5
Grewia simi L.	37.5	37.5	37.5	75	37.5	75	37.5	37.5	18.75	37.5
Jasminum abyssinicum Hochst. Ex DC.	37.5	37.5	37.5	75	37.5	75	37.5	37.5	37.5	37.5
Kedrostis pseudogijef (Gilg) C. Jeffrey	37.5	75	37.5	75	37.5	75	37.5	37.5	18.75	37.5
Loranthus acaciae Zucc.	37.5	37.5	37.5	75	37.5	75	37.5	37.5	37.5	37.5
Ocimum suave Willd.	18.75	37.5	18.75	37.5	18.75	18.75	18.75	18.75	37.5	37.5
Ormocarpum trachycarpum (Taub.) Harms.	75	75	37.5	75	37.5	75	37.5	37.5	37.5	75
Plumbago dawei Rolfe.	9.38	9.38	9.38	18.75	9.38	9.38	9.38	9.38	9.38	9.38
Salvodora persica L.var. *persica*	75	75	37.5	75	37.5	75	37.5	37.5	37.5	37.5
Solanum incanum L.	75	75	37.5	75	37.5	75	37.5	37.5	18.75	18.75
Teclea simplicifolia (Engl.) Verdc.	37.5	37.5	37.5	75	37.5	37.5	37.5	75	18.75	18.75
Thylachium africanum Lour.	18.75	18.75	37.5	37.5	18.75	18.75	37.5	37.5	18.75	18.75
Cefrodoxima +ve Control	18.75	18.75	18.75	18.75	18.75	18.75	18.75	18.75	9.38	9.38

APPENDIX V: TABLES PRESENTING THE DEGREE OF SIGNIFICANCE OF THE MEANS OF THE ZONES OF INHIBITION IN VARIOUS BACTERIAL TEST CULTURES

a) *S. aureas*

SOURCE OF VARIATION	MEAN	STD ERROR	F	P
Acacia ethaaica	101.6667	2.831314	2.1207*	0.00452
Acacia horrida	103.3333	2.831314		
Acacia nilotica	106.6667	2.831314		
Acacia nubica	109	2.831314		
Acacia Senegal	109	2.831314		
Acacia tortilis	107.3333	2.831314		
Acokanthera friesiorum	104	2.831314		
Albizia anthelmitica	115	2.831314		
Aloe secundiflora	108.6667	2.831314		
Balanites aegyptiaca	107.3333	2.831314		
Boscia angustifolia	113.6667	2.831314		
Cordia purpurea	111.3333	2.831314		
Cissus quadrangularis	108.3333	2.831314		
Cissus rotundifolia	109	2.831314		
Clerodendrum myriacoides	104.3333	2.831314		
Commiphora africana	109.6667	2.831314		
Cordia monoica	108.3333	2.831314		
Cordia sinensis	109	2.831314		
Croton macrostachysu	112.6667	2.831314		
Euclea divinoram	109.3333	2.831314		
Euphorbia scarlatica	114	2.831314		
Gomphocarpus fruticosus	110.6667	2.831314		
Grewia simi	105.3333	2.831314		
Jasminum abyssinicum	113.6667	2.831314		
Kedrostis pseudogijef	108.6667	2.831314		
Loranthus acaciae	108	2.831314		
Ocimum suave	107.6667	2.831314		
Ormocarpum trachycarupm	114.3333	2.831314		
Plumbago dawei	117	2.831314		
Salvodora persica	118	2.831314		
Solanum incanum	107	2.831314		
Teclea simplicifolia	115.3333	2.831314		
Thylachium africanum	101.3333	2.831314		
Amoxicillin	108.3333	2.831314		

There was significant difference of the means of the zones of inhibition at $P \leq 0.01$

b. *B. subtilis*

SOURCE OF VARIATION	MEAN	STD ERROR	F	P
Amoxicillin	111	2.711028	1.4049	0.11861
Acacia ethaaica	112	2.711028		
Acacia horrida	112	2.711028		
Acacia nilotica	106.3333	2.711028		
Acacia nubica	111	2.711028		
Acacia senegal	107.6667	2.711028		
Acacia tortilis	113.3333	2.711028		
Acokanthera friesiorum	104	2.711028		
Albizia anthelmitica	108.6667	2.711028		
Aloe secundiflora	107	2.711028		
Balanites aeghptiaca	107	2.711028		
Boscia angustifolia	111.6667	2.711028		
Cadia purpurea	104.3333	2.711028		
Cissus quadrangularis	108.6667	2.711028		
Cissus rotundifolia	112.3333	2.711028		
Clerondendrum myriacoides	109.3333	2.711028		
Commiphora africana	110	2.711028		
Cordia monoica	114.3333	2.711028		
Cordia sinensis	107	2.711028		
Croton macrostachysu	106	2.711028		
Euclea divinoram	110.6667	2.711028		
Euphorbia scarlatica	107.6667	2.711028		
Gomphocarpus fruticosus	110	2.711028		
Grewia simi	108.6667	2.711028		
Jasminum abyssinicum	108	2.711028		
Kedrostis pseudogijef	105.3333	2.711028		
Loranthus acaciae	106	2.711028		
Ocimum suave	106.6667	2.711028		
Ormocarpum trachycarupm	108.3333	2.711028		
Plumbago dawei	116.6667	2.711028		
Salvodora persica	106.3333	2.711028		
Solanum incanum	105	2.711028		
Teclea simplicifolia	109.3333	2.711028		
Thylachium africanum	101.3333	2.711028		

No significant difference of the means of the zones of inhibition at $P \leq 0.05$

c. *S. typhi*

SOURCE OF VARIATION	MEAN	STD ERROR	F	P
Amoxicillin	116.3333	2.893536	1.6499*	0.04129
Acacia ethaaica	110.6667	2.893536		
Acacia horrida	104.3333	2.893536		
Acacia nilotica	109.6667	2.893536		
Acacia nubica	108.6667	2.893536		
Acacia senegal	106.3333	2.893536		
Acacia tortilis	105.6667	2.893536		
Acokanthera friesiorum	103.3333	2.893536		
Albizia anthelmitica	107	2.893536		
Aloe secundiflora	110.6667	2.893536		
Balanites aeghptiaca	102.6667	2.893536		
Boscia angustifolia	110	2.893536		
Cadia purpurea	109.3333	2.893536		
Cissus quadrangularis	108.6667	2.893536		
Cissus rotundifolia	105.6667	2.893536		
Clerondendrum myriacoides	115	2.893536		
Commiphora africana	103.6667	2.893536		
Cordia monoica	106.6667	2.893536		
Cordia sinensis	112.6667	2.893536		
Croton macrostachysu	107.3333	2.893536		
Euclea divinoram	106.3333	2.893536		
Euphorbia scarlatica	111.6667	2.893536		
Gomphocarpus fruticosus	110.6667	2.893536		
Grewia simi	102	2.893536		
Jasminum abyssinicum	106	2.893536		
Kedrostis pseudogijef	107.6667	2.893536		
Loranthus acaciae	115	2.893536		
Ocimum suave	109	2.893536		
Ormocarpum trachycarupm	109	2.893536		
Plumbago dawei	111	2.893536		
Salvodora persica	109.6667	2.893536		
Solanum incanum	106.3333	2.893536		
Teclea simplicifolia	113.6667	2.893536		
Thylachium africanum	102	2.893536		

There was a significant difference between the means for the zones of inhibition against S. typhi at P≤ 0.01.

d. *E. coli*

SOURCE OF VARIATION	MEAN	STD ERROR	F	P
Amoxicillin	114.6667	2.518559	1.4314	0.10639
Acacia ethaaica	105.3333	2.518559		
Acacia horrida	108.6667	2.518559		
Acacia nilotica	113.3333	2.518559		
Acacia nubica	107.6667	2.518559		
Acacia Senegal	112.6667	2.518559		
Acacia tortilis	105.3333	2.518559		
Acokanthera friesiorum	103.3333	2.518559		
Albizia anthelmitica	107	2.518559		
Aloe secundiflora	108.6667	2.518559		
Balanites aeghptiaca	107.3333	2.518559		
Boscia angustifolia	107	2.518559		
Cadia purpurea	109.6667	2.518559		
Cissus quadrangularis	104	2.518559		
Cissus rotundifolia	108.6667	2.518559		
Clerondendrum myriacoides	109.3333	2.518559		
Commiphora africana	107.6667	2.518559		
Cordia monoica	104	2.518559		
Cordia sinensis	104.6667	2.518559		
Croton macrostachysu	111.3333	2.518559		
Euclea divinoram	107.6667	2.518559		
Euphorbia scarlatica	108.6667	2.518559		
Gomphocarpus fruticosus	107	2.518559		
Grewia simi	110	2.518559		
Jasminum abyssinicum	102.6667	2.518559		
Kedrostis pseudogijef	104	2.518559		
Loranthus acaciae	107.3333	2.518559		
Ocimum suave	106	2.518559		
Ormocarpum trachycarupm	109.6667	2.518559		
Plumbago dawei	104.3333	2.518559		
Salvodora persica	105.3333	2.518559		
Solanum incanum	106.3333	2.518559		
Teclea simplicifolia	105	2.518559		
Thylachium africanum	102	2.518559		

There was no significant difference between the means of the zones of inhibition at $P \leq 0.01$

e. *P.aeruginosa*

SOURCE OF VARIATION	MEAN	STD ERROR	F	P
Amoxicillin	108.3333	4.093674	2.2105*	0.00293
Acacia ethaaica	116.3333	4.093674		
Acacia horrida	109	4.093674		
Acacia nilotica	105.3333	4.093674		
Acacia nubica	110	4.093674		
Acacia senegal	108	4.093674		
Acacia tortilis	103.6667	4.093674		
Acokanthera friesiorum	105	4.093674		
Albizia anthelmitica	121	4.093674		
Aloe secundiflora	113.3333	4.093674		
Balanites aeghptiaca	110.6667	4.093674		
Boscia angustifolia	118.6667	4.093674		
Cadia purpurea	115	4.093674		
Cissus quadrangularis	111.3333	4.093674		
Cissus rotundifolia	108.6667	4.093674		
Clerondendrum myriacoides	121	4.093674		
Commiphora africana	107.6667	4.093674		
Cordia monoica	126	4.093674		
Cordia sinensis	114.3333	4.093674		
Croton macrostachysu	112.3333	4.093674		
Euclea divinoram	110.3333	4.093674		
Euphorbia scarlatica	119.3333	4.093674		
Gomphocarpus fruticosus	114.3333	4.093674		
Grewia simi	120	4.093674		
Jasminum abyssinicum	113	4.093674		
Kedrostis pseudogijef	112.6667	4.093674		
Loranthus acaciae	106.3333	4.093674		
Ocimum suave	106.3333	4.093674		
Ormocarpum trachycarupm	120	4.093674		
Plumbago dawei	113.6667	4.093674		
Salvodora persica	108.3333	4.093674		
Solanum incanum	105.3333	4.093674		
Teclea simplicifolia	123.6667	4.093674		
Thylachium africanum	102	4.093674		

There was a significant difference of means of zones of inhibition at $P \leq 0.01$